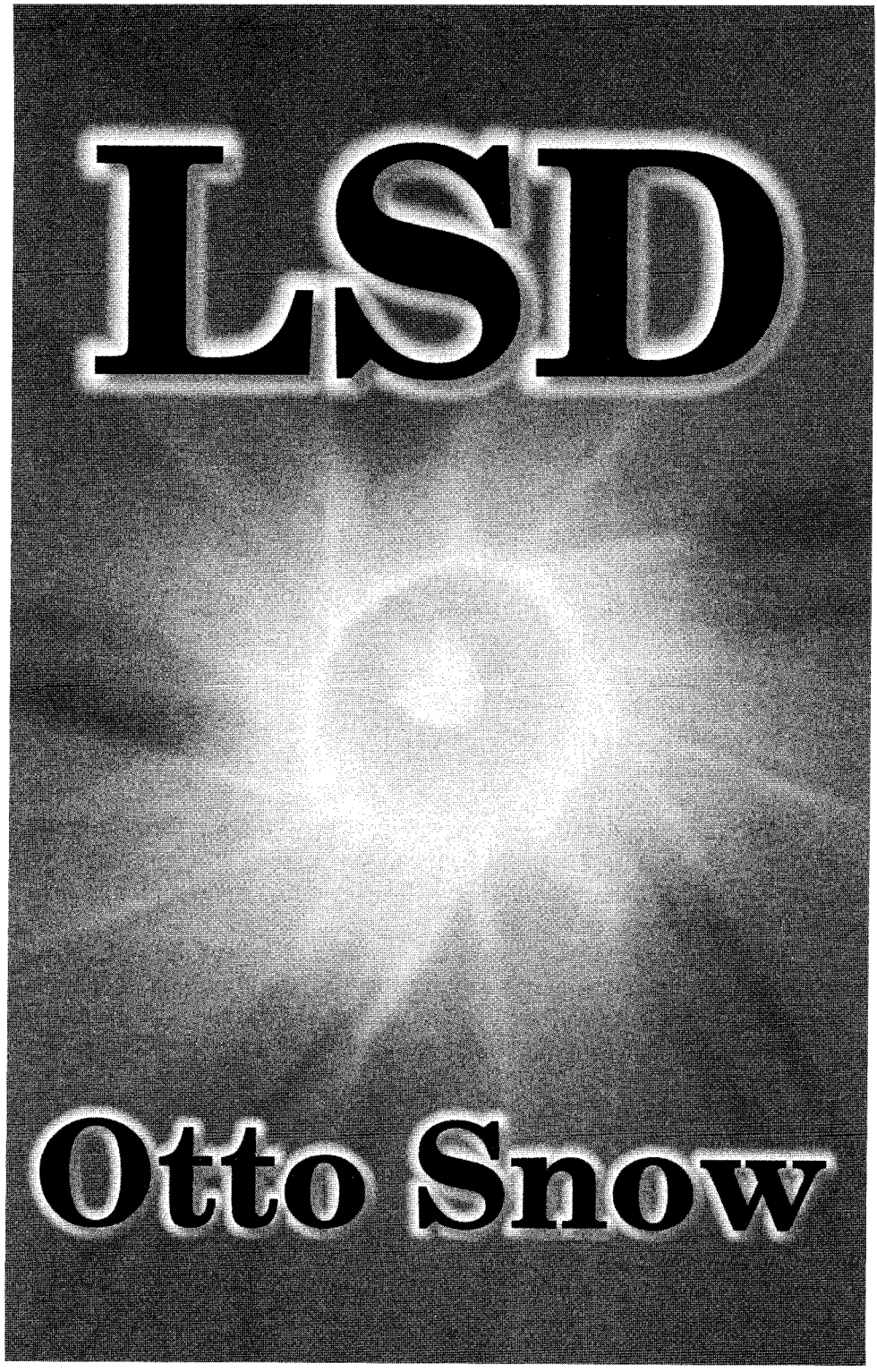

LSD
Otto Snow

Other books by Otto Snow:

Amphetamine Syntheses Industrial Edition
ISBN: 0-9663128-3-X (2002)

OXY
ISBN: 0-9663128-2-1 (2001)

Amphetamine Syntheses: Overview & Reference Guide
ISBN: 0-9663128-0-5 (1998) (Out of Print)

LSD-25 & Tryptamine Syntheses: Overview & Reference Guide
ISBN: 0-9663128-1-3 (1998) (Out of Print)

LSD

ISBN: 0-9663128-4-8
LCCN: 2003113378
Printed in the USA.
Printed November 2003
First Edition

Published by: Thoth Press
P.O. Box 6081
Spring Hill, Fl 34611

Cover graphic and *Argyreia* flower photos by Otto.
Ergot illustrations by Tulasne.
Paspalum illustrations by Agnes Chase and Mary Wright Gill

DEDICATION

I dedicate this book to all those who suffer with migraines, neuroses and alcoholism.

I am in appreciation of: A. Hofmann, A. Shulgin, J. Ott, D. Nichols, R. Manske, S. Cohen, S. Grof, R. Schultes, H. Osmond, T. Leary, R. Metzner, H. Abramson, S. Szára, J. Buckman, A. Kurland, N. Pahnke, S. Unger, R. Alnaes. M. Bishop, R. Sandison, W. Caldwell, B. Aaronson, and all those explorers (too numerous to name) who blazed into the great unknown, unraveling the mysteries of the brain-mind.

I want to thank the Brotherhood of Eternal Love; DEA Office of Intelligence; US, Great Britain, France, and German Patent Offices; US Department of Agriculture; SAMHSA; NIDA; the Josiah Macy, Jr. Foundation and NIMH for their support of brain-mind research.

"From the same jug of whiskey come tears for one
and laughter for another," Sidney Cohen 1964

"If you don't think that it's [LSD] amazing,
just go ahead and try it," Captain Alfred M. Hubbard. (Lee 1985)

"The Greatest psychotherapist in world history was the Buddha,"
Dr. Timothy Leary. (Harrington 1964)

"Progress is a nice word, but change is its motivator
and change has its enemies," Robert F. Kennedy.

"Set and setting, expectation, and atmosphere
account for all specificity of reaction.
There is no "drug reaction" but always setting-plus-drug...
The drug (LSD-25) is just an instrument," T. Leary and R. Alpert.

"It should be our earnest intention to insure that drugs not be
employed to debase mankind, but to serve it," John F. Kennedy.

"'They're like the Romans,' said one LSD promoter, referring to the
legislators. 'They don't realise that this is a religious movement.
Until they make it [the use of psychedelics] legal, we'll find our
sacrament where we can. And no sooner is one made illegal,
we'll come up with another..'" (Tendler 1984)

"I see the true importance of LSD in the possibility of providing
material aid to meditation aimed at the mystical experience of a
deeper, comprehensive reality. Such a use accords entirely with the
essence and working character of LSD as a sacred drug,"
Albert Hofmann 1980

TABLE OF CONTENTS

READER'S NOTICE

This book is a tool for the legal, medical, scientific and political professions and should not be misconstrued as a 'cookbook'. Publisher and author take no responsibility for inaccuracies, omissions, or typographical errors. References and sources are included for those seeking unedited detailed descriptions on the construction of any specific molecule. All chemicals and reactions are potentially toxic, explosive & lethal.

This book is for information purposes only. No person is allowed to produce controlled substances without proper permits and authorization. To take/give substances for human consumption whether legal or illegal without a very thorough knowledge of the substance and the health (mental as well as physical) condition/s of the individual is destined to produce catastrophic results and legal ramifications.

LSD describes the preparation of:
substituted lysergamides,
immediate precursors, and precursors
from common organic sources.

Series and individual reactions are overviewed and extensively referenced. Many different routes are described on altering the molecular structures of known and unknown neurochemicals. The terms and explanations are simplified and interwoven with historical data.

This guide is an asset and a necessity for:
lawmakers, attorneys, teachers, counselors,
law enforcement and students alike.

Baby Woodrose (*Argyreia nervosa*)

Chapter 1
My Personal Introduction to LSD-25

LSD-25 is the generic name (code name) of *d*-lysergic acid diethylamide. 9,10-Didehydro-N,N-6-methyl-ergoline-8ß-carboxamide is another one of the numerous ways to describe the molecule. Delysid is the trade name of the drug originally dispensed for research by Sandoz.

The molecule is the very cornerstone of neurochemistry. LSD has allowed neuroscientists to explore brain biochemistry; e.g. mental illness, serotonin receptors, serotonin receptor subtypes and numerous binding comparisons with other active molecules. Some drugs which have been found to block the effects of LSD are useful antipsychotics. Early studies described LSD as a psychotomimetic, yet this is a general term applied to all phantasmogens.

LSD was invented by Dr. Hofmann in a search for potential medications. There have been few studies on the use of migraines. At fifteen, I had my first hemi-paraesthesia type migraine. There is no known cure. The conventional medications are narcotics, ergot alkaloids, barbiturates, NSAIDs, triptans. All are used chronically and have several toxic adverse effects such as paralysis and death to name a few.

You weigh the risks...

9/15/71

I was 15 at the time. The Vietnam war was raging, the nightly body count aired on the black & white. Hippies, antiwar protests, and riots were the media frenzy. A nation of discontent, on fire.

All I did was study, I had a suit case for all my books. The thing held a dozen books. A suit coat and tie were mandatory in school.

2:30 PM. I unlocked the cellar door to let myself in. Walked upstairs and was greeted by my dog. "Psychodog," a super hyperactive Boston Terrier jumped up to say hello.

I am unsteady and having problems walking. I pat the dog and get her a couple of Dog Bones and let her outside in the yard. My vision is bothering me. I can't focus. It looks like I am seeing from both eyes separately. I was getting this splitting headache, like I never had before. My right arm was having tingling sensations like it was asleep, but it wasn't asleep. I was having a hard time to walk as my right leg was also affected. A glass of water didn't do anything. I couldn't feel my right hand when I opened the door to let the dog in. I called to her and was having a hard time to talk, I couldn't get out the words, they were all garbled. I knew that there was something wrong.

I called my mom at work. She could barely understand me. But said that she would pick me up and was on her way.

My vision was two fields as she pulled the Chevy into the driveway. I was having a hard time to open the car door. I could not feel my right side. My mom was nervous and told me that I would be OK as she bolted to the local hospital.

I was given a room and several physicians looked at me. I couldn't talk coherently, my vision was blurry and I had a screaming head ache, like a knife in my head.

The family doc asks me to follow a pen with my eyes, he takes a light and looks in my eyes.

I was given x-rays, blood tests, throat cultures, and Darvon Compound 65mg. 4 times a day.

9/17/71

Nurses Notes: "complains of headache only when he turns his head."

Dr. Lopez was called in. He did the same thing as my family physician and scribbled in his notes, "Migraine".

9/20/71

Nurses Notes: "has a slight headache."

9/21/71

Discharge: "Remained completely symptom free, while in the hospital," Dr. Dunbar.

I would be given a spinal tap; which came out normal color. A couple of EEG's. The first one didn't take (abnormal) cause I was thinking different emotions to see what it would do on their machine. The second one, I was dosed with chloral hydrate for a sleep EEG and it was normal.

Sept. 20, 1971

Another test. I had spoken with my parents and we decided that I was not going to take any more "monkey tests" after this one.

The hospital didn't have a brain scan machine, so I was sent to another hospital. The light shined into the windows and the people were friendly. The tech that was going to run the test was cute.

"There is nothing to worry about. No one is going to hurt you."

All I could think was she is so cute and what do you want me to do?

"Just tell me what you're going to do."

"I want you to lay down and put your head over here to be scanned. But first you'll have to drink this liquid and it'll taste metallic, but just drink it down. It's safe."

So I take the glass like Socrates and drink it down with a smile. It tasted horrible. Then I notice that she is taking out this huge syringe and has a smile on her face.

"Now if you could hold out your arm."

"No way. You didn't mention needles to me."

"It won't hurt."

"Ya, for you. You're not on the receiving end."

She starts freaking out.

"If I don't give you the injection; the radio-isotope will adhere to your brain".

"Radio-what-the-fuck? You said that this was safe?"

"It is, just let me give you this injection." (10 millicuries technetium 99; half-life of about 6 hours). Fussy girl, needle in her hand. She is fidgeting. The Krypton's gonna make my head glow.

I figured I'd better go for it. She tied my arm with rubber hose, stuck the needle in the vein and released the hose like a pro.

I laid down and put my head in this ancient device. The flickering of the fluorescent lights really bothered me. The lights were strobing. I could feel the mild headache growing into a migraine.

"I can feel a migraine coming on."

The tech operating the gizmo is fidgeting while looking into my head. Her voice is high pitched and excited, "I can actually see the spasm in your the blood vessels!"

10/18/71 Prescription: "Cafergot 20 tablets"

10/18/71 Prescription: "Bellergal 30 tablets"

There are many forms of migraine, there are also many different medications that have been used and are used in the treatment of the disease. Conventional 'migraine' medications approved by the FDA will kill, addict or cripple you.

I was walking in the hallway in school. I was floating off the ground and the books were weighing me down. A twilight world on Bellergal. My arm bothered me, it was the migraine and it was still there. I felt nauseous from the meds. A friend bumped into me and knocked my books everywhere in the hallway. I was in slow motion.

My friend said, "Excuse me," picked up my books for me. "You look really wiped out, what's going on dude?"

"Migraines man, they gave me some goofy pills, I feel real strange. I still have a pain in my head. It effects my speech and movement. My side is stiff, tingly."

"Marvelous cure, too bad we lost the patient. Hemingway. A friend of mine takes some pills for migraines and it works great. He only has to take it once in a while."

"Do you have a couple I could take? Now!"

"You don't take it like that. Plus I don't have any with me."

"Shit. So can you get some for me."

"Sure I want you to read about it first. And the man who invented it, a Dr. Hofmann. Here's your books and will see you in the library at lunch. Be there."

I made it through the next class wondering if Doc Hof has an office locally; with brains in jars; rats in cages with wheels; the bell rang; I bolted to the library. Yota was there. His nose in some books.

"What ya reading?"

"About Doc Hofmann. He works for Sandoz."

"They make these goofy pills I am taking. What the fuck Yota?"

"Hear me out. He found something better. He was working on many drugs and the most advanced one helped my friend. He turned the book and it said LSD.

"Oh my God. People leap out of windows and pregnant women have babies that look like tree stumps."

"Fuck no. That's all bullshit. Well almost. We'll be doing it on ground floor. Your not pregnant are you? You want to get rid of those headaches don't you?"

"Ya, but does your friend have hemiparaesthesia type migraines. Cause that's what I have. And they are no Goddamn fun. They last, they hover like a grim dream and when they hit, I am out of it for days. I am jagged for days after. I can't think right and feel like crap. Then I repeat the whole damn sequence. The pills are just as bad. It sucks."

"All I know is that helps him, he was crippled by them."

"You say when."

"It will be nice and smooth. Have your mom drop you off at my house on Friday and we'll go to the dance. Here, read the book. Don't take anymore of the meds the docs gave you. They don't know or care what they are doing, its just a job."

It was Monday and I read the book through lunch and put the book in between my study books for the rest of the classes. Generally I did my homeworks for the previous class during the next class so that at the end of day I would have time to go for a walk. But this book was more important, so I pushed my homework for the evening.

My mom dropped me off at Yota's house on Friday. His sister was a fox. Long blonde hair. Nice shape. Red puffy lips.

"Pleased to meet you, Otto. That's a unique name."

"Thanks." She shakes my hand. "I know that you will like this band. They are so cool."

"Come here Pavlov," says Yota pulling me by the arm into his

room. "I got the acid."

"How many tablets do I take?"

"No man, you don't do it like that. A whole tablet will put you in orbit. Can you remember Loopy? He took a whole tab and his mom called him for dinner. He said he wasn't coming out of room unless he could have a banana split. He was busted by mom." My friend laughed. "He was jumping around the kitchen chasing trails."

"Trails?"

"You see all those burnt out hippies. They're toast. It's a little bit, just enough," as he cracked off a half. "I've done it before and know what to expect." He looked up and smiled. "It melts in your mind and not in your mouth," and laughs, shows me a piece on his tongue and swallows it.

"Here, I'll give you a third of the tablet, just a little cause we want to see how it goes."

He passes me the third.

"Where did your friend get it?"

"He goes to college and gets it out of a research lab. Its called clinical, white lightening or something like that."

I looked at the small white tablet and images of the spinal tap, the brain scan. The poison meds the docs gave me are making me sick.

"I hope that this helps." I crunched the small piece in my mouth. "Do you think I took enough?"

"Ya. You'll see. It will be fun. Fun is good."

His mom dropped us off at the dance. Many of my friends were there. A joint was being quickly passed around at the door. A friend passes me the joint. Yota puts his arm up against the persons arm and says, "no, we don't want any contamination. The experience must be pure."

The band was great. My friend Gwag came over. He has real thick horn rimmed glasses, skinny, and smelling like cigarettes, he asks, "how do you feel?"

"Ok."

"Do you still have the migraine?"

"Yes, but I can feel something happening."

Gwag went to get something to drink. I was noticing that the lights were a little brighter and the colors of banners in the gym were more vivid. The people dancing were a mass of colorful sticks moving with beat of the music. I am seeing movements of people in the gym like an old fashion movie, when the frames get choppy.

I move my arm an see a series of latent images of my arm and giggle. Gwag brings two paper cups of Sprite.

"Trails, that's what they're called. Have something to drink."

"Thanks." I held the cup and watched the bubbles fizz in the cup. "Witches brew, magic stew," and sipped the beverage. It was effervescent and appealing, the very first sip of something exotic from a far away land. It took on an enchanting appeal. I look at the crowd and sense their feelings, their emotions, I am reading their body language. I listen to various groups of people as we are hanging around on the floor. They are all taking about stupid shit.

I said to Gwag, "they have the consciousness a little bit higher than that of chimps. Monkey people."

"Sleepwalking," says Gwag.

I stop moving to the music and there is a major rush of energy that moves through the crowd and into my body. It feels like the migraine is a veil that drapes my forehead. It is lifting off, from the front of my head to the back, transforming it into sparkle dust in my mind and dissolves (psycholytic effect).

"My migraine is gone." I am experiencing a profound euphoria and bliss for a few minutes.

The experience was safe and effective. We approximated the dosage at 50*ug.* LSD. I speculated that the migraines were caused by adolescent neuroses. The medication kept the migraines at bay, once every few weeks in a variety of environments. Then there was no more LSD available, my friend Yota moved, kids were selling d-Con and scopolomine in capsules as mescaline or acid or what ever you wanted it be.

I was sixteen at the time, and could not obtain LSD from my doctors. The migraines were coming back. So I went looking for the finest LSD that I could obtain for myself. I met many people who were much older than me. I met other migraine sufferers. Several had high positions in the corporate world. Then there were alcoholics and Zen men and women who explored the eternal now with me. LSD is not a 'party drug' to me, it is a medication.

In an area, where the economy is based on high technology, I was able to estimated that 10% of total population used LSD at a 100 *ug.* dosage (mid-seventies).

Over the years, taking LSD at 100 *ug.* every six months, then once a year, then every several years, the crippling migraines were kept at bay for 14 years. Sessions were most effective in appropriate setting with a guide that was one of many good friends. Only those who are experienced and appreciate psycholytic sessions (each and everyone I hold in my heart). Sharing these moments together will always be sacred.

Chapter 2
Medical Applications of LSD-25

Reported Dosages

"At very low doses, 20 mcg or less, very little happens. At 50 mcg, there is an increase in alertness. At 75 mcg some subjects react with a strong experience and others remain very tense and uncomfortable. At 100 mcg about 75 percent of normal subjects become very relaxed and remarkably free of tension. The remainder may require 200 mcg to get the same degree of relaxation. There must be a maximum degree of relaxation before the psychedelic experience is achieved; most subjects have very tense, unpleasant experiences when given too little LSD," Abram Hoffer, M.D. (1967)

"The drug conceived to facilitate recall, reliving, catharsis and abreaction, with the production of associational, dreamlike material for subsequent analysis," Albert Kurland (1967).

"Therapists working with small doses-such as 25-50 μg. of LSD-do so only to facilitate conventional therapy..." Masters; Houston.

"LSD does not act as a true medicament; rather it plays the role of a drug aid in the context of psychoanalytic and psychotherapeutic treatment and serves to channel the treatment more effectively and to shorten its duration." Hofmann 1980

"Very small doses (on the order of 30 μg. of LSD) are sufficient to establish empathic bond.." from: Toward an Individual Psychedelic Psychotherapy, by Masters, R.E.L.; Houston, J.; in *Psychedelics; The Uses and Implications of Hallucinogenic Drugs.*

"Medium doses (100-200 *ug.*) usually suffice to produce regressive phenomena and deeper emotional responses, while larger doses still may take the patient into primal archetypal experiences," R.A. Sandison (1956).

Adjuvant to psychotherapy - 20-40 *ug.* Harold Abramson (1956)

"The use of LSD to enable the patient to shorten this process has been termed psycholytic therapy in Europe. Low doses of LSD are used in psycholytic therapy...." Harold Abramson, M.D. (1967)

"At present, I start treatment with 20 to 100 mcg. of LSD given intravenously. The hysterical personality needs very small doses, while obsessionals need very high ones. The average dose for my patients is between 75 and 150 mcg. and I have never given more than 500 mcg," Dr. John Buckman (1967).

Psycholytic therapy - 30 - 200 *ug.* for treatment of neuroses ref. Van Rhijn 1967

Psychedelic therapy - 400-1500 *ug* for alcoholism.

Alcoholics 200-400 *ug.* 50% improved, (stopped drinking or improved). Alcoholics 400-1500 *ug.* 60% improved (50% remained "totally dry" at follow-up.) 50% remained alcohol free with 200 *ug.* being given every 2 months. 90% of neurotics report "improved" or "much improved". (Unger 1964) Alcoholism - 400 - 1000 *ug.* causes transcendental experience. One year later 50% remain alcohol free. (Caldwell 1968) (MacLean 1961)

"The problem of apathetic, resisting patient is not common in psychedelic therapy, yet I have seen patients in Europe under a dosage of one thousand micrograms... who were completely sober," Dr. W.V. Caldwell (1968)

"Psychedelic therapy, the method usually applied in this country [USA], commonly makes use of doses of at least 300 mcg of LSD and doses may go as high as 2000 *ug*," Harold Abramson, M.D. (1967)

Treatment of Alcoholics

I was seventeen hanging out with a chick in her twenties. Lotus was gorgeous. Long brown hair with a nice figure, deep brown eyes. Knock-out kisses. Lotus told me about Jasmine. She was in her sixties and been in an LSD program for the treatment of alcoholism.

"Cool. Lotus introduce me. Come on Lotus."

"Tomorrow, we'll go over."

We go to Jasmine's apartment. It was on the second story of a building at the top of the hill. A nice quiet location. Inner city rural America. An American flag waved over our heads as we entered the front door.

A little old lady with white hair opens the door. She peered at us through wire rim glasses.

"I am pleased to meet you, Otto." Jasmine held out her hand. "Lotus has told me so much about you, come in."

We walked up the stairs and Jasmine was fully able to make the stairs and prepared some tea. In the living room are pictures of her family. Jasmine smiles and gives us cups of tea.

"So you take LSD for your headaches. I take it for alcoholism," says Jasmine, and smiles with the eyes of child.

"Interesting."

"I told you that she was cool," says Lotus.

"Half my body was partially paralyzed from the migraines.

The LSD keeps it at bay. I can tell when they are coming."

"For me, I have to take it or I won't stop drinking."

"Drinking anything and everything, Otto. I mean it. I saw her drinking cooking wine and not eating," testifies Lotus.

"During the sixties, I was given LSD to stop drinking. I was given a large dose and experienced *satori*. And like you I have to take LSD to keep condition in check."

"How often do you take it?"

"I take 50 micrograms twice a week or one hundred once week. It has kept me from free from alcohol for ten years," as long as I have good LSD."

Jasmine took out a small pill bottle and opened it, reveling 4 purple tablets. She placed them on a white paper. I looked at them more closely. They were molded and not very good.

"And these do it for you?"

"They are very weak."

Over the next year, we would trip together. She was a mentor to me.

One day, I walked over to visit my friend. There was no answer to the doorbell. Strange. I had been over the day before. Was wondering if she was toes up, I should have asked her for the phone number of her daughter.

Then all of sudden I can see something or someone bouncing down the staircase like beach ball, Blam!, slamming against the door. The door cracks open. This odor of someone who hasn't washed in many days cuts into the air, bad, real bad.

"Otto, I am not well. I have been drinking."

Her stomach is distended and she burps.

"Oh my God, does your daughter know?"

"Yes she does, she took my drugs away from me. She is going to send me to a hospital for rehab."

I gave my friend a hug and walked home. I went back a few times and she was gone.

I was coming back from a vacation north. Sixty miles from home. A friend was driving her truck by a head shop.

"Bonnie pull over, I have to check out this head shop."

"I don't know where I'll park."

The truck stalled and I jumped out. Opening the door of the head shop, I smelled patchouli, working behind the counter is my dear friend, Jasmine.

"Otto," her arms outstretched to me.

"Jasmine, you're all right."

Innocence in her eyes, she smiled like a child, we hugged.

"I hooked up with some people in rehab. They are like me and have to LSD for alcoholism. We found some really good LSD. Do you want some Otto?"

"We use doses varying from 400 to 1,500 mcg. given by mouth. The initial dose depends on the psychiatric appraisal of the subject's defense mechanisms. We think that the closer a person is to self-acceptance the less the dosage required, and we use this as a working guide. We usually start with a dose of 400 mcg; experience as the session progresses is used to decide if and when more is required. If after one or two hours the patient shows signs of anxiety because he is holding on desperately to his reality ties, more LSD 25 is needed to induce the psychedelic experience...

Toward the end of the period of hospitalization (mean: two months) the patients were given the LSD treatment. they received 200 mcg. During the session the therapist worked with the patient to bring out repressed memories, abreactions, new insights, and new understanding," Abram Hoffer, M.D.(1967)

"In answer to the question of the need for transcendental experiences in alcoholics, we have found in our experience with around 130 alcoholics that it seems that these people have divorced themselves from the rest of humanity. They must have an experience like this to be able to again come into, and react with, the rest of us humans," Dr. Kenneth E. Godfrey (1967)

Most volunteers (subjects who are not patients) will have an unequivocal reaction when given 100 mcg of LSD by my estimate, based upon several hundred subjects, is that ten percent will have minimal or no LSD reaction with this dose.

In a series of experiments over the past ten years, no subjects have failed to react in the expected manner to 200 mcg. however, perhaps 25 percent of alcoholic subjects will react minimally to 200 mcg and about 90 percent will react to 300 mcg.," Abram Hoffer, MD (1967)

"These studies have shown and indicate that LSD plus brief psychotherapy is of considerable benefit in the treatment of chronic alcoholics. It should be pointed out that it is not the drugs that are therapeutic but the experience the patient has that is of benefit," Oliver O'Reilly, M.D.(1967)

In the treatment of PTSD?

"Psychedelic drugs may not only suspend old imprinted patterns, they may also provide the possibility of reimprinting," Dr. Timothy Leary (1964).

"The liquidation of the traumatic material, by reliving and rational integration with subsequent ecstatic experiences, is accompanied by a heightening of the patient's security and self-esteem and the disappearance of maladaptive patterns and clinical symptoms. A very typical occurrence is the resolution of ambivalent attitudes with a successive narrowing of the oscillation spectrum of contradictory tendencies and a sort of mutual neutralization. Toward the end of the procedure the patients relate that they feel personally free and exempt from various pathological dependencies they suffered from previously," Stanislav Grof, M.D.(1967)

"Love, kindness, patience and security all help to heal trauma. Too bad there is so little of this in the world," Otto.

See also: *Shivitti* by Ka-tzetnik (should be mandatory reading in all high schools).

In the Treatment of Neuroses

LSD has been found to be effective (50 - 66% recovery) in the treatment of obsessional compulsive disorder (Solursh 1966; Sandison 1954; 1956)

LSD has been used on schizophrenic with a recovery rate of 80% (Perrilo 1963)

Neuroses - 55% recovery rate. "We have reason to be satisfied with lysergic acid diethylamide as an aid to treatment, and in many cases the results are so dramatic as to leave one in no doubt as to the value of this remarkable drug," R.A. Sandison (1956).

In the Treatment of Migraines

In clinical studies LSD (the drug) has been found to have a remarkable ability to treat disease from a psychological point of view. It has been extremely successful at aborting migraine headaches (Ling 1963) (Yensen 1989). It's most valued attribute is the prophylactic nature of the substance against migraines. There has been no description on the specific types of migraines that were treated.

In the Treatment of Schizophrenia?

"Morgens Hertz, a Danish physician, described a patient whose long-standing stuttering condition disappeared following LSD treatment (Stafford & Golightly 1967). An American team of researchers found

that schizophrenic children became more communicative following LSD treatment (Bender, Goldschmidt, and Siva Sankar 1956)." pg. 227 In *Psychedelics; The Uses and Implications of Hallucinogenic Drugs*

"It should be noted that when a therapist takes LSD, he enters a state in which he can communicate with schizophrenic patients in a direct, close, empathic fashion. This communication opens the door to effective psychological treatment for schizophrenia. The schizophrenic is lost in time, and a therapist who will enter the paths of his disordered thinking, once he can establish trust, can lead the patient out of the disorder. It is not always sufficient to call out from the forest's edge to rescue someone lost. One must sometimes go in himself."
from: Toward an Individual Psychedelic Psychotherapy, by Masters, R.E.L.; Houston, J.; in *Psychedelics; The Uses and Implications of Hallucinogenic Drugs*

Therapist (Guide) Must Take Drug

"We are engaged in what is called a transactional research design. The researcher sees himself as part of the transaction, and is an active learner in the experiment. Most American psychology today is only a description of what the researcher sees-it is only the report of the researchers experience in observing the subject, rather than what the subject is really experiencing. The subject-object method of research is inadequate for studies of human consciousness," Dr. Timothy Leary (Wakefield 1964).

"We believe that the LSD experience is unique, thus making it necessary for the therapist to take the drug himself, so that he may be, if only in a small measure, able to understand what the patient is talking about," Dr. John Buckman (1967)

The Environments (Settings)

"The session is conducted in a large, beautifully decorated room specifically designed to enhance the drug experience. Music is played during most of the session. The sitter is supportive when required, but the otherwise does not initiate interaction with the subjects." William H. McGlothlin, Ph.D. (1967)

I would be feeling the migraines coming and had to get these down pat, always planning ahead.... Set and setting are paramount. The body can over heat on LSD if the air temperature is too hot. In the winter, I would wear warm clothes outside. During the summer, I would only have a session outside if the temperature was not hot. Usually, in the summer, sessions were conducted outside on a cool sunny day.

Sessions in The Yellow Room

The walls were yellow with a sky light, yellow couch, hardwood floors and an oversized sliding door to a deck that overlooked the forest.

We had large basket of fruits and chips. Sardines and pizza. Walnut pie. Lots of different fruit juices. Valium in case of emergency. The doors were locked.

The sessions would start mid (9AM) morning after a full breakfast. No B-vitamins for 24 hours prior to session. These were standard sessions. A box load of tapes had been stacked for five - six hour session.

During the session, we would sit on the deck and look at the forest. It was beautiful and alive. Usually it meant a walk through the woods.

The forest is alive on LSD. I breath in the forest. It is the Mother Goddess. She breaths oxygen into me. The trees are majestic. Giant air cleaners (GACs) and water purifiers. Organic and safe. I am a dwarf in the woods. The only thing bigger than the GACs are the clouds and they are puffy and white against the deep blue sky.

Each moment is sacred. I can feel my consciousness climbing ever more towards the peak of the session.

At Peak: Noon. It is nice to find a little clearing in the forest, lie down, looking in between the trees up into the sky. I let go. I experience becoming one with the forest totally. She is breathing life into me. I am her child of a million years from day one. We are all biochemically hardwired to Mother Nature.

Sessions in State Parks

Uncle Sam provides sacred areas for us and generations to come. They are ideal for sessions. But remember, follow the rules. What ever goes in comes out. No litter. We are like Star Trek, and we are not supposed to leave garbage in the forest. Day trips in a park are fun.

Sessions in Amusement Parks

I speculate that this may be helpful to those with anhedonia. At 50*ug* it was enjoyable. I went and picked up a friend and he dropped 200*ug*. By the time we got there he was experiencing the drug. I took 50*ug*, and decided to go get something to eat down the road. On the way back, two ladies were thumbing so I picked them up in the puke green Nova.

"So ladies, going to the amusement park?"

One of the ladies is checking my out friend in the back seat, who is curled up in a fetal position laughing.

"Don't worry about him. Just push him over little, he's friendly."
The girls hop in and we are moving to the park.
"What's the matter with him?" asks the girl in the back seat.
"Too much LSD."
"LSD! Is he a drug addict or something?"
"Oh no, he's perfectly safe. He is experiencing the eternal now."
"He looks like he is really out of it."
"Window, you're scaring the ladies, sit up."
"Hi, girls. I'm ok, just the giggles," says Window.
"Oh, we know what that's like."

We go into the amusement park and first ride is the Tilt-a-Whirl. Window and I know can finely sense the weight balance between the spin and speed. Moving from side to side with the girls between us. The seats we are spinning so fast that we can't lift our heads. Window and I are going, ohhhhh, and just letting go. The ladies are freaking and screaming. Window and me are laughing so hard we are ready to pass out. The ride slows down and stops, the ladies loose us fast.

I am grocking the crowd. I am standing there crying. My friend is laughing. I tell him to grock these people. Tears fill his eyes, I am laughing.

We are getting cotton candy in our long hair and laughing hysterically. The day was a blast...

I speculate that the amusement park setting for sessions would be therapeutic for anhedonia. The guide should be a friend, someone kind, playful, behaved in public.

***

Set and setting are of utmost importance (Unger 1963)

According to Drs. T. Leary and R. Alpert (referring to the entheogenic substances, mescaline, psilocybin, LSD):

"(1) these substances do alter consciousness. There is no dispute on this score.

(2) It is meaningless to talk more specifically about the "effect of the drug." Set and setting, expectation, and atmosphere account for all specificity of reaction. There is no "drug reaction" but always setting-plus-drug...

The drug is just an instrument."

Religious Experience

There is no organized religion of LSD. The LSD experience is one of self awareness and discovery. In this context, LSD is used specifically for medical reasons/personal psychotherapy. Many individuals who occasionally take LSD may not smoke marijuana, cigarettes or use any other drugs for that matter.

"Western science is now delineating a new concept of man, not as a solitary ego within a wall of flesh, but as an organism which is what it is by virtue of its inseparability from the rest of the world... medicines which science has discovered... may prove to be the sacraments of this new religion." *The Joyous Cosmology,* by Watts, A. 1970

"The classic psychological assumption is that in the best of circumstances, moving from one level of maturation to the next involves a difficult reorganization and internal crisis, and that failure at any step results in either cessation of growth or distorted development thereafter," Dr. W.V. Caldwell (1968).

"...the mystical experience of union or fusion with its concomitant characteristics has been interpreted in many ways, fading or melting into the universal pool, boundless being, the void, *satori*, nirvana, *samadhi*, the Atman-Brahman identity; the awareness of a "Beyond," "More" or pure "Self;" or union with God. Yet in spite of the particular interpretation, the psychological experience itself is the basis," Walter N. Pahnke (1967).

"The mystics subjective experience of his identity with "the All" is the scientist's objective description of ecological relationship of the organism/environment as a unified field," Alan Watts (1964).

"Dr. W.T. Stace, Professor Emeritus at Princeton University, was asked whether the drug experience [psychedelic] is similar to the mystical experience, he answered, "It's not a matter of its being similar to mystical experience; it is mystical experience," Dr. Huston Smith (1964).

"Are the visions of a prophet revelation or disease? Does schizophrenia encompass both the delusional paranoiacs and the holy men whose trances have provided us with messages which many consider gospel? The psychedelic drugs have a contribution to make in the understanding of such matters," Sidney Cohen, MD (1964).

"There are considerable differences between LSD-induced and schizophrenic symptoms. The characteristic autism and dissociation of schizophrenia are absent with LSD. Perceptual disturbances due to LSD differ from those due to schizophrenia and, as a rule, are not true hallucinations. Finally, disturbances of consciousness following LSD do not resemble those occurring in schizophrenia," Sanford M. Unger (1964).

"Tibetan Buddhists describe this condition as *samadhi*; some mystics (Schjelderup 1961; Stace 1960) as pure consciousness; many patients and volunteers as a condition beyond time and space, with the abolition of all boundaries, and a feeling of being one with the universe (Dunlap 1961; Leary 1964; Newland 1962; Osmond 1957; Stace 1980; Swain 1963). It is a condition without content, consisting of "nothing," in which the patient feels extreme peace or bliss; he experiences a void, yet is not unconscious," Randolf Alnaes, M.D. (1967).

"The differences between the "easy" and the "hard" ways must be similar to the situation in which one man climbs the [mountain] and another takes the ski-lift. The view from the top is the same for both. The mountain climber has sweated and striven against the dangers. His view must be different from the ski-lift rider's because it incorporates the struggle and the triumph. Ski-lift transcendence can approach that of the mountain climber's only if the prior life preparation has also been one of training and self-discipline," Sidney Cohen 1964.

"Patients are apt to describe death and rebirth experiences during the period of drug activity. It is the rigid, punishing superego that dies and then is reborn free of the old guilt. It is a new start with the slate wiped clean. No doubt the process represents the use of strong denial, but this defense might be preferable to the previous manner of handling feelings of shame and self-condemnation." Sidney Cohen, M.D.(1967).

Dr. Leary brings up five common fears that are sometimes generated during a psychedelic session. Fear of loosing rational thinking, fear of doing something shameful, fear of finding out something about one's self that they are unwilling to face, fear of finding out the truth about their phony sham culture that they identify with and the fear that the psychedelic experience will be so pleasant that they will not want to return from this new state (Leary 1964).

"A caged community of chimpanzees reacts very sensitively if a member of the tribe has received LSD. Even though no changes appear in this single animal, the whole cage gets in an uproar because the LSD chimpanzee no longer observes the laws of its finely coordinated hierarchic tribal order," Albert Hofmann.

"Self-identity is completely lost, and the self and that which is outside the self fuse. The ordinary subject-object relationships disappear, along with the conventional separateness of the external object. The extension of this egolessness can culminate in union or communication with the divine," Sidney Cohen, MD (1964).

"Thought must be given to lights flashing at certain frequencies (Hubbard personal communication to Dr. Humphry Osmond, 1955 and 1956).

Opening of the Mandala (100 *ug.*; 9PM drop)

Midnight, I pulled back on the recliner to lift my feet a little, nice and comfortable. I position the strobe away, not at myself or anyone else either. The strobe is snapping out light across the large room and dissolves into darkness. The length of the room is great for this effect. I can see nothing behind the flash, it just merges with the darkness and vanishes, like warm breath on a cold day.

I turn up the strobe until it is rather fast and then I slow it down just a little to were it feels very comfortable. ohhhhhh, very comfortable.

I stare straight ahead and see the primary mandala. It is the focus point from where all the colors are coming from. Colored lights are spinning from the center. They are moving at a constant speed. The colors of the rainbow, they are bright and I notice that there letter T shaped stick-like formations that surround the center and the mandala forms outward. The top of the T's face inward in the circle. There about 50 of them. The T's move and generation the moment of the beads that are spinning along the edges of each T.

There are millions of beads of light spinning around the T's. The T's are moving to the music and the beads flow the movement of the T's. The mandala is about one foot in diameter with multicolored T's moving and colored beads spreading out across my view.

Control of the Mandala

(Step one)

I look at the mandala and think of red. In a little while, the T's turn red. Then I think of blue, and the T's turn blue and then the beads spinning around them turn blue. I think of yellow and colors turn yellow.

(Step Two)

I thought of the secondary colors. Green and the T's turned green and the beads followed. Then Orange and the mandala turned orange. Then I thought of purple. The T's were moving to the music and sensory impulses. They turned purple, the beads were purple. I watched the mandala for a minute.

The diameter of the circle of T's was about 3 feet at 10 feet away. I asked, "Who was I before I was me?" I could feel the mandala is

directly attached to my spirit. It is a visual of the cord of life and I am seeing a cross view. I wanted it to open up. I willed it as I let go.

The mandala opened up like a lotus with millions of petals. It encompassed the room. I was pulled into the vortex. I was moving in a large tunnel. It's walls are time, and I am moving thru. The T's had formed a column and I was in it or it was moving passed, like a car in subterranean tunnel.

The tunnel was metallic. It seemed to travel for ever and then it stopped. I was in darkness. I willed light, and I was in a tomb. There are hieroglyphics on the wall and they are about what I do, what we do, a potion of some sort. It would be 11 years and before I would decipher them...

I am wondering where I am. A wall cracks on the tomb and I see three pyramids in the distance and sand going on forever.

I am pulled back into the tunnel and the am going backwards to where I am now. Sitting in the recliner with the primary mandala back to a three feet diameter at 10 feet away, spinning beads on idle.

Where am I going. The mandala forms a tunnel and I am moving along. It is curving all over, going up and down, being tossed side to side, roller coaster ride on high. Then it stops and I am in a garden. It is full of flowers and butterflies. The trees that surround the garden, are so beautiful. Then all of a sudden I am pulled back into the tunnel and whisked by to my original spot in space and time.

The Ritual of the Million Faces We are All One

Something stuck to me in the tunnel. It wants me to do something. I walk into the bathroom, I shut the door, moonlight is coming through the window and I walk to the mirror.

I have done this several times. But there is something different going on. It is a ritual that I have brought back. The moon makes a reflection in the room. I can see myself in the mirror and look into my eyes. The primary mandala is now surrounding my face and head. The peripheral gets hazy, and out of focus. Then I notice that my body is changing. I am an old man, with long hair and a long beard, wearing animal skins, then it changes, I am shorter and have Asian eyes and I am a woman, then I transform to large man with military clothes, then change again to child, a black child. More and more transformations are taking place, faster and faster until thousands of human forms are passing, we are all connected, we are all one, past, and now all merged

into one. I am all of them, they are me. We are all one. They are all smiling back to me and their faces are moving so fast. My peripheral vision forms into mist and then I am looking back into my eyes, smiling, with beads of light spinning around me.

I open the bathroom door and called a friend, pulled him in and shut the door.

"You've got to check this out. Look at yourself in the mirror. I put my hands on either side of his head."

"Is there something on my face?"

"Shhhhhh, look into your eyes. Keep looking... I am going to be sitting down, but just keep looking at your eyes..."

"Oh my God, what's happening?"

"Let it show you."

Millions of faces appeared, he stood there for several minutes and then turned to me.

"They are all me. We are all one."

"All races, all sexes, all genders, all nationalities, all the love, we are all one."

I turned another person on to the million faces. Same reaction. I practiced the ritual with many people, it was all the same. We are all one. Later, I would find more portals without the use of LSD...

Niacin (NADH-dependent enzyme)

"One of the metabolites of LSD in the human body is 2-oxy-lsd. It is formed by liver microsomes by an NADH-dependent enzyme,"

Nicholas J. Giarman (1967). ref. (Axelrod 1957; Hoffer 1955)

Use of nicotinic acid (niacin, B3) to abort psychosis - "We have given the subject 100 *ug.* of LSD; at the height of the experience we injected intravenously 200 mg. of nicotinic acid. Our experience has been that, within a matter of two to five minutes, almost all of the LSD phenomena disappeared, and the subject claimed that he was entirely normal," Abraham Hoffer (1956)

Satori (17) Temple of the All

Its 6 PM. I am sitting in my room. I flip a plastic bag of LSD/ lactose (5000-6000 *ug.* on a gram of lactose) inside out and pour the power into the jar to add sugar to dose at 75*ug.* a capsule.

I push out the corner with my index finger. The corner of the bag is covered with white powder. I sucked on the corner of the bag and get a very powerful metallic slam in the back of my throat. I visualize the bag turning into a breast.

I knew that I had taken a rather large dose of LSD. Five minutes

later, the wrinkles in the quilt are transforming into spiders, stick-like at first, pulling themselves from the wrinkles, then into hairy spiders by the hundreds, they are jumping off my bed and onto me.

The walls are breathing and my posters are touching my face. The ceiling is dripping onto me. The wood grain in the wooden door is rippling very rapidly.

I get into the kitchen scoop down a 5mg. Librium, 2 large tablets of niacinamide and a large B-complex.

Out the door and into a friend's car. It's pouring rain. I am barely able to stand. Everything is in strobe world, every move was being followed by multi latent images. The ground is covered with massive living colorful mandalas that are vibrating and flowing. I'm still climbing.

The heads of my friends are shrinking, then they are getting large. I am looking at friend's face. His horn rimmed glasses make him look like a giant bug.

It's a torrential downpour, every drop that hits in the ground turns into a rainbow of colors. Every single drop. I couldn't focus, everything glowed. It is as if I were looking at a molecular level, seeing the spin of atomic particles; this is matter/life itself. Everything was made of this material. It was alive and buzzed with energy. Everything is everything. This is the Temple of the All.

I watch cars stretch by, streams of lights, elastic bands of latent images. Whoosh... Elastic bands on tin cans... The water splashes up and is filled with color. It is beautiful. The world is a million mandalas exploding in waves of color. Everything had lost its solid physical shape. The primary mandala had blown off. The whole world is full of energy. It is totally dynamic. All stretched together by buzzing beads of light.

I am looking into a person and seeing their life move by like scanning pages in a book, a moving photo album of their life.

I give a loud belch, my stomach is chambering a rather chunky mix of psychedelic sauce for everyone.

I heave a projectile of dayglow orange psychedelic vomit out the window.

"Stop the car! I am out of here."

I climb out of the coffin box, touching my foot to the ground; the rain stops. There is a long orange streak of vitamins with white potato chuck starbursts that now coat the side of the green car.

I am walking home. It was like coming down from out of clouds in a jet plane, each step I am coming back to earth. Waves of color flare up then shut off. They light up and then they stay off a little longer. The wet road sparkles under the street lights; I am back. One wild two-three hour experience...

Termination of Psychosis

Psychotic reactions triggered by LSD have been reported to be aborted by neuroleptics, beta blockers and pargyline type molecules. These drugs block the neurochemical sequence of events that allow the drug to be active. More study on molecules which block or abort LSD effects should be researched further as it will lead to a better understanding of neurochemical mechanisms.

"The session [300 *ug.*] was terminated with 100 mg of niacin intravenously, and one gram orally," P. Oliver O'Reilly, M.D.(1967).

"Administration of 10-30 mg [Valium], orally to persons under the influence of LSD (whether they are enjoying the drug or suffering from it) results in cessation of LSD effects in about half an hour; hallucinations cease, euphoria wanes, any panic or terror disappears, and (according to patients so treated) the LSD experience comes to a hold while the sedation produced encourages them to seek the sleep that most such patients desire," Dr. Ronald M. Levy (Levy 1971).

"In our experience, 5-20 mg. [Valium] orally suffices, and is safe even in the common situation where a variety of unknown drugs has been self-administered," Dr. Bryanne E.W. Barnett (Barnett 1971).

Warning

Hoffer and Osmond spoke of an unknown substance called anti-S which is proposed as an endogenous anti-schizophrenic substance. Although this substance has not been discovered, the LSD-25 ligand has been found. It occurs to be elevated in psychotics. When psychotics are treated with psychotic drugs, LSD-25 ligand decreases. Although an intriguing molecule to study, this ligand also occurs in normals and has not been linked as the biochemical cause or marker of mental aberrations (Mehl 1977).

"The extremely depressed, the hysterical and the paranoid personalities are poor risks because of the danger of accentuating their depressive, hysterical or paranoid tendencies. The borderline psychotic is a precarious patient because of the danger that he may decompensate and fall into a full-blown psychosis," Sidney Cohen, M.D.(1967).

Seizures have been noted in epileptics... (Fischer 1967).

Anyone who has a cold or a woman near or during their period may experience unusual or distressing menstrual flow and should not take LSD. (Caldwell 1968)

"We found that small doses of Ritalin help to ease some of the anxiety, but often the euphoria produced resulted in depression later. There is also a danger of addiction to any euphoriant drug.... Dr. John Buckman (1967).

Water Spirit (100 *ug*; 9PM drop)

LSD is most colorful if taken at night. I was drinking beer and had some Valium from the day before. It tamed the phenomena some.

It was near peak (11:30 PM approx.). Sitting at the end of a dock on a large lake. I can feel the wind. I can feel the trees moving in the forest. I am one with the forest. The water is all around me. I can sense that I am in the territory of the water.

The moon is out. I gaze across the lake.

The technique of gazing should to be practiced before hand. An easy way is to sit on a lawn and relax your vision till you can see all the leaves of grass at the same time. When the wind blows even a little, you will immediately see the very few leaves of grass move. It is the same vision used in mushroom picking.

I am looking across the water. I can see a green mist a mile across the lake. It starts to move. Then flashes across the lake and appears around the dock and me. I sense that I am in the water's territory. I continue gazing and the green mist flashes across the lake again. So cool. I like this and let go... The green mist starts strobing across the lake and is lifting from around the dock. The mist is floating up around me. It's about 3 feet in the air and I am laughing. I reach my hands out. Its the Mother Goddess and I love her with all my heart.

The strobing and green mist feels so good.

A friend walks onto the dock. We dropped at the same time. She sees nothing. I grab her hand and point out across the lake, "look out there, relax." The green mist is strobing across the lake and my friend sees it too.

"What is it?"

The mist is all around the dock, its up about three feet. The strobing stops, the mist remains. Its getting up to five feet from the water and the water begins to bubble. At that point we both bolted got off the dock and the mist vanished. When we got on the dock it flashed across the lake to us. We stayed off the dock for the rest of the trip.

Several friends have experienced the water spirit, it even works near old water holes that have gone dry. I speculate that horses can see the water spirit. An awareness, like tracking wild animals.

Carlos Castaneda was on the money with this one.

The water spirit feels kinder than Datura, yet I speculate, just as deadly. A friend in law enforcement mentioned that when he was called in to look for missing adolescents from 'acid parties'. If there's water around, they usually end up fishing the body out.

LSD is Love

"What I really discovered under LSD is love. Some call it God, and I like this term too. It is God; it is Love. In the best Christian terminology, God is Love and therefore Love is God. In my own terminology they are also one and the same."

"It's all so simple, isn't it? And we louse it up." Love is such a simple matter, but too often we let social rules and standards get in the way and louse it up." Malden Grange Bishop (1963)

Strobe Candles and the Beads of Lights

I am making love with a girlfriend. The room is set up with candles (candles produce soot, artificial candles might work as well). The candles are strobing in unison in my bedroom/library.

I am falling deeply in love.

Love in her eyes.
Pools of passionate desire,
that I melt into.

I notice that a mandala is forming from my lover, then it is encompassing her body. It is coming from both of us and radiating from our bodies. It fills the room with spinning beads of light. She is seeing it too.

Neither of us are on any drugs. My lover has never taken psychedelics, doesn't smoke cannabis. Waves of beads of light are flowing from us. We are merging into each other and orgasm simultaneously. The orgasm lasted for what seemed to be forever.

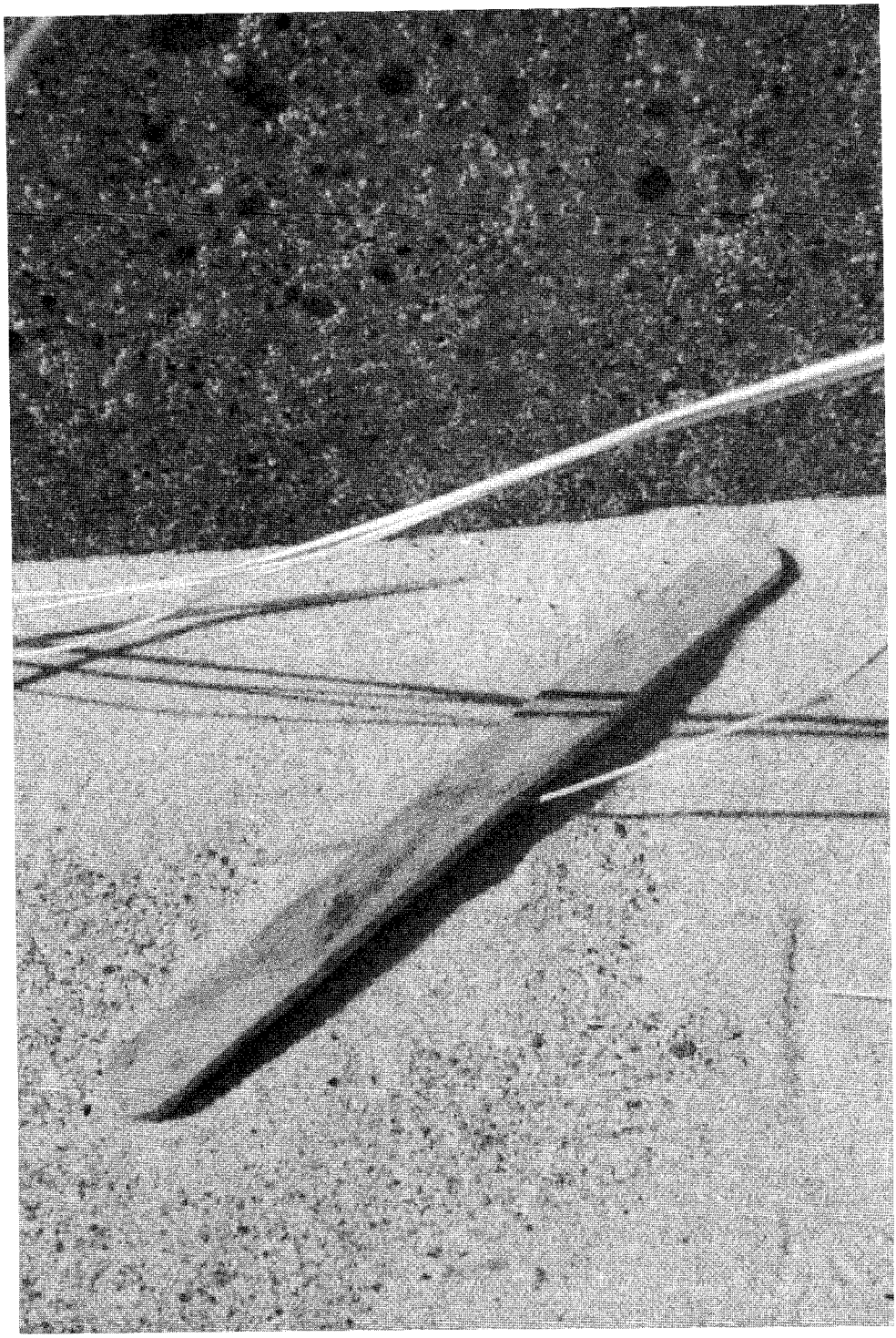
We are laying naked in my bed; the beads of light had stopped. The candles were melted to the base. We went through many candles over the next few months.

LSD can open the experience, but it is not absolutely necessary. It has to do with letting go, to give love and to accept love.

Love is sacred.

"Most people, it seems, who relax and "let go" have the universal experience of discovering a single Reality, a oneness with all things, an identity with God, with the Supreme Being, with the Higher Self, or whatever you wish to call it. Each person arrives at this differently according to his background, his training, his conditioning for the experience, and his attitude toward whatever he discovers."

Malden Grange Bishop (1963)



Chapter 3

Moratorium on LSD-25

In 1967, The President's Commission On Law Enforcement and Administration of Justice compiled a public document titled: *Task Force Report: Narcotics and Drug Abuse, Annotations and Consultants' Papers*; referred to as TFR 1967. I will quote from the various consultants' reports to lay a foundation for a better understanding of prohibitionist policies and the long term effects of these policies.

"Narcotics and Drug Abuse

In 1962 a White House Conference on Narcotic and Drug Abuse was convened in recognition of the fact that drug traffic and abuse were growing and critical national concerns. Large quantities of drugs were moving in illicit traffic despite the best efforts of law enforcement agencies. Addiction to the familiar opiates, especially in big-city ghettos, was widespread. New stimulant, depressant, and hallucinogenic drugs, many of them under loose legal controls, were coming into wide misuse, often by students. The informed public was becoming increasingly aware of the social and economic damage of illicit drug taking.

Organized criminals engaged in drug traffic were making high profits. Drug addicts, to support their habits, were stealing millions of dollars worth of property every year and contributing to the public's fear of robbery and burglary. The police, the courts, the jails and prisons, and social-service agencies of all kinds were devoting great amounts of time, money and manpower to attempts to control drug abuse. Worst of all, thousands of human lives were being wasted.

This Commission has not and could not have undertaken to duplicate the comprehensive study and report on drug abuse so recently completed by another Presidential Commission. Yet any study of law enforcement and the administration of criminal justice must of necessity include some reference to drug abuse and its associated problems. In the course of the discussion in this chapter, recommendations are made where they seem clearly advisable. In many instances these recommendations parallel ones made by the 1963 Commission.

Careful implementation, evaluation, and co-ordination of the new programs, some of which are not yet in operation will be absolutely essential. These are among today's first needs. New ideas are only a

first step. Unless the programs they lead to are provided with sufficient money and manpower and are competently administered, no improvement in drug abuse problems can be expected.

Dangerous Drugs

Drugs in the hallucinogenic class have not yet been proven safe for medical purposes and are not legally available in drugstores. Their sole legitimate use at present is by qualified researchers in connection with investigation reported to and authorized by the Food and Drug Administration (1).

The Hallucinogens

The only legal producer of LSD ceased manufacture in April 1966, and turned over its entire supply of the drug to the Federal Government. A few closely monitored experimental projects involving LSD are still in progress (2).

The hallucinogenic drug traffic appears to be less profit oriented than others (3).

In 1963 the President's Advisory Commission on Narcotic and Drug Abuse found that public and professional education in the field was inadequate. It found the problem clouded by misconceptions and distorted by persistent fallacies (4). Unfortunately these conclusions are as valid today as they were 3 years ago. Misinformation about drugs and their effects is still prevalent, and the measures taken by the Federal Government to correct them are still limited, fragmented, and sporadic. The National Clearinghouse for Mental Health Information within the National Institute of Mental Health (NIMH) collects and disseminates information, but drug abuse is only one of its many concerns, and its audience is largely made up of researchers and other specialists. Similarly, the educational efforts of the Bureau of Narcotics and the Bureau of Drug Abuse Control, while well intended and well executed, are not on the necessary scale. There is a clear present need for a single agency, having a specific mandate for education, to prepare and distribute a broad range of materials, from pamphlets to films, suitable for presentation to target segments of the public, such as college students. The materials must above all be factual.

References

1) Goddard, The Menace of Drug Abuse, Amer. Ed., May 1966.

2) Hearings of §. 2113, §. 2114,§. 2152, supra note 15, at 300 (testimony of Commioner Goddard).

3) Hearings on Organized Crime and Illicit Traffic in Narcotics Before the Permanent Subcommitte on Investigations of the Senate Government Operations Committe, 88th Congress, 1st & 2nd Sessions., pt. 3 (1964); Hearings on §. 2113, §. 2114, § 2152 Before a Special Subcommitte of the Senate Judiciary Committee, 89th Cong., 2nd Sess. (1966); Hearings on H.R. 2, supra note 39.

4) President's Advisory Commission on Narcotic and Drug Abuse, Final Rep. 21-30 (1963).

Mind-Altering Substances

By Richard H. Blum (excerpts from TFR 1967)

Summary of Current Knowledge

There is another fact to consider as part of the evaluation of drug use, drug abuse, and dangerous outcomes. Mind-altering drug use is common to mankind. Such drugs have been employed for millennia in almost all cultures. In our own work we have been able to identify only a few societies in the world today where no mind-altering drugs are used; these are small and isolated cultures.

Hallucinogen Use in the United States

As has been the history with many mind-altering drugs, the pattern of LSD diffusion has been overtime from older prestigeful persons downward to younger less prestigeful ones, also from institutionalized medical and religious (or pseudoreligious) settings to more secular use (2). With secular use, a drug becomes "social," use is subject to less constraint, and greater variety of personalities, settings, and expectations are involved.

Characteristics of Users

LSD, DMT, etc., were first confined to physicians and other research workers and then spread to their subjects, patients, families, and friends. Until a few years ago, LSD remained limited to an "elite" group of successful professionals, artists, and communications industry personnel, their families and friends. These same groups still appear to be using hallucinogens, but the concentration of use appears to have shifted to younger persons. Among teenagers, motorcycle club members, delinquents, urban poor and minorities, etc., there are reports (*Senate Subcommittee on Government Reorganization*, 1966) of spread-

ing interest, suggesting the expected diffusion down the socioeconomic scale. No common psychological or sociological features may be expected among the users of any secular and social drug; different people take drugs for different reasons.

Verified Risks

Crime associated with hallucinogen use appears to have been minimal. Police reports before a California legislative committee emphasized disturbances of the peace (1965) than felonies. It would appear that insofar as decent citizens take hallucinogens their behavior will remain lawful. We expect that with the expansion of hallucinogen use to delinquent groups-and perhaps because it is unlawful in some States, so that its use becomes criminal-a greater frequency of crime will be reported.

Comment

We agree with the present plans of the National Institutes of Health-notably spurred on by Senators Robert Kennedy and Abraham Ribicoff-to conduct epidemiological research on expanding American drug use and to finance further research on the hallucinogens. We also agree with the present policy of the Food and Drug Administration setting up controls over the manufacture and distribution of LSD but not making possession a law violation.

Weighing Risks

With regard to the psychoactive (mind-altering) drugs, what constitutes high gain and what constitutes high risk and who shall decide what these are and how shall that decision affect marketing of the drug? Some tranquilizers which are quite useful in treatment of mental illness produce jaundice-like symptoms and central nervous system (extrapyramidal) symptoms which affect body musculature; yet in the mentally ill (are) these side (toxic) effects... acceptable (?)

Who is to decide what risks a man may take for himself?

LSD

Should it be prohibited from any but experimental medical use with criminal sanctions for possession for any other purpose or, at the other extreme, should it be freely available to anyone to use as he sees fit. Varying positions are held by law enforcement personnel (for control and punitive laws on possession), medical personnel (mostly for medical but no other use), some academicians, theologians, intellectuals, and artist (for nonmedical use but in some controlled setting), members of the government movement (for unrestricted use).

A Narcotics Officer Sample

In another study (1) a small sample of narcotics officers (31 of many more asked to cooperate) were asked about their views on drug offenders and about ideal dispositions for them. Ranking groups on a scale of menace to the community, heroin addicts were ranked as less of a menace than the Communist Party but more of a menace than syndicated crime, burglary rings, and confidence men. Marijuana users were ranked as less of a menace than any of the foregoing but more of a menace than the Mafia, white supremacists, crooked real estate operators, and the like. LSD users raking lower, were more of a menace than the John Birch Society... LSD users came off more easily, being grouped with common drunks, beatniks, homosexuals, adulterers, and speeding drivers...

1) Blum and Whal, "Police Views on Drug Use," in Blum and Associates, *Utopiates*. N.Y.: Atherton Press, 1964

Proposals for Dangerous Drug Legislation

by Michael P. Rosenthal (excerpts from TFR 1967)

Experimental and occasional weekend use of LSD appears to be common (1).

The 1965 amendments (to the Federal Food, Drug, and Cosmetic Act) apply to "depressant or stimulant drugs."

The most important aspect of the coverage of the term "depressant or stimulant drugs," however, is found in that part of the definition which includes:

"any drug which contains any quantity of a substance which the Secretary (of Health, Education, and Welfare), after investigation has found to have, and by regulation designates as having, a potential for abuse because of its depressant or stimulant effect on the central nervous system or its hallucinogenic effect..."

Only recently the Secretary has designated a number of well known tranquilizers and nonbarbiturate sedatives as well as a number of hallucinogens including peyote and mescaline (active ingredient of peyote) and LSD (2). The manufacturers of three of the tranquilizers have challenged the designation, and hearings are currently in progress (3). Two substances used in the manufacture of LSD-lysergic acid and lysergic acid amide have also been designated as "depressant or stimulant drugs" under this part of the definition because they have been found by the FDA to be depressants, and because when either is processed to manufacture LSD a "powerful" hallucinogen is created (4).

Recommendations Dealing With State Law

It is recommended either that the provision of the Federal act which exempts from the prohibition on unauthorized possession, possession "(1) for personal use (of the possessor) or a member of this household, (2) for administration to an animal owned by him or a member of his household" and which puts the burden of proving that the possession was not for any of the purposes mentioned on unauthorized possession with a purpose to sell or otherwise dispose of a "depressant or stimulant drug," but exempting possession (1) for the personal use of a member of the possessor's household, or (2) for administration to an animal owned by the possessor or a member of his household, should be included in any State legislation. State law should not prohibit simple possession or use.

A model State act should also contain a provision to the effect that nothing in it should be deemed to interfere with any right protected by that provision of the State constitution which in substance guarantees the free exercise of religion or with any right protected by the free exercise clause of the first amendment to the United States Constitution.

It is also recommended that unauthorized manufacture of a controlled substance drug should not be the subject of a criminal prohibition under a model act unless it is committed with a purpose to sell or otherwise dispose of such a drug. When it is not committed with such a purpose it may appropriately be a civil violation.

Use and Possession Offenses

The recommendations herein are not based on the view that criminal treatment of use or simple possession is unconstitutional. It is recognized that policy and constitutional considerations may tend to merge. However, the recommendations are based on considerations of what is believed to be proper policy. While it is possible to argue that some of the reasoning Robinson v. California (5) indicates that punishment for use or even simple possession is unconstitutional, the Supreme Court there specifically stated that possession may still be treated as a crime (6). As to use, it was less clear (7). Most States and lower Federal courts have narrowly read Robinson and have held that use may still be made criminal (8).

Possession for Household or Animal Use

It is recommended that the exception to the Federal possession offense for possession for use of household members and for administration to household animals should be retained for controlled drugs which are used in the ordinary practice of medicine. While it is undesirable for a person to give a tranquilizer or barbiturate prescribed for him to another member of his household, the practice is so common that it is not believed the criminal laws should reach it.

LSD

In some respects, whether simple possession or use of LSD should be an offense is a more difficult question to answer than the similar question posed with respect to the commonly used "medically" depressant and stimulant drugs. The possible effects of use may be deemed by some more undesirable than the effects of addiction to barbiturates or nonbarbiturate sedatives or habituation to amphetamines. Upon this question the author does not pass judgment. Unlike the "medically" depressant and stimulant drugs, which have to date been controlled, LSD does not have widespread legitimate use in medical practice. Its medical use is totally experimental (9). It can be introduced or delivered in interstate commerce only under investigational new drug approvals issued to qualified investigators by the FDA (10). Neither would use of LSD be considered normal by most in the community. And though it may be fairly common for a person to give a tranquilizer to a friend or relative, it would not, except in certain groups, be common or considered normal to so distribute LSD.

Even though it is believed that neither simple possession nor use should be prohibited at this time, it must be recognized that if the problem cannot be controlled through trafficking offenses and if adverse affects are found on a large scale, additional legislation may be in order in the future. Such legislation could take the form of a civil violation with a sanction other than interference with personal liberty.

Unauthorized Manufacture

It is recommended that unauthorized manufacture should not be a criminal offense unless it is done with purpose to sell or otherwise dispose of a controlled drug. Illicit manufacturers usually manufacture "depressant or stimulant drugs" to distribute them. However, some controlled drugs may be made on a small scale for personal use. Thus, it

is possible that some individuals may be making LSD solely for their own use. Many of the same reasons which support the exemption of persons who without authorization possess controlled drugs solely for their own use from criminal liability also support their exemption from criminal liability for unauthorized manufacture. Even more than possession, unauthorized manufacture is an offense preparatory to distribution. If the manufacture is not for distribution and if the user is not to be punished for his use, the manufacturer who manufactures for his own use should not be punished either. The mere fact that the user makes the drug himself instead of obtaining it in some other fashion does not stamp him as a more dangerous person. To prove that manufacture for the purpose of sale or other disposition should not ordinarily be a difficult matter. Law enforcement agencies often trace illicit producers through leads furnished by persons who distribute for them or whom these producers otherwise supply. "Simple" unauthorized manufacture, however, may be appropriately treated as a civil violation.

1) See note 313 infra.
2) New York Times, June 28, 1966, p. 50, col. 1.
3) Federal Register, 21 CFR, §166.3, May 18, 1966, pp. 7245, cols. 3 and 7246, col. 1 (proposed).
4) Bulletin on Narcotics, No. 1, 15,21 (1963).
5) In Robinson, 370 U.S. 660 (1962), the Supreme Court held that the cruel and unusual punishment clause of the 8th amendment, made obligatory upon the States by the 14th amendment, barred a State from treating narcotics addiction as a crime. Its reasoning would bar making addiction to dangerous drugs a crime.
6) "A State might impose criminal sanctions, for example, unauthorized manufacture, prescription, sale, purchase, or possession of narcotics within its borders." 370 U.S. at 664.
7) See the dissenting opinion of Mr. Justice White, 370 U.S. at 685, 688.
8) See note, "Alcoholism, public intoxication and the law," 2 Colum. J. of Law and Soc. Prob. 109, n. 142 at 128 (1966).
9) N.Y. Med. Society Report, 22 N.Y. Medicine, No. 9, 3, 5, (May 5, 1966).
10) Statement of Comm'r Goddard before the Subcommittee on Executive Reorganization of the Senate Committee on Government Operations, May 24, 1966 see note 191 supra."

In Response to Moratorium

"The grounds for any possible suppression of these medicines are most entirely superstitious. There is no evidence for their being as deleterious as alcohol or tobacco, nor, indeed, for their being harmful in any way except when used in improper circumstances or, perhaps, with psychotic subjects. They are considerably less dangerous than many of the contents of the family medicine cupboard..." Alan Watts (Harrington 1964).

"We believe that pure LSD ingestion in moderate doses does not damage chromosomes in vivo, does not cause detectable genetic damage, and is not a teratrogen [causing birth defect] or a carcinogen in man," Dishotsky (1971).

LSD-25 has been found not to cause chromosome damage (Irwin 1967) (Loughman 1967) as do many FDA approved psychotropic drugs.

"Contrary to assertions in the popular press, when LSD is administered as part of a therapeutic medical program, "irreversible psychotic changes" and "brain damage" do not occur. Certain irresponsible statements that it does produce such adverse effects have not been supported by valid scientific evidence," Harold A. Abramson, M.D. (1967).

"The blanket suppression of LSD and other psychedelics has been a complete disaster in that (1) it has seriously hindered proper research on these drugs; (2) it has created a profitable black market as it has raised the price; (3) it has embarrassed the police with an impossible assignment; (4) it has created the false fascination with fruit that is forbidden; (5) it has seriously impeded the normal work of courts of justice, and herded thousands of noncriminal types of people into already overcrowded prisons, which, as everyone knows, are schools of sodomy and for crime as a profession; (6) it has made users of psychedelics more susceptible to paranoia more than ever (For purposes of this summary I am including marijuana and hashish as psychedelics, though they do not have the potency of LSD)..." *The Joyous Cosmology*, by Watts, A. 1970

"Therefore, I would like to urge that studies of the effects of LSD in animals and man be intensively pursued under careful control by competent investigators and that current federal and state regulations restricting the use of LSD under such circumstances be reviewed in the length of the published benefits...," William McGlothlin (1967).

"Used [LSD] with ordinary caution by persons trained in its use, we find these substances safe and valuable," Charles Savage, M.D.; James Fadiman, M.A.; Robert Mogar, Ph.D.; Mary Hughes Allen, M.D.(1967).

"Public pressure threatens serious research not only with LSD but with the entire class of hallucinogenic drugs. We cannot put blame on the drugs; we can only put blame on the manner and the ways they are being used. It is my belief that it would be most unfortunate if we were to permit undue hysteria to destroy a valuable tool of science and evaporate an eventual hope for the many hopeless....," Szára 1967.

Many other investigators voiced similar concerns (Cohen 1966); Dahlberg 1966); Freedman 1966; Klee 1966) before congressional committees and other appropriate forums (Szára and Hollister 1973), but the situation remains the same today. Clinical research with these

drugs essentially stopped, with the exception of Strassman's work on DMT (Strassman 1994) and some treatment-oriented work with LSD such as that on dying cancer patients (Yensen 1985)." Szára 1994

Statistics and Anomalies

"To our surprise, half of our subjects (who had never had LSD before), given 125 *ug*. experienced no effects at all during a two-hour stay in a sensory deprivation cubicle. Only after the cubicle door was opened and light and sound began to register on their eyes and ears did they start to feel the effects of LSD. that sensory deprivation can abort the LSD reaction in some people is suggestive evidence that the coding mechanism is one of the affected sites. If there is nothing to code, no changes will occur," Sidney Cohen, MD (1964).

There appears to be two types of people, those who are field-dependent and those who are field-independent (Cohen 1964).

In total darkness, individuals on LSD can see color flashes from two spinning horseshoe magnets (Persinger 1998).

1965 - Leary estimated that 25,000 had tried LSD.
1966 Life Magazine - One million. ref. Tendler 1984

"I soon discovered that the drug "pushers" I had wanted arrested and executed were often our own children who were giving drugs away or selling them because they were "missionaries," rather than evil messengers of some shadowy crime syndicate. Some of our best friends' youngsters were the neighborhood "pusher" in Bel Air because of the status this gave them. Other less affluent boys and girls were supplementing skimpy incomes by small sales that were confined to their own circle of friends..." Art Linkletter.

"Over the period 1977-1992, some 7 to 10 percent of each high school senior class had tried LSD at least once." Henderson (1994)

Those who use LSD are primarily between the ages of 16 to 23. It is a white middle class to white middle-upper-class youth phenomena. Anyone interested in a comprehensive study of this should read: *LSD Still With Us After All These Years*, by Henderson and Glass.

It has been found that the children (10% of high school seniors) of educated parents are more likely to take LSD. The children of uneducated parents and minorities, tent to abuse, narcotics and cocaine. Source: *Monitoring the Future, 1992*; See Leigh A. Henderson (1994)

"The National Household Survey on Drug Abuse for 1993 estimated that 13.2 million Americans 12 years of age or older have used LSD at least once in their lifetime compared to 8.1 million in 1985." Source: DEA 10/1995.

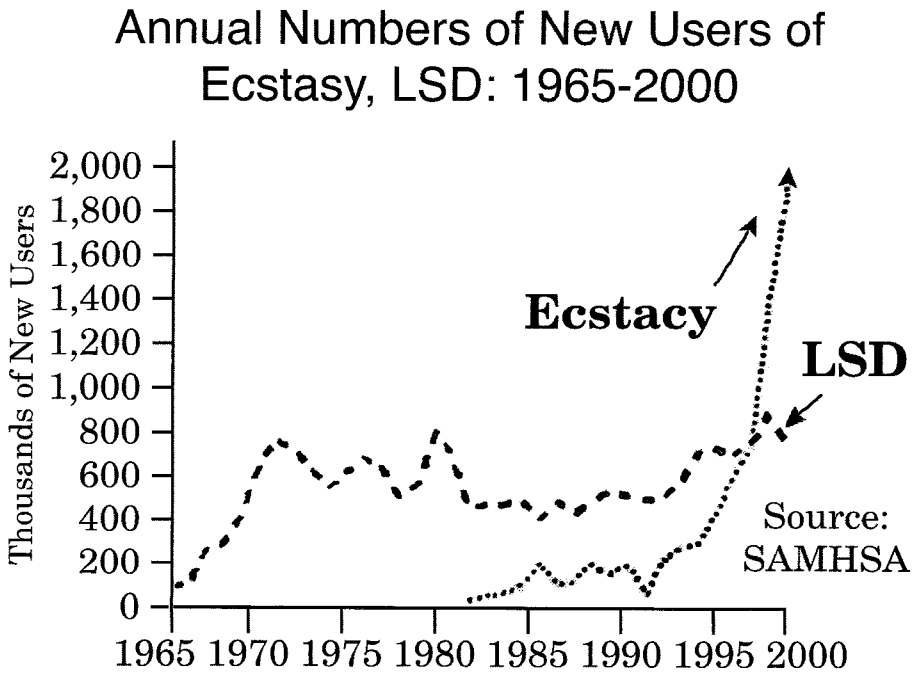
"In 1999, over 12 percent of high school seniors and college students reported that they had used LSD at least once in their lifetime." DEA 2003

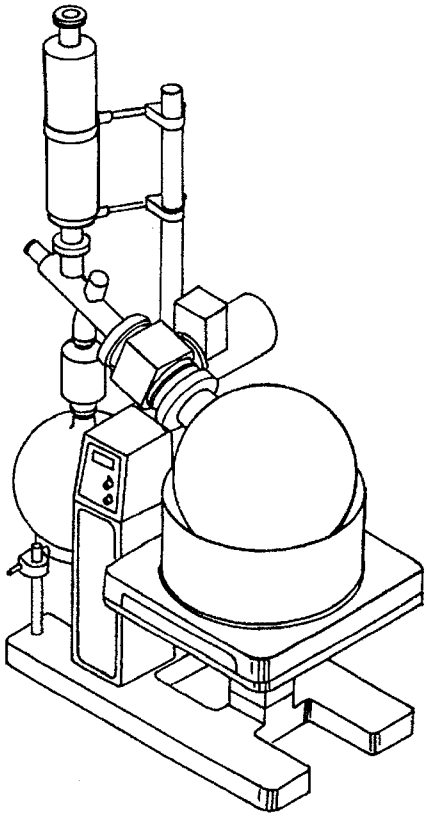
"112,000 Americans aged 12 and older were current LSD users during 2002. Of those surveyed, 10.4 percent indicated using LSD at least once in their lifetime". National Survey on Drug Use and Health

During 2002, 8.6% of college students reported having used LSD within their lifetime, compared to 15.1% of young adults between the ages of 19 to 28 who reported lifetime use of LSD," Drug Abuse Warning Network, Final Estimates 1995–2002, July 2003

"The vast majority of users are middle-class adolescents and young adults". DEA 2003

Individuals who are outside of peak use age range statistics (30 years old plus) generally take LSD for its therapeutic usefulness.





Chapter 4
LSD Laboratories

"LSD manufacturers and traffickers can be separated into two groups. The first, located in northern California, is composed of chemists (commonly referred to as "cooks") and traffickers who work together in close association; typically, they are major producers capable of distributing LSD nationwide. The second group is made up of independent producers who, operating on a comparatively limited scale, can be found throughout the country. As a group, independent producers pose much less of a threat than the northern California group inasmuch as their production is intended for local consumption only." DEA 2003

"Manufacture remains arduous and time-consuming, and is limited by federal chemical controls on the primary precursor chemicals, ergotamine tartrate, lysergic acid and lysergic acid amide. Other limitations include the high price of the precursor chemicals and the high degree of skill required for the manufacture of the drug." (*Chemical Diversion and Synthetic Drug Manufacture*)." DEA 1/2002

List I Chemical	Threshold base weight
Ergonovine and its salts	10 grams
Ergotamine and its salts	20 grams
Ethylamine and its salts	1 kilogram

Special Surveillance List Chemicals:

Ammonia Gas 1,1-Carbonyldiimidazole
Diethylamine and its salts

Equipment:

Tableting Machines Encapsulating Machines
22 Liter Heating Mantels Source: DEA (2002) Chemist's Manual

"Cooking LSD is time consuming; it takes from 2 to 3 days to produce 1 to 4 ounces of crystal. Consequently, it is believed that LSD usually is not produced in large quantities, but rather in a series of small batches. Production of LSD in small batches also minimizes the loss of precursor chemicals should they become contaminated during the synthesis process.

LSD crystal produced clandestinely can be as much as 95- to 100-percent pure. At this purity—and assuming optimum conditions during dilution and application to paper—1 gram of crystal could produce 20,000 dosage units of LSD. However, analysis of LSD crystal seized in California over the past 3 years revealed an average purity of only 62 percent.

Moreover, LSD degrades quickly when exposed to heat, light, and air and is most susceptible to degradation during the application process and once it is in paper form. As a result, under less than optimal, real-life conditions, actual yields are significantly below the theoretically possible yield: 1 gram of LSD crystal generally yields 10,000 dosage units of LSD, or approximately 10 million dosage units per kilogram.

Over the past 30 years, the traditional dilution factor for manufacturing LSD has been 10,000 doses per 1 gram of crystal. Therefore, dosage units yielded from high-purity (95- to 100-percent pure) LSD crystal would contain 100 micrograms. However, dosages currently seen contain closer to 50 micrograms. This discrepancy stems in part from production impurities: during the synthesis process, manufacturers generally fail to perform a final "clean-up" step to remove by-products, thereby lowering the crystal's purity. Further, though average purity of tested LSD crystal samples is, as noted, 62 percent, the average potency of doses analyzed is approximately 50 micrograms rather than 62 micrograms, as would be expected. The diminished potency can be attributed to distributors who, when applying the crystal to paper, often "cheat" by diluting 1 gram of crystal to produce up to 15,000 or more dosage units.

Pure, high-potency LSD is a clear or white, odorless crystalline material that is soluble in water. It is mixed with binding agents, such as spray-dried skim milk, for producing tablets or is dissolved and diluted in a solvent for application onto paper or other materials. Variations in the manufacturing process or the presence of precursors or by-products can cause LSD to range in color from clear or white, in its purest form, to tan or even black, indicating poor quality or degradation. To mask product deficiencies, distributors often apply LSD to off-white, tan, or yellow paper to disguise discoloration.

Compared with methamphetamine, PCP, and other domestically manufactured illicit drugs, few LSD laboratories have been located or seized. Six clandestine LSD synthesis laboratories have been confiscated by DEA since 1981; however, there have been no seizures since 1987."

Source DEA 10/1995

"An analysis of the substances seized from the laboratory revealed 41.3 kilograms of LSD, 23.6 kilograms of iso-LSD, 97.5 kilograms of lysergic acid, and 6.5 kilograms of ergocristine. In the months after the seizure, agents received an additional 13 kilograms of ergocristine, which belonged to the defendants and had been taken by Skinner after the laboratory was moved from the missile base near Salina, Kansas to Wamego. The total amount of ergocristine was 19.5 kilograms."

(Case No. 00-40104-01/02/RDR; 7/20/2003)

"Further trial evidence established that in the history of DEA there have only been four seizures of complete LSD labs and three of these seizures involved Pickard and Apperson including a lab in Mountain View, California in 1998, a lab in Oregon in 1996, and this lab in Wamego, Kansas." (DEA; News Release 4/31/2003)

Investigating LSD Sample

There are many LSD laboratories dotted across the nation which prepare small quantities of LSD for personal use and the use of close friends. There are also 'med labs'. They are run by professionals who continue to produce LSD for patients following the moratorium on human testing. These laboratories generally do not distribute to the public. There are several organized crime laboratories which turn out very large quantities of drugs, not limited to LSD.

Most major universities have a grad student (grad labs) who prepares a small quantity (1-3 grams) as a 'right of passage' of the organic chemist. This LSD is usually given away or is sold at a cost under that of current market prices. Small labs are short lived. They are disorganized, have limited precursor availability and lack a tight active distribution network. Small labs, generally, possess no great risk to society.

During 3 years in the mid 1970's I knew of 6 LSD labs (3 grad labs; 1 idiot lab; 2 med labs). All fizzled in a year, except for one med lab that punched 100*ug* and 200*ug* tablets for aleast a decade.

When a synthesis would take place, I was privileged to be one of the first of many migraine sufferers to obtain relief. Everything is so secretive, no face to face (1970's) with Dr. 'Lysergic Acid' in possession.

"The... code of honor is that you don't turn anybody else in," Doblin continues. "That's lost from the public consciousness. And that's more true from the old days, from the pot dealers. That sort of shifted when the pot dealers got into coke. But that's always been the case with the LSD dealers. That's why the DEA's been very rarely busting labs and major distributors for LSD." Wilkinson 2001

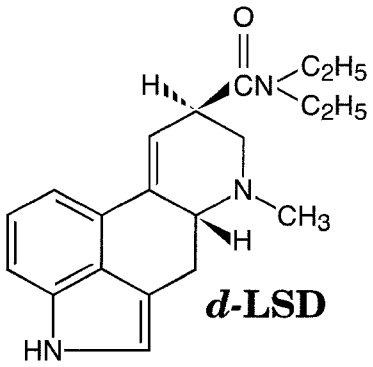
"The sources of supply for most of the LSD available in the United States are believed to be centered in northern California.

During the late 1970's, virtually all LSD tablets analyzed by DEA's Special Testing and Research Laboratory exhibited the same chemical composition and a roughly proportional presence of diluents. The finding suggests a possibility that a single organization manufactured the raw granulated material used in LSD tablet presses nationwide. More probably, however, the analyses indicate that LSD crystal cooks merely have passed on a single recipe for producing the tablets." DEA Oct 1995

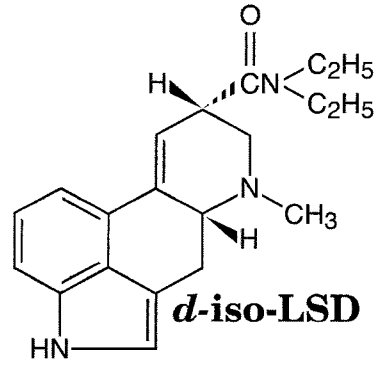
When drug intelligence is given a confiscated sample of LSD there are many tests which can be done to determine more about the source of the drug.

New make shift laboratories for public distribution do not have access to tablet machines (due to cost) and the drug itself tells a lot about the production techniques and knowledge of the chemist/s producing the drug.

The first forms of LSD from a laboratory usually appear on napkins, toilet paper or un-perforated blotter paper. The LSD may not have been triturated properly. By holding a sheet of blotter, impregnated with LSD, in front of a black light will show up imperfections in the trituration. LSD glows under a black light. When holding up a sheet of blotter it will glow evenly if the trituration was done properly, if not, there will be spots that glow brighter than others and not at all in other areas of the paper. This is very dangerous. On one part of the blotter sheet the unit dosages maybe very high and other units, nothing at all. Improper trituration techniques indicates irresponsible sheeting of the blotter.



***d*-LSD**



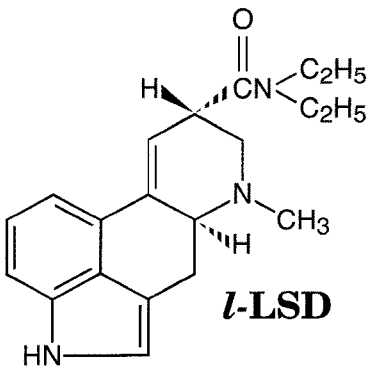
***d*-iso-LSD**

When LSD is in the hands of drug intelligence much can be told about the drug, especially if it is improperly prepared. Each synthesis of LSD creates various isometric forms of the drug.

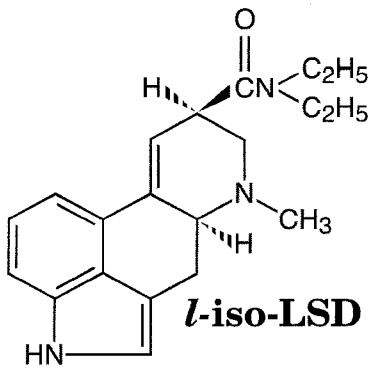
Only the *d*-LSD-25 is active, but improper purification techniques and synthesis lead to impurities and isomers in the end product.

Drug intelligence exists at a federal-international level. Local and state agencies are generally very inadequate at this. Many analytical laboratories are capable of doing very sophisticated microanalysis of drugs, yet the courts and state law enforcement agencies job out testing to determine if a controlled substance is present and usually want to know nothing more about the drug.

Making use of sophisticated analysis by appropriate laboratories at a local level would be a asset for law enforcement; it would allow them to be able to get more insight into the mode of the drug operation.



***l*-LSD**



***l*-iso-LSD**

Profile of Laboratory Chemist

LSD is not made in bathtubs reminiscent of bathtub gin of yesterday's alcohol prohibitionist era. The construction of the LSD molecule is rather complex and can be hazardous. A degree of sophistication and knowledge of techniques to prepare LSD is necessary; LSD is not 'easy to make.'

A person who has no background in chemistry will not be able to understand chemical terms. Someone who is not very familiar with sterile techniques will be unsuccessful at the cultivation of *Claviceps*. (Most purchase ergotamine tartrate through underground channels). Chemists who work with indole molecules must be familiar with manipulating molecules which are sensitive to light, heat and air. This perquisite "knowledge of technique" (being able to work with molecules under inert atmosphere, vacuum and protected from light, aluminum foil, dim lights, etc.) is not for amateurs. Organic chemists capable of mass producing LSD are usually working in cutting edge research (eg. chemical analogs) and are not interested in drug dumping on the population. The primary objective of the 'new age' chemist is to explore the unexplored.

During the early sixties laboratories sprang up across the nation. Many of these laboratories supplied the intellectuals of the day. Today laboratories operate much the same way and the drug does not enter the public market place. Organized crime entered the LSD arena post prohibition of LSD.

Although the law makes no difference between the preparation of and manufacture of LSD, there is a major difference. An individual who makes a few grams of LSD is preparing LSD. An individual or group producing millions of doses of LSD is manufacturing. In the most simplistic of analogies, grandma may bake a few pies for the church bazaar, she is not manufacturing. When grandma goes national and distributes pies throughout the state and nation, she is manufacturing.

A few doses of LSD (for personal use) can not be created by the chemist because reaction products stick to the walls of the labware, filter papers, etc.. Micro synthesis is not something which is easily done. A minimum quantity in the preparation of LSD usually is in amounts from 1 to 3 grams.

LSD is generally sold by one hundred unit amounts. These squares are then broken up among friends. The one hundred quantity is standard for "window pane" and "blotter" types. At this amount the blotter can be examined under black light to determine even or uneven trituration of the drug. The window pane or also called "clearlight" product has also appeared in one hundred and three quantities in small plastic vials (1970's). Several individual dosages can be sent off for analysis to determine dosage, if trituration was done evenly and how the drug was synthesized.

Chemists who only prepare LSD-25 and no other drug are called "psychedelics men." These individuals are more akin to priests than criminals. The pyramid of associates that encompass the "high priest" number into the thousands. At the higher levels of the hierarchy, in close proximity to the priest, are also more psychedelics men and their families.

The procurement of chemicals is done through legal sources and provokes no attention from law enforcement. Drug distribution networks are shut down if drug problems arise in communities that abuse the sacrament.

Psychedelics men can not be called pushers, because they neither want people to abuse the drug, to adulterate (mix amphetamine or PCP with the product) or mishandle the drug. If we were to profile these individuals we could describe them as missionary or modern day apostles.

LSD, its isomers (optical, geometric, positional) and their salts are listed under Schedule 1 as hallucinogenic substances. Schedule 1 meaning: 1) The drug or other substance has a high potential for abuse. 2) The drug or other substance has no currently accepted medical use in treatment in the United States. 3) There is a lack of accepted safety for use of the drug or other substance under medical supervision.

Distribution to the public of analogs, homologs and congeners of the previous molecules maybe subject to controls as described in the Analogue Act of 1986.

Lysergic acid and lysergic acid amide are listed in Schedule 3:

The primary purpose of a scientist is to question, test, and continue researching; no action has any one specific purpose except that of seeking to transcend the unknown into the known. The most a scientist can do is hope to find something which will advance scientific knowledge which in turn will benefit the human race.

The very nature of neurochemistry involves the purchase of drug precursors as they are the precursors of many neurochemicals. Many chemicals that are used in the construction of neurochemicals are also used in clandestine laboratories, the only differences being the end products and their distribution. That's one more reason for thresholds.

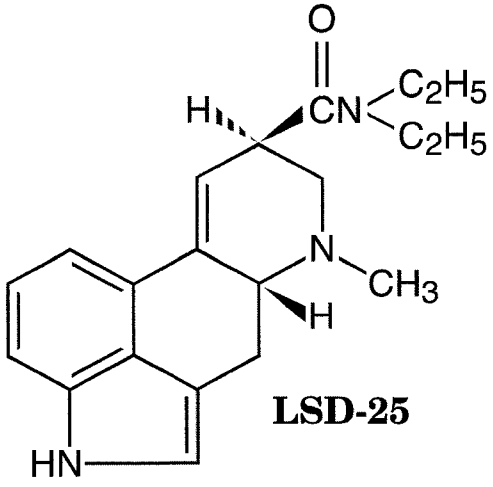
"During clandestine laboratory investigation the forensic chemist may be asked to illustrate the synthetic route used by the defendant(s). For this reason, the forensic chemist should have a clear understanding of the synthetic routes available to the clandestine chemist." Cooper 1984

In all laboratory raids, a forensic chemist must be present to evaluate the chemicals and paperwork. A chemist is also necessary to identify chemicals which may be hazardous and to shut down reactions.

Most laboratories have many chemicals. All individuals (law enforcement officers and suspects) are at risk of exposure to toxic chemicals if they are not contained; safety is paramount. Chemicals are safe as long as they are handled/stored or disposed of properly.

LSD laboratories will have specific equipment for the construction of molecules under an inert atmosphere (eg. gas tanks of nitrogen or argon) and distillation of solvents under reduced pressure (rotary/flash evaporator, vacuum pump, dry ice trap). Although this equipment can be used in the preparation of LSD; this equipment is also used by conscientious chemists so as not to contaminate the environment with solvents or those who are working with heat or air sensitive molecules.

It is the goal of law enforcement to stop illegal dangerous drugs and not to stop budding Einsteins from investigating the unknown. The more law enforcement know about the science of neurochemistry, the more equipped they will be at intercepting and dismantling dangerous illegal drug distribution laboratories.

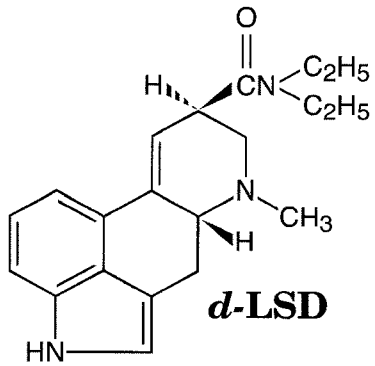


LSD-25

Chapter 5
Lysergamides

d-Lysergic Acid Diethylamide (LSD-25)

LSD-25 was first synthesized in 1938. It was not until 1943 that its powerful psychoactive effects were discovered by Dr. Albert Hofmann.



***d*-LSD**

"...five years after the first synthesis, to produce LSD once again so that a sample could be given to pharmacological department for further tests. This was quite unusual; experimental substances, as a rule were definetly stricken from the research program if once found to be lacking in pharmacological interest." (Hofmann 1980; in LSD)

According to Dr. Hofmann, "Possibly a bit of the LSD solution had contacted my fingertips during the crystallization, and a trace of the substance was absorbed through the skin." (Hofmann 1980; in LSD)

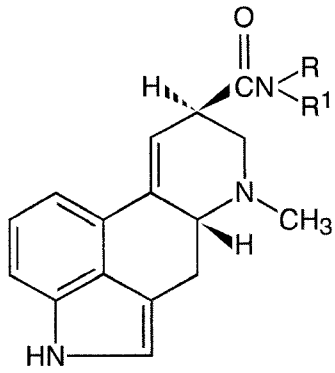
"LSD is in fact not absorbed through skin; Hofmann most likely touched his LSD-contaminated fingers to his mouth." (Henderson 1994)

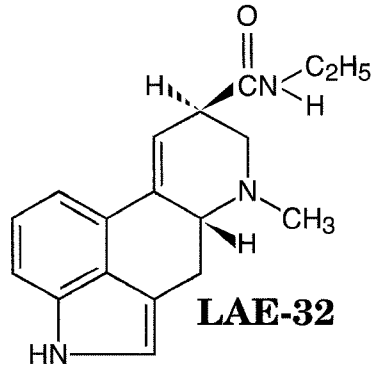
...the rest is history.

"Dr. Hofmann appears to have been one of those individuals who are exceedingly sensitive to the drug. After he experimented with it for a while, he induced his colleagues Rothlin and Stoll to take it. Rothlin got no effects whatsoever. But, luckily, by that time it had been give out to Dr. Stoll's son, a psychiatrist, and he had given it to large numbers of people, and it was obvious that it was having a true pharmacological effect," Dr. Murray E. Jarvik (1967).

"LSD finds an application in medicine, by helping patients in psychoanalysis and psychotherapy to perceive their problems in their true significance." (Hofmann 1980; in LSD)

"Very high doses of LSD, above 500 mcg, evoke quite regularly extreme psychotic episodes, in [Dr.] Leuner's sense, as conditions of catatoniform excitation or stupor, affective-deliriant states with disturbances of consciousness and motor stereotypes," Stanislav Grof, M.D.(1967)

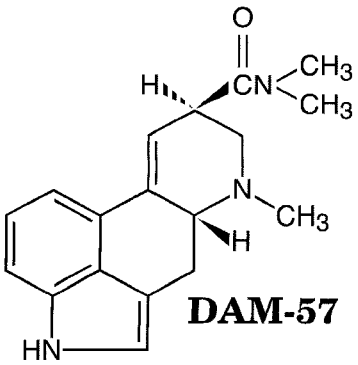




LAE-32

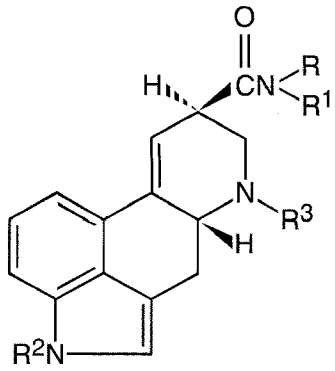
Abbreviated Name		R	R1
LSD-25		CH2CH3	CH2CH3
DAM-57		CH3	CH3
LAE-32		H	CH2CH3
LA-111	Ergine	H	H
LSM-775		-CH2CH2OCH2CH2-	

LAE-32 (*d*-Lysergic acid ethylamide) and DAM-57 (*d*-Lysergic acid dimethylamide) have been reported to be active between 500 to 1,400 µgs. Ott 1993; Jacob 1994). LAE-32 has been reported to be active at 500 ug. (Rothlin 1957). *d*-Lysergic acid monoethylamide; dimethylamide; monopropylamide and dipropylamide are reported to be not active Rothlin 1957a). LA-111 also called ergine (*d*-lysergic acid amide) is the active constituent of an Aztec entheogen called Ololuiqui (*Rivea corymbosa*). It is a feeble psychoactive which is active in humans at 1 mg. LMP-55 (*d*-lysergic acid methylpropylamide) has been reported to be less then 25% the activity of LSD-25. (Abramson). LSM-775 (*d*-lysergic acid morpholide) is active between 300 to 600 µgs. (Grogerty 1957). The *Convolvulaceae* (morning glories) genera of plants contain varying amounts of ergine and the non-active erginine (*d*-iso-lysergic acid amide). Some strains of *Claviceps paspali* also contain varying amounts of both ergine and erginine.



DAM-57

See *LSD - A Total Study* pg. 151 for analog activity comparisons; Jacob 1994; *Pharmacotheon* by Jonathan Ott.



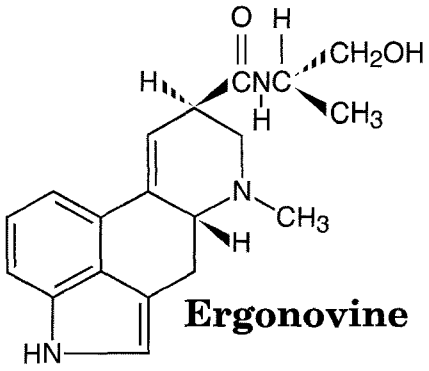
1-Alkyl & N-6 Substituted *d*-Lysergamides

Code Name	R	R1	R2	R3
ALD-52	CH2CH3	CH2CH3	acetyl	CH3
ALA-10	H	CH2CH3	acetyl	CH3
DAM-57	CH3	CH3	H	CH3
LAE-32	H	CH2CH3	H	CH3
MLD-41	CH2CH3	CH2CH3	CH3	CH3
MLA-74	H	CH2CH3	CH3	CH3
ALLYLAD	CH2CH3	CH2CH3	H	ally
BULAD	CH2CH3	CH2CH3	H	butyl
EHLAD	CH2CH3	CH2CH3	H	ethyl
PROLAD	CH2CH3	CH2CH3	H	propyl

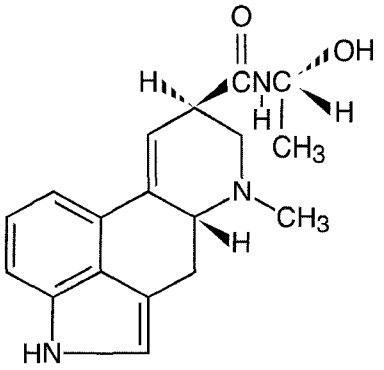
ALD-52 (1-acetyl-*d*-lysergic acid diethylamide) is equally as active a LSD-25. ALA-10 (1-acetyl-*d*-lysergic acid ethylamine) is 1/10 as active as LSD. 1-Alkylation (e.g. R2 = methyl, ethyl) chain lengthening on the LSD-25 molecule decreases the activity of the parent substance (Jacob 1994). MLD-41 binds to serotonin receptors stronger than that of LSD-25. It has reported to be one third as active as LSD (Abramson 1958). N-6 Alkylation (R3) increases activity (Jacob 1994).

See also: Abramson 1958.

Methysergide causes cardiac and pulmonary fibrosis, yet continued to be prescribed for migraines with FDA approval (Graham 1967). For preparation of 6-N substituted lysergamides: (Niwaguchi 1976). Several open chain analogs of LSD and 6-substituted nicotinic acid derivatives have been created: (Lehrfeld 1964) (Whittle 1963). For chloro, nitro and amino analogs of LSD and psilocin see (McKay 1963).



Ergonovine



***d*-Lysergic Acid-α-hydroxy-ethanolamide**

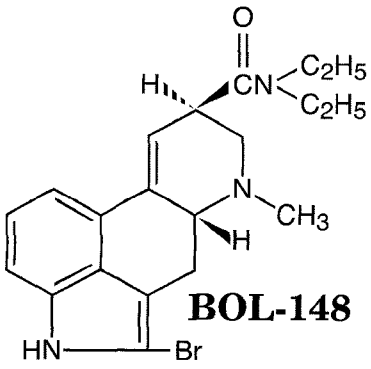
Hydroxy Substituted *d*-Lysergamides

Chemical Name	R	R1
Ergonovine (Ergometrine)	CH3	CH2OH
d-Lysergic acid N-(1-hydroxyethylamide)	CH3	OH

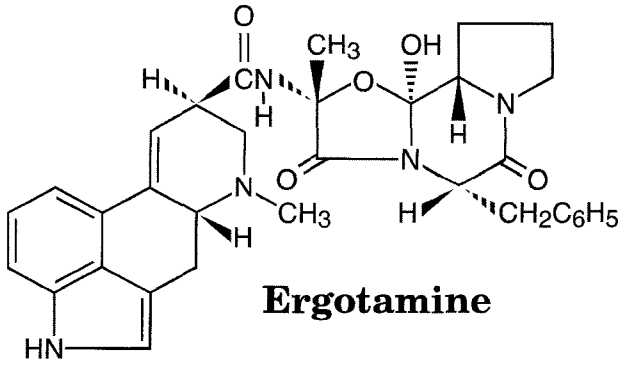
Ergonovine and *d*-lysergic acid N-(1-hydroxyethylamide) occur in some *Convolvulaceae* and also some strains of *Claviceps paspali*. Both produce oxytocic action (contractions of the uterus), mydriasis (dilated pupils) and hyperthermia.

Dihydro-(+)-lysergic acid diethylamide has been reported to be 1.6 times weaker than LSD. Lumi LSD is inactive. (Abramson 1957).

2-Bromlysergic acid diethylamide (BOL-148) is inactive. 2-Oxy lysergic acid diethylamide is the metabolite of LSD-25; it is also inactive.



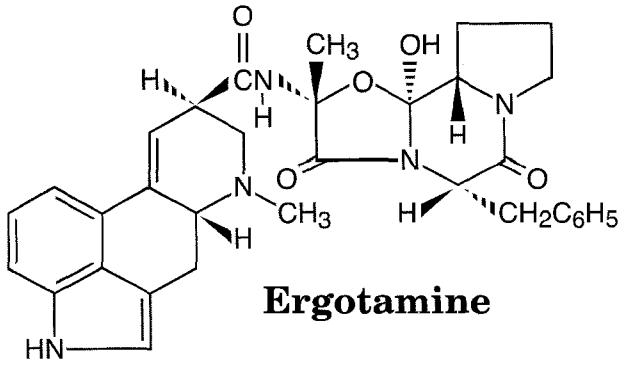
BOL-148



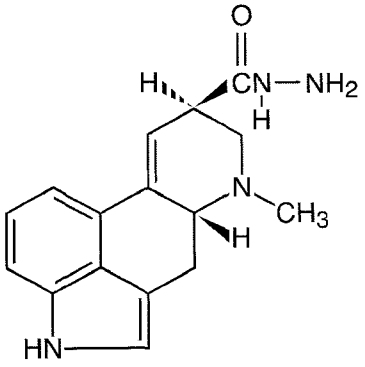
Ergotamine

Ergotamine Tartrate

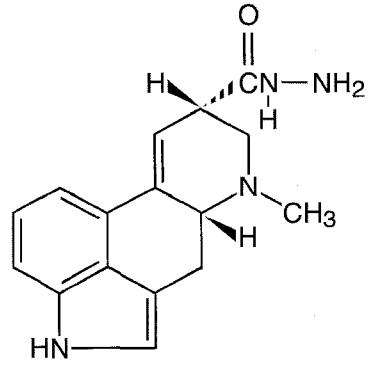
*CAS NUMBER: 379-79-3 *CHEMICAL FORMULA: C33H35N5O5.1/2C4H6O6 *PHYSICAL DESCRIPTIONS: Pale beige powder *MOLECULAR WEIGHT: 1313.56 *MP (DEG C): 203 C (decomposes) *SOLUBILITIES; WATER : <1 mg/mL @ 20 C (RAD); DMSO : >=100 mg/mL @ 18 C (RAD); 95% ETHANOL : <1 mg/mL @ 20 C (RAD); ACETONE : 1-10 mg/mL @ 18 C (RAD) *STABILITY: This chemical is sensitive to prolonged exposure to heat and light. You should protect this container from light and heat; store it under refrigeration. Optical rotation: -125 to -155 degress @ 25 C (c=0.4 in chloroform) *SAX TOXICITY EVALUATION: THR-VERY HIGH via oral route. An experimental teratogen *LABELS REQUIRED: Poison *USES: Used as a vasoconstrictor, specifically for migraines. Has been used as an oxytocic. *ACUTE/CHRONIC HAZARDS: This material is highly toxic by ingestion and parenteral administration. Fatalities have occurred following single injections of only 0.5-1.5 mg. When heated to decomposition it emits toxic fumes. *RECOMMENDED RESPIRATOR: Where the chemical is heated, weighed and diluted, wear a NIOSH-approved half face respirator equipped with a combination filter cartridge, i.e. organic vapor/acid gas/HEPA (specific for organic vapors, HCl, acid gas, SO2 and a high efficiency particulate filter). *SPILLS AND LEAKAGE: If a spill of this chemical occurs, FIRST REMOVE ALL SOURCES OF IGNITION, then you should dampen the solid spill material with acetone and transfer the dampened material to a suitable container. Use absorbent paper dampened with acetone to pick up any remaining material. Seal your contaminated clothing and the absorbent paper in a vapor-tight plastic bag for eventual disposal. Solvent wash all contaminated surfaces with acetone followed by washing with a soap and water solution. Do not reenter the contaminated area until the Safety Officer (or other responsible person) has verified that the area has been properly cleaned.
Source: NTP Chemtrack System November 6, 1990.



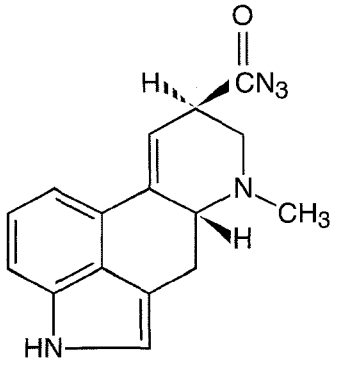
Ergotamine



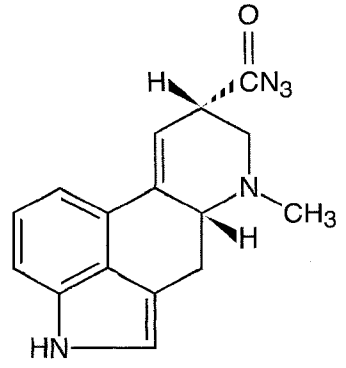
***d*-Lysergic Acid Hydrazide**



***d*-iso-Lysergic Acid Hydrazide**



***d*-Lysergic Acid Azide**



***d*-iso-Lysergic Acid Azide**

Chapter 6
The Curtis Reaction: Lysergic Acid Hydrazine Lysergic Acid Azide and Lysergic Acid Amides

Racemic Lysergic Acid Hydrazide Using Hydrazine Hydrate and Ergot Alkaloids

by Arthur Stoll and Albert Hofmann
Basel, Switzerland December 11, 1935

The complex nature of the alkaloids of ergot is due the fact that on treatment with chemicals they become destroyed to a large extent and that only small quantities of pure degradation products can be isolated. The lysergic acid and its amide is relatively resistant to the action of alkalis and this is the reason why these compounds have been obtained in a relative good yield on alkaline hydrolysis of these alkaloids; but even under optimal conduction of the hydrolysis these compounds are obtained only in a yield of 25-30 per cent of the theory. It was therefore very important to find a process which would give higher yield of these substances.

It has now been found that when suitable derivatives of lysergic acid are subjected to a treatment with hydrazine, lysergic acid hydrazide will be obtained with a very good yield. As starting products for the preparation of lysergic acid hydrazide can be used, for example, the esters and amides of lysergic acid, especially the alkaloids of ergot that contain lysergic acid, whereby for the preparation of the hydrazide the pure alkaloids like ergotamine, ergotoxine, ergotaminine, ergotinine, ergobasine etc. and also raw alkaloids or raw mixtures thereof can be used.

In order to prepare lysergic acid hydrazide, the suitable lysergic acid derivatives are heated with hydrazine hydrate preferably to 80-118° C. under reflux condenser, whereby it is sometimes also advantageous to work in closed vessels under pressure at a temperature of 80-160° C., or in a suitable solvent like propylalcohol, butylalcohol, pyridine, etc., or in an inert atmosphere like in nitrogen gas.

The degradation of alkaloids of ergot with hydrazine to lysergic acid hydrazide is, especially with respect to the obtained yield, much

superior and rational as compared with the known degradation with aqueous or alcoholic alkalis. The fact that by the present process not the lysergic acid, but its hydrazide will be obtained, is a further advantage, because this new derivative of lysergic acid can by one simple recrystallization be obtained in quite pure state and is more stable than the free lysergic acid. Furthermore the hydrazide is a more suitable starting product for synthetical work than the free acid.

A further important advantage of the new process in comparison with the known processes is that it is no more necessary to start from pure ergot alkaloids, as by the hydrazine process raw alkaloids and the noncrystallizable residues of extraction can be used and the lysergic acid hydrazide easily extracted from the reaction medium, owing to its good crystallization properties and the stability of the compound. Instead of direct degradation of ergot alkaloids it is natural that the lysergic acid hydrazide can also be prepared from free lysergic acid by treating same for example with diazomethane and subjecting the methylester of lysergic acid thus obtained to a treatment with hydrazine hydrate. But the direct treatment of alkaloids with hydrazine is naturally simpler and gives also a greater yield than if the hydrazide is prepared by this obvious way.

The lysergic acid hydrazide is very difficultly soluble in water, ether, benzene, chloroform, rather difficultly soluble in pyridine and in hot absolute ethanol. From this last solvent it crystallizes in form of beautiful, compact, clear, on six sides cut crystal plates, that melt under decomposition at 235-240° C. (corr). The hydrazide is easily soluble in acids yielding good crystallizing salts.

One part of ergotamine is suspended in 10 parts of hydrazine hydrate and heated in a nitrogen atmosphere at 100° C. The substance becomes dissolved very rapidly, but as soon as complete dissolution has occurred, precipitation of a solid, nearly colourless compound takes place. The analysis of a test taken out shows that under the action of hydrazine, ergotamine has been transformed into the difficultly soluble ergotaminine. The suspension is then heated further and after about 20 hours it can be observed that complete dissolution has taken place. The brownish solution is then concentrated in vacuo to about 5 parts, whereby the lysergic acid hydrazide begins to separate out in the form of a crystalline greyish powder. In order to complete the precipitation, the reaction mass is left to stand for 1 to 2 days in a cool place in order to complete the crystallization. After this time the crystals are filtered and washed with some absolute alcohol. Yield 0.26 parts, which corresponds to 68 per cent. of the theoretical.

One part of the isomorphous crystallised mixture of ergotamine and ergotaminine, known under the registered Trade Mark of Sensibamine, is dissolved in 5 parts of propyl-alcohol. To this solution are then added 5 parts of hydrazine hydrate and the mixture heated to boiling for 10 hours under a reflux condenser after concentration of the solution in vacuo, the hydrazide is isolated as described above.

Yield 0.2—0.3 parts. Source: Stoll 1937

Lysergic Acid Azides and Amides from Lysergic Acid Hydrazides

Sandoz Basel, Switzerland June 20, 1936

Until now no process was known which allows the physiologically practically inactive lysergic acid to be linked with a basic residue, such as an amino-alcohol or an amino acid, in order to prepare compounds of the type of ergot alkaloids. This was primarily due to the fact that lysergic acid was very difficult to prepare, as it was only obtainable as a degradation product from ergot alkaloids and because its constitution was unknown.

Furthermore this acid is very sensitive like the natural ergot alkaloids themselves, and becomes decomposed by light and the oxygen of the air, and also on treatment with acids. Even in inert organic solvents, such as ethanol, it becomes transformed slowly in the cold and rapidly in warming into a resinous mass, which can no longer be used.

The usual methods for the preparation of acid amides cannot be used with lysergic acid. On trying to transform it into the acid halogenide it becomes transformed into a useless resin. By using lysergic acid methylester, which can be obtained from lysergic acid by treating it with diazomethane, no better result is obtained, as under the conditions necessary for the preparation of acid amides, this compound becomes decomposed.

In our co-pending Specification No. 33855/36 (Serial No. 463,936) we have described the method of preparation of lysergic acid hydrazide by treating with hydrazine alkaloids derived from ergot. By this process it becomes possible to prepare a derivative of lysergic acid with a much superior yield than had been possible until now, and as the lysergic acid hydrazide is a stable and easily crystallisable product, it can easily be obtained in a very pure state. We have now found that by transforming the lysergic acid hydrazide into lysergic acid azide by means of nitrite and acid, a new compound is obtained that is very suitable for the preparation of lysergic acid amides of the type of ergot alkaloids.

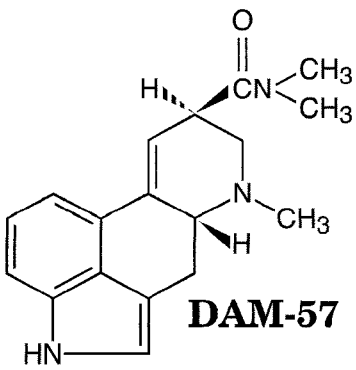
Racemic Lysergic Acid Ethanolamide

rac. Lysergic azide is prepared from the rac. hydrazide as follows: To a cold solution of 10 grams rac. lysergic acid hydrazide in 355 cc. of N/10 hydrochloric acid are added at once and at 0° C. 35.5 cc. of a N/10 sodium nitrite solution previously cooled at 0° C. and with good stirring 365 cc. of a cold N/10 hydrochloric acid solution are added. The solution so obtained has an acid reaction to Congo paper and is left to stand for about 5 minutes at 0° C, in order to complete the formation of the azide.

By neutralizing the solution with an excess of sodium bicarbonate solution, the azide becomes precipitated in clear yellowish voluminous flocks. For further use it can be filtered, washed with water and carefully dried or can be dissolve in solvents, such as ethylether, and the solution used for the condensation with amines. 3 Grams of the azide thus prepared are introduced at -5° C. into a solution of 6 cc. of ethanolamine in 30 cc. of ethanol. The azide dissolves very rapidly and the clear red-brownish solution thus obtained is slowly heated to 30° C. and evaporated in vacuo. By treating the syrup-like residue with 20 cc. of water, a sticky precipitate is obtained that becomes crystalline on standing in the cold for a day. The yield is crude racemic lysergic acid ethanolamide is about 2.3-2.6 grams.

Racemic Lysergic Acid Dimethylamide

To a solution of 1 g. of freshly prepared rac. lysergic acid azide in 200 c.c. of ether are added at 0° C. 5 c.c. of a 5-N alcoholic solution of dimethylamine and the mixture allowed to stand in the dark during one day. The solvent is then evaporated in vacuo and the oily residue stirred with some water until it becomes hard. The dried crude product is then extracted with 100 cc. of boiling benzene, filtered from insoluble impurities, the benzene solution evaporated and the residue crystallized from acetone. In order to obtain the amide in pure form, it is again crystallized from benzene, from which it is obtained in the form of thin plates truncated on six sides, melting with browning at 198° C. (corr.).



DAM-57

Yield about 0.3 g. The sample thus obtained contains one molecule of benzene of crystallization which can be separated by heating the amide at 100° C. in high vacuo. The analysis values obtained correspond to the formula C18H21ON3. The rac. lysergic acid dimethylamide is easily soluble in ethanol and acetone, rather difficultly soluble in benzene and practically insoluble in water. It gives the blue Keller's colour reaction. Source: Sandoz 1938

d-iso-Lysergic Acid-Hydroxybutylamide-2

by Arthur Stoll and Albert Hofmann
Basel, Switzerland June 6, 1939

To a freshly prepared solution of 2 parts of *d*-iso-lysergic acid azide in 300 parts of ether is added an ethereal solution of 2 parts of *d*-2-aminobutanol-1 and the mixture is left to stand at room temperature during 12 hours. The yellowish clear solution is then washed several times with some water, dried over sodium sulphate and the ether evaporated in vacuo. The crystallized residue is treated with a small quantity of acetone and filtered. Yield: 2.2 parts of *d*-iso-lysergic acid-*d,l*-hydroxybutylamide-2. Source: Stoll 1941

Diethylamide of *d*-Lysergic Acid

Sandoz Freiburg, Switzerland April 30, 1943

The present invention is characterised in that the azides of *d* or *d,l*-lysergic acid or *d* or *d,l*-iso-lysergic acid or mixtures of these azides are caused to react with diethylamine and the diethylamides of lysergic acid separated from the reaction products.

In the reaction of the azide of *d*-lysergic acid with diethylamine, there is formed a mixture of the diethylamide of *d*-lysergic acid and the diethylamide of *d*-iso-lysergic acid from which the *d*-lysergic acid derivative is then separated. Similarly from *d*-iso-lysergic acid azide and diethylamine a mixture of the diethylamide of *d*-lysergic acid and the diethylamide of *d*-iso-lysergic acid is formed which is then separated. Moreover, there can be used also the rac lysergic acid azide or the rac. iso-lysergic acid azide whereby mixtures of the diethylamide of *d,l*-lysergic acid and the diethylamide of *d,l*-iso-lysergic acid are formed from which the diethylamide of *d*-lysergic acid can be obtained by suitable means, e.g. by means of the tartrate.

As compared to the known alkaloids of the ergot group which possess an action on the uterus and on the sympathetic branch of the vegetative or autonomic nervous system the new alkaloid prepared according to the present process has a specific action in very small doses of 20—50 *ug.* on the central nervous system.

Example 1

3.0 grams *d*-iso-lysergic acid hydrazide are converted into the azide in the known manner with sodium nitrite in hydrochloric acid solution at 0° C, the acid solution neutralised with sodium bicarbonate and the azide extracted with 300 cc. of ether. 3 cc. of diethylamine are added to the ethereal solution which has been dried for a short while with freshly ignited potassium carbonate and it is allowed to stand in the dark (protect from light) room temperature for 24 hours with occasional shaking. The ether, from which a little dark oil has separated, is evaporated off in a vacuum and the residue-thereby obtained rubbed with 30 cc. water and filtered off with suction. The dark amorphous product has a specific rotation [α]D20 = about 100° (in pyridine) and consists chiefly of a mixture of approximately equal parts of the diethylamide of *d*-lysergic acid and the diethylamide of *d*-iso-lysergic acid.

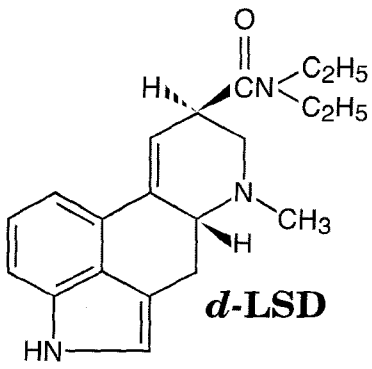
The separation of the two isomers can be carried out e.g. by chromatographic adsorption. The mixture is dissolved in a little chloroform which contains 0.5% of alcohol the solution poured on to a column of aluminium oxide of 4 cm. diameter and 60 cm. length and the chromatogram is produced with the same solvent. Dark impurities soon pass into the filtrate. Then follows a broad zone appearing blue in ultra-violet light which contains the diethylamide of *d*-lysergic acid. Yield 1.0 to 1.3 grams.

The diethylamide of *d*-iso-lysergic acid passes though as a much more slowly moving zone. The residue of the chloroform solution thereof amounting to 0.8 to 1.2 grams, crystallises on taking up in acetone in beautiful prisms of m.p. 182° C (corr.) with decomposition and consists of the pure diethylamide of *d*-iso-lysergic acid [α]D20 = +217° (c=0.4 in pyridine).

The diethylamide of *d*-iso-lysergic acid is converted into the diethylamide of *d*-lysergic acid according to the processes usual with ergot alkaloids, namely by treating them with acid or alkaline compounds. By allowing the iso compound to stand with diluted alcoholic potash for example an equilibrium is soon formed which consists of approximately equal quantities of the lysergic acid and the iso-lysergic acid compounds.

The diethylamide of *d*-lysergic acid separated chromatographically first of all in the amorphous condition crystallises after taking up in a little acetone and dilution with ether is needles united into bundles. From benzene pointed prisms are obtained which melt without sharpness at 80-85° C. (corr.) with decomposition. The new compound is soluble in water with difficulty and is very easily soluble in methanol and ethanol. It possesses a specific rotation [α] D20 = +30° (c=0.4 in pyridine).

3.0 grams of rac. iso-lysergic acid hydrazide are converted into the azide in the known manner and then separated in voluminous light yellow flocks by stirring in an excess of sodium bicarbonate solution, thoroughly filtered off by suction and immediately added at -5° C. to a cooled solution of 3 cc. diethylamine in 30 cc. of alcohol, whereby the azide rapidly dissolves. The brown solution is slowly warmed to 30° C. maintained at this temperature for 1 hour and then the solvent evaporated off in a vacuum. The pasty residue is rubbed with 30 cc. of water and filtered with suction. The crude condensation product, 2.8 grams, which consists of rac. diethylamide of iso-lysergic acid and rac. diethylamide of lysergic acid is chromatographically separated as described in Example 1 with aluminium oxide as adsorption column using chloroform containing 0.5% of alcohol as the solvent. The two main zones are formed having a blue fluorescence in ultra-violet light of which the quickly moving one contains the rac. diethylamide of lysergic acid whereas the more firmly held fraction consists of the rac. diethylamide of iso-lysergic acid. Source: Sandoz 1946



***d*-LSD**

Preparation of *d*-Lysergic Acid Azide

Sandoz Basel Switzerland December 18, 1946

Example 1

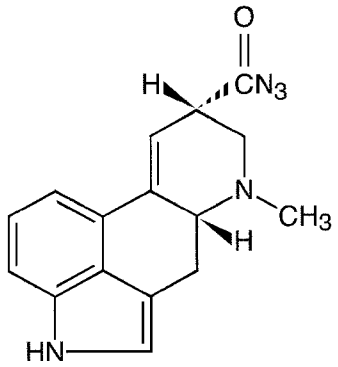
1.14 grams *d*-lysergic acid hydrazide are dissolved in 40 cc. 0.1-N hydrochloric acid, 40 cc. 0.1 N sodium nitrite is added and then 46 cc. of 0.1 N hydrochloric acid added at 0°. Crystallisation of the hydrochloride of lysergic acid azide begins towards the end of the addition of the acid. After 6 minutes 16 cc. of sodium bicarbonate are added and the acid azide taken up in 400 cc. benzene.

Example 2

1.0 gram of the hydrazide of iso-lysergic acid is dissolved in 3.55 cc. 0.1 N hydrochloric acid 3.55 cc. normal sodium nitrite added and 40 cc. 0.1 N normal hydrochloric acid added at 0° C. After 3 minutes 10 cc. N sodium bicarbonate are added and the acid azide taken up in 300 cc. of benzene. The benzene solution is sharply dried with sodium sulphate.

Method 3

0.7 gram *d*-iso-lysergic acid hydrazide is dissolved in 25 cc. 0.1 N hydrochloric acid 5 cc. 0.5 N sodium nitrite added and 28 cc. 0.1 N hydrochloric acid added at 0° C. After 5 minutes, 10 cc. N sodium bicarbonate are added and the acid azide taken up in 500 cc. ether. The ethereal solution is dried with sodium sulphate and carefully evaporated to dryness. The acid azide thus separates for the greater part in the crystallised form.



***d*-iso-Lysergic Acid Azide**

Method 4

1.136 grams *d*-iso-lysergic acid hydrazide are converted into the acid azide as described in method 2 and this is taken up in 400 cc. of toluene. The toluene solution after thorough drying with sodium sulphate...

Source: Sandoz 1950

iso-Lysergic Acid Piperidide

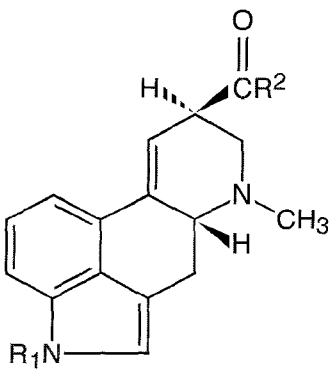
Sandoz Basel Switzerland January 21, 1955

A solution of 10 g of iso-lysergic acid azide in 50 cc. of tetrahydrofuran is mixed with 2 cc of piperidine, the mixture is warmed for two hours to 50°, the solvent is evaporated and a solution of the residue in ethyl acetate is extracted with aqueous sodium bicarbonate solution. The residue resulting from the evaporation of the dried ethyl acetate solution (11 g). Source: Sandoz 1956

Preparation of *d*-Lysergic Acid Hydrazide (the New Process)

by Albert Hofmann; Jürg Rutschmann;
Paul Stadler, Franz Troxler
Basel, Switzerland January 13, 1964

The process consists of reacting an optically active lysergic acid derivative of the formula:



wherein R1 has the above significance and R2 signifies a member selected from the group consisting of alkoxy containing from 1 to 4 carbon atoms inclusive, amino (including a member selected from the group consisting of primary amino alkylamino, hydroxyalkylamino, in which the alkyl portion contains from 1 to 4 carbon atoms inclusive), or a member selected from the group consisting of tripeptides of the type of the natural ergot alkaloids, with excess hydrazine in the presence of at least one equivalent of acid for every mol of compound II, the expression excess meaning that the number of mols of hydrazine must be at least equal to the number of equivalents of acid+2. The acid used for the process may be selected from the inorganic or organic acids of sufficient strength, e.g. with a pK value smaller than 5.

Examples of acids which may be used are inorganic acids, such as hydrochloric, hydrobromic or sulfuric acid, alkyl- or arylsulfonic acids, for example methanesulfonic or benzenesulfonic acid; aliphatic and aromatic carboxylic acids such as formic, acetic or benzoic acid.

The acid may be added to the reaction mixture as such or, simpler, by using a preformed acid addition salt of the alkaloid or of part of the hydrazine.

The use of a solvent is as a rule unnecessary, as the excess hydrazine suffices to bring all reactants into solution. Nevertheless an additional solvent, such as a lower alcohol or a glycol, may be useful in certain cases.

From the (Stoll 1937) as well as from *Hoppe Seyler's Zeitschrift fur physiologische Chemie*, vol. 250, page 7 (1937), and from *Helv. Chimica Acta,* vol. 26, pages 922-928 (1943) it is known that by treating a derivative of the optically active *d*-lysergic acid or *d*-iso-lysergic acid, e.g. one of the naturally occurring ergot alkaloids, *d,l*-iso-lysergic acid hydrazide, an optically inactive product, results. The optically active compound, *d*-iso-Lysergic acid hydrazide, which is the only useful one for the synthesis of products of pharmaceutical interest, must subsequently be separated from the racemic mixture with the aid of an optically active acid, e.g. di-(*p*-toluyl) *l*-tartaric acid as demonstrated e.g. in the *U.S. Patent* 2,447,214. This procedure is experimentally cumbersome and more than half of the original material is lost, mainly as the useless *l*-iso-lysergic acid hydrazide.

On the contrary to this older process, the principal advantage of the new procedure lies in the fact that the cleavage of *d*-lysergic acid derivatives by hydrazine can be effected without racemization in a single, simple step. The yields of useful products are in the range of 80-95% of the theory.

A further advantage of the new process lies in the fact that the reaction proceeds under relatively mild conditions with fewer side reactions occurring concurrently. A reaction temperature of 80 to 90° is as a rule most suitable. The time of reaction at this temperature is in the range of one to a few hours, whereas at e.g. 135° it is only a few minutes. Temperature and time conditions are of course chosen as mild as possible, as with unnecessarily long reaction times and high temperatures partial racemization may occur.

A further technical advantage of the new process is the fact that starting materials may be used which could not be cleaved by hydrazine alone, e.g. the compounds with R2 equal to amino, alkylamino or hydroxyalkylamino (e.g. ergometrine).

It is easily seen that the present process results in the circumvention of the great loss of material in the form of useless L-compound and in a reduction of the necessary chemical steps. The consequence is a considerable saving of time and materials, as well as a further increase in overall yield.

Thus, the new process, for example in preparing the hitherto unknown 1-methyl-*d*-lysergic acid hydrazide, is an important technical advance.

The process in accordance with the invention may, far example, be effected as follows:

One part by weight of compound II in the form of a salt, e.g. ergotamine hydrochloride, is heated with four parts by weight of anhydrous hydrazine for one hour at 90° C. The reaction mixture is then diluted, preferably with water, the excess hydrazine and water distilled off azeotropically, and the residue shaken between aqueous tartaric acid and an inert water-immiscible solvent, e.g. chloroform or ether. The aqueous phase is made alkaline and the final product taken up in a water-immiscible solvent, e.g. chloroform.

Should a compound II, in which R2 signifies NH2 or the radical of an alkylamine or hydroxyalkylamine, having from 1 to 4 carbon atoms inclusive in the alkyl portion, be used, then the reaction mixture, after heating with hydrazine, may be directly diluted with water and extracted with a water-immiscible solvent, e.g. chloroform, if desired, after the addition of ammonia.

The crude product obtained after evaporation of the solvent is generally a mixture of the optically active hydrazides of the lysergic and, predominantly, of the iso-lysergic acid series, which may be separated in accordance with known methods, e.g. by crystallization and/or chromatography or by conversion to a salt with a suitable acid.

Example 1:

1.16 g. of ergotamine hydrochloride are heated with 4 cc. of anhydrous hydrazine for 1 hour to 90°, 20 cc of water are then added, the water and the hydrazine hydrate distilled off and the residue shaken between tartaric acid and ether. The bases liberated after making the aqueous phase alkaline are shaken with chloroform and the crude product resulting after evaporation of the chloroform chromatographed on aluminum oxide.

The resulting *d*-iso-lysergic acid hydrazide is washed into the filtrate with chloroform containing 0.5% of ethanol. Prisms from methanol/ether. Melting point; 202°. [α]D20°=+445° (c.=0.5 in pyridine).

A small quantity of *d*-lysergic acid hydrazide is then washed into the filtrate with chloroform containing 2% of ethanol. Prisms from methanol/ether. Melting point 215°. [α]D20=+10° (c.=0.5 in pyridine).

Example 2

1 g. of iso-lysergic amide hydrochloride is heated with 4 cc. of hydrazine for 50 minutes to 90°, 20 cc. of water and 5 cc. of concentrated ammonia then added and the mixture shaken with chloroform. The crude

product remaining after evaporation of the chloroform is heated with 15 cc. of methanol, the resulting *d*-lysergic acid hydrazide remaining undissolved. Melting point 216° [α]D20=+10° (c.=0.5 in pyridine). The methanol solution is evaporated to dryness and the residue chromatographed on aluminum oxide. *d*-iso-Lysergic hydrazide is washed into the filtrate with chloroform containing 0.5% of ethanol. The melting point and optical rotation are similar to those given in Example 1. (c.=0.5 in pyridine).

Example 3:

A solution of 1.6 g. of 1-methyl-ergotamine hydrochloride in 6.4 cc. of anhydrous hydrazine are heated for 1 hour at 90°, the mixture diluted with 50 cc. of water, the water and the hydrazine hydrate are distilled off and after the addition of a further 6.4 cc. of anhydrous hydrazine the remaining procedure is repeated. The residue is then shaken between a diluted tartaric acid solution and chloroform. The bases liberated after the tartaric acid solution has been made alkaline are shaken with chloroform and the crude product remaining after evaporation of the chloroform is chromatographed on a column of 25 g. of aluminium oxide. 1-methyl-*d*-iso-lysergic acid hydrazide is washed into the filtrate with chloroform containing 0.5% of ethanol. The compound crystallizes from ethanol in the form of nice leaflets. Melting point 201-204°. [α]D20=+400° (c.=0.5 in pyridine). Keller's colour reaction: blue.

The 1-methyl-*d*-lysergic acid hydrazide is then washed into the filtrate with 1 to 2% of ethanol. Prisms from ethanol. Melting point 194-195°. [α]D20°=+14° (c.=0.5 in pyridine). Keller's colour reaction: blue.

Example 4

1 g. of lysergic acid propanolamide (ergometrine) is heated with 5 cc. of anhydrous hydrazine and a solution of 350 mg. of hydrogen bromide in 2 cc. of ethanol to 90° for 1 hour. The reaction mixture is worked up in the manner described in Example 2, the products being *d*-lysergic acid hydrazide and predominantly *d*-iso-lysergic acid hydrazide with the properties indicated in Example 1.

Example 5

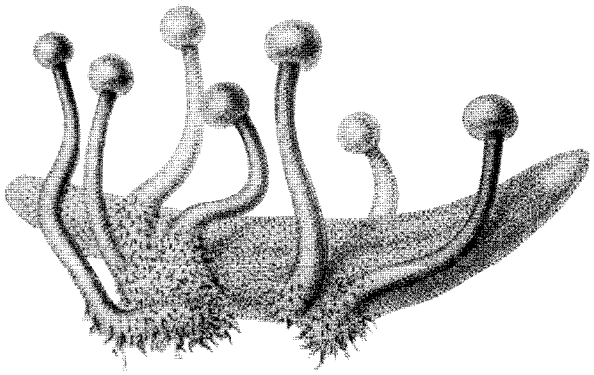
A solution of 1 g. of ergotamine is heated with 5 cc. of anhydrous hydrazine and 1 cc. of glacial acetic acid to 120° for 30 minutes. The mixture is worked up as described in Example 1, the product being essentially *d*-iso-lysergic acid hydrazide with the properties mentioned.
Source: Hofmann 1966a

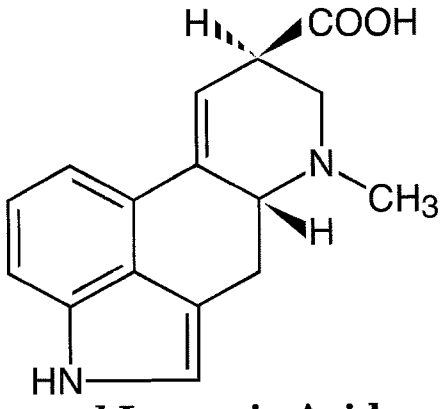
Lysergic Acid-(3'-allyloxy-2'-oxy-propyl)amide

Erzsébet Magó née Karácsony; Jézsef Borsi;
Endre Csányi; Katalin Pik; Lajos Wolf
Budapest, Hungary May 22, 1975

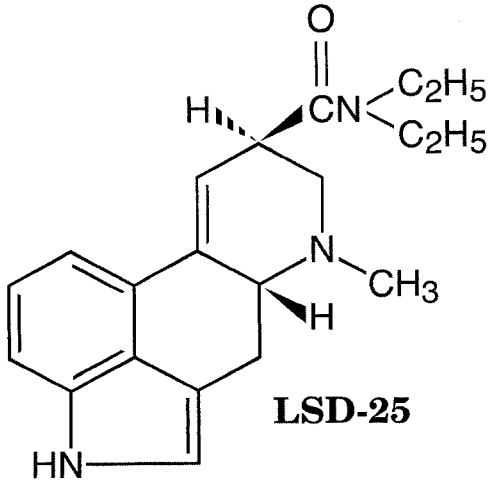
2.81 g of lysergic acid hydrazide, dissolved in 100 mL. of 0.1 N hydrochloric acid, are added to 10 mL. of a 1 N aqueous sodium nitrite solution, and thereafter 15 mL. of 1 N hydrochloric acid are added dropwise to the stirred mixture at 2° to 5° C. The mixture is stirred for an additional 15 minutes at 0° to 5° C Thereafter the mixture is neutralized with saturated sodium bicarbonate solution, and extracted in three portions with a total amount of 2L. of ether. The etheral fractions are combined, dried over anhydrous potassium carbonate, filtered, and a solution of 1.32 g. of 3-allyloxy-2-oxypropylamine in 100 mL. of isopropanol is added to the stirred filtrate. The mixture is stirred at room temperature for 4 hours, and thereafter washed with water. The aqueous phase is extracted with 2x50 mL. of chloroform. The organic solutions are combined, dried over anhydrous potassium carbonate, and evaporated to dryness.

The dry residue is dissolved in ethanol, and converted into its maleate. Source: (Karácsony 1977; 1978) (approx. 70% yield).





***d*-Lysergic Acid**



LSD-25

Chapter 7
The Garbrecht Synthesis
Preparation of Amides of Lysergic Acid

by William L. Garbrecht
Indianapolis, Indiana November 21, 1955

My novel process conveniently can be considered as a two-step process comprising first the formation of a mixed anhydride of lysergic and sulfuric acids, and second the formation of the desired lysergic acid amide by reacting the mixed anhydride with a nitrogenous base. As will be seen from the following disclosure, the intermediate mixed anhydride of lysergic and sulfuric acids can be, but need not be, isolated.

The mixed anhydride of lysergic and sulfuric acids is prepared by reacting a dispersion, i.e., a solution or suspension, of lysergic acid or one of its basic salts with sulfur trioxide. The formation of the mixed anhydride is rapid so that within a matter of minutes, the mixed anhydride is ready for utilization in the second step of the process.

The amide of the lysergic acid is prepared from the mixed anhydride by reacting the latter compound with a nitrogenous base containing at least one active hydrogen attached to nitrogen. This reaction also is quite rapid, especially when the nitrogenous base being reacted is a strongly basic amine, so that the reaction generally is substantially complete within a few minutes. When less basic amines are employed, it is desirable to allow a reaction period of up to about twenty minutes or thereabouts to insure the completion of the reaction.

The temperature at which the reaction between the lysergic acid compound and the sulfur trioxide is carried out is not critical but can be varied over a wide range. Preferably, temperatures substantially in excess of ambient room temperature, that is, about 25° C., should be avoided to reduce likelihood of formation of tarry byproducts. Although temperatures up to about 35° C. are quite suitable for the carrying out of the reaction, lower temperatures in the neighborhood of about 0° C. or below can be advantageous insofar as such temperatures tend to reduce the production of minor amounts of colored reaction products which, although they do not appear to affect the yield adversely, can cause difficulties in the provision of the pure final product. Moreover, the amides of lysergic acid are themselves often quite unstable when they are impure or are present in various reaction mixtures, and hence high

temperatures desirably are avoided to reduce the likelihood of the decomposition of the amides. Temperatures as low as —30° C. can readily be employed, the limiting factor being the temperature at which the dispersing agent solidifies. Since the mixed anhydrides of lysergic and sulfuric acids is relatively unstable, it is essential that a temperature of about 0° C. or preferably substantially lower be employed should the isolation of the anhydride be desired. In such event, the isolation most conveniently is effected by carrying out the reaction between the lysergic acid and the sulfur trioxide in a dispersing agent in which the mixed anhydride is insoluble. Alternatively, if the reaction is carried out in a solvent in which the mixed anhydride is soluble, it can be selectively precipitated by the addition of a solvent in which the mixed anhydride is insoluble. The precipitated mixed anhydride can be isolated by filtration or centrifugation or similar procedures. The anhydride thus obtained is an amorphous solid of tan to brown color. It is unstable and decomposes on standing even at low temperature. As is to be expected, the decomposition of the anhydride is quite rapid when it is exposed to a moist atmosphere.

For the preparation of the mixed anhydride, lysergic acid or a basic, i.e., a metallic or metalloid salt thereof, can be employed. The term "Lysergic acid" as used herein includes the isomers of Lysergic acid, e.g., *d*-lysergic acid, *l*-lysergic acid, *d*-isolysergic acid, and *l*-isolysergic acid. Also included are acids closely related to lysergic acid, for example, 5,6-dihydrolysergic acid and its acyl derivatives and 10,11-dihydro-isolysergic acid. Illustrative examples of salts, both metallic and metalloid, of lysergic acid that are useful for the purpose of this invention include the lithium, potassium, barium, lead, calcium, ammonium, triethylamine, trimethylamine salts, and the like. Although in general the more water-soluble of the lysergic acid salts are productive of better yields of mixed anhydride, those salts which are substantially insoluble, are fully operative. The lysergic acid compound employed can be anhydrous or hydrated. Because of the difficulty of obtaining lysergic acid or its salts in completely anhydrous form, it is more convenient to employ the hydrated forms.

The sulfur trioxide employed for the production of the mixed anhydride desirably is purified and freed from sulfuric acid since the use of sulfur trioxide containing sulfuric acid in any substantial quantity causes a reduction in yield of mixed anhydride as compared to that obtainable with pure sulfur trioxide. Any conventional means of securing pure sulfur trioxide can be employed, as for example, distillation from phosphorous pentoxide. The sulfur trioxide itself can

be prepared by any known method, for example, by distillation from oleum, by the catalytic oxidation of sulfur dioxide or from the commercially available stabilized sulfur trioxide known to the trade as "Sulfan B."

The mixed anhydride of lysergic and sulfuric acids using lysergic acid or a hydrate thereof as a starting material can be prepared by reacting one molecular equivalent of lysergic acid with about one or two molecular equivalents of sulfur trioxide. For the preparation of the mixed anhydride it is preferable to employ a salt of lysergic acid since the use of the salt leads to greater yields of the mixed anhydride. When a salt of Lysergic acid is employed as a starting material, maximum yields of mixed anhydride are obtained when the lysergic acid salt and sulfur trioxide are reacted in a molar ratio of one molecular equivalent of lysergic acid salt to two molecular equivalents of sulfur trioxide. This ratio desirably is employed regardless of whether the salt is hydrated or anhydrous, since substantial variation from this molar ratio results in a decreased yield of mixed anhydride.

The reaction between the lysergic acid compound (whether acid or salt) and the sulfur trioxide is carried out in a dispersing agent, i.e., a solvent or suspending agent. The character of the agent employed is not critical, it being necessary only to use a dispersant which is inert with respect to the reactants, that is, one which will not react with or destroy the lysergic acid compound or the sulfur trioxide. Among suitable dispersants are included the hydrocarbons, for example, hexane, the dialkylformamides, for example, dimethylformamide; the dialkylsulfoxides, for example, dimethylsulfoxide; the alkyl nitriles, for example, acetonitrile, and other solvents such as diethylcyanamide, dioxan, and the like.

As is well known, sulfur trioxide has the ability to form complexes or adducts with many solvents. It readily forms quite stable complexes with dimethylformamide and dioxan, both of which are specifically mentioned above. Such complexes are most conveniently employed in the preparation of the mixed anhydride. The complexes as such can be employed directly in the reaction mixture, or can be dissolved in an excess of the complexing agent, or can be mixed with a different dispersant and then reacted with the lysergic acid compound which also can be suspended or dissolved in a dispersing agent. The use of a sulfur trioxide complex is advantageous since it appears to moderate the course of the reaction and hence reduces the possibility of formation of undesirable by-products. Furthermore, it avoids most of the difficulties inherent in the handling of a dangerous reagent in of

gaseous or concentrated liquid form. Moreover, the amount of sulfur trioxide required to be reacted with the lysergic acid compound can readily be portioned out since the concentration of the sulfur trioxide in the complex or solution of the complex, and hence the amount of sulfur trioxide available for reaction, is easily determined by simple titration an aliquot with a standard alkali solution.

The second step of the process of this invention, which comprises reacting the mixed anhydride with a nitrogenous base containing at least one active hydrogen attached to the nitrogen, is carried out simply by mixing the anhydride and nitrogenous base. The amine can be dispersed in an organic or aqueous dispersant, even water itself, and the dispersion added to the mixed anhydride or the amine itself can simply be added to a dispersion of the mixed anhydride. As is obvious, the dispersant should not contain highly reactive functional group which will react competitively with the mixed anhydride and so reduce the yield of the desired amide.

The temperature at which amide formation is carried out is not critical. The upper temperature limit is governed largely by the stability range of the mixed anhydride, and the lower temperature limit by the freezing point of the dispersant employed. Most conveniently the temperature employed is that which was used for the preparation of the mixed anhydride, since the invention is carried out most expeditiously by preparing the mixed anhydride and then adding the nitrogenous base to the anhydride without separating the anhydride.

The complete utilization of the mixed anhydride requires about five mols of nitrogenous base per mol of mixed anhydride. The mixed anhydride appears to contain in ionic association with it, a molecule of sulfur trioxide or sulfuric acid, which accounts for the requirement of an apparent excess of base. The use of less than five mols of base per mol of anhydride is operative but results in a lower yield of amide than that obtainable with five mols of base. Although greater molar proportions of nitrogenous base can be employed, no advantage accompanies their use.

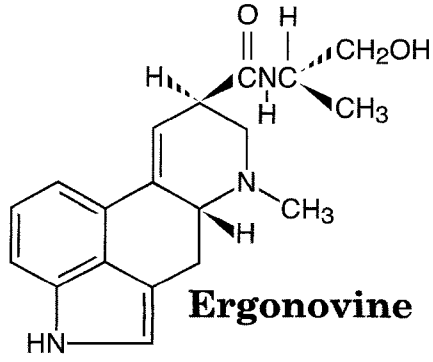
Nitrogenous bases useful in my process include ammonia, either as liquid ammonia or as ammonium hydroxide, and hydrazine; primary amines, such as ethylamine, glycine, propylamine, aniline, and the like, secondary amines, such as morpholine, diphenylamine, methylaniline, diethylamine, and the like; aminoalcohols, such as 2-aminopropan-1-ol, isovalinol, ephedrine, 2-(N-benzylamino)-propan-l-ol, and the like. A specific aminoalcohol, 1-(+)-2-aminopropan-l-ol, is especially usefully employed in my invention since its reaction with the mixed anhydride of *d*-lysergic and sulfuric acids produces the pharmacologically active

compound ergonovine. It is a particular advantage of my process, and quite unforeseen, that the reaction product of an aminoalcohol and the mixed anhydride of lysergic and sulfuric acids consists solely of the desired amide with no undesired ester byproduct. For example, when 1-(+)-2-aminopropan-l-ol is reacted with the mixed anhydride of *d*-lysergic and sulfuric acids, the only lysergic acid derivatives produced in isolatable quantities are ergonovine and its isomeric amide, ergonovinine.

The lysergic acid amides produced in accordance with this invention are readily isolated by a conventional method which comprises treating the reaction mixture with water to obtain an aqueous solution of the lysergic acid amide, extracting the amide into a water-immiscible solvent and then crystallizing the amide or a salt thereof. Other conventional isolation and purification procedures known to the art are also applicable. As is well recognized by those skilled in the ergot alkaloid field, many of the ergot alkaloids and related compounds are crystallizable only with difficulty, and hence careful manipulation is often required to obtain crystalline products.

Preparation of Ergonovine

7.15 g. of *d*-lysergic acid monohydrate and 1.05 g. of lithium hydroxide monohydrate are dissolved in 100 mL. of methanol. The methanol solution is evaporated to a syrup in vacuo, and to the syrup are added 500 mL. of anhydrous dimethylformamide. The solution is concentrated in vacuo at a temperature of about 50° C. to a volume of about 150 mL. to distill off methanol and water. The remaining solution of the lithium salt of *d*-lysergic acid is cooled to about 10° C. and 74.5 mL. of a 0.67 molar solution of sulfur trioxide-dimethylformamide complex in dimethylformamide are added. The mixture is stirred thoroughly and is allowed to stand for about five minutes to assure the complete formation of the mixed anhydride of lysergic and sulfuric acids. To the solution are then added with stirring 9.4 g. of 1-(+)-2-aminopropan-l-ol. The mixture is allowed to stand for five minutes during which time the anhydride and the amine react together to produce ergonovine. 300 mL. of 20 percent aqueous sodium chloride solution are added to the reaction mixture, and the



Ergonovine

aqueous mixture is extracted five times with 300 mL. portions of ethylene dichloride. The ethylene dichloride extracts which contain ergonovine and some isomeric ergonovinine formed during the reaction are combined and evaporated to a syrup in the cold in vacuo. The syrup is dissolved in a minimum amount of methanol and sufficient maleic acid is added to make the solution slightly acidic. The solution is treated with a small amount of decolorizing carbon and is filtered to remove the carbon. To the filtrate are added about three volumes of ether. The mixture is allowed to stand for several hours at about 0° C. whereupon ergonovine maleate separates in crystalline form. The maleate salt is filtered off and dried in air.

From the filtrate, ergonovinine, the amide of the isomeric isolysergic acid, is recovered as follows:

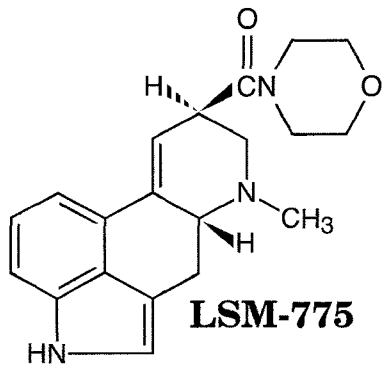
The filtrate is evaporated to a syrup and to the syrup are added about 200 mL. of saturated aqueous sodium chloride solution. Sufficient aqueous ammonium hydroxide is added to make the solution slightly basic and the basic solution is extracted several times with 100 mL. portions of ethylene dichloride. The ethylene dichloride extracts are combined and evaporated in vacuo yielding a residue comprising ergonovinine.

The ergonovinine can, if desired, be crystallized in the form of its nitrate or some other salt or can be isomerized to ergonovine by the procedure disclosed by Stoll and Hofmann in *Helv. Chim. Acta.* 26, 944 (1943).

Preparation of *d*-Lysergic Acid Morpholide

3.24 g. of potassium *d*-lysergate monohydrate are dissolved in 25 mL. of anhydrous dimethylformamide. The solution is cooled to about 10° C. and is treated with 18.9 mL. of a 1.06 molar solution of sulfur trioxide-dimethylformamide complex in dimethylformamide. After a few minutes 4.3 g. of morpholine are added to the reaction mixture with stirring. The mixture is allowed to stand for a few minutes during which time the formation of the morpholine amide of lysergic acid is completed. The mixture is treated with 100 mL. of saturated sodium chloride containing 5 mL. of concentrated ammonium hydroxide. The lysergic acid amide is recovered from the aqueous mixture by extracting repeatedly with ethylene dichloride until tests of the ethylene dichloride extracts with Van Urk reagent indicates that the extraction is substantially complete. The combined extracts are dried with anhydrous magnesium sulfate and are concentrated by evaporation in vacuo in the cold. The residual syrup comprising *d*-lysergic acid morpholide is

dissolved in 25 mL. of methanol, the solution is acidified with excess maleic acid, and is diluted with ether to incipient turbidity. The mixture is allowed to stand in a refrigerator for several hours, whereupon off-white, needle-like crystals of *d*-lysergic acid morpholide acid maleate are formed and precipitate from the solution.



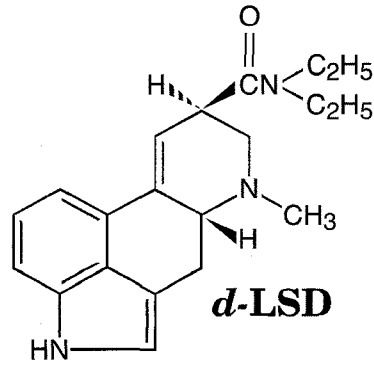
LSM-775

The above procedure yields about 1.5 g. of crystals which melt indefinitely with decomposition on a Fisher John block at about 195° C.

From the crystallization mother liquors, after concentration, neutralization, and re-extraction with ethylene dichloride, there can be obtained about 0.8 g. of amorphous *d*-isolysergic acid morpholide. This material can be isomerized to *d*-lysergic acid morpholide by the procedure disclosed by Smith and Timmis in *J. Chem. Soc.* 139, II, 1168 (1936).

Preparation of *d*-Lysergic Acid Diethylamide

About 1.64 g. of potassium *d*-lysergic acid hydrate are suspended in about 25 mL. of anhydrous hexane. To the suspension is added a solution of 0.8 g. of sulfur trioxide dissolved in 25 mL. of acetonitrile, the addition being carried out with the reagents maintained at about 5° C., and with sufficient stirring. To the mixture is added a solution of about 1.82 g. of diethylamine dissolved in 25 mL. of ether. After standing for about five minutes the solution is extracted about five times with 100 mL. portions of water. The aqueous extracts are combined and are saturated with sodium chloride. The saturated solution is extracted five times with 100 mL. portions of ethylene dichloride. The ethylene dichloride extracts are combined and are evaporated in vacuo leaving a residual syrup comprising, a mixture of the diethylamides of *d*-lysergic and *d*-isolysergic acids.



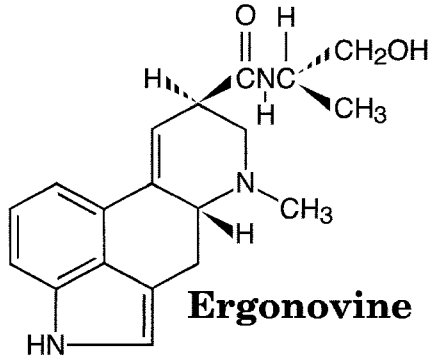
***d*-LSD**

Preparation of Ergonovine

1.62 g. of racemic potassium lysergate are dissolved in 25 mL. of anhydrous dimethylformamide. 7.9 mL. of a 1.26 molar solution of sulfur trioxide-dimethylformamide complex dissolved in dimethylformamide are added and the mixture is maintained at about 10° C. with stirring for a few minutes. 1.88 g. of 1-(+)-2-aminopropan-1-ol are added to the solution of the mixed anhydride and the mixture is stirred for five minutes while maintaining the temperature at about 10° C. 100 mL. of saturated sodium chloride solution and 5 mL. of concentrated ammonium hydroxide are added to the reaction mixture, and the aqueous mixture is extracted five times with 50 mL. portions of ethylene dichloride. The ethylene dichloride extracts are combined and dried, and the ethylene dichloride is removed by evaporation in vacuo. The residual syrup containing the two l-(+)-propanol amides of *d,l*-lysergic acid is treated with methanol and excess maleic acid to convert the two amides into their maleate salts. The mixture of amide maleates is precipitated from the methanol solution by the addition of ethyl ether, and the precipitated mixture is filtered off and dried in air.

Source: Garbrecht 1956

Reference: Garbrecht 1959



Ergonovine

Sulfan B (trade name) or *gamma* sulfuric acid anhydride: "May be prepared in a laboratory by heating fuming sulfuric acid and collecting the sublimate in a cooled receiver. If the vapor is condensed above 27° the *gamma*-form is obtained as a liquid... Melted SO3 exists in the *gamma*-form and on solidifying tends to the *alpha*-form.... Combines with water with explosive violence... On contact with wood shavings the heat produced by dehydration is sufficient to cause fire. mp 16.8°." Source: Merck Index 12th Edition.

Lysergic Acid Amide Derivatives

by Albert Hofmann and Franz Troxler
Baselland, Switzerland November 7, 1961

1-Methyl-*d*-lysergic Acid (+) butanolamide-(2)

1.0 g of 1-methyl-D-lysergic acid and 86 mg. of lithium hydroxide (89%) are dissolved with shaking in 150 cc of absolute methanol, 0.5 g of activated charcoal are added, the mixture filtered through a talc layer, the filtrate evaporated to dryness and the residue subsequently heated to 80° for two hours in a high vacuum. The dry lithium salt of 1-methyl-D-lysergic acid is then dissolved in 30 cc of dimethyl formamide and a solution of 547 mg of sulphur trioxide in 5.5 cc of dimethyl formamide is added to the solution at 0°. The mixture is kept at 0° for 10 minutes and then cooled to -10°, 1.5 g of (+)-butanolamine(2) are added and the mixture kept at -10° for a further 10 minutes. To decompose the sulphur trioxide complex 250 cc of water are added at -60° and the mixture shaken with ethyl acetate. Upon evaporation of the ethyl acetate solution, which has been dried over potassium carbonate a crude product results from which the pure 1-methyl-D-lysergic acid (+)-butanolamide-(2) is obtained as the bimaleate in the following manner: The crude product is dissolved in 5 cc of methanol, a solution of 350 mg of maleic acid in 2 cc of methanol is added and 15 cc of ether are slowly added to the mixture which crystallizes spontaneously. Needles from methanol. Non-characteristic melting point, decomposition above 165°. [α]D20= +44° (c=0.3 in water). Keller's colour reaction: blue.

The 1-methyl-D-lysergic acid used as a starting material is produced by heating a methanolic solution of 1-methyl-ergotamine with barium hydroxide, precipitating the barium ions with sulphuric acid, adding ammonia after filtration and concentrating the solution to a small volume, the 1-methyl-D-lysergic acid crystallizing. Melting point 224°.

1-Ethyl-D-lysergic Acid Ethylamide

2.5 g of 1-ethyl-D-lysergic acid (melting point 219—220°) and 221 mg of lithium hydroxide (89%) are dissolved with shaking in 150 cc of methanol, 0.5 g of activated charcoal are added, the mixture filtered through a talc layer, the filtrate evaporated to dryness and subsequently

heated for a further 2 hours to 80° in a high vacuum. The dry lithium salt of 1-ethyl-D-lysergic acid is then dissolved in 60 cc of dimethyl formamide and 14.7 cc of a sulphur trioxide dimethyl formamide complex solution (content 94 mg SO3 per cc dimethyl formamide) are added and 15 cc of ether are added slowly added to the solution at 0°. The mixture is kept at 0° for 10 minutes, then cooled to -10°, 1.91 g of ethylamine added and the mixture kept at -10° for a further 10 minutes. To decompose the sulphur trioxide complex 250 cc of water are then added at -60° and the mixture shaken with chloroform. Upon evaporation of the chloroform solution dried over potassium carbonate a crude product, from which the pure 1-ethyl-D-lysergic acid ethylamide is obtained as the bimaleate remains. The crude product is dissolved in 10 cc of methanol, a solution of 805 mg of maleic acid in 5 cc of methanol is added and 15 cc of ether are added slowly to the mixture which crystallizes spontaneously. Needles from methanol, having a melting point of 179—180° (decomposition) result. Keller's colour reaction: blue
.

1-n-Propyl-D-lysergic Acid (+) Butanolamide-(2)

A solution of 1.2 g of sulphur trioxide in 12 cc of dimethyl formamide is added to a solution of 2.39 g of lithium salt of 1-n-propyl-D-lysergic acid in 30 cc of dimethyl formamide at 0°. The mixture is kept at 0° for 10 minutes, 3.37 g of (+)-butanolamine-(2) are added at -10° and the mixture kept at -10° for a further 10 minutes. To decompose the sulphur trioxide complex 200 cc of water are added to the reaction mixture at -60° and the mixture is shaken with chloroform. The chloroform/dimethyl formamide solution, which has been dried over potassium carbonate, is then evaporated at 40° and finally at a pressure of approximately 0.01 mm of Hg and is evaporated to dryness. The residue, the 1-n-propyl-D-lysergic acid (+)-butanolamide-(2), is converted to the crystalline maleate in the following manner: The amide is dissolved in 40 cc of methanol, a solution of 1 g of maleic acid in 10 cc of methanol is added and approximately 50 cc of ether carefully added, the 1-propyl-D-lysergic acid (+)-butanolamide-(2) bimaleate crystallizing in the form of colourless needles having a melting point of 194—197°. [α]D20 = +36° (c=0.25 in water). Keller's colour reaction: blue. Source: Hofmann 1965

Alternative Syntheses of Lysergamides

Alternative sythneses produce mixed esters, amides, etc. and are generally non specific in acylatation. Yet illegal drug laboratories use what they have available. In many cases the end products are mixures of inactive lysergamides and active lysergamides. These impurites are chemical 'finger prints' for the forensic chemist which not only tells what chemicals were used in the synthesis, but can also be useful in determaining the knowledge of the chemist.

Starting Molecule: Lysergic acid
Rgts: Trifluoroacetic acid anhydride Diethylamine Acetonitrile
Product: *d*-iso-Lysergic acid diethylamide Ref.: Pioch 1956

Starting Molecule: *d*-Lysergic acid monohydrate
Rgts: Methansulfonic Acid Anhydride L-2-amino-1-propanol DMF
Product: Ergonovine Ref.: Garbretch 1958

Starting Molecule: *d*-iso-Lysergic acid
Rgts: N,N-Carbonyldiimidazole Dimethylformamide Diethylamine
Product: N,N-Diethyl-*d*-lysergamide Ref.: Cerny 1962

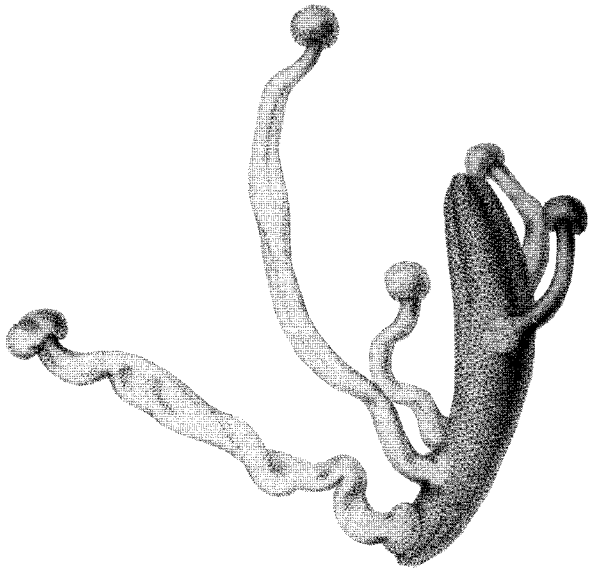
Starting Molecule: *d*-iso-Lysergic acid hydrazide
Reagents: Acetylacetone *d*-2-Aminobutanol-1
Prdt: *d*-iso-Lysergic acid-*d*-1-hydroxybutylamide-2 Ref.: Hofmann 1963

Starting Molecule: anhydrous Lysergic acid
Reagents: Phosgene Diethylamine Dimethylformamide
Product: N,N-Diethyl-*d*-lysergamide Reference: Patelli 1964

Starting Molecule: Lysergic acid monohydrate
Reagents: Cyclohexylamine L-2-Amino-1-propanol
Phosphorous oxychloride
Prdt: *d*-N-CyclohexyllysergamideRefs.: Johnson 1973; Shulgin, *TIHKAL*

Starting Molecule: Lysergic acid
Reagents: Trimethylaluminum Amine
Product: Lysergic Acid Alkylamides Reference: Neef 1982

Starting Molecule: *d*-Lysergic acid See also: *US* 6,221,870 B1
Rgts: Propanephosphoric acid anhydride, Diethylamine, Triethylamine
Product: *d*-Lysergic acid diethylamide Ref.: Galen 1998



Chapter 8
Fractional Crystallization of Lysergamides

Fractional Crystallization of *d*-Lysergic Acid-Hydroxybutylamide-2

Arthur Stoll and Albert Hofmann
Basel, Switzerland June 6, 1939

The alcoholic ethereal filtrate is then dried over calcined potassium carbonate and the solution evaporated, whereby 0.9-1 part of a mixture of *d*-lysergic acid-*d,l*-hydroxybutylamide-2 and of *d*-iso-lysergic acid-*d,l*-hydroxybutylamide-2 is obtained.

In order to separate the isomers, the residue is dissolved in 15 parts of hot chloroform and filtered from the small quantity of inorganic salt, whereby on cooling down, the difficultly soluble chloroform compound of *d*-lysergic acid-*d,l*-hydroxybutylamide-2 crystallizes out. Yield: 0.4 part.

This base gives easily water-soluble salts with inorganic and organic acids, such as hydrochloric, phosphoric, acetic, tartaric, oxalic acids. The neutral tartrate of the base can be prepared for example by treating a methanol solution of the base with the equivalent quantity of tartaric acid, whereby the neutral salt crystallizes out in white needles formed to bushes. This salt is easily soluble in water. When dried in high vacuo, its composition is (C20H25O2N3)2.C4H6O6.
Source: Stoll 1941

Optically Active Salts of Lysergic and iso-Lysergic Acids

Arthur Stoll and Albert Hofmann
Basel, Switzerland September 16, 1942

The preparation and the isolation of the therapeutically valuable and active derivative contained in ergot is a problem that has occupied chemistry and pharmacy for more than 120 years. Actually it is known that the action of ergot is due to the alkaloids contained therein, which have been isolated in recent years and which are always present as pairs of isomers. Chronologically the following alkaloids have become known up to now:

Ergotinine (1878)	Ergotoxine (1906)
Ergotamine (1918)	Ergotaminine (1918)
Ergobasine (1935)	Ergobasinine (1935)
Ergosinine (1936)	Ergosine (1936)
Ergocristine (1937)	Ergocristinine (1937)

The long period which has been necessary in order to isolate the active compounds of ergot shows the great difficulties which are encountered with regard to their isolation and preparation in a pure form. These difficulties are especially due to the complication and especially to the easy decomposition of the ergot alkaloids which become transformed into dark amorphous products already under the action of light and of the air-oxygen. Also acids as well as alkalis and in some case even solvents are capable of transforming the ergot alkaloids, this being proved by the fact that they become dark colored and lose their crystallization power. Further difficulties lay in the fact of the easy transposition of the levo-rotary form into the dextro-rotary form of the alkaloids this leading again to mixtures which may be crystallized only with difficulties.

The ergot alkaloids having the properties of producing mixed crystals between the levo- and dextro-rotary form, often false conclusions have been made, so that mixtures of different products have often been considered as pure individuals. It will be seen in the following that the property of alkaloids consisting in giving isomorph crystallizations has also led to the fact that preparations from ergot have been considered as uniform compounds, whereas in truth they were mixtures of different alkaloids.

The great decomposition power of the ergot alkaloids is based on the liability of the basic radical which is characteristic for all alkaloids. This radical is the lysergic acid of the formula C15H15N2COOH. The said compound consists of an unsaturated nitrogen-containing ring system, the constitution of which is hitherto not completely cleared up and which is present in two isomer forms turning easily into each other. The ergot alkaloids known up to now correspond to the general formula C15H15N2COR; therefore, the individual ergot alkaloid parts differ from each other only in the constitution of the substitute R which is linked to the carboxylic group of the lysergic acid. Source: Stoll 1948

Fractional Crystallization *d*-Lysergic Acid Diethylamide *d*-Tartrate from Racemic Lysergic Acid Diethylamide

Sandoz Freiburg, Switzerland April 30, 1943

From the rac. diethylamide lysergic acid, the diethylamide of *d*-lysergic acid can be separated e.g. by means of the neutral tartrate. For this purpose, 3.2 grams diethylamide of rac. lysergic acid (1/100 mol.) are dissolved in 6 cc. methanol and treated with 0.75 grams *d*-tartaric acid (1/200 mol.) in 2 cc. of the same solvent by inoculation with the *d*-tartrate of the diethylamide of lysergic acid this crystallises in almost colourless needles united in bundles. Yield 1.0 to 1.2 grams.

Source: Sandoz 1946. See also pages 73-74.

Separating and Purifying Alkaloids of Ergot

Sandoz Freiburg, Switzerland Sept. 16, 1943

The separation of the natural as well as of the synthetic lysergic acid derivatives has been effected up till now chiefly by conversion into inorganic or organic salts. Thus, e.g. ergotoxine, decades before it could be obtained as base in crystalline form, was purified as phosphate which crystallises in bundles of needles. Furthermore, salts of ergot alkaloids with sulphuric acid, hydrochloric acid, oxalic acid, tartaric acid, picric acid etc. are described in the literature. Although some of these salts crystallise very well they are nevertheless so easily decomposed that repeated recrystallisation is not advantageous. They are therefore only useful to a limited extent for the preparation of lysergic acid derivatives in a pure state. It is not possible to prepare with the usual acids crystallised salts of dextro-rotary isomers of the ergot alkaloids which are derivatives of iso-lysergic acid.

The ergotoxine preparation which was used for the separation into its components as described hereinafter was recrystallised twice from benzene and possessed the following properties corresponding with those given in the literature for ergotoxine. When crystallised from benzene it formed homogeneous glittering, highly refractive plates which by drying in a high vacuum to 80° C. lose 21% of their weight as solvent of crystallisation. Easily soluble in ethyl alcohol, methyl alcohol, acetone, chloroform and acetic acid ester; sparingly soluble in ether and not crystallisable in any of these solvents. The product dried in a high vacuum melted between 170° and 200° C. with decomposition. Sandoz 1945

Crystallization of *d*-Lysergic Acid Diethylamide Tartrate by Sandoz Basle, Switzerland April 30, 1943

By dissolving 1 equivalent of the base with 1 equivalent of *d*-tartaric acid in a little methanol, the neutral tartrate of the diethylamine of *d*-lysergic acid crystallises in needles united in bundles. The salt is very readily soluble in water and melts without sharpness and with decomposition at 200° C. (corr.). Source: Sandoz 1946

Ergot alkaloids and their salts form solvent complexes with the solvent of crystallization. This will cause breakdown of the alkaloid. Solvent complexes are broken off (dried) by heating the alkaloid in high vacuum till constant weight or for approximately one hour.

Examples: "The sample (rac. lysergic acid dimethylamide) thus obtained contains one molecule of benzene of crystallization which can be separated by heating the amide at 100° C. in high vacuo." Sandoz 1936

"The residue (mixture of *d*-lysergic acid amide and *d*-isolysergic acid amide) is dissolved in methanol containing slightly over a molar equivalent of maleic acid, ether is added to the solution to the point of turbidity, and ether is added to the solution to the point of turbidity, and the mixture is chilled to about 0° C. A quantity of fine, colorless needles of ergine maleate are thus obtained which are purified by recrystallization form a mixture of ethanol and ethyl ether. Recrystallized ergine acid maleate prepared by the above method was found to exist as the mono-methanol solvate. Source: Garbrecht 1955.

Fractional Crystallization of *d*-Lysergic Acid Piperidide *d*-Tartrate by Sandoz Basle, Switzerland January 21, 1955

On mixing equivalent amounts of lysergic acid piperidide and *d*-tartaric acid in 5 times the amount of methanol the *d*-tartrate crystallizes in the form of needles having a melting point of 145-150° $[\alpha]D20=+15°$ (0.4% concentration in water). Source: Sandoz 1956

Fractional Crystallization of *d*-Lysergic Acid Cyclobutylamide

by Miroslav Semonsky, Viktor Zikan
Prague, Czechoslovakia August 9, 1956

Acid maleates of all *d*-lysergic acid cycloalkylamides were prepared in the following way: The crystalline base was dissolved in the minimum amount of ethanol, if necessary with mild heating for a brief period. Maleic acid in an excess of 10 per cent and dissolved in a small amount of absolute ethanol was then added, and the mixture was shaken and left standing for crystallization. After 3 hours standing at room temperature the precipitated colourless crystals of the acid maleate were sucked off, washed several times with a small amount of absolute ethanol and dried in darkness at room temperature. The yield was 90 per cent.

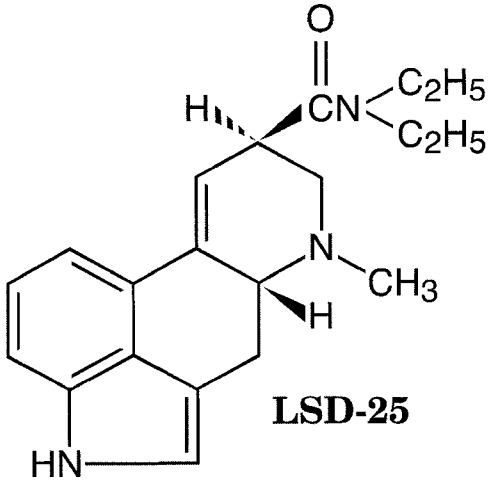
d-Lysergic acid cyclobutylamide having been recrystallised from acetone-benzene the substance forms colourless massive prisms. The substance contains 15.44 percent crystal solvents, which maybe removed by drying firstly at 100° C. (0.5 mm pressure) and then at 117° C. (0.5 mm). Melting at 100° C. (0.5 mm pressure) and then at 117° C. (0.5 mm). Its [α] D20 is -16.4° (c=0.488, pyridine).

The acid maleate forms colourless small needles melting gradually at about 209° C. with decomposition. The acid salt is free of solvents.

Source: Semonsky 1956, 1959. See also pages 73, 74, 95, 96

"*d*-isolysergic acid cyclobutylamide forms colourless, star-shaped joint prisms melting at 202-204° C. with decomposition. After having been recrystallized from ether-ethanol the substance contains no crystal solvent" Source: Sandoz 1936

"After recrystallization (*d*-lysergic acid cyclopentylamide) from benzene the substance contains 13.52 percent of crystal solvent which may be removed by drying at 100° C. (0.5 mm)... No completely solvent-free substance (*d*-lysergic acid cyclohexylamide) could be obtained even after drying for a long time at 117° C. (0.5 mm.)... Its acid maleate forms coulourless, needle-like solvent-free crystals... melting at about 225° C. with decomposition, when previously recrystallized from ethanol. The corresponding *d*-isolysergic acid derivative forms colourless solvent-free prisms melting at 204-205° C. with decomposition Source: Semonsky 1956



LSD-25

Chapter 9
Chromotography of Ergot Alkaloids

Separation of Pure Crystallized Ergot Alkaloids

Arthur Stoll and Ernst Burokhardt
Basel, Switzerland February 25, 1935

It is known that by using the chromatographic adsorption-analysis, suggested by Tswett in 1906 and developed for laboratory purposes by R. Kuhn, A. Winterstein and P. Karrer, it is possible to separate the dyestuffs of the leaves. For instance the preparation of chlorophylls and of carotinoids in pure state has also been realized by this method. It is further possible with the aid of ultraviolet rays, which allow to differentiate unsaturated hydrocarbons of high molecular weight from one another on account of their fluorescence, to prepare same in a very pure state.

The present invention relates to the manufacture, separation and preparation of ergot-alkaloids in state of high purity, by subjecting mixtures of the very sensitive ergot-alkaloids to chromatographic adsorption analysis.

Until now only two pairs of well defined ergot alkaloids are known. These are: the pair of ergotoxin and ergotinin, which can be transformed one into the other, and the pair of ergotamin and ergotaminin, which can also be transformed one into the other. Recently, two new well crystallized ergot-alkaloid-preparations, called Sensibamin (see British patent No. 388,529 and Ergoclavin (see German patent No. 606,778) have been described.

By systematic tests with known mixtures of ergot-alkaloids it has been found, that when solutions of such mixtures in inert solvents are allowed to pass through an adsorption column, the more easily soluble and active alkaloids of ergot, the ergotamin and ergotoxin, are retained by the adsorption media and not, as it ought have been expected, the difficultly soluble and physiologically less active alkaloids, ergotaminin and ergotinin. By using this method it becomes, therefore, possible to separate the active and valuable alkaloids from the impurities or less active principles and to obtain these alkaloids in pure crystallized form.

Another possibility was that the amphoteric reacting alkaloids ergotamin and ergotoxin would react with the basic adsorption media,

such as aluminium oxide, and give salt-like compounds, but this does not occur, as can easily be shown by the, following test. By shaking a chloroform-solution of a mixture consisting of the levorotary and easily soluble ergotamin and of the dextrorotary and difficultly soluble ergotaminin with aluminium oxide, only a small part of the alkaloids is retained by superficial adsorption, as is shown by a small decrease of the optical rotation; a selective adsorption of the levorotary ergotamin would have occasioned a substantial increase of the dextrorotation of the solution.

By subjecting the same solution to the chromatographic adsorption process, it is found that the levorotary ergotamin is retained within the adsorption column, whereas the difficultly soluble dextrorotary ergotaminin runs first through it. As the optical activity of these isomers in chloroform is very different—

(Ergotamin [α]20°/D = -155° and Ergotaminin [α]20°/D =+381°), it is easy to control the separation of the two alkaloids by means of the polarimetric method. It is also possible to control the separation of the alkaloids by irradiating their solutions with ultraviolet light, as it is known that in this light the ergot-alkaloids possess a violet to blue fluorescence.

If, therefore, the adsorption column containing the solution of a mixture of the alkaloids ergotamin-ergotaminin is irradiated with ultraviolet light, it will be seen that the lower part of the column contains a zone rich in alkaloid, the middle part is very poor in alkaloids and the upper part contains again a zone rich in alkaloid visible by its violet-white fluorescence. If the separation of the zones in the column is not distinct, the chromatographic process should be repeated with the single fractions, until quite pure compounds are obtained.

According to the present invention it becomes possible not only to separate pure mixtures of ergotamin-ergotaminin or of ergotoxin-ergotinin into their components in a high degree of purity, but also to work up such raw extracts of ergot, that contain impurities of a non-alkaloidic character.

The examination of the ergot-preparation called Sensibamin, which possesses in chloroform the optical rotation of [α]30°/D=+125°, has shown that this preparation can easily be separated by means: of the chromatographic method into a dextro- and a levorotary fraction. The levorotary fraction obtained thereby contains ergotamin, whereas the dextrorotary fraction contains ergotaminin.

As the arithmetical middle of—

[α] 20°/D calculated from the levo-rotation of the ergotamin and from

the dextro-rotation of ergotaminin is about +113°, this calculated value approaches very closely the value given for Sensibamin and confirms the fact that this product consists of the above cited two alkaloids.

In the same manner it is also possible to separate the product called Ergoclavin into a levorotary and a dextrorotary component.

The chromatographic process also makes it possible to prepare crystallized pure ergotoxin, which crystallizes very difficultly and which generally has been obtained in an amorphous impure state.

All the known processes heretofore used for the separation and preparation of pure ergot alkaloids are completely different from the process herein described, as indifferent adsorption media used in the process of Tswett have never been used for this purpose. The possibility of separating and of preparing the very sensitive ergot-alkaloids even from impure mixtures by the chromatographic process and without the use of any chemical reagents, represents a very valuable process and a great improvement of the art.

In order to carry out the present process, raw extracts or solutions of ergot-alkaloids are dissolved in indifferent solvents, for example aromatic hydrocarbons, such as benzene, toluene and/or homologues; halogenated hydrocarbons, such as chloroform, dichlorethylene, tetrachlorethane, trichlorethylene or mixtures of such solvents, and these solutions are allowed to flow through a column containing absorbents, such as sugar, milk-sugar, aluminium oxide, ammonium hydroxide, silicon dioxide, calcium oxide, calcium carbonate, asbestos, fibrous aluminium hydroxide, that are insoluble in the solvents used. On addition of further amounts of solvents, the chromatogram becomes developed in the adsorption column and can be seen on irradiation with ultra-violet light.

One object of the present invention is, therefore, a process for the manufacture, separation and preparation of pure ergot-alkaloids, consisting in subjecting raw or pure mixtures of ergot alkaloids or impure ergot-alkaloids to the chromatographic adsorption process.

Another object of the present invention is the method allowing the separation and preparation of pure alkaloids of ergot, consisting in subjecting the solutions of the above said mixtures of ergot-alkaloids in inert solvents to the chromatographic adsorption process.

Another object of the present invention is the use of the chromatographic adsorption process for the preparation of ergot-alkaloids in pure state.

Still another-object of the present invention is the use of adsorption columns containing such absorbents, that are insoluble in

the solvents, in which the mixtures of ergot-alkaloids are dissolved for the purpose of subjecting them, to the chromatographic adsorption process.

The following examples, without being limitative, illustrate the manner in which the present process can be carried out and show the preparation of pure alkaloids from raw-extracts or from mixtures of known alkaloids. The process described in the examples can be carried out an technical scale with greater quantities of alkaloids with any difficulty.

Separation of a Mixture of Ergotamin and Ergotaminin

A vertical glass tube of 40 cm. length and 22 mm. internal diameter, provided at the lower part with a filter and connected to a rubber tube, is filled under suction and light pressing successively with 120 g. of aluminium oxide (prepared according to Brockmann). In this manner a column of about 34 cm. height of adsorption medium is obtained, which is first impregnated with chloroform, the chloroform being allowed to run through the lower opening of the tube, until it reaches a level at a height of 2 to 3 mm. above the top of the adsorbent medium. Thereupon a solution of 0.5 g. of ergotamin and of 0.5 g. of ergotaminin in 100 ccm. of chloroform is introduced into the tube. As soon as the whole alkaloid solution has completely leaked in the adsorption column, a further quantity of chloroform is added and on irradiation of the tube with ultra-violet light a formation of layers in the adsorption medium can be observed. A relatively small layer rich in alkaloid is pushed forward and runs first out of the tube, then follows a zone poor in alkaloids, and in the upper part of the tube a second, large alkaloid layer, recognized by its violet-white fluorescence will be present.

The solution flowing out of the tube is carefully collected in fractions and each fraction is examined. The first 50 ccm. of the solution show in a polarimetric tube of 2 dm. a rotation of +8.38°. In the following 200 ccm. of the solution the dextrorotation becomes lower and lower as the concentration in alkaloid is only very small. After these intermediate fractions, which are poor in alkaloids, fractions follow which are levorotary and contain ergotamin. In spite of the fact that ergotamin is more easily soluble in chloroform than ergotaminin, it will be extracted only later from the absorption column and is found in the last fractions. The dextrorotary fractions are then evaporated in vacuo at a low temperature and yield 0.52 g. of a product, which on single recrystallization from pyridine gives pure ergotaminin with a high yield. The levo-rotary

fractions give after careful evaporation 0.33 g. of pure ergotamin, which on crystallization from aqueous acetone yields the typical columns of the ergotamin-acetone-water-crystals.

Pure Ergotamin from a Raw Extract

100 ccm. of a benzene extract from ergot, prepared as described in Example 2 of German Patent No. 357,272 and containing, besides ergotonin, ergot-oil and other impurities are allowed to pass through an adsorption column containing 120 g. of a finely powdered milk-sugar, which has previously been impregnated with benzene. On addition of further quantities of benzene, the first fractions flowing out contain only ergot-oil, but no alkaloids, the following fractions contain only traces of dextrorotary ergotaminin. The main alkaloid fraction follows thereupon and contains the levorotary ergotamin in a high degree of purity. After careful evaporation In vacuo and at low temperature, the residue of these last fractions is recrystallized from aqueous acetone and yields water-clear, strongly light breaking rhombic prisms and tables of ergotamln-acetone-water.

Source: Stoll 1937

Separation of Diethylamide of *d*-Lysergic Acid

Sandoz Freiburg, Switzerland April 30, 1943

The separation of the two isomers can be carried out e.g. by chromatographic adsorption. The mixture is dissolved in a little chloroform which contains 0.5% of alcohol the solution poured on to a column of aluminium oxide of 4 cm. diameter and 60 cm. length and the chromatogram is produced with the same solvent. Dark impurities soon pass into the filtrate. Then follows a broad zone appearing blue in ultra-violet light which contains the diethylamide of *d*-lysergic acid. Yield 1.0 to 1.3 grams.

The diethylamide of *d*-isolysergic aid passes though as a much more slowly moving zone. The residue of the chloroform solution thereof amounting to 0.8 to 1.2 grams, crystallises on taking up in acetone in beautiful prisms of m.p. 182° C (corr.) with decomposition and consists of the pure diethylamide of *d*-isolysergic acid [α] D20 = +217° (c=0.4 in pyridine).

Source: Sandoz 1946

Isolation of the Alkaloids from Culture of Ergot (*Claviceps purpurea* [Fr] Tul.)

Arthur Stoll, Arthur Brack, Albert Hofmann, Hans Kobel Switzerland April 16, 1953

According to this invention, a suitable strain of ergot (*Claviceps purpurea* [Fr] Tul.) can be cultured in vitro on a suitable nutrient medium under certain prescribed conditions in such a way that it produces ergotamine, ergotaminine and ergometrine in quantities large enough for isolation in a pure crystalline form on a preparative scale. This invention is of fundamental importance because it is capable of rendering the production of medicinally important ergot alkaloids independent of natural occurrence and of the artificial culture of ergot in the field.

The saprophytic culture of ergot has been reported several times in the literature and it has been claimed that ergot alkaloids have been detected both in the mycelium and in the culture medium. This claim rests on the results of color tests, principally upon the reaction to the test of van Urk with *p*-dimethylaminobenzaldehyde (van Urk, *Pharm. Weekbl.* 66, 437 [1929]). However, this reaction is not specific, since indole and many indole derivatives also give a reddish-violet coloration with this reagent (Ehrlich, *Pharm. Zentralh.*, 1918, 143 and may be mistaken for ergot alkaloids. It is known that various micro-organisms are capable of producing indole or indole derivatives in nutrient medium. For this reason a positive van Urk reaction cannot be taken as definite evidence of the presence of ergot alkaloids in total extracts.

In many cases, however, the saprophytic cultures of ergot described in the literature were tested for the presence of ergot alkaloids not only by means of color reactions, but also by biological methods (e.g. uterotonic activity). Nevertheless, when applied to complex material such as culture filtrates or extracts of mycelium, these methods of examination cannot be regarded as specific either, since other substances, e.g. histamine, also exert a more or less marked uterotonic action.

That the methods of culturing ergot in vitro described in the literature have not so far led to the successful production of ergot alkaloids on a preparative scale is convincingly demonstrated by the fact that the ergot employed for the manufacture of the active principles

is still obtained exclusively by artificial culture in the field or gathered from places where it occurs wild. Nevertheless, brief mention may be made of the various attempts of this nature which have been described in the literature. McCrea (*Amer. J. Bot.* 18, 50 [1931]; U. S. Patent No. 2,056,360) claimed that he had succeeded for the first time in producing ergotoxine, histamine and tyramine by saprophytic culture of ergot. This claim was based on biological tests (cock's comb test, oxytocic activity and pressor action), without isolation of the substances mentioned.

Jaretzky (*Arch. Pharm. Berl.* 273, 348 [1935]) reported the saprophytic culture of ergot on a suitable medium and claimed that the cornutine test (*p*-dimethylaminobenzaldehyde) and the biological test of Broom and Clark both gave positive results. The formation of alkaloids by *Claviceps purpurea* in saprophytic culture was also described by de Tempe (Thesis, Amsterdam 1945), but again a red coloration with van Urk's reagent was the only basis for this claim.

Burlet, Meyer and Chadue (*Therapie* 7, 144 [1952]) have recently reported experiments on the saprophytic culture of ergot on a special nutrient medium, the composition of which is not given. On the basis of a positive Freudweiler color test with vanillin in sulphuric acid (Goris, Liot, Janot and Goris: *Pharmacie - Galenique, Paris*, 2, 1231 [1949]) and a positive biological test on the uterus of the guinea pig, the authors assume the presence of alkaloids to have been proved. Sim and Youngken (*J. Amer. Pharm. Ass.* 40; 434 [1951]) claim to have been able to detect traces of ergot alkaloids in the mycelium of *Claviceps purpurea* cultured in vitro, this claim again being based on the use of the van Urk color test. Since a purplish coloration was taken as positive, it may be assumed that the compounds responsible were certainly- not alkaloids of ergot, as these give a blue coloration with van Urk's reagent. The authors themselves describe the reaction in the following words:

"All the extracts from the experimental mycelial tissue materials gave a purplish color which indicated the presence of ergot alkaloids or similar compounds possessing an indole nucleus."

In addition to the color reaction, the authors also carried out biological tests, but here again the results are not conclusive.

In a Japanese patent application (application No. 1676/50, publicized on June 5, 1950) a process was described according to which a certain strain or ergot (*Claviceps purpurea parriesta katagirii*), which is obtained from grasses and not from rye, can be made to produce alkaloids in vitro. In the course of this work it appears that the authors did actually succeed in isolating a crystalline alkaloid on a preparative scale from cultures of this fungus. The alkaloid so obtained was not known

previously and has been named agroclavine. In a more recent publication, the Japanese authors Abe, Yamano, Kozu and Kusumoto (*J. Agric. Chem. Soc., Japan*, 25, 458 [1952]) describe how a nutrient medium containing mannite as a source of carbon and ammonium succinate as a source of nitrogen, was inoculated with a strain of ergot found in Japan as a parasite on *Elymus mollis* Tri., and how they were subsequently able to isolate from the medium the following alkaloids: ergokryptinine, agroclavine, and traces of ergokryptine and ergosine, as well as a new water-soluble alkaloid which they named elymoclavine.

It is thus clear that with the exception of the work described in the last two publications, crystalline preparations have never been isolated from ergot grown by saprophytic culture. The Japanese workers describe only the isolation of ergokryptine and ergokryptinine in addition to previously unknown alkaloids. Up till now, however, no one has succeeded in isolating from an in vitro culture of ergot the alkaloid ergotamine which has so many important uses in obstetrics, gynaecology and internal medicine.

According to the present invention, by using a suitable strain of *Claviceps purpurea* and a suitable nutrient medium, and by observing certain prescribed conditions, it is possible to grow the fungus in vitro in such a way that it produces ergotamine and other ergot alkaloids, such as ergotaminine and ergobasine, in quantities sufficient to enable them to be isolated in a pure crystalline form on a preparative scale.

The culture medium, the exact composition of which is given hereinafter, is sterilized and inoculated with spores of *Claviceps purpurea* (Fr.) Tul. and then incubated. After some time the solution becomes covered by a layer of mycelium which usually has a dark brownish violet color. The mycelium is removed, dried and the alkaloids isolated as described below. The filtered culture medium is extracted separately, and yields pure ergotamine together with small quantities of ergotaminine and ergobasine. To obtain the alkaloids, the following procedure may be applied both to the mycelium and to the culture solution. The fatty constituents are first extracted with an organic solvent which leaves the alkaloids undissolved, and the alkaloid-containing material is then made into a paste with aqueous soda (sodium carbonate) solution. This liberates the alkaloids from their salt-like combination and they can then be extracted with an organic solvent such as ether. The alkaloids are removed from the ether solution by extraction with an acid, e.g. tartaric acid, and the aqueous extract purified by shaking out with an organic solvent. The entire aqueous solution is then made alkaline, e.g. by addition of sodium bicarbonate, and the alkaloids taken up in an

organic solvent immiscible with water. The separation and purification of the crude bases obtained in this way can be carried out, for example, by chromatography on alumina. The ergot alkaloids ergotamine, ergotaminine and ergobasine so obtained agree in all their properties with the preparations obtained from natural ergot.

To demonstrate the presence of the alkaloids, both the mycelium and the culture medium are made alkaline, extracted with ether and the ethereal solution shaken out with a 1% solution of tartaric acid. The aqueous solution of the tartrates is then treated with van Urk's reagent (*p*-dimethylaminobenzaldehyde in sulphuric acid solution) and the color developed under the quartz lamp (Smith, *U.S. Public Health Reports* 45, 1466 (1930); Schlemmer, Wirth and Peters, *Arch. Pharm., Berl.* 274, 16 (1936)). The intensity of the blue coloration corresponds to an alkaloid content of 0.1% in the dry mycelium and 0.0076 mg. per cc. in the culture solution (based on a molecular weight of 600).

The isolation of the active principles from a larger quantity of mycelium obtained in this way and from the corresponding culture solution is carried out in the following manner:

260 grams of dried mycelium are defatted by shaking for 1/2 hour with 2.6 liters of petroleum ether, filtering off and shaking with two further portions of 1.3 liters of petroleum ether.

The defatted material is now made into a paste with 260 cc. of 5% aqueous sodium carbonate solution and then extracted with ether by shaking for 1/2 hour on the shaking machine, first with 2.6 liters of ether and then twice more with 1.3 liters of ether. The alkaloids are removed from the combined ether extracts by shaking out with a 1% aqueous solution of tartaric acid, first with 1.3 liters and then with four further portions of 0.65 liter of the solution. In order to remove neutral and acid impurities, the combined aqueous extracts are shaken out three times with 300 cc. of ether and each ether extract washed twice with 100 cc. of a 1% aqueous solution of tartaric acid. The tartaric acid washings are combined with the main tartaric acid extract which is then made alkaline by addition of sodium bicarbonate. The alkaloidal bases are thus liberated and the fraction insoluble in water is extracted by shaking out with 500 cc. of ether and then with three further portions of 250 cc. of ether. The combined ether extracts are dried over sodium sulphate and then evaporated to dryness under reduced pressure. The residue of crude alkaloids thus obtained weighs 0.31 gram.

This preparation is purified by dissolving in chloroform containing 1% alcohol and allowing the solution to percolate through a column containing 31 grams of Brockmann's alumina. The fractions which

fluoresce blue in ultraviolet light, showing that they contain alkaloid, are collected separately and evaporated to dryness. The residue from the more rapidly traveling blue zone weighs 0.039 gram. It is taken up in 0.5 cc. of methanol from which the alkaloid immediately crystallizes. Yield: 0.030 gram of ergotaminine. After recrystallizing once from methanol, in which the compound is very sparingly soluble, it is obtained in the form of triangular platelets which melt at 240° with decomposition and exhibit a specific rotation [α]20°/D=+367° (+3°) (c=0.5 in chloroform).

The residue obtained on evaporation of the more slowly traveling zone of the chromatogram weighs 0.137 gram. On taking up in 0.5 cc. of 90% acetone, the alkaloid crystallizes in the horizontally truncated, polyhedral, highly refringent prisms typical of ergotamine, M.P. 180° with decomposition. Yield: 0.133 gram of ergotamine. On drying in high vacuum at 80° the crystals lose 20% of their weight, corresponding to a content of solvent of crystallization of 2 mols acetone arid 2 mols water, according to the formula C33H35O5N5.2CH3COCH3.2H2O

The specific rotation of the substance dried in this way is [α]20°/D= -160° (c=0.6 in chloroform). The alkaloid also agrees completely with authentic ergotamine in all other properties, e.g. in its solubility in various organic solvents blue coloration with Keller's reagent, etc.

In order to isolate the water-soluble alkaloids, the aqueous solution from which the ergotamine and ergotaminine have been extracted is saturated with solid sodium chloride, made alkaline by addition of 10 gram of sodium hydroxide, and extracted first with 2 liters of ether and then with 2 further portions of 1 liter of ether. On drying the ether extract over sodium sulphate and evaporating to dryness, a residue weighing 90 mg. is obtained. The alkaloidal content as determined colorimetrically is 29 mg. (calculated on a molecular weight of 600). On taking up in a little methanol, 50 mg. of fatty, non-alkaloidal impurities remain undissolved and are filtered off. The residue which remains on evaporation of the filtrate is taken up in chloroform containing 1% alcohol and purified by chromatography on a small column of alumina. The residue obtained after evolution of the column and evaporation to dryness of the chromatographed solution is taken up in 0.2 cc. of chloroform from which 6 mg. of ergometrine crystallizes out as the sparingly soluble chloroform compound. On recrystallizing from benzene, the product is obtained in the form of soft needles melting at 159-162°. The compound thus obtained agrees in all its properties with authentic ergometrine.

The 7 liters of culture filtrate, having a pH of 4.8 which are obtained after filtering off the mycelium in the above experiment, are

brought to a pH of 8.0 by addition of 2 N aqueous sodium bicarbonate solution and shaken out twice with 1.4 liters of ether. The alkaloidal portion of the combined-ether extracts is removed by shaking out twice with 400 cc. of 1% aqueous tartaric acid. The aqueous extract is then made alkaline with soda arid extracted once with 250 cc. of chloroform and three times more with 100 cc. of chloroform. After drying over sodium sulphate, the chloroform solution is evaporated to dryness giving a residue weighing 68 mg. the alkaloidal content as determined colorimetrically according to the method of van Urk and Smith is 56 mg. (calculated on a molecular weight of 600).

The separation and purification of the crude alkaloids is effected by chromatography on a column of alumina using chloroform containing 1% alcohol as solvent. Two separate zones which fluoresce blue in ultraviolet light are formed.

The residue obtained on evaporation of the more rapidly traveling zone weighs 32 mg. On taking up in 4 cc. of methanol, 20 mg. of a very sparingly soluble alkaloid separate out. This melts at 235-240° (decomp.) and exhibits a specific rotation of [α]20°/D= +365° (+5°) in chloroform), which identifies it as ergotaminine. In all other properties this alkaloid also agrees with authentic ergotaminine.

The solution obtained by elution of the more slowly traveling zone of the chromatogram yields a residue weighing 13 mg. on evaporation to dryness. When this is taken up in 0.1 cc. of 90% acetone, 10 mg. of ergotamine crystallize out in the horizontally truncated polyhedral prisms typical of this alkaloid. The melting point of 80° (decomp.) and the specific rotation [α]20°/D= -160° in chloroform), as well as all the other properties, agree with those of authentic ergotamine.

Source: Stoll 1957

Chromatography of Lysergic Acid Diethylamide

by Richard P. Pioch
Indianapolis, Indiana December 6, 1954

A solid residue of 3.45 g. comprising the "normal" and "iso", forms of *d*-lysergic acid N,N-diethyl amide is obtained. This material is dissolved in 160 mL. of a 3-to-1 mixture of benzene and chloroform, and is chromatographed over 240 g. of basic alumina. As the chromatogram is developed with the same solvent, two blue fluorescing zones appear on the alumina column. The more rapidly moving zone is *d*-lysergic acid N,N-diethyl amide which is eluted with about 3000 mL. of the same

solvent as above, the course of the elution being followed by watching the downward movement of the more rapidly moving blue fluorescing zone. The eluate is treated with tartaric acid to form the acid tartrate of *d*-lysergic acid N,N-diethyl amide which is isolated. The acid tartrate of *d*-lysergic acid N,N-diethyl amide melts with decomposition at about 190-196° C.

The *d*-iso-lysergic acid N,N-diethyl amide which remains adsorbed on the alumina column as the second fluorescent zone is removed from the column by elution with chloroform. The "iso" form of the amide is recovered by evaporating the chloroform eluate to dryness in vacuo. Source: Pioch 1956

Chromatography of *d*-Lysergic Acid Piperidide

by Sandoz Basel, Switzerland January 21, 1955

(11 g) is dissolved in benzene and is chromatographed on a column of aluminium oxide. 7.2 g of lysergic acid piperidide are eluted by means of absolute benzene. It is not possible to obtain lysergic acid piperidide in the form of the free base as a crystalline substance. The amorphous compound is very easily soluble in most organic solvents but is practically insoluble in water. It forms crystalline water soluble salts. Sandoz 1956

Chromatography of Amides of Lysergic Acid

William L. Garbrecht
Indianapolis, Indiana November 21, 1955

The residual syrup comprising *d*-lysergic acid morpholide is dissolved in 25 mL. of methanol, the solution is acidified with excess maleic acid, and is diluted with ether to incipient turbidity. The mixture is allowed to stand in a refrigerator for several hours, whereupon off-white, needle-like crystals of *d*-lysergic acid morpholide acid maleate are formed and precipitate from the solution.

The above procedure yields about 1.5 g. of crystals which melt indefinitely with decomposition on a Fisher John block at about 195° C.

From the crystallization mother liquors, after concentration, neutralization, and re-extraction with ethylene dichloride, there can be obtained about 0.8 g. of amorphous *d*-isolysergic acid morpholide. This material can be isomerized to *d*-lysergic acid morpholide by the procedure disclosed by Smith and Timmis in *J. Chem. Soc.* 139, II, 1168 (1936).

The ethylene dichloride extracts are combined and are evaporated in vacuo leaving a residual syrup comprising, a mixture of the diethyl amides of *d*-lysergic and *d*-isolysergic acids.

The two amides can be separated as follows:

The syrup is dissolved in a mixture of 60 mL. of benzene and 20 mL. of chloroform and the solution is passed over a chromatographic column of 150 g. of basic alumina. The chromatogram is developed with the same solvent mixture. The more rapidly moving of the two blue fluorescing bands consists of the diethylamide of *d*-lysergic acid. About 2 liters of solvent mixture are required to elute the first band. The eluate is treated with a sufficient amount of tartaric acid to convert the amide to the tartrate salt, and the salt is isolated by evaporating the solution to a low volume to cause separation of the tartrate salt of the diethylamide of *d*-lysergic acid.

The diethylamide of *d*-isolysergic acid is recovered by eluting it from the alumina column with chloroform, and evaporating the chloroform eluate.

The residual syrup containing the two *l*-(+)-propanol amides of *d,l*-lysergic acid is treated with methanol and excess maleic acid to convert the two amides into their maleate salts. The mixture of amide maleates is precipitated from the methanol solution by the addition of ethyl ether, and the precipitated mixture is filtered off and dried in air.
Source: Garbrecht 1956

Chromatography of Lysergic Acid Morpholide

by Richard P. Pioch
Indianapolis, Indiana March 5, 1956

In carrying out the process for the preparation of the new compounds, it should be noted that whenever solutions containing lysergic acid or its derivatives are prepared, they are preferably kept cool and protected from light. As is well known, compounds containing the lysergic acid structure are unstable in the presence of heat, light and air and may undergo irreversible decomposition even to the extent of forming dark colored products which are difficult to remove during the purification of the desired end products. It is likewise known that in the course of the preparation of derivatives of lysergic acid a mixture of the isomeric lysergic and isolysergic acid derivatives are commonly formed. A separation of these isomers is readily effected chromatographically. Conveniently, columns of aluminum oxide, ionexchange resin or

the like are employed, and the mixture of isomers is placed upon the column using a suitable solvent. The desired products are then separated by elution with other and more polar solvents or solvent mixtures. The eluates containing the individual isomers can be evaporated to dryness and the residue crystallized in the usual way by dissolving it in a minimum amount of warm solvent and cooling. The isomers can be rechromatographed as such or in the form of an acid addition salt for further purification. Alternatively, the mixture of isomers resulting from the process of preparation of the new compounds can be separated by evaporating the reaction mixture to dryness, redissolving the residue in a suitable water-immiscible solvent and washing the solution with water to remove unreacted lysergic acid in the form of an amine salt. After evaporation of the solvent, the residue is converted to an acid addition salt, such as the maleic acid addition salt, of the lysergic acid amide, and then the lysergic acid amide salt is separated from the isolysergic acid amide salt by fractional crystallization. The processes of separation of mixtures of isomers and purification by chromatography are readily followed by color tests such as the Van Urk test, or, more simply, by examination under ultraviolet light which causes marked fluorescence of the compounds.

The following examples more specifically illustrate the preparation of the novel amides of lysergic acid which are included within the scope of the invention.

d-lysergic acid morpholide (maleate salt) thus prepared melts at about 205-207° C. with decomposition and weighs 870 mg.

Analysis.—Calculated for C24H27N3O6: C, 63.56; H, 6.00; N, 9.27 Found: C, 63.22; H, 5.75; N, 9.04

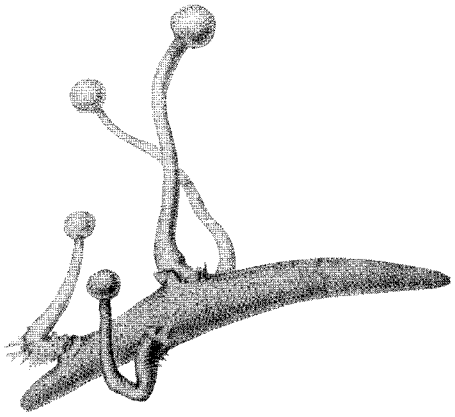
The maleate salt is dissolved in a minimum volume of water and neutralized with sodium carbonate. The resulting mixture is extracted with three 100 mL. portions of chloroform, the combined chloroform extracts are dried with sodium sulfate, are filtered, and are evaporated to dryness in vacuo. The residue is taken up in a small volume of benzene and cooled, whereupon crystals of *d*-lysergic acid morpholide are formed.

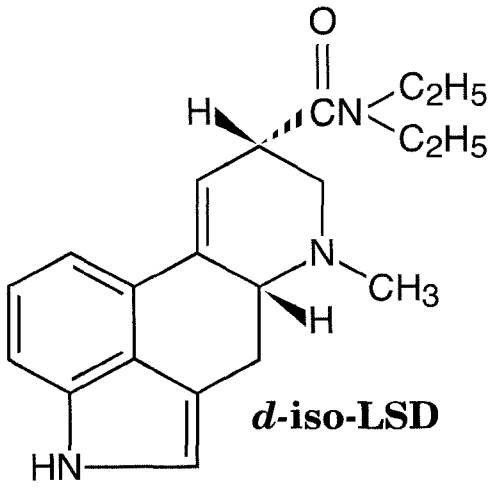
The combined filtrate and washings from the isolation of *d*-lysergic acid morpholide acid maleate are evaporated to dryness. The acid maleate salt of *d*-isolysergic acid morpholide contained in the residue is dissolved in water, is converted to the corresponding free base by treatment with sodium carbonate and is extracted with chloroform as set forth hereinabove. The chloroform solution containing the free

base is evaporated to small volume, is placed on a column of 50 g. of basic alumina and is chromatographed using a 1:1 benzene-chloroform mixture as the eluant. The fractions of the eluate giving a positive Van Urk test are combined and are evaporated to dryness. Crystallization of the resulting residue from acetone yields about 550 mg. of *d*-isolysergic acid morpholide melting at about 199-200° C. with decomposition.

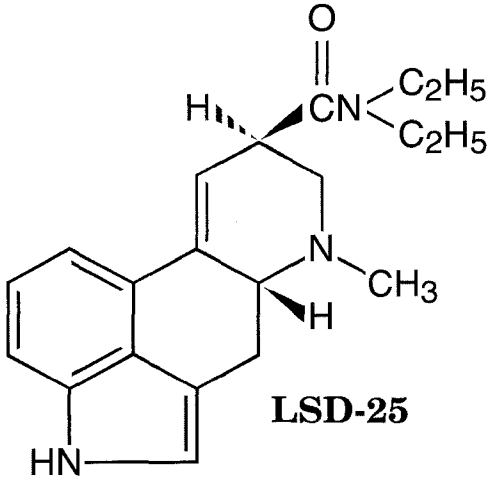
Chromatography of *d*-Lysergic Acid N-methyl-N-isopropylamide

A solution of 3.5 g. of the amine mixture ("normal" and "iso" *d*-lysergic acid N-methyl-N-isopropylamides) in about 100 mL. of a 1:9 chloroform-benzene solvent mixture is placed on a chromatographic adsorption column containing about 110 g. of basic alumina. The first liter of eluate, using the same solvent mixture, after evaporation of the eluting solvent and recrystallization of the resulting residue from ethyl acetate, yields about 340 mg. of *d*-lysergic acid N-methyl-N-isopropylamide, melting at about 196-197° C. with decomposition. The chromatogram is further developed with another one liter portion of the same eluent followed by 100 mL. of pure chloroform, and these eluates comprise mixtures of the "normal and "iso" amides. Further elution with 200 mL. of pure chloroform results in an eluate which yields a solid residue of *d*-isolysergic acid N-methyl-N-isopropylamide after evaporation of the eluting solvent. Recrystallization of this residue from ethyl acetate yields about 860 mg. of *d*-isolysergic acid N-methyl N-isopropylamide melting about 194-195° C. Source: Pioch 1961





***d*-iso-LSD**



LSD-25

Chapter 10
Isomerization of *d*-iso-Lysergic Acid Amides

Isomerization of *d*-iso-Lysergic Acid-Hydroxybutylamide-2

Arthur Stoll and Albert Hofmann
Basel, Switzerland June 6, 1939

The transposition of the iso-lysergic acid derivative into its respective compound of the lysergic acid series can be carried out by treating the above cited compound with acids, such as acetic acid, phosphoric acid, or alkalies, such as sodium and potassium hydroxide. This transposition can be carried out for instance in the following way:

One part of the iso-compound is dissolved in 10 parts of absolute ethanol and an alcoholic potassium hydroxide solution is added thereto. The mixture is left to stand at room temperature during 45 minutes. After this time equilibrium is reached between lysergic acid and the iso-lysergic acid forms, which can be checked by determination of the constancy of the optical rotation of the solution. When this point is reached, potassium hydroxide is transformed into potassium carbonate by bubbling through the solution a stream of carbon dioxide; the thick crystal paste of potassium carbonate is then diluted with 50 parts of ether, filtered and washed again with 50 parts of ether.

The alcoholic ethereal filtrate is then dried over calcined potassium carbonate and the solution evaporated, whereby 0.9-1 part of a mixture of *d*-lysergic acid-*d,l*-hydroxybutylamide-2 and of *d*-iso-lysergic acid-*d,l*-hydroxybutylamide-2 is obtained. Source: Stoll 1941

Isomerization of *d*-iso-Lysergic Acid Cyclopropylamide

by Miroslav Semonsky and Viktor Zikan
Prague, Czechoslovakia August 9, 1956

The intermediate thus obtained (*d*-iso-lysergic acid cyclopropylamide) was dissolved in a tenfold amount of absolute ethanol and the clear solution was mixed at room temperature with one part of 4N-potassium hydroxide solution in aqueous ethanol (50 per cent). After 45 minutes standing at room temperature and in darkness potassium carbonate was precipitated by introducing carbon dioxide. The initial volume was restored by adding ethanol and the solution was diluted with five volumes of ether. Potassium carbonate was filtered off and washed with ethanol-ether (1:5). Joint filtrates were made free from solvents by evaporating under slightly reduced pressure in a nitrogen atmosphere. The equilibrium mixture of *d*-iso-lysergic and *d*-lysergic acid cyclopropylamides thus attained. Source: Semonsky 1959

Epimerization of *d*-iso-LSD into *d*-LSD

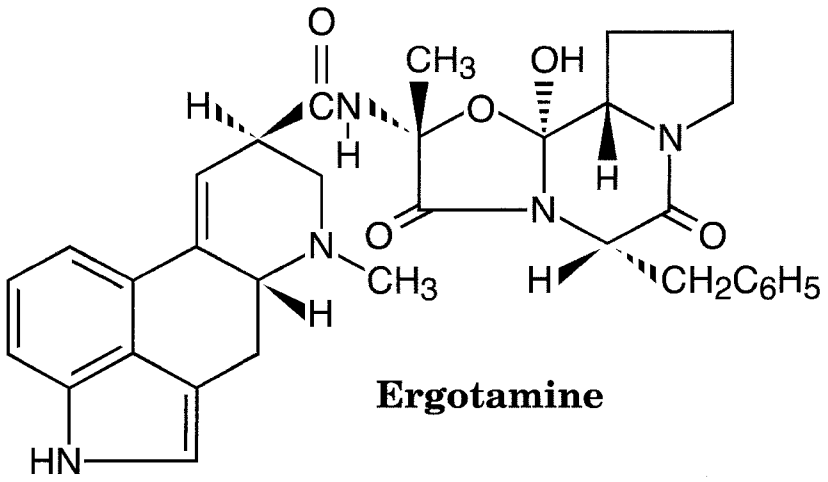
d-iso-Lysergic acid diethylamide is dissolved in a 0.4 molar methanolic solution of potassium hydroxide and allowed to stand in the dark for approximately 1 to 2 hours. Carbon dioxide gas is bubbled through the solution forming a paste of potassium carbonate. The paste of potassium carbonate/alcohol/LSD is mixed with 50 parts ether and filtered; this is repeated. The filtered solutions are dried and evaporated to leave a mixture of *d*-lysergic acid diethylamide and *d*-iso-lysergic acid diethylamide which can be separated by fractional crystallization or chromatography.

For epimerization of *d*-iso-lysergic acid to *d*-lysergic acid see (Semonsky 1965). For epimerization of *d*-iso-LSD to *d*-LSD (Cerny 1968).

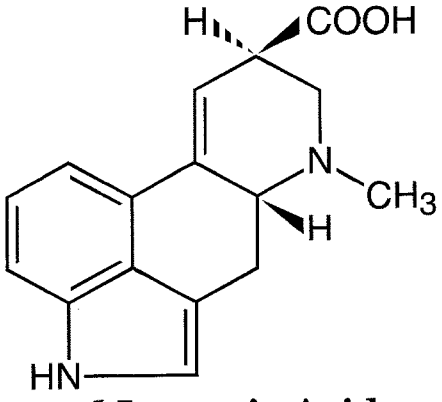
Free Base Ergot Alkaloids from Their Salts (ergobasine)

by Arthur Stoll and Albert Hofmann
Basel Switzerland September 16, 1942

In order to prepare the free base, the salt is suspended in water, then an excess of a sodium bicarbonate solution is added thereto and the base extracted with ether. After evaporation of the ether and treating the residue with a small quantity of acetone the residue crystallizes. Source: Stoll 1948



Ergotamine



***d*-Lysergic Acid**

Chapter 11
Lysergic Acid

The annual production of lysergic acid exceeds 12 thousand kilos. In 1976 the kilogram price was between $3000 to $4000 according to Heinz G. Floss. Lysergic acid is the precursor for molecules which:

1) increase cerebral blood circulation
2) antimigraine medications
3) affect activity of hypothalmic-pituitary system including the regulation of prolactin from the pituitary.
4) the construction of molecules of unknown activity for use in research and potential medications.

Lysergic acid can be created:

1) semisyntheticly or totally synthetic, but it is not cost effective.
2) by isolation from field cultivated ergot (from ergot alkaloids).
3) by fermentation of *Claviceps* species (from ergot alkaloids).
4) by extraction from *Convolvulaceae* seeds (from ergoline alkaloids).

Arcamone of Farmitalia S.A. (*Claviceps paspali*), and Kobel at Sandoz Laboratories (*Claviceps purpurea*) developed the fermentation of *Claviceps* fungus for industrial production.

Ergot contains approximately 1% alkaloids. *Convolvulaceae* seeds contain varying amounts of alkaloids. Submerged fermentation of *Claviceps* is the most economical source for ergot alkaloids.

References: (Floss 1976) (Ott 1993)

Date	Price	Item Amount	Source
1965?	$20,000	Lysergic acid 500 grams (Owsley)	(Tendler 1984)
1968	$100,000?	Lysergic acid 5 kilos Ergotamine tartrate 10-20 kgs (Nicks Sands, Tim Scully)	(Tendler 1984)
1968	$5,000	Ergotamine tartrate 1 kilo	(Lee 1978)
1993	$75	Ergotamine tartrate 1 gram (retail)	
1993	$300	Ergotamine tartrate 5 grams (retail)	
2001	$100,000?	Ergocristine kilogram (Pickard)	(Wilkinson 2001)
2003	$110	Ergotamine tartrate 1 gram (retail)	

Lysergic Acid from Ergot Alkaloids

by Ernst Boris Chain, Cesare Bonino and Antonio Tonolo Rome, Italy July 19, 1960

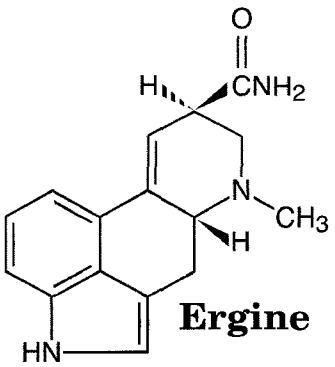
236 mg. of alkaloid derivative of *d*-lysergic acid are heated in a methanolic 1 N KOH for one hour. Ammonia is evolved from the mixture. The solution is acidified with 4 N acetic acid: 121 mg. of *d*-lysergic acid are obtained. Source: Chain 1964. See also page 191

"Stoll later admitted that the American researchers were more bold then him since, he, knowing the sensitivity of the ergotamine, would have never thought of treating them with an aqueous solution of boiling 7% potash (Stoll 1965)," in *Ergot* by Kren 1999.

Preparation of Lysergic Acid

Ergot alkaloid (eg. 2 grams) can be transformed into lysergic acid when dissolved in (50 mL) normal methyl alcoholic potassium hydroxide solution and refluxed under inert gas. A small amount of water (approx. 50 mL) is added and the alcohol is evaporated under reduced pressure. The base is extracted with ether and the aqueous layer is acidified with sulfuric acid to precipitate the crude lysergic acid, which is then purified.

Alkaloid	Reflux Time	Yield	References
Ergine	75-80 minutes	80%	Jacob 1934
Ergometrine	135 minutes	75%	Smith 1932; 1936

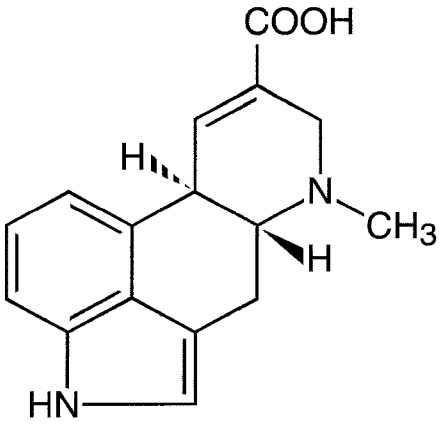


Ergine

Recrystallization of Lysergic Acid

Lysergic acid monohydrate crystallizes in very thin hexagonal leaflets when recrystallized from water. It decomposes at 238 degrees, but varies with rate of heating. Lysergic acid monohydrate, when dried (140 degrees at 2 mm.), forms anhydrous lysergic acid.

Reference: (Jacobs 1934)



6-Methyl-Δ-8,9-ergoline-8-carboxylic Acid

Lysergic Acid from 6-Methyl-Δ-8,9-ergolene-8-carboxylic Acid

Jürg Rutschmann, Hans Kobel, Emil Schreier
Basel, Switzerland August 29, 1963

500 mg. of the crude, crystalline 6-methyl-Δ-8,9-ergolene-8-carboxylic acid, are heated in 10 mL. of 2N-sodium hydroxide solution for 2 hours on a water bath; the hot solution is treated with active carbon and the filtrate adjusted to pH=5.5 with dilute hydrochloride acid and glacial acetic acid. After a few hours, the resulting crystalline lysergic acid is filtered off, washed with water and methanol and dried at 80° C. under vacuum.

Melting point: 245-247° C. with decomposition.

The lysergic acid prepared in this manner is identical with the compound described in the literature.

Source: Rutschmann 1967 See also page 127.

Chapter 12
Ergot Alkaloids and Ergolines from *Convolvulacea*

Convolvulaceae is a widely distributed family of plants. I would suggest readers pick up a copy of *The Botany and Chemistry of Hallucinogens* and also *Pharmacotheon* for a more detailed look at *Convolvulaceae* history and constituents. The seeds of various species (*Argyreia*, *Ipomoea*, *Stictocardia*, and *Cuscuta*) have been analyzed to contain ergolines:

Chemical Name	Alternative Names
d-Lysergic acid amide	Ergine
d-iso-Lysergic acid amide	iso-Ergine
Lysergic acid-*alpha*-hydroxyethylamine	
iso-Lysergic acid-*alpha*-hydroxyethylamine	
Ergonovine	Ergometrine
Ergonovinine	Ergometrinine
Chanoclavine-I	
Chanoclavine-II	
Penniclavine	
Elymoclavine	
Agroclavine	

Rivea corymobosa

The Aztecs used the seeds of several species for divination purposes in religious ceremonies. "*Ololiuqui*" is the seeds from *Rivea corymbosa* (also called *Turbina corymbosa*). The plant itself is called "*coaxihuitl*," "*the snake-plant*."

Rivea corymbosa produces beautiful white flowers and grows in Mexico, West Indies, Texas, Southern California, Central and Southern Florida. The alkaloid content of the seeds range from a low of 0.021% to a high of 0.060% according to Marderosian (1966) and Youngken.

Ref.: (Taber 1962)

Morning Glories

The seeds of *Ipomoea violacea* were used by the Aztecs in religious ceremonies. They were called "*tlitliltzin*". These seeds are used religiously/medicinally by the Zapotecs, Mazatecs, Mixtecs, Chinantecs in Oaxaca and are called "*Badoh negro.*" Other species (*Ipomoea rubro-caerulea praecox, Ipomoea purpurea* have tested positive for indole alkaloids.) *Ipomoea violacea* is commercially available in many horticultural varieties:

Heavenly Blue
Pearly Gates
Flying Saucers
Wedding Bells
Summer Skies
Blue Star

Alkaloids vary from a low of 0.005% to a high of 0.079%.
References: Genest 1966; Marderosian 1964; 1966; Nikolin 1972; Niwaguchi 1969; Taber 1963.

Argyreia nervosa

Hawaiian Baby Woodrose (*Argyreia nervosa*) also called woolly wood roses are beautiful vines that grow in Hawaii, Mexico, and the southern parts of Texas, California and Florida. The plant is believed to originate from India. The Hindus used the roots in the treatment of inflammatory disease. The alkaloid constituents of seeds range from a low of 0.5% to a high of 0.9%. Ergine and isoergine make up approximately 54% of the total alkaloids.

The leaves of the morning glory contain only traces of ergolines. References: (Chao 1973) (Hylin 1965). Ingestion of seeds described produces lethargy, nauseousness and vomiting.

"Although theoretically possible, manufacture of LSD from morning glory seeds is not economically feasible and these seeds never have been found to be a successful starting material for LSD production." DEA 2003

Extraction of Ergoline Alkaloids From Seeds

Method A

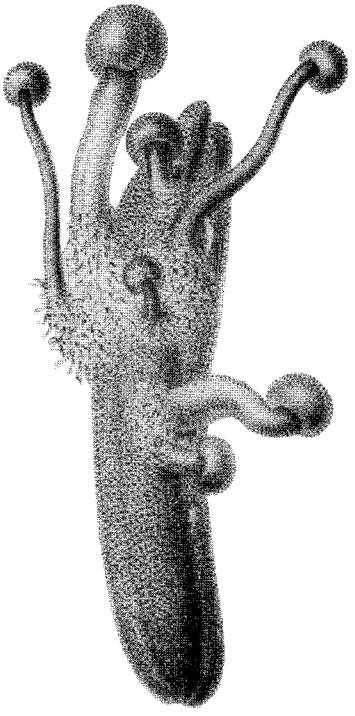
Pulverized seeds (100 grams) must be defatted before extraction of alkaloids. Naphtha or petroleum ether are suitable solvents for fat extraction of the seeds. The seeds can be refluxed in the solvent or they can be refluxed in a Soxhlet extractor. The seed mash is then filtered from the solvent. Total extraction of fats is accomplished when new solvent extract leaves no greasy residue on evaporation.

The seed mush is then allowed to dry of solvent, mixed with 500 mL of 10% ammonium hydroxide (strong ammonia water) and extracted with ether or appropriate solvent. Evaporation of the solvent leaves the alkaloids. Reference: (Genest 1965)

Method B

100 Grams of pulverized seeds is mixed with 50 grams of sodium bicarbonate and 100 mL of water. 100 Grams of anhydrous sodium sulfate are mixed to leave the mass dry and granular. The mass is extracted three times with one liter of ethyl acetate. The ethyl acetate solutions are combined and evaporated to leave the alkaloid residue. Reference: (Marderosian 1966)

All extractions should be done under inert atmosphere. Ergot alkaloids will decompose in light, heat and air. Tartrate and maleate salts are less susceptible to destruction.



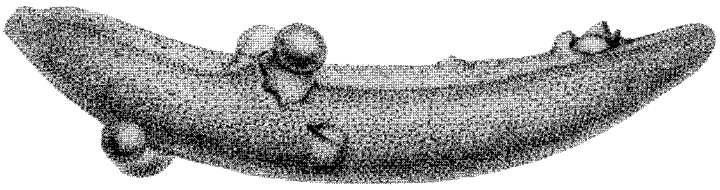
Chapter 13
Method of Developing *Claviceps purpurea*
by H.H. Whetzel and Donald Reddick

Since the publication of the beautiful illustrations of *Claviceps purpurea* by Tulasne in 1853, this fungus has been a favorite type used by authors of text books as representative of fleshy pyrenomycetes. Sclerotia of this fungus are found commonly enough but the students rarely see the perithecial stage. This is probably not because stromata are not formed commonly, but because they are not sought at the right time, and because of their small size. In an attempt to develop stromata for class demonstration and use, we have met with such abundant success that our methods of procedure may be of interest both as to method and scientifically as well. Some earlier attempts by one of us to develop the ascigerous stage from dried sclerotia had proven failures and taking our cue from nature we thought to simulate natural conditions to as great an extent as possible.

About August 10, 1907, one of us collected quantities of the sclerotia of *Claviceps purpurea* Tul. in the heads of rye (Secale cereals) which had come up "volunteer" in a field of oats near Swan, Noble Co., IN.

On the later date quantities of sclerotia of the several collections were enclosed separately in ordinary screen wire and put on the ground under a grape arbor. They were not disturbed until April 6, 1908. On that date, they were brought to the laboratory, placed on moist sand in a covered slender dish and kept at room temperature.

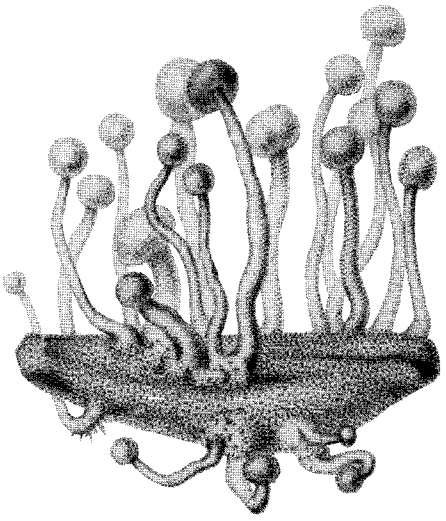
On April 18, 1908, we noticed evidence of germination in the sclerotia from the rye. There were tardy developments in all cases so that it was May 23rd, before all stromata had developed.



Stroma Sprouting from *Claviceps purpurea*

Refs.: Christensen 1939; Cooke 1966; Cooke 1967; Cooke 1970; Hadley 1968; McCrea 1931; Mitchel 1968; Nisbet 1977

April 19, 1908 (some of the stromata at least 24 hours old). The first indication of development is the rupture of the cortex of the sclerotium and appearance of a white globose head 0.5 mm. in diameter. This ascigerous portion is pushed up on a stem, increases in diameter and is sharply differentiated from the stem. The stem is pale lilac; broad at the base and tapering toward the apex.



Germination of Ergot

April 22, 1908, "no indications of perithecia at this date; the head has enlarged slightly and has become pale straw color; the stem has lengthened perceptibly."

April, 24, 1908, "yesterday the old stromata began to show punctures indicating the ostiola of the perithecia; today these are quite distinct, but the asci are still decidedly immature. The ascigerous portion is flesh color to pale fawn; up to 1.5 mm. in diameter. The stems are lilac at the apex and fade out nearly white at the base; up to 1 cm. long. One sclerotium has 12 stromata developing from it." A white radiating tuft of hyphae developed about the base of many of the stems, especially after the stromata were nearly mature.

On April 19, 1908, sclerotia from the same collection, kept dry in the laboratory over winter, were placed on moist sand in a slender dish. May 23, 1908, there were no indications of development in any case.

At that time we had not seen an excellent paper by Rostowzew which is written in Russian and in which he makes the point, by experiment, "the sclerotia of ergot (*Claviceps purpurea* Tul.) preserve their vitality for one year only. This viability is lost in less than one year, if they are subjected to complete drying out while in the resting stage."

In attempting to make photographs we have noticed the very decided tendency of the stem to twist and turn. In order to obtain a good photograph without blurring, it was necessary to keep the stromata on a wet background and covered with a thin glass dish while the process of focusing was performed. The cover was removed and the water taken away with a blotter only long enough to make the exposure.

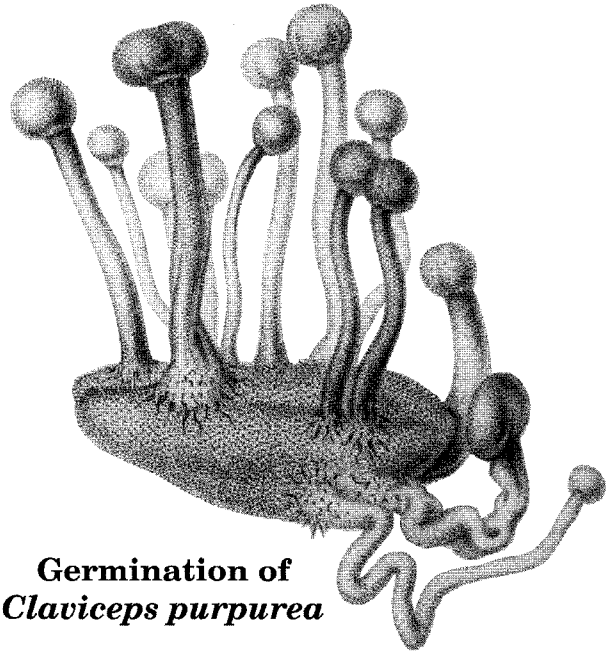
The twisting was also noticed in the culture dishes, but it was not given any study. Rostowzew studied this carefully and made some extremely interesting observations. He finds that this movement is an adaptation for the discharge of spores in a vertical direction. That the discharge of spores is only in a vertical direction was demonstrated by the placing of cover glasses in various positions near a mature stroma. Spores were obtained only on the glass suspended directly over the stroma, never at the sides nor beneath.

No inoculation experiments were made by us as no grasses were in flower as early as May 24th at Ithaca. Quantities of the sphacelial stage on rye were found in June, 1908, by one of us, in the locality from which sclerotia were obtained in 1907. Cornell Univ., Ithaca, NY"

Source: Whetzel 1911

A simple classroom germination is described in *Molds, Mushrooms and Mycotoxins* by Christensen, pub. by Minn. Press which follows:

For the class demonstration of germinating sclerotia, I have collected the sclerotia of ergot from Minnesota rye in the fall and have put these on the surface of moist sand, then have put them in an incubator at 4 to 5° C (40-42° F) and have left them until spring, at which time they were exposed to outdoor weather; after a few weeks, they began to germinate. If the sclerotia are kept moist and at 3 to 4° C (about 40° F) for a couple of months, then held at 14° C (57° F), they will germinate by mid-December. By manipulation of the temperature-time schedule, that is, the sclerotia can be induced to germinate at various times, but in nature they germinate when it is their time to germinate - when their host plants are flowering.



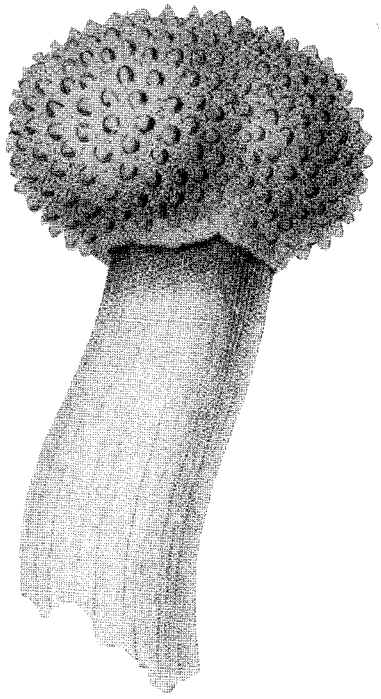
Germination of *Claviceps purpurea*

References: Henson 1940; Lewis 1962; Schwarting 1945.

Field Inoculation with *Claviceps purpurea*

The field inoculation of rye with *Claviceps* can be achieved by using an artificial spore suspension similar to natural honey dew suspension. A sterilized solution of 34 to 66 percent beet sugar is most effective. Maple syrup, corn syrup and honey all proved ineffective.

Beet sugar solution (34 to 66%) met all the following criteria of natural honey dew suspension. According to Ralph W. Lewis:



"(1) prevent immediate germination,
(2) protect the spores from death by desiccation after application, (spores remained viable for 5 days after the solution had been allowed to dry in air or over calcium chloride.)
(3) attract insects,
(4) allow germination once the spores come in contact with the pistils of the rye flowers."

Artificial Honey Dew-Preparation of Spore Suspension in Quart Canning Jars (Method by Hayes)

250 mL of wheat grain and 250 mL. of water were poured in each quart canning jar and allowed to set overnight. The jars were sterilized by autoclaving for one hour at 15 lbs. Jars were inoculated with a sporulated (conidia) culture of *Claviceps* and allowed to grow at room temperature for six weeks. Cultures were then mixed with 500 mL of water and blended for two minutes. The cultures were screened through a 16 mesh and then a 40 mesh screen. To this is added one liter of beet sugar and stirred until dissolved.

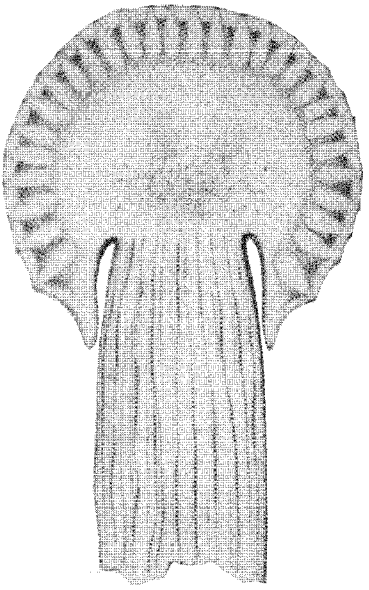
Artificial honey dew suspension is stored at -18 to 0° C. 1.75 quarts of suspension is produced from each quart of culture.

Spraying of the Rye Field

The rye flowers must be sprayed on a dry day as the solution will be washed off in the rain. The rye must also be sprayed when the rye flowers are in bloom. Rye flowers in 15 minute cycles every 45 minutes throughout the morning. Flowers begin opening when the sun first strikes the field until noontime. Weather and temperature also effects the flowering. The best time in which to spray occurs from 7 A.M. to 11 A.M.

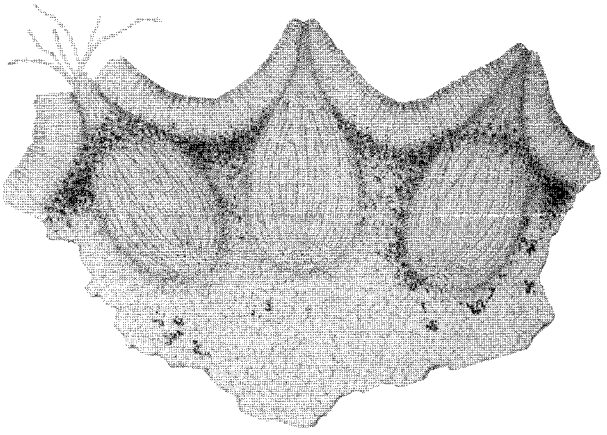
In 1943, Ralph Lewis power sprayed his rye fields three times each morning for a period of three days. He used a 1:7 dilution of the suspension and 60% of the rye heads became infected with ergot.

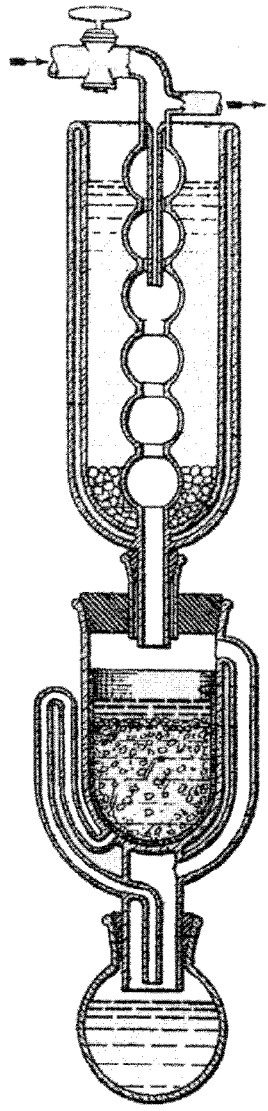
According to Heinz G. Floss approximately 95% of all peptide alkaloids are produced by extraction of field inoculated ergot.



Cross Section of Stroma

Refs.: Lewis 1945; 1962.
Claviceps gigantea Fuentes 1964.
Alkaloid extraction from ergot: Arcamone 1961; Bankovskii 1969





Chapter 14
Ergot Alkaloid Extraction from *Claviceps* Species

Ergot Alkaloids from Commercial Ergot

Elmer H. Stuart
Indianapolis, Indiana November 18, 1936

To 20 pounds of ergot, conveniently whole and unground but not necessarily so, are added about 25 pounds of liquid ammonia. The ergot thus treated may be used directly as obtained on the market, but preferably I first defat it, as by extraction with gasoline, as I find that that gives an increased yield; but such preliminary defatting operation is not essential.

After the liquid ammonia has stood on the ergot for an hour or two, it is drawn off. Then another 25 pounds or so of liquid ammonia are added to the already once-extracted ergot, and allowed to stand for another hour or two, and drawn off, and may be added to the first liquid ammonia extract obtained. If desired, the extraction may be carried further, by further treatment of the already twice-extracted ergot with liquid ammonia, but usually two or at most three extractions are sufficient to obtain as great a yield as is economically practical.

The extraction of the ergot with the liquid ammonia requires no special apparatus. It may be carried out in any ordinary open-top percolator or other receptacle, but desirably one of the usual type of percolator which is provided with a valved opening at the bottom for drawing off the extract. The evaporation of some of the ammonia keeps the remainder of the ammonia sufficiently cold to remain liquid, so that no increase in pressure beyond atmospheric pressure is needed to maintain such liquidity. The walls of the percolator or other container are conveniently heat-insulated, in any usual manner, to minimize absorption of heat from, the surrounding atmosphere and thus to minimize the loss in volume of liquid ammonia by reason of evaporation. The apparatus is desirably located under a hood, to dispose of that portion of the ammonia which evaporates.

The combined liquid-ammonia extract, which is reddish-brown in color, contains a physiologically active principle (or principles) of the ergot. If desired, this combined extract may be simply evaporated to dryness, to give a solid residue which contains such active principle (or

principles). Such evaporation is desirably under vacuum in order to obtain a solid residue substantially free from ammonia. The evaporation may be carried out in any desired apparatus, with or without recovery of the evaporated ammonia. The heat for the evaporation may be simply that from the atmosphere, if slow evaporation is all that is wanted; or may be accelerated by supplying heat in any usual manner of supplying heat for evaporation. Of course, for acceleration of the evaporation it is desirable that the container in which the evaporation is carried out shall not have the insulated walls which are desirable for the container in which the extraction is carried out. To ensure stability, I prefer to acidify before all the ammonia has been removed conveniently by adding tartaric acid when the total volume of the extract has been reduced under vacuum to about one-twentieth of the volume of the original extractive.

The solid residue thus obtained from 20 lbs. of ergot ordinarily weighs about 2 lbs. It is very crude, and contains much contaminant. But it can be used to make a liquid extract highly potent in ergot activity, and physiological assay by the usual methods, such as the cock's-comb method (the official method of the *U. S. Pharmacopoeia, Tenth) Decennial Revision*, 1926) and the Broom and Clark method, shows that the yield in units of potency is ordinarily close to the full activity of the original ergot.

To get the alkaloids (for I consider the hermorrhage-controlling active principles of ergot to be alkaloids) relatively free from contaminant, I prefer to proceed as follows: Instead of evaporating the combined liquid-ammonia extract to dryness, I first merely reduce its volume until most of the ammonia is evaporated off, to leave a rather thick extract. I extract this thick liquid-ammonia extract with pure ether, desirably two or three times, conveniently shaking to facilitate the extraction. For each ether-extraction I use about a gallon of pure ether; although the amount of ether may vary over a considerable range. The ether removes most if not all of the active principle (or principles) of the ergot from the liquid ammonia. On standing, the ether, which now contains the active principle (or principles) in the form of the alkaloidal base, separates from the liquid ammonia by rising to the top, and may easily be poured off.

This ether solution containing the active principle is then desirably treated to remove any residual traces of ammonia. This may be done by warming the ether; or it may be done by adding a little water, which on shaking absorbs any ammonia present but on standing promptly separates from the ether. The small amount of water necessary to remove the ammonia removes little if any of the active principle.

The ether solution contains the active principle (or principles) of the ergot, but also contains some of the contaminants that were present in the liquid-ammonia solution. For further purification, I extract the ether solution with weakly acidulated water, desirably several times. While other acids may be used for acidulating the water, I prefer tartaric acid, about 10 grams of it in a liter of water for the first extraction and usually only about one or two grams of it in 200 to 500 cc. of water for the second and third extractions. The acid neutralizes the alkaloidal bases present in the ether solution, to form salts which are soluble in water but insoluble in ether; so that these salts are taken up by the water. These salts include the active principle. On standing, the water and the ether separate; and the water may be drawn off through a separatory funnel.

I now add an equal volume (about 1.5 to 2 liters) of ether to the water solution, and make the mixture barely alkaline to litmus, as by adding sodium bicarbonate. On this alkalinization, the salt of the desired active principle is decomposed to free the alkaloidal base, which is taken up by the ether; but by making the alkalinization just barely sufficient to turn litmus blue the greater part of the other salts which are present in the water solution remain in the water. The water and the ether are allowed to separate, by standing, and the ether is decanted off. This ether solution contains the active principle (or principles) in the form of the alkaloidal base, in fairly pure state.

If desired, the ether extraction of the water solution may be repeated once or twice, with about 800 to 1000 cc. of ether after the first extraction. All the ether extracts are combined, and washed With a small amount of water to remove any contaminating salts which may have been carried into the ether from the water solution.

The combined ether solution is now dried, as with anhydrous sodium sulphate, to make it substantially water-free. This combined ether solution, which has a total volume of about 2.5 liters, is reduced in volume by evaporation to about 750 cc. Then an ether solution of tartaric acid is added until no more precipitate forms. The precipitate is separated from the liquid, as by filtration followed by drying in a vacuum desiccator to remove any residual ether.

The solid thus obtained from 20 pounds of a good grade of ergot (say one assaying 200% U. S. P.) ordinarily weighs about six grams; but the amount of this solid will be less for poorer grades of ergot. This solid is the tartrate of the alkaloid base (or bases). It is an amorphous powder slightly brownish in color.

Source: Stuart 1937

Isolation of Lysergic Acid from *Claviceps paspali* Cultures

by Werner Schlientz and Benno Sutter
Switzerland Januanry 11, 1965
translation by Otto Snow

The process which consists of adsorbing the acids on activated carbon in presence of a reducer in an inert gas atmosphere then eluted with aqueous-alcoholic solutions of very volatile bases. The adsorption of the acids on the activated carbon can be carried out in a wide field of pH, for example between pH 1 and pH 13. With the values extremes of the pH, for example at pH 1 and for pH 13, it is simply necessary to use more activated carbon to obtain an adsorption complete.

An advantageous manner to carry out the adsorption of the acids of formula I starting from their aqueous solutions consists in operating in a following way: to the aqueous solutions in question, which can be for example filtrates of culture or mother liquors, acids or alkaline, coming from the complementary treatment of filtrates of culture, or from the solutions in which one carried out the isomerisation of the isolysergic acid in lysergic acid until the equilibrium using a caustic soda detergent, one approximately twice adds the activated carbon in the form of a mixture with this quantity of an auxiliary of filtrage, such as for example Celite, the aqueous solution to treat being added, before or during the addition of the activated carbon, certain reducers, such as for example of dithionites, of alkaline metals or hydroquinone.

It is perfectly possible also to carry out adsorption and filtration in an inert atmosphere of gas, for example under nitrogen or carbon dioxide, the activated carbon used having to be heated with approximately 300° under an inert gas before being used, then, adsorption and filtration carried out, the activated carbon charged with the adsorbed product is eluated, for example by means of an aqueous-methanolic ammoniacal solution.

Example 1 - 100 Liters of a culture filtrate of the new stock of *Claviceps paspali* Stevens and Hall [*Helvetica Chimica Acta* 47, 4, 1052 (1964)] containing according to the colorimetric analysis 870 mg/liter of alkaloids (relatively has a molecular weight of 268) one adds 800 grams of potassium metabisulfite then 2.5 kg of activated carbon, for example of charcoal or of coal of blood, one agitates the whole during approximately fifteen minutes and one separates by filtration the activated carbon.

One washes this carbon with 20 liters of distilled water and then eluates with 120 liters of methanol containing 10% of concentrated ammonia. The eluate is concentrated with 2 liters under 20 mm of mercury and at 30° and the carboxylic acid, which crystallises at the end of twenty-four hours, is dried at 80° under 1 mm of mercury.

One collects 81 grams of alkaloids, quantity which corresponds to an output of 93%. The chromatography on paper one finds that the gross product has the following composition:

70% 6-methyl-Δ-8,9-ergolene-8-carboxylic acid
21% *d*-lysergic acid;
1% *d*-iso-lysergic and
2% clavines.

Example 2 - One runs a nitrogen current during half an hour through 60 liters of an aqueous solution, according to the colorimetric analysis, 830 alkaloid mg per liter (relative with a molecular weight of 268). Then one filters the solution through a filter with pressure, while operating under a pressure of 1.5 atmosphere, on a layer of a mixture of 1 kg of activated carbon and 2 kg an auxiliary (eg. Celite) of filtration (which, before being utilized, were heated to 300° in an atmosphere of nitrogen): the acids are then adsorbed on coal.

When adsorption is finished, one washes carbon with 15 liters of distilled water until one cannot detect any more, in the filtrate, of water soluble impurities, and then one subjects this coal to an elution with 50 liters of methanol added with 10% of concentrated ammonia. The eluate is concentrated to 1 liter under 20 torr at 30° and the acid carboxylic, which crystallized at the end of twenty-four hours, is dried under 1 torr at 80°. The recovery is 45.3 grams is 91% of the theoretical quantity.

According to the chromatography on paper, the gross product has the following composition: 99% of *d*-lysergic acid and 1% of *d*-iso-lysergic acid

Example 6 - 100 Liters of a very acid filtrate of culture (pH from approximately 2) of *Claviceps paspali* Stevens and Hall, whose content, determined by colorimetry, is 1.2 grams per liter (relative with a molecular weight of 268), one adds, while agitating, 500 grams of sodium dithionite and 2.0 kg of activated carbon (Carboraffin). One separates coal by drying, one washes it with 20 liters of water and one subjects it to an elution with 120 liters of methanol added with 10% concentrated ammonia. One concentrates the eluate to 8 liters. One separates by filtration the lysergic acid which crystallized, one washes

it with 1 liter of water and one dries it with 80° under 1 mm of mercury. One thus collects 105 grams of crude lysergic acid whose content, determined by colorimetry, is 83% (relative with a molecular weight of 268): the output is thus 72.6%.

According to the chromatography on paper, this product consists of 87% of lysergic acid and 13% *d*-iso-lysergic acid.

Source: Schientz 1966

Extracting Alkaloids from Ergot of Rye

LEK Tovarna Farmacevtskih
Ljubljana, Yugoslavia December 13, 1972

When isolating the ergot-alkaloids, their decomposability and conversion into inactive isomers has to be taken into account. For this reason the process should be accomplished rapidly, in order that the large quantity of ballast substances, mainly fats, do not effect the process.

Prior art procedures utilize a preliminary purification, e.g. extraction. This prolongs the reaction times, but causes considerable losses of alkaloids and undesirable isomerisation.

More recent processes omit the preliminary extraction. However, this has numerous other drawbacks. Whether the extracts are concentrated or various additives are used to prevent the formation of emulsions, the alkaloids are exposed to the action of heat and various reagents. The removal of the ballast substances requires a repeated transfer of the alkaloids from one solvent into another which prolongs the isolation procedure, lowers the yield and reduces the quality of the product.

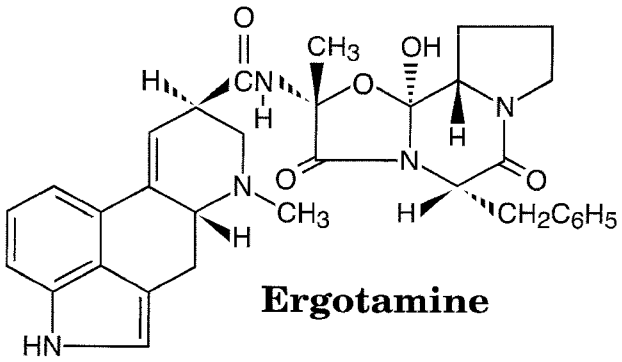
The process according to the invention as hereinafter exemplified avoids the above mentioned drawbacks to a high degree. The preliminary extraction is not necessary and separation of the alkaloids from the ballast substances proceeds easily and rapidly.

According to the present invention there is provided a process for the isolation of ergot alkaloids which process comprises extracting ground ergot of rye with an organic water immiscible solvent, contacting the resultant extract with an adsorbent material in order to reversibly adsorb the alkaloids, desorbing the alkaloids by means of a solvent which is more polar than the solvent used for the extraction, concentrating the resultant eluate in vacuo and thereafter precipitating the alkaloid by the addition of petroleum ether.

The drug is extracted with an organic, water-immiscible solvent, such as: chloroform, benzene, trichloroethylene, toluene, methylene chloride, or dichloroethane. The extract is filtered through a column of a suitable adsorbent, preferably alumina, whereby the alkaloids and small quantities of the ballast substances are adsorbed, whereas the greater part of the ballast substances are transferred into the filtrate. The adsorbed alkaloids are then eluted with a much smaller quantity of a more polar solvent or a mixture of the above mentioned solvents with methanol or ethanol. Subsequently the eluate is concentrated to 1/20 of its volume and separated from the major part of the ballast substances remaining on the adsorbent. The alkaloids are isolated from the eluate by careful evaporation of the solvent in vacuo and precipitation in an excess of petroleum ether. The process of adsorption and desorption of the alkaloids can be followed in UV-light. The same effect is attained by suspending the adsorbent in the extract or eluate and separating it by filtration.

The process according to the invention is illustrated in detail by the following example:

10 Kg. of ground ergot of rye is extracted in the usual manner with trichloroethylene. 50 litres of the extract is passed through two 75 g. columns of active alumina over a period of 2 hours. The active alumina is contained in two glass columns with a diameter of 10 cm. and a length of 50 cm. The alkaloids are eluted with 2 litres of ethyl acetate and the eluate is concentrated to a volume of about 150 cc. The alkaloid-bases are precipitated by pouring the concentrate into a tenfold quantity of petroleum ether filtered off and dried in a vacuum drier.



Ergotamine

The yield amounts to 90% of the alkaloids contained in the drug, in the form of a whitish amorphous powder containing 85 to 90%, with respect to the ergotamin-base. The percentage of the dextrorotatory isomers is practically the same as in the drug.

Source: LEK 1972

Isolation of Ergot Alkaloids from *Claviceps purpurea* Culture Suspensions Using Clay

Werner Grawert; Ludwig Schiedt, Brigitte Neumann, Karlheinz Heidenbluth; Christoph Dauth; Rudolf Schirutschke; Monika Müller, of Radebeul and Dresden, German Democratic Rep. December 20, 1978

It has been found surprisingly, that adsorbent clays such as bentonite, nontronite, bleaching earth, and Fuller's earth have an unusually high adsorption capacity at the natural pH value of the culture suspension not only for the ergometrin, but for all the investigated ergot alkaloids of the most diverse structures.

It has further been discovered that the mycella-adsorbent mixture can be separated from the liquid component in a relatively facile manner through filtration or other physical separation means.

A still further surprising discovery was that after physical separation, the mycella-adsorbent mixture containing the alkaloids can be subjected to fluidized bed drying according to the known physical parameters at the normally unfavorably high temperature for the alkaloids of 80°-90° C. without isomerization or decomposition of the compounds. After a washing with a weak aqueous alkaline solution, the alkaloids can then be extracted easily and virtually completely from the dry mycella-adsorbent mixture with an organic aprotic solvent.

In further investigations it was determined that this drying process was also applicable to those cultures in which the alkaloids are contained in the cell mass; thus, the addition of the adsorbent in these systems was not necessary. In this case, after mechanical separation and drying in the fluidized bed, dry mycella containing the alkaloid are obtained, the further work-up of which is not dependent upon the time of the termination of the drying process, unlike in the prior art.

This inventive process can be used to isolate a whole spectrum of ergot alkaloids, such as ergocryptine, ergotamine, ergocristine, ergocornine, ergometrine, lysergic acid, agroclavine, ergosine and others. This is achieved through the addition of 4-5% by weight, based on the water soluble recovered material, of an adsorbent clay and stirring for about 30 minutes. After mechanical separation of the solid material, for

example through filtration, the wet mycella-adsorbent mixture is placed in a fluidized drying bed at 80°-90° C. for about 20 to 40 minutes until an exhaust temperature of between 50° and 70° C. and a residual moisture content of no more than about 15% is achieved. The mycella-adsorbent mixture is then mixed with an aqueous, weakly alkaline solution, for example, 15% ammonia water, and then extracted with an apropriate organic solvent, for example, a lower carboxylic acid ester, acetone or a halogenated hydrocarbon. This extract is then further treated by liquid-liquid extraction with an aqueous acidic phase-in a ratio of 2:1 to 1:1 by volume, in order to eliminate the non-basic residue. The aqueous solution containing the total alkaloid content is then made basic to a pH in the range of 8 to 9.5 and the alkaloids are extracted with an organic solvent not miscible with water, preferably ethyl acetate, in a ratio of 1:1 to 3:1 by volume.

The further treatment of the extract follows the known procedure with recognition of the chemical and physical properties of the alkaloids.

Bentonite is a montmorillonite-containing clay, named after its source, Fort Benton, Mont. It is among the group of clays commonly referred to as bleaching earths which require activation by an acidic treatment process. Fuller's earth, another montmorillonite-containing clay, is not treated by any activation process before use; hence its description as a "raw bleaching earth". These and other adsorbent clays may be employed in the inventive process.

Example 1

200 L. of culture suspension of *Claviceps purpurea* (Fr.), Tul. IMET PA 130 (obtained from the Jena Central Institute for Microbiology and Experimental Therapy, GDR) is stirred for 30 minutes with 8 kg bentonite. It is then filtered through a 10 mm layer of calcium sulfate dihydrate over a rotary vacuum cell filter; the practically alkaloid-free filtrate is discarded. The recovered mycella-bentonite mixture is dried in a fluidized bed at an influx temperature of 90° C. until an exhaust temperature of 60° C. is achieved. This takes approximately 30 minutes. There is recovered 20-24 kg of mycella-bentonite mixture with a residual moisture content of from 3-5%; this mixture contains 95-100% of the ergotoxin and 92-96% of the ergometrin contained in the culture suspension.

Example 2

100 L. of the culture suspension of Example 1 is stirred for 1 hour with 5 kg of bleaching earth, filtered, and the mycella-adsorbate mixture is dried in the fluidized bed at 80°-90° C. for about 15 minutes until an exhaust temperature of 50° C. is reached. There is recovered 12 kg of the dried mycella-adsorbent mixture with a residual moisture content of 10-12% and an alkaloid content of 92-100% based on the culture suspension.

Example 4

60 Kg. of the dry mycella-adsorbent mixture of Example 1 or 2 is mixed with 7.5 L. of 1:1 diluted ammonia, extracted in a mechanical extractor with 150 L. chloroform or methylene chloride over 45 minutes, filtered under pressure and the process repeated with the same volume of solvent for 30 minutes. The total extract is added to an equal volume of 5% aqueous acetic acid and processed in a separator, the aqueous phase constantly adjusted to pH 9; the process is repeated a second time with the same solvent or with ethylene acetate at a double volume. After evaporation and isolation of the ergometrin and ergotoxin, the adducts and yields of an alkaloid content of 96 to 103% calculated as the bimaleinate. The yield is (*approx.*) 351.6 g ergotoxin-toluol adduct, 72% calculated from the culture suspension and 243 g ergometrin-chloroform adduct, 82% from the suspension.

Example 5

30 L. culture solution of *Claviceps purpurea* IMET PA 134 at its natural pH value (5-6) is stirred for 30 minutes with 1.2 kg bentonite. It is then filtered through a layer of calcium sulfate dihydrate and the filtrate discarded. Drying of the mycella-adsorbent mixture according to Example 1 yields 2.8 kg of the dry mixture, which contains practically all of the alkaloid content of the culture suspension (ergosine, ergosinine, traces of chanoclavine). Rotary extraction of the alkaloids with a 10 to 12 fold volume of ethyl acetate yields up to 90% of the alkaloid content of the dry mycella-bentonite mixture.

Source: Grawert 1980

The previous mothods can be used in the extraction of ergot alkaloids from *Claviceps paspali* cultures.

Preparing Lysergic Acid from Paspalic Acid

Jean-Claude Gaullier
Gournay sur Marne, France October, 26, 1999

It is known that lysergic acid can be prepared by isomerizing paspalic acid, using potassium hydroxide (*Helvetica Chimica Acta*, 64, 47, 478 (1981)) or sodium hydroxide (*Helvetica Chimica Acta*, 47, 115, 1052 (1964) and JP70013302), but these processes do not allow either good yields or a product comprising small quantities of isolysergic acid to be obtained industrially.

A process has now been found, which is the subject of the present application, allowing lysergic acid of sufficient purity to be obtained in very good yields by isomerizing paspalic acid.

The alkali metal hydroxide used may be in particular sodium hydroxide or potassium hydroxide.

Preference is given to the use of sodium hydroxide.

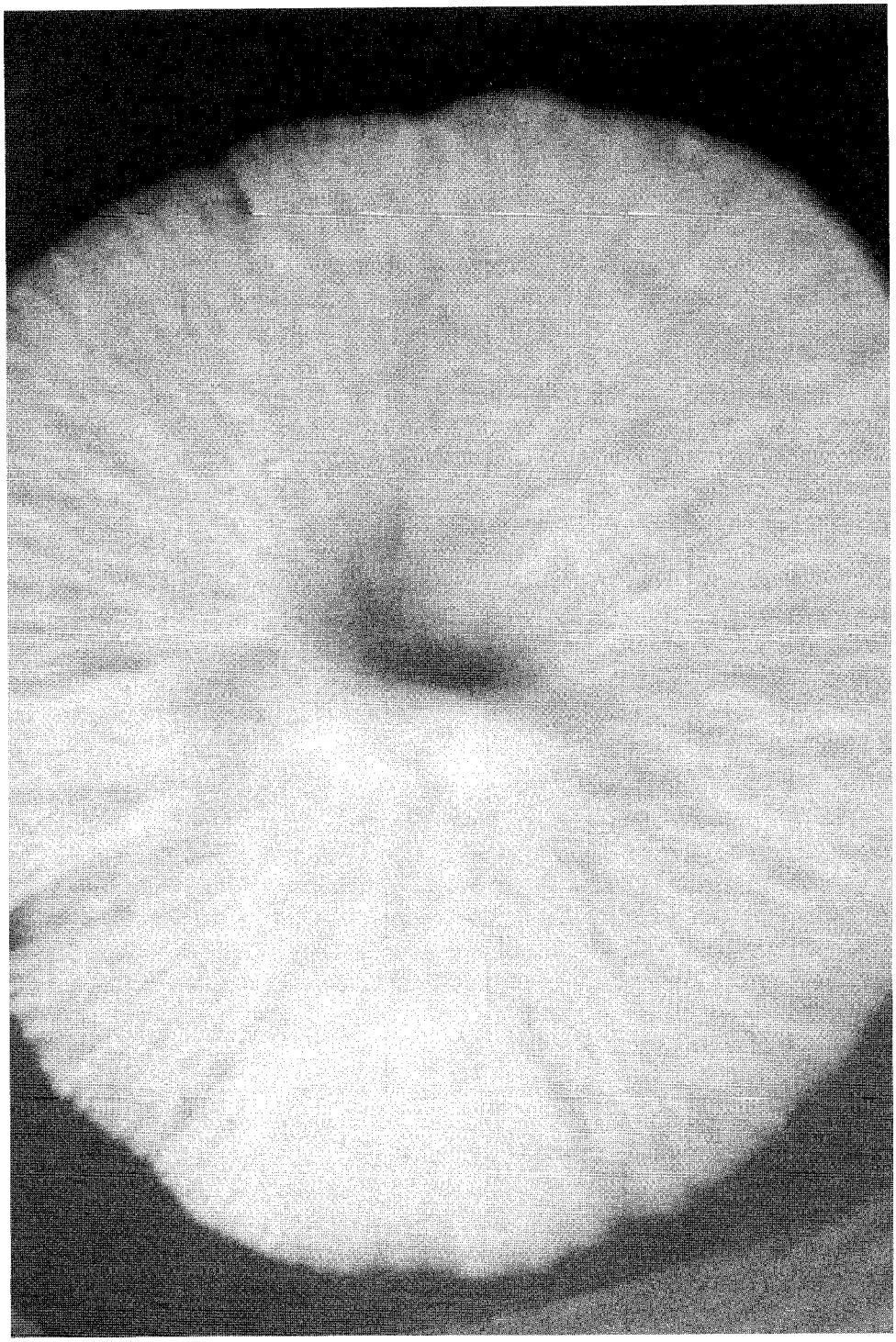
The process is generally carried out in an inert solvent, such as water or an aliphatic (C1-C4) alcohol (methanol or ethanol, for example), or in a mixture of these solvents, at a temperature between 20° C. and 60° C. and preferably between 25 and 35° C. It is advantageous to leave the reaction medium at this temperature for from 20 to 30 hours and in particular for 24 hours.

A—Preparation of Lysergic Acid with Sodium Hydroxide Alone

5 g of paspalic acid in 100 mL. of a 2N aqueous solution of sodium hydroxide are heated at reflux for 2 hours. After cooling, the pH of the reaction medium is brought to 5.5 by adding an aqueous solution of hydrochloric acid and glacial acetic acid (20 ml of water, 10 mL. of hydrochloric acid and 10 mL. of acetic acid). The precipitate is filtered, washed with 3 times 20 mL. of a water/methanol (50:50) mixture, then dried in vacuo at 75° C. This gives 3.15 g of lysergic acid comprising 6.8% of isolysergic acid and the RRi is 59.3%.

B—Preparation of Lysergic Acid with Potassium Hydroxide Alone

5 g of paspalic acid in 360 g of a 0.5N solution of potassium hydroxide in a water/ethanol (50:50) mixture are heated at reflux for 1 hour. After cooling, the pH of the reaction medium is brought to 5.5 by adding 1N hydrochloric acid. The precipitate is filtered, washed with 3 times 20 mL. of a water/methanol (50:50) mixture, then dried in vacuo at 75° C. This gives 2.86 g of lysergic acid comprising 1% of isolysergic acid and the RRi is 49.8%. Source: Gaullier 2001



Chapter 15
Claviceps purpurea Cultivation and Strain Selection

Ergots are obtained from the field and stored in the refrigerator in a tightly sealed container so as not to infect anything in the refrigerator. Ergot is poisonous.

Several dozen Petri dishes are sterilized by heat. 350 degrees F for three and one half hours. The dishes are then allowed to cool.

Preparation of Media

Claviceps will grow on various organic substrates. Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) are the most popular.

Malt Extract Agar (MEA)

Malt Extract: --------- 10 grams
Peptone: ---------- 2.5 grams
Agar: ---------- 7.5 grams
Distilled Water: ----- 500 mL

Potato Dextrose Agar

150 Grams of diced potatoes are boiled in 250 mL of water until cooked. The cooked potatoes are strained through cheese cloth and water is added to bring the solution to 500 mL 7.5 Grams of agar are dissolved in the water with heating. 10 Grams of glucose (corn syrup) are added.

The media is sterilized in an autoclave (pressure cooker) for 30 minutes and allowed to cool in the pressure cooker. When the sides of the pressure cooker are just a little warm to touch the media is ready to pour into the sterilized Petri dishes. If the media is not allowed to cool it will form water condensation in the dishes and increases the rate of contamination. If the media is allowed to cool too much it will gel and then will not pour.

The media should be poured as rapidly as possible so as not to contaminate the dishes with air born microbes. The dishes are allowed to gel and then stored in a refrigerator until ready for inoculation with ergot.

Inoculation of Cultures

Sterile conditions are a must. Several scalpels or Exacto knifes are placed in a drinking glass and the glass is then filled with alcohol (wood, denatured, rubbing) to sterilize the knives. An ergot is grasped at its ends by thumb and index fingers of both hands. The ergot is then snapped in half. Inside the ergot is a white, gray or pinkish material, this is the mycelium. Take one of the scalpels from the alcohol solution and allow the alcohol to drip free from the blade. A small piece of the mycelium is then taken from one half of the ergot and placed onto the media. This technique must be done rapidly as opening of the culture dishes for extended periods will produce contamination. Several dozen dishes must be cultured as contamination will occur and during strain selection many of the cultures will be discarded. See page 190.

Strain Selection

The sclerotial form of *Claviceps purpurea*, ergot, is a heterokaryotic fungus, that is a multi strain fungus containing multinucleated cells. Sclerotial forms of the fungus produce alkaloids. Condial forms of the fungus are uninucleated. They do not produce alkaloids unless the condia are germinated and the hyphia are mated. Petri dishes inoculated with an inner piece from the ergot will form many cultures, many will be contaminated, others will be non-heterokaryotic, many will form spores (condia) and not form alkaloids. Strain selection is necessary to isolate a strain which will be a high alkaloid producer. Sectors will form in some of the cultures. These sectors may be yellowish white, white, cream, violet, brown etc. Some of the cultures will form large colonies with no sectors, or late forming sectors, these are generally the heterokaryotic cultures. Heterokaryotic cultures are most like the original sclerotial form of the fungus and will generally produce the highest percentage of alkaloids in submerged cultures. Sectors that fluoresce under UV produce 10 fold increase in alkaloids in submerged cultures compared to non-fluorescing sectors. Alkaloid estimate of cultures (Vining 1959).

Refs: Abou-Chaar 1961; Adams 1964; Amici 1966; 1967; 1969; Arcamone 1961; Berman 1954; Brady 1960; Cherewick 1956; Hareven 1970; Kelleher 1969; 1971; Mantle 1969; 1976; Michener 1950; Mizrahi 1968; Ogunlana 1969; Pacifici 1962; 1963; Paul 1954; Ramstad 1955; Societa Farmaceutici Italia 1961; Taber 1966; Tyler 1954) After several strains have been obtained, they are then cultivated industrially in large fermenters, which are just large pressure cookers. Large equipment is impractical for most individuals. Canning jars or pressure cookers can be successfully used. Industrial Fermentation Equipment: (Cleverdon 1955) (Dworschack 1954) (Fuld 1957).

Ergot Preparation

Adelia McCrea
Detroit, Michigan January, 4, 1933

The prior literature reveals the fact that *Claviceps purpurea* is a fungus which attacks the naturally growing rye and develops the material which is used commercially on a large scale for the manufacture of ergot extracts. The prior literature also reveals the fact that the fungus can be cultured upon certain laboratory media but there is nothing prior to my research which has convincingly demonstrated the possibility of producing on a commercial scale an artificially prepared substance useful as a substitute for the natural ergot of commerce.

I have discovered that by properly controlling the surrounding conditions it is possible to saprophytically grow the fungus *Claviceps* purpurea and obtain a material containing the chief characteristics of the natural ergot product including the particular alkaloids ergotoxine and ergotamine. In carrying out my process it is necessary to first obtain a culture of the fungus, then preferably to develop a "single spore" strain and finally to transplant the strain on a suitable medium which will cause sufficient development to provide a final product having the desired characteristics. The cultures of the fungus may be obtained in several ways.

1. From the ascopores (perfect stage) of germinating sclerotia.
2. From the conidia ("perfect" or "sphacelial" stage, the "honey-dew" of rye infection).
3. From bits of tissue taken from the inner portion of matured (but living) sclerotia, the "ergot" of commerce.

The first two methods are of course strictly seasonal due to the fact that they depend upon the growing rye, while the last method can be used at any time when living sclerotia can be obtained. Since it is possible to secure such sclerotia alive and active in the field each summer, it will be seen that by the third method cultures of the fungus are always available.

By whatever procedure the original culture is obtained the subsequent steps of the process are the same. After isolation has given a pure culture, it is advisable to develop a "single spore" strain from which all future plantings are then made although, so far as known, *Claviceps purpurea* is homothallic. This pure, single spore strain is then grown saprophytically ("artificially") on any suitable medium either of liquid composition or of moist cereal composition, e.g. bran or ground rye. One

formula that has been found to give good growth contains the following ingredients:

Media A

Magnesium sulphate	grams	0.625
Peptone	grams	0.625
Dihydrogen potassium phosphate	grams	1.25
Maltose	grams	6.25
Malt extract	grams	6.25
Water, distilled	1000 cc.	

Other media capable of sustaining growth of *Claviceps purpurea* are given below:

Media B

Magnesium sulphate	grams	0.5
Dihydrogen potassium phosphate	grams	1.0
Sodium chloride	grams	0.5
Dextrose	grams	10.0
Ferrous sulphate	grams	0.01
Gelatin	grams	20.0
Distilled water	cc. 1000	

Media C

Ammonium nitrate	grams	4.0
Ammonium phosphate	grams	0.6
Potassium carbonate	grams	0.6
Magnesium sulphate	grams	0.6
Ferrous sulphate	grams	0.07
Saccharose	grams	70.0
Water, distilled	cc. 1500	

Rye bran mixed with thirty per cent; distilled water in which one per cent asparagin is dissolved.

(ammonium nitrate can be used instead of asparagin see following articles)

When the cereal media are used, e.g., ground rye, rye bran, etc., growth is allowed to proceed as far as possible in order that the material may be almost completely utilized by the fungus. The mass is then dried, ground if necessary, extracted and tested exactly as for crude drug.

When a liquid media is seeded with a spore suspension of the fungus, a heavy ring of growth develops at the margin of the flasks and scattered discrete colonies form over the surface of the liquid which later join to make a mat of varying thickness according to the available nutrients and the length of time allowed for growth. At least three weeks should be given, better four, to permit full maturity but this varies somewhat with the food.

Cultures are then combined, pressed with a spatula to free from excess medium, left in petroleum ether several hours, drained on filter paper and rendered brittle by vacuum drying. This material is then ground, extracted, and tested exactly as for crude drug.

This fungus is not unduly exacting so far as physical factors are concerned. Ordinary room temperature is suitable, as it has an optimal range of 20° to 28° C., but a temperature range of from 18° to 30° C. can be employed. Outside of these ranges the results are ordinarily not as good although the organism is not completely destroyed at temperatures which are considerably outside of this range. An abundant moisture supply must be available to permit normal vegetative growth before drying checks further development. Light exerts a marked effect upon the production of color which appears to be closely associated with the production of ergosterol but it has not appeared that this factor increases the content or activity of alkaloids. Ample aeration is a requisite for good growth. Oxygenation is a very definite stimulation to more rapid metabolism and increased growth, provided that other factors are kept at optimal conditions. The time necessary for securing what may be termed nature mycelial mats corresponds approximately to the period of growth in rye heads under natural conditions, i.e., about a month from date of inoculation.

When properly isolated and grown under suitable conditions, this saprophytic fungus growth develops the characteristic active principles, i.e. the alkaloid constituents of ergot sclerotia grown naturally as a parasite of rye. This has been repeatedly shown by tests that conform strictly to the methods in the *U.S.P.* X. Extracts of this material, therefore, exhibit the physiological activity and medicinal value of extracts made from the crude drug sclerotia, the "ergot" of commerce.

Source: McCrea 1936

Production of Ergot Alkaloids

Siegfried Windisch and Walther Bronn
Berlin, Germany March 3, 1955

For many years attempts have been made to cultivate ergot saprophytically in an artificial nutrient medium. The establishment of such cultures created no significant difficulties but the fungus could not be induced to produce the valuable alkaloids under artificial conditions. Although G. Schweitzer, *phytopathol. Z.*, 13; 317 (1941), claimed, in saprophytic cultivation, to have obtained a sclerotial form similar to the sclerotia developed by the fungus in natural growth and to have found an alkaloid content equal to that of the commercially available product, these results could not be confirmed by other investigators who later repeated the tests. In this connection reference is made to the following papers: H. D. Michener and N. Snell, *Americ. Journ. Botany*, 37, 52 (1950), S. K, Sim and H. W. Youngken, *J. Amer. Pharm. Assoc., Sci. Ed.*, 40, 434 (1951), and V.E. Tyler, and A.E. Schwarting, *ibid.*, 41 590 (1952). In view of this fact the investigation of ergot has, for many years, been largely confined to the parasitic culturing of the fungus.

Many years were spent by the present inventors in investigating the nutritional requirements and the metabolism of a large number of saprophytically cultivated *Claviceps* spec. of European and extra-European origin and they were successful at last in finding cultural conditions in which the biosynthesis of alkaloids was induced to occur. Basically, it was found that these fungi never form alkaloids when the conditions of cultivation permit intensive cellular respiration. During the period of growth, that is to say during the period of active cell multiplication, cellular respiration is very intense. Respiration is necessary for cell division to take place at all. Similarly, autolysis of the cells of the fungus at the end of the period of growth is coupled with pronounced respiration so that in the course of development of a normal culture, which includes cellular growth and autolysis, no alkaloids can be formed and accumulated. To promote the biosynthesis and the accumulation of alkaloids, the prerequisite condition is for respiration of the cells of the fungus to be severely suppressed by measures calculated to maintain the respiratory metabolism of the cells in a state of quiescence.

It is therefore proposed according to the present invention to cultivate *Claviceps* spec. saprophytically in nutrient substrates which contain assimilable sources of carbon, nitrogen, as well as of essential mineral salts, the cultivation being conducted at low pH values, prefer-

ably between pH 3.0 and 5.0, and under aerobic conditions, and then, for the purpose of inducing the production of alkaloids, to maintain the cell growth of the fungus in a static condition of pH-values between approximately 5.5 and 7.0 in an environment wherein respiration is largely suppressed. According to the invention, the reduction in the rate of respiration is achieved either by producing anaerobic conditions at rH values of the substrate equal to 18 or less, or by creating an artificial nutrient deficiency, or by using specific toxic inhibitors of cellular respiration. The two latter methods bring about a reduction in respiratory activity even in aerobic conditions.

Further features of the present invention will emerge in the course of the following particularized description of the method herein proposed.

A large number of samples of ergot of different origin of the species *Claviceps purp.* Tul. and other species of the genus *Claviceps* were examined and their alkaloid content as well as the composition of the alkaloids determined. Pure cultures were grown on saccharose-peptone-mineral salt-agar by the well-known method of isolating pseudo-parenchymatous tissue from inside the sclerotia. It is important to segregate the species and strains, before cultivation, according to the type and quantity of the alkaloids in the sclerotia because some strains are found to contain only traces of alkaloids and these latter fail to produce them even in the conditions that have been described. Consequently, only selected strains with satisfactory alkaloid forming potential are suitable for the production of alkaloids in saprophytic cultivation.

Growth Conditions for *Claviceps* Spec.

These are similar to those required for growing most moulds. For the purpose of synthesizing the purely cellular tissues an assimilable organic source of carbon, a similarly assimilable organic or inorganic source of nitrogen, and certain mineral salts are required. The examined strains did not depend upon the presence of special growth factors although such additions had an accelerating effect. In regard to cultural methods, both surface and submerged cultures can be used in a similar way to the manner in which moulds are grown.

Assimilable carbon sources are arabinose, xylose, glucose, fructose, galactose, mannose, saccharose, dextrine, starch, mannite, fatty oils (such as olive oil) and the intermediate acids of glycolysis and of the tricarboxylic acid series, preferably glucose, fructose, and mannite. Lactose could not be utilized by any of the strains that were examined.

Assimilable nitrogen sources readily utilized by all the tested strains were peptone, hydrolysed casein, yeast extract, glutamic acid,

asparagine, glycocoll, leucine, *dl*-alanine, guanidine and urea. Ammonium salts and nitrates were likewise easily assimilated but measures had to be taken to prevent the ammonium salt anions and the nitrate cations from impairing the growth of the fungus. When feeding sulphate of ammonium, for instance, the progressive assimilation of nitrogen by the fungus releases an equivalent quantity of sulphuric acid which gradually retards growth and finally inhibits it altogether. When using alkali nitrates, a corresponding process of alkalinisation occurs. Ammonium nitrate acidifies because the ammonium ions are assimilated first. However, if when using nitrates or ammonium salts the substrate is continually neutralised, the fungus takes advantage of these sources of nitrogen as readily as when organic sources of nitrogen are available. Essential mineral salts are phosphates, magnesium, iron, and a few trace elements. The following mineral salt concentrations were found to be both suitable and sufficient to promote optimum growth:

KH2PO4 500-100 mg. percent, MgSO4.7H2O 100 mg. percent, FeSO4.7H2O 10 mg. percent, ZnSO4.7H2O 1 mg.percent, CuSO4.5H2O gamma percent, (NH4)Mo7O24.4H2O 5 gamma percent, MnSO4.5H2O 8 gamma percent, H3BO2 2 gamma percent. The mineral content of tap water used for preparing the substrate is generally adequate to satisfy the demand for trace elements, so that nothing more is required than an addition of phosphate and magnesium salt.

Growth factors are not essential but an addition of organ or vegetable extracts, such as malt extract, yeast extract, and the like, slightly accelerates growth. The best culture temperature is 25° to 30° C., and optimum hydrogen ion concentration (pH) in the substrate for promoting growth lies between pH 3.5 and 5.0. Optimum redox potential (rH) of the substrate for growth promotion is at least rH 24 and higher. In surface cultivation, this may be obtained by using maximum obtainable surface area of the culture medium, in shaker flasks, by using a sufficiently high frequency of shaking and by filling the flask with not more than 1/4 its volume with substrate, and when effecting deep cultivation in fermenters by stirring and vigorous aeration.

Relationship Between Growth and Nutrient Concentration

For optimum growth both the absolute and relative quantities of the nutrients must be available in certain minimum amounts. The limiting proportions are approximately carbon:nitrogen:phosphate as 40:1:1. In aerobic conditions, the synthesis of carbon into cellular

substance then reaches optimum values. If the relative quantity of nitrogen or phosphorus is increased or the relative carbon quantity lowered, incorporation of the latter into the cellular structure continues at optimum values. The efficiency of carbon assimilation does not therefore change very appreciably. However, this does not apply if the relative carbon quantity is considerably increased for instance by using a proportion of 100:1:1, or if there is a considerable deficiency of both nitrogen and phosphorus. The efficiency of carbon utilization as well as the speed of assimilation falls off at once. At the same time, the composition of the mycelium undergoes a change. There is considerable storage of fats in the cells and simultaneously respiration begins to be strongly reduced. With reference to the absolute quantities of nutrients, the general rule appears to be that in surface cultivation the carbon concentration required for optimum cellular synthesis must not exceed 4%. This is equivalent to 10% glucose. In submerged cultivation, the maximum is about 1.2% carbon which corresponds with 3% glucose. Higher concentrations lead to over crowded conditions of growth.

Conditions for Alkaloid Synthesis

The production of alkaloids in saprophytic cultivation of *Claviceps* spec. is confined to selected strains with pronounced alkaloid-forming ability. The fundamental requirement for the accumulation of alkaloids in such cultures is the restriction of the intensity of cellular respiration and, at the same time, the maintenance of a pH-value in the substrate of between 5.5 and 7.0. Since these conditions preclude growth and the quantity of alkaloid formed depends upon the volume of the available fungal material, cellular respiration must not be impeded for the purpose of alkaloid formation until the culture has first been allowed to pass through a growth phase. Both phases can be so controlled that one merges into the other, or alternatively, they may be conducted in two separate cultural stages. The means of restricting cellular respiration comprise either the imposition of anaerobic conditions, or the creation of a deficient nutritive environment, or the addition of specific respiratory toxins. The addition of indole or of its derivatives, such as indoleacetic acid and tryptophane, is not essential but it does increase the alkaloid yield.

Alkaloid Production by Inhibition of Respiration in Anaerobic Conditions

The necessary reduction in the rate of respiration that is required

for the production of alkaloids was achieved by successive or sudden reductions in the redox potential of the substrate to rH values of about 18 or less. To this end, well-known reducing agents such as ascorbic acid, sulphite, hydroquinone, organ extracts, were added to the substrate, and/or atmospheric oxygen was prevented from coming into contact with the cultures.

Production of Alkaloids When Inhibiting Respiration by the Creation of a Nutrient Deficiency

A number of research workers have pointed out that the cells of various micro-organisms appear to enter a rest stage in regard to their respiratory metabolism after they have stored considerable reserves of fats and carbohydrates. This was discovered for instance in the case of yeast by C. C. Lindgren, *Arch. Biochem.*, 8, 119 (1945). Culture methods which lead to an accumulation of fats and carbohydrates in the cells of many microorganisms are to-day widely known. Nearly all these methods rely on creating deficiencies in assimilable nitrogen, phosphate, or sulphate in the nourishment of the organisms, whilst at the same time maintaining adequate supplies of carbohydrates. Among the many papers which have been published on this subject it will be sufficient to refer to general surveys published by F.F. Nord, *Advances Enzymol.*, 9, 653 (1949); A. Kleinzeller, *Advances Enzymol.*, 8, 299 (1948), and K. Bernauer, *Erg. Enzymforsch.*, 9, 297 (1943). That the metabolism of Claviceps spec. similarly undergoes a change to adjust itself to deficiency conditions is for the first time disclosed by the inventors. Experiments show that from a certain threshold value downwards increasing deficiencies of nitrogen, phosphate, or sulphate, in the substrate lead to a continuous reduction in the turnover of carbohydrates, a decrease in utilization efficiency, as well as a decrease in the protein content of the cells, whereas the storage of fats and carbohydrates is stimulated. As a result the intensity of respiration diminishes in proportion. Since respiration was recognized to be the limiting factor in the biosynthesis of alkaloids by ergot, it is proposed to exploit the method of creating a nutrient deficiency for the purpose of inducing the manufacture of alkaloids by the fungus. The transition from the growth phase (intensive respiration) to the alkaloid forming phase (severely reduced respiration) may be continuous. By taking suitable measures the process can, of course, be discontinuously conducted in two separate stages, for instance by transferring normally-grown mycelium to a substrate in which the nutrient elements are present in the extreme proportions that create deficiency conditions.

The features of this method according to the present invention will be more particularly hereinafter described.

Nitrogen Deficiency

Nitrogen is deficient in the nutrient substrate for cultivating *Claviceps* spec. if the relative quantities of nitrogen and carbon (N/C) are considerably reduced to ratios less than 1:40 in surface cultures and 1:25 in submerged cultures. The figures given represent approximate limiting ratios in which the available nitrogen in the substrate is just sufficient to support normal growth of the fungus and the cells will show optimum protein content coupled with minimum storage of fat as well as optimum respiration under aerobic conditions. If the carbon concentration in the substrate is raised or the nitrogen concentration lowered, that is to say, if the ratio of N/C is reduced the symptoms of nitrogen deficiency will forthwith appear. These consist in a reduction in the rate of metabolism, a reduction in the utilization efficiency, a decrease in the protein content of the cells, a diminution in the rate of cellular respiration, and a concomitant increase in the storage of fats and carbohydrates within the cells, and in the synthesis of alkaloids. Metabolism continuously changes until the most extreme N/C conditions are reached. However, in practice there is little advantage in creating very extreme conditions because, as has been explained, the rate of metabolism is concurrently reduced. However, cell multiplication practically ceases under extreme N/C conditions in view of the fact that the cells need a minimum nitrogen content amounting to about 2.5% N (by dry weight), and that a certain quantity of nitrogen is also required for the biosynthesis of alkaloids. Experiments with various strains of ergot have shown that N/C ratios between about 1:70 and 1:100 constitute optimum conditions for the formation of alkaloids.

Example III

Culture of a *Claviceps* spec. strain (from a sclerotium isolated on *Elymus mollis*) at 27° C. in an Erlenmeyer flask on 100 ml. substrate in surface culture.

Substrate A:	Substrate B:
N/C=1:18	N/C=1:80
8.00% saccharose	8.00% saccharose
0.43% urea	0.086% urea
0.20% KH_2PO_4	0.20% KH_2PO_4
0.10% $MgSO_4$	0.10% $MgSO_4$
plus trace elements	plus trace elements

Corresponding submerged cultures produce substantially similar results but the alkaloid yields are rather less.

Phosphate Deficiency

The limiting ratio of phosphate and carbon which offers just enough phosphate for a saturated nutrition of ergot is approximately PO_4/C=1:80 to 1:100, provided other nutrients are available in adequate quantities. As soon as the phosphates are reduced to smaller proportions similar symptomatic changes in metabolism as occur when nitrogen is deficient at once become apparent.With regard to the production of alkaloids phosphate deficiency adduces more favorable results than the nitrogen deficiency method. Again, an excessive deficiency of phosphate is not an advantage. The optimum ratio for alkaloid synthesis was found to be PO_4/C approximately equal to 1:200 to 1:280.

Example IV

Substrate A:	Substrate B:
PO_4/C=1:29	PO_4/C=1:235
5.00% glucose	5.00% glucose
0.43% urea	0.043% urea
0.10% KH_2PO_4	0.012% KH_2PO_4
0.10% $MgSO_4$	0.10% $MgSO_4$
plus trace elements	plus trace elements

Substantially the results obtained in submerged culture are similar to the above, but the alkaloid yields are less.

Sulphate Deficiency

In effect, the reaction of *Claviceps* spec. to sulphate deficiencies is similar to the change in metabolism that occurs in the case of nitrogen and phosphate deficiencies. Again, an accumulation of fats is observed in the cellular tissues in conjunction with a reduction in protein content and a diminution in cellular respiration, and this is accompanied by the synthesis of alkaloids. The limiting ratio of sulphate and carbon which is just sufficient to cover the sulphate demand of the strains that were examined was found to lie approximately between SO_4/C=1:200 and 1:300. The most favorable ratio for stimulating alkaloid formation was between 1:500 and 1:1000.

Example V: The figures relating to a control culture have here been omitted since the comparative results are substantially equivalent to those obtained in nitrogen and phosphate deficiency tests. The example therefore merely gives a substrate upon which, in conditions similar to those already described in the foregoing examples, a total of 36.2 mg. percent of alkaloids (calculated on the basis of ergometrine) was obtained from surface cultures at the end of 25 days.

Substrate: 5.00% glucose
0.43% urea
0.20 % KH_2PO_4.
0.01% $MgSO_4.7H_2O$ SO_4/C approx. 1:500
0.04% $MgCl_2$
Plus trace elements Source: Windisch 1960

Preparation of Ergot Alkaloids

Richter Gedeon Vegyeszeti Gyar R.T.
Budapest Hungary March 17, 1964

The present invention relates to the production of ergot alkaloids, especially of ergometrine and of ergotoxine of high ergocristine content by biosynthesis; more particularly it provides a process for the preparation of such ergot alkaloids by cultivating a specific strain of *Claviceps purpurea* on a synthetic nutrient medium.

We have surprisingly found, that a strain isolated by systematic selection from a culture of *Claviceps purpurea* irradiated by a dose of 10,700r with radioactive cobalt ((60Co) and cultivated under saprophytic conditions on a synthetic nutrient medium containing saccharose as carbon source, ammonium succinate as nitrogen source and inorganic salts, is able to produce highly valuable ergot alkaloids, mainly ergometrine, ergocryptine and ergocornine in concentrations sufficient for economic commercial recovery. In order to obtain a genetically homogeneous culture of stable productivity from this *Claviceps purpurea* strain, which has been registered with the Hungarian National Institute for Public Health under Nr. OKI-620, 125-1

Example 1

6 Litres of a nutrient medium of the composition given below were charged into an aerated laboratory fermenter of 10 litres capacity, equipped with a stirring device:

Saccharose	10%	Citric acid	1%
KH_2PO_4	0.25%	$MgSO_4$	0.1%
$Ca(NO_3)_2$	0.1%		

The pH-value of the medium was adjusted with aqueous ammo-

nia solution to 5.6 The nutrient medium was sterilized for 20 minutes at 110°C, then inoculated with a 3 days old shaken culture of the strain *Claviceps purpurea* OKI 22/1963 and the fermentation was performed at 24°C, under aeration with an air stream of 1 litre/litre/minute and under stirring at 330 R.P.M. for six days. The culture obtained in this way has been analysed by paper chromatography and then layer chromatography and has been found to contain chiefly ergometrine and an ergotoxine rich in ergocristine, further some chanoclavine, elymoclavine, penniclavine, alkaloid ME-87, setoclavine, costaclavine, agroclavine and lysergic acid. These have been determined by thin layer-chromatography, in the following way:

The fermentation broth was made alkaline and the alkaloids and the lysergic acid were extracted with chloroform, this solution was purified by repeated phase-change between chloroform and aqueous 1% tartaric acid; the components were then separated by thin-layer chromatography on alumina and assayed by colour reactions with amphiindicators (cf. *Planta Med.*, 11, 169, 1963). 1 mL. of the fermentation broth contained on the average 100 mcg of ergotoxine and ergometrine, further 200 mcg of clavine alkaloids and lysergic acid.

Another Nutrient Media

The culture (300 L.) was transferred into 3000 litres of a nutrient medium of the following composition in an acid-resistant fermenter of 5 m3 capacity:

Saccharose	10.0%
Succinic acid	1.0%
Ca(NO3)2	0.1%
KH2PO4	0.025%
MgSO4	0.025%
CaCl2	0.012%

The pH-value of the medium was adjusted with aqueous ammonia solution to 5.6 The sterilization was carried out in two steps as described above. The fermentation was continued for 7 days under aeration of 1 litre/litre/minute and stirring with 280 R.P.M. at 24°C, with systematic addition of foam-inhibitors.
Source: Richter 1967

Preparation of Ergotamine and Ergocryptine

Alba-Maria Amici; Anacieto Minghetti; Tollio Scotti; Celestino Spalla Milan, Italy July 17,1968

According to the invention, a strain of *Claviceps purpurea* FI 32/17 is cultivated under aerobic conditions in submerged culture in a

liquid nutrient medium containing an assimilable source of carbon, an assimilable source of nitrogen and mineral salts. The carbon sources are preferably glucose, saccharose, dextrin, sorbite, mannite, glycerin, citric acid, or succinic acid. The nitrogen source may for example be ammonia, asparagine, peptone, casein hydrolyzates, yeast extract, meat extract, soya meal, agar, or an ammonium salt such as the nitrate, sulphate or chloride. The mineral salts may for example be chlorides, nitrates; carbonates, sulphates, phosphates of alkali metals, of magnesium, iron, zinc or manganese. The culture ran be carried out in flasks or in laboratory or industrial fermenters at a pH of from 4.5 to 6.5 at from 20° to 35° C and generally takes from 4 to 20 days. The alkaloids obtained can be separated and purified by extraction with solvents and chromatography.

	C4	19	T25D	Potato Glucosate	S
Glucose, g	40	7.5		20	40
Saccharose, g.			150		
Citric acid, g.			30		
Peptone, g.	4				8
Meat Extract, g.	4				
Yeast Extract, g.		1.0	0.1		
Diammonium phosphate (NH4)HPO4), g.	5				
Bipotassium phosphate (K2HPO4), g	1				
Monopotassium phosphate (KH2PO4), g.			0.5		
Magnesium sulfate (MgSO4.7H2SO4), g.	2.5		0.5		
Potassium chloride (KCl), g.	0.5		0.125		
Sodium chloride (NaCl), g.	0.5				
Ferrous sulfate (FeSO4.7H2O), mg.	10		3.5		
Zinc sulfate (ZnSO4.7H2O), mg.	10		3		
Ammonia, pH at			5.2		
Agar, g.	18	18	18	18	18
Aqueous potato extract, 1 cc.	150		500		
Distilled water, cc., to	1,000	1,000	1,000	1,000	1000
Tap water, cc. to		1,000			1,000
pH before sterilization	6.7	7.4	5.2	7.2	6.4

Sterilization: C4=100°Cx20'; 19=120°Cx20'; T25D=115°Cx20'
Potato Glucosate=120°Cx20'; S=110°Cx20'

1) Preparation of the aqueous potato extract: 200 g. of peeled potatoes are cut into pieces and boiled for 45 minutes in 500 cc. of tap water. The mixture is filtered through gauze and taken up to the initial volume.

Example 1

From a stock of cultures on slants of C+ medium (Table 1) an inoculum is made on a slant of the same medium. The inoculum is incubated at 28° C for 8 days and the culture thus obtained is employed to inoculate two 300 cc flasks, each containing 50 cc of the following SC medium:

Glucose	80 g
Peptone	16 g
Tap water to	1,000 cc

pH 5.9
Sterilization at 110° C for 20 minutes.

The flasks are incubated for 4 days at 24° C on a rotary shaker at 220 r.p.m. with a stroke of 3.5 cm. The cultures thus obtained are used, in the amount of 10%, to inoculate 300 cc flasks containing 50 cc of the following 668 medium:

Saccharose	60 g
Glycerin	60 g
Glucose	80 g
Yeast extract	0.1 g
Citric acid	15 g
Potassium chloride (KCl)	0.125 g
mono Potassium phosphate (KH_2PO_4)	0.5 g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.5 g
Ferrous sulphate ($FeSO_4,7H_2O$)	7 mg
Zinc sulphate ($ZnSO_4.7H_2O$	6 mg
Distilled water	to 1,000 cc

pH 5.2 with ammonia
Sterilization at 100° C for 20 minutes.

After 13 days of incubation under the conditions described for the vegetative culture, the cultures contain 2200 y/cc of a mixture of alkaloids consisting of 48% of ergotamine and 52% of ergocryptine.

Example 2

From a stock of cultures on slants of TS medium (Table 1) an inoculum is made on a slant of T25D medium (Table 1). The inoculum is

incubated at 28° C for 10 days and the culture thus obtained is used to inoculate two 300 cc flasks, each containing 50 cc of the following TG medium:

Glucose	100 g
Citric acid	10 g
Yeast extract	0. 1 g
mono Potassium phosphate (KH2PO4)	0.5 g
Magnesium sulphate (MgSO4.7H2O)	0.3 g.
Ferrous sulphate (FeSO4.7H2O)	7 mg
Zinc sulphate (ZnSO4.7H2O)	6 mg

Distilled water to 1,000 cc pH 5.2 with ammonia

Sterilization at 120° C for 20 minutes.

The flasks are incubated for 6 days at 24° C on a rotary shaker at 220 r.p.m. with a stroke of 3.5 cm. The cultures thus obtained are used, in the amounts of 10%, to inoculate 300 cc flasks containing 40 cc of the following 668B medium:

Saccharose	75 g
Glycerin	75 g
Glucose	100 g
Yeast extract	0.125 g
Citric acid	18.75 g
Potassium chloride (KCl)	0.156 g
mono Potassium phosphate (KH2PO4)	0.625 g
Magnesium sulphate (MgSO4.7H2O)	0.625 g
Ferrous sulphate (FeSO4,7H2O)	9 mg
Zinc sulphate (ZnSO4.7H2O)	7.5 mg

Distilled water to 1,000 cc

pH 5.2 with ammonia

Sterilization at 120° C for 20 minutes.

After 14 days of incubation under the conditions reported for the vegetative phase, the cultures contain 3800 *ug.*/cc of a mixture of alkaloids consisting of 45% of ergocryptine and 55% of ergotamine.

Example 3

An inoculum is made on a slant of C4 medium (Table 1) from a stock of cultures on slants of potato glucosate medium (Table 1). The inoculum is incubated at 28° C for 6 days and the culture thus obtained is used to inoculate two 300 cc flasks, each containing 50 cc of SC medium (Example 1).

Thereafter, the flasks are incubated for 4 days at 24° C on a rotary shaker at 220 r.p.m. with a stroke of 3.5 cm. The cultures thus obtained are used, in the amount of 10%, to inoculate 300 cc flasks containing 45 cc of the following T25 medium:

Saccharose	300 g
Citric acid	15 g
Yeast extract	0.1 g
Potassium chloride (KCl)	0.125 g
monopotassium phosphate (KH2PO4)	0.5 g
Magnesium sulphate (MgSO4.7H2O)	0.5 g
Ferrous sulphate (FeSO4.7H2O)	7 mg
Zinc sulphate (ZnSO4.7H2O)	6 mg

Distilled water to 1,000 cc

pH 5.2 with ammonia Sterilization at 100° C for 20 minutes.

After 13 days of incubation under the conditions described for the vegetative phase, the cultures contain 1800 y/cc of a mixture in equal parts of ergocryptine and ergotamine.

Purification of the Product

The contents of 120 flasks produced as in Example 1 having a titer between 2000 and 2500 y/cc are combined. 5 liters of the culture obtained are filtered. The filtrate and the mycelium are separately extracted. The filtrate is adjusted to pH 9 with sodium carbonate and twice extracted with 3 liters of chloroform each time. The chloroform is concentrated in vacuo at 20°-30° C to about one fifth of the starting volume and extracted with a 2% aqueous solution of tartaric acid. The tartaric solution is made alkaline at pH 9 and extracted with chloroform. The mycelium is stirred with 50% of aqueous acetone containing 2% of tartaric acid, filtered and the filtrate is made alkaline to pH 9 and extracted with chloroform. The chloroform extracts of the mycelium and of the filtrate are combined, evaporated to a small volume and precipitated by addition of hexane. A crude product is obtained which, after drying in vacuo, weighs 11.2 g. This material is dissolved in 13 cc of glacial acetic acid. 110 cc of methanol are then added. 15 cc of 5% sulphuric acid in methanol are added and the reaction mixture is allowed to stand overnight at 3° C. Thus 3.9 g of crystalline ergotamine sulphate, melting at 206° C and corresponding to 3.6 g of base, are obtained.

The mother liquors are concentrated under nitrogen atmosphere, reduced pressure to a small volume. The residue is adjusted to 20 cc with water, made alkaline at pH 9.5 with ammonium hydroxide and extracted with chloroform. The chloroform extract concentrated in vacuo at 30° C to small volume is passed through a column of 400 g of silica gel in chloroform. By eluting with chloroform, a fraction is obtained containing ergocryptine which is evaporated to dryness. The residue is

dissolved in benzene at the ratio of 1:20 and concentrated to a quarter of the starting volume. The reaction mixture is allowed to stand overnight at 5° C. 4.8 g of ergocryptine base are obtained, which after recrystallization from methyl alcohol and drying melts at 212° C; [α]20/D=187° (c = 1% in chloroform). By carrying on the elution of the silica column with chloroform and 4% methanol, another fraction is obtained which on evaporation to dryness yields 0.7 g of product. This is dissolved in 7 cc of 80% of aqueous acetone and 0.7 g of ergotamine solvated with 2 molecules of water and 2 molecules of acetone precipitate, melting at 180°C; [α]20/D = -124° (c = 1% in chloroform).

Example 5

From a stock of cultures on slants of T25D medium (Table 1) an inoculum on 5 slants of the same medium is carried out which are incubated at 28° C for 10 days. The mycelium of the cultures thus obtained is suspended in 60 cc of water. It is homogenized and then used to inoculate 6 liters of TG medium (Example 2) contained in a 10 liter glass fermenter and sterilized at 120° C for 30 minutes. The inoculum is incubated for 5 days at 24° C with an aeration corresponding to an air flow of 4 liters per minute under shaking at 300 r.p.m. of a rotary shaker provided with 6 paddles. The cultures thus obtained serve to inoculate, in the amount of 10%, 6 liters of T25 medium (Example 3) prepared and sterilized in another 10 liter fermenter. This is incubated at 24° C with an aeration corresponding to an air flow of 6 liters per minute under shaking of 350 r.p.m. of a rotary shaker having 6 paddles. After 10 days of incubation, the culture contains 1200 y/cc of a mixture in equal parts of ergotamine and ergocryptine. Source: Amici 1972

Fermentation Process

Géza Wack, Lajós Nagy, Dénes Szekély,
József Szolnoky, Eva Udvardy-Nagy, Erzsébet Zsóka
Budapest, Hungary June 27, 1973

After experiments of theoretical value for the production of saprophytic alkaloids, the fermentation process for the preparation of lysergic amide, constituting the basic skeleton of the classical ergot alkaloids, was the first one which could be realized in industrial scale (British Patent Specifications Nos. 883,229 and 1,041,246). The elaboration of these processes was significantly enhanced by two facts: *Claviceps paspali*, a lysergic acid producing microorganism originating from a grass species, possesses particularly advantageous cultivation

properties in comparison with the *Claviceps* species originating from rye, and on the other hand, the thus-formed lysergic amide is water soluble, and accordingly, when excreted by the microorganism cells, it can accumulate in the liquid part of the fermentation broth.

The first fermentation process producing a valuable alkaloid, the water-soluble ergometrine (see British Patent Specification No. 1,071,846) was elaborated on the basis of the observations obtained in connection with the lysergic amide producing processes. The microorganism strain used in this process was a new representative of the genus *Claviceps paspali* mentioned above. Soon thereafter a commercial-scale fermentation process producing proper alkaloid levels has been elaborated for the production of ergometrine, in which the rye-parasitic *Claviceps purpurea*, a characteristic strain of the open-air rye cultivation, was utilized (British Patent Specification No. 1,170,600).

Despite the large number of literature data all the fermentation processes for the production of ergot alkaloids utilizing *Claviceps purpurea* and producing hardly water-soluble peptide-type alkaloids (such as the members of the ergotoxine group, or ergotamine) show several uncertainties in their reproducibility. The cyclic side-chain of the peptide-type alkaloids consisting of three amino acids renders the biosynthetic process more complicated. Moreover, several difficulties arise from the fact that the hardly water-soluble alkaloids accumulate in the cells. This feature is disadvantageous both as regards the alkaloid formation and with respect to the separation of the alkaloid from the culture broth.

The biosynthesis of peptide-type alkaloids is favorably influenced by ensuring the presence of a proper nitrogen source in the whole fermentation process. According to the earlier procedures appropriate amino acids, such as asparagine (German Patent Specification No. 1,007,949), or complex nitrogen sources of natural origin, such as yeast extract, peptone, vegetable extracts, etc. (see German Patent Specification No. 1,120,128) were added to the culture broth usually in an amount of 1%.

The use of these substances, however, involves the disadvantage that they accelerate the growth of the respective microorganisms, too. Rapidly growing cultures, however, produce no or only little amounts of such metabolism products as the ergot alkaloids. As it is known, ergot alkaloids are produced during the so called secondary metabolism, i.e. when the microorganism growth is retarded.

When separating the peptide-type alkaloids accumulated in the microorganism cells a part of the lipid and pigment content of the cells also enters into the extract. Due to the fact that these components have

solubility and distribution conditions similar to those of the ergot alkaloids, their removal from the products is extraordinarily difficult. No directives can be found in the previous publications for reducing the pigment production during the fermentation, even more, according to some references the retarded growth necessary for the secondary metabolism is attained by adjusting the phosphorous content of the culture broth to a low value (0.25 g. of potassium dihydrophosphate per litre). As it is known, these low amounts of phosphorous are consumed and the growth rate is decreased within some days, due to the lack of phosphorous, however, a strong pigment formation sets in.(See British Patent Specification No. 1,064,764.)

In order to avoid the disadvantages mentioned above, our work aimed at the isolation of a *Claviceps purpurea* strain capable of producing a peptide-type alkaloid by cultivation, well utilizing the inorganic nitrogen sources, producing alkaloid also on media of elevated phosphorous content, and producing the alkaloid relatively quickly (i.e. the alkaloid level of the culture tends to reach the maximum on the 6th to 7th day of cultivation).

Claviceps purpurea strain is cultivated from a sclerotium of high alkaloid content, and the aimed variant is gradually enriched from this culture using three inoculation steps in repetitive cycles. In the individual transinoculation cycles an inoculum is produced on a liquid culture medium using the colonies removed from a solid culture medium, thereafter, using this inoculum, a liquid alkaloid-producing culture is prepared. Finally, the cells produced in this latter stage are transinoculated again onto a solid culture medium.

Ammonium nitrate is added to each of the individual culture media in increasing amounts of 1.0 to 10.0 g./L. corresponding to the decreasing sensibility of the microorganism. To the liquid culture medium of the alkaloid producing cultivation there are also added 0.5 g./L. of potassium dihydrophosphate together with 20.0 g./L. Of sodium chloride (this latter substance has already been used in earlier processes, see for example British Patent Specification No. 1,170,600).

The sharp-edged, conically emerging colonies showing no conidium formation are removed from the solid culture medium. Among the alkaloid-producing liquid cultures those are transinoculated to the solid culture media which produce the highest alkaloid level on the 6th to 7th day of cultivation, and show the most favorable ratios of peptide-type alkaloids. Using elevated amounts of phosphorous, the growth retardation necessary for the alkaloid production is ensured by the sodium chloride additive.

Using the procedure as outlined above a new strain is isolated. This strain is a variant of *Claviceps purpurea* capable of producing ergocryptine and ergocornine, and possessing the required favorable properties.

Culture medium SC 101 has the following composition:

Saccharose	100.0 g.
Citric acid	10.0 g.
Sodium chloride	10.0 g.
Ammonium nitrate	1.0 g.
Potassium dihydrophosphate	0.3 g.
Magnesium sulfate	ad pH 5.2
Ammonium hydroxide	
Fibrous agar	20.0 g.
Water	ad 1000 mL.

Effect of organic nitrogen sources on the alkaloid production of the strain:

Nitrogen Source	Concentration g./L.	Alkaloid content on the 7th day, *ug*/mL.
Asparagin	3.0	945
Peptone	3.0	402
Casein	3,0	730
Corn steep liquor	3.0	595
Ammonium nitrate (control)	3.0	1042

Effect of ammonium nitrate on the alkaloid production of the strain:

Ammonium nitrate g./L.	Alkaloid content on the 7th day, *ug*./mL.	Relative amount of g./L. peptide-type alkaloids on the 7th day, %
0	1072	52
2.0	1482	66
3.0	1530	71
6.0	1121	69
10.0	1036	61
15.0	981	64

The S2C culture medium has the following composition.

Saccharose	200.0 g.
Citric acid	15.0 g.
Potassium dihydrophosphate	0.5 g.
Magnesium sulfate	0.3 g.
Ammonium hydroxide	ad pH 5.2
Water	ad 1000.0 mL.

The composition of the SC 100 culture medium is the following:

Saccharose	100.0 g.
Citric acid	10.0 g.
Magnesium sulfate	0.3 g.
Potassium dihydrophosphate	0.5 g.
Sodium chloride	10.0 g.
Ammonium hydroxide	ad pH 5.2
Water	ad 1000.0 mL.

Source: Wack 1975

Fermentation of Ergoline Derivatives

Eva Borowski, Klaus Braun; Klaus Breuel; Christoph Dauth; Dieter Erge; Werner Grawert; Detlev Gröger; Liselotte Höhne; Edda Knothe; Monika Müller; Gisela Nordmann; Rudolf Schirutschke; Klaus-Dieter Volzke, of Radebeul, Dresden and Halle, Germany

November 8, 1976

The nutrient medium in this case was a solid medium (pH 6.8 to 6.9) which contained 3% saccharose, 0.3% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4.7H_2O$, 0.05% KCl, 0.001% $FeSO_4.H_2O$, 1% corn steep liquor and 3% agar. The incubation was effected in slants in 500 mL. industrial glass narrow neck bottles and produced after 14 days incubation at 24° C, 3 x 10 *(to the)* 9 *(th)* spores per culture vessel which were suited as substrate for submerged cultures for the alkaloid synthesis during 10 months as far as stored at 4° C.

Cultures for the Alkaloid Production

Preferably the incubation of the cultures is effected, generally, at a pH from 6.5 to 4.0, a time from 3 to 18 days, a temperature from 15° to 28° C and aerobic conditions. Further details will appear from the following examples. The first three examples illustrate obtaining an extract directly from the culture medium.

Example 4

A part of the spores of an inoculum grown as described in Example 1 and stored for 9 months at 4.° C was suspended and equal amounts thereof were placed into round bottom flasks of 500 mL. contents. Each flask received 120 mL. of a culture medium containing 10% saccharose, 1.5% ammonium citrate, 0.1% Ca(NO3)2, 0.025% KH2PO4, 0.01% KCl, 0.03% MgSO4, 0.001% FeSO4, 0.0004% ZnSO4; distilled water; pH 5.5; sterilization 30 min at 0.5 at. above atmospheric.

The flasks were subjected to shaking at 24° C on a rotary shaking machine at 175 rpm under aerobic conditions. After 7 days the contents of the flasks were distributed in a ratio of 1:10 into Erlenmeyer flasks of 500 ml contents of which each received 120 mL. of a culture medium containing 20% saccharose, 1% ammonium citrate, 0.1% Ca(NO3)2, 0.025% KH2PO4, 0.01% KCl, 0.03% MgSO4, 0.001% FeSO4, 0.0004% ZnSO4; distilled water; pH 5.5; sterilization 30 min at 0.5 at. above atmospheric.

The flasks were then subjected to shaking under the same condition as before.

By means of the van Urk reaction 2470 mg alkaloids per liter were found to be in the culture medium after 14 days, calculated for ergotoxine bimaleinate. The alkaloids were then extracted with methylene chloride and were separated according to the method of Hofmann & Rochelmeyer (*Arch. Pharmazie* 297 (1965) p. 186/187) by thin layer chromatography through formamide impregnated kieselguhr. The UV spectrum was then determined photometrically. The medium was found to contain 1280 mg of ergotoxine 340 mg of ergometrine per liter. The remainder were two hitherto not defined native peptide alkaloids and simple ergoline derivatives such as agroclavin, chanoclavin, etc.

Parallel to this test there were determined the contents of alkaloids of the mycelium which had been separated by suction filtration and of the filtrate. It was found that the ergometrine was completely contained in the filtrate and that in addition 90% of the total ergotoxines was in the filtrate.

Example 5

Ten glass fermentors of 2 L. contents were inoculated with a submerged spore culture made as in Example 2. The fermentors contained each 1.3 L. nutrient solution A containing 10% saccharose, 1.5% ammonium citrate, 0.05% KH2PO4, 0.03% MgSO4, 0.001% FeSO4 and 0.0004% ZnSO4 in tap water (pH 5.5; sterilization 30 min. at 0.5 at above atmospheric).

The fermentation was effected at 24° C while stirring at 400 rpm with aeration of 0.3 L. air/min. The contents of one of the fermentors was transferred after seven days into an 18 liter special steel fermentor containing 10 liters of a nutrient solution B. The latter solution contained 10% saccharose, 1.4% citric acid, 1.0% ammonium hydroxide (25%), 0.05% Ca(NO3)2, 0.025% KH2PO4, 0.01% KCl, 0.03% MgSO4, 0.001% FeSO4 and 0.0004% ZnSO4 in tap water; pH 5.5.

After an incubation at 24° C, 360 rpm and 2.5 L. air/min. half of the contents of the fermentor was employed on the fifth day as inoculum for a 63 L. special steel fermentor containing 40 L. of a nutrient solution C. The solution C contained 20% saccharose, 1.75% citric acid, 0.075% KH2PO4, 0.02% KCl, 0.03% MgSO4, 0.003% FeSO4, 0.0012% ZnSO4 in tap water; addition of ammonium hydroxide at pH 5.3; sterilization 60 min. at 110° C.

The culture was subjected to stirring for 7 days at 24° C and 300 rpm and was aerated with 27 L. of air/min. If necessary an antifoaming agent was added. At the end of the incubation the culture liquid contained 2640 mg of total alkaloids per liter.

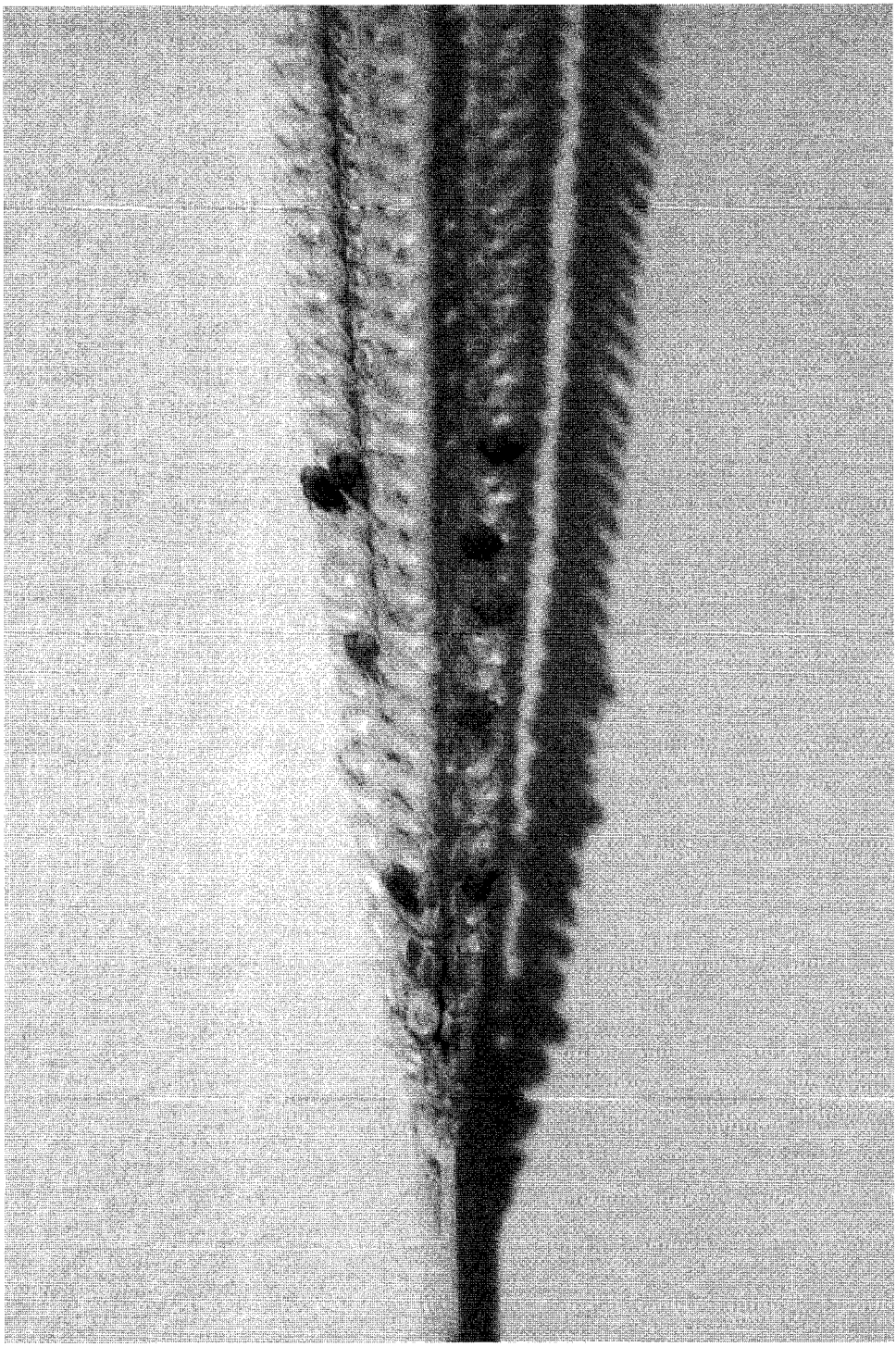
An analysis carried out as in Example 4 showed that the contents comprised 1300 mg ergotoxine and 550 mg ergometrine.

Source: Borowski 1978

The following culture media uses mannitol as a carbon source. Mannitol maybe replaced by sucrose or glucose but should be sterilized by running through a Seitz filter.

Media by A. Tonolo (1966): 20% mannitol
3% peptone
tap water

Adjust pH to 6.2 Incubation at 24 degrees C. for 8 to 10 days produces 800 to 1,400 μg ergotamine per mL from *Claviceps purpurea*.



Chapter 16
Life History of *Claviceps paspali*
By H.B. Brown

During the last decade *Paspalum dilatatum* Poir. has attained considerable prominence as a forge grass in various parts of the South. One serious objection to its use, however, is that forge poisoning frequently results among cattle feeding on it. Brown and Ranck showed that the poisonous property is due to *Claviceps paspali* Stevens and Hall, a fungus that infects the grass very generally. This species was described by Stevens and Hall in 1910. Norton observed this fungus on *P. dilatatum* in Maryland in 1902. He suspected that it was poisonous, but carried on no feeding experiments to determine this.

Since September, 1914, the writer has been making a study of the life history of *Claviceps paspali* and its growth and distribution in the region about the Mississippi Agricultural College. In this region the fungus infects *Paspalum dilatatum* very generally, a few weeks after the grass heads out at least 90 per cent of the old heads showing infection.

Life History of The Fungus

Sclerotia produced during the summer and autumn drop to the ground when the old grass head sheds it spikelets, and lie on the ground until spring. They may be found at any time during the winter and spring by searching in the litter on the ground where infected *Paspalum dilatatum* grew the season before. Sclerotia gathered during the winter and placed in moist chambers kept at room temperature will germinate in 20 to 30 days, but it is the writer's experience that sclerotia forced in this way do not produce as many nor as large and vigorous stromata as those that germinate in the normal way. After a few days on rainy weather about the middle of May, sclerotia germinating on the ground may be expected. They were first found on May 10 in 1915 and on May 21 in 1906. In each case this was just after the host plant had begun to flower.

Sclerotia of *Claviceps paspali* when mature are globular in shape, 2 to 4 mm. in diameter, irregularly roughened on the surface, and yellowish gray in color; the interior is homogeneous in structure and contains a considerable quantity of oil. Germinating sclerotia produce

from one to several stromata, usually two or three, with slender whitish stalks 3 to 15 mm. in length, and heads about 1 mm. in diameter. The heads are roughened over the surface owing to projecting perithecial necks and are at first whitish in color, later becoming rather bright yellow, and finally brownish.

A vertical section of a stromatic head shows numerous flask-shaped perithecia embedded in the outer part of the head. Thus forming small pimple-like projections. Each perithecium contains numerous slender, cylindrical asci, 150 to 170μ in length; at the outer end of each ascus there is a thimble-like knob fitting over the end. The wall of the ascus is so thin that it can not be distinguished clearly. The ascospores are filiform and hyalin, being a little less than 1μ in diameter and 70 to 100μ in length. There are probably eight spores in an ascus, although not more than seven were counted with certainty. It was not possible to count the spores when inside an ascus, as they are hyalin and packed together closely, and it was a rather difficult matter to count them as the ascus disintegrated.

Mature stromatic heads from sclerotia just gathered from the field when allowed to dry slightly and then moistened exuded asci very freely. The asci go to pieces quickly after escaping from the perithecia and liberate the spores. A change of moisture conditions in the field will cause spores to be deposited on the surface of the stromatic head, where they are in position to be picked up by insects and chance to rub against the head. The stromata are somewhat tough and leathery and last for several days. If the ground becomes dry during their regular period they dry out, but revive with the coming of moisture and again shed spores. No stromata were found in the field after July 2.

Flowers of *Paspalum dilatatum* inoculated with ascospores by rubbing stromatic heads against stigmas and spikelets of the grass heads showed abundant evidence of infection in seven days. Flowers on control plants showed no infection. (Both inoculated plants and controls were kept under bell jars). In the field, infected heads are not found for several days after the sclerotia germinate. They are first noticed on June 8 in 1915 and on June 12 in 1916, being, respectively, 29 and 22 days after germinating sclerotia were first found. In 1915, infected or diseased heads were not plentiful in the fields until about July 12. Preceding this date there were several days of rainy weather. In 1916, similar observations were made. Diseased heads became very common during July, following several weeks of rain. On August 1, 1916, they were more plentiful than since the autumn of 1914.

In the fields the first infection of the season is doubtless carried by insects. Running over the ground, they are likely to rub against the stromatic heads, which are covered with ascospores, and, climbing up the grass clumps to take flight, may carry ascospores to the grass flowers and produce infection. That infection does not take place often is evidenced by the fact that the disease is slow in getting a start after the sclerotia germinate.

The infecting fungus attacks the pistil of the grass flower, and in a few days the ovary is almost entirely destroyed, a mass of fungus tissue filling the space it occupied. There is a mass of fungus tissue between the glumes of a grass spikelet a week after infection. The central part of the grass flower has been replaced by homogeneous tissue, while around the edge are numerous tufts of hyphae standing at right angels to the central mass. Each tuft contains a number of hyphae. The digital ends of these hyphae, or certain of them, enlarge and for conidia or sphacelia spores. The spores are hyalin but show granules when stained, oblong, about 5μ wide x 15μ long. They are produced in great abundance and are carried from the hyphae on which they were produced by a droplet of honeydew, a sticky, sweetish exudation of the fungus tissue. Insects of many kinds feed on this honeydew and carry infection by means of spores clinging to their bodies. Hand inoculations, which were made by smearing honeydew containing sphacelia spores on flower stigmas, produced infections that were exuding honeydew and sphacelia spores freely within the space of a week. This result was obtained in the case of plants kept under bell jars, and also with plants inoculated in the field. Sphacelia spores frequently germinate in the droplet of honeydew and give it a whitish appearance.

The sphacelia stage in which honeydew is exuded lasts but a few days. If the weather is dry, the whole grass head is likely to become dry and dead, and no further development occurs. Or, again, honeydew may become infected with a species of *Fusarium* or *Cladosporium* and growth be stopped. If weather conditions are favorable, the solid mass of fungus tissue, constituting the bulk of the sphacelia tissue, continues to enlarge and soon forces the glumes of the spikelets apart. These masses are young sclerotia. In some cases within a week after the sphacelia stage was at its height the young sclerotia were projecting from between the glumes of the spikelet and were 1-2 mm. in diameter. Following this, some of the sclerotia continue to enlarge, attaining a maximum dia. of about 4 mm. and characters as outlined above. During Sept. and Oct. the largest sclerotia are to be found; and are also most plentiful then. Source: Brown 1916

Host Plants to *Claviceps paspali*

Latin Name	Common Name	
P. ciliatifolium		
P. distichum	Knotgrass	
P. dilatatum	Dallis-Grass	Perennial
P. floridanum	Florida Paspalum	
P. intermedium		
P. langei	Rustyseed Paspalum	Perennial
P. laeve		Perennial
P. longipilum		
P. notatum	Bahia Grass	Perennial
P. pubescens		
P. pubiflorum	Hairy-Seed Paspalum	Perennial
P. urvillei	Vasey-Grass	Perennial

References: Burton 1948; Gieger 1939; Gröger 1961; LeFebvre 1939; Luntrell 1977; Stevens 1910. See also: *The Story of Ergot*

Host Plants Resistant to Artificial Innoculation of *Claviceps paspali*

Latin Name	Common Name	Perennial
P. lividum	Long-Tom	Perennial
P. malacophyllum	Ribbed	Perennial
P. notatum	Bahia Grass	Perennial
P. supinum		

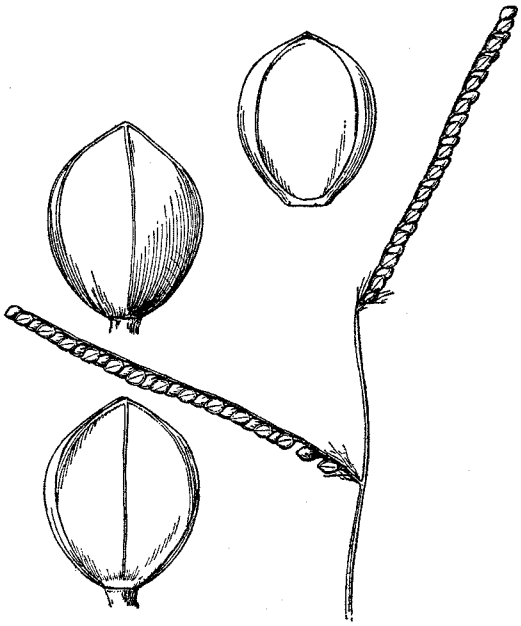
Ergot Size in Reference to Size of Spikelet

Paspalum Species	Spikelet Length	Size of Ergot Length x Diameter
dilatatum	3-4 mm.	3-4.5 mm. in diameter
laeve	2.4-3.4 mm.	3-4.5 mm. in diameter
urvillei	2.2-2.7 mm.	1-2 mm. x 1-1.5 mm.

Paspalum longipilum

Description

Similar to *P. laeve*, the culms usually ascending or spreading, the leafy shoots at base mostly fewer, a raceme-bearing branch often borne (usually hidden) in next to the lowest sheath; sheaths pilose with long hairs, often conspicuously so, but sometimes very sparsely; blades usually flat, pilose on both surfaces or glabrous or nearly so beneath, commonly less erect than in *P. laeve*; racemes 2 to 6, commonly 2 or 3, on the average more lax and spreading than in *P. laeve*; spikelets 2.5 to 2.8, rarely to 3 mm. long, 2 to 2.4 mm. wide, the fruit usually covered at maturity, the sterile lemma often tinged with russet.



Paspalum longipilum

This species is fairly distinct from *P. laeve*, but a few specimens grade into the form represented by the type of *P. australe*. A few others, with spikelets 2.8 to 3 mm. long, are scarcely distinguishable from *P. circulare*, but the spikelets are less rounded and the glume and sterile lemma rather thicker.

This species is common in the savannas and flatwoods of Florida where it is an important constituent of native pastures.

Distribution

Damp mostly sandy soil, savannas, open woods, and wet pine barrens, New York to Tennessee and Florida and west to Texas.

Paspalum dilatatum

Paspalum dilatatum (Dallis Grass)

A rather stout perennial, in clumps of few to several culms and leafy sterile shoots from a knotted base of very short rhizomes; culms ascending to suberect from a curved or decumbent base, or some of the culms of a clump widely spreading, 40 to 175 cm. tall, simple, or sparingly branching from the lower nodes, compressed, glabrous; nodes glabrous or the lower sparsely pubescent; sheaths commonly overlapping, rather loose, compressed, the lower harshly pilose toward the base, sometimes conspicuously so, otherwise glabrous or ciliate at the summit; ligule about 3 mm. long; blades flat, ascending to spreading, 6 to 45 cm., commonly 10 to 25 cm., long, 3 to 12 mm. wide (the uppermost reduced), at base about as wide as the summit of the sheath, usually sparsely ciliate at base, otherwise glabrous, the margin scabrous; panicle erect or nodding, of 2 to 11, commonly 3 to 5, ascending to drooping rather broad racemes, the lower 4 to 11 cm., commonly 6 to 8 cm., long, the slender flattened common axis 2 to 20 cm. long; rachis narrowly winged, about 1.2 mm. wide, bearing numerous long white hairs at the base, the margin seabrous; spikelets on slender flattened pedicels, closely imbricate, 2.8 to 3.8 mm. long, about 2 mm. wide (excluding the hairs), ovate, pointed, depressed plano-convex or almost concavo-convex; glume slightly exceeding the sterile lemma, both pointed beyond the fruit, 5 to 9 nerved (lateral nerves obscure), sparsely covered with silky hairs on the surface, the glume in addition bearing on the marginal internerves a fringe of long white silky hairs, from rather scant to copious and woolly; fruit 2.4 to 2.6 mm. long broadly elliptic, pale, minutely papillose-striate.

This species, commonly known as *paspalum* or *paspalum* grass and recently as Dallis grass, is introduced in the Southern States, where it is considered a valuable pasture grass.

"Panicles from which the spikelets have been stripped are tied into whisk brooms and used in South Carolina for brushing cotton lint from clothing, being much better for that purpose than ordinary whisk brooms."—J. B. Norton.

In low ground, from rather dry prairie to marshy meadows, New Jersey to Tennessee and Florida, and west to Arkansas and Texas; also adventive in Oregon, Colorado, Arizona, and California; sparingly introduced in the West Indies and in Central America; native of South America from Brazil to Argentina; also in Chile, probably introduced. Also escaped in Hawaii and Guam, the Philippines, India, Africa, and Australia, and naturalized in southern France. Ref. Luttrell 1977.

Paspalum uervilli
Vasey Grass

A stout erect perennial in clumps of few to many culms, purplish below; culms 75 cm. to 2.5 meters tall, simple or branching from the lower, sometimes from the middle nodes, subcompressed, glabrous; nodes glabrous; sheaths keeled toward the summit, the lower loose, coarsely hirsute or glabrescent toward the summit, the upper glabrous or sometimes ciliate on the margin or with a few hairs at the summit, rarely sparsely hirsute, often somewhat auricled; ligule 3 to 5 mm. long; blades flat, ascending, relatively firm, 12 to 48 cm., commonly 20 to 30 cm., long, 3 to 15 mm. wide, rarely to 65 cm. long and 2 cm. wide (the uppermost reduced), slightly rounded at base or narrowed to the width of the sheath, densely long-pilose at the very base on the inside, otherwise glabrous, the margin seabrous; panicle erect, 10 to 42 cm. long, of 6 to 25, commonly 12 to 18, ascending to slightly drooping racemes, the lower 7 to 14 cm. long, the upper gradually shorter, narrowly ascending, the slender common axis angled, glabrous; rachis narrowly winged, about 0.8 mm. wide, with a few long hairs at the base, the margin seabrous; spikelets on slender flattened pedicels, imbricate, 2 to 3 mm., commonly 2.2 to 2.7 mm. long, 1.2 to 1.5 mm. wide (excluding the hairs), ovate, abruptly pointed, depressed plano-convex; glume and sterile lemma. equal, pointed beyond the fruit, thin in texture, 3 to 5-nerved, both copiously edged with long silky white hairs, the glume sparsely clothed with appressed silky hairs throughout, the lemma glabrous or nearly so in the middle; fruit 1.8 to 2 mm. long, elliptie, pale, nearly smooth.

This species, known as Vasey grass, is readily grazed while young, and in some sections of the South is cut for hay.

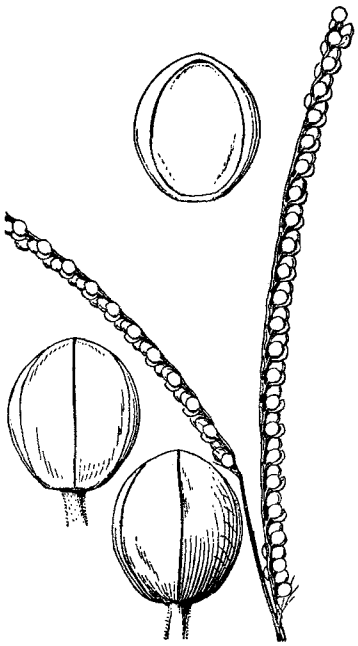
Distribution

Along ditches and roadsides, and in waste ground, mostly in rather moist soil; North Carolina to Florida and west to Texas; also in southern California, Guatemala, and Cuba; native from Brazil to Argentina; introduced in Bolivia, Chile, and the Hawaiian Islands.

Paspalum urvillei

Paspalum pubenscens

A slender perennial in dense tufts, rather yellowish-green to olivaceous, culms slender, ascending to spreading, 45 to 90 cm. tall, strongly compressed, glabrous; nodes glabrous; sheaths keeled, pilose toward the summit or at least on the keel and along the margin, sometimes throughout, or the lower, rarely all, glabrous except along the margin; ligule a minute membrane with a dense row of white hairs 3 to 4 mm. long back of it; blades flat, 8 to 20 cm., rarely to 30 cm., long 2 to 10 mm., rarely to 15 mm., wide, mostly linear, slightly narrowed to a base scarcely wider than the sheath or the upper rounded at base (in the wider-leaved specimens all more or less rounded), from sparsely to conspicuously pilose on both surfaces, sometimes minutely puberulent beneath the long hairs on the upper surface; peduncles slender, flat, finally elongate, often pilose toward the summit, the axillary 1 or 2 from upper and middle nodes; racemes 1 to 3, more commonly solitary, mostly arching, 4 to 15 cm., rarely to 17 cm., long, the rachis long-pilose at base, the common axis occasionally pilose; spikelets in pairs, crowded on short pedicels, the lower often winged at base, 1.9 to 2.1 mm. long. 1.7 to 1.9 mm. wide, suborbicular to broadly obovate, the glume and sterile lemma subequal, 3-nerved, or the glume rarely 4 or 5 nerved, or the mid nerve of the lemma suppressed, glabrous, the glume rarely sparsely pubescent; fruit about the size and shape of the spikelet.



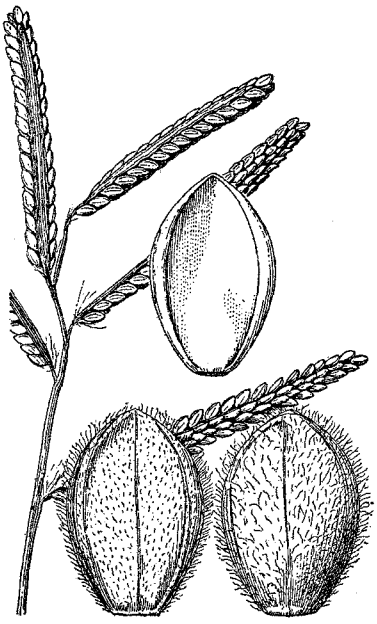
P. pubenscens

This species varies in the amount of pubescence. The spikelets are more uniform in shape and size than in most species of this group. Plants with very narrow blades resemble *P. setaceum* but the spikelets are larger. *Paspalum pubescens* is common in pastures and old fields and is reported to be of considerable forage value in Indiana, Tennessee, Alabama, and Florida.

Dry or moist open ground or open woods, more common in sandy regions, Vermont and Massachusetts to Florida, and west to Michigan, Oklahoma, and Texas.

Paspalum pubiflorum

A glaucous to olivaceous perennial, decumbent at base, often rooting at the nodes and bearing erect flowering branches, the internodes of the decumbent part short, the nodes swollen; culms rather robust, 40 cm. to 1 meter long, ascending or erect from the decumbent base, compressed, often sulcate in drying, glabrous, the ascending part simple or bearing a few leafy shoots, rarely a flowering branch; nodes dark, at least the lower pilose; sheaths mostly shorter than the internodes, rather loose, at least the lower sparsely papillose-pilose, the margins membranaceous, brown; ligule 1 to 3 mm. long; blades flat, rather lax, 6 to 25 cm. long, commonly 10 to 15 cm. long (the uppermost reduced), 6 to 14 mm. wide, acuminate, usually with a few stiff hairs at the rounded base, otherwise glabrous, the margin scabrous, rarely ciliate toward the base; racemes 2 to 8, usually 3 to 5, the lowest sometimes distant, 2 to 10 cm. long, rather thick, from erect to horizontally spreading, a tuft of long white hairs in the axils; rachis 1.2 to 2 mm. wide, green, glabrous, scabrous on the margins, the lowest often naked at base; spikelets in pairs, rarely solitary, usually crowded, 3 to 3.2 mm. long, about 2 mm. wide, obovate, rather turgid, obtuse, yellowish green or sometimes purplish; second glume and sterile lemma subequal, 3 to 5 nerved, the glume softly pubescent with spreading hairs, the sterile lemma minutely appressed-pubescent; fruit pale, about 3 mm. long and 1.9 mm. wide, minutely striate-roughened.



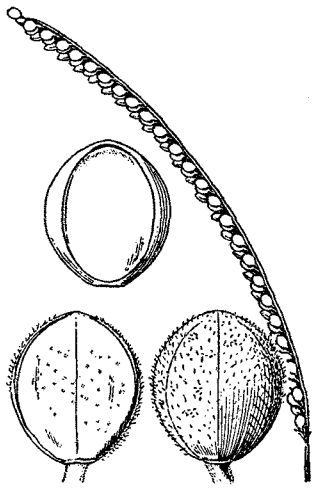
Paspalum pubiflorum

Occasional plants have slightly smaller spikelets. Rarely, as in Hitchcock 5608 and Holway 3416, the blades are sparsely pilose on the lower surface. A specimen collected by Brother Arsène in Morelia, Mexico, in 1909, and Léon 1968 have spikelets only obscurely pubescent, as in the type of *P. planifolium* Fourn.

Moist open ground, banks, low woods, along streams and irrigation ditches, especially in alkaline clay soil, Louisiana and Texas, throughout Mexico, up to 2,000 meters altitude; also in western Cuba.

Paspalum ciliatifolium

A slender perennial in rather open tufts, purplish brown toward the base; culms erect to spreading, 35 to 90 cm. tall, glabrous; nodes glabrous; sheaths keeled, rather broad, glabrous or pubescent along the margin, or the lower from puberulent to appressed-pubescent; ligule minute, membranaceous, with a dense row of hairs 2 to 3 mm. long back of it, commonly produced into an erect auricle 0.5 to 1 mm. long on one or both margins; blades flat, from lax to rather firm, ascending to spreading, 10 to 35 cm. long, 7 to 20 mm. wide (rarely as much as 48 cm. long and 25 mm. wide), rounded to subcordate at base or narrowed to the width of the sheath, typically strongly ciliate along a minutely undulate cartilaginous margin, but sometimes ciliate toward the base only, very rarely not at all ciliate, otherwise glabrous or pilose along the mid nerve below or minutely pubescent toward the apex, rarely throughout; racemes 1 to 3, rarely 4, slender, arching, 4 to 15 cm., commonly 7 to 10 cm., long, the slender rachis with a tuft of hairs at base; spikelets in pairs, crowded, 1.9 to 2.1 mm. long, 1.3 to 1.7 mm. wide, from elliptic-obovate to suborbicular, strongly plano-convex, the glume slightly shorter than the sterile lemma exposing the summit of the fruit at maturity, both 3-nerved, or the midnerve of the lemma suppressed, glabrous, or, especially the glume, minutely pubescent with obscurely capitate hairs, commonly minutely speckled; fruit about the size and shape of the spikelet, pale, smooth and shining.



P. ciliatifolium

Paspalum ciliatifolium is a polymorphic species. Study of a great amount of material has made it impossible to recognize as distinct the groups segregated by Nash. Pubescence on foliage and spikelets varies in a single plant. Rather stout, somewhat paler seacoast plants, with firmer blades scarcely ciliate, are the form described as *P. epile*. Plants with softly pubescent lower sheaths, and blades but slightly ciliate, are the form described as *P. eggertii*. The shape of the spikelet varies in a single raceme from elliptic-obovate to suborbicular. The spikelets tend to become rounder at maturity, but both mature and immature are found of both shapes. The spikelet shown in figure was drawn in the Paris

Herbarium from the Michaux specimen. In the majority of specimens the spikelets are less rounded than this.

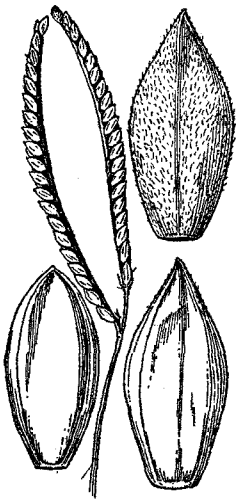
Besides the above variations there are a number of specimens, otherwise typical or fairly typical *P. ciliatifolium*, in which the spikelets are only 1.7 to 1.9 mm. long. These and the specimens of *P. longepeduncalatum* with unusually large spikelets approach, but the blades in those referred to *P. ciliatifolium* are larger and not aggregate toward the base.

The most characteristic form is greener than the other species of this group and less leafy at base, the lower blades reduced.

Open ground or open woods, mostly sandy, New Jersey to Florida, Tennessee, Arkansas, and Texas; also in Honduras and the West Indies.

Paspalum distichum L.
Knot-grass, Joint Grass
Fort Townsend Grass

A widely creeping perennial with slender rhizomes, extensively stoloniferous, often forming loose mats, the stolons usually slender, subcompressed, sometimes as much as 1 meter long, the sheaths on the average less loose than in *P. vaginatum*, the blades usually well developed; branches erect or ascending, most of them finally flowering, 6 to 50 cm. tall, often sparingly branching, the culms subcompressed, the dark nodes often with a few ascending hairs; sheaths loose, keeled, commonly pilose on the margin toward the summit; ligule membranaceous, about 0.5 mm. long; blades flat, ascending, 3 to 12 cm. long, 2 to 6 mm. wide at the rounded ciliate base, tapering to an acuminate sometimes involute apex, dull green, relatively soft in texture, occasionally minutely pubescent on the upper surface; peduncles commonly short, often included; racemes usually 2, rarely as many as 4, from erect to reflexed, commonly incurved, 1.5 to 7 cm. long, rarely longer; rachis slightly pedunculate in one, sometimes in both racemes, usually a few long white hairs in the axils, 1 to 1.5 mm., rarely 2 mm. wide, triangular, minutely scabrous on the margin; spikelets solitary (rarely in pairs in the middle of the raceme), imbricate, 2.5



P. distichum

to 3.5 mm., rarely 4 mm. long, 1.3 to 1.5 mm. wide (the variation in size sometimes found in the same plant) elliptic, abruptly acute, pale green; first glume frequently developed; second glume and sterile lemma equal, 3 to 5 nerved, the midnerve relatively prominent, the glume minutely appressed-pubescent, sometimes obscurely so; fruit 2.5 to 2.8 mm. long, about 1.2 mm. wide, elliptic.

This is the most variable of the three allied species, sometimes closely resembling *P. vaginatum* but distinguishable by the slightly more turgid spikelets, the glume and sterile lemma not papery, the midnerve evident, the glume at least obscurely pubescent.

Paspalum distichum, known as knotgrass, jointgrass, and Fort Thompson grass, is a valuable soil binder along streams subject to erosion in the Tropics and sub-Tropics. It furnishes excellent grazing in flat regions near the coast. It is sometimes a bad weed in the cotton fields of the Black Belt of Alabama. In the West it is spreading along irrigation ditches and sometimes invades rice fields in California. In Australia it is known as water-couch and siltgrass. It is there regarded as a valuable pasture grass on allavial flats.

Distribution

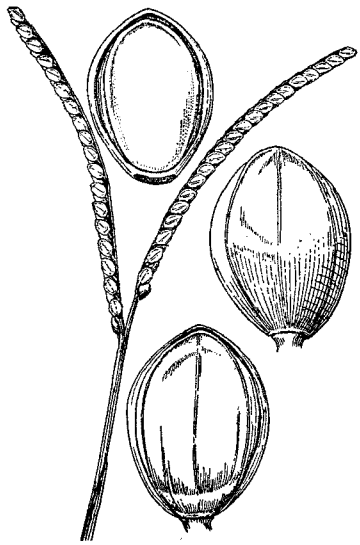
Ditches and wet, rarely brackish, places, New Jersey to Florida, Tennessee and Arkansas, and west to California and north along the coast to Washington, south through Mexico and the West Indies to Argentina and Chile; also near warm-temperate coasts of the Eastern Hemisphere.

Paspalum notatum
Bahia Grass

An ascending perennial with short stout woody horizontal rhizomes, forming tough but not extensive sods, the rhizome clothed with the firm persistent bases of old sheaths; culms simple, 15 to 50 cm. tall, rarely taller, glabrous, flattened, the nodes dark; leaves crowded at the base, the overlapping sheaths usually short and reaching to a common height, the blades stiffly spreading, thus forming a rather conspiouous distichous tuft near the base of the culm; sheaths compressed, keeled, rather glossy, glabrous or ciliate toward the summit or rarely pubescent throughout; ligule a minute membrane with a dense row of white hairs about 1 mm. long back of it; blades flat, or folded at base, 2 to 30 cm. long (the uppermost reduced to a mere point), 3 to 10 mm. wide, linear, usually sparsely ciliate toward the base, sometimes almost to the summit, otherwise glabrous; racemes 2, rarely 3,

subconjugate (the common axis about 5 mm. long), recurved-ascending, 2.5 to 12 cm. long (usually 4 to 7 cm.), relatively rather thick; rachis about 1 mm. wide, glabrous, usually flexuous toward the summit; spikelets solitary, 2.5 to 3.8 mm. long, 2 to 2.8 mm. wide, ovate to obovate; glume and sterile lemma equal, firm in texture, smooth and shining, 5-nerved (the intermediate nerves often obscure); fruit 2.5 to 3.5 mm. long, 1.8 to 2.5 mm. wide, oval.

In what is presumably the typical form from the West Indies the spikelets are not more than 3 mm. long (except in three collections from Havana), while in the continental specimens the spikelets are 3 to 3.8 mm., rarely 4 mm., long. The continental specimens are on the average larger than those of the West Indies and the racemes longer. Except in size the continental form, to which belongs *P. saltense* Arech., does not differ in any way from the Antillean form. The West Indian form is about half way between *P. saltense* and *P. minus*, which Fournier differentiated from the large form of Mexico. From the largest spikelets of this form to the smallest of *P. minus* there is an almost unbroken series.



Paspalum notatum

The following specimens from Havana belong to the continental form: Leon 117b, 928-1/2 and Tracy 9118. Hitchcock 6962 and 6984 from Uruápan, Mexico, are the Antillean form. In North American specimens the foliage is nearly glabrous. In a few specimens from Venezuela and Brazil the foliage is conspicuously pilose: Bailey 247; Chase 8360, 9092; Pittier 7212, 7242. Hitchcock's no. 5765 is exceptional in having four racemes, a short pair above the ordinary pair.

This species has been introduced as a pasture grass in the Gulf States under the name of Bahia grass. It is proving of value, thriving on both clay and sandy soil and being readily grazed. It forms the main constituent of native pasture in Cuba and Porto Rico and in parts of Costa Rica, Brazil, Uruguay, and Argentina.

Open ground, savannas, and pastures from sea level to 2,000 meters, from central eastern Mexico to Argentina and throughout the West Indies; introduced in the southern United States, and rare as a ballast plant northward. Ref. Burton 1948

Paspalum laeve

Paspalum laeve
Field Paspalum

A tufted perennial commonly with numerous erect or ascending leafy shoots at the base; culms erect or ascending, 0.4 to 1 meter tall, rarely taller, simple or rarely with concealed or short-exserted raceme-bearing branches in the lower sheaths, compressed, glabrous; sheaths compressed-keeled, several usually crowded: at the base, glabrous or pilose on the margins or sometimes on the back toward the summit; ligule brown, 2 to 3 mm. long; blades usually folded at base, flat or folded above, rather firm, commonly erect or nearly so, sometimes glaucous, 5 to 30 cm. long, rarely longer, 3 to 10 mm., rarely 12 mm., wide, the uppermost often reduced, glabrous to ciliate or sparsely pilose on the upper surface, or sometimes toward the base beneath; racemes 2 to 5, commonly 3 or 4, rarely 6 to 8, spreading or ascending, 3 to 10 cm. long, rarely longer, the common axis slender, glabrous; rachis about 1 mm. wide, with a tuft of long hairs at the base, spikelets not crowded, 2.5 to 3 mm. long, 2 to 2.5 mm. wide, broadly oval to suborbicular; glume and sterile lemma equal, toward maturity the tip of the fruit usually exposed, 5-nerved, the middle of the lemma commonly russet-brownish; fruit nearly the size and form of the spikelet.

Where it grows plentifully this species affords a nutritious hay. Together with the allied species it is called field paspalum.

Fields, meadows, open woods, and waste ground, especially common in red clay soil, New Jersey and Pennsylvania to Florida and eastern Texas.

Paspalum langei

A rather slender olivaceous perennial in tufts of few to several culms; culms ascending, 30 to 100 cm. tall, mostly simple, but occasionally with a single leafy branch, compressed, glabrous; nodes glabrous; sheaths keeled, pubescent along the margin and often on the collar, otherwise glabrous to sparsely papillose pubescent; ligule membranaceous, about 1 mm. long; blades flat, ascending, usually rather thin, 10 to 40 cm. long, 6 to 15 mm. wide, tapering to a narrow base or, especially the upper, rounded at base, scabrous and often sparsely ascending ciliate on the margin, and with long hairs on the upper surface at the very base, otherwise from glabrous to appressed papillose-pubescent on the upper surface and sparsely pubescent below (more commonly nearly glabrous);

peduncles slender, 1 to 3 rather short-exserted from the upper sheath, the axillary ones usually appearing late, the racemes often partly included, axillary racemes also often borne in the middle sheaths, mostly partly or wholly included; racemes 2 to 5 (rarely to 7), arcuate or subflexuous, ascending to spreading, 3.5 to 11 cm. long, mostly rather distant on a slender channeled axis 7 to 14 cm. long, the rachis with a few long hairs at the base and occasionally along the margin; spikelets in pairs, not crowded, 2.2 to 2.6 mm. long (rarely only 2 mm. or as much as 2.8 mm.), 1.3 to 1.4 mm. wide, elliptic-obovate, olive-green, turning brown at maturity and in drying, the first glume minute (rarely nearly obsolete) on the primary spikelet, with a ciliate brownish hyaline margin, usually acuminate and one-fourth to one-third as long as the spikelet on the secondary, occasionally alike on both spikelets; second glume and sterile lemma 5-nerved, finely pubescent and sparsely to copiously speckled with brown glandular spots, the glume slightly shorter than the sterile lemma, the summit of the fruit exposed at maturity; fruit 2.1 to 2.3 mm. long, pale, minutely papillose-striate.

Paspalum langei

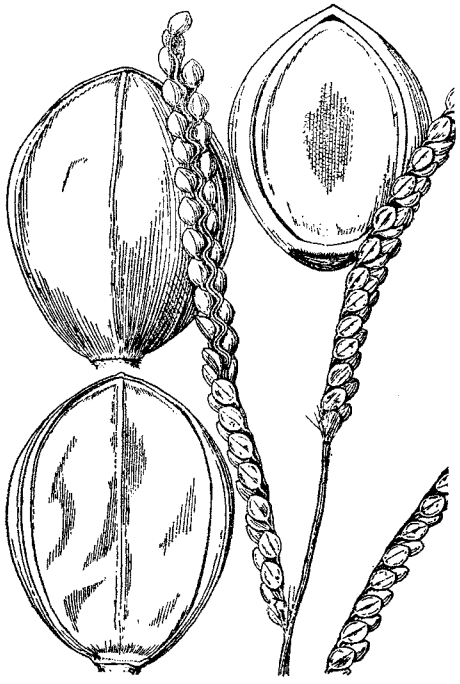
This species varies from the relatively small, nearly glabrous form with shorter blades, like the type specimens of *Dimorphostachys drummondii* and *Paspalum oricola*, to the tall, long-leaved, pubescent form, like the type of *Dimorphostachys* cillifera, but no two characters separating these extremes remain coupled. Only two specimens with glabrous sterile lemmas, given by Nash 3 as a character of *P. langei*, have been seen, Seaton 112a and Hitchcock 9078 The latter has pubescent blades, more than 40 cm. long (characters assigned to *P. ciliiferum*). No specimens have been seen with spikelets having glabrous second glumes.

Hitchcock's nos. 8630, 8681, 8686, and 8744, all from Nicaragua, are coarse, rather robust plants resembling *Paspalum botterii*.

Moist woods and shaded slopes and banks, occasionally in open ground, mostly at low altitudes, Florida, Texas, and the Greater Antilles to Venezuela.

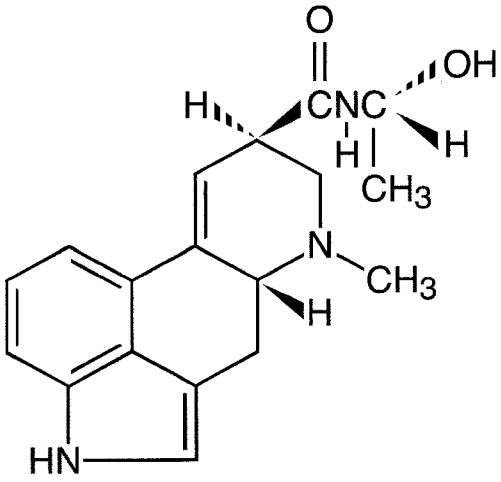
Paspalum floridanum

An erect perennial, the culms solitary or few together from short stout scaly rhizomes, simple, from rather slender to stout, 0.8 to 2 meters, commonly 1 to 1.5 meters, tall, compressed, glabrous; leaves rather numerous, several crowded at the base, the sheaths, especially the lower, mostly overlapping, keeled, from nearly or quite glabrous to densely rather harshly tawny-villous; ligule firm, 2 to 3 mm. long; blades firm, commonly folded at base and flat above, sometimes folded throughout or subinvolute, mostly ascending or with the summits spreading, 12 to 50 cm. long, 4 to 10 mm. wide, the upper reduced, from at least pilose on the upper surface at base to densely villous like the sheaths, usually villous on the upper surface and glabrate beneath; racemes usually 2 to 4, sometimes 5 or 6, rarely more or solitary, 4 to 12 cm. long, thick, usually suberect or ascending, or the whole inflorescenee slightly nodding, the common axis slender; rachis 1 to 1.5 mm. wide, usually strongly zigzag, pilose at the base, scabrous and sometimes sparsely long-ciliate throughout; spikelets in pairs (one of the pair sometimes rudimentary), crowded, 3.6 to 4 mm. long, 2.8 to 3.1 mm. wide, usually 4 mm. long and 3 mm. wide, oval, pale; glume and sterile lemma equal, scarcely covering the fruit at maturity, firm and papery, slightly inflated, irregularly wrinkled, 5-nerved; fruit about 3.5 mm. long and 2.5 mm. wide, oval, light brown, under a lens minutely papillose-striate.



Paspalum floridanum

Distribution: Low moist sandy soil, pine woods, flatwoods, savannas, and low prairies, in the Coastal Plain from Virginia to central Florida and along the Gulf to Texas, and north in the valleys to Missouri and Oklahoma. Source: Chase 1929



***d*-Lysergic Acid-α-hydroxy-ethanolamide**

Chapter 17
Cultivation of *Claviceps paspali*
Alkaloid Derivatives of Lysergic Acids

Ernst Boris Chain, Cesare Bonino, Antonio Tonolo,
Rome, Italy July 19, 1960

Nowadays the alkaloid derivatives of lysergic acid are generally obtained from ergot, that is from natural sclerotia of *Claviceps purpurea* (Fr.) Tul. A. The investigators Stoll *et al.* (1957) have recently reported the production of such alkaloids by saprophytic surface culture of a suitable strain of *Claviceps purpurea* (Fr) Tul. isolated from rye. Others (Abe et al.: *J Agric. Chem. Soc. Japan* 25, 1952, p. 458; Taber et al: *Canad. J. of Microbiology* 4, 1958, p. 611) have described processes for the preparation of alkaloids by saprophytic surface culture of some particular strains of *Claviceps*. However such alkaloids do not contain lysergic acid in their molecule and are different from those obtained by natural sclerotia of *Claviceps purpurea* (Fr.) Tul.

In all these investigations, carried out over many years, the production of the alkaloids occurs only by saprophytic surface culture after 20-40 days of incubation and besides the unitary production is so low as to be impractical.

More recently, Spruson et al. (*Australian P.* 34,313/58) have described a process for the biosynthetic production of ergot alkaloids by the cultivation of *Claviceps purpurea* Tul., under essentially anaerobic conditions and with a substantial reduction of cell respiration. Such conditions present a number of evident disadvantages.

The process of the present invention facilitates the production of lysergic acid derivatives alkaloids in high yields, through a submerged culture of new strains of *Claviceps*, under aerobic conditions and stirring, without causing a reduction of the cell respiration. This signifies that the formation of alkaloids of lysergic acid can be carried out by an industrial fermentation.

The organisms employed for the process of the present invention are new strains of *Claviceps paspali* Stevens and Hall. It had been found that the strains *Claviceps paspali* Stevens and Hall, which do not produce the lysergic acid derivatives alkaloids by submerged culture, may be virulented artificially, to give new strains of *Claviceps paspali*, which in turn allow said production.

The artificial virulentation occurred as follows. Strain F. 97 was isolated from sclerotia grown on plants of *Paspalum distichum*, collected at Tivoli (Rome) and identified and classified as *Claviceps paspali* Stevens and Hall. Embryos of Rosen 4 n rye were inoculated, before germination, with the strain F. 97 and then cultivated "in vitro." The new virulented subspecies were isolated from sclerotia obtained on said embryos.

The strains which are used in the process of the present invention and are described as new strains of *Claviceps paspali* Stevens and Hall in this specification and the claims thereof have been filed at the Istituto Superiore di Sanita, Viale Regina Elena 299, Rome (Italy), and denominated by the marks: F-140; F-S13/1; F-237; F-240. The American Type Culture Collection of Washington has assigned to the strains F/s 13/1, F/237, F/240 and F/140 of *Claviceps paspali* ATCC numbers 13892, 13893, 13894 and 13895, respectively.

The process of the invention is therefore one for the production of alkaloid derivatives of lysergic acid which comprises fermenting under aerobic conditions an aqueous nutrient medium containing a source of carbon, nitrogen and mineral salt with a new strain of *Claviceps paspali* Stevens and Hall as herein before defined.

The above-said strains have the following morphological characteristics: the colonies, obtained in agar glucose potato on Petri dishes, have a diameter of 1.5-3 cm. after 10-15 days of cultivation at 27° C; they are round, having a continuous border and smooth surface, showing a white-grey aerial mycelium and a brown or dark vegetative mycelium. The aerial mycelium, velvety and somewhat fasciculated, is constituted by either simple or synnematic hyphae, which have a diameter of 3-4u and septa at a distance of 20-50u, containing droplets of fat. The vegetative mycelium is a mat of compact hyphae which have changed their original structure in a pseudoparenchyma with selerotal structure. In fact the cells have a polygonal form with a diameter of 3-4 x 10-15u, being tightly bound and showing a great number of droplets of fat material. The presence of conidia or clamydospores has never been observed. Sporulation has never been obtained even by changing the sources of carbon or nitrogen in the media.

If the colony surface is scratched by a needle the vegetative mycelium, which lies under the aerial mycelium, presents a ink or fleshy color. The above-said characteristics represent a particular feature of these strains, that have never been observed in other strains of *Claviceps* isolated.

In submerged culture the mycelium forms groups of small round or irregular pellets, having sizes of 0.1-1 x 0.5-3 cm., somewhat loose, which are constituted by synnemata formed by tightly bound hyphae. The hyphae have a diameter of 3-5*u* and are straight with very few lateral branching. The hyphae contain a great number of droplets of fat, even at the early stages. The mycelium, in submerged culture, may have a yellow, brown, grey-green or green color, according to the different media and to the age.

As regards the production of alkaloid derivatives of lysergic acid, the present invention is not limited to the use of the described strains, but comprises also the mutants thereof, which may be obtained, e.g., by means of either a selection or a mutation by the action of U.V. rays or Roentgen rays or any other mutagenous substance or, particularly, by artificial infection of either embryos or grasses cultivated in vitro or plants of grasses cultivated both in vivo or in vitro and the said mutants are to be included in the definition of a new strain of *Claviceps paspali* Stevens and Hall.

According to our invention the process is preferably carried out by cultivating the above described organisms, in aerobic conditions and in submerged culture, both in laboratory flasks and in industrial fermentors, in an aqueous nutrient solution which contains: in organic salts, nitrogen sources and carbohydrates or their suitable compounds acting as carbon sources, until a high yield of alkaloids is obtained.

As regards the inorganic salts, they may be chlorides and/or nitrates and/or carbonates and/or sulphates and/or phosphates of alkaline metals, earth alkaline metals, magnesium, iron, zinc and manganese but preferably MgSO4 and KH2H2PO4.

The behavior of the strains described in the present invention when grown in presence of Fe++ and Zn++ in the medium, is different from that of the strains of *Claviceps purpurea* described by Stoll *et al.* (1957). These two elements may decrease the production of alkaloids markedly.

The nitrogen sources may be ammonium salts such as citrate, tartrate, malate, succinate, oxalate, acetate and the like; amino acids and their mixtures, peptides or proteins, their hydrolysates, meat extracts, hydrosoluble fractions of cereal-like corn or wheat; corn malt extract, corn steep liquor, soya-bean meal, peanut meal, chick-pea meal, cotton bean meal.

The carbohydrates may be glucose, sucrose, starch, dextrins, sorbitol, mannitol, lactose and the like.

The cultivation can be accomplished under aerobic conditions, in

surface culture or preferably in submerged culture; it may be carried out either in laboratory flasks or in fermentors, under stirring or still conditions and maintaining aerobiosis with air or oxygen. The fermentation is carried out at a temperature from 22° to 30° C., preferably at 27° C. and at pH range from 4.2 to 6, preferably at 5.2. The production of the alkaloids generally starts after two days of culture, reaching the optimum after 7-9 days.

The evaluation of alkaloids content may be effected on the basis of color tests by the Van Urk reaction (*Pharm. Weekbled* 66, 1929, p. 473) after extraction as follows: the culture broth is alkalinized to pH 8 and extracted first with chloroform and then re-extracted with the aqueous acidic solution (e.g., 1% H_2SO_4 or 2% tartaric acid) which is used for the colorimetric analysis of alkaloids.

The usual procedures of extraction with suitable organic solvents, such as benzene, chloroform, methylene chloride and the like, or absorption with the known absorbent means, such as charcoal, bentonite and the like, under alkaline conditions, may be used for the separation and isolation of the mixture or the obtained alkaloids. The mixture, in which lysergic acid amide and isolysergic acid amide are prevalently present, can then be hydrolyzed with alkali, in known manner, to lysergic and isolysergic acid (*J. Chem. Society*, 1934, p. 674, and 1936, p. 1440).

The details of the cultivation will be illustrated by the following examples.

Example 1

The process is carried out in 500 mL. flasks containing 100 mL. of a suitable nutrient medium. The flasks are shaken by a rotary shaker (200 revolutions/minute; eccentric throw: 10 cm.). The optimal incubation temperature is at 27° C. The relative moisture is 85-90%. The cultivation is carried out in the dark. A flask is inoculated with the mycelium which is obtained from a 10 days' culture in potato-glucose-agar of one of the above-described new strains of *Claviceps paspali* Stevens and Hall. The nutrient medium is the following:

	Percent
Mannitol	5
Succinic acid	1
KH_2PO_4	0.1
$MgSO_4.7H_2O$	0.03
Chick-pea meal	0.1

Distilled water.

The pH is adjusted to 5.2 with aqueous ammonia solution.

A homogeneous culture is generally formed on a rotary shaker after 7-10 days' incubation and it is constituted by a mass of synnematic hyphae. A part, 10%, of such a culture is used as a seed for the prefermentation culture which is carried out in flasks containing the same medium. After 4 days' cultivation the fermentation flasks are inoculated with 10% of the mycelium grown in the prefermentation flask.

The fermentation medium for the production of the alkaloids has the following composition:

	Percent
Mannitol	5
Succinic acid	3
KH_2PO_4	0.1
$MgSO_4.7H_2O$	0.03

Distilled water.

The pH is adjusted to 5.2 with aqueous ammonia solution.

In this medium, the average production of alkaloids reaches 1000 *ug.*/mL. after 7-9 days' incubation.

Ten liters of culture broth, obtained by 110 fermentating flasks, are filtered and the mycelium is discarded since it contains a very low amount of alkaloids. The filtered dark colored broth (which contains about 1000 *ug.*/mL. of alkaloids) is made alkaline by adding sodium carbonate or sodium hydroxide solution and extracted with 10 L. of a mixture chloroform-isobutanol (4:1). The organic extract is re-extracted with an aqueous 2% tartaric acid solution. The aqueous acidic solution is then concentrated under vacuum and at 20 - 40 C. to a small volume (about one-tenth of the original volume). The residual solution is made alkaline, extracted with chloroform and the solvent evaporated. A white crystalline powder is obtained, from which, by alkaline hydrolysis in known manner, lysergic and isolysergic acid are obtained.

Example 2

The cultivation is carried out with the following nutritive media:

Mannitol	5
Malic acid	3
KH_2PO_4	0.1
$MgSO_4.7H_2O$	0.03

Distilled water.

The pH is adjusted to 5.2 with aqueous ammonia solution.

The fermentation is carried out according to the procedure in Example 1. After 7-9 days' incubation, the production of alkaloids reaches the value of 1000 *ug.*/mL. The same yield is obtained if tartaric acid, citric acid, malic acid, acetic acid, fumaric acid, succinic acid are used.

Example 3

The cultivation is carried out with the following nutrient medium:

	Percent
Sorbitol	5
Malic acid	3
KH_2PO_4	0.1
$MgSO_4.7H_2O$	0.03
Distilled water.	

The pH is adjusted to 5.2 with aqueous ammonia solution. The fermentation is carried out according to the procedure described in Example 1. After 7-9 days' incubation, the production of alkaloids reaches 1000 *ug.*/mL.

Other suitable nitrogen sources are: soybean meal, peanut meal, bean meal, lentil meal, pea meal, potato meal, hydrolyzed casein, yeast extract, corn-steep liquor and the like.

4 Litre Fermentor

The sterilization is effected in an autoclave for 20 minutes at 100° C. and for 40 minutes at 120° C. The fermentors are aerated from the bottom through a sintered glass sparger. The foam is controlled by adding the usual anti-foaming agents such as Vaseline oil (Vaseline is a registered trademark) containing 6% Alkaterge and the like. The incubation temperature is kept at 27° C.

The inoculum is constituted by 400 mL. of the culture.

Example 6

The nutrient medium has the following composition:

	Percent
Sorbitol	5
Succinic acid	1
KH_2PO_4	0.1
$MgSO_4.7H_2O$	0.03
Chick-pea meal	1
Distilled water.	

The pH is adjusted to 5.2 with aqueous ammonia solution. The fermentation is carried out as described earlier. After 4-7 days' culture the production of alkaloids reaches 1400-1600 *ug.*/mL.

Obtaining the New Alkaloids

460 mL. of a filtered (to remove mycelium) culture broth, obtained according to the process described in said copending application and containing 450 *ug.*/cm.3 of alkaloids (determined colorimetrically as ergometrine), are cooled to 5° C. and the pH is adjusted to 8 by adding sodium bicarbonate. The alkaloids are extracted with chloroform cooled to 2 to 5° C. (about 500 mL.) and the chloroform extract is extracted with 50 mL. of aqueous 0.1 M tartaric acid. Several transfers (3-4) are carried out between the organic solvent and the dilute tartaric acid and, by operating in the temperature range of 0° to 10° C. and by adjusting the pH of the aqueous phase before the extraction with organic solvent between 7 and 9, a final chloroform extract of about 20 mL. is obtained. Source: Chain 1964

Microbiological Production of Lysergic Acid Derivatives

Jürg Rutschmann, Hans Kobel,
Basel, Switzerland August 30, 1962

It has now been found that a new strain of *Claviceps paspali* Stevens and Hall, found in New Guinea in the form of sclerotica *Paspalum dilatatum* Poir., and to which has been accorded the number NRRL 3027 by the United States Department of Agriculture (Northern Utilization Research and Development Division), Peoria (Il.), can be used to produce lysergic acid amide and isolysergic acid amide, with only minor admixture of other lysergic acid derivatives, by cultivation in a nutrient medium. It is a characteristic of this new strain that it will produce conidia when cultivated in vitro, and the use of these conidia, rather than mycelial fragments, greatly facilitates inoculation. This new strain has been deposited at the above mentioned U.S. Dept. of Agriculture.

The process of the invention, therefore, for the production of lysergic acid derivatives comprises cultivating the strain of *Claviceps paspali* Stevens and Hall, NRRL 3027, in a nutrient medium and isolating lysergic acid derivatives from the said medium.

The ready formation of conidia by the new strain confers other advantages besides ease of inoculation. Thus, single spores can be isolated by the usual techniques and genetically homogeneous material thus obtained.

Moreover, it is easier to produce mutants from such spores by artificial methods, such as X-ray or ultra-violet irradiation or the use of certain chemicals, than from sterile mycelium. The conidia can be freeze-dried and thus stored indefinitely. Finally, should the strain begin to lose its vigor it can be restored by growth on *Paspalum* plants which may be readily infected with conidia.

The sclerotica formed by the new strain of *Claviceps paspali* Stevens and Hall on Paspalum plants contain alkaloids, unlike those formed by other strains of this fungus.

The alkaloid content of the sclerotica amounts to 0.04%, calculated on an average molecular weight of 300. The composition of the alkaloid mixture is as follows:

	Percent
Lysergic acid amide	40
Isolysergic acid amide	20
Ergobasine	15
Ergobasinine	10
Chanoclavine	10
Unknown	5

The isolation and propagation of the new strain of fungus may be carried out as follows.

A small piece of tissue is extracted under sterile conditions from the interior of a sclerotium and transferred to a beer wort agar [composition: 250 mL. of unhopped light beer wort (solids content 17%); 18 g. of agar; distilled water to 1 liter; pH 5.2]. A circular colony develops, which reaches a diameter of 15 mm. after 14 days at 24° C. It consists of a 1 mm. thick film of pseudosclerotial structure lying on the agar, and above this a cushion of white aerial mycelium. A brown colouring substance diffuses into the agar. No conidia are formed.

This colony is divided into pieces with a spatula and transferred into a test tube with 12 cc. of the following agar nutrient medium: bear wort 500 mL., corn steep solids 60 g., lactic acid 1 mL., ammonium chloride solution to pH 4.8, agar 20 g., distilled water to 1 liter.

There forms around each fragment of inoculant a small colony of initially white, and later reddish-brown, mycelium. After 10 days, conidia begin to separate off at the hyphal tips. After 20 days, sufficient conidia are present to produce therefrom an aqueous suspension with which

20 sloped agar tubules containing the same agar as above can be inoculated. These cultures are incubated at 24° C. The conidia germinate after 24-36 hours. After 6 days, the agar surface is uniformly coated with a fine white mycelium, after 10 days a brownish-grey, finely furrowed mycelium covering has formed, which lies closely on the agar and has only short serial hyphae. Conidia are formed on the latter. After 12 days, there are formed at a number of points in the mycelium, points at which small droplets of a reddish-brown liquid are precipitated. The droplets reach a diameter of 1-3 mm. and soon become cloudy from the presence of very numerous conidia. After 16-18 days, the formation of conidia has substantially ceased. A sloped agar culture in a test tube, 2 cm. in diameter and containing 12 mL. of agar substrate, contains about 10 (*to the*) 9 (*th*) conidia.

The conidia produced in this way may he used to inoculate culture media used for the production of lysergic acid derivatives. For this purpose the submerged culture technique is preferably used.

The initial culture is first prepared as follows:

As medium, a 4.5% aqueous malt extract solution having a pH of 5.4 is employed. One liter of this solution is sterilised in a 2-liter Erlenmeyer flask for 20 minutes at 110° C., and then inoculated with about 5 x 10 (*to the*) 8 (*th*) conidia of a 20-day old agar culture of the new strain and incubated for 3 days at 23° C. on a rotating shaking machine. A compact culture of fine mycelium flakes is formed. The flakes consist of loose clusters of hyphae and have a diameter of 2-4 mm. No alkaloids can be detected.

Larger quantities of initial culture may be prepared in glass fermentation vessels each containing 10 liters of the same medium and inoculated with about 5 x 10 (*to the*) 9 (th) conidia. Incubation is continued for 3 days at 23° C. with aeration with 6 L. of air per minute and stirring at 200 r.p.m. In order to prevent foaming, a silicone emulsion is added. The fermentation cultures thus obtained have the same characteristics as the shake cultures.

For the main culture, the following nutrient solution, which contained in 1 liter of distilled water, 50 g. of sorbitol, 36 g. of succinic acid, 2 g. of KH_2PO_4, 0.3 g. of $MgSO_4$, 1 mg. of $FeSO_4.7H_2O$ and 10 mg. of $ZnSO_4.7H_2O$ and had been adjusted to a pH of 5.4 with NHOH, is particularly suitable.

This nutrient solution is inoculated with 10% of a 3-day old initial culture and incubated in portions of 100 mL. in 500-mL. Erlenmeyer flasks at 23 ° C. on a reciprocating shaking machine. A large scale culture may be grown in a similar manner in a stainless steel fermentation vessel containing 170 L. of nutrient medium. Aeration is effected

with 170 L. of air per minute and stirring, initially at 70 r.p.m., and subsequently at 80 r.p.m. In order to prevent foaming, a silicone emulsion is employed.

In this way, cultures consisting of numerous mycelium particles are obtained. These particles have a diameter of about 5 mm. and have a spherical compact nucleus consisting of pseudoparenchymatous tissue about 1 mm. in diameter. This nucleus has radial extensions about 2 mm. long consisting of parallel hyphae. After culture for about 10 days, the mycelium is dark brown, and the filtrate is a dark reddish-brown. The pH varies only to a minor extent.

The isolation of the alkaloids from this culture may be effected as follows: 20 liters of the culture filtrate are brought to a pH value of 9.75 by adding 1.2 kg. of sodium carbonate. The mixture is then extracted 3 times, each time with 20 liters of ethylene chloride. The organic phase is washed with 5 liters of water and then concentrated, in a vacuum, to total of 4 liters. The concentrate is extracted 3 times, each time with 1 1iter of 5% tartaric acid, the tartaric acid extracts brought to a pH value of 9.0 by the addition of sodium carbonate and then extracted 3 times, each time with 2 liters of ethylene chloride. The extracts are washed with a little water, dried over sodium sulphate, and evaporated in a vacuum. By means of investigations in accordance with Van Urk (*Pharm. Weekblad* 66, 473, 1929), these materials were found to contain 12.2 g. of indole compounds, whilst in accordance with paper chromatographical investigations, 11.3 g. of saponifiable indoles were found to be present.

The mixture of alkaloids so obtained has the following composition:

	Percent
Lysergic acid amide	45
Isolysergic acid amide	42
Ergobasine	3
Ergobasinine	3
Chanoclavine	3
Elymoclavine	2
Penniclavine	2

The analysis is effected by means of paper chromatography and the paper irradiated with ultraviolet light whereupon the varying intensities of.the colours on the paper may be taken as a measure of the quantities of the various constituents present.

When the culture is carried out in the fermentation vessel, 145 L. of culture filtrate are obtained after culture for 12 days. This culture filtrate is made alkaline with a 2 N sodium hydroxide solution and then extracted 5 times at a pH of 7.4 and 3 times at a pH value of 10, each time with 200 liters of ethyl acetate. The combined ethyl acetate extracts are then concentrated to 50 liters and the alkaloids removed from the concentrate with the aid of an aqueous tartaric acid solution. These aqueous extracts are made alkaline and again extracted at pH values of 7.4 and 10 with ethyl acetate. After washing until neutral with a little water, the solutions are evaporated to dryness.

112.7 g. of total alkaloids are isolated, which is equivalent to a concentration of 805 mg./L. The isolation is effected in accordance with one of the above described methods.

The alkaloid mixture has the following composition:

	Percent
Lysergic acid amide	66
isoLysergic acid amide	21
Ergobasine	4
Ergobasinine	2
Chanoclavine	2
Elymoclavine	2
Agroclavine	2
Penniclavine	1

The analysis was again effected by means of paper chromatography in the above described manner. Source: Rutschmann 1965

Production of Ergot Alkaloids

Varro E. Tyler, Jr.
Seattle, Washington May, 6, 1963

The strain employed in the instant invention was isolated from a sclerotium of *Claviceps paspali* parasitic upon *Paspalum dilatatum* Poir. of Australian origin, and a culture thereof has been placed on permanent deposit with the American Type Culture Collection, Washington, D.C. The said culture was submitted under the strain number 247/61 and has been assigned the ATCC accession number 14988.

Taxonomic studies carried out with *Claviceps paspali* ATCC 14988 are summarized in the paragraphs which follow. Observations were made at 7 and 14 days on plate cultures incubated at 26° C. Cultural

observations were based on colony characteristics since there appear to be no significant microscopic structures such as conidia, chlamydospores or sclerotia.

All cultures appear to contain polygonal cells, and fat droplets are present in most hyphal cells. Observation of fat droplets in the cycelia was carried out on cultures grown on potato-glucose agar by Burdon's method as described on page 20 of Leaflet IV of the *Manual of Methods for Pure Culture Study of Bacteria Biotech Publications* (1951). In the descriptions which follow, numbers in parentheses refer to color blocks in Maerz and Paul, *Dictionary of Color* (1950).

Colony Characteristics of *Claviceps paspali* ATCC 14988

Corn steep medium.—Vegetative growth abundant, compact, smooth, deep red-brown (15-1OE); margin radiative; aerial mycelium fasciculate, light brown (11-2E); colorless exudate; brown soluble pigment.

The medium is prepared by combining the following ingredients and adjusting to pH 5.6:

G. Corn steep solids	10
Cerelose (Dextrose)	5
Sucrose	10
KH_2PO_4	0.5
$Mg_2SO_4.7H_2O$	0.3
Agar	20

Water, q.s., 1000 mL.

Potato-glucose medium.—Vegetative growth moderate, red-brown (14-11E3); aerial mycelium floccose, white; neither exudate nor soluble pigment produced.

The medium is prepared by steaming 40 g. of potatoes for 30 minutes in 500 mL. of water. To the resulting mixture are added 5 g. of glucose, 17 g. of agar and sufficient water to make the total volume 1000 mL.

Sasamino acid-sucrose medium.—Vegetative growth fair, restricted, deep brown (16-1OA), margin radiative aerial mycelium floccose, dark brown (12-6E); brown soluble pigment.

Alphacel-coconut milk medium.—Vegetative growth fair, smooth, brown (14-8F); aerial mycelium white, velutinose; brown soluble pigment.

This is a modification of the medium of Sloan et al., *Mycologia* 52, 47-63 (1960). The composition of the medium is as follows:

Alphacel (Cellulose)	g. 20
$MgSO_4.7H_2O$	g. 1
KH_2PO_4	g. 1.5
$NaNO_3$	g. 1.5
Coconut milk *	50 mL.
Tomato paste	g. 2.5
Baby oatmeal (Heinz)	g. 2.5
Agar	g 20

Water, q.s., 1000 mL.

Cellulose (Nutritional Biochemical Corp.).
* Marga-Rita coconut juice (Dairy Fresh Products Co.).

For the preparation of alkaloid derivatives of lysergic acid, the hitherto unknown strain of *C. paspali* is cultivated in a culture medium containing assimilable sources of carbon, nitrogen and inorganic salts. The organisms are isolated from the dry sclerotia obtained from the parasitized *Paspalum dilatatum* after the sclerotia have been subjected to surface sterilization. A preferred procedure consists in thoroughly brushing the dry sclerotia and then shaking successively with portions of aqueous n-propanol, aqueous formaldehyde, and sterile distilled water. Thin segments are then cut from the dried sclerotia and are placed on agar slants to germinate. After a suitable incubation period, the vegetative mycelium is scraped from the agar slant and is introduced into a small volume of a preculture medium in which growth is permitted to continue. After a period of several days, usually from about 4 to 8 days, abundant proliferation of the mycelium is apparent. The mycelium so obtained is transferred aseptically to the production medium, whereupon a further incubation period of between about 5 and about 15 days produces an abundant yield of mycelium, which is separated by filtration. The mixture of lysergic acid alkaloids is recovered from the culture broth filtrate by the usual solvent extraction techniques, and can be separated into the individual alkaloids by known procedures. Alternatively and preferably, the alkaloid mixture can be converted by hydrolysis with alkali to lysergic and isolysergic acids, which can be employed to prepare desired derivatives of these acids by synthetic procedures.

The culture medium for producing the lysergic acid alkaloids by cultivation of the new strain of *C. paspali* be any one of several media, since the organism is capable of utilizing different energy sources.

However, for economy of production, maximum yields of alkaloids, and ease of recovery of the products, certain culture media containing relatively simple nutrient sources are preferred. For example, the media which are useful in the production of the alkaloids include an assimilable source of carbon such as glucose, sucrose, starch, molasses, dextrins, corn steep solids, corn syrup liquor, sorbitol, mannitol, lactose, and the like. A preferred source of carbon is mannitol. Additionally, the media employed contain a source of assimilable nitrogen such as oatmeal meat extracts, peptones, amino acids and their mixtures, proteins and their hydrolysates, corn steep liquor, soybean meal, peanut meal and ammonium salts of organic acids such as the citrate, acetate, malate, oxalate, succinate, tartrate and like salts.

Mineral salts, for example those providing chloride, nitrate, carbonate, sulfate, phosphate, calcium, magnesium, sodium, potassium, iron, zinc, manganese and like ions are also incorporated in the media with beneficial results. As is necessary for the growth and development of other microorganisms, essential trace elements should also be included in the culture medium for growing the organisms employed in this invention. Such trace elements are commonly supplied as impurities incidental to the addition of the other constituents of the medium.

Submerged aerobic cultural conditions are the conditions of choice for the production of the lysergic acid alkaloids by the processes of this invention. For preparation of relatively small amounts, shake flasks and surface culture in bottles can be employed, but for the preparation of larger quantities, submerged aerobic culture in sterile tanks is preferred. The medium in the tank can be inoculated directly with the mycelium obtained from the agar slant. However, in order to avoid the growth lag experienced when this procedure is employed and the relatively inefficient use of the fermentation equipment resulting therefrom, an alternative procedure is preferably employed. Furthermore, it has been found that higher yields of the lysergic acid alkaloids ultimately result when a vegetative inoculum is grown in a suitable preculture medium, the composition of which differs from that of the final production medium. Accordingly, it is desirable to transfer the mycelium from the agar slant into a preculture medium favorable for rapid mycelial development and, after a well-developed vegetative inoculum has been so obtained, to transfer the vegetative inoculum under suitable conditions to the production medium in the large tank. Thus, for example, a preculture medium containing corn steep solids and/or corn syrup solids is especially suitable for the production of the vegetative inoculum since large quantities of mycelium are produced in submerged culture in a short time and excellent alkaloid yields result when this

mycelium is used as inoculum. However, the presence of corn steep or corn syrup solids in the production medium has a detrimental effect upon the yield of alkaloids produced in some production media. Consequently, it is usually desirable to filter and wash the mycelium produced in such a preculture medium prior to the inoculation of the production medium therewith.

As is customary in submerged culture processes, sterile air is blown through the culture medium. For efficient growth of the organism and optimum alkaloid production, the volume of air employed in tank production is preferably at least about 0.1 volume of air per minute per volume of culture medium, and will generally range from about 0.2 to about 2 volumes/volume/minute.

The organisms grow best at temperatures in the range of about 22° C. to about 28° C. Optimal production of alkaloids appears to occur at a temperature of about 23° C. to 24° C.

The initial pH of the culture medium can vary some; what. However, it has been found desirable that the initial pH of the medium be between about pH 4 and about pH 6, preferably from about pH 5 to about pH 6. As is observed in other fermentation processes the pH of the medium changes gradually throughout the growth period of the organism, the final pH being dependent at least in part upon the initial pH of the medium, the buffers present in the medium, and the period of time the organism is permitted to grow.

For optimum production of alkaloids, it is important that the mycelium employed for the inoculation of a liquid culture medium be maintained on solid media exclusively prior to transfer to submerged culture. Thus, for example, yields are significantly depressed when the mycelium has been transferred from a submerged culture to surface culture on solid media prior to inoculation into submerged culture. The optimum route for preparation of inoculum therefore is from surface culture to submerged medium or from surface culture, through a series of transfers on surface culture, to submerged medium...

It is of interest to note that addition of tryptophan to the culture medium does not appear to enhance the production of alkaloids by the new strain of *Claviceps paspali* employed herein. Also noteworthy is the fact that relatively high concentrations of iron appear to promote, or at least support, alkaloid synthesis by this strain. Thus, for example, concentrations of FeSO4.7H2O in the range of 100 - 200 mg. per liter appear to be without detrimental effect.

Although the present description has been directed primarily to one strain, it is to be understood that natural or artificial mutants thereof are within the scope of the invention. Such mutants are obtained by

methods well known in the art, such as by natural strain selection, by chemically induced mutation, or by mutation induced by irradiation with Ultraviolet or X-radiation.

The practice of the invention is illustrated by the examples which follow.

Example 1

Dry sclerotia of *Claviceps paspali*, ATTC No. 14988 are brushed thoroughly and are then shaken successively for two-minute periods with 50 percent aqueous n-propanol and 4 percent aqueous formaldehyde solutions to effect sterilization. The sclerotia are then rinsed thoroughly with three portions of sterile distilled water. Thin segments are cut from the treated sclerotia with a razor blade and are then grown for seven days at room temperature in 1 x 6 inch test tubes on an agar slant medium having the following composition:

G. Corn Steep solids	10
Cerelose	5
Sucrose	10
KH2PO4	0.5
MgSO4.7H2O	0.3
Bacto-Agar (Difco)	20.0

Distilled water, q.s. 1000 mL.

The pH of the slant medium is adjusted to 5.6 with concentrated ammonium hydroxide.

The mycelia from each slant are harvested and suspended in about 10 mL. of distilled water. The suspension is divided into 2.5 mL. portions, each of which is employed to inoculate 50 ml. of a preculture medium contained in a 250 mL. of wide-mouth Erlenmeyer flask fitted with a milk filter cap. The composition of the preculture medium is as follows:

G. Corn steep solids	20
Cerelose	10
Sucrose	20
KH2PO4	1
MgSO4.7H2O	0.3

Distilled water, q.s. 1000 mL.

The medium is sterilized by autoclaving at 121° C. for twenty minutes. The pH of the sterilized medium is about 5.5. The flasks containing the inoculated preculture medium are incubated for about seven days at about 24° C. on a gyratory shaker having a 1.5-inch throw at 250 r.p.m.

The mycelia from each flask are filtered, washed with sterile distilled water, transferred to a similar flask containing about 50 mL. of a production medium, and incubated under the same conditions for seven additional days. The production medium has the following composition:

Instead of separating the various components of the alkaloid mixture by chromatography, the total alkaloids can be hydrolyzed to yield a mixture containing lysergic and isolysergic acids from which lysergic acid is recovered as follows: Five grams of the crude alkaloid mixture are dissolved in 100 mL. of aqueous 10 percent potassium hydroxide solution and the solution is heated at reflux temperature for one hour under a nitrogen atmosphere. The reaction mixture is cooled and acidified to Congo red with dilute aqueous sulfuric acid. The mixture is filtered and the dark solid obtained is triturated with several 100-mL. portions of ammoniacal ethanol. The remaining insoluble material is discarded. The filtrate is evaporated to dryness under diminished pressure and the residue is digested with 20 mL. of cold methanol in order to remove some resinous colored material. The mixture is cooled and filtered to separate the crude lysergic acid which, after recrystallization from water, melts with decomposition at about 238° C.
Source: Tyler 1963

Heterocyclic Carboxylic Acids Production

Jürg Rutschmann, Hans Kobel, Emil Schreier
Basel, Switzerland August 29, 1963

The isolation and culture of *Claviceps paspali* Stevens et Hall NRRL 3080 may be carried out, for example, in the following manner:

A small specimen of tissue is removed from the interior of a sclerotium under sterile conditions and transferred to beer wort agar. [Composition: 250 mL. of unhopped, light-colored beer wort (17% solids), 18 g. of agar-agar, distilled water to make 1 litre (pH 5.2).] A circular colony develops which after 14 days at 24° C. has a diameter of 15 mm.; it consists of a skin, about 1 mm. thick, of pseudo-sclerotial structure lying on the agar and on top of it there is a cushion of white aerial mycelium. A brown color diffuses into the agar. No conidia are formed.

This colony is broken up with a spatula into fractions which are transferred to a test tube with 12 cc. of the following agar culture medium:

Beer wort	500 mL.
Cornsteep solids	60 g.
Lactic acid	1 mL.
Ammonium chloride solution to pH	4.8
Agar-agar	20 g.
Distilled water to make	1 Litre

A small colony of white, subsequently reddish brown, mycelium forms round every inoculation specimen. 10 days later conidia begin to be formed by constriction at the tips of the hyphae. After 20 days a sufficient number of conidia is present to permit the preparation of an aqueous suspension with which 20 agar slant tubes (same agar as above) can be inoculated. Those cultures are incubated at 24° C. The conidia germinate after 24 to 36 hours. After 6 days, the surface of the agar is evenly covered with a fine, white mycelium, and after 10 days a brownish grey, finely wrinkled mycelial blanket has developed which is in intimate contact with the agar and has only short aerial hyphae, from which conidia form by constriction. After 12 days, there appear at various spots in the mycelium centers which exude small droplets of a reddish-brown liquid. The droplets reach a diameter of 1 to 3 mm. and soon turn turbid owing to the presence of very numerous conidia. After 16 to 18 days the formation of conidia is practically complete. An agar slant culture in a test tube 2 cm. in diameter filled with 12 mL. of agar nutrient medium contains about 10 (*to the*) 9 (*th*) conidia.

For cultivation by the submerged culture technique, a preculture is first prepared as follows. The medium used is a 4.5% aqueous malt extract solution of pH 5. One litre of this solution is sterilised for 20 minutes at 110° C. in a 2-litre conical flask, then inoculated with 6.10 (*to the*) 8 (*th*) conidia of a 15-days old agar culture and incubated for 3 days on a rotating shaking machine at 24° C. A dense culture of fine mycelial flocks forms, each flock consisting of a loose bunch of hyphae of diameter 2 to 4 mm. No alkaloids can be identified.

To manufacture a large amount of preculture, glass fermenters containing 10 litres each of the same medium, are inoculated with 6.10 (*to the*) 9 (*th*) conidia each and incubated for 3 days at 23° C. while being aerated with 6 litres of air per minute and stirred at 300 r.p.m. To inhibit foaming a silicone emulsion is used. The resulting fermenter cultures are identical with the shaken cultures. Particularly good results in preparing the main culture have been obtained with a nutrient solution that contains in 1 litre of distilled water.

Sorbitol	g. 50
Succinic acid	g. 36
KH_2PO_4	g. 2
$MgSO_4$	g. 0 3
$FeSO_4.7H_2O$	mg. 1
$ZnSO.7H_2O$	mg. 10

and has been adjusted with ammonia to pH 5.4. This nutrient solution was inoculated with 10% of a 3-days old preculture and incubated in portions of 100 mL. each in 500 mL. conical flasks at 23° C. in a reciprocal shaking machine. Other cultures were grown in a similar manner in a stainless steel fermenter containing 170 litres of nutrient medium while aerating with 170 litres of air per minute and stirring, first at 70 and then at 180 r.p.m. Foaming was inhibited with a silicone emulsion.

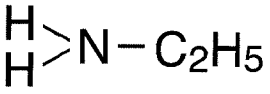
In this manner cultures consisting of numerous identical mycelium particles are obtained; they have a diameter of about 5 mm. and have a globular, compact core of about 1 mm. diameter of pseudoparenchymatic tissue. This core has appendices of about 2 mm length, arranged in a star shape, consisting of parallel hyphae. On completion of the cultivation after about 10 days, the mycelium is dark brown and the filtrate is an intense reddish brown color. The pH changes only insignificantly.

The culture filtrate prepared in this manner has a total alkaloid content (determined by colorimetry) of 620 mg. per litre, assuming an average molecular weight of 300. The composition of the alkaloid mixture, determined by paper-chromotography, is as follows:

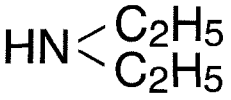
	Percent
6-Methyl-Δ-8,9-ergolene-8-carboxylic acid mixed with minor proportions of Lysergic acid and isolysergic acid	86.5
Lysergic acid amide	3.9
isoLysergic acid amide	3.9
Ergobasine	1.0
Ergobasinine	0.5
Clavine alkaloids	4.2

Alternatively, the culture filtrate can be processed directly to yield lysergic acid for example in the following manner: The solution is evaporated, 6-methyl-Δ-8,9-ergolene-8-carboxylic acid is extracted from the residue with an alcoholic ammonia solution, the extract is heated to isomerise the 6-methyl-Δ-8,9-ergolene-8-carboxylic acid to lysergic acid and the latter is then isolated from the solution as described above. Rutschmann 1967

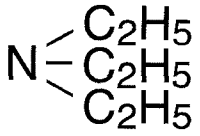
Ethylamine



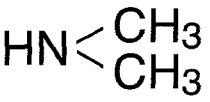
Diethylamine



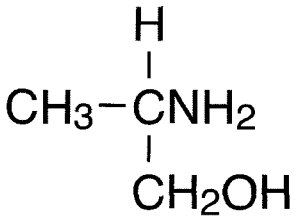
Triethylamine



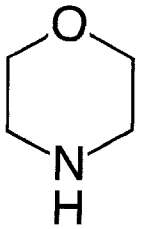
Dimethylamine



2-Amino-1-propanol



Morpholine



Chapter 18
Preparation of Diethylamine from Ethanol

by Robert Bottoms
Louisville, Kentucky June 18, 1935

The reaction between the alcohol and the ammonium chloride or other halide is preferably carried out with proportions about those which are necessary to form the secondary amine, since the secondary amine is the most desirable commercially...

The reaction mixture is preferably placed in an autoclave or closed reaction vessel capable of withstanding high pressure and capable of enabling a relatively high temperature being applied, as for example, substantially above 200° and, if desired, up to 400° C, with an optimum range between 275° and 325°.

The alkyl amines as prepared are usually in the form of their hydrochlorides or other hydrohalides, and consist of a mixture of primary, secondary and tertiary amines... I have disclosed methods (see below) by which the primary, secondary and tertiary amines may be separated from each other.

920 grams (20 moles) ethyl alcohol, 535 grams (10 moles) ammonium chloride, 500 cc. water and 5 grams ferric chloride are placed in an autoclave and heated to 275°-280° C for 2 hours. The autoclave is then cooled, and the reaction mixture neutralized and made alkaline by adding a 50% water solution of 1,000 grams sodium hydroxide. The excess alkali is not essential, but serves to "salt out" the free amine which is separated and distilled. Diethylamine in better than 80% yield results. Source: Bottoms 1937

Separation of Diethylamine

by Robert Rogers Bottoms
Birmingham, Alabama October 22, 1936

In processes of producing alkylamines, and particularly methylamines, by reacting the corresponding alkyl chlorides, halides, hydroxides and so forth, with ammonia or ammonium salts such as ammonium chloride, the reaction product usually contains substantial quantities of all three of the primary, secondary and tertiary amines, even though the temperature, pressure, proportions and other reaction conditions may be controlled so as to give rise to a preponderance of one of said amines.

These amines being of similar behavior and having only slightly different chemical and physical properties are difficult to separate, whether present as free bases or in the form of their hydrohalide salts.

I am aware that various special methods have been proposed for separating these amines, such as fractional distillation of azeotropic mixtures under superatmospheric pressure, with or without the addition of large quantities of ammonia, and fractional crystallization of the hydrohalide salts from a solvent such as, alcohol. None of these methods is as simple or direct, nor does it produce as sharp a separation, as that which I am about to describe.

I have found that the lower alkylamines may be separated one from the other by taking advantage of their differences in basicity. In my preferred process, the amines. preferably the alkylamines or methylamines, desirably in the form of their hydrohalide, or hydrochloride salts, are treated with an alkaline material in sufficient quantity and of sufficient strength to displace one or more of the amines from its salt.

The dissociation constants of the amines are a measure of their basicity. For the purpose of my invention, these dissociation constants are given as follows:

	Methyl	Ethyl	Propyl
Mono	0.00050	0.00056	0.00047
Di	0.00074	0.00126	0.00102
Tri	0.000074	0.00064	0.00055
Ammonium hydroxide	0.000018		

It is evident from the above that each of the amines of a group has a different dissociation constant and therefore a different basicity. I have found that this fact enables me to accomplish a separation of certain of the amines from the others. Even though each of the primary, secondary and tertiary amines in one group has a different basicity, it is not economically feasible with any process to separate those which have approximately the same basicity, for example, in the case of the methylamines, it is entirely practical to treat a mixture of the three amine salts with a quantity of alkali stoichiometrically equivalent to the trimethylamine present in the mixture and subsequently to boil out or otherwise remove the liberated trimethylamine. It is not, however, economically possible to obtain a sharp separation by treating the resulting residue of mono and dimethylamine saits with a further quantity of alkali equivalent to the monomethylamine and boil - the solution to obtain monomethylamine. The difference in basicity between the mono and dimethylamines is so small that good separation is not obtained.

In the case of the ethyl and propyl amines it is not economically possible to treat mixtures of the salts with a quantity of alkali equivalent to the primary amine present and then boil out this amine in pure condition. It is however, entirely practical to treat such a mixture with a quantity of alkali equivalent to both the primary and tertiary amines present and then boil the solution so as to drive out these amines and leave only the secondary amine salt in the solution.

It is practical to treat mixtures of the salts of the amines with a quantity of alkali which is equivalent stoichiometrically to the alkylamines present in the mixture which have dissociation constants smaller than 60% of the dissociation constant of the secondary amine present in the mixture.

It is also obvious from the above discussion that the amines in their free state may be treated with an acid in sufficient quantity to combine with the stronger amines and thus effect separation from the weaker amines.

The preferred alkaline compounds for carrying out my invention are the alkali metal and alkaline earth metal oxides, hydroxides and carbonates, and these compounds are utilized in just sufficient amount to displace the desired amine or amines. It is obvious, of course, that if the mixture of amine salts also contains free mineral acid or an ammonium salt, an additional amount of alkali stoichiometrically equivalent to these substances must be added. I have also found that as alkaline reagents for accomplishing this separation it is possible to use other alkylamines or methylamines of different basicity. These amines may be used for displacement together with or in place of the alkalies above mentioned. For example, dimethylamine being more basic than trimethylamine may be utilized to displace triethylamine from its hydrohalide salts, when utilized in stoichiometrical proportions. Similarly, diethylamine may be utilized to displace mono and triethylamine from their hydrohalide combinations.

In the case of the ethyl and propyl amines the secondary amines can be separated from the others by my process and the primary and tertiary amines may then be separated from each other by fractional distillation.

A mixture containing 400 grams of monoethylamine, 400 grams of diethylamine and 200 grams of triethylamine is dissolved in water containing 650 grams of HCl. This solution is then treated with 693 grams of caustic potash, which is just sufficient to neutralize the excess HCl and to liberate the mono and triethylamines. These are distilled from the solution, an additional 310 grams of caustic potash added, and the diethylamine distilled and recovered.

650 grams of dimethylamine hydrochloride and 150 grams of trimethylamine hydrochloride is... dissolved in water and treated with 83 grams sodium carbonate. The liberated trimethylamine is distilled from the solution, and the dimethylamine hydrochloride remaining in solution is recovered by evaporating the solution to dryness and extracting the hydrochloride with ethyl alcohol. Source: Bottoms 1937a

Process of Separating Lower Alkylamines

by Robert Rogers Bottoms
Birmingham, Alabama October 22, 1936

A mixture containing 400 grams of monoethylamine, 400 grams of diethylamine and 200 grams of triethylamine is dissolved in water containing 268 grams of H2SO4. The solution, after complete agitation, is heated to approximately its boiling point, whereby all of the mono and triethylamines are removed from the solution, the diethylamine remaining fixed as a sulfate.

A mixture of 120 grams of monoethylamine, 700 grams of dimethylamine and 140 grams of trimethylamine, all by weight, is dissolved in water containing 710 grams of HCl. After thorough agitation, the solution is heated to approximately its boiling point, whereby the trimethylamine originally present is distilled out of the mixture. The mono and dimethylamines remain in solution as hydrochlorides and can be separated by the usual means.
Source; Bottoms 1937b Refs. Reddie 1938; 1938a

Preparation of Diethylamine

by William Edward Garner and Daniel Tyrer

A mixture of 8000 mL. of ethanol and 3000 grams of ethyl bromide was saturated with ammonia several times during the day; the temperature of the mixture gradually rose to about 30° C, and after some time the ammonium bromide began to crystallize out. After twenty-four hours the alcoholic ammonia solution was separated from the crystals of ammonium bromide, and the alcohol and unchanged ethyl bromide were distilled off. Water was added to the residue and the last traces of alcohol were removed by boiling. The hydrobromides of the mixed bases were then decomposed by a very concentrated solution of sodium hydroxide and the liberated amines distilled off. The alcoholic ammonia containing the ethyl bromide was used again for the prepara-

tion of more of the mixed hydrobromides. By using a fractionating column with ten bulbs there is no difficulty in obtaining an effective separation of the bases.

The yields were:	Monoethylamine.....	10.9 %
	Diethylamine.....	17.9 %
	Triethylamine.....	19.1 %

About 80 percent of the diethylamine (boiling within 1 degree) can be obtained by two fractional distillations. A further quantity of the mixed bases can be produced from the monoethylamine by treating it with more ethyl bromide.

Five hundred grams of crude monoethylamine (containing about 10 per cent of diethylamine) were dissolved in 2500 mL of alcohol and 1000 grams of ethyl bromide were added. The mixture must be cooled in ice, since much heat is evolved. After twenty-four hours 200 grams of ammonium bromide were added to fix any free bases, and the solution was treated as described above.

The yields were:	Monoethylamine.....	38.7 %
	Diethylamine.....	30.1 %
	Triethylamine.....	17.3 %

As the monoethylamine can be again used for the preparation of diethylamine, about 50 percent of the monoethylamine can be converted into diethylamine. Source: Garner 1916

Preparation of Ethylamine and Diethylamine
by Emil Alphonse Werner

Five liters of 90 percent ethanol were saturated with ammonia (compare this volume p. 698) until 490 grams of the gas had been dissolved, 200 grams of ethyl bromide were added (ratio ethyl bromide to ammonia approximately 1 to 16), after which, at successive intervals of two days, fresh quantities of the alkyl haloid were added in the following amounts: 180, 170, 150, 130, 110, 100, 80, and, finally, 66 grams. Preliminary experiments had shown that with the above ratio of ammonia the whole of the ethyl bromide was decomposed after two days, hence the successive quantities were regulated so as to maintain the desired excess of ammonia throughout the progress of the change. In all, 1186 grams of ethyl bromide were used; ammonium bromide began to separate on the twelfth day, and on the sixteenth day the preparation was stopped.

Test experiments on a small scale with pure alcohol had shown that when ammonium bromide separated in quantity in the early stage of the process, the formation of triethylamine was promoted when the reaction was prolonged. The reason is fairly obvious when the probable mechanism of the process is considered, hence it was found advantageous to use alcohol containing 10 percent water.

The alcoholic solution, separated from ammonium bromide, was concentrated by distillation (the ammonia evolved was used to charge more alcohol) until nearly all the ammonium bromide formed had separated, 362 grams of which were recovered.

The solution of the hydrobromides of the mixed amines was distilled until the temperature reached 130° C, in order to remove the last traces of alcohol. Where it was not found convenient to liberate the entire quantity of the mixed amines by the addition of aqueous sodium hydroxide to the residue, chloroform was used as a solvent for their separation.

Ethylammonium bromide is dissolved by chloroform to the extent of only 0.163 gram in 100 mL. at 14° C, whilst the same volume of chloroform dissolves 42 grams of diethylammonium bromide. By this means, 465 grams of pure ethylammonium bromide and 510 grams of diethylammonium bromide, containing slightly more than 5 percent of triethylammonium bromide, were obtained. After the separation of triethylamine (14 grams) by treatment with the requisite proportion of sodium hydroxide, 226 grams of diethylamine, collected at 56-57.5° C and dried over potassium hydroxide were obtained.
Source: Werner 1918

Amines can be produced by many different chemical syntheses, so numerous that I will list only a few references. A thorough search of German, Russian, and American scientific literature is waiting should the reader wish to look into it further.
References: (Davies 1952) (Lemon 1962) (Price 1916) (Rakshit 1913) (Watt 1947)

For the preparation of diethyl and triethylamine from ethyl chloride see British Celanese 1951.

Ethylamines; by heating ethanol and ammonium chloride to 300° see Berthelot 1853; from the decomposition of yeast and flour see Hesse 1857; Sullivan 1858; from the electrolytic reduction of nitroethane see Pierron 1899.

Preparation of Ethyl Bromide (1-Bromoethane) from Sodium Bromide and Ethyl Alcohol

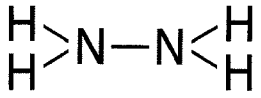
$$H_2SO_4 + NaBr \rightarrow$$
$$HBr + NaHSO_4 \rightarrow$$
$$C_2H_5OH + HBr \rightarrow C_2H_5Br + H_2O$$

270 mL. of water is poured into a one liter boiling flask equipped with a long condenser set downward for distillation. 300 Grams of finely powdered (ground into a fine powder in a mortar and pestle) sodium bromide are added with stirring. 110 mL. of ethanol are added and then 400 grams (218 mL.) of concentrated sulfuric acid are gradually added through a dropping funnel. The mixture is not refluxed but is slowly distilled. The end of the condenser is equipped with a adaptor tube that is very slightly immersed in a beaker of ice water. The distillate is collected in the ice water. The water insoluble layer contains the ethyl bromide. It is separated from the water and washed with water.

Purification

The crude ethyl bromide can be purified by washing with 60 grams of cold concentrated sulfuric acid and then washed (dried) with a sodium carbonate solution (15 grams of sodium carbonate in 150 mL. of water). Ethyl bromide can be further purified by distilling at 38.5-39.5° C. Boiling chips (porous plate chips) must be added to the boiling flask to prevent superheating and bumping. Yields are 90 to 95% theoretical.

References: Kamm 1941; Vogel 1943



Anhydrous Hydrazine

Chapter 19
Anhydrous Hydrazine from Hydrazine Sulfate

****

CAUTION!
HYDRAZINE AND AMMONIA ARE BOTH VIOLENT POISONS!
BOTH CAN CAUSE SEVERE LUNG IRRITATION,
LIVER AND KIDNEY DAMAGE
WHICH MAY NOT APPEAR FOR DAYS.

****

Production of Hydrazine Sulfate

by Friedrich Raschig
Ludwigshafen-on-the-Rhine October 31, 1907

It has generally been assumed that upon the addition of hypochlorites to ammonia or ammonium salts, ammonium hypochlorite NH4OCl is formed. It has only once been suggested that during this reaction a product, monochloramin NH2Cl, containing one molecule less water, is formed (*Proc. Chem. Soc.* 1890, p. 21). It can easily be proved that this suggestion is correct, for a solution of sodium hypochlorite to which ammonia has been added no longer shows the general reaction of hypochlorites of imparting a violet coloration to anilin-water. It therefore no longer contains hypochlorous acid and actually by distillation in vacuo a compound of the composition NH2Cl can be isolated in the form of a very volatile oil which is easily soluble in water and possesses a disagreeable odor of chlorid of nitrogen.

I have now discovered that monochloramin can by treatment with ammonia be made to form hydrazin according to the equation

$$NH_2Cl + NH_3 = NH_2NH_2.HCl$$

In order to prepare hydrazin with advantage according to this reaction, it is unnecessary and, indeed, inadvisable to isolate the extremely decomposable and poisonous monochloramin and subsequently treatment with ammonia, but I prefer to treat a hypochlorite directly with an excess of ammonia. The formation of monochloramin takes place practically at once and subsequently two reactions tend to take place of which the first is represented by the equation given above and leads to the formation of hydrazin, while the second reaction leads to the

formation of ammonium chlorid and the liberation of free nitrogen according to the equation

$$3NH_2Cl + 2NH_3 = N_2 + 3NH_4Cl$$

I have discovered that the second and undesirable reaction tends to preponderate if the mixture of the monochloramin with excess of ammonia be allowed to stand in the cold, or if a catalyser such for instance as small quantities of iron and copper, or a compound such as acetone which lowers the viscosity of the solution be present. On the other hand, if the mixture containing the monochloramin and ammonia is heated without delay, preferably to the boiling point, or if, either when hot, or when cold, a compound which increases the viscosity of the solution such for instance as glycerin, sugar, starch, casein, albumen, gum, and glue, is present, the first reaction resulting in the production of hydrazin is favored and yields up to from 70 to 80% of the theoretical amount of hydrazin can be obtained. The addition of formaldehyde solution also influences favorably the production of hydrazin, in all probability because of the formation of carbohydrates by the reaction on the formaldehyde of alkali formed during the production of the monochloramin. In all cases an excess of ammonia tends to increase the yield of hydrazin.

The following examples will serve to illustrate further the nature of my invention and the method of carrying it into practical effect, but my invention is not confined to these examples.

Example 1. Add one (1) liter of sodium hypochlorite solution containing about seventy (70) grams of active chlorin to one (1) liter of twenty (20) per cent. ammonia solution, the temperature being preferably kept below fifteen (15) degrees centigrade. The formation of the monochloramin takes place very rapidly and the mixture obtained contains no trace of hypochlorite, but only chloramin and an excess of ammonia. The mixture should without delay be heated to the boiling point, whereupon a reaction according to the equation

$$NH_2Cl + NH_3 = N_2H_4.HCl$$

takes place side by side with the formation of nitrogen. Hydrazin is formed and can easily be isolated in the form of the very difficultly soluble sulfate by boiling away the excess of ammonia, acidifying with sulfuric

acid, and evaporating till crystallization commences. From twenty-five (25), to thirty (30), grams of hydrazin sulfate, that is, from twenty (20), to twenty-five-(25), per cent. of the theoretical yield, are obtained.

Example 2. Stir together three hundred (300) grams of bleaching powder with one (1) liter of twenty (20) per cent. ammonia solution and then rapidly heat the mixture to the boiling point, whereupon considerable quantities of hydrazin are formed. In this case also it can be isolated in the form of hydrazin sulfate by boiling off the excess of ammonia, acidifying with sulfuric acid, filtering off the calcium sulfate formed and evaporating till crystallization commences.

Example 3. Add twelve (12) cubic centimeters of a five (5) per cent. glue solution to three (3) liters of twenty (20) per cent. ammonia solution, then add further one (1) liter of sodium hypochlorite solution containing about seventy (70) grams of active chlorin, and then heat up the mixture finally driving off the excess of ammonia. The residue contains hydrazin which can be conveniently isolated by evaporating the residue and precipitating the hydrazin in the form of its sulfate.

Source: Raschig 1909

Hydrazine Sulfate

Prepared by Roger Adams and B. K. Brown
Checked by J. B. Conant and W. L. Hanaway

$$2NH_3 + NaOCl \rightarrow NH_2NH_2 + H_2O + NaCl$$

1. Procedure

A NORMAL solution of sodium hypochlorite is prepared as follows: in a 5-L. round-bottom flask are placed 1800 g. of sodium hydroxide solution (300 g. of sodium hydroxide to 1500 g. of water) and 1500 g. of ice. Chlorine gas is then passed into the solution until it has gained in weight approximately 213 g. During this addition, the solution must be kept thoroughly cooled with ice, in order that chlorates will not be formed. After all the chlorine has been passed in, it is necessary to be certain that the mixture is slightly alkaline, since any excess of free chlorine in the solution prevents the formation of hydrazine.

In a 14-inch evaporating dish are placed 1500 cc. of c. p. ammonia water (sp. gr. 0.90), 900 cc. of distilled water, 375 cc. of 10 per cent gelatine solution, and 1200 cc. of the normal sodium hypochlorite

solution prepared as above. This mixture is heated as rapidly as possible and boiled down until one-third of the original volume is left. This solution is then cooled thoroughly with ice and filtered with suction, first through two layers of toweling and then through one thickness of ordinary filter paper over cloth, in order to remove finely divided solid impurities. The solution is then placed in a precipitating jar, and cooled down thoroughly (0°) with ice and salt; 10 cc. of concentrated sulfuric acid for each 100 cc. of solution are gradually added with constant stirring.

A precipitate of hydrazine sulfate ($NH_2NH_2.H_2SO_4$) forms. The mixture is allowed to stand in the cold for a few hours in order to complete the precipitation, and is then filtered by suction in the usual way and washed with cold alcohol. The yield varies from 53 g. to 58 g. per 1500 cc. of ammonia water (34-37 per cent of the theoretical amount). The product is perfectly white and crystalline, and satisfactory for almost any purpose. If an absolutely pure product is desired, it must be recrystallized from water. For every 21 g. of crude product, 100 g. of boiling water are used. If the crude hydrazine is brown, it is advisable to use a little bone-black. After the mixture has been filtered and cooled to 0°, 19 g. of pure white crystals are obtained.

2. Notes

In the preparation of the sodium hypochlorite solution it is quite necessary that the mixture be kept cold and be alkaline to red litmus paper at the end of the reaction, if good yields of hydrazine are to be obtained. Since iron is an anti-catalyzer, it is necessary to use distilled water throughout the process. As a viscolizer, a substance such as starch, glycerol, glue or gelatine may be used; the last, however, gives by far the most satisfactory results.

In order to obtain a pure white hydrazine sulfate as the first precipitate, it is necessary to cool the hydrazine solution thoroughly and filter it twice before the sulfuric acid is added. Moreover, the sulfuric acid must be added slowly and with stirring. If these conditions are not followed, material containing brown particles results.

The mother liquor obtained from the crystallized hydrazine sulfate contains a small amount of hydrazine. If 200 g. of copper sulfate are dissolved in water and added to 10 L of the filtrates from the above processes, a light-blue crystalline precipitate of the double salt of copper sulfate and hydrazine sulfate will be formed after ten hours. This salt, when suspended in ten times its weight of distilled water and treated with hydrogen sulfide, decomposes into copper sulfide and hydrazine sulfate. After the copper salt has been filtered off, the solution is

concentrated until the hydrazine sulfate crystallizes. The yield of product is small, so that it is hardly advisable to undertake this recovery in the laboratory.

It is possible for one man, simultaneously evaporating six dishes of the hydrazine mixture, to turn out from 20 to 25 runs in nine hours. The time for the evaporation of a solution, such as is mentioned in the experimental part, with a four-flame Bunsen burner, is two to three hours; if the evaporation is carried out more slowly than this, the yield of product is distinctly diminished.

3. Other Methods of Preparation

Hydrazine salts have been prepared by the action of hypochlorites on ammonia (1) or urea (2) by the hydrolysis of salts of sulfohydrazimethylene disulfonic acid (3) by the hydrolysis of triazoacetic acid (4) by the reduction of diazoacetic ester (5) by the reduction of nitroguanidine followed by hydrolysis (6) by the reduction of the nitroso derivatives of hexamethylene tetramine (7) by the reduction of nitrates or nitrites with zinc in neutral solution (8) by the action of sodium bisulfite on hyponitrous acid followed by reduction (9) by the reduction of K2SO3N2O2 (10) by the action of ammonia on dichlorourea (11) by the reduction of nitrosoparaldimin (12) by the action of copper sulfate on ammonia at high temperatures (13) by the reduction of methylene diisonitrosoamine (14) by the hydrolysis of the addition product of diazoacetic ester and fumaric or cinnamic esters (15).

1) *D. R. P.* 192,783; *Chem. Zentr.* 1908 (1), 427; *Chem. Ztg.* 31, 926 (1907); *D. R. P.* 198,307; *Chem. Zentr.* 1908 (1), 1957; *Eng. Pat.* 22,957; *C.A.* 2, 1999 (1908); *U. S. Pat.* 910,858; *C.A.* 3, 1065 (1909); *French Pat.* 382,357; *C.A.* 3, 2358 (1909); *Ber.* 40, 4588 (1907); *Laboratory Manual of Inorganic Preparations, by* A. B. Lamb, Harvard University, Cambridge, Mass.

2) *J. Russ. Phys. Chem. Soc.* 37, 1 (1905); *Chem. Zentr.* 1905 (1) 1227; *D. R. P.* 164,755; *Frdl.* 8, 53 (1905); *French Pat.* 329,430; *J. Soc. Chem.* Ind. 22, 1063 (1903); *Chem. Zentr.* 1905 (1) 1227.

3) *D. R. P.* 79,885; *Frdl.* 4, 26 (1895); *Ber.* 28, 2381 (1895).

4) *Ber.* 20, 1632 (1887); *Chem. News* 55, 288 (1887); *D. R. P.* 47,600; *Frdl.* 2, 554(1889); *J. prakt. Chem.* (2) 39, 27(1889).

5) *Ber* 27, 775 (1894); 28, 1848 (1895); *D. R. P.* 58,751; *Frdl.* 3, 15 (1891); *D. R. P.* 87,131; *Frdl.* 4, 28 (1896).

6) *Ann.* 270, 31 (1892); *D. R. P.* 59,241; *Frdl.* 3, 16 (1891); *Eng. Pat.* 6,786; *J. Soc. Chem. Ind.* 11, 370 (1892).

7) *D. R. P.* 80,466; *Frdl.* 4, 27 (1895); *Ann.* 288, 232 (1895).

8) *Eng. Pat.* 11,216; *J. Soc. Chem. Ind.* 14, 595 (1895).

9) *Ber.* 33, 2115 (1900); *Ann.* 288, 301(1895).

10) *Ber.* 27, 3498 (1894).

11) *J. Chem. Soc.* 95, 235 (1909); *Chem. News* 98, 166 (1908).
12) *Ber.* 23, 752 (1890).
13) *Chem. News* 66, 223 (1892).
14) *Ber.* 27, 3292 (1894);
15) *Ber.* 21, 2637 (1888).
Source: Adams 1922

Anhydrous Hydrazine

Anhydrous hydrazine can be obtained from hydrazine salts by various procedures. The most simplified involves the use of liquid ammonia and two thermos bottles, under a fume hood.

Liquid ammonia can be obtained by condensing ammonia gas (using a dry ice cold trap) into liquid ammonia.

The liquid ammonia is poured into a thermos bottle. 50 Grams of hydrazine sulfate are gradually added with rapid stirring (mechanical). The solution is stirred for another half hour after addition is complete. The solution is filtered rapidly through a fluted filter paper, any remaining solids are transferred back to the original thermos bottle and liquid ammonia is added and then run through the fluted filter paper again. The combined solutions of liquid ammonia containing anhydrous hydrazine are evaporated to leave colorless (90 plus %) anhydrous hydrazine. The yield is 6 to 8 grams of anhydrous hydrazine.

Anhydrous hydrazine must be stored in a tightly sealed amber bottle. It will remain viable for many years if stored in a cool dark place.

Refs.:

(Adams 1941) (Barber 1948) (Brown 1911) (Elgin 1929) (Friedrichs 1913) (Hurd 1929) (*Organic Syntheses* (1941) 21: 70)(*Organic Syntheses* 24: 53-55) (*Organic Syntheses Col.* (2): 86) (Penneman 1949) (Raschig 1927) (Schenk, P.W.; in *Handbook of Preparative Inorganic Chemistry* (1) 469-472) (Troyan 1953) (Wenner 1932)

Chapter 20
Anhydrous Hydrazine from Hydrazine Hydrate

****

CAUTION!
HYDRAZINE IS A VIOLENT POISON! CAN CAUSE SEVERE LUNG IRRITATION, LIVER AND KIDNEY DAMAGE WHICH MAY NOT APPEAR FOR DAYS. DISTILLATION IN THE PRESENCE OF AIR WILL RESULT IN EXPLOSION!

****

Manufacture of Hydrazine
by Olin Baltimore USA Feb. 6, 1952

Hydrazine, as ordinarily manufactured, is obtained in relatively dilute aqueous solution. Hydrazine and water form a constant boiling mixture so that the dilute solutions obtained cannot be concentrated by ordinary fractional distillation to a hydrazine content greater than about 64% to 70%. In order to obtain higher concentrations of hydrazine by further distillation it is necessary to include in the distill and a dehydrating agent, such as sodium hydroxide, barium oxide, or calcium oxide. In order to obtain higher yields of substantially anhydrous hydrazine and to avoid the hazards of explosion occasioned by high distillation temperatures, such further distillation is carried out at reduced pressure. However, even with such precaution explosions occur. In the absence of oxygen, hydrazine will, under suitable circumstances, decompose violently. Such explosions may be attributed to the presence of small amounts of impurities such as iron oxide, electric sparks, and the like which initiate the autodecomposition of the hydrazine.

It is therefore an object of this invention to provide a safer method for manufacturing hydrazine. It is another object of the invention to provide an economical process for safely producing hydrazine of high concentration without the requirement of reduced pressure during distillation. A further object is to provide an improved economical efficient method for producing substantially anhydrous hydrazine. A still further object is to render hydrazine vapor sufficiently insensitive to electric sparks and the like that mass decomposition thereof is prevented.

The foregoing objects and advantages are attained in accordance with this invention by desensitizing hydrazine with a hydrocarbon. This is accomplished by distilling the aqueous hydrazine solution in contact with a dehydrating agent and the hydrocarbon. Sodium hydroxide is the

preferred dehydrating agent although barium hydroxide, calcium oxide and the like may be used. At least one mole of sodium hydroxide should be included for each mole of water to be removed. When sodium hydroxide is used in an amount equimolar with the water present, a completely liquid system is obtained above a temperature of about 60°C. composed of an upper layer of liquid rich in hydrazine and a lower layer of liquid rich in sodium hydroxide monohydrate. Although the hydrazine may be distilled directly from this mixture, if desired these liquid layers may be separated prior to distillation, with the upper layer being subsequently distilled to recover the hydrazine. The hydrocarbon included during the distillation process in accordance with this invention is a saturated hydrocarbon having a boiling point in the range of about 90°C. to about 150°C. at atmospheric pressure. When the mixture is distilled, the distillate is composed of substantially anhydrous hydrazine and the hydrocarbon. Inasmuch as the liquid hydrocarbon is immiscible with the hydrazine, the distillate separates into two layers and the hydrocarbon layer may be decanted and returned for reuse in concentrating more hydrazine.

It has been found that the hydrazine solution may be distilled safely at atmospheric pressure due to the fact that in the presence of the hydrocarbon the hydrazine distills over at a lower temperature and the fact that the amount of such hydrocarbon vaporized with the hydrazine is sufficient to prevent violent decomposition of the hydrazine, thereby greatly reducing the explosive hazard. Any hydrocarbon which is saturated and boils within the range of about 90°C. to 150°C. at atmospheric pressure is satisfactory for the purpose.

Hydrocarbons having a boiling point substantially above 150°C. are not in general satisfactory for the purpose since the distillation temperature at atmospheric pressure then reaches a level engendering hazard from hydrazine decomposition and the amount of the hydrocarbon distilled over may be insufficient to prevent violent decomposition of the hydrazine in the event of a spark or the like. Hydrocarbons having a boiling point substantially below about 90°C. are not in general suited for the purpose due to the impractically large amount of such hydrocarbon distilled over with a given amount of hydrazine. Minor amounts of such higher and lower boiling hydrocarbons may, of course, be included in a hydrocarbon mixture for the purpose, provided the resulting mixture boils within the range of about 90°C. to about 150°C.

The amount of the saturated hydrocarbon distilled over with the hydrazine will, of course, vary with different hydrocarbons but the hydrocarbons boiling in the range from 90°C. to 150°C. distill over in

ample amount to prevent hazardous decomposition of the accompanying hydrazine.

In the absence of the hydrocarbon even a small amount of air in the hydrazine vapor is sufficient at elevated temperature to initiate a violent explosion. Source: Olin 1956

Hydrazine Hydrate 40-45%

University of Illinois October 9, 1921

$$NH_2NH_2.H_2SO_4+2NaOH \rightarrow NH_2NH_2.H_2O+Na_2SO_4+H_2O$$

l. Procedure

In a 750 cc. copper flask fitted with a cork stopper (covered with tin foil) holding a reflux condenser are placed 200 g. (1 mole) of hydrazine sulfate and 160 g. (2.6 moles) of technical sodium hydroxide. Through the condenser, 75 cc. of water are added gradually (five minutes); the reaction becomes quite vigorous and care must be taken that none of the vapors escape from the condenser. After all the water has been added, the mixture is refluxed for one and a half hours. The reflux condenser is then removed and set downward for distillation. The flask is heated with a free flame and the product is distilled. It is necessary to heat the flask quite strongly toward the end in order to drive over the last of the hydrazine hydrate. The distillate is a clear liquid weighing 170 to 180 g., and contains 40 - 45 percent of hydrazine hydrate ($NH_2NH_2.H_2O$), as shown by titration with standard acid. The actual yield of the hydrazine hydrate present in the solution is 69 to 73 g. (90-95 percent theory).

2. Notes

Glass flasks cannot be used in the preparation because of the high temperature required in the distillation. The amount of water given in the procedure was found to be the most satisfactory. If less water is used, the yield is lowered while more water dilutes the product without increasing the yield.

The product is sometimes contaminated with silicic acid from the glass of the condenser. This may be removed by filtration. All of the corks should be covered with tin foil in order to avoid having them attacked by the hydrazine hydrate.

Hydrazine hydrate may be titrated against standard acid using methyl orange as an indicator, or it may be titrated against iodine with

starch as an indicator. If iodine is used in the titration, 0.1 of a cc. of the hydrazine hydrate solution is diluted with about 100 cc. of water, 2 to 3 cc. of starch solution are added and immediately before titration 5 g. of sodium bicarbonate, Rapid titration with iodine gives a distinct end point. The reaction is:

$$5\ NH_2NH_2.H_2O+2I_2 \rightarrow$$
$$4\ NH_2NH_2.HI+5\ H_2O+N_2$$

Titration by iodine and by acid give the same results, showing that practically no ammonia is present in the hydrazine hydrate prepared by this method.

The method of preparing hydrazine hydrate by treating the sulfate with concentrated aqueous sodium hydroxide, distilling off the water, precipitating the sodium sulfate with alcohol, filtering, distilling the alcohol, then vacuum distilling the residue, gives yields of about 50-60 percent of the theory. The percent of the hydrazine hydrate in the product is about 50 percent. This method has the advantage of requiring only glass apparatus, but it is a little longer and does not give so good a yield as the method described above.

3. Other Methods of Preparation

Hydrazine hydrate has been prepared by the distillation of hydrazine sulfate or hydrochloride with water-free potassium hydroxide (1); by the distillation of hydrazine sulfate with water and potassium hydroxide (2); by treating a concentrated solution of hydrazine sulfate or hydrobromide with concentrated potassium hydroxide, adding alcohol to throw out the potassium salts, filtering the alcoholic solution, concentrating and distilling the residue under reduced pressure (3).

References: 1) *J. prakt. Chem.* (2) 39, 42 (1889).
2) *J. prakt. Chem.* (2) 42, 522 (1890).
3) *Rec. trav. chim.* 14, 82 (1895); 15, 175 (1896).

Source: Hydrazine Hydrate 1921

Preparation of 65% Hydrazine

by Charles D. Hurd and C.W. Bennett
Evanston, Illinois August 3, 1928

A mixture of 150 g. hydrazine hydrate solution containing 41.8% of hydrazine hydrate by weight and 150 g. of xylene was distilled using a 500-cc. flask fitted with a cork and a Hempel column of beads. The dimensions of the column were 35 mm. diameter and 17 cm. length. From the top of this column a side arm led though a condenser into a receiver. Wherever corks were exposed to hydrazine vapors, they were protected by tin foil. The distillation consumed only fifteen minutes and the vapors came over at 92-100 degrees. All the xylene (150 g.) and 62.5 g. of water had distilled over. The watery distillate analysed 10.5% by weight of hydrazine hydrate, whereas the residual solution in the flask, which weighed 85 g., analyzed for 65.2%. In other words, about 87% of the original hydrazine hydrate still remained in the concentrated residue. Source: Hurd 1929

Preparation of Anhydrous Hydrazine

Etat Francais Paris, France Aug. 14, 1958

Hydrazine is generally obtained by reaction in the aqueous phase. It is subsequently concentrated by successive evaporations and rectifications and then, in order to eliminate the water to the maximum extent, one of the methods most recently employed consists in using the dehydrating power of alkaline or alkaline-earth substances, such as caustic soda, potash, or alkaline-earth oxides (quicklime of barium oxide). It is then separated from the dehydrating agent by distillation.

However, this distillation cannot be carried out without extensive precautions, because the vapour of anhydrous hydrazine (an endothermic substance) is in a metastable state and, above a vapour pressure of 39 mms. of mercury, its decomposition, which is started at one point, is propagated throughout the whole of the volume of the gas and gives rise to cracking phenomena which are more dangerous the more violently the gas is brought into contact with air at high temperature, as then the mixture detonates. The starting of the self-decomposition may be caused by any local and/or transitory excitation: overheating at a point, electrical discharge, or by the presence of an oxide reducible by hydrazine (particularly rust).

In order to eliminate this disadvantage and to prevent the propagation of the self-decomposition prior to the dehydrating and distilling it has already been proposed to dilute hydrazine with inert gases such as hydrogen, nitrogen and argon, or with aliphatic hydrocarbons, so that the dehydrating and distilling take place in an inert gaseous atmosphere. Fairly good results are thus obtained, but, however, anhydrous hydrazine at a concentration of only 95% can be obtained by this process.

The object of the present invention is, especially, to obtain anhydrous hydrazine at a concentration of 98—99% at least, with the same safety conditions as when for example aliphatic hydrocarbons are used.

According to the present invention, a process for obtaining and preserving anhydrous hydrazine having a degree of dryness of 98—99% at least, comprises contacting hydrazine with a dehydrating agent, followed by distillation of the resulting mixture, in which the two operations are effected in the presence solely of an unsaturated liquid aromatic hydrocarbon, such for example as benzene, xylene or toluene, or a mixture of any two or the three of these substances.

In the specification of (Olin 1956)—which is concerned with an improved process for concentrating aqueous hydrazine by distilling said hydrazine in contact with a dehydrating agent and a saturated hydrocarbon—it is pointed out that low-cost commercially available saturated hydrocarbons can be used, even if they contain minor amounts of unsaturated aromatics, as such amounts are permissible, but that unsaturated aromatic hydrocarbons in general are not suitable for the purpose, due to their tendency to react with the hydrazine.

In contradistinction to this and to what has hitherto been thought, the Inventors have ascertained that when used according to the process of the present invention hydrazine does not act upon the said unsaturated liquid aromatic hydrocarbons. On the contrary, it has been found that, by their use according to the process of the invention, anhydrous hydrazine can be obtained at a much greater degree of dryness...

... the percentages by weight of benzene, toluene and xylene, on a percentage basis of hydrazine plus hydrocarbon which is necessary to use under atmospheric pressure to eliminate the decomposition phenomenon of N2H4: Benzene 27.9% Toluene 24.0% Xylene 20.0%

It has been observed that if there is introduced into the mixture a third mineral substance without vapour pressure, nothing is changed. There can then be added to the mixture dehydrating agents such as quicklime, barium oxides, caustic soda or caustic potash, it being possible to have the last two dehydrating agents, if necessary, in the form of an approximately saturated aqueous solution.

There will always be safety conditions as long as liquid hydrocarbon is present. The minimum theoretical quantity of hydrocarbon to be used for a given quantity of hydrazine can easily be deduced from the table of partial pressures of inhibitors and anhydrous hydrazine given above. In practice, in order to extract from the dehydrating agents the whole of the hydrazine, the quantity of hydrocarbon used will at all times exceed the minimum theoretical quantity necessary for stabilisation according to the table, (*see actual patent*) so that the partial pressure of the anhydrous hydrazine is well below the maximum safe partial pressure, thus making for greater security.

There is given below, purely by way of illustration, an example of the preparation of anhydrous hydrazine in the presence of benzene.

Example:

600 gms. of quicklime and a litre of commercial benzene are introduced into a two-litre flask. After powerful agitation in order to impregnate the lime, 100 gms. of hydrazine hydrate containing 63% by weight of N2H4 are added. The ternary mixture is powerfully agitated for one hour. There is first produced a slight heating which indicates the hydration of the lime. After homogenisation, the ternary mixture is in the form of a fairly consistent pasty mass. A cooler is connected to the flask and then the distillation of the anhydrous hydrazine and of the benzene is commenced. The temperature is at 78—79°C. for the major part of the distillation. Towards the end of the operation, it is necessary to raise the temperature of the lime paste to 92—96°C., in order to recover the maximum quantity of benzene.

Under these conditions, 95% of the hydrazine introduced is recovered, and this has a concentration of about 99%. In addition 96% of the benzene used, is recovered.

It is interesting to note that the hydrocarbons mentioned have a specific gravity which is less than that of the hydrazine. During the condensation, the liquid hydrazine will always be under a layer of hydrocarbon which will insulate it from contact with the air.
Source: Francais 1963

High Purity Anhydrous Hydrazine

by Harry James Barber of Essex, UK and William Robert Wragg of Hertsfordshire, UK October 17, 1949

Methods heretofore known for the preparation of hydrazine in anhydrous condition were both tedious and uneconomic. It is the object of the present invention to provide a simple process for obtaining anhydrous hydrazine in high yield.

The process of this invention is based upon the discovery that phthalyl hydrazides, sparingly soluble and relatively nonvolatile acids of high melting point, combine with hydrazine to form well-defined nonhydrated salts.

The present invention, therefore, comprises a process for the isolation of anhydrous hydrazine which consists in dissociating an anhydrous phthalyl hydrazide salt of hydrazine, by distillation, preferably under reduced pressure, and collecting the hydrazine thus liberated.

The phthalyl hydrazide salts of hydrazine may be prepared by various methods, e.g. by direct combination of a phthalyl hydrazide and hydrazine.

An important feature of this invention consists in the preparation of anhydrous hydrazine by dissociating the anhydrous hydrazine salt of phthalyl hydrazide itself. This salt can be prepared in anhydrous condition by the addition of ethyl alcohol to an aqueous solution of phthalylhydrazide in excess hydrazine hydrate and drying the resultant precipitate. Heat treatment of the anhydrous salt up to 180°C. under a pressure of 0.05 mm. of mercury in a vacuum distillation apparatus connected to a receiver cooled to about -40°C. gives a 92% yield of anhydrous hydrazine.

Alternatively, the required anhydrous salts may be obtained by reacting a phthalimide with about 2.5 molecular proportions of hydrazine hydrate in boiling ethyl alcohol until all the ammonia formed has been eliminated. From the reaction mixture a precipitate, consisting of the required phthalyl hydrazide salt of hydrazine, separates. Dissociation of the dried salt in the manner hereinbefore described yields the anhydrous hydrazine.

The invention includes within its scope not only the use of phthalyl hydrazide itself but also nuclear substituted derivatives of phthalyl hydrazide.

Phthalyl hydrazide (48.6 g.) was dissolved at 90°C. in aqueous hydrazine hydrate (292 cc. of a 16% w/v. solution; 3.1 mol.). The solution was filtered and stirred into ethyl alcohol (800 cc.). The white bulky precipitated salt was collected at 0°C., washed with ethyl alcohol and dried over caustic potash at 25°C./25 mm., giving 43 g. (74%), m.p. 340-344°C. (Found: N, 28.8%. Calculated for C8H6O2N2, N2H4:N, 28.85%.

In the step of dissociating the phthalyl hydrazide salt there was used an apparatus consisting of an all-glass distillation flask immersed in a heating bath and sealed to a U-shaped receiver connected to an oil pump, there being constrictions in the two limbs of the receiver at which it could be sealed off and removed while still evacuated. Finely powdered anhydrous phthalylhydrazide salt of hydrazine (9.7 g.) was filled into the flask and covered with a plug of glass wool. The apparatus was evacuated to 0.01 mm. and the receiver placed in a solid carbon dioxide-acetone bath. The temperature of the salt was raised slowly to 180°C., by which time the anhydrous hydrazine had collected as a practically colourless solid, 1.47 g. (92%: 99.5% pure by the iodate method described by Kolthoff, *J.A.C.S.* 1924, 46, 2009).

The phthalylhydrazide salt employed in this example: may also be prepared in the following manner:—

Hydrazine hydrate (25 cc. of a 50% w/v. aqueous solution; 2.5 mol.) was added to a suspension of phthalimide (14.7 g.) in boiling ethyl alcohol (178 cc.). After 6 hours refluxing, the white bulky product was collected at 0°C. and washed with ethyl alcohol. The anhydrous salt (17.5 g.; 90%) was obtained by drying this material over caustic potash at 25°C./25 mm. (Found: N, 28.6%).Source: Barber 1949 Ref: Barber 1948

The Stabilisation of Hydrazine

by Olin Baltimore, USA July 8, 1952

Hydrazine has been known for a long time. It has usually been available only in the form of its salts and as hydrazine hydrate, a constant boiling composition comprising approximately 2 parts by weight of hydrazine to 1 part by weight of water. More recently, however, substantially anhydrous hydrazine comprising at least 95% N2H4 has become an article of commerce. Hydrazine hydrate is comparatively stable in storage and use compared to 95% hydrazine. The latter may decompose in storage, particularly in the presence of certain metals or metal compounds. Stabilisation of the concentrated hydrazine with respect to decomposition is important in order to avoid extensive losses and hazard in manufacture, in storage and in use.

It has now been discovered that the stability of concentrated hydrazine, particularly as prepared by the Raschig process and its corrosiveness to metals are associated with a property most simply explained as its potential acidity which can be neutralised, treating the hydrazine as the dissociating medium or solvent, by the addition of certain substances which are weakly basic and which are non-reactive with hydrazine.

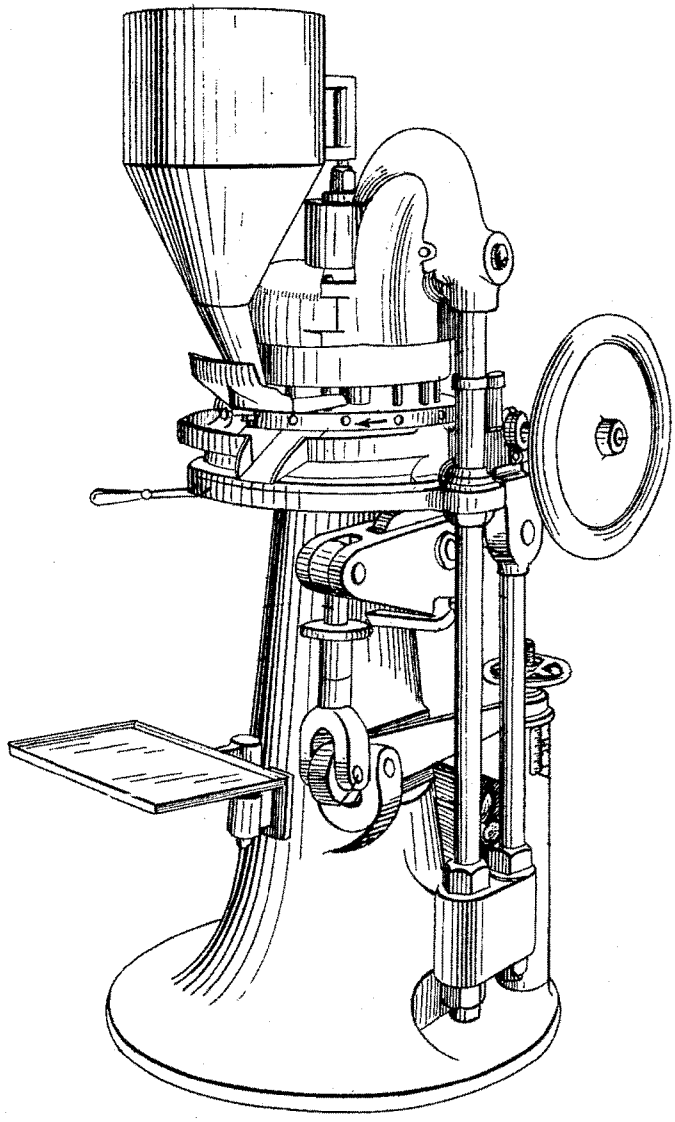
According to the invention, there is provided a method of stabilising concentrated hydrazine against decomposition, which comprises adding to concentrated hydrazine magnesium, calcium, zinc or aluminium, or an oxide or a carbonate of such metal. It has been found that the potential acidity causing undesirable instability and corrosiveness in concentrated hydrazine can be satisfactorily neutralised and buffered by such an addition. Hydrazine solutions of a greater concentration than hydrazine hydrate up to substantially anhydrous 100% hydrazine may be stabilised by this means.

Suitable proportions of zinc oxide, which is a particularly effective substance, may vary from 0.2 to 5% by weight. Ordinarily about 1% of zinc oxide gives satisfactory results. Less than the lower limit recited may be insufficient and more than the upper limit does not appear to be necessary. This zinc oxide may be a chemically pure grade or may be an ordinary commercial grade.

The zinc oxide may be added to the hydrazine during manufacture to prevent its decomposition at any stage where the concentration is greater than that of hydrazine hydrate. It may be added to finished 95% hydrazine as it is loaded into drums or tank cars for storage or shipment. The insoluble excess zinc oxide is easily removed by settling and decanting or by filtration, and the hydrazine product may be distilled prior to use if necessary or desirable.

...in the absence of metals 96.8% hydrazine is a relatively stable substance even when heated at its boiling point under atmospheric pressure. Even so, the addition of 2% of zinc oxide reduces the apparent rate of decomposition to about 1/30 of that obtained in the absence of zinc oxide. Source: Olin 1955 Reference: Olin 1957

HYDRAZINE CAS No.: 302-01-2 Chemical Formula: H4N2 *PHYSICAL DESCRIPTIONS: Colorless oily liquid - fuming. White crystals in air. Penetrating ammonia odor *MW: 32.05 *MP 1.4°C *BP 113.5°C *SOLUBILITIES: Water: Miscible; Alcohol: Miscible; Chloroform: Insoluble; ETHER : Insoluble *Flash point: 38 C degrees. Flammable. Fires involving this chemical should be extinguished with alcohol foam, carbon dioxide, and/or dry chemical extinguishers. A fire in your laboratory involving this chemical should be extinguished with a dry chemical, carbon dioxide or halon extinguisher. *Disaster hazards: Dangerous when heated to decomposition: Emits highly toxic fumes of Nitrogen compounds; May explode by heat or chemical reactions with alkali metals. *REACTIVITY: Dangerous when exposed to heat, flame or oxidizing agents. Violent poison. Burns with a violet flame. Explodes during distillation if traces of air are present affected by uv and metal ion catalysis. Powerful reducing agent; Weak base. *SAX TOXICITY EVALUATION: High via oral, intravenous, and dermal routes. Systemic poisoning. Damage to liver and destruction of red blood cells. An experimental carcinogen of lung, nervous system, liver, kidney, heamatopoietic organs, breast, and subcutaneous tissue. *Recommended Exposure Limit to this compound-air: Ceiling Limit 0.03 ppm/120M *USES: Rocket fuel and jet fuel, cleaning agent, rubber chemical, drugs, agricultural chemicals. *MINIMUM PROTECTIVE CLOTHING: If Tyvek-type disposable protective clothing is not worn during handling of this chemical, wear disposable Tyvek-type sleeves taped to your gloves. * SUGGESTED GLOVES: Butyl rubber, Nitrile, PVC, Neoprene *RECOMMENDED RESPIRATOR: When working with this chemical, wear a NIOSH-approved full face positive pressure supplied-air respirator or a self-contained breathing apparatus (SCBA). *STORAGE PRECAUTIONS: You should keep this material in a tightly-closed container under an inert atmosphere, and store it at refrigerated temperatures. *SPILLS: If you should spill this chemical, use absorbent paper to pick up all liquid spill material. Seal the absorbent paper, as well as any of your clothing which may be contaminated, in a vapor-tight plastic bag for eventual disposal. Wash any surfaces you may have contaminated with a strong soap and water solution. Do not reenter the contaminated area until the Safety Officer (or other responsible person) has verified that the area has been properly cleaned. *DISPOSAL AND WASTE TREATMENT: It is suggested that your contaminated materials should be destroyed by incineration in a special, high temperature (>2000 degrees F), chemical incinerator facility. *SKIN CONTACT: IMMEDIATELY flood affected skin with water while removing and isolating all contaminated clothing. Gently wash all affected skin areas thoroughly with soap and water. IMMEDIATELY call a hospital or poison control center even if no symptoms (such as redness or irritation) develop. IMMEDIATELY transport the victim to a hospital for treatment after washing the affected areas. *INHALATION: IMMEDIATELY leave the contaminated area; take deep breaths of fresh air.*EYE CONTACT: First check the victim for contact lenses and remove if present. Flush victim's eyes with water or normal saline solution for 20 to 30 minutes while simultaneously calling a hospital or poison control center. Source: NTP Chemical Repository; Last revised: 8/13/01



Chapter 21
Tablet and Clearlight Manufacture

LSD has been dispersed in numerous forms. Delysid (LSD-25) from Sandoz was distributed in ampules and also tablets containing 100 μg.. LSD that appeared outside of research circles was usually triturated (dosed) on sugar cubes and in tablet form (both as compressed tablets and molded tablets) of many sizes and colors. LSD that is dispersed into films (clearlight, window pane) of gelatin, agarose or cellulose also appears in a variety of colors and shapes. The most common form of LSD appears in blotter paper form.

During the nineteen sixties dosages of LSD were extremely high (e.g. 500 μg. Owls, sugar cubes). Today the blotter form (which is most prevalent) of LSD ranges from approximately 20 to 80 μg.

The shape, color and dosage are the trademark of the laboratory.

Tablet Manufacture in History
by Joseph R. Wood

The present era of compressed-tablet making dates back but a few years, (ref. 1) and during this period the quality of the tablets, as made by the highest exponents of the art, has gradually improved until it has reached a high standard. In the early stages, the chief problem was to construct a machine which would economically compress powders or granulations. When, after a number of years, this was accomplished, compressed tablets were on a commercial basis; that is, they could be made to sell at a profit to the manufacturer. From that time to the present, countless different machines have been constructed, many of them cumbersome and defective in other ways, until today a number of machines are well-nigh perfect.

The early tablets (and, unfortunately, many of to-day may be classed with them) were compressed hard, and made without reference to their solubility or to their power to disintegrate, and little skill was required in their preparation. On the other hand, the proper manipulation of the medicinal ingredients, and the choice, proportioning, and manipulation of excipients best suited to use with the different formulas, require a considerable degree of skill, as well as an intimate knowledge of the physical and chemical properties of the ingredients. During

the past fifteen years, a great advance has been made in respect to solubility and disintegration of tablets, and at the present time there are on the market many tablets closely attaining perfection.

Compressed tablets are believed to have been made first in 1844, by Professor Brockeden, in England, who, in using a machine for the compression of lead for use in pencils, conceived the idea that the same principle could be applied to the compression of drugs and chemicals into tablet form. He thus compressed potassium bicarbonate and sodium bicarbonate. These tablets had a considerable sale both in England and in this country, where they were sold by Mr. Frederick Brown, of Philadelphia.

In 1871 Professor Brockeden's business was purchased by the Messrs. Newbery.

About this time, Mr. Jacob Dunton, of Philadelphia, began to compress a variety of formulas, including quinine tablets, on a machine of the Brockeden pattern.

Shortly after this, about 1872, Messrs. John Wyeth and Brother, in conjunction with Mr. Henry Bower, succeeded in producing a machine which, while still a handpress, was so much of an advance over the previous patterns, that the cost of compression was materially reduced, and the resulting tablets were successfully exploited.

Since that time, the preparation of compressed tablets has been taken up by a host of manufacturers and pharmacists.

The manufacture of moulded tablets is a still more modern art. The idea of filling moulds with medicated milk sugar was first proposed by Dr. Robert M. Fuller, of New York, who, on February the twenty-first, 1878, read a paper presenting the subject before the American Medical Society. Some time later, Dr. Fuller laid the details before Mr. Horatio N. Fraser, who was at that time in charge of the prescription department of a large New York pharmacy. Dr. Fuller requested that nothing concerning the tablets or their manufacture be made the subject of a patent, in order that all pharmacists should be free to make the tablets.

After vain attempts to induce his employers to embark in the new enterprise, Mr. Fraser started, in 1883, in an extremely modest way, to manufacture moulded tablets; with what result, every pharmacist knows.

It is worthy of note, that, after more than twenty years, inventors have yet to perfect a machine which will successfully replace the moulds of hard rubber and the spatulas with which the best tablets are still made.

Compressed Tablets

Compressed Tablets (abbreviated C.T.) are small bodies made by the compression of medicinal substances by appropriate apparatus, usually a compressing machine. They are of various shapes: cylindrical, square, octagonal, oval, etc., and with convex, flat, or other form of upper and lower surfaces. The usual form is cylindrical, with convex upper and lower surfaces. They range in size from one-eighth inch in diameter to three-fourths inch or more. Those having a diameter of 7-32 inch or less are termed Compressed Triturate Tablets (C.T.T.), and in most cases contain one grain or less of medicament. Tablets having properties characteristic of lozenges are termed Compressed Lozenges.

Properly made Compressed Tablets possess the following properties:

1. Accuracy of dose.
2. Perfect subdivision of ingredients.
3. Uniformity in weight and appearance.
4. Rapid solubility if composed of freely soluble substances.
5. Rapid disintegration when immersed in tepid water, if composed of difficultly soluble or insoluble substances.
6. Sufficient firmness to prevent crumbling or wearing away of edges when ordinarily handled.

Most compressed tablets require in their preparation a certain routine, briefly described as follows: The ingredients are mixed, moistened, forced through a sieve to form granules, and dried. The dry granulation is then lubricated and compressed into tablets.

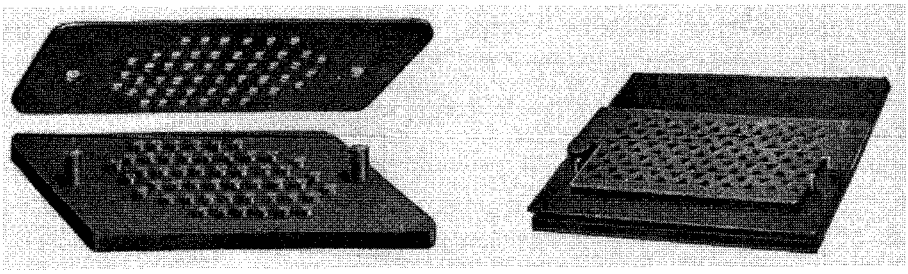
These steps are conveniently considered separately and will be taken up in order:

1. Triturating.
2. Mixing, Granulating, and Drying.
3. Lubricating.
4. Compressing.

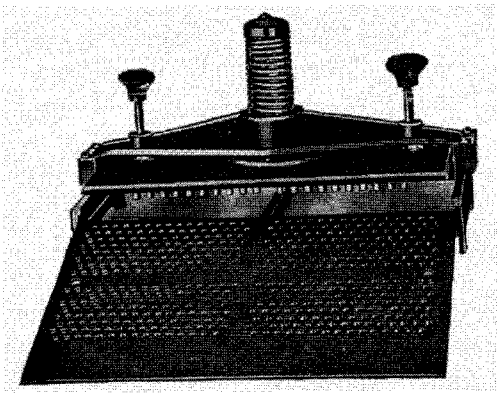
ref. 1. "Stamps have been found in England which have been shown to have been used by the Romans to stamp remedies for producing clearness of vision, or for doing away with dimness of sight. The object aimed at by the medicament was specified in the stamp. It is noteworthy that the stamps so far discovered were designed for remedies for ocular diseases. The preparations were hardened with gum or some viscid substance and were thus ready to be liquefied at any time. Thus our supposedly very modern device of triturates or compressed tablets is only a revival of an ancient Roman custom." (American Medicine.)

Source: Wood 1906 see also *Tablet Making* by Arthur Little

Molded Tablets (Tablet Triturates)



Tablet Triturate Machine



Tablets can be molded using a mixture of sucrose, lactose and/or dextrose. A predetermined amount of active ingredient is mixed with a pre-weighed amount of tablet mixture. This mixture is pushed onto the upper plate mold and tablets are ejected from the mold by gently pressing it onto the pegboard and allowed to dry.

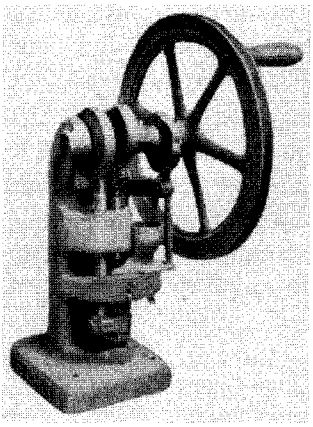
The hardness of a tablet is manipulated by adjusting the constituent proportions of sugars. Two forms of these tablets can be made; one is called a hypodermic tablet, it easily dissolves in water and is not a hard tablet. These types of tablets break down (mechanically) very easily during storage and transport. The second type is harder and retains its shape in transport. A general formula for hard tablets appears in Remington's Practice of Pharmacy: five parts lactose to one part sucrose. The composition is moistened with 70% alcohol and mixed thoroughly.

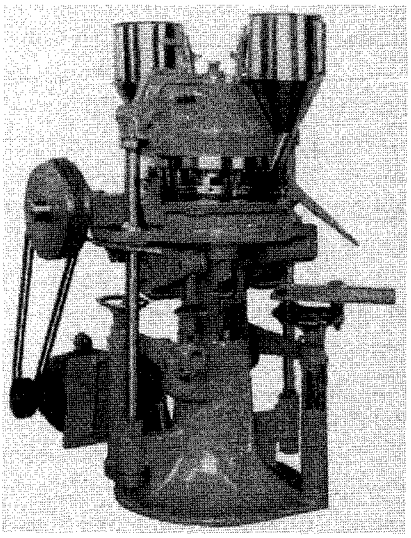
When the mixture is moistened too much, the consistency is too liquid to form tablets; the tablets come out looking like a blob of gooey dough. If the mixture is not moistened enough the tablets will crack and fall apart.

Trituration of Tablets

The upper plate is placed on a flat surface. The mixture (consistency of thick paste) is then pushed into the perforations on the upper plate with a spatula and the excess scrapped off. It is then dusted with a fine powder of sucrose and allowed to dry. When the tablet sheet is almost dry, the plate is pressed onto the lower peg board to push the tablets through the perforations (pegs must be higher then thickness of mold). The tablets are allowed to dry a little further (not completely) on the top of the pegs and then are poured into a container for complete drying.

Tablets were invented by Brockedon in 1843. By 1894 almost every known disease or affliction was being 'treated' with worthless ineffective tablets. Fraud was a common practice. Physicians began making their own tablets for patients to guarantee the quality and standardize the dosage of constituents in tablets.





Compressed Tablets

Compressed tablets are formed with a tablet machine. The LSD is diluted into sugar, binder and lubricants and "punched" into tablets. LSD in this form was most prevalent during the 1960's thru 1980's. The cost of tablet equipment, size and weight (tonnage) tends to make this form prohibitive for security reasons. Tablets take up space and can not be easily concealed. Transportation of large quantities of tablets is subject to discovery by law enforcement and breakdown of tablets.

Tableting Lysergic Acid Amides

by Albert Hofmann, Franz Troxler; Hans Ott Hanover, New Jersey February 7, 1963

Tablets are prepared by mixing together:

d-Lysergic acid-(+)-hexanolamide-(2') 500 mg.
Lactose 29 g.
Starch 20 g.

and passing the mixture through a fine sieve. Stearic acid (500 mg.) is then mixed in and the mixture is compressed in tabletting dies to form 1000 tablets weighing 50 mg. each.
Source: Hofmann 1967

Blotter Carrier

"LSD most often is found in the form of small paper squares or, on occasion, in tablets. On occasion, authorities have encountered the drug in others forms—including powder or crystal, liquid, gelatin square, and capsule—and laced on sugar cubes and other substances. LSD is sold under more than 80 street names including acid, blotter acid, doses, and trips, as well as names that reflect the designs on sheets of paper. More than 200 types of LSD tablets have been encountered since 1969 and more than 350 paper designs have been acquired since 1975. Designs range from simple five-point stars in black and white to exotic artwork in full four-color print. Inexpensiveness (prices range from $2 to $5 per dosage unit or "hit,"; wholesale lots often sell for as little as $1 or less), ready availability. Alleged "mind-expanding" properties, and intriguing paper designs make LSD especially attractive to junior high school and high school students." Source: DEA Oct 1995

"The printed sheets are dipped into shallow pans containing LSD crystal dissolved in methanol, ethanol, or other solvent (water can be used; however, its slower evaporation rate increases the likelihood of degradation) and then are laid out or hung up to dry. The printing inks generally are insoluble in the solvents to ensure that the image does not run. Because this production procedure is inexact, the potency of LSD can vary from sheet to sheet and even from square to square."

DEA Oct 1995

"LSD that has been applied in solution onto sugar cubes or blotting paper - decompose in the course of weeks or a few months,"

Albert Hofmann

Thin Film Carrier: "Clearlight" or "Gel"

Clearlight, also called window pane, has appeared in a carrier of small film pyramids (many colors). This form was achieved by spraying a mixture of jelling agent on to plastic light covers of small pyramids. Small film squares (tiles) appeared containing 210 μg. of LSD salt (1974).

Preparation of Clearlight Carrier: "Sheeting"

Clearlight is formed by several different ways. A mixture of an appropriate solvent is heated with a jelling agent (more glycerin makes sheets more flexible). The jelling mixture is sprayed on to plastic molds or sheeted using a apparatus that makes thin layer films for chromatography applications. The heated solution can also be poured onto waxed glass or a porcelain plate, allowed to cool, peeled and cut.

The gel sheets are then absorbed with a solvent containing a predetermined amount of LSD and dried. The individual dosages can be cut using a large paper cutter or agarose film cutter (eg. large pasta machine along with a large paper cutter).

The tiles are cut into plastic garbage barrels, scale weighed into units of 103 tiles per mini-plastic vial (1974).

Tiles that miss the barrel are picked up at clean up with the quick wave of a UV light (Trout 2002). A small shop vacuum works fine.

Lamellae

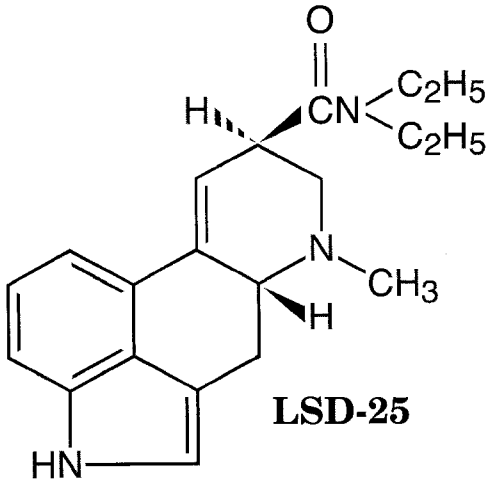
Lamellae also called lamels or eye discs is a small medicinal gelatin disc containing a specific amount of a drug.

Formulas in parts by weight:

	Gelatin	Water	Glycerin
Lamel	9 parts	44 parts	1 part
Gelatin Capsule	1 part	2 parts	1 part.
Gelatin Capsule	16 parts	20 parts	15 parts.

Reference: *Formulas For Profit* 1939

"LSD is an unusualy fragile molecule... As a salt, in water, cold, and free from air and light exposure, its is stable indefinitely... Oh yes, and often overlooked, there may be only an infinitesimal amount of chlorine in treated tap water, but then there is only an infinitesimal amount of LSD in a typical LSD solution. And since chlorine will destroy LSD on contact, the dissolving of LSD in tap water is not appropriate," Shulgin in *TIHKAL*.



LSD-25

Suggested Reading

Aaronson, B. ed.: *The Use of LSD in Psychotherapy; Transactions of a Conference on d-Lysergic Acid Diethyalmde (LSD-25) April 22, 23 and 24, 1959*; Josiah Macy, Jr. Foundation; (1960) LC: 60-14409

Aaronson, B. ed.; *The Use of LSD in Psychotherapy and Alcoholism*; The Bobbs-Merrill Co., Inc. (1967) LC: 66-25284

Aaronson, B.; Osmond, H.; *Psychedelics; The Uses and Implications of Hallucinogenic Drugs*; Anchor Books; (1970) LC: 70-103788

Barnett, G.; Trsic, M.; Willette, R. eds.; *'QuaSAR' Research Monograph No. 22, ed.*; NIDA (1978).

Bennett, H.; *Formulas For Profit*; World Publishing (1939)

Bishop, M.G.; *The Discovery of Love; A Psychedelic Experience with LSD-25*; A Torquil Book (1963) LC: 63-19719

Black, D; *Acid: The Secret History of LSD*; Vision Paperbacks (1998) ISBN: 1-901250-11-3

Blum, R. & Associates; *Utopiates*; Atherton Press; (1964) LC: 63-23746

Bove, F.J.; *The Story of Ergot*; S. Karger (1970)

Bureau of Justice Assistance; *Developing a Strategy for Multiagency Response to Clandestine Drug Laboratories*

Caldwell, W.V.; *LSD Psychotherapy; An Exploration of Psychedelic and Psycholytic Therapy*; Grove Press (1968) LC: 68-22006

Calne, D.B.; Horowski R.; McDonald, J.; Wuttke, W.; *Lisuride and Other Dopamine Agonists*; Raven Press (1983) ISBN: 0-89004-867-3

Cavanna, R.; Ullman, M.; *PSI and Altered States of Consciousness*; Garrett Press (1968) LC: 68-8909

Cayce, H.L.; *Venture Inward*; Harper & Row; 1964; LC: 64-10284

Chase, A.; *The North American Species of Paspalum*; Contributions from the United States National Herbarium; Volume 28, Part 1; 1929; United States Government Printing Office

Cholden, L.; *Lysergic Acid Diethylamide and Mescaline in Experimental Psychiatry*; Grune & Stratton (1956) LC: 56-8731

Christensen, C.M.; *Molds, Mushrooms and Mycotoxins*; Minn. Press (1975) ISBN: 0-8166-0743-5

Cohen, S.; *The Beyond Within: The L.S.D. Story*; pub. by Atheneum; LC: 64-25848

Cook, E.F.; LaWall, C.H.; *Remington's Practice of Pharmacy (1936)*; J.B. Lippincott Co.

DeBold, R.C.; Leaf, R.; *LSD, Man & Society* (1967) Wesleyan University Press; LC: 67-24111

Dunlap, J.; *Exploring Inner Space*; Harcourt Brace and World (1961) LC: 61-8979

Efron, D.H.; Holmstedt, B.; Kline, N.S. eds.; *Ethnopharmacologic Search for Psychoactive Drugs; Proceedings of a Symposium held in San Francisco, CA 1/28-30/1967*; Sponsored by the Psychopharmacology Research Branch, NIMH.
Efron, D.E.; Usdid, E.; eds.; *Psychotropic Drugs and Related Compounds, 2 nd Ed.*; U.S. Dept. of Health, Education and Welfare; NIMH
Fuller, J.G.; *The Day of St. Anthony's Fire*; Signet Books; (1968)
LC: 68-23632
Gardner, T.J.; Manian, V.; *Principles and Cases of the Law of Arrest, Search, and Seizure*; McGraw-Hill Book Company; (1974) ISBN: 0-07-022837-X
Garattini S.; Ghetti, V. eds.; *Psychotropic Drugs;* Elsevier Publishing Co. (1957) LC: 57-13476
Gowan, J.C.; *Development of the Psychedelic Individual*; Colleague, Creative Education Foundation (1974)
Grof, S.; *LSD Psychotherapy*; Borgo Press (1986) ISBN: 0-8095-6300-2
Grof, S.; *LSD Psychotherapy: Exploring the Frontiers of the Hidden Mind;* Hunter House (1994) ISBN: 0-89793-158-0
Grof, S.; *Realms of the Human Unconscious; Observations from LSD Research;* Viking Press (1975)
Grof, S.; Halifax, J.; *The Human Encounter with Death*; E.P. Dutton Pub. (1977)
Henderson, L.A.; Glass, W.J.; *LSD: Still With Us After All These Years*; Lexington Books, (1994)
Hofmann, A.; *LSD, My Problem Child*; McGraw-Hill; (1980)
ISBN: 0-07-029325-2
Hofmann, A.; *Insight Outlook*; Humanics; (1986) ISBN: 0-89334-191-6
Ka-tzetnik; *Shivitti: A Vision*; Gateways Consciousness Classics (1998) ISBN: 0-89556-113-1
Kren, V.; Cvak, L.; *Ergot: The Genus Claviceps*; Harwood Academic Publisher (1999) ISBN: 90-5702-375-X
Leary, T.F.; *High Priest*; The World Publishing Co. (1968) LC: 68-9031
Leary, T.; Metzner, R.; Alpert, R.; *The Psychedelic Experience: A Manual Based on the Tibetan Book of the Dead*; University Books (1964) LC: 64-19705
Leary, T.; *Neurologic* (1973)
Lee, D.; Pratt, C.; *Operation Julie; How the Undercover Police Team Smashed the World's Greatest Drugs Ring* (1978)W.H Allen & Co.Ltd.;ISBN:0491021763
Lee, M.; *Acid Dreams* (1982) Grove Press: ISBN: 0-8021-3062-3
Lin G.C.; Glennon, R.A. eds; *Hallucinogens: An Update*; Research Monograph 146; (NIDA) 1994: NIH Pub. No. 94-3872
Ling, T.M.; Buckman, J.; *Lysergic Acid (LSD 25) and Ritalin in the Treatment of Neurosis*; Lambarde Press (1963)
Linkletter, A.; *Drugs at My Door Step*; Word Books (1973) LC: 73-84575
Lipton, M.A.; DiMascio, A.; Killam, K.F., eds.; *Psychopharmacology a Generation of Progress*; Raven Press; 1978; ISBN: 0-89004-191-1
Little, A.; Mitchell, K.A.; *Tablet Making* (1949) The Northern Publishing Co., Ltd.

Lyttle, T.; *Psychedelic Monographs and Essays 5*; PM & E Publishing Group; (1990) ISSN: 0-892-371X
Lyttle, T.; *Psychedelics ReImagined*; Autonomedia; (1997)
Marks, J.; *The Search for the Manchurian Candidate; The CIA and Mind Control;* Dell Publishing; (1979) ISBN: 0-440-20137-3
Newland, C.A.; *My Self and I*; Coward-McCann, Inc. (1962) LC: 62-10953
Ogg, B.A.; *The Chemistry of Hydrazine*; Audrieth, L.F.;Wiley (1951)
Osol, A.; Farrar, G.E.; *The Dispensatory of the United States of America 24th ed.*; (1947); J.B. Lippincott Company
Ott, J.; *Pharmacotheon; Entheogenic Drugs, Their Plant Sources and History;* Natural Products Co. (1993); ISBN: 0-9614234-3-9
Pletscher, A.; Landewig, D.; eds.; *50 Years of LSD* (1993) The Partheon Publishing Group; ISBN: 1-85070-569-0
Ratsch, C.; *Gateway to Inner Space*; Prism Press; (1989); ISBN: 1-85327-037-7
Ridgway, K.; *Hard Capsules; Developement & Technology*; The Pharmaceutical Press (1987) ISBN:0 85369 159 2
Sand, N.; *Psychedelic Secrets* (available soon).
Schultes, R.E,; *Hallucinogenic Plants*; Golden Press; (1976)
LC: 74-21666
Schultes, R.E.; Hofmann, A.; *The Botany and Chemistry of Hallucinogens*; Charles C. Thomas Publisher (1980); ISBN: 0-398-03863-5
Shulgin, A.; Shulgin, A.; ed. by Joy, D.; *TIHKAL; The Continuation;* Transform Press; (1997); ISBN: 0-9630096-9-9
Snow, O.; *Amphetamine Syntheses: Industrial Edition*; Thoth Press (2002); ISBN: 0-9663128-3-X
Soloman, D.; *LSD; The Consciousness-Expanding Drug* (1964) G.P. Putnam's Sons; LC: 64-18016
Stahl, E.; *Thin-Layer Chromotography: A Laboratory Handbook; ed.*; Acedemic Press; (1965); LC: 63-19323
Stevens, J.; *Storming Heaven: LSD and the American Dream;* Harper Collins Pubs. (1988) ISBN: 0-06-097172-x, PL-7172, PL
Stillman & Willette eds.; *Psychopharmacology of Hallucinogens*, NIDA, Pergamon Press, 1978, ISBN:0-08-021938-1
Szára, S., ed.; *LSD - A Total Study*; PJD Publications (1975)
ISBN: 0-9600290-3-6
Tendler, S.; May, D.; *The Brotherhood of Eternal Love*; Panther Books (1984) ISBN: 0-586-04909-6
Thomas, G.; *Journey into Madness; the True Story of Secret CIA Mind Control and Medical Abuse*; Batam Books; (1989) ISBN: 0-553-28413-4
Wasson, R.G.; Ruck, C.A.; Hofmann, A.; *Road To Eleusis: Unveiling the Secret of the Mysteries*; Harcourt Brace Jovanovich; (1978) ISBN: 0-15-525279-1
Watts, A.W.; *The Joyous Cosmology*; Vintage Books; (1962) LC: 62-100080; ISBN: 394-70299-9
Wood, J.; R.; *Tablet Manufacture; Its History, Pharmacy and Practice* (1904) J.B. Lippincott Company

Bibliography

Abou-Chaar, C.I., Brady, L.R., Tyler V.E.; Occurrence of Lysergic Acid in Saprophytic Cultures of *Claviceps*; *Lloydia* (1961) 24 (2): 89-93

Abou-Chaar, C.I., Gröger, D., Brady, L.R., Tyler V.E.; Interactions of Ergot Strains in Saprophytic Culture; *Lloydia* (1961)24: 159-167

Abramson, H.A.; Some Observations on Normal Volunteers and Patients in Lysergic Acid Diethylamide and Mescaline in *Experimental Psychiatry* (1956)

Abramson, H.A.; Lysergic Acid Diethylamide (LSD-25): XXIX. The Response Index As a Measure of Threshold Activity of Psychotropic Drugs in Man; *J. Psych* (1959) 48: 65-78

Abramson, H.A.; Sklarofsky, B.; Baron, M.O.; Fremeont-Smith, N.; Lysergic Acid Diethylamide (LSD-25) Antagonists; *Arch. of Neurology and Psychiatry* (1958) 79: 201-207

Adams, R.A.; Ergot Alkaloids; *US Patent* 3,117,917; *Chemical Abstracts* (1964) 11345 a-b

Adams, R. and B. K. Brown, B.K.; Hydrazine Sulfate; *Organic Syntheses* Vol. 2 (1922)

Adams, R., Brown, B.K.; Hydrazine sulfate; *Organic Syntheses Col.* (1941) (1) 309-310

Agurell, S., Ramstad, E.; Analysis of Clavine Alkaloids of *Pennisetum* Ergot; *Lloydia* (1962) 25: 67-77

Alnaes, R.; Therapeutic Application of the Change in Consciousness Produced by Psycholytica (LSD, Psilocybin, etc.) in *The Use of LSD in Psychotherapy and Alcoholism* (1967)

Axelrod, J.; Brady, R.O.; Witkop, B.; Evarts, E.V.; The Distribution and Metabolism of Lysergic Acid Diethylamide. *Ann. N.Y. Acad. Sci.*, 1957, 66: 435

Amici, A.; Minghetti, A.; Scotti, T.; Spalla, C.; Process for the Preparation of Ergotamine and Ergocryptine; 1972; *US* 3,658,653

Amici, A.M., Minghetti, A., Scotti, T., Spalla, C., Tognoli, L.; Ergotamine Production in Submerged Culture and Physiology of *Claviceps purpurea*; *Applied Microbiology* (1967) 15 (3): 597-602

Amici, A.M., Minghetti, A., Scotti, T., Spalla, C., Tognoli, L.; Production of Peptide Ergot Alkaloids in Submerged Culture by Three Isolates of *Claviceps purpurea*; *Applied Microbiology* (1969) 18: 464-468

Amici, A.M., Minghetti, A, Tonolo, A., Spalla, C.; Preparation of Lysergic acid; *Chemical Abstracts* (19??) 15314 e-g

Amici, A.M., Scotti, T., Spalla, C., Tognoli, L.; Heterokaryosis and Alkaloid Production in *Claviceps purpurea*; *Applied Microbiology* (1967) 15 (3): 611-615

Arcamone, F.; Chain, E.B.; F.R.S.; Ferretti, A.; Minghetti, A.; Pennella, P.; Tonolo, A.; Vero, L.; Production of a New Lysergic acid Derivative in Submerged Culture by a Strain of *Claviceps paspali* Stevens & Hall; *Proc. Roy. Soc. (London)* (1961) Ser. B 155: 26-54

Bankovskii, A.I., Composition of Ergot Alkaloids of an Ergotamine Strain; Lekarstv. Rasteniya 1969, No. 15, 368-375 (Russ.); *Chemical Abstracts* (1971) 137422g

Barbeau, A.; The Last Ten Years of Progress in Clinical Pharmacology of Extrapyramidal Symptoms, pgs. 771-776; in *Psychopharmacology a Generation of Progress* (1978).

Barber, H.J., Wragg, W.R.; A Convenient Laboratory Preparation of Anhydrous Hydrazine; *Journal of the Chemical Society* (1948) 1458

Barber, H.; Wragg, W.; Improvements in or relating to the preparation of Hydrazine; 1949 *GB* 620,713

Barnett, B.E.W.; Diazepam Treatment for L.S.D. Intoxication; *Lancet* (1971) 2: 270.

Berman, M.L.; Youngken, H.W.; The Use of Certain Rye Homogenates for Supporting Growth of *Claviceps purpurea* (Fries) Tulasne; *Journal of the American Pharmaceutical Association* (1954) 43: 200-204.

Berthelot; *Ann. Chim. Phys.*; 1853 [v], 23, 317

Bishop, G.A.; *The Discovery of Love* ; Dodd and Mead Co.; (1963)

Bonns, W.; A Preliminary Study of *Claviceps purpurea* in Culture; *American Journal of Botany* (1922) 9: (7) 339-353.

Borowski, E; Braun, K.; Breuel, K; Dauth, C.; Erge, D.; Grawert, W.; Gröger, D.; Höhne, L.; Knothe, E.; Müller, M.; Nordmann, G.; Schirutschke, R.; Volzke, K.; Process for the Fermentation Production of Ergoline Derivatives; 1978; *US* 4,086,141

Bottoms, R.R.; Production of Alkylamines; 1937; *US* 2,085,785

Bottoms, R.R.; 1937a; Process of Separating Lower Alkylamines; *US* 2,085,786

Bottoms, R.R.; 1937b; Process of Separating Lower Alkylamines; *US* 2,085,787

Brady, L.R., Tyler, V.E.; Alkaloid Accumulation in Two Clavine-Producing Strains of *Claviceps*: *Lloydia* (1960) 23 (1) 8-20

British Celanese Limited; Improvements in the Production of Saturated Aliphatic Secondary and Tertiary Amines; 1951; *GB* 656,154

Brown, A.W., Houlehan, A.E.; Behavior of the Hydronitrogens and Their Derivatives in Liquid Ammonia 2. Ammonolysis of Certain Hydrazine Salts; *Journal of the American Chemical Society* (1911) 33: 1734-1742

Brown, A.W., Welsh, T.W.; Behavior of the Hydronitrogens and Their Derivatives in Liquid Ammonia 1. Ammonolysis of Hydrazine Sulfate; *Journal of the American Chemical Society* (1911) 33: 1728-1734

Brown, H.B.; Life History and Poisonus Properties of *Claviceps paspali*; *Journal of Agricultural Research* (1916) 7: (2) 401-407

Buckman, J.; Theoretical Aspects of LSD Therapy in *The Use of LSD in Psychotherapy and Alcoholism* (1967)

Burton, G.; LeFebvre, C.L.; Ergot Sterility in Bahia Grass; *Phytopathology* (1948) 38: 556-559.

Case No. 00-40104-01/02/RDR; USA vs. William Leonard Pickard and Clyde Apperson (7/20/2003)

Castagnoli, N.; Mantle, P.G.; Occurrence of *d*-Lysergic Acid and 6-Methyl-ergol-8-ene-8-carboxylic Acid in Cultures of *Claviceps purpurea*; *Nature* (1966) 211: 859-860.

Cerletti, A.; Doepfner, W.; Comparative Study on the Serotonin Antagonism of Amide Derivatives of Lysergic Acid and of Ergot Alkaloids; *J. Pharmacol Exp. Ther.* (1958) 122: 124-136

Cerny, A.; Semonsky, M.; Ergot Alkaloids. XXXIII. Epimerization of The Simpler Amides of D-Lysergic, D-isoLysergic and 1-Methyl-D-Lysergic Acids; *Collection Czechoslov. Chem. Commun.* (1968) 34: 694-698

Cerny, A.; Semonsky, M.; *Collection Czechoslov. Chem. Commun.* (1962) 27,1585; *Chemical Abstracts* (1971) 75, 77110?

Chain, E.B.; Bonino, C.; Tonolo, A.; Alkaloid Derivatives of Lysergic Acids; 1964; *US* 3,162,640

Chao, J. and Der Maderosian, A.H.; Ergoline Alkaloidal Constituents of Hawaiian Baby Wood Rose, *Argyreia nervosa* (Burm. f.) Bojer; *Journal of Pharmaceutical Sciences*, (1973) 62 (4) 588-591

Chao, J. and Der Maderosian, A.H.; Identificaton of Ergoline Alkaloids in the Genus *Argyreia* and Related Genera and Their Chemotaxonomic Implications in the *Convolvulaceae*; *Phytochemistry* (1976) 12: 2435-2440.

Christensen, B.V.; Reese, J.A.; Changes in Ergot with Various Moisture Contents under Different Conditions of Storage; *Journal of the American Pharmaceutical Association* (1939) 28: 343-360

Cleverdon, R.C.; A Laboratory Shaking Machine; ? (1955) 4: 66-68

Cohen, S.; Suicide Following Morning Glory Seed Ingestion; *American Journal of Psychiatry* (1964) 120: 1024-1025

Cohen, S.; *The Beyond Within; The LSD Story* (1964)

Cohen, S.; Psychotherapy with LSD: Pro and Con in *The Use of LSD in Psychotherapy and Alcoholism* (1967)

Cohen, S.; Statement to the Senate Subcommittee on Executive Reorganization Hearing on Organization of Government Programs Relating to LSD. Washington, DC, May 24-26, 1966

Cooke, R.C.; Carbohydrate Physiology of Sclerotia of *Claviceps Purpurea* During Dormancy and Germination; *Transactions of the British Mycological Society* (1970) 54 (1): 93-99

Cooke, R.C.; Mitchell, D.T.; Sclerotium Size and Germination in *Claviceps purpurea*; *Transactions of the British Mycological Society* (1966) 49 (1): 95-100

Cooke, R.C.; Mitchell, D.T.; Germination Pattern and Capacity for Repeated Stroma Formation in *Claviceps purpurea*; *Transactions of the British Mycological Society* (1967) 50 (2): 275-283

Cooper, D.A.; Allen, A.C.; Synthetic Cocaine Impurities; *Journal of Forensic Sciences* (1984) 29 (4); 1045-1055

Dahlberg, C.C.; Statement to the Senate Subcommittee on Executive Reorganization Hearing on Organization of Government Programs Relating to LSD. Washington, DC, May 24-26, 1966

Davies, P., Reynolds, P.W., Coats, R.R. Taylor, W.C.; Production of Amines; *U.S. Patent* 2,609,394 (1952)

DEA News Release; March 31, 2003; Pickard and Apperson Convicted of LSD Charges; Largest LSD Lab Seizure In DEA History.

Der Marderosian, A.H.; The Comparitve Morphology and Indole Alkaloid Constituents of Certain Species and Varities of Morning Glories (*Convolvulaceae*), (1964), Ph.D. Thesis, University of Rhode Island.

Der Marderosian, A.H., Guarino, A.M., DeFeo, J.J., Youngken, H.W.; A Uterine Stimulant Effect of Extracts of Morning Glory Seeds; *Psychedelic Review* (1964) 1: 317-323

Der Marderosian, A.H., Guarino, A.M., DeFeo, J.J., Youngken, H.W.; A Note on a Uterine Stimulant Principle in Extracts of the "*Ololiuqui*" Morning Glory; *American Journal of Pharm.* (1965) 137: 24-27

Der Marderosian, A.H., Hauke, R.L; Youngken, H.W.; Preliminary Studies of the Comparative Morphology and Certain Indoles of *Ipomoea* Seeds; *Economic Botany* (1964) 18: 67-76

Der Marderosian, A.H., Youngken, H.W.; The Distribution of Indole Alkaloids Among Certain Species and Varities of *Ipomoea*, *Rivea* and *Convolvulus* (*Convolvulaceae*); *Lloydia* (1966) 29 (1): 35-42

Dishotsky, N.I.; Laughman, W.D.; Mogar, R.E.; Lipscomb, W.R.; LSD and Genetic Damage: Is LSD Chromosome Damaging, Carcinogenic, Mutagenic, or Teratogenic?; *Science* (1971) 172(3982), 431-440

Dunlap, J.; *Exploring Inner Space. Personal Experience Under LSD 25.* New York: Harcourt, Brace & World, 1961

Dworschack, R.G.; Lagoda, A.A.; Jackson, R.W.; Fermenter for Small-Scale Submerged Fermentations; *Appl. Microbiol.* (1954) 2: 190-197

Elgin, J.C., Taylor, H.S.; Hydrazine Hydrate; *Journal of the American Chemical Society* (1929) 51: 2062

Eszterhas, J.; The Strange Case of the Hippie Mafia; *Rolling Stone* December 7, 1972 Issue 123

Eszterhas, J.; Hippie Mafia II; *Rolling Stone* December 21, 1972 Issue 124

Fischer, D.D. et al.; Grand Mal Seizures Following Ingestion of LSD; *California Medicine* (1967) 106: 210-211

Floss, H.G.; Biosynthesis of Ergot Alkaloids and Related Compounds; *Tetrahedron* (1976) 32: 873-912

Francais, E.; 1963; Improvements in the preparation and in the Preservation of Anhydrous Hydrazine; *GB* 932,712

Freedman, D.X.; Statement to the Senate Subcommittee on Executive Reorganization Hearing on Organization of Government Programs Relating to LSD. Washington, DC, May 24-26, 1966

Friedrichs, F.; On Extraction with Liquified Gases and the Ammonolysis of Hydrazine Sulfate; *Journal of the American Chemical Society* (1913) 35: 244-247

Fuentes, S.F.; Isla, M.; Ullstrup, A.J.; Rodriques, A.E.; *Claviceps gigantea*, a New Pathogen of Maise in Mexico; *Phytophatology* (1964) 54: 379-381.

Fuld, G.J.; Dunn, C.G.; A 50-Gallon Pilot Plant Fermentor for Classroom Instruction; ? (1957) 6: 15-22

Galen, A.S.; Bulej, P.; Faustman, J.; Cvak, L.; Cibulka, R.; Guttmann M.; Zpusob pripravy substituovanych amidu kyseliny *d*-lysergove a *d*-dihydrolysergove jejich derivatu; *CZ* 287,047 (1998)

Garbrecht, W.L.; Preparation of Amides of Lysergic Acid; *US Patent* 2,774,763 (1956)

Garbrecht, W.L.; Synthesis of Amides of Lysergic Acid; *Journal of Organic Chemistry* (1959) 24: 368-372

Garner, W.E.; Tyrer, D.; Preparation of Diethylamine: *Journal of the Chemical Society* (1916) 109: 174-175

Garner, W.E., Tyrer, D.; Note on the Preparation of Diethylamine; *Journal of the Chemical Society* (1916) 109: 174-175

Gaullier, J.; Process of Preparing Lysergic Acid (2001)*US*6,242,603 B1

Genest, K.; A Direct Densitometric Method on Thin-layer Plates for the Determinatin of Lysergic Acid Amide, Isolysergic Acid Amide and Clavine Alkaloids in Morning Glory Seeds; *Journal of Chromotography* (1965) 19: 531-539

Genest, K.; Rice, W.B., Farmilo; Psychotomimetic substances in Morning Glory Seed; *Proc. Can. Soc. Forensic Sci.* (1965) 4: 167-186

Genest, K.; Sahasrabudhe, M.R.; Alkaloids and Lipids of *Ipomoea*, *Rivea* and *Convolvulus* and Their Application to Chemotaxomy; *Economic Botany* (1966) 20: 416-428

Gertz, B.; Horror Weapons; *Air Force Magazine*; Jan. 1996 pgs. 44-48

Giarman, N.J.; *The Pharmacology of LSD in LSD, Man & Society* (1967)

Gieger, M.; Barrentine, B.F.; Isolation of the Active Principle in *Claviceps paspali* - A Progress Report; *Journal of the American Chemical Society* (1939) 61: 966-967

Godfrey, K.E.; The Metamorphosis of an LSD Psychotherapist in *The Use of LSD in Psychotherapy and Alcoholism* (1967)

Gogerty, J.H.; Dille, J.M.; Pharmacology of *d*-Lysergic Acid Morpholide (LSM). *J. Pharmacol Exp. Ther.* (1957) 120: 340-348

Graham, J.R.; Cardiac and Pulmonary Fibrosis During Methysergide Therapy for Headache; *American J. of Med. Sci.*; (1967) 254: 1-12

Graham, J.R.; Suby, H.I.; Le Compte, P.R.; Sadowsky, N.L.; Fibrotic Disorders Associated with Methysergide Therapy for Headache; *New England Journal of Medicine* (1966) 274: 359-368

Grawert, W; Schiedt, L.; Neumann, B.; Heidenbluth, K; Dauth, C; Schirutschke, R.; Müller, M.; Process for the Isolation of Ergot Alkaloids from Culture Suspensions; 1980; *US* 4,237,291

Groff, S.: The Use of LSD 25 in Personality Diagnostics in *The Use of LSD in Psychotherapy and Alcoholism* (1967)

Gröger, D.; Tyler, V.E.; Dusenberry J.E.; Investigation of the Alkaloids of *Paspalum* Ergot; *Lloydia* (1961)24 (1) 97-102

Hadley, G.; Development of Stromata in *Claviceps Purpurea*; *Transactions of the British Mycological Society* (1968) 51 (5); 763-769

Hareven, D.; Koltin, Y.; Nuclear Distribution in the Mycelium of *Claviceps* and the Problem of Strain Selection; *Applied Microbiology* (1970) 19 (6) 1005-1006

Harrington, A.; *A Visit to Inner Space; in LSD: The Consciousness-Expanding Drug* (1964).

Henderson, L.A.; Glass, W.J.; *LSD: Still with us After all These Years* (1994)

Henson, L.; Valleau, W.D.; The Production of Apothecia of *Sclerotinia Sclerotiorum* and *S. Triforliorum* in Culture; *Phytopathology* (1940) 30: 869-873

Hesse; *Jahresber* 1857: 403

Hoffer, A.; A Program for the Treatment of Alcoholism: LSD, Malvaria and Nicotinic Acid in *The Use of LSD in Psychotherapy and Alcoholism* (1967)

Hoffer, A; Agnew, N.; Nicotinic Acid Modified LSD-25 Psychosis; *J. Mental Sci* (1955) 101: 12-27

Hofmann, A.; The Active Principles of the Seeds of *Rivea corymobsa* and *Ipomoea Vviolacea*; *Harvard University Botanical Museum Leafl.* (1963) 20: 194-212

Hofmann, A.; Rutschmann, J.; Stadler, P., Troxler, F.; Process for Lysergic Acid Hydrazides; 1966a; *US* 3,239,530

Hofmann, A.; Stadler, P.; Troxler, F.; Process for Lysergic Acid Hydrazides; *US Patent* 3,239,530 (1966)

Hofmann, A.; Troxler, F.; Esters of Indoles; *US Patent* 3,075,992 (1963)

Hofmann, A.; Troxler, F.; Preparation of Lysergic Acid Derivatives, and Intermediates; *US Patent* 3,085,092 (1963)

Hofmann, A. ; Troxler, F.; Improvements in or Relating to Lysergic Acid Amide Derivatives; 1965; *GB* 994,486

Hofmann, A. ; Troxler, F.; Ott, H.; Lysergic Acid Amides; 1967; *GB* 1,054,572

House, H.D.; The North American Species of the Genus *Ipomoea*; *Ann. of the New York Academy of Sciences* (1908) 18: 259-260

Hurd, C.D., Bennett, C.W.; Concentration of Hydrazine Hydrate Solutions; *Journal of the American Chem. Society* (1929) 51: 265-269

Hydrazine Hydrate; *University of Illinois Bulletin*; Oct. 9, 1921

Hylin, J.W., Watson, D.P.; Ergoline Alkaloids in Tropical Wood Roses; *Science* (1965) 148: 499-500

Irwin, S.; Egozcue, J.; Chromosome Damage not Found in Leukocytes of Children Treated with LSD-25; *Science* (1968) 159: 749

Isbell, H.; Miner, E.J.; Logan, C.R.; Relationships of Psychotomimetic to Antiserotonin Potencies of Congeners of Lysergic Acid Diethylamide; *Psychopharmacologia* (1959) 1: 20-28

Jacob III, P.; Shulgin, A.T.; Structure-Activity Relationships of the Classic Hallucinogens and Their Analogs; in *Hallucinogens: An Update* 1994

Jacobs, W.A.; Craig, L.C.; The Ergot Alkaloids 2. The Degradation of Ergotinine with Alkali, Lysergic Acid; *Journal of Biological Chemistry* (1934) 104: 547-551

Jarvik, M.E.; The Behavioral Effects of Psychotogens in *LSD, Man & Society* (1967)

Johnson, F.N.; Ary, I.E.; Teiger, D.G.; Kassel, R.J.; Emetic Activity of Reduced Lysergamides; *Journal of Medicinal Chemistry* (1973) 16(5) 532-537

Kamm, O., Marvel, C.S.; Alkyl and Alkylene Bromides; *Organic Syntheses Collective* Volume 1 (1941) 25-35

Karácsony, E.M..; Borsi, J.; Csányi, E.; Pik, K.; Wolf, L.; Compounds with Ergoline Skeleton; 1977; *US* 4,005,089

Karácsony, E.M.; Borsi, J.; Csányi, E.; Pik, K.; Wolf, L.; Compounds with Ergoline Skeleton; 1977; *US* 4,064,249

Karácsony, E.M.; Borsi, J.; Tardos, L.; Király, I.; Elek, S.; István Elekes; Novel Lysergic Acid Amides and Process for Preparing Same; 1978; *US* 4,101,552

Kurland, A.A.; The Therapeutic Potential of LSD in Medicine in *LSD, Man & Society* (1967)

Kelleher, W.J., Kim, B.K., Schwarting, A.E.; Production of Lysergic Acid Derivatives in Submerged Culture. 5. Effect of Surfactants on Alkaloid Accumulation; *Llyodia* (1969) 32 (3): 327-333

Kelleher, W.J., Kruger, R.J., Rosazza, J.P.;
The Violet Pigment of Lysergic Acid Alkaloid-producing Cultures of *Claviceps Paspali*: Fe (3) Complex of 2,3-Dihydroxybenzoic Acid; *Lloydia* (1971) 34 (2): 188-194

Klee, G.D.; Statement to the Senate Subcommittee on Executive Reorganization Hearing on Organization of Government Programs Relating to LSD. Washington, DC, May 24-26, 1966

Leary, T.; Introduction in *LSD: The Consciousness-Expanding Drug* (1964).

Leary, T.; Metzner, A.; Alpert, R.; *The Psychedelic Experience: A Manual Based on the Tibetan Book of the Dead*, New Hyde Park, N.Y.; University Books, 1964

Lee, D.; Pratt, C.; *Operation Julie*; W.H. Allen & Co.; (1978) ISBN: 0 491 02176 3

Lefebvre, C.L.; Ergot of *Paspalum*; *Phytopathology* (1939) 29: 365-367

Lehrfeld, J.; Burkman, A.M.; Gearien, J.E.; Synthesis of 6-Substituted Nicotinic Acid Derivatives as Analogs of Ergot Alkaloids; *Journal of Medicinal Chemistry* (1964) 7: 150-154

LEK Tovarna Farmacevtskih; Improvements in or Relating to Extracting Alkaloids from Ergot of Rye; 1972; *GB* 1,299,557

Lemon, R.C., Depot, S., Myerly R.C.; Production of Amines; *US Patent* 3,022,349 (1962)

Levy, R.M.; Diazepam for LSD Intoxication; *Lancet* (1971) 1: 1297.

Lewis, R.W.; The Field Inoculation of Rye with *Claviceps purpurea*; *Phytopathology* (1945) 35: 353-360.

Lewis, R.W.; Development of Conidia and Sclerotia of the Ergot Fungus on Inoculated Rye Seedlings; *Phytopathology* (1956) 46: 295-297.

Lewis, R.W.; Guttation Fluid: Effects on Growth of *Claviceps purpurea* in Vitro; *Science* (1962) 690-691

Lin, G.C.; Preface; in *Hallucinogens: An Update* 1994

Ling, T.M.; Buckman, J.; The Use of LSD in Individual Psychotherapy; *Proceedings of the Royal Society of Medicine* (1963) 53: 927-937

Loughman, W.D.; Sargent, T.W.; Israelstam, D.M.; Leukocytes of Humans Exposed to Lysergic Acid Diethylamide: Lack of Chromosomal Damage; *Science* (1967) 158: 508-510

Luttrell, E.S.; The Disease Cycle and Fungus-Host Relationships in Dallisgrass Ergot; *Phytophathology* (1977) 76: 1461-1468.

MacDougal, T.; *Ipomoea Tricolor*, a Hallucinogenic Plant of the Zapotecs; *Bol. Centr. Invest. Anthropol. Mex.*, No. 6, p. 6

MacLean, J.R.; MacDonald, D.C.; Byrne, V.P.; Hubbard, A.M.; The Use of LSD-25 in the Treatment of Alcoholism and Other Psychiatric Problems Quart. J. Stud. Alcohol (1961) 22 (1): 34-45

Mantle, P.G.; Nisbet, L.J.; Differentiation of *Claviceps purpurea* in Axenic Culture; *Journal of General Microbiology* (1976) 93: 321-334.

Mary, N.Y., Kelleher, W.J., Schwarting, A.E.; Production of Lysergic Acid Derivatives in Submerged Culture. 3. Strain Selection on Defined Media; *Lloydia* (1965) 28 (3); 218-229

Masters, R.E.L.; Houston, J.; Toward an Individual Psychedelic Psychotherapy; in *Psychedelics: The Uses and Implicatons of Hallucinogenic Drugs.*

McCrea, A.; Ergot Preparation and Progress of Obtaining Same; 1936, *US* 2,050,360

McGlothlin, W.H.; Social and Para-Medical Aspects of Hallucinogenic Drugs in *The Use of LSD in Psychotherapy and Alcoholism* (1967)

McCrea, A.; The Reactions of *Claviceps purpurea* to Variations of Environment; *American Journal of Botany*; (1931) 18 (1) 50-78

McKay, J.B.; Parkhurst, R.M.; Silverstein, R.M.; Skinner, W.A.; Analogs of Psilocin and Lysergic Acid Diethylamide I. Chloro, Nitro, and Amino Derivatives of 3-Substituted Indoles; *Canadian Journal of Chemistry* (1963) 41: 2585-2590

Mehl, E.; Rüther, E.; Redemann, J.; Endogenous Ligands of a Putative LSD-Serotonin Receptor in the Cerebrospinal Fluid: Higher Level of LSD-Displacing Factors (LDF) in Unmedicated Psychotic Patients; *Psychopharmacology* (1977) 54: 9-16

Michener, H.D.; Snell, N.; Studies on Cultural Requirements of *Claviceps purpurea* and Inactivation of Ergtomine; *American Journal of Botany* (1950) 37 (1): 52-59

Mitchell, D.T.; Cooke, R.C.; Water Uptake, Respiration Pattern and Lipid Utilization in Sclerotia of *Claviceps purpurea* During Germination; *Transactions of the British Mycological Society* (1968) 51 (5); 731-736

Mitchell, D.T.; Cooke, R.C.; Some Effects of Temperature on Germination and Longevity of Sclerotia in *Claviceps purpurea*; *Transactions of the British Mycological Society* (1968) 51 (5); 721-729

Mizrahi, A., Miller, G.; Long-term Preservation of a Nonsporulating Strain of *Claviceps paspali*; *Applied Microbiology* (1968) 16: 1100-1101

Morris, L.J.; Hall, S.W.; Fatty Acid Composition of Sphacelial and Sclerotial Growth Forms of *Claviceps purpurea* in Relation to the Production of Ergoline Alkaloids in Culture; *Trans. Br. Mycol. Soc.* (1969) 53 (3): 441-447.

Neef, G. Eder, U.; Saurer, G.; Ast, G.; Schröder, G.; Process for the Preparation of Ergoline Derivatives; *CZ* 229,948.

Newland, C.; *Myself and I.* New York: Coward McCann, 1962

Nikolin, A; Nikolin, B.; The Separation of Ergot and Clavine Alkaloids by Gel Filtration, *Phytochemistry* (1972) 11: 1479-1480

Nisbet, L.J.; Dickerson, A.G.; Mantle, P.G.; Changes in Respiration Patterns of *Claviceps purpurea* During Parasitic Development; *Transactions of the British Mycological Society* (1977) 69 (1); 11-14

Niwaguchi, T.; Inoue, T.; Chromatographic Separation of Lysergic Acid Amide and Isolysergic Acid Amide in Morning Glory Seeds; *Journal of Chromatography* (1969) 43: 510-512

Niwaguchi, T.; Nakahara, Y.; Ishii, H.; Studies on Lysergic Acid Diethylamide and Related Compounds. IV. Syntheses of Various Amide Derivatives of Norlysergic Acid and Related Compounds; *Yakugaku Zasshi* (1976) 96 (5): 673-678

Ogunlana, O.E., Ramstad, E., Tyler, V.E.; Effects of Some Substances on Ergot Alkaloid Production; *Journal of Pharmaceutical Sciences* (1969) 58 (1): 143-

Olin; 1955; The Stablisation of Hydrazine *GB* 738,441

Olin; Improvements in or relating to Manufacture of Hydrazine; 1956 *GB* 7,569,545

Olin; 1957; Stabilized Concentrated Hydrazine and method of Stabilizing same; *GB* 778,357

O'Reilly, P.O.; Brief Psychotherapy, LSD and the Alcoholic in *The Use of LSD in Psychotherapy and Alcoholism* (1967)

Osmond, H.; Ololiuqui; The Ancient Aztec Narcotic; *Journal Mental Sci.* (1955) 101: 526-537

Osmond, H.; A Review of the Clinical Effects of Psychotomimetic Agents; *Ann. N.Y. Acad. Sci.* 66: 418 (1957)

Ott, J.; LSD, *Ololiuhqui*, Kykeon: The Ergoline Complex; In *Pharmacotheon: Entheogenic Drugs, Their Plant Sources and History* (1993)

Pacifici, L.R., Kelleher, W.J. and Schwarting, A.E.; Production of Lysergic Acid Derivatives in Submerged Culture. 1. Fermentation Studies; *Lloydia* (1962) 25 (1); 37-45

Pacifici, L.R., Kelleher, W.J. and Schwarting, A.E.; Production of Lysergic Acid Derivatives in Submerged Culture. 2. Fermentation Studies; *Lloydia* (1963) 26 (3); 161-173

Pahnke, W.N.; LSD and Religious Experience in *LSD, Man & Society* (1967)

Patelli, B.; Bernardi, L.; Process for the Preparation of Lysergic Acid Amides; *US Patent* 3,141,887

Paul, A.G.; Kelleher, W.J.; Schwarting, A.E.; The Culture of *Claviceps purpurea*; *Journal of the American Pharmaceutical Assoc.* (1954) 43: 208-207

Penneman, R.A., Audrieth, L.F.: The Ternary System; Hydrazine-Water-Sodium Hydroxide; *Journal of the American Chemical Society* (1949) 71: 1644-1647

Perrilo, A.E.; de la Villa, A.G.; Experiencia Farmacológica en el Discutido Terreno de la Demenica Esquizofrenica; *Acta Psiquiatrica y Psicologica Argentina* (1963) 9 (1): 49-52

Perry, C.; Augustus Owsley Stanley III; *Rolling Stone*; Novemeber 25, 1982 Issue 383

Persinger M.A.; Putative Perception of Rotating Permanent Magnetic Fields Following Ingestion of LSD; *Percept. Mot. Skills* (1998) Oct;87(2):601-2;

Pfaff, R.C.; Huang, X.; Marona-Lewicka, D.; Oberlender, R.; Nichols, D.E.; Lysergamides Revisited; in *Hallucinogens: An Update* (1994)

Pierron; *Bull. Soc. chim.* 1899 [iii], 21, 784

Pioch, R.P.; Preparation of Lysergic Acid Amides; *US Patent* 2,736,728 (1956); *Chemical Abstracts* (1956) 50: 10803

Pioch, R.; Lysergic Acid Amides; 1961; *US* 2,997,470

Price, T.S., Brazier, S.A., Wood, A.S.; The Preparation of Diethylamine on a Large Scale in the Laboratory; *Journal of the Society of Chemical Industry* (1916) 35: 147-149 (*p*-nitrosodiethylaniline is carcinogenic)

Rakshit J.N.; A Modified Method for the Preparation of Triethylamine; *Journal of the American Chemical Society* (1913) 35: 1781-1783

Ramstad, E.; Gjerstad, G.; The Parasitic Growth of *Claviceps purpurea* (Fries) Tulasne on Rye and its Relation to Alkaloid Formation (1955) 44: 741-743.

Raschig, F.; Prep. of Anhydrous Hydrazine; *Chemical Abstracts* 4: 2615

Raschig, F.; Production of Hydrazine; (1909) *US* 910,858

Reddie, L.N.; Process of Separating Lower Alkylamines; 1938; *GB* 486,821

Reddie, L.N.; Process of Separating Lower Alkylamines; 1938a; *GB* 486,822

Rice, W.B., Genest, K.; Acute Toxicity of Extracts of Morning Glory Seeds in Mice; *Nature* (1965) 207: 302-303

Richards, W.A.; Mystical and Archetypal Experiences of Terminal Patients in DPT Assisted Psychotherapy; *Journal of Religion and Health* (1978) 17 (2) 117-126

Richter Gedeon Vegyeszeti Gyar R.T.; Preparation of Ergot Alkaloids; 1967; *GB* 1,064,764

Rothlin, E.; Lysergic Acid Diethylamide and Related Substances; *Ann. NY Acad. Sci.* (1957) 66: 668-685

Rothlin, E.; Pharmacology of Lysergic Acid Diethylamide and Some of its Related Compounds; *J. of Pharmacy and Pharmacology* (1957) 9: 569-587

Rutschamann, J., Kobel, H.; Obtaining Deriviatives of Lysergic Acid by a Microbiological Process; *Chemical Abstracts* (19??) 15314 e-g

Rutschmann, R.; Kobel, H.; Microbiological Production of Lysergic Acid Derivatives; 1965; *US* 3,219,545

Rutschmann, R.; Kobel, H.; Schreier, E.; 1967; Heterocyclic Carboxylic Acids and Their Production; 1967: *US* 3,314,961

Sahasrabudhe, M.R., Genest, K.; Fatty Acid Composition of Morning Glory Seed Oil; *Journal American Oil Chemi. Soc.* (1965) 42: 814

Sandison, R.A.; The Clinical Uses of LSD in *Lysergic Acid Diethylamide and Mescaline in Experimental Psychiatry* (1956)

Sandison, R.A.; Spencer, A.M.; Whitelaw, J.D.A.; The Therapeutic Value of Lysergic Acid Diethylamide; *Journal of Mental Science* (1954) 100: 491-507

Sandoz; The Preparation of Lysergic Acid Hydrazide; 1937; *GB* 463,936

Sandoz; The Preparation of Synthetic Derivatives of Lysergic Acid; 1938; *GB* 480,822

Sandoz; Process for Separating and Purifying the Alkaloids of Ergot; (1945) *GB* 573,514

Sandoz Ltd.; Diethylamide of *d*-Lysergic Acid; *British Patent* 579,484 (1946); *Chemical Abstracts*: 2450

Sandoz Ltd.; Urethan Derivatives of the Lysergic Acid Ring System; *British Patent* 639,887; *Chemical Abstracts* (1950) 44: 10740-10741

Sandoz; Amide-Like Derivatives of Lysergic and Isolysergic Acids; 1956; *GB* 785,173

Sandoz Ltd.; Hydrazides; *Belg. Patent* 609,011 (1962); *British Patent* 609,011; *Chemical Abstracts* 57: 12568

Savage, C.; Fadiman, J.; Mogar, R.; Allen, M.H.; Process Outcome Variables in Psychedelic (LSD) Therpy; in *The Use of LSD in Psychotherapy and Alcoholism*, (1967)

Schenk, P.W.; Hydrazine Hydrate, Hydrazine; *Handbook of Preparative Inorganic Chemistry* 1: 469-472

Schjelderup, H.; *Det Skjulte menneske*, Oslo, Cappelen, 1961

Schlientz, W.; Sutter, B.; Procédé permettant d'isoler des composés de la famille de l'acide lysergique; 1966; *FR* 1,463,426

Schlientz, W., Sutter, B.; Lysergic Acid Isolation; *Chemical Abstracts* (1967) 84858e; *French Patent* 1,463,426

Schultes, R.E.; A Contribution to our Knowledge of Rivea corymbosa the Narcotic Ololiuqui of the Aztecs; *Botanical Museum of Harvard University*, Cambridge, Massachusetts (45 pp.) (1941).

Schultes, R.E.; Lecture 1, Jungle Search for New Drug Plants in the Amazon: Lecture 2, Native Narcotics of the New World; Lecture 3, Botany Attacks the Hallucinogens., University of Texas, Third Lecture Series, *Pharm. Sci.* Part 5, pp. 138-185

Schultes, R.E.; The Nomenclature of Two Mexican Narcotics; *Taxon.* (1964) 13: 65-66

Schwarting, A.E.; Hiner, L.D.; A Study of Domestic Ergot of Wheat and Rye; *Journal of the Pharmaceutical Association* (1945) 34: 11-16.

Semonsky, M.; Zikan, V.; Cycloalkyamines of *d*-Lysergic Acid and Method for Producing the Same; 1959; *GB* 816,273

Semonsky, M.; Zikan, V.; Beran, M.; Preparing D-Lysergic and D-isolysergic Acid or Mixtures therof. *Czech.* 123,689; *Chemical Abstracts* (1968) 68: 36323w

Shinners, L.; Correct Nomenclature of Two Mexican Narcotics; *Taxon.* (1965) 14: 103-104

Smith, H.; Do Drugs Have Religious Import? in *LSD: The Consciousness-Expanding Drug* (1964).

Smith, L.I., Howard, K.L.; Anhydrous Hydrazine; *Organic Syntheses* (1944) 24; 53-54

Smith, S.; Timmis, G.M.; The Alkaloids of Ergot. Part 3. Ergine, a New Base Obtained by the Degration of Ergotoxine and Ergotinine; *Journal of the Chemical Society* (1932) 763-766

Smith, S.; Timmis, G.M.; The Alkaloids of Ergot. Part 4. A Complex Group Common to Ergotoxine and Ergotinine; *Journal of the Chemical Society* (1932) 1543-1544

Smith, S.; Timmis, G.M.; The Alkaloids of Ergot. Part 6. Ergometrine; *Journal of the Chemical Society* (1936) 1166-1169

Societa Farmaceutici Italia; Lysergic Acid Amide or Derivatives; *British Patent* 883,329; *Chemical Abstracts* (1962) 13021 c-e;

Solursh, L.P.; The Use of LSD-25; Psychotherapy: An Evaluation: *International Journal of Neuropsychiatry* (1966) 2 (2): 651-656

Stace, W.T.; *Philosophy and Mysticism*. Philadelphia: Lippincott, 1960

Stevens, F.L.; Hall, J.G.; Three Interesting Species of *Claviceps*; *Botanical Gazette* (1910) 50: 460-463

Stoll, A.; Process for the Manufacture, Separation and Preparation of Pure Crystallized Ergot-Alkaloids; 1937; *US* 2,080,954

Stoll, A.: Ergot - A Treasure House for Drugs; *The Pharm. Journal*; (1965) 194: 605-613.

Stoll, A.; Brack, A.; Hofmann, A.; Kobel, H.; Process for the Preparation of Ergotamine, Ergotaminine and Ergometrine by Saprophytic Culture of Ergot (*Claviceps purpurea* [Fr] Tul.) in Vitro and Isolation of the Alkaloids thus Produced; 1957; *US* 2,809,920

Stoll, A.; Burokhardt, E.; Process for the Manufacture, Separation, and Preparation of Pure Crystallized Ergot Alkaloids; 1937; *US* 2,080,954

Stoll, A.; Hofmann, A.; Lysergic Acid Hydrazide and a Process for its Manufacture; 1937; *US* 2,090,429

Stoll, A.; Hofmann, A.; D-Lysergic Acid-D-L-hydroxybutylamide-2 and a Process for its Preparation; *US Patent* 2,265,207 (1941)

Stoll, A.; Hofmann, A.; Ergot Alkaloids. IV. Optically Active Hydrazides of Lysergic and isoLysergic Acids; *Helv. Chim. Acta.* (1943) 26: 922-928; *Chemical Abstracts* 38: 1499-1500

Stoll, A.; Hofmann, A.; Partialsynthese von Alkaloiden vom Typus des Ergobasins; *Helv. Chim. Acta.* (1943) 26: 944-933

Stoll, A; Hofmann, A.; Optically Active Salts of the Lysergic Acid Derivatives and a Process for Their Preparation and Isolation; 1948; *US* 2,447,214.

Strassman, R.J.; Human Hallucinogenic Drug Research: Regulatory, Clinical, and Scientific Issues; in *Hallucinogens: An Update* (1994)

Stuart, E,H.; Ergot-Derived Product and Process of Obtaining It; 1937; *US* 2,067,866

Sullivan; *Jahresber* 1858: 231

Swain, F. et al.; Four Psilocybin Experiences. *Psychedelic Review.* 1: 21 (1963)

Szára, S.; Holliarwe, L.; "NIMH and Legal Drug Control." *Report Prepared for the National Institute of Mental Health, 1973.*

Szára, S.; Are Hallucinogens Psychoheuristic?; in *Hallucinogens: An Update* (1994)

Taber, W.A., Heacock, R.A.; Location of Ergot Alkaloid and Fungi in the Seed of *Rivea corymbosa* (L.) Hall. f., *"Ololiuqui"*; *Canadian Journal of Microbiology* (1962) 8: 137-143

Taber, W.A., Heacock, R.A., Mahon, M.E.; Ergot-type Alkaloids in Vegetative Tissue of *Rivea Corymbosa* (L.) Hall. f.; *Phytochemistry* (1963) 2: 99

Taber, W.A., Siepmann, R.; Comparison of Growth and Primary Shunt Production Formation by *Claviceps purpurea* Cultured on Succinic Acid and Glucose as Carbon; *Applied Microbiology* (1966) 14: 320-327

Taber, W.A., Vining, L.C.; A Comparison of Isolates of *Claviceps* spp. for the Ability to Grow and to Produce Ergot Alkaloids on Certain Nutrients; *Can J. Microbiology* (1960) 6: 355-365

Taber, W.A., Vining, L.C., Heacock, R.A.; Clavine and Lysergic acid Alkaloids in Varieties of Morning Glory; *Phytochemistry* (1963) 2: 65-70

Tendler, S.; May, D.; *Brotherhood of Eternal Love* (1984) Granada Publishing

Tonolo, A.; Production of Peptide Alkaloids in Submerged Culture by a Strain of *Claviceps purpurea* (Fr.) Tul.; *Nature* (1966) 209: No. 5028

Troyan, J.E.; Properties, Production, and Uses of Hydrazine; *Industrial and Engineering Chemistry* (1953) 45 (12) 2608-2612

Trout, K.; *Some Simple Trytamines* (2002) 272 pgs.; pg. 154; Mydriatic Productions

Tyler, V.E., Jr.; Schwarting, A.E.; *Journal of the American Pharmaceutical Association* (1954) 43: 207-211.

Tyler, V.E., Jr.; Process for the Production of Ergot Alkaloids; 1965: *US* 3,224,945

Unger, S.; Mescaline, LSD, Psilocybin and Personality Change: A R eview; Psychiatry: *Journal for the Study of Interpersonal Processes* (1963) 26 (2): 111-125

Unger, S.M.; Mescaline, *LSD, Psilocybin and Personality in LSD: The Consciousness-Expanding Drug* (1964).; from *Delysid (LSD), Annotated Bibliography*, Addendum No. 3, mimeographed, Sandoz Pharmaceuticals, 1961 p 307

Van Rhijn, C.H.; Variables in Psycholytic Treatment; in *The Use of LSD in Psychotherapy and Alcoholism* (1967)

Vining, L.C., Taber, W.A.; Estimation of Ergot Alkaloids in Cultures of *Claviceps purpurea*; *Canadian Journal of Microbiology* (1959) 5: 441-451

Vogel, A.I.; Physical Properties and Chemical Constitution. Part VIII. Alkyl Chlorides, Bromides and Iodides; *Journal of the Chemical Society* (1943) 636-647

Von Felsinger, J.; Lasagna, L.; Beecher, H.K.; The Response of Normal men to Lysergic Acid Derivatives (Di and Mono-ethyl Amides: Correlation of Personality and Drug Reaction; *J. Clinical & Experimental Psychopathology* (1956) 17: 414-428

Wack, G.; Nagy, L.; Szekély, D.; Szolnoky, J.; Udvardy-Nagy, E.; Zsóka, E.; Fermentation Process for the Preparation of Ergot Alkaloids; 1975; *US* 3,884,762

Watts, A.; A Psychedelic Experience: Fact or Fantasy? in *LSD: The Consciousness-Expanding Drug* (1964).

Wakefield, D.; The Hallucinogens: A Reporter's Objective View in *LSD: The Consciousness-Expanding Drug* (1964).

Wasson, R.G.; Notes on the Present Status of *Ololiuqui* and the other Hallucinogens of Mexico; *Harvard University Botanical Museum Leaf.* (1963) 20: 161-193

Watt, G.W., Otto, J.B.; The Ammonolysis of Ethyl Iodide by Liquid Ammonia; *J. of the American Chemical Society* (1947) 69: 836-838

Wenner, R.R., Beckman, A.O.; The Quantum Yield in the Photochemical Decompositon of Gaseous Hydrazine; *Journal of the American Chemical Society* (1932) 54: 2787

Werner, E.A.; The Preparation of Ethylamine and of Diethylamine; *Journal of the Chemical Society* (1918) 113: 899-902

Whetzel, H.H.; Reddick, D.; A Method of Developing *Claviceps purpurea*; *Phytopathology* (1911) 1:(2) 50-53

Whittle, C.W.; Castle, R.N.; Open-Chain Analogs of LSD II. Synthesis of Some 2-(3-Indolylethyl)- and 2-(3-Methyl-2-indolyl-ethyl)piperidines; *Journal of Phamraceutical Sciences* (1963) 52: 645-648

Wilkinson, P.; The Acid King; *Rolling Stone* July 5, 2001 Issue 872

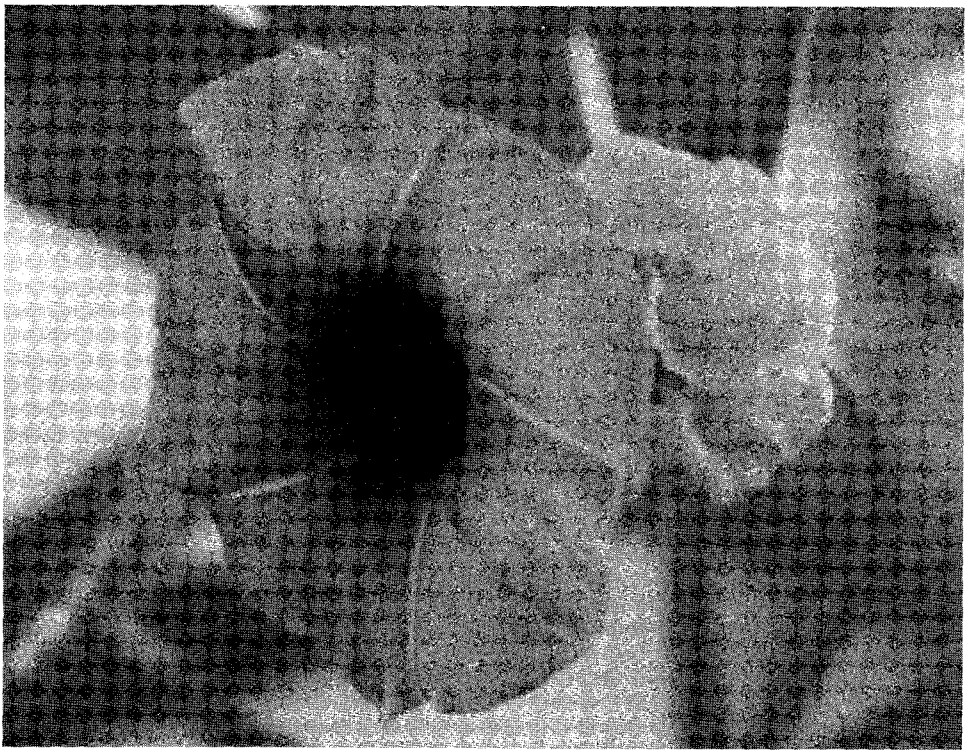
Wilson, K.A.; The Genera of *Convolvulaceae* in the Southeastern United States; Journal of the Arnold Arboretum (1960) 41: 298-317

Windisch, W.; Bronn, W.; Production of Ergot Alkaloids; 1960; *US* 2,936,266

Yensen, R.; LSD and Psychotherapy; *Journal of Psychoactive Drugs* (1985) 17 (4): 267-277

Yensen, R.; From Mysteries to Paradigms: Humanity's Journey from Sacred Plants to Psychedelic Drugs; in *Gateway to Inner Space* 1989

"Legitimate human investigation with classical hallucinogens was severely curtailed about 25 years ago. During the ensuing period, a significant body of information has been accrued primarily on the basis of animal studies. Novel agents have been identified, mechanisms of action have been proposed, new animal models have been described. New clinical data are now required to challenge or validate the results of these studies." R.A. Glennon (1994)



"Jesus emphasized that God is within us. And gradually we have refined this idea to where we say that God is within our hearts. Although we still think that God is something apart, a Power from outside, we insist that God dwells in our hearts, thus God is wherever we are. And conversely we are wherever God is. Under LSD you come to know that God is not apart and aside from Man but that God is within Man, and is Man, and that Man is within God, and is God. Thus God and Man become identical. This is the oneness with God," Malden Grange Bishop (1963).