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SYNTHESIS AND CHEMISTRY OF LYSERGIC ACID DERIVATIVES (U)

Part I

by

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October 1964

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US ARMY EDGEWOOD ARSENAL
CHEMICAL RESEARCH AND DEVELOPMENT LABORATORIES
EDGEWOOD ARSENAL, MARYLAND 21010

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Chemical Research and Development Laboratories Special Pu

SYNTHESIS AND CHEMISTRY OF LYSERGIC ACID DERIV

Part I

Task No.: 1C522301A06001
Notebook Nos.: 6687, 6779, 6908,
6915, 6931

Date Started: June 1961
Date Completed: February 1964

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Part I

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(U)

FOREWORD

This work was conducted under Task 1C522301A06001, New Agents Research (U). The experimental data are contained in notebooks 6687, 6779, 6908, 6915, and 6931. The work was started in June 1961 and completed in February 1964.

Acknowledgments

The authors are indebted to the Analytical Research Branch of the Chemical Research Division for the analyses and absorption spectra, to the Agents Properties Branch of the Physicochemical Research Division for the mass spectral analyses, and to the Human Investigations Facility of the Directorate of Medical Research for the fluorometric analyses.

(C)

DIGEST

(C) Because D-lysergic acid diethylamide (LSD) is one of the most powerful psychotomimetic compounds known, the synthesis and chemistry of this compound have been investigated to establish procedures for its large-scale preparation.

(C) The following conclusions were reached:

1. (C) LSD acid maleate can be prepared in overall yields of 75% from D-lysergic acid.

2. (C) An inexpensive and readily available source for D-lysergic acid is still not available.

3. (C) LSD with a higher melting point (160° to 162°C) than previously reported can be prepared by crystallizing it from ether.

4. (U) The tartrate and acid maleate salts of LSD have similar solubilities in organic solvents, but the tartrate salt is more water-soluble. LSD is insoluble in water but very soluble in most organic solvents.

5. (U) The isomerization equilibrium between n- and iso-LSD is not a serious stability problem, since the normal form predominates (88% normal to 12% iso).

6. (U) The addition of water to the 9,10-double bond of LSD under ultraviolet irradiation is one of the most serious stability problems.

7. (U) LSD reacts rapidly with bromine or bleach.

8. (U) LSD acid maleate in aqueous solutions was 90% intact after 20 hr of reflux.

9. (U) Thin-layer chromatography is a powerful tool for the study of the synthesis and chemistry of LSD.

(U)

CONTENTS

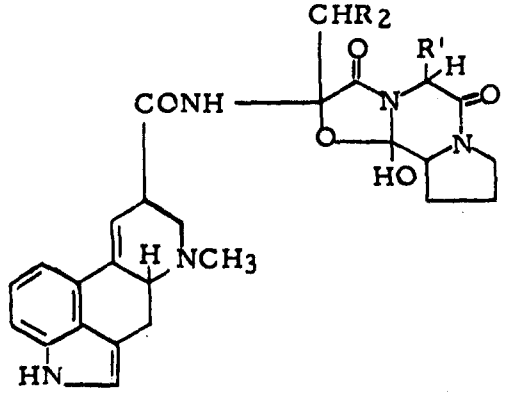
(C) SYNTHESIS AND CHEMISTRY OF LYSERGIC ACID DERIV.

Part I

1. (C) INTRODUCTION.

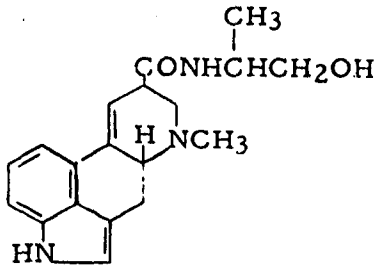
A. (C) History.

(U) The physiological effects of ergot, the sclerotium f of the fungus Claviceps purpurea on rye, wheat, oats, barley, grasses, were recorded in pre-Christian times. It was identif the causative agent of the medieval gangrenous scourge known fire, and its capacity to induce uterine contractions was recor 1582.[1-4] The isolation and characterization of the active princ led to six related bases, all of which are amides of the same s gic acid.[5,6] Five of the six bases are shown in I.[3] The sixth the ergot alkaloids, ergonovine (ergobasine, ergometrine), an the hydrolysis product of these alkaloids, are shown in II and I

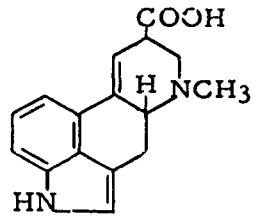


I. Ergot Alkaloids

	R	R'
Ergotamine	-H	$-CH_2C_6H_5$
Ergosine	-H	$-CH_2CH(CH_3)_2$
Ergocristine	$-CH_3$	$-CH_2C_6H_5$
Ergocryptine	$-CH_3$	$-CH_2CH(CH_3)_2$
Ergocornine	$-CH_3$	$-CH(CH_3)_2$

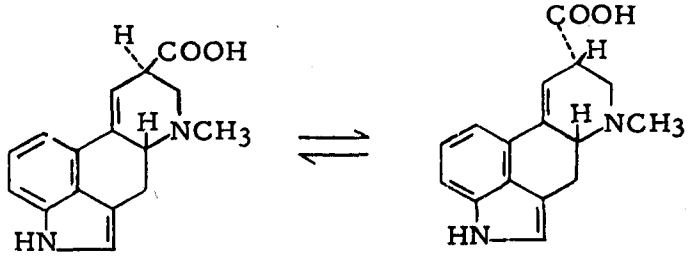


II. Ergonovine



III. Lysergic Acid

(U) The existence of isomeric pairs of these ergot alkaloids was noted by Kraft[7] and by Barger and Karr.[8] The pairs are interconverted in hydrolytic solvents by the action of hydrogen and hydroxyl ions. The levorotatory, pharmacologically active alkaloids (-ines) were derived from a lysergic acid structure, and the dextrorotatory, pharmacologically inactive alkaloids (-inines) were derived from the isolysergic acid structure. The nature of the isomerization was explained by Stoll, Hofmann, and Troxler,[9] who published evidence that indicated the two acids differed only in the configuration at C_8; that is, the two acids were epimers at C_8 and the mutarotation consisted of an epimerization at this center (IV). The configurations for D-lysergic acid shown in the text are based on the configurations recently given by Stadler and Hofmann.[10]



normal iso

IV. Isomerization of Lysergic Acid

(U) The synthesis of D-lysergic acid diethylamide (LSD) from natural D-lysergic acid by Stoll and Hofmann[11] and the accidental ingestion of a small amount of this material by Hofmann, as reported by Kornfeld,[2] Stoll,[12] and others,[3] led to the discovery of the unusual capacity of this drug to cause abnormal psychic phenomena in man. The active dose for man was found to

be 0.02 to 0.05 mg per man[13] (MED, 0.0005 mg/kg). The LD50 showed strong species specificity — 46 mg/kg in the mouse, 16 mg/kg in the rat, and 0.3 mg/kg in the rabbit.[13] The chemical and pharmacological relationships of LSD to other derivatives of lysergic acid have been discussed by Cerletti[14] and by Hofmann,[13, 15] who have compared the in vivo and in vitro effects of LSD, ergot alkaloids, and 18 lysergic acid derivatives closely related to LSD. Numerous papers on the effects of LSD on animals and on man have been published and are noted in two excellent publications from Sandoz Pharmaceuticals.[16, 17]

(C) The low dose required for production of mental aberrations in man aroused interest in the use of LSD or lysergic acid derivatives as potential incapacitating chemical agents. The potential use of LSD as a psychochemical agent is discussed in the Final Report of Task Blow Top,[18] which contains extensive information on the chemistry of lysergic acid derivatives. The report contains a bibliography, but the references are not cited in the text and the sources of information are difficult to establish.

B. (U) Production of D-Lysergic Acid and Derivatives.

The major problem in the use of lysergic acid derivatives has been a source of supply of lysergic acid.[18] Until recently, the principal source of lysergic acid was ergot. Although ergot forms on rye, wheat, oats, barley, rice, and grasses, in pharmaceutical practice the name ergot is used to refer to the product obtained from rye plants. Based on previous information,[18] 500 kg of ergot was assumed to yield about 1 kg of ergot alkaloids. The ergot alkaloids expressed as ergotoxine, an averaged mixture of ergocristine, ergocryptine, and ergocornine, would have the approximate composition $C_{33}H_{40}N_5O_5$ (molecular weight, 586). Since each alkaloid molecule contains 1 molecule of lysergic acid (molecular weight, 268), the theoretical yield of lysergic acid is 268/586, or 47% of the alkaloid. Thus, 1 kg of ergot alkaloids would yield a maximum of 0.47 kg of lysergic acid. The production of 1 kg of lysergic acid would require over 1,000 kg of ergot, and, ignoring conversion costs, the ergot alone would cost $2,200 (assuming a cost of $1/lb). Aspects of the worldwide production of ergot have been reported by Kleiderer[19] and by Smith.[20]

Another biological source for lysergic acid is the seeds of Rivera corymbosa,[21] recently discovered to contain D-lysergic acid amide. Taber and Heacock[22] have shown that the alkaloids were present in the embryo of the

seed but not in the seed coat, and that the Claviceps species, the previously known source of ergot, were not detected in the seed. They concluded that the seed, rather than a fungus or bacterium in the seed, is the source of alkaloid.

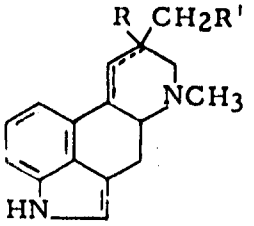
The total synthesis of lysergic acid has been accomplished by researchers at Eli Lilly and Co. and at Harvard University,[23,24] but the overall yield from N-benzoylindoline-3-propionic acid has been reported to be less than 1% of D, L-lysergic acid. Pilot-plant studies at Eli Lilly and Co. have indicated that improvement in some of the latter steps of the 15-step synthesis might lead to overall yields of about 2%,[18] but that the cost of synthetic D-lysergic acid would be similar to that for D-lysergic acid produced from ergot.[25] They estimated the cost to be $4,000 to $5,000/kg of D-lysergic acid.

The production of lysergic acid, or lysergic acid derivatives, by the saprophytic culture of the Claviceps species has been extensively investigated.[19,20,26-28] Although processes for production of ergot alkaloids had been patented,[26,27,29] the yield of alkaloids had been low and the development of large-scale fermentations had not been successful.[20] In 1960, Arcamone et al.[30] published a paper claiming the production of lysergic acid derivatives by the saprophytic culture of a strain of Claviceps paspali Stevens and Hall. The production of total alkaloids (calculated as lysergic acid) at a level of 2 mg/ml of fermentation broth was reported. The main products of the fermentation were lysergic acid amide, isolysergic acid amide, lysergic acid α-hydroxyethylamide, and isolysergic acid α-hydroxyethylamide. The latter products had not been reported previously to occur in nature. A more complete paper from Arcamone et al.[31] gives details on the Claviceps paspali strain and its development, the fermentation process, and the isolation and characterization of the fermentation products. The paper states that the only lysergic acid derivative produced by the Claviceps paspali strain was the α-hydroxyethylamide, which is unstable and readily decomposes into lysergic acid amide and acetaldehyde during extraction of the alkaloids. A United States patent assigned to Societa Farmaceutici Italia (Farmitalia) covering this process was granted in June 1962.[32] The patent claims the production of alkaloids at levels of 1.4 to 1.6 mg/ml in 90-ℓ fermentations. Chain[33] reported that under optimal conditions an alkaloid concentration of 5 mg/ml was produced, and that at this production rate the alkaloid is a cheap, readily available precursor for the semisynthetic ergot alkaloid industry. Production levels of 5 to 6 mg/ml of lysergic acid in fermentation tanks of 30,000 ℓ were also reported by Tonolo.[34] Since the presence of lysergic acid in these fermentations was not stated definitely, these production levels could be in error or could mean 5 to 6 mg/ml of alkaloids expressed as lysergic acid.[30] The

present lysergic acid production by this process is being done by Farmitalia. Farmitalia-quoted prices as of May 1963 for the fermentation-produced alkaloids were $12.31/gm for D-lysergic acid, $15.65/gm for D-lysergic acid amide, and $16.78/gm for D-lysergic acid α-hydroxyethylamide.

Kelleher,[35] in October 1962, made an estimate of the cost of fermentation-produced alkaloid. Using a yield of total alkaloid of 0.5 to 0.6 mg/ml (the level of alkaloid production at the University of Connecticut), he estimated the cost of total alkaloids to be approximately $500/kg. Cost data in a report on the commercial production of riboflavin by a submerged fermentation process and information in the "Exhibits of the Kefauver Hearings" on the cost to produce tetracycline by a fermentation process were used. Assuming this estimate to be correct and using the production level of 5 to 6 mg/ml of total alkaloids, the cost for the total alkaloids could be as low as $50/kg. Kelleher[36] also reported that more than 75% of the total alkaloids produced by the strain used at the University of Connecticut are lysergic acid derivatives. Carney[37] has stated that at a production level of 2 mg/ml of alkaloids, the fermentation process would be commercially feasible, and that in his opinion the synthetic process for lysergic acid production developed by Eli Lilly and Co. could not compete. Carney also stated that Lilly had obtained an ergonovine sample from Farmitalia that had been produced by the fermentation process.

In 1960, a contract for the study of the saprophytic culture production of lysergic acid derivatives was negotiated with the School of Pharmacy at the University of Connecticut, under the direction of Schwarting. The intent of this contract was the investigation of the biological conversion of clavine alkaloids to lysergic acid or lysergic acid derivatives. Clavine alkaloids (V) are the alkaloids normally produced by the saprophytic culture of Claviceps purpurea.[3, 20, 28] Because the Schwarting group had a Claviceps purpurea strain that produced elymoclavine in concentrations of 2 mg/ml of fermentation broth, the initial study was the attempted conversion of elymoclavine to lysergic acid.



V. Clavine Alkaloids

	Δ	R	R'
Agroclavine	8, 9	—	-H
Elymoclavine	8, 9	—	-OH
Penniclavine	9, 10	-OH	-OH
Triseclavine	9, 10	-OH	-H

Rochelmeyer[38] had proposed that elymoclavine was a precursor of lysergic acid in the biosynthesis of ergot alkaloids. The bio-oxidation study by Schwarting was just beginning when the original paper by Arcamone et al.[30] appeared. When a strain of Claviceps paspali (designated CD-3), one of the strains developed at the Istituto Superiore di Sanita, Rome, Italy, became available, the research emphasis at the University of Connecticut was shifted to studies using that strain. There was a possibility that Claviceps paspali was supporting a biological oxidation, because Arcamone et al.[30] claimed that an excess of oxygen in the culture fluid was essential for production of alkaloids. The transformation of elymoclavine into lysergic acid using a strain of Claviceps paspali has recently been reported.[39] The biogenetic relationship between the ergot alkaloids and the clavine alkaloids has been discussed in a review article by Weygand and Floss.[40]

Research on the production of ergot alkaloids by saprophytic culture under Contracts DA18-108-405-Cml-734 and DA18-108-AMC-42A with the University of Connecticut, the work performed by the Crops Division, U. S. Army Biological Laboratories, Fort Detrick, Md.,[41] and the work done under Contract DA18-064-404-Cml-510 with Michigan State University are described in the reports issued under these contracts. The contract research confirmed the report[20] that the available Claviceps paspali strains developed by Tonolo et al.[31] gave erratic production of ergot alkaloids. The Claviceps paspali strain CD-3 was very sensitive to changes in the composition of solutes in the tap water[42,43] used in the fermentation broth and of trace elements in

the broth.[43] Tonolo[44] states that the strain is so sensitive to trace elements that during strain-selection experiments the chemicals used in the media must always come from the same bottle. Use of another bottle of the chemical could lead to loss of alkaloid production.

Analysis at the University of Connecticut[45] of a sample of the alkaloids produced by the Farmitalia fermentation process indicated the following composition: n- and isolysergic acid amide, ergonovine, elymoclavine, chanoclavine, penniclavine, and agroclavine. The lysergic acid derivatives and elymoclavine together accounted for approximately 90% of the total alkaloids. Analysis[46] of alkaloids produced by CD-3 strains indicated the following composition: chanoclavine, n- and isolysergic acid amide, ergonovine, ergonovinine, n- and isolysergic acid α-hydroxyethylamide, and five unidentified chromatographically distinct alkaloids. The lysergic acid derivatives appeared to make up 85% to 90% of the total alkaloids. An alkaloid level of 1.2 mg/ml has been reached in isolated experiments in 1-ℓ fermentations, but in larger fermentations (20 to 40 ℓ) the total alkaloids produced varied from 0.3 to 0.5 mg/ml.[47] Addition of organic supplements, such as glycine, acetamide, and tryptophan, is very effective in increasing alkaloid production in cultures that produce 0.1 to 0.2 mg/ml on unsupplemented media. These same supplements, singly or in combination, lose their effectiveness when added to cultures capable of producing high alkaloid yields on unsupplemented, defined media.[43] Some of the research conducted at the University of Connecticut has been recently published.[48,49]

Another process for the production of lysergic acid and derivatives has been the aerobic fermentation of strains of Aspergillus, recently claimed by Sandoz S.A. in Belgian Patent No. 629,158. No information on yield of alkaloids was given, but the process is purported to be more economic than processes using natural ergot or by total synthesis, and apparently Aspergillus grows better than Claviceps.

The ergot alkaloids either from field-grown ergot or from saprophytic fermentations must be isolated and converted to lysergic acid, the starting material for the synthetic lysergic acid derivatives such as LSD. The process for isolation of field-grown ergot has been reviewed.[18] Alkaloids can be isolated from the fermentation broth by extraction with suitable solvents, such as benzene, chloroform, and methylene chloride, or by absorption or charcoal or bentonite.[31,32]

The extracted alkaloids can be hydrolyzed with alkali to give lysergic and isolysergic acid.[51-52]

A process for the conversion of peptide alkaloids to lysergic acid has been developed by Eli Lilly and Co.[53] The process is as follows: 50 gm of peptide alkaloid, calculated to be the equivalent of 70% ergotoxin, is dissolved with gentle warming in 125 ml of methanol. Separately, 50 gm of caustic soda is dissolved in 500 ml of water, and this alkaline solution is added to the methanol solution of the alkaloid. The mixture is actively refluxed for 1.5 hr and then chilled until it is ice cold. To the reaction mixture is added an ice-cold solution of 40 ml of concentrated sulfuric acid in 400 ml of water. The reaction mixture should not be allowed to become hot during the acidification. The reaction mixture is chilled overnight and filtered by suction. Residue on the filter funnel is pulled as dry as possible. A large amount of residue will remain on the sides of the flask. Residue on the filter funnel is transferred back to the flask containing the unfiltered residue and stirred for 30 min with 400 ml of 95% ethanol containing 40 ml of ammonium hydroxide. The reaction mixture is then filtered through talc and the filtrate is held. The residue is reextracted, with stirring, using 200 ml of 95% ethanol containing 20 ml of ammonium hydroxide. The material is again filtered and the two filtrates are combined. To the filtrate is added 25 gm of Darco G-60, and the mixture is stirred for 0.5 hr. It is then filtered through talc and the filtrate is held. The residue is reslurried with 200 ml of 95% ethanol containing 20 ml of ammonium hydroxide. (Note: This one reslurry is all that should be used.) The reslurried mixture is filtered through talc, and the two filtrates are combined and concentrated, preferably in vacuo, to about 250 ml volume. Crystals will usually start to form at about 500 ml volume. The concentrate is chilled overnight, then filtered, washed with cold ethanol, and dried in vacuo at about 50°C. This first crop of D-lysergic acid should weigh about 12 gm. The filtrate from this crop of crystals can be further concentrated to about 50 to 25 ml volume, then chilled. A second crop will result, weighing about 1 to 2 gm, and will contain D-lysergic acid with some isolysergic acid. The quantity of isolysergic acid in this second crop was not given.

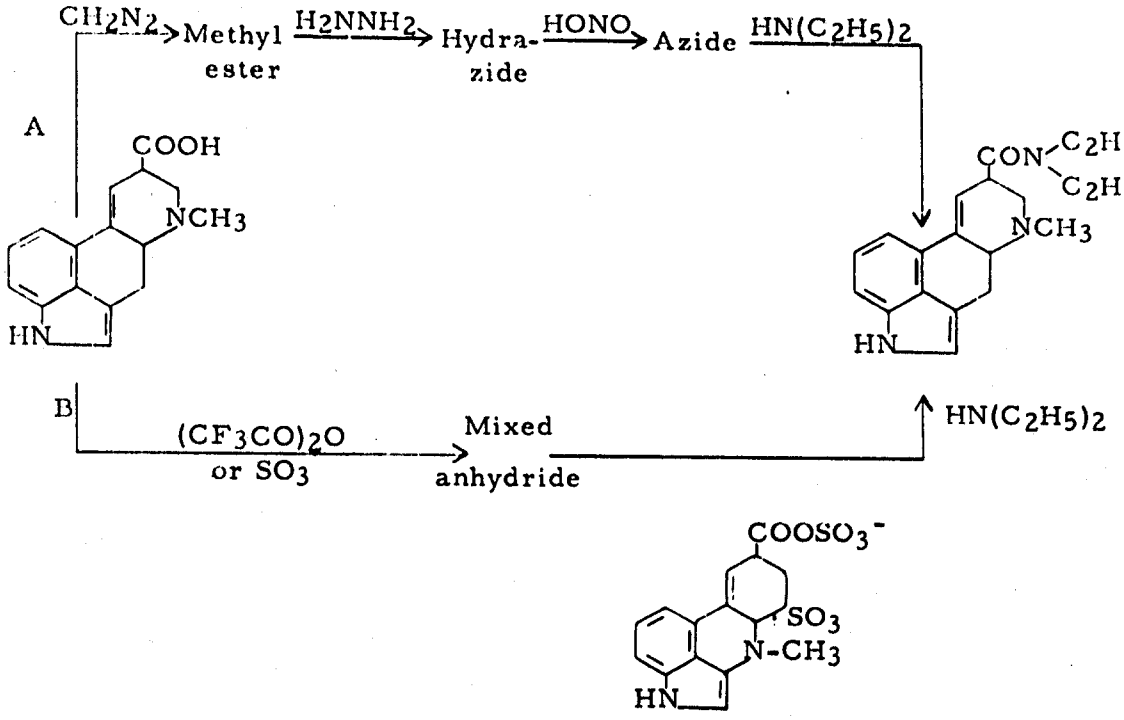
The Lilly workers state that the concentrations specified and the use of the relatively large quantity of Darco G-60 are important. Darco G-60 absorbs preferentially the isolysergic acid, which otherwise would interfere materially with the purification. Only one reslurry of the Darco G-60 with 95% ethanol and ammonium hydroxide is used, because more than one reslurry would remove the absorbed isolysergic acid. The average yield of first-crop D-lysergic acid is reported to be 73%, with an additional 6% to 12% of impure acid (contaminated with isolysergic acid) obtained from the second crop. Garbrecht, of Eli Lilly and Co., has recently stated that the claim that Darco G-60 removes isolysergic acid is not correct. Darco G-60 is important in the

process to remove impurities that would interfere with the crystallization of the lysergic acid, but Garbrecht has never been able to recover isolysergic acid from Darco G-60, and doubts that little if any isolysergic acid is absorbed.[54]

The physical constants[55] for D-lysergic acid as a monohydrate are: mp 240°C (dec); $[\alpha]_D^{20}$ +40° (c 0.5, pyridine); λ_{max} 229, 239, and 310 mμ; pK_a 3.44, pK_b 7.68. The physical constants for isolysergic acid as the dihydrate are: mp 218°C (dec); $[\alpha]_D^{20}$ +281° (c 1, pyridine); pK_a 3.44, pK_b 8.61.

C. (U) Synthesis of LSD and Other Lysergic Acid Amides.

The methods for the conversion of lysergic acid to the physiologically active amides are outlined below (VI).[3] In the method of Stoll and Hofmann [11,56-59] (A in VI), lysergic acid is converted to the ester by treatment with diazomethane, the ester is reacted with hydrazine to give the hydrazide, the hydrazide is treated with nitrous acid to yield the azide, and the azide is reacted with a suitable amine to give the corresponding amide. The hydrazide can be prepared directly from the ergot alkaloid. Garbrecht[60] states that although the procedure is frequently capable of producing the amide product in good yields, certain inherent difficulties reduce its practical value. Of these, the most important is that the necessary reaction conditions for preparing the lysergic acid hydrazide result in a racemized and isomerized material, D,L-isolysergic acid hydrazide. Further, the method leaves much to be desired in terms of operational ease, since the azide must be collected in a relatively large volume of ether, and several hours are required to perform the acetylation step. (A yield of 70% pure racemic isolysergic acid hydrazide from ergotamine has been reported,[56] and this has been converted to the LSD tartrate in a yield of 12%.[58]) The Pioch[61] and Garbrecht[60,62] methods both use D-lysergic acid as the starting material, which is converted to a mixed anhydride of lysergic acid and trifluoroacetic acid (Pioch), or lysergic acid and sulfuric acid (Garbrecht). The mixed anhydride is treated with a suitable amine to yield the corresponding amide: In the Garbrecht procedure, the lithium salt of D-lysergic acid monohydrate was preferred to free lysergic acid, because the salt gave better yields of amide product.[60] The products from both of these methods were free from racemization, but contained both n- and isolysergic acid amides that could be separated by chromatography or by suitable methods of fractional crystallization. Garbrecht[60] reports a first-crop yield of ergonovine maleate of 65%. The yield of LSD by this method was 25%.[63] The Task Blow Top Report,[18] in a figure identical to VI, indicates a yield of 53.4% of mixed diethylamides. Pioch[61] reports a 53.4% yield of residue from the chloroform extract of the reaction that comprises the normal and iso forms of LSD.



VI. Synthesis of Lysergic Acid Amides

Garbrecht,[60] reviews the reaction systems applied to lysergic acid amide synthesis, and reports that attempts to prepare lysergic acid chloride yield only decomposition products. Hofmann and coworkers,[64] however, report the preparation of ergotamine by the reaction of the peptide portion of ergotamine with lysergic acid chloride hydrochloride.

The report does not contain experimental details, but references to Swiss Patent Application No. 11132 are made for lysergic acid chloride hydrochloride, and it is stated in a footnote that the detailed publication will appear later in Helvetica Chimica Acta. Detailed publications[65, 66] have recently appeared that give experimental details for the preparation of the peptide portion of ergotamine and the synthesis of ergotamine; the synthesis of lysergic acid chloride hydrochloride is not given, but reference is made again to the Swiss patent application. An abstract[67] of a Belgian patent reports the preparation of 9, 10-dihydrolysergic acid chloride hydrochloride by treatment of 9, 10-dihydrolysergic acid with phosphorus pentachloride and phosphorus oxychloride at 90°C. The dihydrolysergic acid chloride was used to prepare dihydroergotamine. The statement is made that ergotamine can be similarly prepared.

An abstract[68] of a French patent describes the preparation of lysergic acid amides, including LSD, by the treatment of lysergic acid with a phosgene dimethylformamide complex followed by treatment with an appropriate amine. No yield of product was given.

The amides of lysergic acid are usually obtained as the crystalline maleate or tartrate salt. The amides of isolysergic acid generally fail to form such crystalline salts and remain in the mother liquors.[60]

The physical constants for n- and iso-LSD, the acid maleate, and the tartrate are found in table 1.

TABLE 1

PHYSICAL CONSTANTS OF LSD AND ITS SALTS

Compound	Melting point	$[\alpha]_D^{20}$	pK_a	Ultraviolet maxima (λmax)
	°C			mμ
LSD	80 - 85 (dec) a/	+17° (c 0.5, pyridine) b/	6.37 c/	313 (ε 9,330) d/
LSD acid maleate e/	145 e/	-4° (c 1.16, methanol) a/	–	314 (ε 8,600) e/
	195 (dec) f/	–	–	314 (ε 8,700) f/
$(LSD)_2$ tartrate	198 - 200 (dec) b/	+30° (c 1, water) b/	–	–
iso-LSD	182 (dec) a/	+217° (c 0.45, pyridine) a/	7.52 c/	–

a/ Stoll, A., and Hofmann, A. Helv. Chim. Acta 26, 944 (1943).

b/ Ibid. 38, 421 (1955).

c/ Stoll, A., et al. Ibid. 37, 2039 (1954).

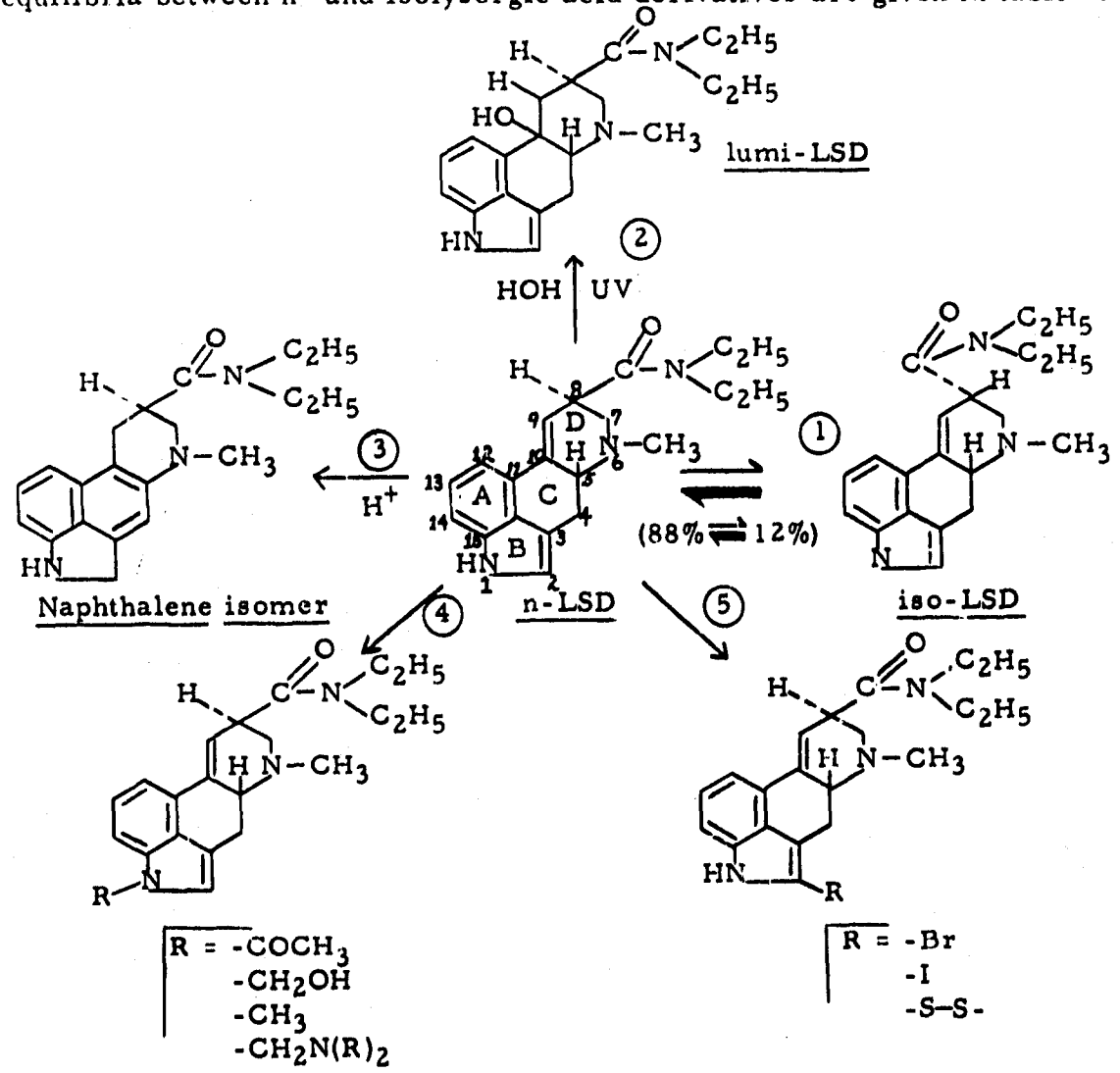
d/ Troxler, F., and Hofmann, A. Ibid. 40, 1706 (1957).

e/ Data obtained on sample from W. L. Garbrecht, Eli Lilly and Co.

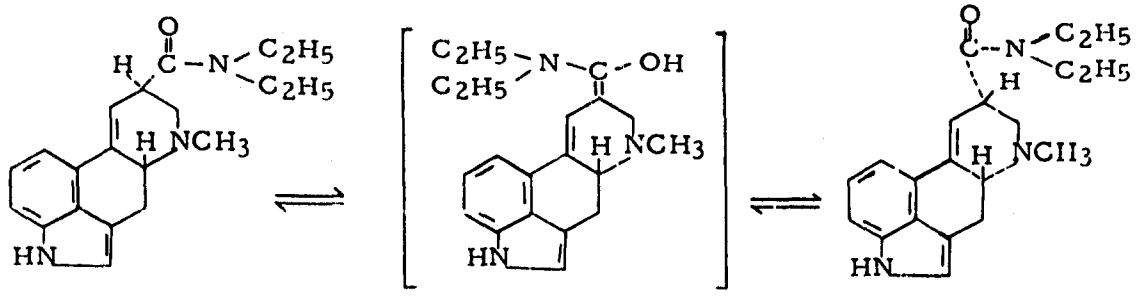
f/ After precipitation from methanol with ether.

D. (U) Chemistry of LSD and Related Compounds.

Some of the important reactions of LSD are illustrated in VII. Reaction (1) is the isomerization reaction that, as previously indicated, involves the configuration at C_8. The mechanism of this isomerization is explained by the enolization of the carbonyl group at position C_8, leading to intermediate formation of an acid enolate that rearranges to the acid form (VIII).[9] The extent of the isomerization for lysergic acid and various derivatives of lysergic acid has been studied by Smith and Timmis[51,69] and by Stoll et al.[70] The equilibria between n- and isolysergic acid derivatives are given in table 2.



VII. Reactions of LSD



VIII. Isomerization of LSD

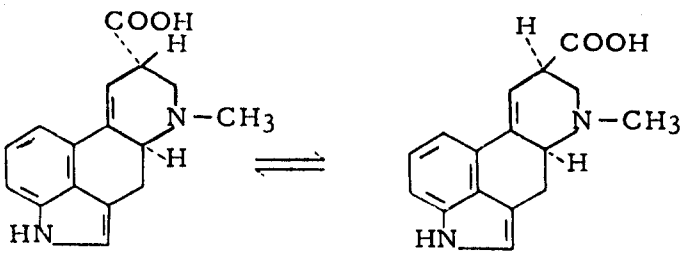
TABLE 2

ISOMERIZATION EQUILIBRIA BETWEEN n- AND ISOLYSERGIC ACID DERIVATIVES

Compound	n-Lysergic acid form	Isolysergic acid form
	%	
Lysergic acid*	43	57
Ergosine	42	58
Ergocryptine	48	52
Ergobasine	52	48
Lysergic acid ethylamide	54	46
Lysergic acid dimethylamide	84	16
LSD	88	12

* Calculated from data of Smith, S., and Timmis, G. M. J. Chem. Soc. 1956, 1440 (1956).

Lysergic acid and its derivatives can also exist in an L- configuration (IX). The L- form of lysergic acid does not exist in nature, and was formed by treatment of the natural D-lysergic acid with an alcoholic aqueous barium hydroxide solution at 150°C.[51] The L-lysergic acid derivatives are physiologically inactive.[5,11] Stoll and Hofmann[71] isolated the levorotatory L- isomers and made a study of their stereochemistry.[56]



normal iso

IX. Configurations of L-Lysergic Acid

Ergot alkaloids and other lysergic acid derivatives are sensitive to light. Stoll and Schlientz[72] studied this light reaction and isolated two light-exposure products, which they designated with the prefix lumi. The new compounds were prepared by irradiation of the alkaloids in aqueous acid solutions with ultraviolet light. In the absence of ultraviolet radiation under otherwise similar conditions, no reaction occurs. The lumialkaloids lack the characteristic blue fluorescence of lysergic acid in ultraviolet light. Since the double bond in the 9, 10- position is responsible for the blue fluorescence, this double bond was assumed to be saturated upon irradiation. Analysis of the lumi product of ergotamine gave an empirical formula that corresponded to the addition of 1 molecule of water. It was concluded that under ultraviolet irradiation, water adds to the 9, 10- double bond and that the hydroxyl is attached to the C_{10} [reaction (2), VII]. Hellberg[73] has studied this reaction and states that suitable, strongly polar constituents must be in the solution in sufficient quantities. In pure water or in solutions of neutral salts, no transformation occurs on the basis of the change in the maxima at 315 mμ, during 1 hr of irradiation. Hellberg[74-76] also reports the preparation and isolation of the four possible lumilysergic acids. The physical constants for lumilysergic acid diethylamide (I) are: mp 223°C; $[\alpha]_D^{20}$ -29° (c 0.3, pyridine); λ_{max} 223 mμ (log ε 4.55), 284 mμ (log ε 3.89), 293 mμ (log ε 3.88); and for lumilysergic acid diethylamide (II) are: mp 256°C; $[\alpha]_D^{20}$ -75° (c 0.2, pyridine); λ_{max} 225 mμ (log ε 4.51), 285 mμ (log ε 3.83), 293 mμ (log ε 3.82).

In acid reagents, lysergic acid and its derivatives are changed and no longer give the color reactions (van Urk, Keller) characteristic for the indole system. Stoll and Petrzilka[77] investigated this change and showed that it was the conversion of the lysergic acid system into the corresponding isomer, the benz[c, d]indoline derivative [reaction (3), VII], in which rings A and C form

a naphthalene system. The benz[c,d]indoline was isolated and crystallized in the form of the stable N-acetate, and the structure was established by synthesis. The rearrangement of lysergic acid into the benzindoline form is irreversible, and the naphthalene form was shown to be thermodynamically favored. The inability to reverse this reaction was of great significance in the attempts to synthesize lysergic acid. The rearrangement is very temperature-dependent—the reaction at 25°C requires 48 hr, and the reaction at 100°C is complete after only a few minutes. The rearrangement studies were performed in glacial acetic acid, chloroform, methanol, or ethanol saturated with hydrochloric acid. The physical constants for the naphthalene isomer of LSD as the N-acetate are: mp 164° to 167°C; $[\alpha]_D^{20}$ -12° (c 0.3, pyridine).

Troxler and Hofmann[78-80] have prepared lysergic acid derivatives substituted on the ring system of the lysergic acid [reactions (4) and (5), VII], and give the infrared and ultraviolet spectra of LSD and these derivatives. Table 3 contains a list of the ring-system substituents of LSD and their physical properties. The derivatives substituted in the 2- position do not give the van Urk test, which requires the 2- position to be free,[81] and 1-dimethylaminomethyl LSD also fails to give this test.[80] Reaction of LSD with an excess of potassium amide and methyl iodide yields 8-methyl- and 1,8-dimethyl-D-isolysergic acid diethylamide.

Lysergic acid and its derivatives are easily reduced with sodium and amyl alcohol,[82] or by catalytic hydrogenation,[83] to give the 9,10-dihydro-derivative. The similarity of the ultraviolet spectra of the dihydro- and lumi-derivatives of ergotamine is shown in figure 1.[70] The physical constants of dihydrolysergic acid diethylamide prepared from D-dihydroisolysergic acid azide and diethylamine are: mp 130° to 135°C; $[\alpha]_D^{20}$ -114° (c 0.5, pyridine); those for the tartrate salt are: mp 180° to 185°C; $[\alpha]_D^{20}$ -79° (c 0.9, water).

TABLE 3

DERIVATIVES OF LSD

Derivative	Empirical formula	Melting point	$[\alpha]_D^{20}$
		°C	
1-Acetyl- a/	$C_{22}H_{27}O_2N_3$	— b/	-14° (c 0.5, pyridine)
1-Acetyl- as acid tartrate a/	$C_{22}H_{27}O_2N_3 \cdot C_4H_6O_6$	186 - 190	-6° (c 0.5, pyridine)
1-Hydroxymethyl- a/	$C_{21}H_{27}O_2N_3$	164 - 166	+23° (c 0.5, pyridine)
1-Dimethylamino-methyl- a/	$C_{23}H_{32}ON_4$	— b/	+15° (c 0.5, pyridine)
1-Methyl- c/	$C_{21}H_{27}ON_3$	— b/	+20° (c 0.5, pyridine)
1-Methyl- as acid tartrate c/	$C_{21}H_{27}ON_3 \cdot C_4H_6O_6$	110 - 120	+25° (c 0.5, water)
2-Bromo- d/	$C_{20}H_{24}ON_3Br$	120 - 127	+53° (c 0.5, chloroform)
2-Bromo- as acid tartrate d/	$C_{20}H_{24}ON_3Br \cdot C_4H_6O_6$	130 - 140	+34° (c 0.5, water)
2-Iodo- d/	$C_{20}H_{24}ON_3I$	206 - 208	+22° (c 0.5, pyridine)
Disulfide e/	$C_{40}H_{48}N_6O_2S_2 \cdot 2H_2O$	184	-1,020° (pyridine)

a/ Noncrystalline product.

b/ Troxler, F., and Hofmann, A. Helv. Chim. Acta 40, 1706 (1957).

c/ Ibid., 1721 (1957).

d/ Ibid., 2160 (1957).

e/ Reported in J. Am. Chem. Soc. 79, 3191 (1957), as being prepared by A. Hofmann. Physical constants given are for Hofmann compound; no concentration in pyridine for $[\alpha]_D^{20}$ given.

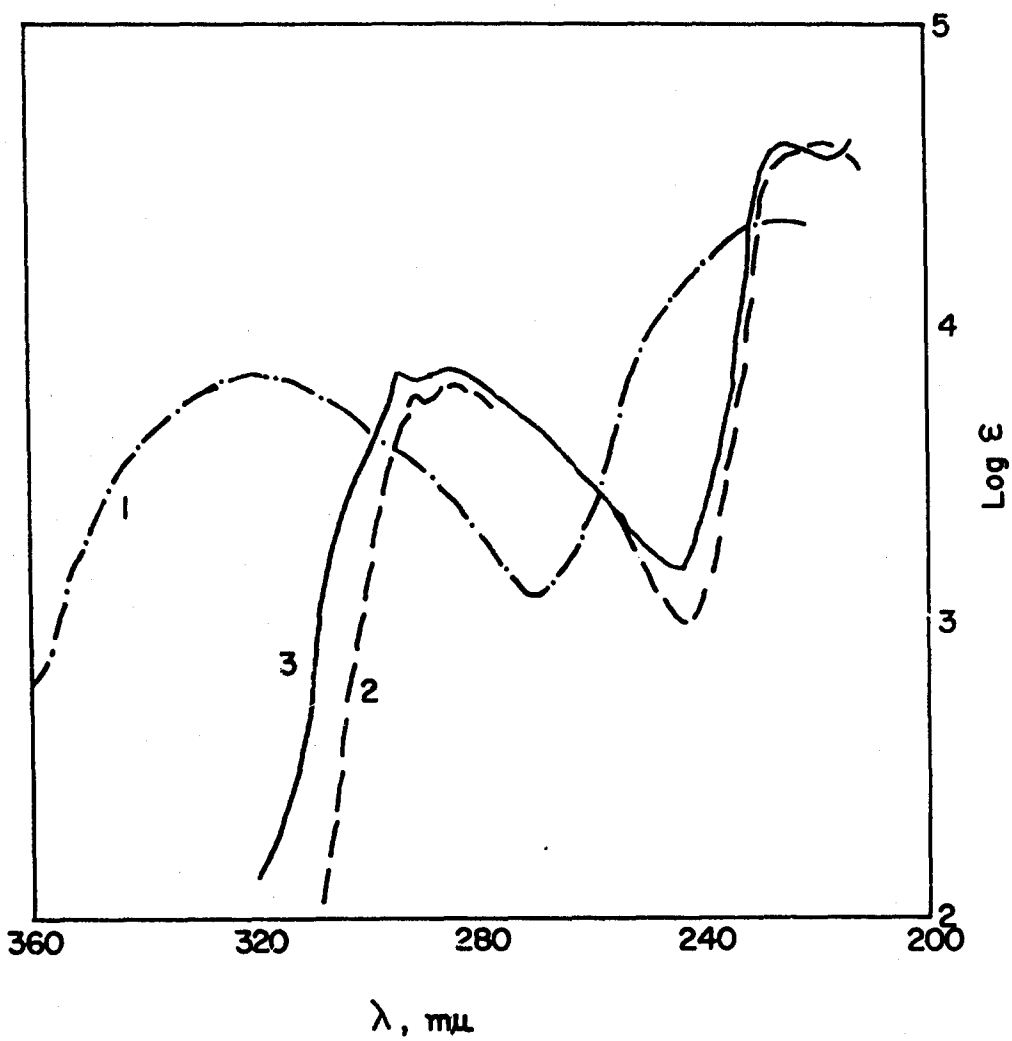
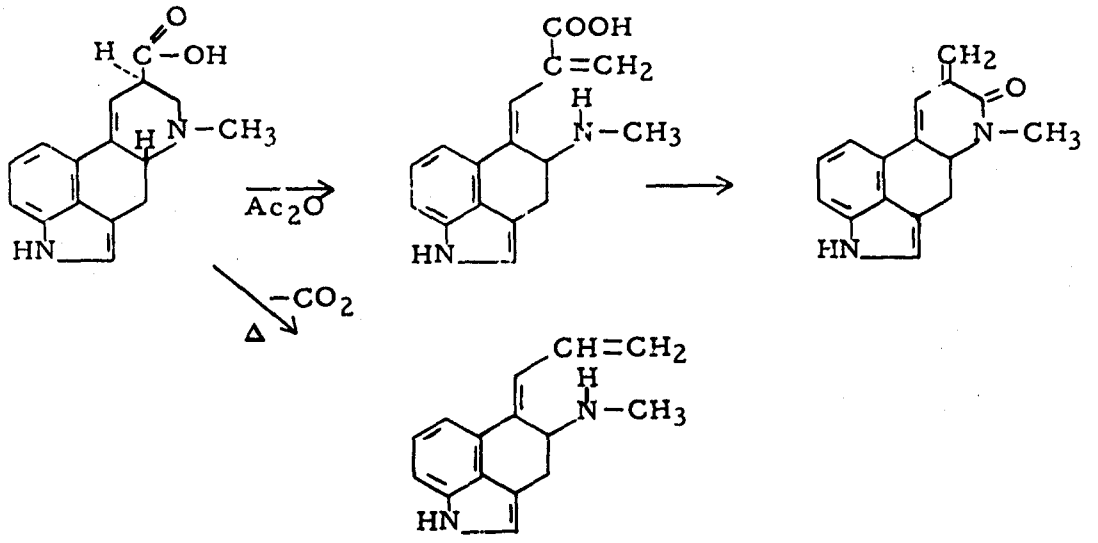


FIGURE 1

ULTRAVIOLET SPECTRA OF LYSERGIC ACID DERIVATIVES

Curve 1 - Ergotamine
Curve 2 - Dihydroergotamine
Curve 3 - Lumiergotamine

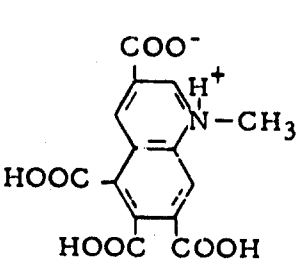
Lysergic acid and its derivatives are thermally unstable and decompose at their melting points (table 1). When lysergic acid and isolysergic acid are heated with acetic anhydride, they react like β-aminocarboxylic acids to give a lactam (X). This reaction was important in assigning the double bond in ring D to the 9, 10- position.[9]



X. Lactam Formation and Decarboxylation of Lysergic Acid

Decarboxylation[9] of the lysergic and isolysergic acids resulted also in the rupture of the bond between N_6 and C_7, as shown in structure X.

The oxidation of lysergic acid with nitric acid was studied by Jacobs.[84] The products of this oxidation were phenol, p-nitrobenzoic acid, and a crystalline substance, $C_{14}H_9O_8N$, whose structure is A in XI.[6]



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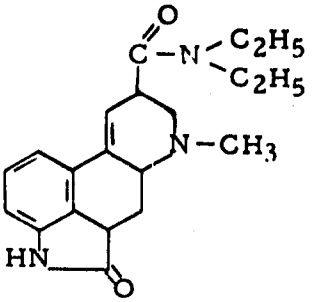

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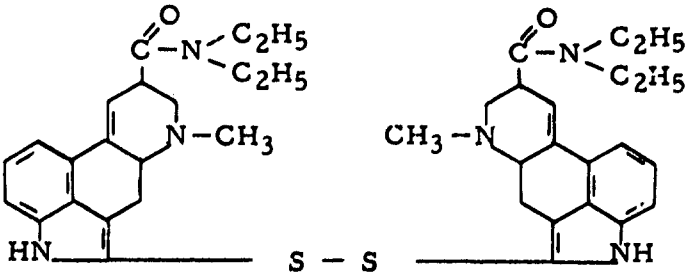

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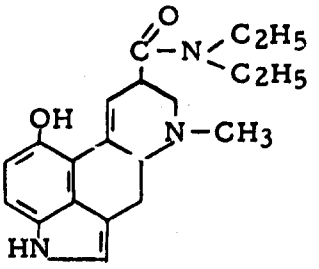

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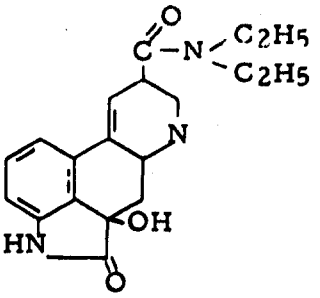

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XI. Oxidation Products of Lysergic Acid

Axelrod et al.[85] claimed that metabolism of LSD gave the 2-oxindole derivative (structure B, XI). A later paper[86] reports the synthesis of this compound by the hydrolysis of the disulfide of LSD (structure C, XI). Oxidation of LSD with peracetic acid gave, using paper chromatography, two spots of R_f 0.5 and 0.85. The spot R_f 0.5 gave a positive van Urk test and fluorescence under ultraviolet light. On standing in solution, or, more rapidly when treated with acid, the spot at R_f 0.5 disappeared to give the van Urk-negative compound, R_f 0.85. The hydrolysis product of the disulfide, structure B, also gave a van Urk-negative spot at R_f 0.85.

Slaytor and Wright[87] have recently reported that the metabolite of LSD in rat bile is 12-hydroxy-D-lysergic acid diethylamide (structure D in XI). Hofmann and Troxler[88] have reported that the careful treatment of LSD with calcium hypochlorite gave 2-oxo-3-hydroxy-2,3-dihydro-D-lysergic acid diethylamide (structure E in XI). Reduction of this compound gave 2-oxo-2,3-dihydro-D-lysergic acid diethylamide (structure B in XI), previously reported by Axelrod[85] to be a biological oxidation product of LSD.

The section "Physical and Chemical Properties of LSD" in the Task Blow Top Report[18] states that dilute oxidizing agents attack the ring structure, producing a "psychoto-mimetically inactive compound." The same statement is made for reaction with chlorine, hypochlorites, and chloramides. The information in this report is not referenced; hence, the source of information is not known.

E. (U) Stability of Lysergic Acid Derivatives.

The Task Blow Top Report[18] also states that solid samples of LSD are stable in cool, moisture-free, light-tight containers and that aqueous solutions are stable for about 7 days.

The only published information on solution stability of lysergic acid derivatives is found in a paper by Schlientz et al.[89] Additional information on stability of lysergic acid amides was obtained from Eli Lilly and Co.[90] The paper reports that aqueous injection solutions of 0.02% D-lysergic acid (+)-butan-1-ol-amide (2)-hydrogen maleate at pH 3.2 were 88% intact at the end of 11.2 yr. The products found after 11.2 yr were 88% D-lysergic acid butanolamide, 10% D-isolysergic acid butanolamide, 1% D-lysergic acid, and 1% D-isolysergic acid. The information obtained from Eli Lilly and Co. concerned the storage stability of ampoules (type 1 amber glass) containing 0.215 mg/ml of ergonovine maleate at pH 2.8 to 3.0. The solution contained

0.1% lactic acid and 0.1% ethyl lactate, which served as mild antioxidants. The ampoules were sealed in air, sealed with exclusion of air, and sealed with exclusion of air by a special seal. It was felt that the special seal gave a sealed ampoule with less air. The data obtained by the workers at Lilly are reported in table 4. The loss of ergonovine is believed to be caused by oxidation, since only 0.04 mg of oxygen would be required for oxidation of the ergonovine in an ampoule. Ampoules of ergonovine solutions at pH 4 to 5 showed the formation of ergonovinine in 0.5 yr, and those at pH 7 indicated the establishment of equilibrium between ergonovine and ergonovinine in 1 hr. The storage stability of solid ergonovine samples has not been studied, but the researchers at Lilly feel that solids should have good storage stability, since ergonovine tablets left from World War II and stored in Italy were 90% intact after years of storage.

TABLE 4

STORAGE STABILITY OF ERGONOVINE MALEATE SOLUTIONS

Type of ampoule seal	Temperature	Time	Ergonovine
	°C	yr	%
Air	5	4	61
Exclusion of air	5	4	95
Exclusion of air by special seal	5	4	100
Air	50	0.5	50
Exclusion of air	50	0.5	73.5
Exclusion of air by special seal	50	0.5	82.5

F. (U) Analysis of Lysergic Acid Derivatives.

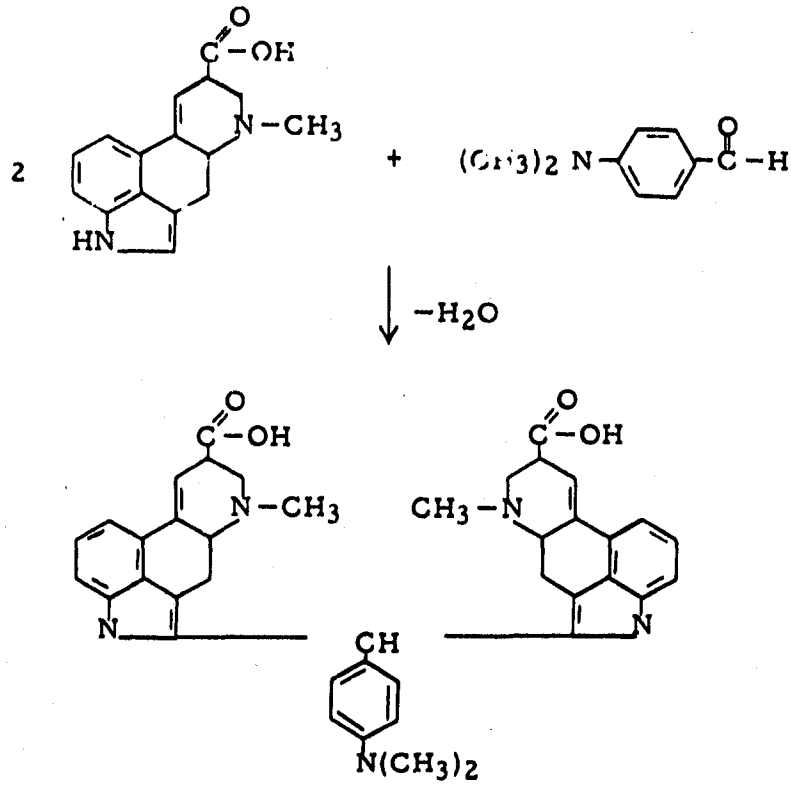
The qualitative detection of lysergic acid and lysergic acid derivatives is based on observation of the characteristic blue fluorescence of the compounds in solution, and on the Keller or van Urk color test. The characteristic blue fluorescence under ultraviolet irradiation is caused by the 9, 10- double bond in conjugation with the indole ring system of lysergic acid, and changes in this conjugated system lead to the loss, or modification, of the characteristic fluorescence. The Keller test is based on the development of a blue ring at the interface of an aqueous sample mixed with acetic acid and concentrated sulfuric acid containing ferric chloride. The test is not specific for lysergic acid. The van Urk test[91] is based on the reaction of p-dimethylaminobenzaldehyde (PDAB) in a mineral acid solution with lysergic acid derivatives to yield a blue color.

Quantitatively, the van Urk test is the official colorimetric assay procedure in the U. S. Pharmacopoeia, with which all determinations are made at a wavelength of 550 mμ.[92] The van Urk test is dependent on an unblocked 2- position. Pohm[81] has described the mechanism of the color reaction (XII). Excellent reviews of the van Urk method are given in the paper by Michelon,[93] the report by Kelleher and by Schwarting,[94] and the papers by Voigt.[95, 96] The Michelon paper describes the use of sodium nitrite to enhance the color produced by the reaction between PDAB and the ergot alkaloids.

Leeman and Weller[97] describe the use of p-toluenesulfonic acid as a color reagent for indole compounds. This reaction can be used for quantitative estimation.

The lysergic acid derivatives can be quantitatively analyzed by ultraviolet spectrophotometry by measuring the adsorption at 310 mμ. This method can detect as little as 5 μg/ml of LSD.[85] The most sensitive method is spectrophotofluorometry, which can detect concentrations as low as 0.001 μg/ml.[84] The fluorescence is measured at 445 mμ when the compound is activated with 325-mμ light.

Biological methods are also available for the quantitative determination of LSD.[3, 18]



XII. Color Reaction of Lysergic Acid to Form Blue Compound of Rosindole Type

Ergot alkaloids, lysergic acid, lysergic acid derivatives, and clavine alkaloids can be separated and purified by both adsorption and partition chromatography. The ergot alkaloids have been separated by paper chromatography[87, 98-100] and by thin-layer chromatography (TLC).[101, 102]

The chromatography of lysergic acid[103] and its separation from isolysergic acid have been described.[89, 104] The quantitative estimation of the ergot alkaloids by paper chromatography is also given.[89] Lysergic acid derivatives have been separated by paper[73, 89] and by thin-layer systems.[21, 46] The paper chromatography of LSD has been reported in several papers.[86, 87] The separation of clavine-type alkaloids has been investigated by Voigt.[96]

C. (C) Route of Administration of Lysergic Acid Derivatives.

(U) The Task Blow Top Report[18] states that LSD was effective by injection, by the oral route, and should be effective by inhalation. A recent review article[105] reports that the use of orally inhaled ergotamine tartrate in the treatment of vascular headaches, including migraine and histamine cephalalgia, has been clinically demonstrated.[106, 107]

(C) Because of the continuing interest in LSD as an incapacitating agent, the synthesis and chemistry of the compound and its derivatives were investigated, as described below.

II. (U) EXPERIMENTATION.

A. Materials and Equipment.

D-Lysergic acid monohydrate, lot No. 37924, D-lysergic acid amide, lot No. 44709, and D-lysergic acid α-hydroxyethylamide, lot No. 44602, had the following Farmitalia specfications:

	D-Lysergic acid		
	Monohydrate	Amide	α-Hydroxyethylamide
Loss on drying, %	6.8 (80°C, 1 mm Hg)	2.2 (80°C, 3 hr in vacuo)	0.58 (65°C in vacuo)
Melting point, °C (all decomposed)	226 - 228.5	204 -209	190 - 195
$[\alpha]_D^{20}$ (c 0.5, pyridine)	+32°	—	0.0
Purity (van Urk test), %	89.1	92.50	96.3
Ultraviolet spectrum, mμ	max at 229, 241, 310; min at 269	See figure 2	See figure 3
Isolysergic acid present, %	ca. 2.5	—	—

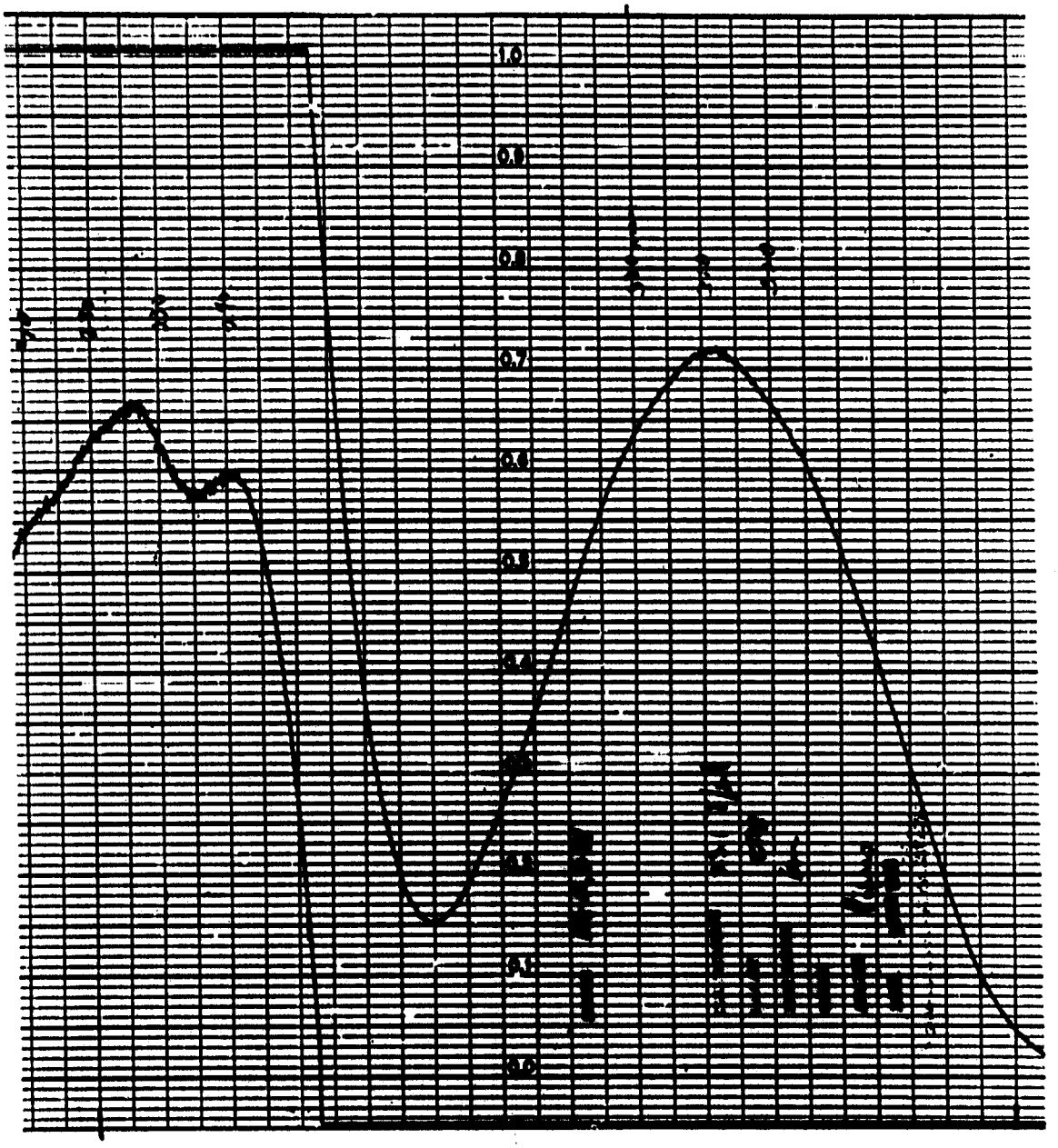


FIGURE 2

ULTRAVIOLET SPECTRUM OF D-LYSERGIC ACID AMIDE

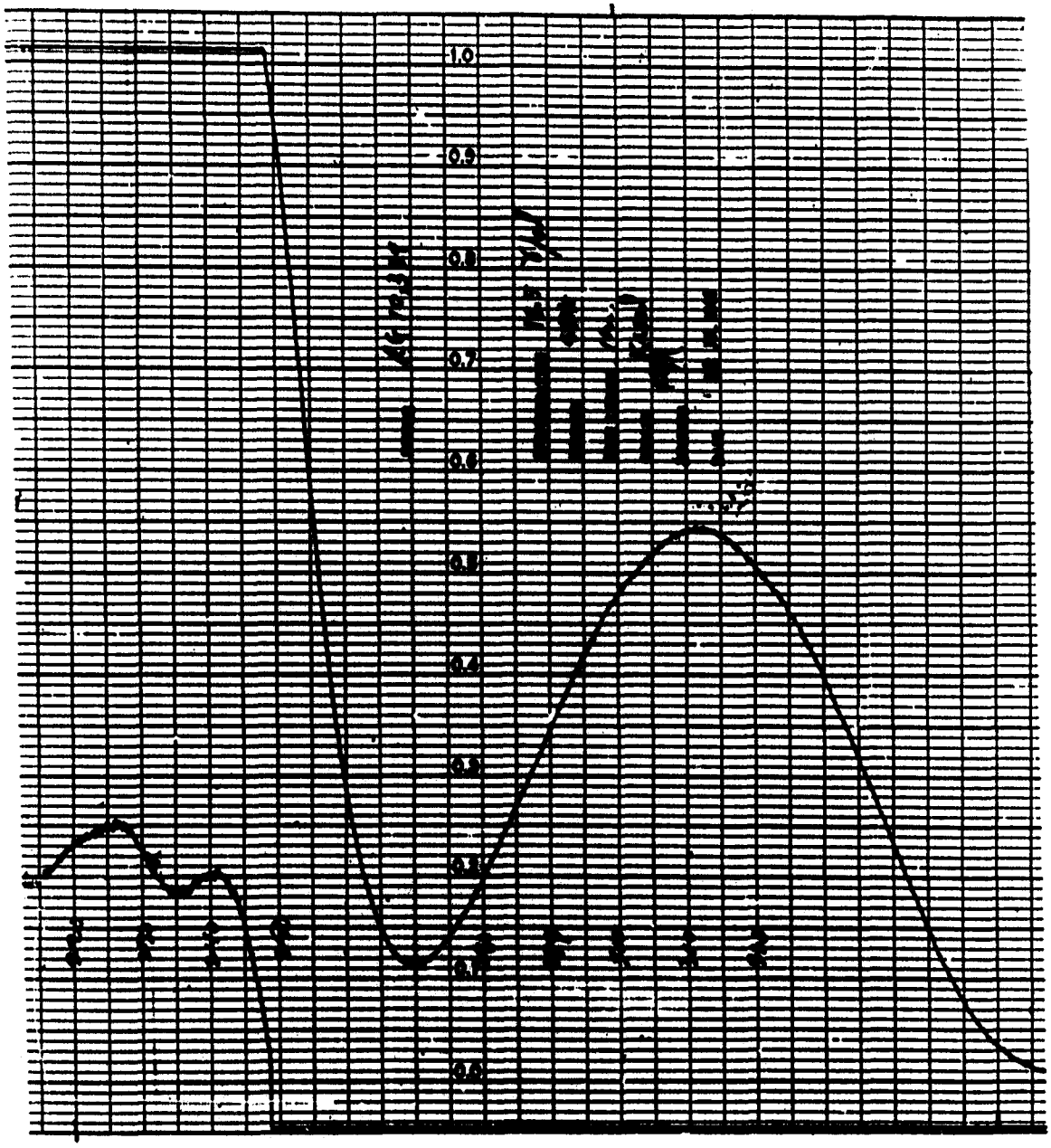


FIGURE 3

ULTRAVIOLET SPECTRUM OF D-LYSERGIC ACID
α-HYDROXYETHYLAMIDE

All the synthetic runs and the chemical studies were performed in ordinary ground-joint laboratory glass equipment. In the experiments for the preparation of lumi-LSD, Vycor glass equipment was used.

The thin-layer chromatoplates were prepared using the Brinkmann Instruments, Inc., apparatus, and the layers used were the standard 250-mμ thickness unless otherwise noted. The silica-gel chromatoplates were prepared using silica gel G prepared according to Stahl and obtained from Brinkmann Instruments, Inc. The cellulose powder, MN 300 G, and the aluminum oxide, G, were also obtained from Brinkmann Instruments, Inc.

B. Procedures.

1. Isolation of D-Lysergic Acid.

a. Isolation of D-Lysergic Acid From Ergot Alkaloids.

The procedures for the hydrolysis of the ergot alkaloids and the isolation of D-lysergic acid have been reported by Eli Lilly and Co.[53] A 50-gm sample of cristamine was dissolved with gentle warming in 125 ml of methanol. Separately, 50 gm of sodium hydroxide was dissolved in 500 ml of water and added to the methanol solution of the alkaloid. The mixture was refluxed for 1.5 hr and then chilled with a dry ice-trichloroethylene mixture to -10°C. An ice-chilled solution of 40 ml of concentrated sulfuric acid in 400 ml of water was added to the reaction mixture while the reaction temperature was maintained at 0° to 10°C. The acidified reaction mixture was cooled overnight at 3°C and filtered by suction. The solid residue on the filter paper was sucked as dry as possible and transferred back to the flask that contained unfiltered residue; this mixture was stirred for 30 min with 400 ml of 95% ethanol containing 40 ml of concentrated ammonium hydroxide. The reaction mixture was filtered through talc and the filtrate was held. The residue was resuspended, with stirring, in 200 ml of 95% ethanol containing 20 ml of concentrated ammonium hydroxide and again filtered through talc. Darco G-60 (25 gm) was added to the combined filtrates, and the mixture was stirred for 0.5 hr and filtered through talc. The Darco G-60 residue was reslurried with 200 ml of 95% ethanol containing 20 ml of concentrated ammonium hydroxide and filtered through talc. The two filtrates were combined and concentrated in vacuo to about 250 ml. The concentrate was allowed to stand overnight at 3°C and then filtered to yield 7.67 gm of D-lysergic acid: mp 226° to 227°C (dec after drying for 2 hr at 67°C/0.2 mm); $[\alpha]_D^{20}$ +35° (c 0.5, pyridine); λ_{max} 308 mμ (ε 9,280); yield 15.3% (based on 50 gm of cristamine). The

mother liquor was concentrated to 50 ml and an additional 1.2 gm of impure D-lysergic acid was obtained. An attempt to isolate crystalline isolysergic acid from the Darco G-60 residue was unsuccessful. Pertinent physical constants for commercial samples are as follows:

	Lysergic acid	Lysergic acid monohydrate
Melting point, °C	230 - 233 (dec)	226 - 228.5 (dec)
$[\alpha]_D^{20}$ (c 0.5, pyridine)	+36°	+32°
ϵ_{max} (310 mμ)	9,169	–
Purity (van Urk test), %	98	89.1
Isolysergic acid present, %	–	2.5

The procedure was repeated and the results of these runs are as follows:

Batch	Yield gm	Melting point °C	ϵ_{max} (312 mμ)
2	8.3	219 - 220 (dec)	8,800
3	7.3	222 - 225 (dec)	8,630
4	6.4	221 - 222 (dec)	8,800
5	7.7	226 - 227 (dec)	9,070

Optical rotation of the above materials varied from 33° to 41° at 25° to 26°C.

b. Recovery of D-Lysergic Acid From Simulated Hydrolysis Mixture.

D-Lysergic acid monohydrate (15.0 gm) was dissolved in a mixture of 415 ml of water, 100 ml of methanol, and 41.5 gm of sodium hydroxide. After standing for 0.5 hr, the mixture was cooled to 5°C and treated with a cold (5°C) mixture of 33.5 ml of sulfuric acid in 335 ml of water, which caused immediate precipitation of the lysergic acid sulfate. After the mixture was refrigerated overnight at 3°C, the solid was collected by filtration and stirred with 300 ml of a mixture of 10 volumes of 95% ethanol and 1 volume of concentrated ammonium hydroxide for 0.5 hr. The mixture was filtered through talc,

the filtrate was held, and the residue was stirred with 150 ml of the ammoniacal ethanol solution and filtered. The filtrates were then combined, stirred with 20 gm of Darco G-60 for 0.5 hr, and then filtered through talc. The filtrate was held and the residue was stirred with 150 ml of the ammoniacal ethanol for 0.5 hr. After this mixture was filtered through talc, the combined filtrates were concentrated in vacuo to about 150 ml. After this solution was refrigerated overnight, the precipitate was collected by filtration to yield 9.3 gm of D-lysergic acid monohydrate: mp 228° to 230°C (dec); $[\alpha]_D^{20}$ +36.1° (c 0.504, pyridine); λ_{max} 312 mμ (ε 9,070). Further concentration of the mother liquor yielded a second crop of 1.4 gm: mp 224° to 229°C (dec); $[\alpha]_D^{20}$ +132.9° (c 0.510, pyridine); λ_{max} 313 mμ (ε 8,630); and a third crop of 0.3 gm: mp 195° to 225°C (dec); λ_{max} 310 mμ (ε 7,000). This third crop was too dark for an optical-rotation reading to be made, as was the residue of 0.5 gm of material obtained by evaporation of the mother liquor until there was a constant weight of residue.

The materials obtained above were subjected to analysis on cellulose thin-layer chromatoplates. The developing solvent was prepared by shaking 800 ml of an 0.1 N aqueous potassium hydroxide solution with 200 ml of dimethyl phthalate in a separatory funnel. After standing for 1 hr, the two phases were separated and 200 ml of formamide was added to the upper (aqueous) phase. Pure lysergic acid has an R_f of 0.67 with this system.

Smith and Timmis[52] report that lysergic acid can be partially epimerized to isolysergic acid by the action of boiling water. An aqueous solution of lysergic acid was refluxed in a nitrogen atmosphere for 6 hr and chromatographed in the manner described above. Other than the spot for n-lysergic acid, the only spot observed on the plate was at R_f 0.75, believed to be isolysergic acid. The first crop gave only one spot corresponding to lysergic acid, but the second, third, and fourth crops gave spots corresponding to both the normal and iso acids. The total recovery of both n- and isolysergic acid was 77%, and that of pure n-D-lysergic acid was 62%.

c. Thin-Film Chromatography of D-Lysergic Acid and D-Isolysergic Acid.

A sample containing a mixture of normal and iso D-lysergic acids, obtained from the isomerization of lysergic acid with boiling water under a nitrogen atmosphere, was dissolved in a solution of ammoniacal ethanol (95% ethanol:concentrated ammonium hydroxide, 10:1, v/v), spotted on a silica-gel plate impregnated with silver nitrate (silica gel G:silver nitrate, 10:1, w/w),

and developed with the ammoniacal ethanol. Two fluorescent spots were observed under ultraviolet irradiation at R_f 0.41 and 0.47. A known sample of D-lysergic acid gave an R_f 0.47. TLC of the mixture on silica gel G with ammoniacal ethanol yielded one fluorescent spot at R_f 0.82.

d. Conversion of D-Lysergic Acid Amides to D-Lysergic Acid.

A mixture (calculated to be equivalent to 83 gm of cristamine) of 15 gm of D-lysergic acid amide and 15 gm of D-lysergic acid α-hydroxyethylamide was warmed gently with 200 ml of methanol, and to this mixture was added a solution of 83 gm of sodium hydroxide in 830 ml of water. The mixture was refluxed for 1.5 hr and then cooled to 5°C with an ice-brine bath. A chilled solution of 67 ml of concentrated sulfuric acid in 670 ml of water was added to the mixture, causing precipitation of D-lysergic acid sulfate. The mixture was refrigerated overnight at 3°C, and the lysergic acid sulfate was collected by suction filtration, pulling the solid residue as dry as possible. The solid was transferred back to the reaction vessel, which contained some solid residue, and was stirred for 0.5 hr with 625 ml of a mixture of 95% ethanol:concentrated ammonium hydroxide (10:1, v/v). The mixture was filtered through talc and the filtrate was held. The residue was stirred for 0.5 hr with 300 ml of the ammoniacal ethanol solution, the mixture was filtered through talc, and the combined filtrates were stirred for 0.5 hr with 40 gm of Darco G-60. After this mixture was filtered through talc, the filter cake was stirred with 300 ml of the ammoniacal ethanol solution for 0.5 hr, and the mixture was refiltered through talc. The combined filtrates were concentrated in vacuo to about 400 ml, causing precipitation of the product. After standing overnight at 3°C, the mixture was filtered and washed with cold ethanol to yield 14.7 gm of D-lysergic acid monohydrate: mp 226° to 228°C (dec); $[\alpha]_D^{20}$ +30° (c 0.488, pyridine); λ_{max} 312 mμ (ε 8,800).

Further concentration of the filtrate to about 75 ml resulted in the deposition of an additional 3.2 gm of acid (total yield, 60%): mp 220° to 223°C (dec); $[\alpha]_D^{20}$ +136° (c 0.486, pyridine); λ_{max} 312 mμ (ε 8,400).

e. Conversion of Crude Alkaloids Produced by Saprophytic Culture to Lysergic Acid.

Mixed alkaloids in the form of a black tarry mass isolated from saprophytic cultures by Schwarting and estimated by him to be equivalent to 5 to 8 gm of ergonovine maleate (molecular weight, 441.47) were hydrolyzed in the manner described for ergot alkaloids, employing 75 ml of methanol and

a solution of 25 gm of sodium hydroxide in 250 ml of water. Lysergic acid sulfate was precipitated using a solution of 20 ml of concentrated sulfuric acid in 200 ml of water. The precipitate was stirred for 0.5 hr with 200 ml of the ammoniacal ethanol solution described in section 1, a. The mixture was filtered through talc, the solid residue was stirred again for 0.5 hr with 100 ml of the ammoniacal ethanol solution, and the mixture was filtered through talc. The filtrates were combined and stirred for 0.5 hr with 12.5 gm of Darco G-60. After this mixture was filtered, the filter pad was stirred for 0.5 hr with 100 ml of the ammoniacal ethanol solution, and the mixture was filtered. Concentration of the combined filtrates to about 75 ml yielded 1.6 gm of D-lysergic acid monohydrate (31% to 51% based on 5 to 8 gm of ergonovine maleate): mp 227° to 228°C (dec); $[\alpha]_D^{24}$ +38° (c 0.5, pyridine); λ_{max} 312 mμ (ε 9,280). An ultraviolet spectrum of this material is shown in figure 4.

f. Colorimetric Method for Assay of Lysergic Acid Derivatives.

The method of Michelon and Kelleher[93] was employed for the assay of lysergic acid derivatives. To 2 ml of an aqueous solution of lysergic acid derivatives (containing 5 to 100 μg of total alkaloids per sample) was added 2 ml of 0.1% PDAB in sulfuric acid:water (1:1, v/v). This mixture was stirred thoroughly and allowed to stand for 10 min. Then, 0.1 ml of a freshly prepared 0.1% aqueous sodium nitrite solution was added, mixed, and the optical density at 590 mμ was determined. Exposure of all samples to direct sunlight was avoided. The standard curve obtained for LSD acid maleate, employing a Coleman Junior spectrophotometer, Model 6A, is shown in figure 5.

g. Isolation of Saprophytically Produced Alkaloids and Their Conversion to D-Lysergic Acid.

A fermentation broth from the saprophytic culture of Claviceps paspali, containing a mixture of lysergic acid derivatives and clavine-type alkaloids, was obtained from Schwarting to study the isolation of the desired alkaloids and their subsequent conversion to D-lysergic acid. After the 40-ℓ culture broth (estimated to contain the equivalent of 12 gm of ergonovine maleate) had been filtered free of mycelia, it was concentrated to approximately 6 ℓ, filtered free of precipitated nutrients, and transported to these Laboratories. The mixture was further concentrated to a volume of 4 ℓ and filtered by gravity through glass wool that was washed with two 100-ml portions of water. The combined filtrate and washings were estimated to have an alkaloid content equivalent to 2.33 mg/ml (a total of 9.3 gm) of alkaloids expressed as LSD acid maleate by the method described in section 1, f. All alkaloid concentrations reported in the following sections were estimated with this method.

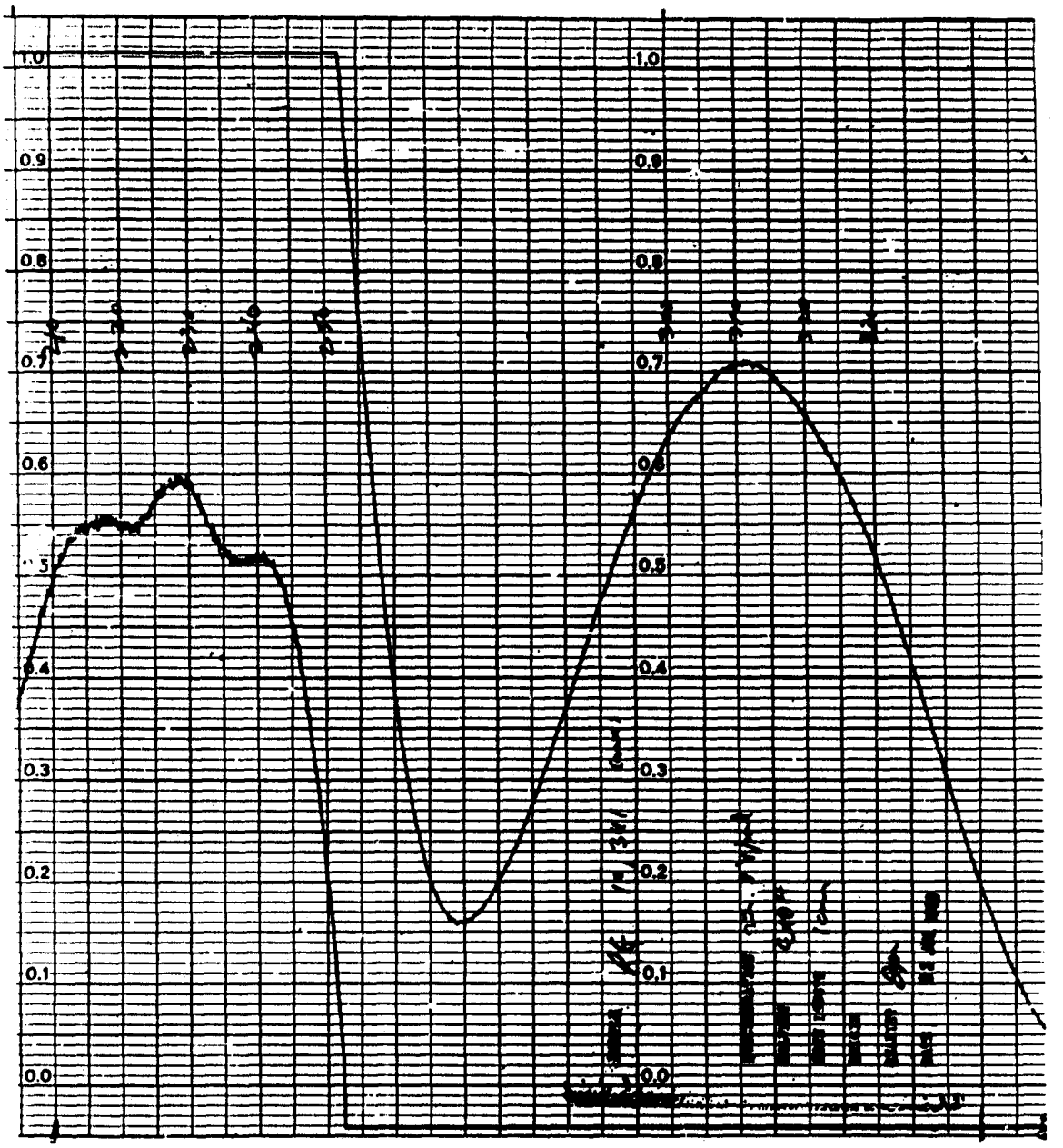


FIGURE 4

ULTRAVIOLET SPECTRUM OF D-LYSERGIC ACID MONOHYDRATE

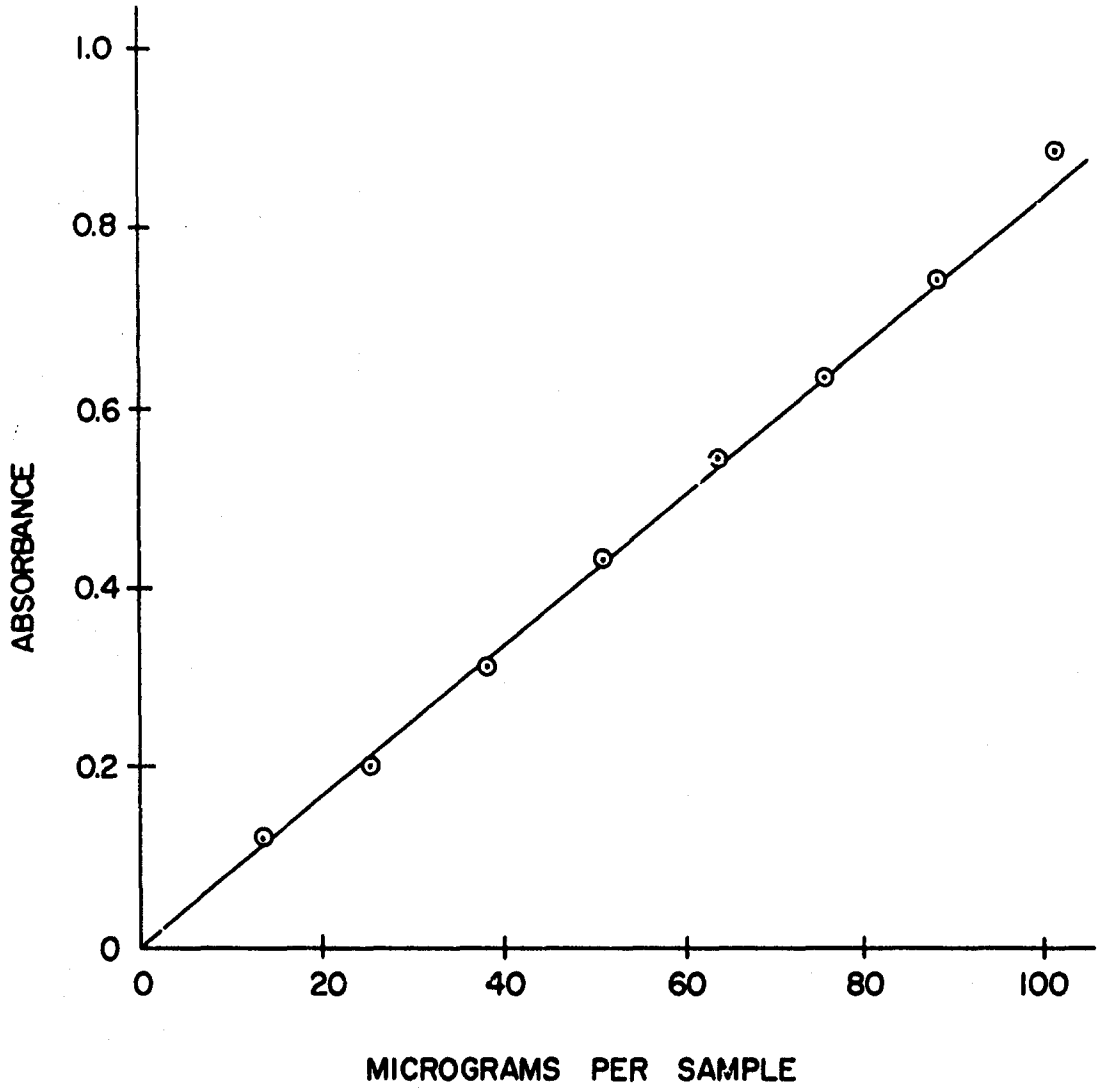


FIGURE 5

STANDARD CURVE OF LSD ACID MALEATE

After the pH of the solution was adjusted to 8.5 with concentrated ammonium hydroxide, four 800-ml portions were extracted with the following solvents: (A) isobutyl alcohol; (B) n-butanol; (C) chloroform:isobutyl alcohol, 4:1; and (D) chloroform:n-butanol, 4:1. The results of these extractions are listed in table 5.

TABLE 5

EXTRACTION OF ALKALOIDS WITH FOUR SOLVENT SYSTEMS

Solvent system	Volume of extracts	Concentration of alkaloids in aqueous phases	
		After first extraction	After second extraction
	ml	mg/ml	
Isobutyl alcohol	200	0.230	0.135
n-Butanol	200	0.220	0.140
Chloroform: isobutyl alcohol, 4:1	800	0.290	0.230
Chloroform:n-butanol, 4:1	800	0.260	0.220

The extraction mixture was centrifuged to separate the organic phase after all extractions employing systems A and B, but only after the first extractions employing systems C and D.

TLC of the various extracts on silica gel using a benzene:ethanol mixture (1:1, v/v) revealed no differences in the composition of their alkaloid contents. The alkaloids were primarily n- and isolysergic acid amide, with some n- and isolysergic acid methylcarbinolamide and some clavine alkaloids, based on R_f value assignments made by Kelleher and Schwarting.[46]

The first and second extracts of system C were combined and the solvent was removed in vacuo at a maximum bath temperature of 40°C until a dark, viscous residue containing only a slight amount of alcohol remained.

The crude residue was converted, without further purification, to D-lysergic acid monohydrate employing the method described in section 1, a, using a mixture of 15 ml of methanol, 50 ml of water, and 5.5 gm of sodium hydroxide. The lysergic acid sulfate was precipitated by adding a solution of 4.5 ml of concentrated sulfuric acid in 45 ml of water. After the mixture was refrigerated overnight at 3 °C, the lysergic acid sulfate was collected by vacuum filtration, and the filtrate was made basic by the addition of concentrated ammonium hydroxide. This solution was extracted with four 100-ml portions of chloroform. The combined extracts were dried over magnesium sulfate, and the solvent was removed in vacuo to give a constant weight of 310 mg (26% of the alkaloids estimated to be in the 800 ml of fermentation broth) of unreacted alkaloids. TLC showed these to be primarily clavine alkaloids with only small amounts of lysergic acid derivatives present.

The lysergic acid sulfate was stirred with 40 ml of the ammoniacal ethanol solution described in section 1, a, for 0.5 hr, and, after the mixture was filtered through talc, the filter pad was stirred with 20 ml of the ammoniacal ethanol solution for 0.5 hr. After this mixture was filtered through talc, the combined filtrates were stirred for 0.5 hr with 2.6 gm of Darco G-60. The mixture was filtered through talc, the filter pad was stirred for 0.5 hr with 20 ml of the ammoniacal ethanol solution, and the mixture was refiltered through talc. Concentration of the combined filtrates to about 15 ml yielded 320 mg of D-lysergic acid monohydrate (28% of the alkaloids estimated to be in the aqueous phase before extraction). The mother liquor (which TLC showed to contain lysergic acid and isolysergic acid), on concentration in vacuo, yielded 300 mg (25%) of an amorphous material. It has not been possible to isolate crystalline lysergic acid or isolysergic acid from this material.

Extracts from system D, when treated in the same manner as system C, resulted in the recovery of 330 mg (28%) of unreacted alkaloids, 300 mg (25%) of D-lysergic acid monohydrate, and an additional 300 mg (25%) of amorphous material similar to that described for system C.

Evaporation of the solvent in vacuo from the combined isobutyl alcohol extracts (system A) resulted in the deposition of 3.8 gm of material believed to be nutrients from the culture mixture along with the desired alkaloids. This residue has not yet been investigated.

In order to obtain alkaloids free of nonalkaloidal material, a method similar to that of Arcamone et al.[31] was employed. The combined n-butanol extracts (system B) were diluted with 400 ml of hexane and extracted

with three 80-ml portions of water. The aqueous phases were adjusted to pH 3.5 with 10% sulfuric acid during the extractions. The combined aqueous extracts were treated with 350 ml of a saturated aqueous sodium bicarbonate solution and 100 mg of Versene, and then extracted with three 300-ml portions of chloroform. The combined chloroform extracts were dried over magnesium sulfate, and the solvent was removed in vacuo to constant weight to yield 810 mg (66% assuming a 1:1 mixture of lysergic acid amides and methylcarbinolamides to be present) of alkaloids in the form of a viscous oil. Arcamone reported a yield of 70% by a similar method.

This material was hydrolyzed by refluxing it for 1.5 hr in a mixture of 2.2 gm of sodium hydroxide, 22 ml of water, and 5.3 ml of methanol; then, lysergic acid sulfate was precipitated with a solution of 1.8 ml of concentrated sulfuric acid in 18 ml of water. After this mixture was refrigerated overnight, the precipitate was collected by suction filtration and then stirred for 0.5 hr with 17 ml of the ammoniacal ethanol solution. After this mixture was filtered through talc, the filter pad was stirred for 0.5 hr with 8.0 ml of the ammoniacal ethanol solution, and the mixture was filtered through talc. The combined filtrates were stirred for 0.5 hr with 1.0 gm of Darco G-60, and the mixture was filtered through talc. The pad was stirred for 0.5 hr with 8.0 ml of the ammoniacal ethanol solution, and the mixture was filtered through talc. Concentration of the combined filtrates to about 10 ml yielded 280 mg of D-lysergic acid monohydrate. Further concentration of the mother liquor yielded another 90 mg of acid (total yield 31%).

2. Preparation of Dimethylformamide-Sulfur Trioxide (DMF-SO_3) Complex.

A dry, 12-ℓ, three-necked, round-bottomed flask was charged with 6 kg of DMF, which was distilled through an 18-in. bubble-cap column at a pressure of 22 mm. Cuts were taken periodically to determine the water content of the distillate. After approximately 600 ml had distilled, a fraction was obtained that contained 0.1% water (Karl Fischer). The distillation was stopped, and the distillation flask was fitted with a Drierite drying tube. The following day the flask was fitted with a dropping funnel charged with 1 lb of Sulfan B, a mechanical stirrer, a condenser with a drying tube, and a thermometer. Silicon grease was used on all joints. The DMF was cooled to 2°C by a brine-ice bath, and the Sulfan B was added at a rate to maintain a temperature between 0° and 6°C over a 3-hr period. After addition was complete, the mixture was stirred for 1 hr more to dissolve the solid complex that coated the sides of the flask. The resulting solution was transferred to 1-ℓ reagent bottles and stored at 3°C. An aliquot dissolved in water and titrated to a phenolphthalein end point with sodium hydroxide had a molarity of 1.09.

3. Preparation of LSD.

a. In the first experiment (run No. 1), a solution of D-lysergic acid monohydrate (1.43 gm, 0.005 mole) and lithium hydroxide hydrate (0.21 gm, 0.005 mole) in 150 ml of methanol was prepared. The solvent was removed under reduced pressure while being heated by steam. The glasslike residue was dissolved in 200 ml of DMF, and about 100 ml of the solvent was removed by distillation at 20 mm pressure through a 12-in. column. The resulting solution was cooled to 10°C, and 8.54 ml of a previously prepared 1.17 M solution of a DMF-SO_3 complex in DMF was added from a buret. After this solution was stirred for 5 min, 3 ml of diethylamine was added rapidly; after 5 min, 80 ml of water was added, followed by 40 ml of a saturated sodium chloride solution. The mixture was extracted with one 200-ml portion and five 100-ml portions of ethylene chloride. After the combined extracts were dried for 10 min over magnesium sulfate, the solvent was removed in vacuo. The residue was dissolved in 80 ml of a benzene:chloroform mixture (3:1, v/v), and chromatographed over 150 gm of Fisher alumina. The column was developed with the same solvent mixture. After about 1,500 ml of eluate had been collected, a band, fluorescing blue under ultraviolet radiation, came off the column. Approximately 2,500 ml of solvent was required to elute this band. The first 1,500 ml of eluate was evaporated to dryness in vacuo, and the residue was triturated with ligroine to yield 200 mg (12%) of product: mp 80°C (dec); λ_{max} 310 mμ (ε 8,200); [literature values: 80° to 85°C (dec)[11]; 313 mμ (ε 9,300)[78]]. The infrared spectrum was consistent with that of the authentic material.

The remaining eluate was evaporated to dryness in vacuo; the residue was dissolved in 25 ml of benzene:alcohol (99:1) and chromatographed over 60 gm of Fisher alumina. The column was developed with the same solvent mixture. After 400 ml of eluate had been collected, a fraction of 100 ml contained the desired product on the basis of its blue fluorescence. The solvent was removed in vacuo, and the residue was triturated with ligroine to yield 100 mg (6%) of product: mp 80° to 85°C (dec); $[\alpha]_D^{22}$ +22° (c 0.41, pyridine); λ_{max} 310 mμ (ε 9,100); [literature values: 80° to 85°C (dec)[11]; +17° (c 0.5, pyridine)[108]; 313 mμ (ε 9,330)[78]].

b. A second experiment (run No. 2) using 7.15 gm (0.025 mole) of D-lysergic acid monohydrate, 1.06 gm (0.025 mole) of lithium hydroxide hydrate, and 15 ml of diethylamine was performed in the same manner as run No. 1, using 400 ml of methanol, 500 ml of DMF, 43 ml of 1.17 M DMF-SO_3, 400 ml of water, and 200 ml of saturated

aqueous sodium chloride solution. The extraction was done using one 500-ml portion and eight 250-ml portions of ethylene chloride. After drying and removal in vacuo of the combined extracts, the crude product was chromatographed over 750 gm of alumina using benzene:ethanol (99:1, v/v) as the developing solvent. The eluate was evaporated in vacuo, the residue was dissolved in 30 ml of chloroform, and petroleum ether was added until the solution was quite turbid. After the solution was refrigerated overnight, the product was filtered to yield 3.6 gm of tan product: mp 80° to 84°C (dec). TLC of this material on alumina using 3% ethanol in benzene showed two major components, n-LSD, R_f 0.69, and a smaller amount of iso-LSD, R_f 0.48. Attempted rechromatography of this material on alumina resulted in its loss when the column was allowed to run dry of developing solvent.

c. An identical run (No. 3) was performed, and chromatography of the extract on 750 gm of alumina using 1% ethanol in chloroform gave 3.2 gm of material, which TLC showed to be similar to that from run No. 2. Of this material, 2.0 gm was chromatographed over 700 gm of alumina using benzene:acetone (9:1, v/v) as the developing solvent. The blue fluorescing fractions coming off the column were concentrated in vacuo and taken up in chloroform. Addition of petroleum ether to the chloroform solutions gave the following results:

Cut	Volume, ml	Amount of product isolated, mg	ϵ max (310 mμ)
A	500	300*	7,000
B	750	500*	7,500
C	1,000	400*	8,080
D	750	200**	—
E	1,250	—	—

* One spot on TLC corresponding to LSD.

** Two spots on TLC corresponding to LSD and iso-LSD.

After collection of cut E, the benzene:acetone developing-solvent ratio was changed to 7:3, and 1,500 ml of eluate yielded a dark, crystalline material upon evaporation in vacuo. The material was recrystallized from acetone to yield 25 mg of pale-yellow iso-LSD: mp 182° to 183°C (dec); [literature value: 182°C (dec)[11]].

d. Three further runs (Nos. 4, 5, and 6), performed identically to runs Nos. 2 and 3, yielded 1.0, 0.8, and 0.6 gm of LSD, respectively.

e. As a result of the poor yields obtained from runs Nos. 4, 5, and 6, a fresh sample of DMF-SO_3 reagent was prepared for subsequent runs. The fresh DMF-SO_3 complex, 1.4 M, was used in run No. 7 (method A).

Method A. A solution of 7.15 gm (0.025 mole) of D-lysergic acid monohydrate and 1.06 gm (0.025 mole) of lithium hydroxide hydrate in 75 ml of methanol was prepared. Of this solution, 0.5 ml was diluted with 5 ml of water, and the pH was found to be 9.4. The solvent was removed in vacuo and the residue dissolved in 250 ml of DMF. The solvent was distilled under reduced pressure with a maximum head temperature of 40°C to remove water present in the reaction mixture. After 75 ml of the distillate had been removed, the water content of the distillate was less than 0.1%. Distillation was stopped at this point, and, after the reaction was cooled to 5°C with an ice bath, DMF-SO_3 reagent (36 ml, 1.4 M) was added rapidly, followed by 15 ml of diethylamine 5 min later. After 5 min, 400 ml of water was added, followed by 200 ml of saturated sodium chloride solution. The resulting mixture was then extracted with one 500-ml portion and six 250-ml portions of ethylene chloride until the extracts gave only a pale-blue color when a small portion of the extract was shaken with an equal volume of van Urk's reagent. After the extracts were dried over magnesium sulfate for 15 min, they were evaporated to dryness in vacuo with a rotary evaporator. The bath temperature was not allowed to exceed 40°C. The residue was dissolved in a benzene:chloroform mixture (3:1) and chromatographed over 750 gm of alumina. When the product-bearing eluate began to come off the column (after about 7 to 8 ℓ had passed through), the ratio of benzene to chloroform was changed to 2:1. Approximately 10 ℓ of this solvent was needed to elute the product. After the solvent was removed in vacuo and the oily residue taken up in 100 ml of methanol, 2.0 gm of maleic acid was added to the solution. After the maleic acid was dissolved, 300 ml of ether was added and crystallization started immediately. After the crystalline material was refrigerated overnight, it was filtered to yield 5.0 gm (45%) of tan product: mp 195°C (dec); λ_{max} 314 mμ (ε 8,350). This product was reprecipitated from methanol with ether to yield 4.65 gm (42%) of LSD acid maleate (I): mp 195°C (dec); $[\alpha]_D^{27}$ -8° (c 1.04, methanol); λ_{max} 314 mμ (ε 8,700). TLC showed one spot corresponding to n-LSD acid maleate. Authentic material obtained from Eli Lilly and Co. had the following physical constants: mp 145°C (dec); $[\alpha]_D^{26}$ -4° (c 1.16, methanol); λ_{max} 314 mμ (ε 8,600). The Lilly material used for the optical rotation was precipitated from the methanol with ether to yield a material (II) [mp 195°C (dec)] with an infrared spectrum identical with (I). A mixed melting point of (I) and (II) was not depressed.

f. A total of 28 additional runs (Nos. 8 through 35) was made to investigate the synthesis and purification of LSD acid maleate further (table 6). These runs were performed in essentially the same manner as method A (run No. 7) with the following two modifications:

(1) A 6% excess of D-lysergic acid was generally needed to bring the pH of the diluted methanolic lithium lysergate solution to a range of 9.2 to 9.6. This did not, however, require a change in the amount of DMF-SO_3 reagent or diethylamine used.

(2) The method of purification and crystallization was varied from that used for method A (run No. 7) in some cases, and these methods are described below.

Method B. The combined, dried, ethylene chloride extracts from run No. 9 were evaporated to dryness in vacuo. The residue was dissolved in 500 ml of ethylene chloride, 2.5 gm of maleic acid was added, and the solution was filtered and extracted with four 75-ml portions of water. The combined aqueous extracts were made basic with ammonium hydroxide and extracted with one 400-ml and four 200-ml portions of ethylene chloride. The combined extracts were dried for 10 min over magnesium sulfate, and the solvent was removed in vacuo. The residue was dissolved in 15 ml of acetone, filtered, and treated with 0.5 gm of maleic acid and then 10 ml of ether. After approximately 1.5 hr, crystallization was well underway and the flask was refrigerated overnight. The following day the precipitate was filtered to yield 0.8 gm of a dark material that was recrystallized from acetone-methanol to yield 0.6 gm of a tan product: mp 194° to 198°C (dec); λ_{max} 314 mμ (ϵ 8,800).

Acidification of the aqueous reaction mixture of run No. 9, after ethylene chloride extraction was complete, caused a precipitate to form. This mixture was refrigerated overnight and then filtered; the precipitate was air-dried to yield 4.0 gm of gray solid. The solid was dissolved in a mixture of 40 ml of ethanol and 4 ml of concentrated ammonium hydroxide; this solution was filtered, stirred with 2.5 gm of Darco G-60 for 30 min, and filtered. The filtrate was concentrated in vacuo until crystallization began. The flask was refrigerated overnight and its contents were filtered to yield 3.1 gm of white solid [mp 226° to 228°C (dec)] that was identical with lysergic acid in its solubility properties. The aqueous reaction mixture in subsequent runs was usually acidified as a matter of routine.

TABLE 6

SYNTHESIS OF LSD

Run number	Size of run	Method of isolation	Yield	
	mole		gm	%
7	0.025	A	4.7	42
8	0.025	A	4.6	42
9	0.025	B	0.6	5.5 a/
10	0.025	C	0.2	1 b/
11	0.025	C	–	– c/
12	0.025	A	3.2	29
13	0.050	D d/	4.3	29
		E e/	1.6	25
14	0.025	A	5.6	51
15	0.025	A	4.6	42
16	0.025	A	5.5	50
17	0.025	D	4.4	40
18	0.025	D	3.0	27
19	0.025	D	3.7	34
20	0.050	D	7.0	32
21	0.050	D	9.4	43
22	0.050	D	8.2	37
23	0.050	D	10.0	45
24	0.100	D	12.2	28
25	0.025	D	2.8	25
26	0.025	E	3.0	27
27	0.050	D	2.2	20
28	0.100	D	6.2	14
29	0.025	D	0.4	4
30	0.025	E	5.6	51
31	0.050	D	13.7	62
32	0.050	D	14.6	66
33	0.100	D	26.4	60
34	0.200	D	45.0	51
35	0.025	D f/	0.751	69
		F f/	0.706	64
		G f/	0.588	53
		H f/	0.698	64
		I f/	0.493	61
		I f/	0.487	60
36	0.025	D g/	7.8	71

a/ 4.0 Gm lysergic acid sulfate recovered.

b/ 5.2 Gm lysergic acid sulfate recovered.

c/ 5.9 Gm lysergic acid sulfate recovered.

d/ 67% of run.

e/ 33% of run.

f/ 10% of run.

g/ DMF-SO_3 complex 13 mo old.

Method C. Runs Nos. 10 and 11 produced a high yield of lysergic acid sulfate upon acidification of the extracted reaction mixtures. The residues remaining after evaporation of the ethylene chloride extracts were combined, dissolved in a mixture of 100 ml of DMF and 75 ml of methanol, and treated with Darco G-60 until after filtration to remove the Darco G-60. The solution was a pale-red color. To the filtered solution was added 1.5 gm of maleic acid and ether to bring the volume to approximately 3 ℓ, and the mixture was refrigerated overnight. The following day, the mother liquor was decanted from the solid that had formed on the walls of the flask. The solid was recrystallized from acetone-methanol to yield 0.2 gm of LSD acid maleate: mp 191° to 193°C (dec).

Method D. The combined extracts from run No. 17 (0.025 mole) were concentrated until the odor of ethylene chloride could no longer be detected in the residue. The resulting DMF solution was diluted with 400 ml of benzene, passed over 60 gm of alumina, and then eluted with 500 ml of a benzene:chloroform (1:1) mixture. The combined eluates were concentrated in vacuo until the evaporation rate was very low and the odor of chloroform and benzene could not be detected in the residue. The resulting DMF solution was treated with a solution of 2.5 gm of maleic acid in 75 ml of methanol, and then with 600 ml of ether. After several minutes, crystallization started and the mixture was refrigerated overnight. The following day the precipitate was filtered and washed with three 50-ml portions of ether to yield 4.4 gm of product: mp 185° to 190°C (dec); λ_{max} 314 mμ (ε 8,500).

Method E. The combined ethylene chloride extracts from run No. 13 (0.050 mole) were concentrated to a volume of 1,800 ml. Of this, a 600-ml portion was stirred for 15 min with 1 gm of Darco G-60, the mixture was filtered, and the charcoal was washed with 75 ml of ethylene chloride. To the combined filtrate and wash was added a solution of 2.0 gm of maleic acid in 15 ml of methanol, followed by 1,000 ml of ether. After the solution was refrigerated overnight, the mother liquor was decanted from the solid that had formed on the sides of the flask, and the solid was recrystallized from acetone-methanol to yield 1.6 gm of product: mp 190° to 192°C (dec). The remaining 1,200 ml of extract was worked up by method D to yield 4.3 gm of product: mp 191° to 194°C (dec).

Aliquots of the ethylene chloride extract (total extract, 2.2 ℓ) of run No. 35 (0.025 mole) were subjected to the isolation procedures described below.

A 220-ml aliquot, 10% of the total extract (I), was subjected to method D to yield 761 mg (69%) of LSD acid maleate: λ_{max} 313 mμ (ε 8,350).

Method F. A 220-ml aliquot, 10% of the total extract, was concentrated in vacuo to a viscous brown oil that, after solution in 220 ml of benzene, was stirred with 2.5 gm of alumina at room temperature for 0.5 hr. The reaction mixture was filtered, concentrated in vacuo to a brown oil, treated with 16 ml of a methanolic solution of 286 mg of maleic acid followed by ether to turbidity, and cooled at 0°C to yield 0.706 gm (64%) of LSD acid maleate. The alumina, after it was washed with benzene and ethanol, yielded the equivalent of an additional 33 mg (3%) of product (as determined by colorimetric assay).

Method G. A 220-ml aliquot, 10% of the extract, was treated with 125 mg of Darco G-60, stirred for 0.5 hr at room temperature, and filtered. The filter cake was washed with ethylene chloride, and the combined filtrates were concentrated to 88 ml and retreated with 125 mg of Darco G-60 followed by 10 ml of a methanolic solution of maleic acid (286 mg). LSD acid maleate could not be isolated from this mixture. This mixture was then concentrated to a viscous oil, treated with methanol followed by ether to the point of slight turbidity, seeded with a known sample of LSD acid maleate, and cooled to 0°C to yield 588 mg (53%) of LSD acid maleate: λ_{max} 313 mμ (ε 8,410).

Method H. A 220-ml aliquot, 10% of the extract, was concentrated in vacuo to a viscous oil, then treated with 15 ml of a methanolic solution of 286 mg of maleic acid followed by 50 to 60 ml of ether to the point of slight turbidity, seeded with a known sample of LSD acid maleate, and cooled to 0°C to yield 698 mg (64%) of LSD acid maleate: λ_{max} 313 mμ (ε 8,250).

Method I. A 220-ml aliquot, 10% of the extract, was concentrated in vacuo to a viscous brown oil, dissolved in 10 ml of methanol, treated with water to the point of turbidity, and seeded with a known sample of LSD. After the mixture was cooled at 0°C overnight, 164 mg of a grey solid was obtained. The mother liquor was concentrated in vacuo to a brown oil, dissolved in 3 ml of methanol, treated with water to turbidity, and cooled overnight at 0°C to yield an additional 329 mg of solid, making a total of 493 mg (61%) of LSD: mp 90° to 99°C. TLC showed only a small amount of iso-LSD in the product.

Duplication of this procedure resulted in the isolation of 0.487 gm (60%) of product.

4. Recrystallization of LSD Acid Maleate.

A solution of 72 gm of crude LSD acid maleate (from runs Nos. 8, 12, 13, and 15 to 26) in 750 ml of hot methanol was treated with 1 gm of Darco G-60 for 5 min and filtered. The solution was evaporated to about 600 ml on a steam bath and treated with 200 ml of ether. After standing 1 hr at room temperature, the flask was refrigerated overnight.

The next day the precipitate was filtered and washed with two 50-ml portions of 25% methanol in ether to yield 61 gm (85%) of tan product: mp 194° to 197°C (dec); $[\alpha]_D^{25}$ -7° (c 1.00, methanol); λ_{max} 314 mμ (ε 8,800). The ultraviolet and infrared spectra of this material are found in figures 6 and 7.

Analysis of $C_{24}H_{29}N_3O_5$:

Calculated: C, 65.58; H, 6.65; O, 18.20
Found: C, 65.56; H, 6.63; O, 18.16

5. Reequilibration of LSD.

This procedure is based on that used by Eli Lilly and Co. in the equilibration of ergonovinine to ergonovine.

The combined mother liquors from runs Nos. 31 and 32 were combined and the solvents removed in vacuo until the odor of methanol or ether could not be detected in the residue. To this solution was added 2 ℓ of ethylene chloride, 1 ℓ of water, and concentrated ammonium hydroxide until the mixture was basic. The mixture was shaken in a separatory funnel, and the organic phase was drawn off. After 400 gm of sodium chloride was added to the aqueous phase, it was extracted with 1-ℓ portions of ethylene chloride until the extracts gave a negative test with van Urk's reagent (three extractions were necessary), and the aqueous phase was discarded. The combined extracts were dried over magnesium sulfate for 15 min, and, after removal of the desiccant, the solvent was removed in vacuo until the odor of ethylene chloride could not be detected in the residue. The remaining DMF solution was treated with a mixture of ethanol (200 ml), water (60 ml), and potassium hydroxide (32 gm), and allowed to stand for 4 hr at room temperature.

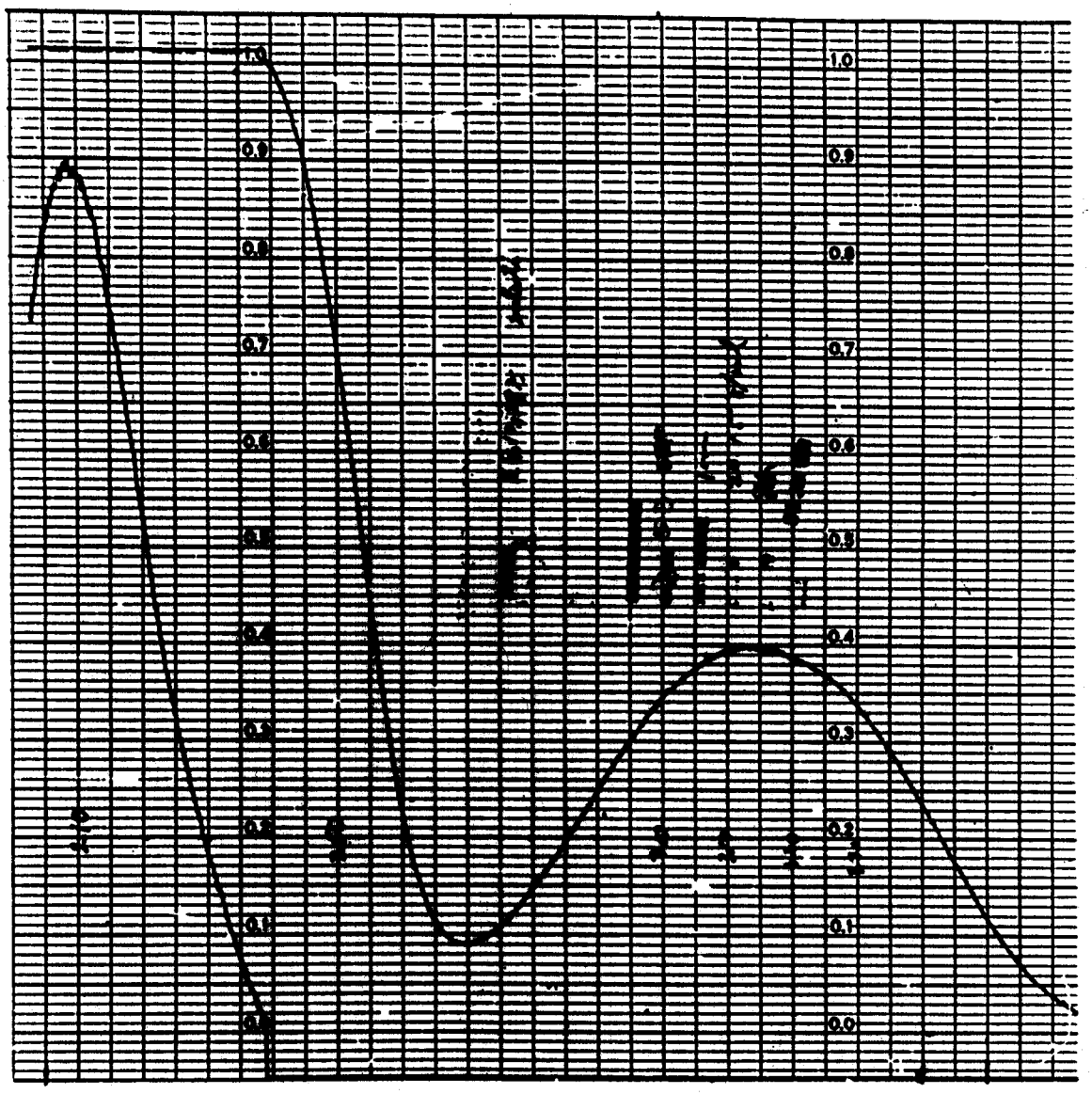


FIGURE 6

ULTRAVIOLET SPECTRUM OF LSD ACID MALEATE

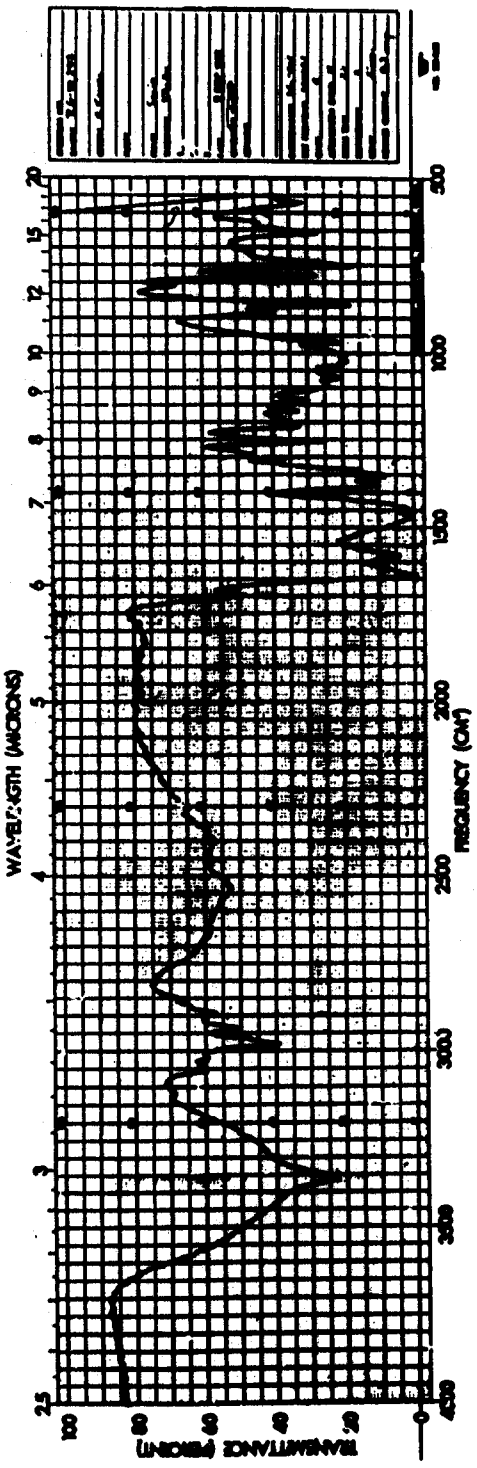


FIGURE 7

INFRARED SPECTRUM OF LSD ACID MALEATE

To the basic solution was added a mixture of 1,000 ml of water and 20 ml of acetic acid until a pH of 8.0 was achieved. To this solution was added 5 ℓ of water and 1,400 gm of sodium chloride. This solution was extracted with two 4-ℓ portions of ethylene chloride. The first extract took almost all the color from the aqueous phase and gave a strong test with van Urk's reagent. The second extract was almost colorless and gave a negative test. The aqueous phase also gave a negative test. The aqueous phase and the second extract were discarded. TLC showed the normal derivative to be the principal constituent of the first extract.

After the extract was dried over magnesium sulfate for 15 min, the solvent was removed in vacuo until the odor of ethylene chloride could not be detected in the residue. The resulting DMF solution was treated with 100 ml of methanol and filtered. The evaporation flask and filter paper were washed with another 25 ml of methanol and the solutions were combined. To the combined solution were added 3.0 gm of maleic acid and 1 ℓ of ether, which caused turbidity. After the mixture was refrigerated overnight, the precipitate was filtered and washed with 40 ml of acetone to yield 3.9 gm of crude product, which corresponds to 9% of the theoretical amount of product expected to be obtained from runs Nos. 31 and 32.

6. Conversion of LSD Acid Maleate to Free Base.

A mixture of 1.0 gm of LSD acid maleate, 200 ml of water, 5 ml of 1 N ammonium hydroxide, and 200 ml of ethylene chloride was shaken in a separatory funnel until the solid had completely dissolved. The organic phase was drawn off and the aqueous phase was extracted three times with 200-ml portions of ethylene chloride. After the combined extracts were dried over magnesium sulfate, they were evaporated to an oil in vacuo. Attempts to crystallize the base by precipitation from a chloroform solution with ether as described in the literature[11] failed; however, treatment of the chloroform solution with ligroine caused precipitation. After the mixture was refrigerated overnight, it was filtered to yield 650 mg of tan material: mp 80° to 82°C (dec): λ_{max} 312 mμ (ε 7,000). A sodium fusion test gave a positive test for chlorine.

Analysis of $C_{20}H_{25}N_3O$-$CHCl_3$:

Calculated: Cl, 24.02
Found: Cl, 20.8

The ϵ_{312} based on a 1:1 chloroform adduct is 9,600. Of this material, 56 mg was dissolved in 1 ml of methanol and the solution was treated with 1 ml of water. After 15 min, crystallization had started and the mixture was refrigerated for 0.5 hr. The white precipitate was filtered, washed with two 0.5-ml

portions of a 1:1 methanol:water mixture, and then dried in vacuo over phosphorus pentoxide to yield 25 mg of LSD, mp 83° to 86°C (dec); $[\alpha]_D^{26}$ +20° (c 0.39, pyridine); λ_{max} 312 mμ (ε 9,120). To a solution of 5.0 gm of LSD acid maleate in 125 ml of methanol and 25 ml of 1 N ammonium hydroxide was added 1 ℓ of water, which caused immediate turbidity and crystallization. After the mixture was refrigerated for 2.5 hr, the white precipitate was filtered, washed with three 50-ml portions of water, and dried in a vacuum desiccator over phosphorus pentoxide for 24 hr to yield 3.45 gm of material that had turned pale yellow: mp 88° to 90°C (the melt darkens slowly as the temperature is raised to 140°C at which point it is quite black); $[\alpha]_D^{26}$ +19° (c 0.506, pyridine); λ_{max} 312 mμ (ε 9,320). TLC gave one spot corresponding to that of LSD.

Analysis of $C_{20}H_{25}N_3O$:

Calculated: C, 74.25; H, 7.79; N, 13.00
Found: C, 72.9; H, 7.5; N, 12.4

Another portion of LSD (mp 80° to 85°C) was recrystallized twice by dissolving the material in boiling ether, filtering the hot solution, and evaporating the ether on a steam bath to the point of turbidity. Refrigerating the mixture until crystallization appeared to be complete yielded a pale-green product: mp 160° to 162°C (dec); $[\alpha]_D^{26}$ +18.5° (c 0.496, pyridine); λ_{max} 312 mμ (ε 9,320). TLC of the material gave one spot corresponding to that of authentic LSD, and the infrared spectrum was identical with the spectrum reported in the literature[78] for LSD.

Analysis of $C_{20}H_{25}N_3O$:

Calculated: C, 74.25; H, 7.79; N, 13.00
Found: C, 74.5; H, 7.8; N, 12.8

Recrystallization of LSD (mp 88° to 90°C) from ether yielded a white material [λ_{max} 312 mμ (ε 9,320)] identical in every respect to the product: mp 160° to 162°C (dec). TLC gave one spot corresponding to that of authentic LSD.

Analysis of $C_{20}H_{25}N_3O$:

Calculated: C, 74.25; H, 7.79, N, 13.00
Found: C, 74.4; H, 7.9, N, 12.8

Mass-spectrometric analysis of the LSD base [mp 80° to 85°C (dec)] and the LSD [mp 160° to 162°C (dec)] gave mass spectra from 15 to 350 mass units. The samples produced identical spectra and indicated a molecular weight of 323 for the compounds. A rather small mass peak at 251 indicates a cleavage of the $(C_2H_5)_2N$ fragment from the parent. An intense mass peak at 221 indicated that a very probable and stable fragment is formed by the loss of the $(C_2H_5)_2N$-C=O group plus two hydrogen atoms. Other prominent fragmentation peaks were believed to originate from the loss of the fragment $(C_2H_5)_2N$-C=O plus H plus a methyl group, and this fragment plus a fragment of mass 26, which probably indicates a splitting of the pyrrole ring and the loss of the C-N group.

7. Conversion of LSD Acid Maleate to Free Base.

LSD acid maleate (20 gm) was dissolved in a mixture of 250 ml of methanol and 100 ml of a 1 N aqueous ammonium hydroxide solution. To this mixture was added 1,500 ml of water, causing a white precipitate to form with the evolution of heat. After refrigeration of the mixture overnight, the solid was collected by filtration and washed with three 50-ml portions of water. The precipitate was dried overnight in a vacuum desiccator over magnesium perchlorate. The dried solid was then dissolved in about 1,300 ml of boiling ether, and the solution was filtered and reduced to a volume of about 1 *l* on a steam bath, causing crystallization. The mixture was removed from the steam bath, allowed to come to room temperature, and then refrigerated overnight. The precipitate was collected by filtration to yield 7.7 gm of product: mp 160° to 162°C (dec); λ_{max} 312 mμ (ε 9,260). The ultraviolet and infrared spectra of this material are shown in figures 8 and 9. Concentration of the mother liquor resulted in the recovery of two additional crops of product, amounting to 3.9 gm [mp 160° to 162°C (dec); λ_{max} 312 mμ (ε 9,300)] and 1.4 gm [mp 160° to 162°C (dec); λ_{max} 312 mμ (ε 9,200)]. A total of 13.0 gm (88%) of product was obtained.

8. Preparation of LSD Acid Maleate.

A solution of 140 mg of maleic acid in 2 ml of methanol was added to a solution of 300 mg of LSD (mp 155° to 158°C) in 2 ml of methanol. The mixture was treated with 3 ml of ether and refrigerated for 2 hr. The precipitate was collected by filtration, washed with a methanol:ether mixture (1:1, v/v), and dried in a vacuum desiccator over phosphorus pentoxide to yield 300 mg of LSD acid maleate: mp 195° to 198°C (dec); λ_{max} 314 mμ (ε 8,680). A mixed-melting-point determination with an authentic sample was not depressed.

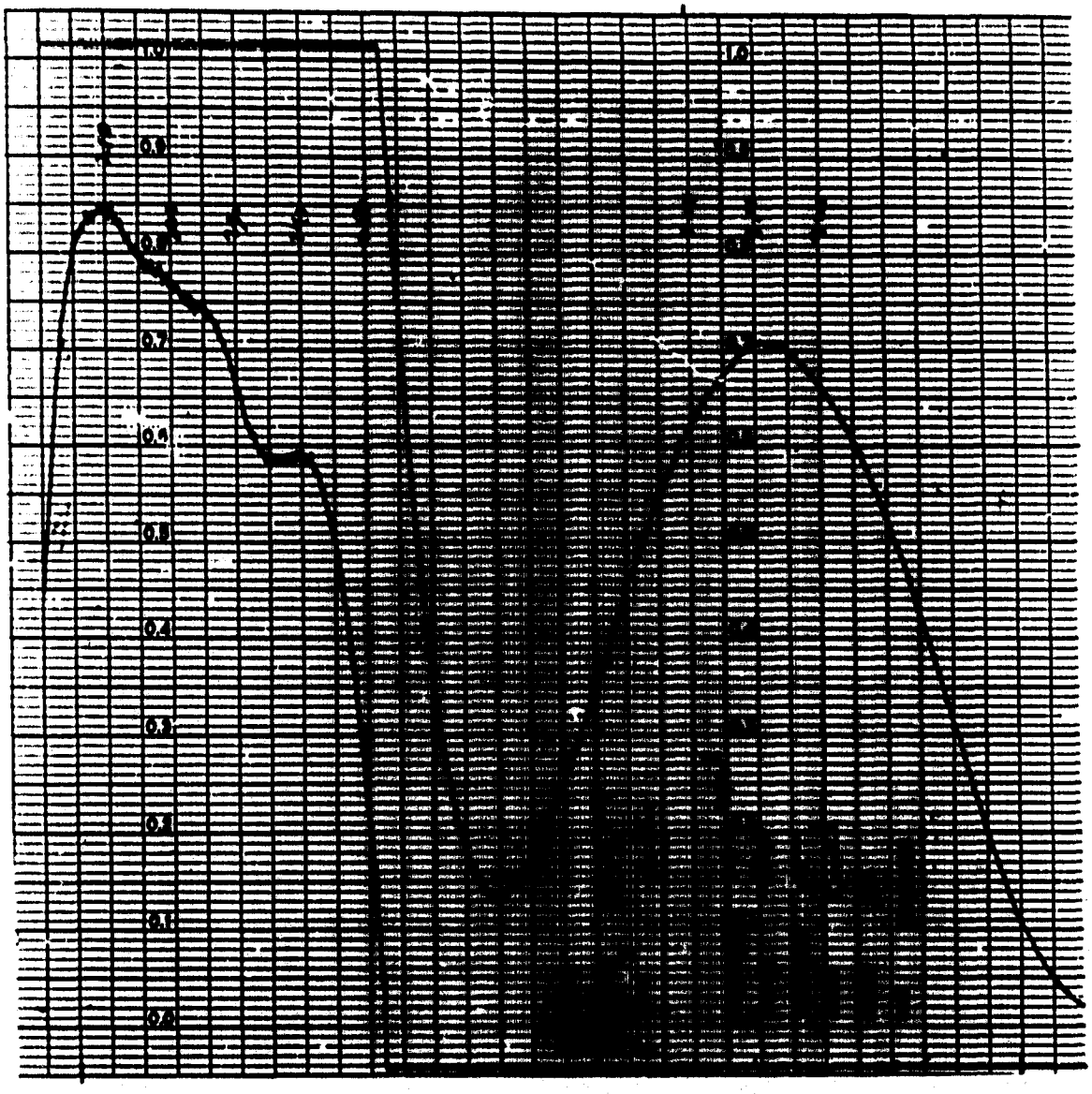


FIGURE 8

ULTRAVIOLET SPECTRUM OF LSD

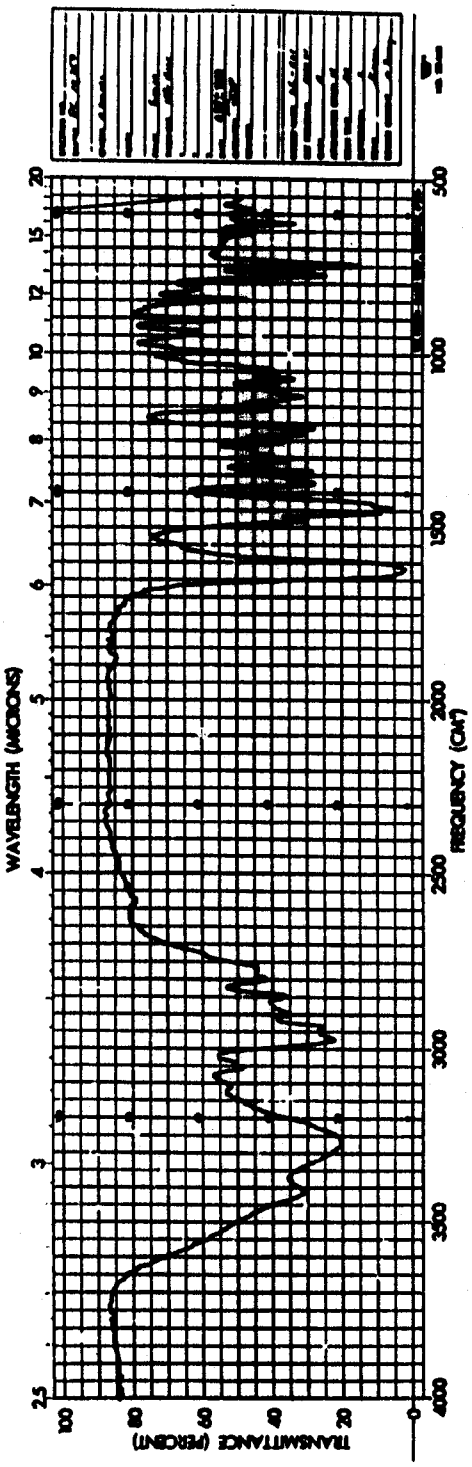


FIGURE 9

INFRARED SPECTRUM OF LSD

9. Preparation of LSD Tartrate.

A solution of 160 mg of *D*-tartaric acid in 1 ml of methanol was added to a solution of 500 mg of LSD (mp 155° to 160°C) in 1 ml of methanol, and the mixture was refrigerated overnight. The white precipitate was collected by filtration, washed with four 1-ml portions of a methanol:ether mixture (3:1, v/v), washed with one 2-ml portion of ether, and then dried in a vacuum desiccator over phosphorus pentoxide to yield 320 mg of LSD tartrate: mp 193° to 197°C (dec); λ_{max} 313 mμ (ε 9,840). A mixed-melting-point determination with an authentic sample was not depressed.

10. Studies on Isolation of LSD.

The remaining ethylene chloride extract (856 ml, 9.73 mmoles) from run No. 35 (table 6) was concentrated in vacuo at room temperature to 39.5 ml of dark-brown solution. The solution was treated with 6.5 ml of methanol, followed by addition of water until the reaction mixture became slightly turbid. After the turbid solution was seeded with a known sample of LSD (mp 158° to 160°C), the solution was cooled to 0°C for 1 hr and filtered to yield 1.246 gm of a dark-gray solid. The addition of more water to the filtrate led to an additional 0.271 gm of dark-gray solid. The filtrate from this solid was concentrated in vacuo to a dark-brown viscous oil. The oil was dissolved in 5.0 ml of methanol and treated with water to turbidity. The turbid solution was cooled to 0°C to yield 0.482 gm of a gray-black solid. Total solids recovered were 1.999 gm (yield, 61%). TLC of these products on silica gel G using 15% methanol in chloroform (v/v) indicated the presence of LSD in all the products, with iso-LSD absent in the first product but present in the other two products.

The 1.999 gm of product was dissolved in 800 ml of hot, absolute ethyl ether, treated with 2 gm of Darco G-60, and heated to reflux on a steam bath for 5 min. The solution was filtered and concentrated on the steam bath to 20 ml. The cooled solution (0°C) was filtered to yield 1.264 gm (after drying in vacuo for 6 hr) of cream-colored solid: mp 159° to 161°C (dec); λ_{max} 312 mμ (ε 9,310). The best sample of LSD has given λ_{max} 312 mμ (ε 9,320). An additional 0.111 gm of LSD [mp 153° to 155°C (dec)] was obtained on concentrating and cooling the ether filtrate. Total recovery on recrystallization (1.375 gm, 4.26 mmoles) was 69%; the overall yield of LSD after one recrystallization was 43.7%.

11. Preparation of LSD Tartrate (LSD-25).

A solution of 0.30 gm of D-tartaric acid in 3 ml of methanol was added to a solution of 1.3 gm of LSD (mp 85° to 90°C) in 4 ml of methanol, and the mixture was heated on a steam bath for a few minutes, causing crystallization. After the mixture was refrigerated for 3 hr, the solid was filtered and washed with several milliliters of methanol to yield 0.98 gm of product. Concentration of the mother liquor yielded two more crops of 0.20 and 0.05 gm. The combined precipitates were recrystallized from 6 ml of hot methanol to yield 1.0 gm of product: $[\alpha]_D^{27}$ +29° (c 1.0, water); literature value: $[\alpha]_D^{20}$ +30° (c 1, water)[108]; λ_{max} 313 mμ (ε 9,620). The ultraviolet and infrared spectra of this material are shown in figures 10 and 11.

Analysis of $(C_{20}H_{25}N_3O) \cdot C_4H_6O_6 \cdot 2CH_3OH$:

Calculated: C, 64.17; H, 7.19; N, 9.76
Found: C, 64.2; H, 7.4; N, 9.8

Upon introducing a melting-point tube with the LSD-25 into an oil bath at 175°C and slowly raising the temperature (2°C/min), darkening occurs at 183°C, the material sinters at 192°C, and a definite melt is formed at 196° to 199°C [literature value: mp 198° to 200°C (dec)[108]]. Physical constants for authentic material sold commercially by Sandoz are: mp 182° to 186°C (dec); λ_{max} 313 mμ (ε 9,460).

12. Epimerization of LSD and Iso-LSD.

LSD and iso-LSD were epimerized in 0.1 N methanolic potassium hydroxide, and optical rotation was used to follow the reactions. Figure 12 illustrates the epimerization and table 7 gives the observed rotations and the time they occurred. The equilibrium ratio of approximately 88:12 of the normal to iso derivative agrees with the literature.[70]

13. Studies on Solubility of LSD and Its Tartrate and Acid Maleate Salts.

The solubility of the tartrate and acid maleate salts of LSD in various solvents was determined as follows: Five milliliters of solvent was poured in a test tube and warmed to 40°C. The salt was added with stirring until a saturated solution was obtained. After standing at room temperature for 2 hr, the mixture was centrifuged and the solution was decanted into a tared flask and weighed. The solvent was removed in vacuo until a constant weight was obtained, and the solubilities were calculated.

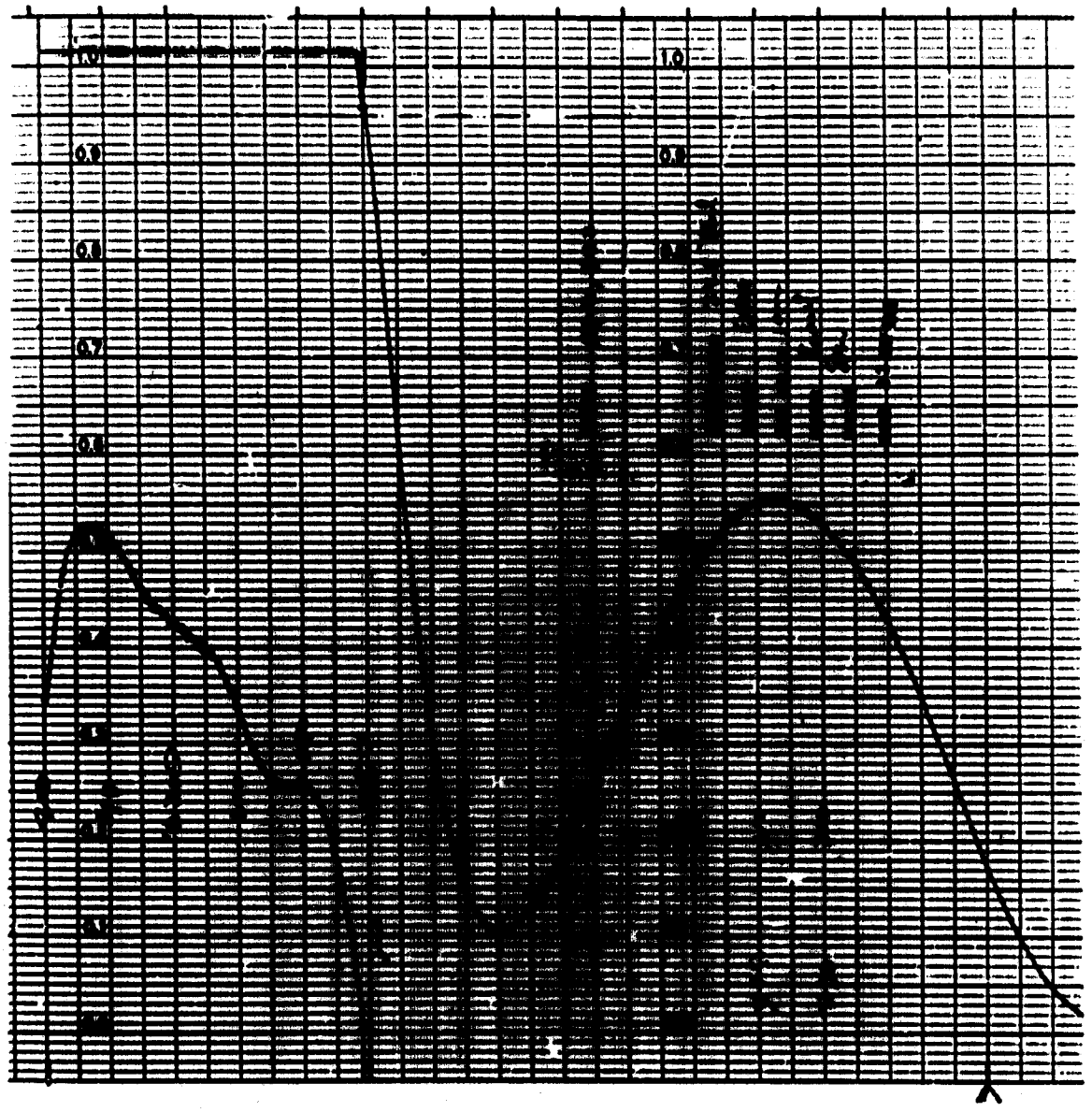


FIGURE 10

ULTRAVIOLET SPECTRUM OF LSD TARTRATE

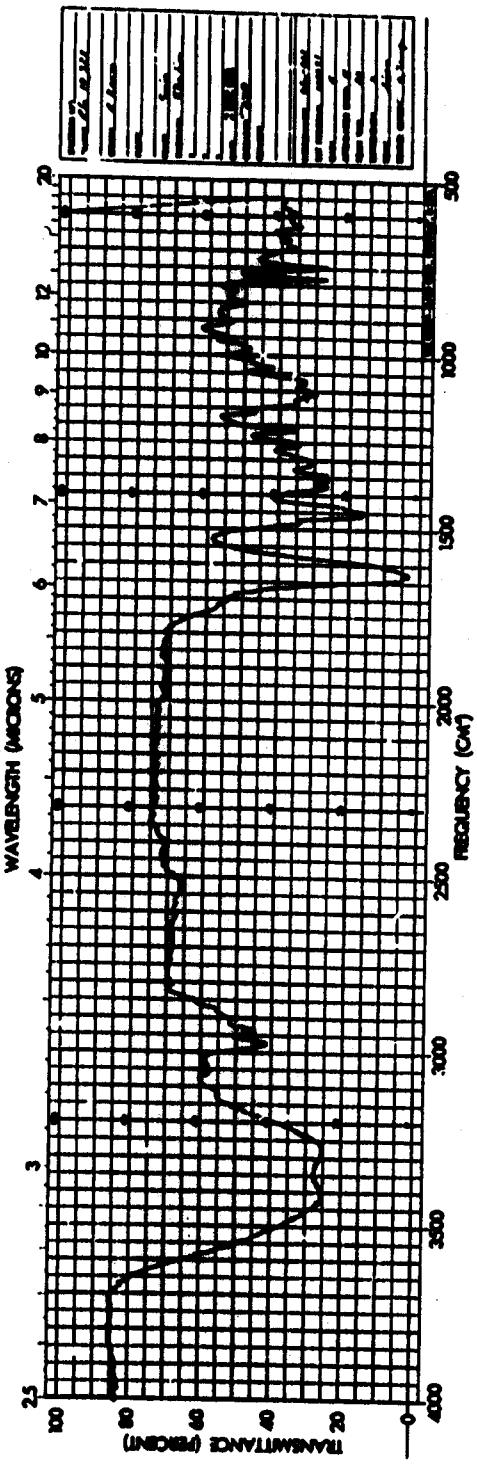


FIGURE 11

INFRARED SPECTRUM OF LSD TARTRATE

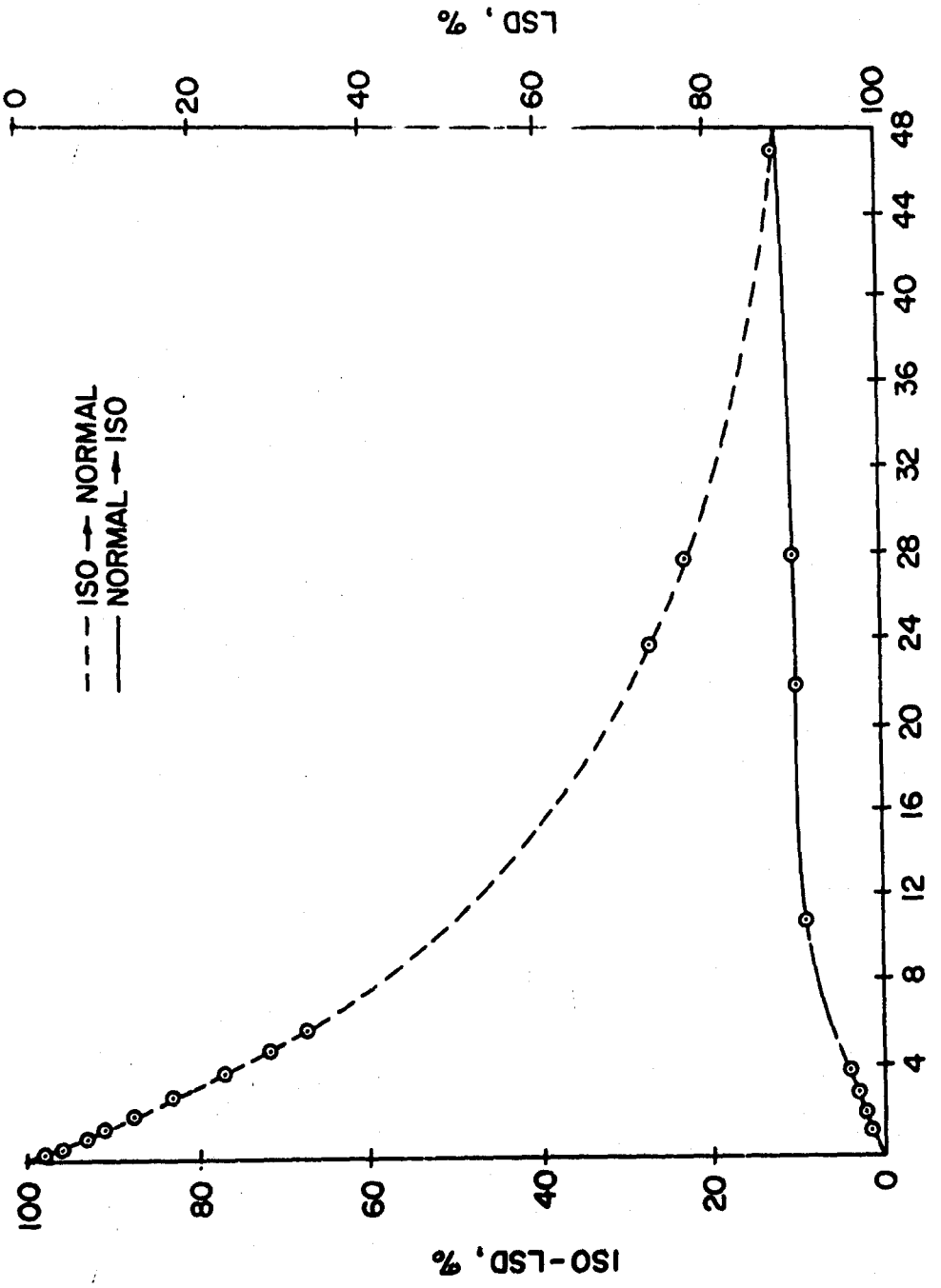


FIGURE 12

EPIMERIZATION OF LSD AND ISO-LSD

TABLE 7

EPIMERIZATION OF LSD AND ISO-LSD IN 0.1 N METHANOLIC POTASSIUM HYDROXIDE

LSD			Iso-LSD		
Time	$[\alpha]_D^{27}$ (c 0.202)	LSD a/	Time	$[\alpha]_D^{27}$ (c 0.510)	LSD
hr		%	hr		%
0 b/	+62°	100.0	0 b/	+186°	0.0
1	+63.1°	91.1	0.18	+183.7°	1.9
2	+64.5°	98.0	0.50	+181.7°	3.5
3	+65.3°	97.3	1	+177.2°	7.1
4	+66.6°	96.8	1.5	+174.8°	9.0
11	+72.9°	91.2	2	+170.8°	12.3
22	+74.1°	90.2	3	+164.9°	17.0
28	+74.7°	89.8	4	+156.9°	23.5
			5	+151.0°	28.2
			6	+145.0°	33.1
			24	+95.5°	73.0
			28	+90.1°	77.3
			47	+76.7°	88.1

a/ Calculated from the equation: $62X + 186(100 - X) = 100([\alpha]_D^{27})$, where X = percent n-LSD and 100 - X = percent iso-LSD.

b/ Values at zero time obtained by extrapolation.

Because of the small amount of LSD available and the generally high solubity of the free base in organic solvents, the following method was employed: To the solute (ca. 50 mg) was added 10-µl portions of the solvent until solution was complete. The solubility was expressed in terms of a range in which the true solubility falls. In the special case in which water was used as a solvent for the free base, 13.3 mg was not completely dissolved by 300 ml.

The results of the solubility studies are found in table 8.

TABLE 8

SOLUBILITIES OF LSD AND TARTRATE AND MALEATE SALTS

Solvent	Solubilities		
	Free base	Tartrate	Acid maleate
	gm/ 100 ml of solvent		
Ethanol	62 - 71	1.65	0.55
Methanol	105 - 130	3.94	2.05
Water	$<4.4 \times 10^{-6}$*	23.8	0.61
Dimethylformamide	135 - 200	30.7	31.8
Acetonitrile	52 - 58	0.35	0.42
Chloroform	88 - 105	0.31	0.33

* 12×10^{-6} gm in 100 ml at 75°C.

14. Studies on Stability of LSD.

a. Thermal Stability of Solutions of LSD.

(1) An aqueous solution of LSD acid maleate (27 μg/ml) was refluxed in the dark, and samples were taken periodically for colorimetric determinations. On the basis of colorimetric assay, after 5 hr of heating, 100% of the starting material remained, after 10 hr, 83%, and after 20 hr, 27%. The material was extracted with chloroform, and silica gel TLC plates were run, employing a solution of 15% methanol in chloroform. Four spots were observed: R_f 0.85, red fluorescence; R_f 0.7, blue fluorescence and a positive van Urk test (corresponding to starting material); R_f 0.65, red fluorescence; and R_f 0.4, no fluorescence but a positive van Urk test.

(2) An aqueous solution of LSD acid maleate (30 μg/ml) was made acidic to pH 1 with concentrated hydrochloric acid and refluxed in the dark. On the basis of colorimetric assay, after 20 hr of heating, 95% of the starting material remained, and after 40 hr, 65%. TLC, using the system described above, showed the following four spots: R_f 0.75, red fluorescence; R_f 0.7, white fluorescence; R_f 0.65, blue fluorescence and a positive van Urk test (starting material); and R_f 0.1, blue fluorescence and a positive van Urk test.

(3) An aqueous solution of LSD acid maleate (0.079 mg/ml) was refluxed in a nitrogen atmosphere in the dark. Colorimetric determinations (van Urk) were made with the following results:

Time of reflux hr	Concentration mg/ml
0	0.079
6.5	0.074
14.0	0.073
20.0	0.073

(4) LSD acid maleate (10.0 mg) was added to 100 ml of water and brought to pH 1 with concentrated hydrochloric acid. The initial absorbance of a fourfold dilution (25%) was 0.37. After 2 hr of refluxing, the absorbance was still 0.37. After a total of 6.75 hr of refluxing, the heating was stopped for the day. Refluxing was begun again, and, after a total of 8.25 hr, the absorbance was 0.39. At this time the solution had a slight bluish cast. Refluxing was continued until the end of the working day. The next day, after a total of 21.25 hr of refluxing, a thin-layer plate (silica gel with 15% methanol in chloroform) showed a faint blue color at the origin, no fluorescence, and a negative van Urk test; at R_f 0.16, a blue fluorescent spot and a positive van Urk test; and at R_f 0.48, a blue fluorescent spot and a positive van Urk test (unreacted LSD). The solution was refluxed for a total of 22.75 hr, when heating was stopped. The solution then remained at room temperature for 3 days and became a more intense blue in color. A van Urk test gave an absorbance of 0.55, and a thin-layer plate showed a blue spot at the origin; at R_f 0.11, a faint fluorescent spot and a weakly positive van Urk test; at R_f 0.15, a faint fluorescent spot and a weakly positive van Urk test; at R_f 0.5, fluorescence and a positive van Urk test (the LSD); and at R_f 0.57 and 0.69, very faint fluorescence and a negative van Urk test.

(5) A DMF solution of the LSD acid maleate (30 gm/ml) was heated to reflux at 153°C. Within 15 min the solution had turned a deep brown, and a colorimetric assay indicated the starting material was completely decomposed.

(6) LSD samples (5 mg) were added to 50 ml of DMF and the solutions were heated to 100°C. The initial colorimetric assay (van Urk test) gave an absorbance of 0.22 for LSD acid maleate and 0.15 for LSD.

After the solutions were heated to 100°C for 1 hr, the temperature of the free base solution climbed to 115°C during the next hour, and the solution became more yellow than the maleate solution. The temperature was then adjusted back to 100°C and heating was continued for 4 hr. At the end of this time, the color of the two solutions appeared to be of equal intensity, and the absorbances were 0.12 for the free base and 0.07 for the maleate. Thin-layer plates run on material from the experiments with DMF at 100°C (the material having been concentrated in vacuo at room temperature) showed about 15 spots for both the free base and the maleate. The spots in both cases were in the same order and had the same color and fluorescence.

(7) A similar run gave initial absorbances of 0.15 for LSD and 0.22 for LSD acid maleate. On heating the solutions to 100°C, the LSD acid maleate solution began to darken in color within 15 min. After 30 min of heating, the absorbances were 0.15 for LSD and 0.21 for LSD acid maleate. After 3 hr of heating, the absorbances were 0.15 for LSD and 0.13 for the LSD acid maleate.

(8) A solution of DMF containing 30 μg/ml of LSD acid maleate was heated for 2 hr at 75°C. No darkening of the solution was noticed, and only one spot was obtained on a thin-layer plate corresponding to the standard.

b. Thermal Stability of Solid LSD Acid Maleate.

(1) LSD acid maleate (10.3 mg) was placed in a melting-point tube and a standard melting-point determination was made. As expected, the material blackened and decomposed. The material was then extracted with methanol, and a colorimetric assay showed 92% of the original material to be present.

(2) Another sample was heated in a melting-point tube at 225°C for 0.5 hr; the assay showed 16% of the material to be present. TLC (silica gel with 15% methanol and 85% chloroform) of this material gave the following spots: R_f 0.92, red fluorescence; R_f 0.84, blue fluorescence and a negative van Urk test; R_f 0.64, blue fluorescence and a positive van Urk test (undecomposed LSD acid maleate); R_f 0.16, blue fluorescence and a positive van Urk test; R_f 0.03, blue fluorescence and a positive van Urk test; and at the origin, light-yellow to orange fluorescence and a positive van Urk test.

15. Rearrangement of LSD in Strongly Acidic Solution.

A solution of 25 mg of LSD acid maleate in 5 ml of glacial acetic acid saturated with hydrogen chloride was heated to 50°C. Samples were removed at intervals and examined by TLC (silica gel G with 15% methanol and 85% chloroform). After 4 hr of heating at 50°C, a purple color developed in the solution that increased in intensity as the heating continued. A thin-layer chromatogram run prior to the appearance of color in the solution indicated a second spot (R_f 0.75, yellow fluorescence) in addition to the spot for LSD acid maleate (R_f 0.50, blue fluorescence). After 52 hr of heating, no LSD acid maleate could be detected by TLC. The thin-layer plate contained six spots: R_f 0.75, yellow fluorescence; R_f 0.50, no fluorescence; R_f 0.33, red fluorescence; R_f 0.28, purple color; R_f 0.20, blue color; and R_f 0.13, purple color. A blue color was also noted at the origin.

16. Preliminary Studies on Action of Oxygen, Bromine, and Sodium Hypochlorite on LSD.

a. LSD acid maleate (5.09 mg) was dissolved in 100 ml of water, and a portion was placed in a tube equipped with a fine fritted-glass disk through which oxygen was bubbled in the dark. After 20 hr, no noticeable change had occurred in the sample on the basis of colorimetric assay and TLC. There was, however, an increase in the value of the ϵ_{min} at 268 mμ that has not yet been explained.

b. Oxygen was bubbled through a solution of 50 mg of LSD in 250 ml of ethanol while the solution was irradiated with an ultraviolet lamp placed at a distance of 12 in. Colorimetric assays taken each hour showed a decrease in absorbance, with complete disappearance of a positive test after 22 hr. TLC of the solution showed a fluorescing material with the same R_f as the starting material, but it did not give a positive van Urk test.

c. To a solution of LSD in Merck carbon tetrachloride (0.5 mg/ml) was added an equimolar amount of bromine in the same solvent (0.0112 M), and the mixture was examined by TLC employing 15% methanol in chloroform with silica gel. The following three spots were observed within seconds: R_f 0.74, yellow fluorescence; R_f 0.64, quenches ultraviolet radiation; and R_f 0.51, quenches ultraviolet radiation. None of the spots gave positive van Urk tests. The starting material disappeared after 30 to 45 min. Baker carbon tetrachloride was originally used in this experiment, but solutions of bromine in this solvent became colorless after a few hours, indicating that impurities most likely were reacting with the bromine. Bromine solutions in the Merck solvent were found to be stable.

d. LSD (0.005 gm) was dissolved in a 15% solution of methanol in chloroform. To this a small amount of commercial bleach was added, and a silica gel plate was run immediately. The plate was developed with 15% methanol in chloroform. Three spots that quenched under ultraviolet irradiation were observed. A spot corresponding to LSD was not observed.

17. Studies on Conversion of LSD to Its Lumi Derivatives.

a. A solution of 0.9 gm of LSD acid maleate in 90 ml of 10% acetic acid was placed in a Vycor No. 7900 tube and irradiated under a nitrogen atmosphere at room temperature with a 30-w General Electric ultraviolet germicidal lamp at a distance of 20 cm from the tube. Samples were taken every 0.5 hr for the first 3 hr, and then over longer periods of time because the reaction under these conditions was not as rapid as had been anticipated. The method of sampling was as follows: Five milliliters of the solution was made basic with ammonium hydroxide and then extracted with 5 ml of chloroform. The extract was evaporated to dryness, 2 or 3 drops of chloroform were added to the residue, and the resulting solution was spotted on a silica gel chromatoplate that was developed with 15% methanol in chloroform. The starting material (R_f 0.69) was determined by fluorescence and gave a positive van Urk test. A spot with an R_f of 0.26, believed to be the lumi derivative, did not fluoresce but did give a positive van Urk test. Based on the disappearance of the starting material, the reaction took 13.5 hr to go to completion.

b. LSD acid maleate (400 mg) was dissolved in 400 ml of 10% acetic acid and irradiated for 6 hr with a 100-w General Electric S-4 lamp while being stirred in a 500-ml round-bottomed Vycor flask. The aqueous solution was made basic with concentrated ammonium hydroxide and extracted with chloroform. After the dried extract was evaporated, which TLC showed to contain a small amount of LSD, the resulting crude oil was purified by column chromatography on Fisher alumina with chloroform. The major fraction consisted of an oil (150 mg) whose behavior on TLC was identical with that described above and whose ultraviolet spectrum was identical with that of lumi-LSD reported in the literature[70]: λ_{max} 223, 284, and 293 mμ.

This material was rechromatographed on Fisher alumina, utilizing benzene:chloroform (1:1) as the eluate, to yield an oil that crystallized on standing in a concentrated methyl ethyl ketone solution at 3 °C for several months.

c. In a manner similar to Hellberg's work[73] with ergotamine tartrate, aqueous solutions of LSD acid maleate (19.09 μg/ml) and LSD tartrate (25.28 μg/ml) in quartz cells were irradiated with a Mineralight ultraviolet lamp, the radiation of which was limited to the region of 366 mμ by a filter. Ultraviolet absorption spectroscopy was used to follow any possible reaction. Absorbances at 311 and 292 mμ after various times of irradiation are recorded in table 9, and absorbance curves are given in figures 13 and 14.

TABLE 9

EFFECT OF ULTRAVIOLET RADIATION ON LSD SALTS

Irradiation time	Absorbances for two salts irradiated at two wavelengths			
	Tartrate		Acid maleate	
	311 mμ	292 mμ	311 mμ	292 mμ
hr				
0	0.52	0.39	0.38	0.28
1	0.50	—	0.36	0.27
2	0.48	—	0.34	0.265
3	0.47	0.38	0.33	0.26
4	0.455	—	0.305	0.26
21	—	—	0.25	0.30
22	0.335	0.42	—	—

On the basis of the shapes of the curves after nearly 1 day of irradiation, it would appear that some lumi formation occurred.

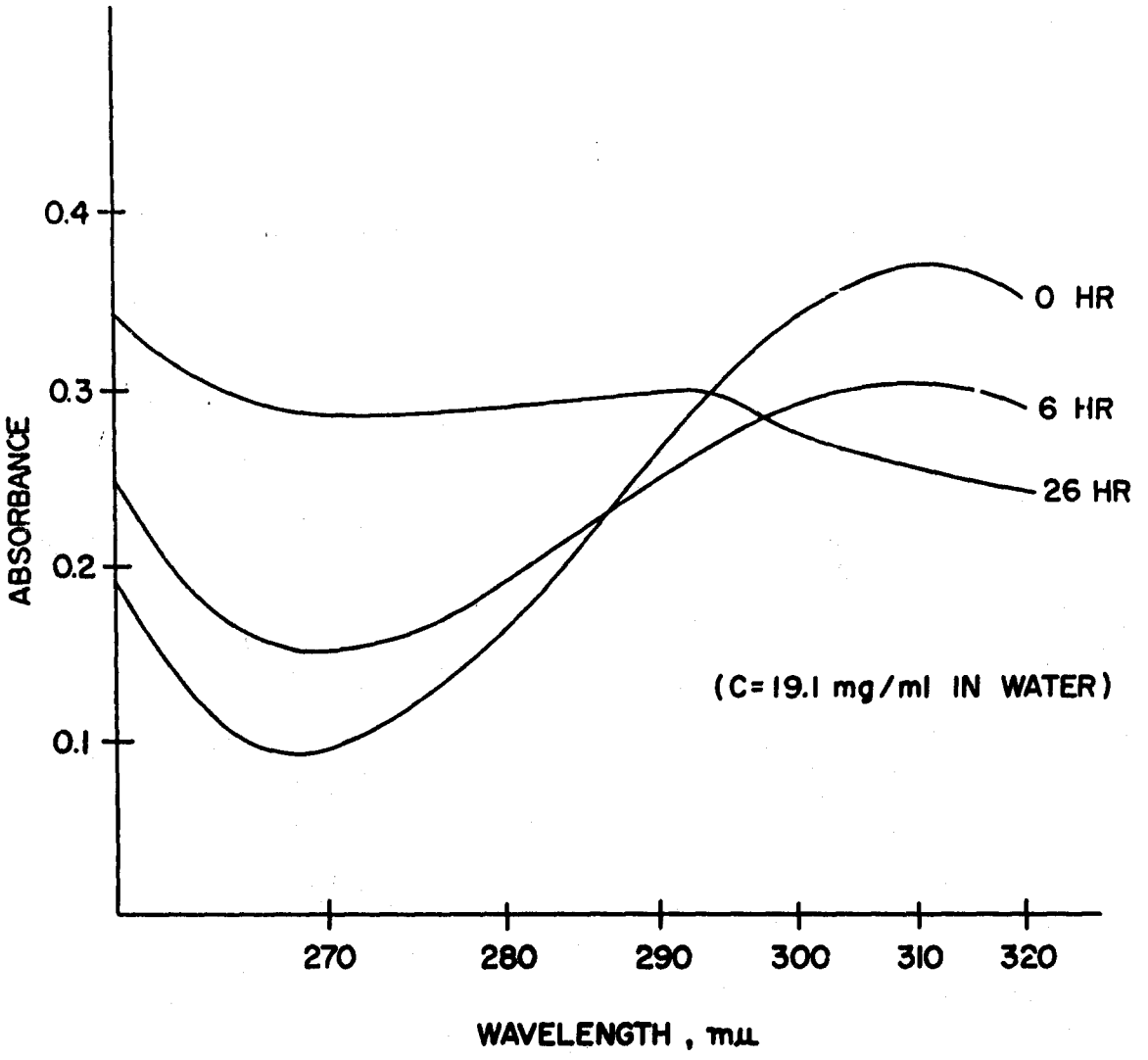


FIGURE 13

ULTRAVIOLET SPECTRA OF LSD ACID MALEATE IRRADIATED AT 366 mμ

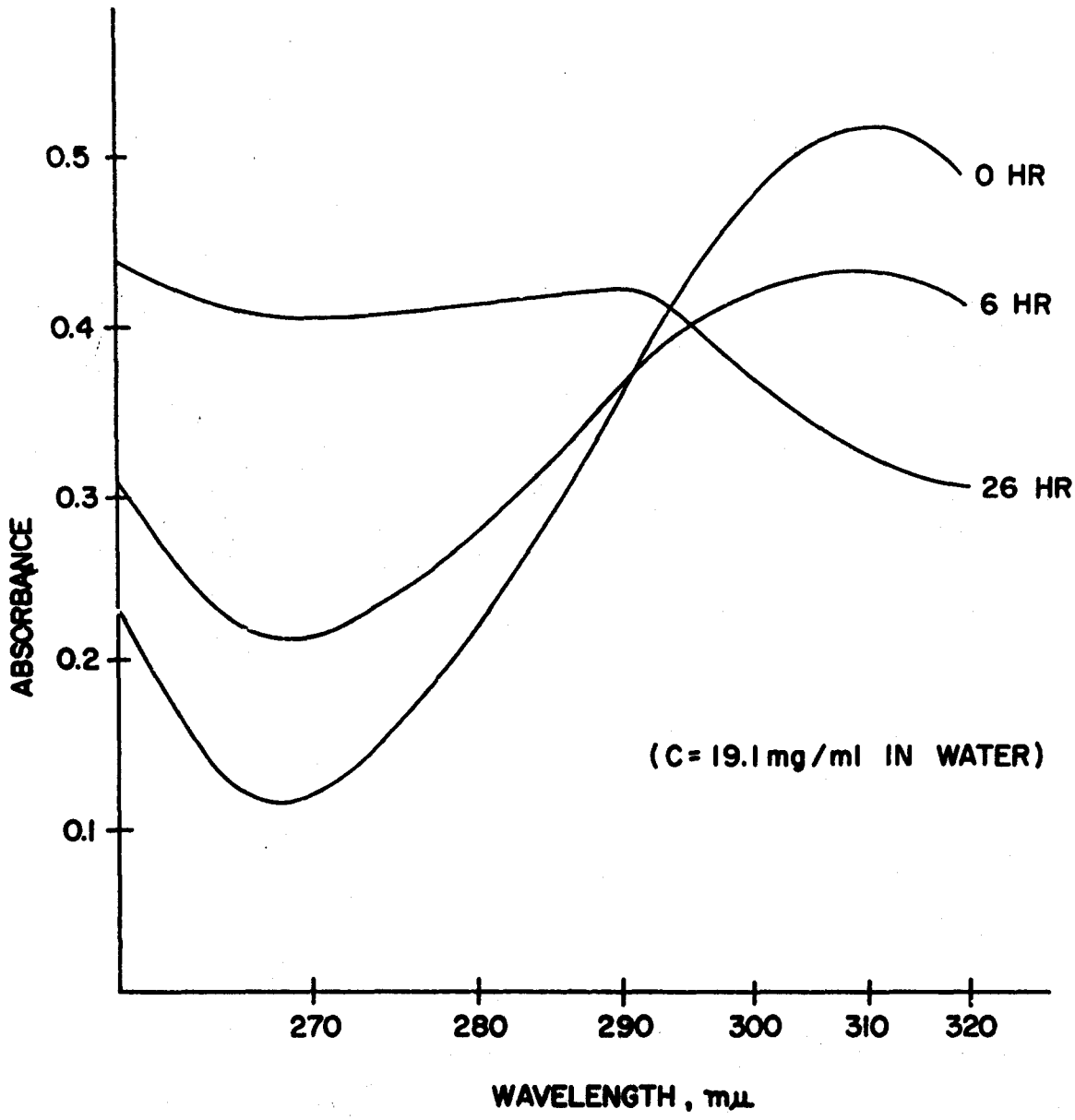


FIGURE 14

ULTRAVIOLET SPECTRA OF LSD TARTRATE IRRADIATED AT 366 mμ

18. Effect of Sunlight on Solid LSD.

A Petri dish containing powdered LSD(mp 160° to 162°C; ϵ_{312} 9,260) was placed on a window sill facing south,and samples were taken periodically for ultraviolet and TLC analyses. After 7 days, the material turned tan in color and gave one spot corresponding to LSD on TLC [mp 154° to 155°C (dec); ϵ_{312} 9,410]. After 14 days the material was gold in color [mp 150° to 158°C (dec); ϵ_{312} 9,260]. TLC on silica gel (15% methanol in chloroform) revealed the following three spots: R_f 0.0, faint-yellow fluorescence; R_f 0.46, faint-pink fluorescence; and R_f 0.66, LSD. Fluorometric analysis of a sample taken after 22 days gave a spectrum identical with the starting material, but that had an estimated 7% less fluorescence than the starting material. After 24 days, the material had an ϵ_{312} 9,120; after 46 days the material had an mp 148° to 155°C (dec); ϵ_{312} 8,820.

III. (C) DISCUSSION.

(U) The synthesis and some aspects of the chemistry of LSD have been investigated. The isolation and conversion of ergot alkaloids and saprophytically produced alkaloids to D-lysergic acid have also been studied. The investigation of the synthesis of LSD by the Garbrecht method[60] has been completed, but research on the isolation of D-lysergic acid, other methods of synthesis of LSD, and the chemistry of LSD is continuing.

(U) The method described by Garbrecht[62] was used with minor modifications for the synthesis of LSD. Garbrecht had reported a 25% yield of LSD acid maleate by this method, but suggested that a study of the method should lead to better yields. Yields of up to 71% of LSD acid maleate have been obtained in synthetic runs of 0.005 to 0.20 mole during the course of this study. The mother liquors from the crystallization of the LSD acid maleate also contained the iso-LSD formed during the synthesis. This iso-LSD can be recovered and converted to n-LSD in alkaline solutions. LSD is obtained in good yields, since the isomerization equilibrium strongly favors n-LSD (88%)over iso-LSD (12%). Thus, an additional 9% of LSD acid maleate was obtained from two runs averaging 66%, giving an overall yield of 75%.

(U) This reaction seemed to proceed to completion with little decomposition, on the basis of the assay for total amide produced. Garbrecht explained the low yields obtained as a consequence of the difficulties encountered in the isolation of these sensitive materials. The sensitive portion of the LSD

molecule, however, is the lysergic acid portion, which is common to the other lysergic acid amides, such as D-lysergic acid benzylamide, that had been prepared in 75% yield.[62] The other reaction that could cause loss of LSD product was the formation of the iso form, but, as previously noted, in the case of LSD the normal form is favored. LSD is not inherently different from the other amides synthesized by this method, as demonstrated by the yields of LSD obtained in this study.

(U) In the laboratory synthesis of LSD, the control of the pH of the methanol solution of lysergic acid monohydrate and lithium hydroxide between 9.2 to 9.6 was very important.[86] To obtain this pH, it was necessary to use a 6% mole excess of the D-lysergic acid monohydrate. In runs where the pH was not controlled between 9.2 to 9.6, the yield of product was poor, and unreacted lysergic acid was recovered as lysergic acid sulfate from the aqueous reaction mixture on acidification with sulfuric acid.

(U) The quality of DMF-SO_3 complex was also found to be important. The complex is reported to be stable for at least 3 to 4 mo, but, in some cases, a poor yield of product was obtained with complexes stored for only short periods of time. A method of quality control for the DMF-SO_3 complex is not available, and, in the laboratory, the only means of checking quality was to determine the molarity and to use the material in a small-scale, explorative run to see if LSD was produced. The storage stability of the DMF-SO_3 complex for larger operations may not be a problem because the complex would be used in a shorter period of time. In the laboratory, the methanol solution of lithium lysergate was concentrated in vacuo to a syrup and DMF was added. The DMF solution was distilled in vacuo at or below 40°C until the water content of the DMF distillation cuts was less than 0.5%. In the laboratory, a water content of 0.1% was readily obtained. The resulting anhydrous solution of lithium lysergate in DMF was cooled to 0° to 10°C in preparation for addition of the DMF-SO_3 complex.

(U) The DMF-SO_3 complex and diethylamine were added very quickly, the materials being poured into the reaction mixture one after the other. Diethylamine was used in a 5 M equivalent. The reaction mixture was then treated with an excess of aqueous sodium chloride solution. The first indication of a successful reaction was obtained at this step. A light-yellow colloidal solution was observed for those runs that gave LSD in satisfactory yields. The alkaline aqueous sodium chloride solution was extracted with ethylene chloride until a van Urk test of the aqueous solution gave only a pale-blue color. The ethylene chloride extracts were combined, treated with

anhydrous magnesium sulfate, and allowed to stand overnight at 3 °C. The laboratory synthesis, from the weighing of the lysergic acid to the completion of the ethylene chloride extraction, required about 7 hr, or 1 working day. The dried ethylene chloride extract, after all the ethylene chloride was removed, was concentrated in vacuo at room temperature to an oil.

(U) The concentrate was diluted with benzene and placed on a short-pass alumina column. The long alumina column reported in the literature for LSD gave an excellent product, but required days to develop and to elute, and the product was contained in a large volume of solvent. An alumina column containing approximately one-twelfth (short-pass column) of the amount of alumina required for the recommended column was sufficient to give a product that could be obtained as a crystalline acid maleate salt. The alumina used in the laboratory and recommended by Garbrecht was a Fisher Chemical Co. alumina of activity grade 2.5 to 3.0. It is important to use a low-activity alumina, because lysergic acid and lysergic acid derivatives are strongly adsorbed on high-activity alumina (grade 1) and cannot be readily eluted. The alumina column was developed and eluted with a chloroform-benzene mixture. The progress of the products on the column can be followed by observing the blue fluorescence under ultraviolet irradiation. The exposure of the column and the fractions from the column to ultraviolet radiation must be kept to a minimum to avoid the formation of lumi-LSD. The eluted column always contained a black band at the top that did not develop with benzene-chloroform. The fractions containing the LSD were combined and concentrated in vacuo to remove most of the benzene and chloroform, which was indicated by the change in the rate of concentration. The concentrated solution that appeared to be the desired product in DMF was treated with a methanol solution of maleic acid and then with ethyl ether. The solution was allowed to stand overnight at 3 °C, and the resulting crystalline LSD acid maleate was filtered and washed with ether. The LSD acid maleate gave one major spot, with a small spot corresponding to iso-LSD acid maleate on TLC, and had the following properties: mp 185° to 190 °C (dec); λ_{max} 314 mμ (ε 8,500); 96.5% purity based on ε 8,800. This LSD acid maleate was dissolved in boiling methanol, treated with activated charcoal (Darco G-60), and filtered; about 20% of the methanol was distilled off. Ethyl ether was added and the solution was cooled overnight at 3 °C. The recrystallized LSD acid maleate was recovered in 85% yield and had the following properties: one spot on TLC; mp 194° to 197 °C (dec); $[\alpha]_D^{25}$ -7° (c 1.00, methanol); λ_{max} 314 mμ (ε 8,800).

(U) The determination of the purity of LSD samples leaves much to be desired. The purity has been based on the ϵ_{max} at 314 mμ, but, since no absolute purity standards are available, the percent purity must be based on reported ϵ_{maxima}, or on ϵ_{maxima} obtained in the laboratory on biologically active samples. The adsorption at 314 mμ does not differentiate between the n- or isolysergic acid amides (figure 15).[31] The lack of a doublet at 230 mμ for lysergic acid amide would indicate presence of isolysergic acid amide, but this cannot be used quantitatively. It is necessary to determine the optical rotation of the sample, and to compare the value with those reported in the literature or experimentally determined on samples with high extinction coefficients and known to be biologically active. In practice, a product like the acid maleate, giving one spot on TLC, having an ϵ_{max} of 8,700 or better, and an optical rotation of -7° ±2°, is considered pure. The melting points of LSD, its salts, lysergic acid, and other lysergic acid derivatives are useful for identification. However, because these compounds do not have sharp melting points but melt with decomposition over a range of temperatures, the melting point offers only an indication of a degree of purity.

(U) The majority of the LSD prepared has been isolated as the acid maleate salt. Garbrecht had recommended the maleate salt of the lysergic acid derivatives as the one most readily obtained as a pure crystalline product. The acid maleate salt has been found in the laboratory to be the most readily handled LSD salt.

(U) In table 6, the results of the numerous synthetic runs for the preparation of LSD are compiled. Isolation method D, employing the short alumina column, was used in the majority of the preparations and was the preferred method. In method A, a long alumina column was used that gave a quality product, but it required days to develop and to elute and gave the product in a large volume of solvent. Methods B, C, and E attempted to go directly to the LSD acid maleate without passage through an alumina column. These methods did not give good yields of quality product, and indicated that the LSD acid maleate could not be directly obtained by treatment of the ethylene chloride extract with maleic acid, as was done in the preparation and isolation of ergonovine maleate at Eli Lilly and Co. The variation in yield of product noted in the table was caused by the isolation method used, poor control of the pH of the methanol solution of lysergic acid monohydrate and lithium hydroxide (runs Nos. 1 through 6), poor-quality DMF-SO_3 complex (runs Nos. 11, 12, 18, and 19), unexplained operator variations (one operator consistently obtained better yields than another operator), and, in some runs, a combination of these causes. Starting with run No. 30, all of the variables were under better control and the yields were more consistent.

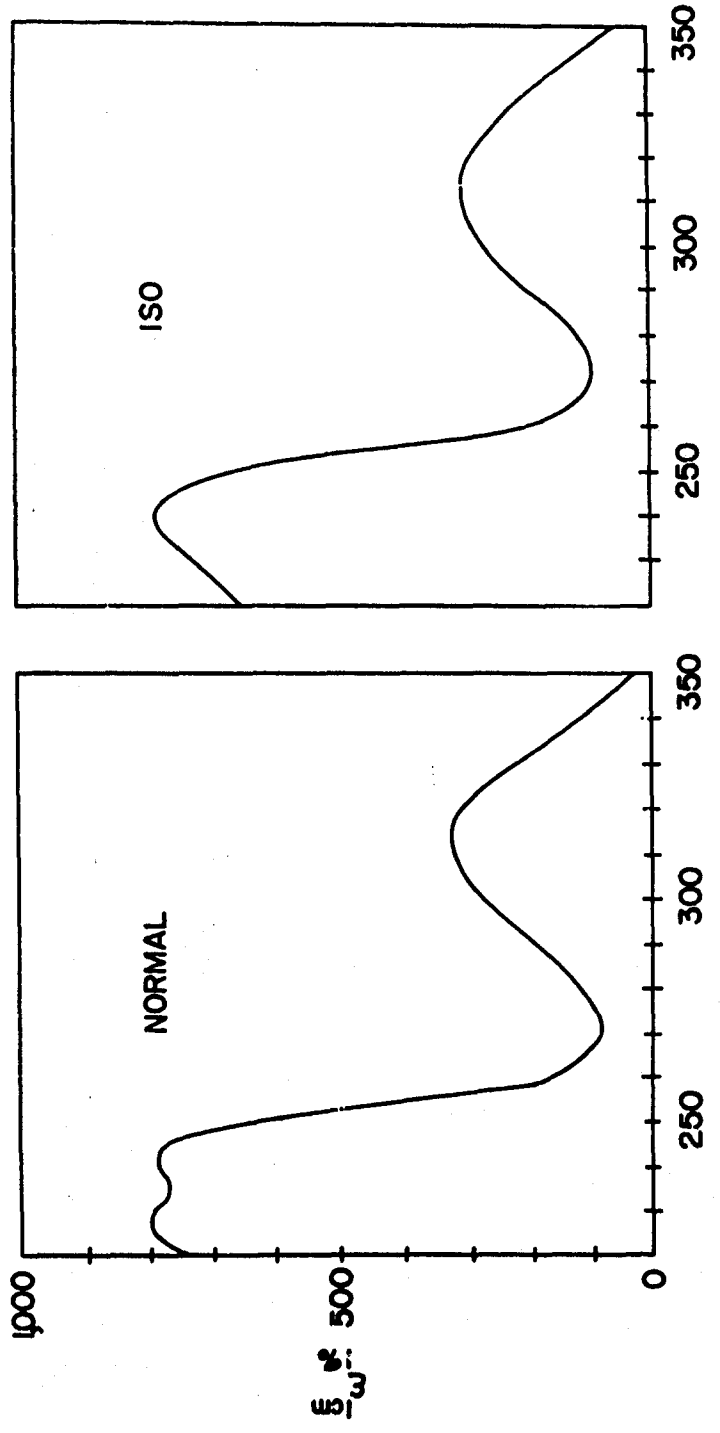


FIGURE 15

ULTRAVIOLET SPECTRA OF D-n- AND D-ISOLYSERGIC ACID AMIDE

(U) The isolation of the LSD tartrate (LSD-25) as a pure product and in comparable yields to the LSD acid maleate has to date not been achieved. To obtain a pure LSD tartrate sample, it has been necessary to convert the maleate salt to the free base and treat it with tartaric acid. The direct isolation of LSD tartrate by a method similar to that used for the LSD acid maleate is being studied.

(U) The maleate salt was converted to LSD base by treatment with aqueous ammonium hydroxide to give a product with a melting point in the range reported for LSD, 80° to 85°C. This material on TLC gave only one spot. LSD had previously been isolated from fractions obtained from long-column chromatography on alumina with melting points in the same range, but TLC indicated that this material was not pure, the major impurity being iso-LSD.

(U) The LSD obtained from the maleate salt was recrystallized from ethyl ether to give a product with the following properties: mp 160° to 162°C (dec); $[\alpha]_D^{20}$ 18.0°; λ_{max} 312 mμ (ε 9,320). This high-melting LSD gave only one spot on TLC, gave an excellent elemental analysis, and was physiologically active. Both the low-melting LSD (mp 82°C) and the high-melting LSD could be converted to identical acid maleate and tartrate salts. Both bases gave identical mass spectra and indicated a molecular weight of 323 on mass-spectrometric analysis. The low-melting base obtained from LSD acid maleate in basic water solution, when dried in vacuo for 15 hr, gave a material with a melting point of 87° to 92°C (ϵ_{312} 9,320). The high-melting LSD has not been reported in the literature, but, on the basis of the excellent elemental analysis and the increased melting point of the low-melting LSD on drying, it is believed to be a highly pure nonhydrated LSD.

(C) Some confusion exists for the designations for D-lysergic acid diethylamide. Stoll and Hofmann[108] state that the water-soluble tartrate is designated as LSD-25, or Delysid. However, literature from Sandoz Pharmaceuticals[16,17] designates D-lysergic acid diethylamide as LSD-25, or Delysid, and states that the material is soluble in water, a process that is facilitated by adding crystalline tartaric acid. It is doubtful if any of the reported clinical studies with LSD were performed with the free base, which is insoluble in water (less than 4.4×10^{-6} gm/100 ml water), and, to our knowledge, not commercially available. The LSD was most likely used as the water-soluble tartrate salt (2 molecules of LSD to 1 molecule of tartaric acid), or the acid maleate salt (1 molecule of LSD to 1 molecule of maleic acid, with one carboxyl group of the maleic acid being free). In these Laboratories, LSD was coded as EA 1729, the tartrate salt (LSD-25) as EA 1653, and the acid maleate as EA 3528.

(U) The study of the isolation of LSD or LSD salts by techniques other than purification by chromatography on an alumina column indicates that a crude LSD acid maleate, ϵ_{max} in the range 8,200 to 8,400, with a melting point ranging from 180° to 190°C, can be obtained in yields of 53% to 64% using the alumina as a slurry (method F), using Darco G-60 as a slurry (method G), and using no adsorption step prior to crystallization (method H). It was possible to isolate the crystalline LSD base by these methods (method I). The high-melting LSD has also been isolated directly from an ethylene chloride extract of the basic reaction mixture in 43% yield [experiment No. 10 (run No. 35)]. The necessity to use ethyl ether as the crystallizing solvent for the high-melting LSD complicates this isolation. Ether is not only a dangerous solvent, but its low boiling point, 36°C, leads to loss of product in processing. The use of other crystallizing solvents is being investigated. No attempt has been made to determine the optimum conditions for obtaining LSD acid maleate or LSD by these methods.

(U) The largest laboratory preparation of LSD was 0.20 mole, and no information on an LSD run larger than this is available. Eli Lilly and Co. regularly prepared ergonovine maleate by the Garbrecht method in 1-mole synthetic runs. These 1-mole runs are batch operations similar in all respects to the laboratory synthesis, and involve one operator using large semiprocess equipment. Although Lilly has done these preparations for many years, the water content of the distilled DMF and the molarity of the DMF-SO_3 complex are determined for every production run. Lilly officials claim that they have not studied the scale-up of this method for continuous or plant operation and have no plans to do such a study.

(U) The batch operation for ergonovine maleate requires approximately four 8-hr shifts to complete (one operator per shift). The most time-consuming operation (two of the four shifts) was the extraction of the product from the reaction mixture using ethylene chloride. The complete extraction of the product normally required 15 to 20 extractions (in these Laboratories 5 to 6 were sufficient) and was complicated by the formation of emulsions. Emulsion formation has not been a problem in the laboratory extractions except when the yields of LSD were poor. The ergonovine maleate was obtained by concentrating the ethylene chloride solution, treating the concentrate with Darco G-60, filtering, and adding a methanol solution of maleic acid to the filtrate. Crystallization occurs when the mixture is cooled. Lilly claims a yield of 60% to 65% of ergonovine maleate from batch operations. The mother liquors from three to five runs are combined and concentrated for recovery and conversion of the ergonovinine to ergonovine.

(U) No other method available at this time appears to offer the ease of laboratory operation, the low cost and ready availability of the chemicals required, and the high yield of product found for the Garbrecht method.

(U) The reported synthesis of D-lysergic acid chloride-hydrochloride might offer a method preferable to the Garbrecht method, but to date no experimental details are available that would allow a comparison of the methods. The possibility of exchange reactions between D-lysergic acid or acid amides with simple organic acid amides, such as diethylacetamide, has not been investigated. However, the high reaction temperature for such reactions, 150°C or more, makes their use for the synthesis of the temperature-sensitive D-lysergic acid amides very doubtful.

(U) The possible production of LSD by the saprophytic culture method will be investigated at the University of Connecticut. It has been reported that Claviceps paspali strains have produced lysergic acid, ergonovine, and D-lysergic acid α-hydroxyethylamide; other amides, such as diethylamide, might possibly be produced by strain modification, or by the use of suitable organic precursors (such as diethylamine) in the fermentation broth.

(U) At present, the most important problem in LSD synthesis is the supply of D-lysergic acid. The material is not readily available in over kilogram quantities, and the cost as of May 1963 was $12,000/kg. If Kelleher's claimed production figures and cost estimates per kilogram for large-scale production are used, the cost should be as low as $50/kg. It was assumed in this estimate that the sensitive lysergic acid derivative could be extracted and converted to lysergic acid in good yield. Unexpected problems may be encountered in the extraction and conversion of the product.

(U) Although the production levels claimed for Farmitalia fermentations are 5 to 6 gm/ℓ, the production level in 40-ℓ fermentations at the University of Connecticut have not exceeded 0.5 gm/ℓ; although individual strains have produced 1.2 gm/ℓ in 1-ℓ fermentations. Strain-selection experiments using strain CD-3 (C.05 gm of total alkaloids/ℓ), believed to be similar to strain F-140 reported in the paper by Arcamone,[31] had given on the third selection 6 isolates out of 24 that produced 0.5 to 0.65 gm/ℓ of total alkaloids. This steadily increasing alkaloid production was lost on the fourth selection because of contamination of the University water supply. The strain, after loss of production to an average of 0.2 gm/ℓ, did not respond to further strain selection but did give yields up to 1.2 gm/ℓ when organic supplements (tryptophan and acetamide) were added to the fermentation broth. A strain

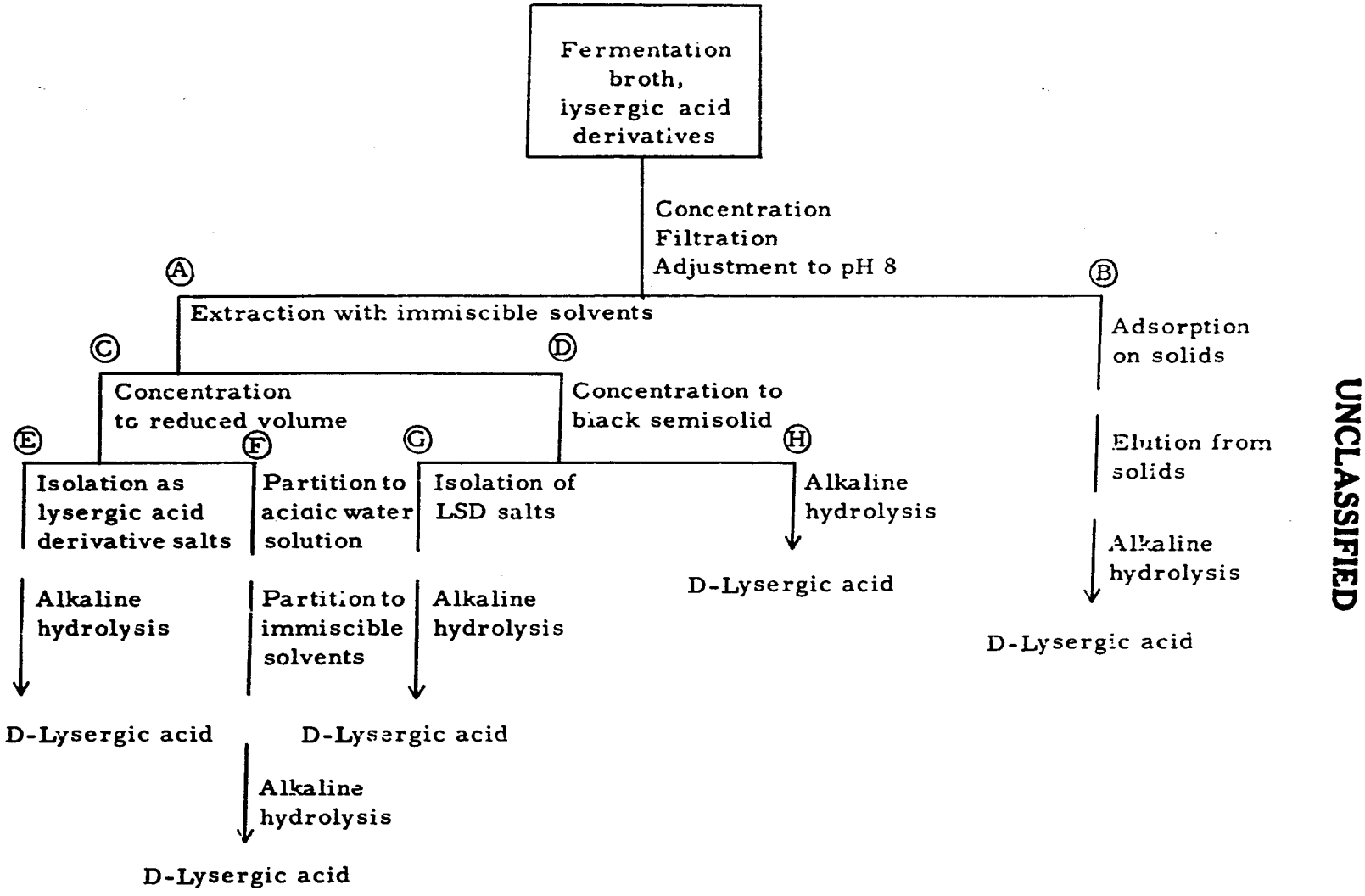
that gave a peak alkaloid concentration of 0.73 gm/ℓ as a control, however, did not give a higher alkaloid concentration when the same supplements (tryptophan and acetamide, 0.72 gm/ℓ; glycine, 0.83 gm/ℓ) were used. Strain-selection experiments with CD-3 strains at the University of Connecticut have been resumed, incorporating the information obtained from Tonolo[44] and employing defined media. To date, a stable, high-producing strain and the optimum growth conditions for continued high alkaloid productions have not been obtained at the University of Connecticut.

(U) The Garbrecht method requires D-lysergic acid that must be obtained either from ergot or the saprophytically produced alkaloids. Using a method devised by Eli Lilly and Co., cristamine, obtained from ergot and calculated on the basis of colorimetric assay to contain 70% ergotoxine, was successfully converted to D-lysergic acid in yields of 12.8% to 16.6%, based on cristamine. A second crop of 1 to 2 gm of impure D-lysergic acid was also obtained. The claimed yield for this material was 18% to 20% of the cristamine weight, and the workers at Eli Lilly and Co. stated that this represented a yield of 94% to 99% of the lysergic acid available in the cristamine.[83] On this basis, it was anticipated that the conversion of the saprophytically produced alkaloids, estimated to be 85% to 90% simple lysergic acid derivatives, could be performed using the same procedure. To date, a similar recovery and conversion of the fermentation-produced alkaloids has not been realized.

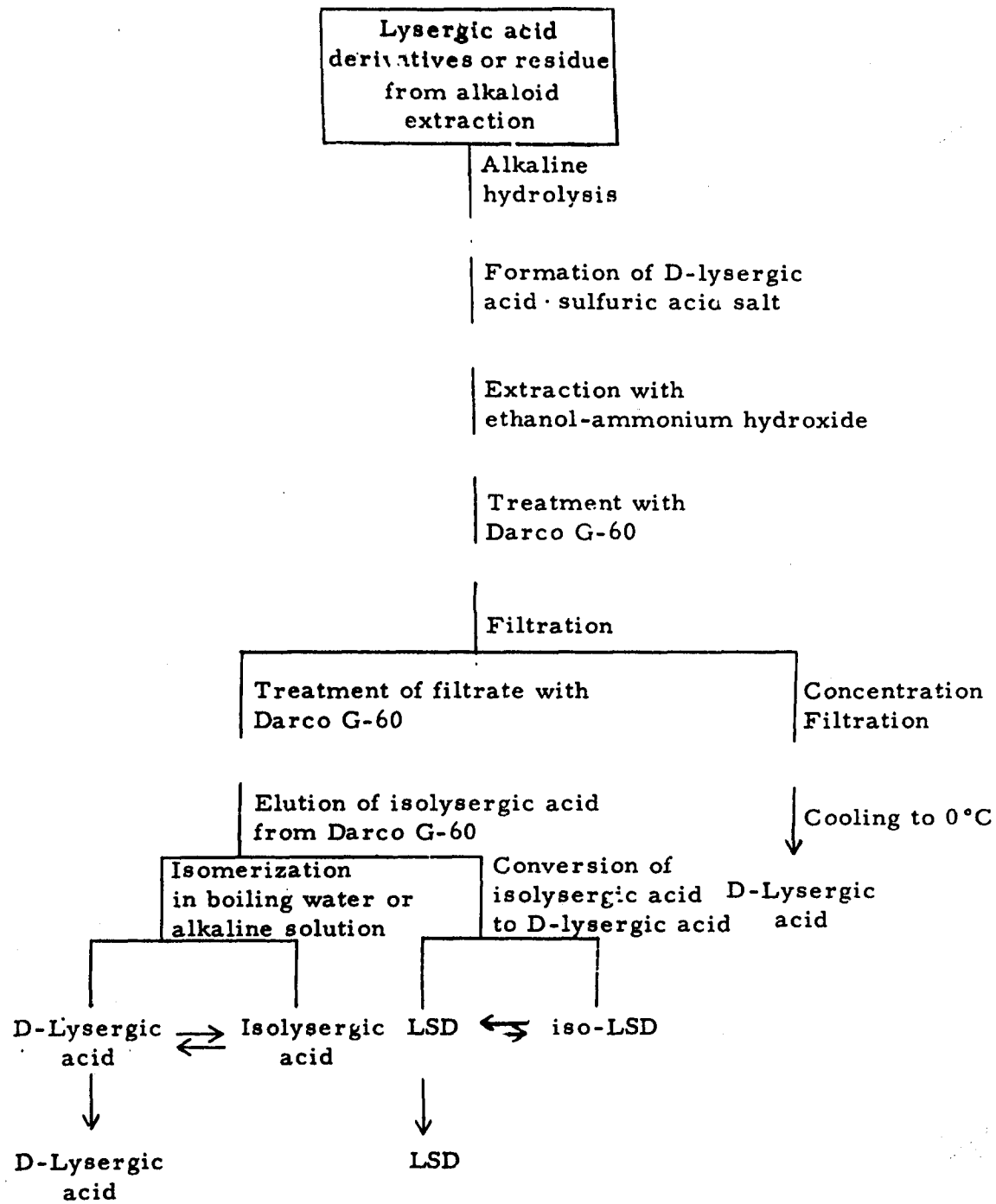
(U) The flow diagram below (I) indicates various routes from the fermentation broth containing the alkaloids to the desired D-lysergic acid. At the completion of the fermentation, the fermentation broth is separated from the mycelial growth, concentrated in volume, and filtered. The adsorption (B) of the alkaloids on solids such as charcoal, bentonite, and celite has been suggested, but little information is available on the use of solid adsorption for the ergot alkaloids. The alkaloids would have to be eluted from the solid, and either concentrated to solid residue or precipitated from the eluate. Researchers at Eli Lilly and Co. have stated that adsorption on solids would only be used if liquid extraction failed. The recovered alkaloids would then be converted to lysergic acid using the method outlined in the flow diagram (II). The extraction (A) of the alkaloids from the alkaline fermentation broth using immiscible solvents, such as chloroform, benzene, ethylene chloride, butanol, etc., has been reported by Chain and his associates[31] and has been used at the University of Connecticut. Since information was available on this approach, the extraction of the broth with butanol, isobutyl alcohol, a chloroform-butanol mixture, and a chloroform-isobutyl alcohol mixture was studied. All of these systems appear to give good recovery of the alkaloids on the basis of the colorimetric

assay of the extracted fermentation broth. The butanol and isobutyl alcohol extracts required centrifugation to separate the phases, and appeared to remove considerable amounts of nonalkaloid material from the broth, which may complicate the conversion to D-lysergic acid. On the basis of TLC of the extracts, the composition of the alkaloids extracted by all four systems was identical.

(U) An examination of flow diagram (I) shows that the shortest route to D-lysergic acid is represented by (D) and (H). Therefore, this route, with the concentration of the extraction solutions to a black semisolid residue and the conversion of this material to the D-lysergic acid, has been studied in the laboratory. To date, only a 25% yield of D-lysergic acid on the basis of total alkaloids in the broth has been obtained by this method. If the total alkaloids are 85% to 90% lysergic acid derivatives (D-lysergic acid amide, D-lysergic acid α-hydroxyethylamide, ergonovine, and the iso forms of these amides), as claimed by Kelleher,[35] this is a poor recovery. To obtain good recovery of the acid, it may be necessary to purify the lysergic acid derivatives by isolation as salts (G) or as crystalline lysergic acid derivatives. Also, concentration of the extracts to a smaller volume (C) and isolation (E) of the derivatives or salts of the derivatives or partition (F) of the derivatives between water and other immiscible solvents offer other approaches to recovery of the lysergic acid. The routes (A) to (D) to (B), (A) to (C) to (E), and (A) to (C) to (F) have been successfully used to obtain solid lysergic acid derivatives, but these routes are complicated and little information is available on the yields obtained. With the exception of route (A) to (C) to (F), they have not been studied in the laboratory. The use of Versene during the partitions (F) of lysergic acid derivatives between water and chloroform was recommended in the paper by Arcamone et al.[31] to stabilize the lysergic acid derivatives against light exposure. The transformation on light exposure of the lysergic acid derivatives to an intractable dark-brown resin occurred much more rapidly if Versene was omitted, which indicated that the reaction was catalyzed by traces of metal. This comment is of interest in relation to the claim by Hellberg that lumilysergic acid formation is dependent on the presence of suitable, strongly polar constituents in sufficient quantities.



I. Extraction and Conversion of Fermentation-Produced Alkaloids



II. Conversion of Alkaloids to D-Lysergic Acid

(U) Using the partition method (A) to (C) to (F) [flow diagram (I)], reported by Arcamone, a 66% recovery of the alkaloids (70% reported by Arcamone) was obtained. This material has been converted to D-lysergic acid in a 31% yield, based on the total alkaloids estimated to be present in the fermentation broth, and is similar to that obtained from the semisolid extraction residue. The colorimetric assay of the fermentation broth represents the clavine-type alkaloids as well as the lysergic acid derivatives. Some of the clavine alkaloids (elymoclavine, etc.) give a green color (useful for identification) with the van Urk test, but adsorb at 590 mμ, the wavelength used for the van Urk assay. Since no quantitative information on the composition of the alkaloids produced in these fermentations was available, the mixture was assumed to be 50% D-lysergic acid amide and 50% D-lysergic acid α-hydroxyethylamide for the purpose of determining percent recovery. The standard curve used in the van Urk colorimetric assay was prepared with LSD acid maleate and the concentrations were expressed as LSD acid maleate. The most common standards for the van Urk assay are ergonovine maleate or ergotamine tartrate. The fermentation-produced alkaloids, if expressed as ergotamine tartrate (molecular weight, 1,313.46), would be almost 1-1/2 times the weight of the same alkaloids expressed as ergonovine maleate (molecular weight, 441.47). Also, the color developed in the van Urk test is affected by organic solvents such as DMF, and to obtain accurate results, a standard curve incorporating the solvents present must be prepared. While the extraction and conversion study was in progress, Schwarting reported that the main fermentation product, D-lysergic acid α-hydroxyethylamide, obtained from Farmitalia, contained 40% D-isolysergic acid α-hydroxyethylamide, 30% lysergic acid amide, and 10% isolysergic acid amide, and contained only an estimated 20% of the D-lysergic acid α-hydroxyethylamide. If large amounts of the iso form are present in the fermentation-produced alkaloids, they may be complicating the D-lysergic acid isolation. These estimates were made from various thin-layer systems and must not be considered as quantitative. In the Eli Lilly and Co. procedure for converting ergot alkaloids to D-lysergic acid [flow diagram (II)], the recovery of the acid is dependent on removal of the isolysergic acid by Darco G-60. In this method, 25 gm of Darco G-60 is used to remove the isolysergic acid from a 50-gm sample of cristamine said to contain 70% ergotoxine, which averages 47% lysergic acid. Thus, a theoretical 16.5 gm of lysergic acid (iso or normal) is treated with 25 gm of Darco G-60 and produces a purported yield of 12 gm of first-crop D-lysergic acid and 1 to 2 gm of second-crop D-lysergic acid contaminated with its iso form (a 79% to 85% recovery of available D-lysergic acid). It requires a large amount of Darco G-60 (25 gm) to remove a small amount of isolysergic acid (2.5 to 3.5 gm). Thus, if the fermentation-produced alkaloids contain large amounts of the isolysergic acid derivative, it may be necessary to use larger amounts

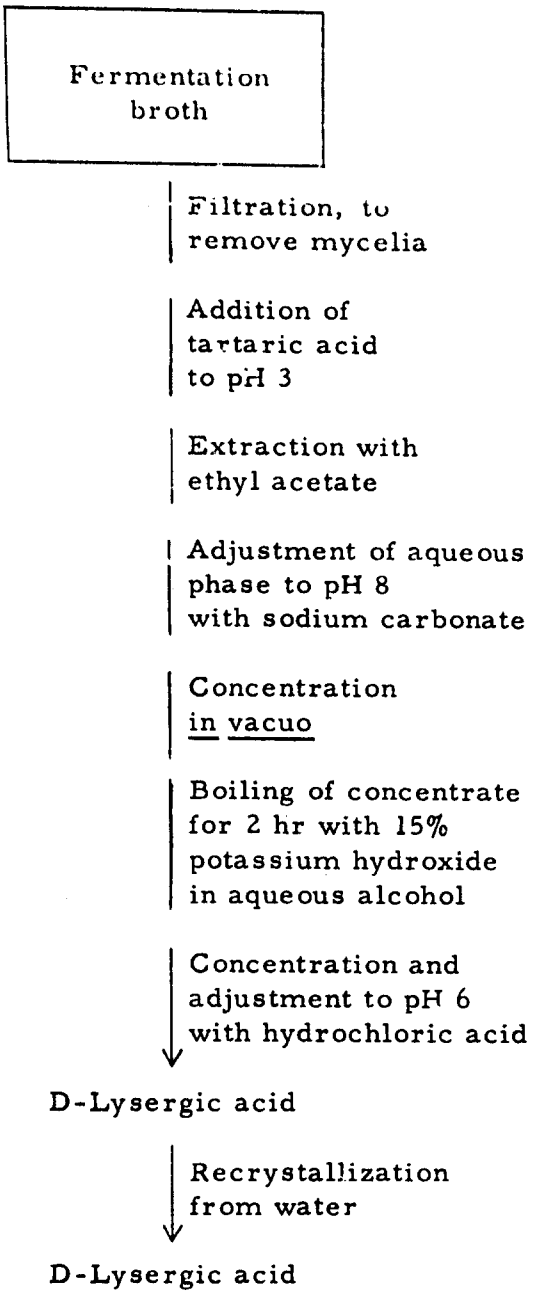
of Darco G-60 to obtain the available D-n-lysergic acid in good yield. To obtain a good yield based on total alkaloids produced, it would be necessary to isolate and recover the isolysergic acid from the Darco G-60 and convert it to a useful product. Isolysergic acid can be converted in alkaline solution or in boiling water to n-lysergic acid, but the isomerization equilibrium is estimated to be 43% normal and 57% isolysergic acid (table 2). In flow diagram (II), the conversion of isolysergic acid to either n- or iso-LSD is shown. If n- or iso-LSD can be synthesized in good yield from isolysergic acid, the recovery of isolysergic acid would be simplified because the isomerization equilibrium for LSD is 88% n-LSD and 12% iso-LSD.

(U) Garbrecht and Kornfeld, of Eli Lilly and Co., however, have questioned the presence of isolysergic acid under the conditions used for hydrolysis of the amides. They feel that, under the alkaline hydrolysis conditions used for the conversion of the ergot alkaloid cristamine, the isomerization equilibrium is strongly shifted towards the n-lysergic acid, and that very little isolysergic acid is present. They have been unable to recover isolysergic acid from hydrolysis mixtures and were unable to obtain it from the Darco G-60 used in their process. They have stated that the claim that Darco G-60 removes isolysergic acid in the Lilly process is not correct. Darco G-60 may remove traces of isolysergic acid, but its importance is in the removal of other impurities that would complicate recovery of the acid. Garbrecht also stated that lysergic acid amides could be prepared from isolysergic acid. Kornfeld has suggested a study of the hydrolysis conditions for fermentation-produced alkaloids; since they are simpler amides than the peptide alkaloids, less drastic hydrolysis conditions could be used. Kornfeld states that L-lysergic acid has been found in lysergic acid obtained from Farmitalia, and feels that the L-lysergic acid could arise from too drastic hydrolysis conditions.

(U) The isomerization equilibrium of D-lysergic acid as a function of pH will be studied. Also, the study of the extraction and conversion of the fermentation-produced alkaloids to lysergic acid will be continued.

(U) Farmitalia personnel had indicated to Hoffmann, of these Laboratories, that initially they had encountered difficulties in obtaining D-lysergic acid from the fermentation-produced alkaloids, but that the problems had been solved.

(U) In the abstract of the Belgium Patent No. 629, 158, the production of lysergic acid derivatives by strains of Aspergillus was reported, and a rather simple method of isolation and conversion of the alkaloids to lysergic acid was given. This method, outlined in flow diagram (III), is being investigated to see whether it can be applied to the alkaloids from a Claviceps paspali fermentation.



III. Isolation and Conversion of Aspergillus-Produced Alkaloids

(U) Certain aspects of the chemistry of LSD were investigated to obtain information on some of the known reactions of LSD, and on reactions where little or no data were available. The plan was to isolate and identify the reaction products, if possible, or to establish TLC systems that could be used to identify the reactions taking place. If the investigated reactions could be characterized, the reactions that lead to loss of LSD during synthesis, processing, and purification might be identified and minimized.

(U) Probably the most important reaction of LSD and all lysergic acid derivatives is the formation of the lumilysergic acid derivative. In an acidic aqueous solution, at least 85% LSD was reported to be converted to the lumi derivative in 6 hr when illuminated with an analytical quartz lamp; in direct sunlight, at least 80% of an acidic aqueous ergotamine solution had reacted in 30 hr. In the laboratory, an acidic solution of 0.9 gm of LSD in a Vycor tube was irradiated with a 30-w General Electric germicidal lamp at a distance of 20 cm; 13.5 hr of irradiation were required to complete the conversion of LSD on the basis of TLC. A spot at R_f 0.26, which did not fluoresce but gave a positive van Urk test, was believed to be the lumi-LSD. In an attempt to prepare and isolate lumi-LSD, 400 mg of LSD maleate in an acidic solution in a Vycor flask was irradiated with a 100-w General Electric S-4 bulb. On the basis of TLC, this transformation required 6 hr. The spot at R_f 0.26 was present. Chromatography on alumina gave an oil that had an R_f 0.26 on TLC and an ultraviolet spectrum identical with that for lumi-LSD reported in the literature.[72] A pure crystalline product has not been obtained. Preliminary studies on the stability of LSD acid maleate in deionized water indicate that lumi-LSD was being formed, but the rate and extent of the reaction have not been determined. The formation of lumi-LSD by irradiation of solid samples of LSD has not been investigated. Work on the effects of ultraviolet irradiation on LSD is continuing.

(U) The formation of the naphthalene isomer of LSD has also been studied. A sample of LSD maleate in glacial acetic acid saturated with hydrogen chloride was maintained at 50°C, and, after 52 hr, LSD could not be detected on thin-layer chromatograms. Because this was a rather drastic treatment not normally encountered, no other studies of this reaction have been attempted.

(U) The reported isomerization equilibrium for LSD (88% n-LSD, 12% iso-LSD) was experimentally confirmed and indicates that this is not a serious stability problem for LSD.

(U) The thermal stability of the LSD maleate, as a solid and in solution, has been investigated. Solid samples of LSD acid maleate held at 225 °C for 0.5 hr were 84% decomposed. The LSD acid maleate in boiling water and in a solution of pH 1 (hydrochloric acid) heated to boiling produced, after 20 hr of heating, 92.4% and 95% of the intact LSD, respectively. A DMF solution of LSD acid maleate heated to 153 °C caused decomposition of the LSD in 15 min, but at 75 °C the material was stable over a 2-hr heating period. Research is continuing on the problem of thermal stability of LSD and its salts.

(U) The preliminary studies of the reaction of LSD with bromine, bleach, and oxygen indicated that LSD is very sensitive to bromine and bleach, because both caused the complete disappearance of LSD in several minutes. LSD appeared to be stable to oxygen at room temperature over a 20-hr period on the basis of the van Urk test and TLC. Ultraviolet spectra run on samples taken during the oxidation study were not identical to untreated LSD, but showed an increase in the ϵ_{min} at 268 mμ, which has not been explained.

(U) Solubility studies of LSD, the acid maleate, and the tartrate salts in methanol, ethanol, water, DMF, acetonitrile, and chloroform were made. LSD was very soluble in the organic solvents, but insoluble in water (less than 4.4×10^{-6} gm/100 ml at 25 °C, 12×10^{-6} gm/100 ml at 75 °C). The solubilities of the tartrate and maleate salts in organic solvents were similar, but the tartrate salt was more soluble in water (23.8 gm/100 ml) than the acid maleate salt (0.6 gm/100 ml).

(U) Throughout this investigation, the use of TLC as a method of quality control, to follow the course of reactions, to study extractions and the composition of the extracts, and as a method of qualitatively identifying types of decomposition has been invaluable. The R_f values obtained on TLC are not so reproducible as those obtained on paper chromatography, but this can be corrected by the use of reference dyes or by running known LSD or LSD derivatives along with the sample to be investigated.

IV. (C) CONCLUSIONS.

(C) The following conclusions were reached:

1. (C) LSD acid maleate can be prepared in overall yields of 75% from D-lysergic acid.

2. (C) An inexpensive and readily available source for D-lysergic acid is still not available.

3. (C) LSD with a higher melting point (160° to 162°C) than previously reported can be prepared by crystallizing it from ether.

4. (U) The tartrate and acid maleate salts of LSD have similar solubilities in organic solvents, but the tartrate salt is more water-soluble. LSD is insoluble in water but very soluble in most organic solvents.

5. (U) The isomerization equilibrium between n- and iso-LSD is not a serious stability problem, since the normal form predominates (88% normal to 12% iso).

6. (U) The addition of water to the 9, 10- double bond of LSD under ultraviolet irradiation is one of the most serious stability problems.

7. (U) LSD reacts rapidly with bromine or bleach.

8. (U) LSD acid maleate in aqueous solutions was 90% intact after 20 hr of reflux.

9. (U) Thin-layer chromatography is a powerful tool for the study of the synthesis and chemistry of LSD.

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13. ABSTRACT

(U) The synthesis of D-lysergic acid diethylamide (LSD) from natural D-lysergic acid reported by investigators in 1943, and the accidental isolation of a small amount of this material reported in 1947, led to the discovery of the unusual capacity of this drug to cause abnormal psychic phenomena in man. The document describes the production of LSD and its derivatives, methods of synthesis of LSD and other lysergic acid amides, the chemistry of LSD and related compounds, stability and analysis and its derivatives of LSD 107 literature citations are listed.

14. KEYWORDS

Reaction	Stability	LSD
Derivatives	Isolation	Amides
Configuration	Oxidation	Synthesis
Isomerization	Monohydrates	Properties
Decarboxylation	Diethylamide	Chemical agent
Clavine alkaloids	Chromatography	Lumilysergic acid
Physical constants	Lactam formation	D-isolysergic acid
Ultraviolet spectrum	Ergonovine maleate	

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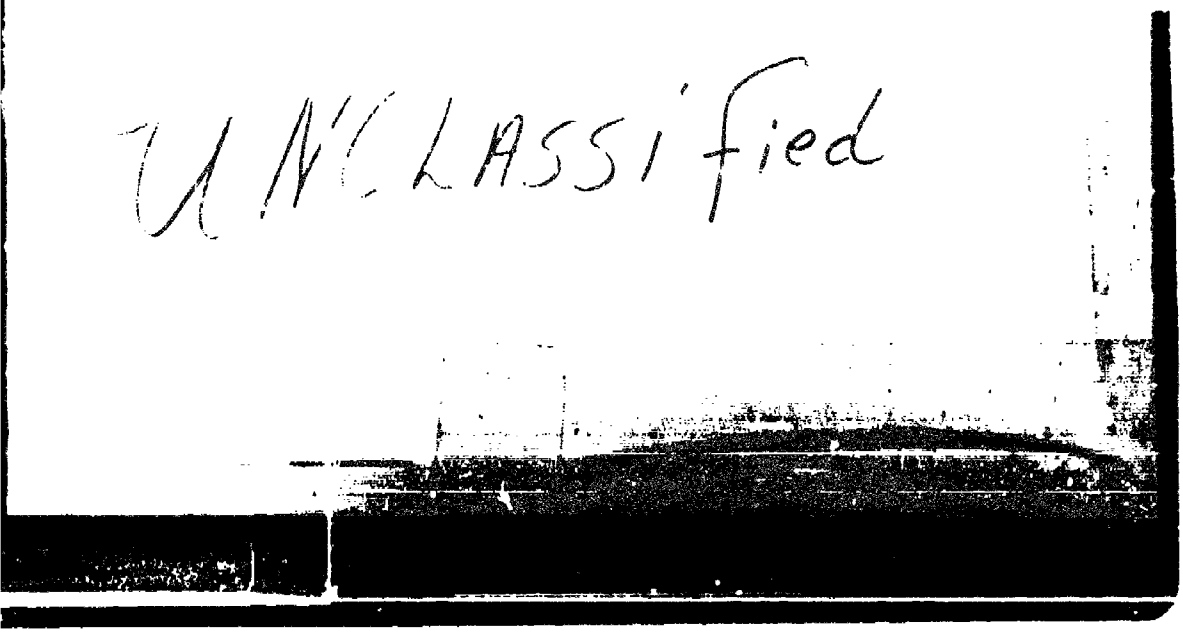

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US ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND
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REPLY TO
ATTENTION OF:

APR 14 2015

RDCB-DPS-RS

MEMORANDUM THRU Director, Edgewood Chemical Biological Center (ECBC), (RDCB-D, Mr. Joseph L. Corriveau), 5183 Blackhawk Road, Aberdeen Proving Ground, MD 21010-5424

FOR Office of the Chief Counsel, US Army Research, Development and Engineering Command (RDECOM), (AMSRD-CCF/Ms. Kelly Knapp), 3071 Aberdeen Boulevard, Aberdeen Proving Ground, MD 21005-5424

SUBJECT: Operations Security/Freedom of Information Act (FOIA) Review Request

1. The purpose of this memorandum is to recommend the release of information in regard to request to RDECOM FOIA Requests FA-15-0072.

2. ECBC received the request from Ms. Kelly Knapp, the RDECOM FOIA Officer. The request originated from [redacted].

3. The following document was reviewed by Subject Matter Experts within ECBC:

a. Synthesis and Chemistry of Lysergic Acid Derivatives - Part 1; AD0354374; dated 20 Nov 1918, 11 pages.

4. ECBC has determined that the reviewed document is suitable for release, however, it must have the classification/distribution changed through the Defense Technical Information Center prior to any release.

5. The point of contact is Mr. Ronald L. Stafford, ECBC Security Manager, (410) 436-1999 or ronald.l.stafford.civ@mail.mil.

RONALD L. STAFFORD
Security Manager