[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES AND FROM THE NATIONAL INSTITUTE OF MENTAL HEALTH, NATIONAL INSTITUTES OF HEALTH]

Studies on the Chemical and Enzymatic Oxidation of Lysergic Acid Diethylamide[1]

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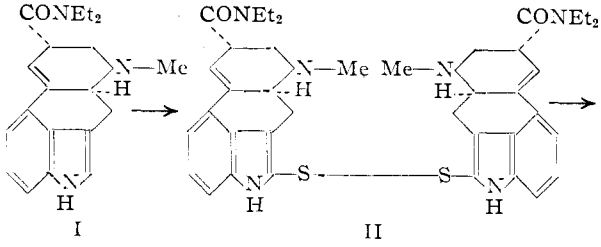
The reaction of the trichloroacetate of lysergic acid diethylamide (I) with disulfur dichloride in benzene yielded the symmetric disulfide II which on reductive acid hydrolysis and purification by multiple countercurrent distribution gave a non-crystalline compound, which was identical in all respects with a product obtained by enzymatic oxidation of I. On the basis of color reactions, alkaline opening to a diazotizable amine and ultraviolet absorption spectrum the oxindole structure III is suggested for this oxidation product.

Introduction

Whereas studies on the metabolic fate of the hallucinogenic (psychotomimetic) lysergic acid diethylamide LSD in intact animals[2-4] have been handicapped by the great potency of I, the formation of a physiologically inactive metabolite (presumably III) was observed in the oxidation by an enzyme present in liver microsomes.[5] This paper describes the formation of III from I by reductive hydrolysis of the disulfide II.



Experimental

Purification of the LSD-Metabolite Obtained by Enzymatic Oxidation.—The solution of the crude metabolite from 5 mg. of LSD[5] in 0.1 *N* hydrochloric acid was evaporated to dryness at 20° in a vacuum desiccator. The residue was taken up in as little methanol as possible and filtered. The concentrated solution was evenly distributed (micropipet) along the starting line of several sheets of Whatman No. 1 filter paper. After completion of the chromatographic process (ascending technique, mixture of 2,4-lutidine–*t*-amyl alcohol, 1:1, saturated with water) the sheets were dried. Stripes of the area corresponding to an R_F-value of 0.85 were cut out under an ultraviolet light and eluted by a descending solvent front of methanol. After evaporation of the solvent there remained about 3 mg. of colorless residue which defied crystallization. No crystalline salts could be obtained. The ultraviolet absorption spectrum showed a sharp peak at 259 mμ.

Conversion of Lysergic Acid Diethylamide (I) to the Disulfide II.—To a suspension of 300 mg. of LSD (I) in 100 ml. of anhydrous benzene was added 500 mg. of anhydrous trichloroacetic acid. A clear solution was obtained by gentle warming. To this solution which was cooled to the point of near-solidification was added within 15 minutes a solution of 69 mg. of disulfur dichloride (S_2Cl_2) in 10 ml. of benzene. After ten more minutes the strongly yellow reaction mixture was poured into 700 ml. of petroleum ether (30–40°). The trichloroacetate of the disulfide II which separated in yellow flakes was collected and washed thoroughly with petroleum ether and ether. The salt was then transferred into a separatory funnel, decomposed with *N* alkali and the free disulfide II exhaustively extracted into ethyl acetate. The orange-colored solution was washed, dried and concentrated. The disulfide was recrystallized for analysis from dioxane–water, m.p. 184°, yield 200 mg. (60% theor.), R_F 0.95 descending technique, 2,4-lutidine–amyl alcohol, 1:1, saturated with water, Fig. 1. The Ehr-

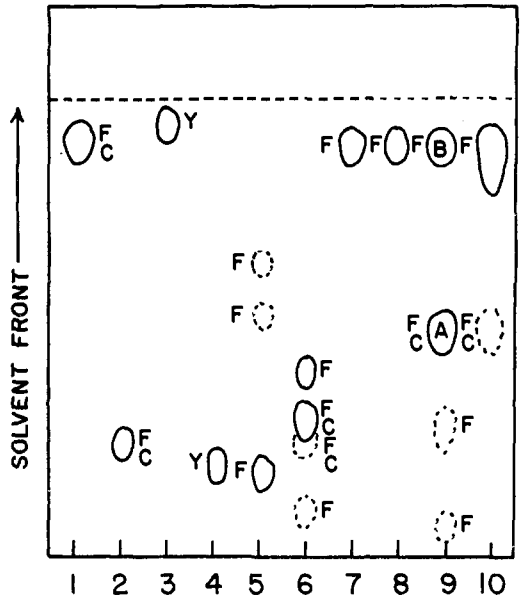


Fig. 1.—R_F-values and analytical determination of the various compounds; solvent system 2,4-lutidine–*t*-amyl alcohol (1:1, saturated with water): 1 = LSD (I); 2 = lysergic acid; 3 = disulfide (II) of LSD; 4 = disulfide of lysergic acid; 5 = hydrolysate of disulfide of lysergic acid; 6 = oxidation of lysergic acid; 7 = hydrolysate of II; 8 = enzymatic oxidation product of I; 9 = oxidation of LSD (I) by peracetic acid to a labile Ehrlich-positive compound A and a stable Ehrlich-negative compound B; 10 = stable Ehrlich-negative oxidation product B of I from Ehrlich-positive oxidation product A by the action of acid. Methods for characterization: F = fluorescence under ultraviolet light; C = color developing after spraying with cinnamaldehyde solution; Y = yellow original color.

(1) Oxidation Mechanisms. XVIII. Previous paper in this series, THIS JOURNAL, **78**, 2873 (1956).

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(4) V. Lanz, A. Cerletti and E. Rothlin, *Helv. Physiol. Pharmacol. Acta*, **13**, 207 (1955).

(5) J. Axelrod, R. O. Brady, B. Witkop and E. V. Evarts, *Nature*, **178**, 143 (1956); Symposium on Psychotomimetic and Psychotherapeutic Agents, Annals of the New York Academy of Sciences, in press.

lich reaction was negative, the Keller reaction (concentrated sulfuric acid containing a trace of ferric ion) gave a green color, as a result of the combination of the blue color, characteristic of β-substituted indoles, and of the yellow color of the disulfide II. Reduction with zinc dust in pyridine–glacial acetic acid according to Kuhn and Winterstein[6] gave a colorless solution which formed an insoluble red-brown mercaptide with lead acetate and on standing reoxidized to the yellow solution of the disulfide. The ultraviolet spectrum of II showed the expected displacement to longer wave length: $\lambda_{max}^{CH_3OH}$ 324 (log ε 3.54); lysergic acid tartrate, $\lambda_{max}^{CH_3OH}$ 312 (log ε 3.93).

Anal. Calcd. for $C_{40}H_{48}N_6O_2S_2 \cdot 2H_2O$: C, 64.53; H, 6.98; N, 11.31; S, 8.60. Found: C, 64.72; H, 6.79; N, 11.46; S, 8.83.

No weight loss was observed when the material was dried at 100° *in vacuo*.

Dr. A. Hofmann, Sandoz A. G., Basle, Switzerland, had the kindness to inform us that the same disulfide, m.p. 182°, $[\alpha]^{20}D$ −1020° (pyridine), analyzing for $C_{40}H_{48}N_6O_2S_2$ (recrystallized from dioxane–water, dried at 90° *in vacuo*) has been obtained in his laboratory. A direct comparison of our, with his, sample established the identity.

Hydrolysis of the Disulfide II.—Refluxing of II with 20% acetic acid after a short time led to the evolution of hydrogen sulfide which was trapped as lead sulfide. Although the rate of liberation of H_2S decreased markedly it was still noticeable after 6 hr. On a preparative scale, 200 mg. of disulfide II was dissolved in 30% acetic acid and equidistributed into 5 small glass tubes each of which contained 100 mg. of C.P. zinc dust. The tubes were sealed *in vacuo* and heated to 110° for 16 hr. The contents of the tubes was cooled, lyophilized and the residue taken up in a small volume of methanol, filtered, mixed with a fivefold volume of ether, and filtered again. The filtrate was extracted with dilute HCl, the acid solution made alkaline and extracted with ethyl acetate. After washing, drying and concentration, petroleum ether precipitated from the organic solution 30 mg. of a greyish-brown powder, whose color reactions and R_F-value were the same as those of the metabolite. However, the analysis by ultraviolet fluorescence spectrophotometry in a Bowman-Aminco spectrofluorophotometer[7] indicated the presence of impurities, among them residual disulfide. The ultraviolet spectrum of this crude hydrolysis product showed λ_{max} 320 mμ with a shoulder at 260 mμ. All attempts at further purification by crystallization, salt formation or fractional precipitation failed. The close R_F-values of the impurities in various solvent combinations (Fig. 1) prevented successful resolution by paper chromatography. The crude hydrolysate was then purified by countercurrent distribution in a 25 unit Craig machine utilizing as stationary lower phase 10 ml. of 0.2 *M* phosphate buffer, *p*H 7.4 and as moving upper phase heptane containing 7% of *t*-amyl alcohol (both solvent systems being mutually saturated). The crude hydrolysis product (30 mg.) showed the following distribution: tubes 1–5 contained a brown impurity which was not investigated; tubes 8–15 contained the colorless solution of III, λ_{max} 259 mμ; tubes 20–25 contained yellow solutions of disulfide. The solution in tubes 8–15 was the same as the product of enzymatic oxidation of I in the following respects:

Ultraviolet absorption spectrum: $\lambda_{max}^{CH_3OH}$ 259 mμ, identical with the enzymatic product.

Ultraviolet fluorescence spectrum: identical; λ_{max} of fluorescence 430 mμ with λ_{max} of the exciting beam at 335 mμ.

Distribution Coefficients.—The relative concentrations in aqueous solutions of varying *p*H before and after extraction with various organic solvents were determined by measurement of the per cent. transmission of the maximum of fluorescence at 430 mμ in a Bowman–Aminco spectrofluorophotometer,[7] adjusting the transmission to 100 at the beginning of extraction.

Biphasic systems		Relative concn. of metabolite III	
Inorganic	Organic	By hydrolysis of II	By enzymatic oxidation of I
Phosphate buffer *p*H 7.8	Heptane–5% isoamyl alcohol	70	69
Phosphate buffer *p*H 7.8	Ethylene dichloride	14	15
0.1 *N* hydrochloric acid	Ethyl acetate	42	39

R_F-**Values**: identical, 0.85, *cf.* Fig. 1.

Ehrlich Reaction: both negative.

Azotest.—Both compounds, after treatment with hot aqueous alcoholic alkali, but not before, showed a diazotizable amino group which coupled after diazotization with an alkaline solution of β-naphthol.

Reaction of Folin and Ciocalteu[8]: both positive.

Oxidation of LSD (I) with Peracetic Acid.—Five milligram samples of LSD (I) were oxidized with 1 ml. of a mixture of glacial acetic acid and 30% hydrogen peroxide ("Superoxol," C.P.) in a ratio of 5:1 at room temperature for ten minutes up to one hour. The reaction mixtures were freeze-dried and chromatographed using the usual solvent mixture. Two discrete spots were observed (Fig. 1). The compound A with R_F 0.5 was obtained on brief oxidation. It gave a positive Ehrlich reaction and had $\lambda\lambda_{max}$ 249 and 310 mμ. On standing in solution or, more rapidly on treatment with acid, rearrangement to an Ehrlich-negative compound B, R_F 0.85 occurred.

Similarly, the action of peracetic acid on lysergic acid produced two different compounds with R_F-values of 0.3 (Ehrlich-positive) and 0.4 (Ehrlich-negative, Fig. 1).

Lysergic Acid Disulfide.—The reaction of lysergic acid with disulfur dichloride in benzene in the presence of trichloroacetic acid was carried out as described for the preparation of II. The crude product separated during the reaction as a yellow microcrystalline powder, showing increasing decomposition above 150°, soluble in alcohol, slightly soluble in water, insoluble in ethyl acetate, acetone and benzene; the Ehrlich reaction was negative. The crude product contained 9.66% of sulfur, calculated 10.70%. A satisfactory process for recrystallization was not found. The hydrolysis of this disulfide under the same conditions as used for II gave several products (chromatogram, see Fig. 1) none of which was identical with either product from the oxidation of lysergic acid with peracetic acid.

Results and Discussion

In principle there can be visualized three simple pathways to effect the conversion of LSD (I) to the oxindole derivative III: (1) oxidation of I with peracetic acid[9], a process which is known to oxidize skatole (IV) to atroxindole (VII). The first intermediate is believed to be the β-hydroxyindolenine V[10] which in aqueous peracetic acid may add the elements of water to yield the glycol VI (R = H) which undergoes acid-catalyzed loss of water (*via* a benzylcarbonium ion) to the oxindole VII. With perbenzoic acid in chloroform the common intermediate V may add peracid to form VI (R = $OCOC_6H_5$) undergoing ring-fission with loss of benzoate anion to give VIII.[11] Two products are formed from I and peracetic acid; an Ehrlich-positive compound A rearranging on standing or by the action of acid to an Ehrlich-negative compound B. If the glycol analog of VI in the lysergic acid series were stable one would have to assume that any possible reaction with *p*-dimethylaminobenzaldehyde would be faster than rearrangement catalyzed by the acid present. No stable α-unsubstituted β-hydroxyindolenines are known and so one does not know whether such compounds may give a color with Ehrlich reagent. However, the scheme glycol (VI) → oxindole (VII) seemed unlikely since it was found that the acid-catalyzed rearrangement of the Ehrlich-positive

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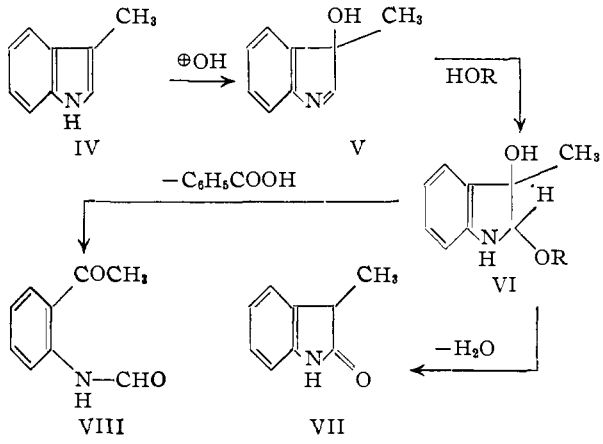

oxidation product of lysergic acid gave a product whose R_F-value was definitely different from the R_F of the presumable oxindole prepared by a more direct method. Such a distinction by paper chromatography was not possible for the analogous products from LSD.

(2) The attempted solvolysis of the commercially available bromination product of LSD, believed to be 2-bromo-LSD because of the negative Hopkins–Cole and Ehrlich reactions, was without success. Even heating with silver oxide for several hours failed to produce silver bromide.

(3) Finally, the reaction of LSD with disulfur dichloride (S_2Cl_2) was investigated. Pyrroles with free α and β-positions[12] and indoles with free α-position[13] are converted by this reagent to symmetrical disulfides. This procedure was applied to the trichloroacetate of LSD in order to prevent the basic tertiary nitrogen from reacting with the disulfur dichloride. The product obtained in this way had all the properties expected from a compound of the disulfide structure II. II is no longer physiologically active.[14] Whereas hydrolysis of the disulfide from tryptophan proceeds smoothly even with water alone, II had to be heated under fairly vigorous conditions with dilute acetic acid and zinc dust. Mineral acid led to resinification, and base could not be used without the danger of losing the amide group. The purification of the hydrolysis mixture by fractionation and countercurrent distribution finally yielded a pure compound which, though not obtained crystalline, was identical with regard to ultraviolet and fluorescence spectra, color reactions, distribution coefficients and R_F-values with the metabolite obtained by enzymatic oxidation of LSD. In addition to the negative Ehrlich and the positive Folin reactions the formation of a diazotizable amino group on treatment with base and the sharp absorption maximum at 259 mμ[15] support the oxindole structure III for this metabolite.

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(15) For a discussion of oxindole spectra, *cf.* R. Goutarel, M. M. Janot, V. Prelog, R. P. A. Sneeden and W. I. Taylor, *Helv. Chim. Acta*, **34**, 1145 (1951).

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[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

Quebrachamine. I

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Dehydrogenation of quebrachamine, $C_{19}H_{26}N_2$ (II), furnished a mixture of homologous indoles, a mixture of 3-methyl-5-ethyl- and 3,5-diethylpyridine, a mixture of homologous carbazoles and a propyl derivative of α- or β-carboline. Two major oxidation products, the hydroxy base $C_{19}H_{26}N_2O$, m.p. 188° (V), and an N(a)-acetyldihydroindole base, $C_{21}H_{28}N_2O_2$, m.p. 213° (IV), were obtained on oxidation of II with ozone or hydrogen peroxide in acetic acid. The hydroperoxide VI, formed by catalytic oxygenation of II, on sublimation or warming in polar solvents disproportionated to V and oxygen. V, on catalytic hydrogenation, yielded the hexahydrohydroxy base $C_{19}H_{32}N_2O$, m.p. 177° (IX), which on dehydrogenation with palladium gave a base, $C_{11}H_{17}N$, isolated as the picrate, which also has been obtained from aspidospermine.

Quebrachamine has been isolated from the bark of *Aspidosperma quebracho blanco*,[1] together with aspidospermine and yohimbine or quebrachine, from *Aspidosperma chakensis*, together with spegazzinine,[2] and from the bark of *Gonioma kamassi*.[3,4] It belongs to the small group of crystalline oxygen-free plant bases. It contains an indole system, possibly unsubstituted at the α-position (positive Hopkins–Cole and Ehrlich reactions, red picrate),[5,6] and, judging from its resistance to mild catalytic hydrogenation, no other unsaturation. In addition to the indolic non-basic nitrogen N(a), there is a basic (pK 6.76, Table II) nitrogen N(b) which must be tertiary. The structure of a N-(3-indolylethyl)-1,2,3,4-tetrahydroisoquinoline (I, ring junction *cis* or *trans*)[7] is ruled out for, at least, two reasons: (i) the Hofmann degradation of compounds of type I, *e.g.*, of *chano*desoxyyohimbol[8] gives a volatile base, optically active N-methyl-*trans*-decahydroisoquinoline, in good yield. (ii) Catalytic dehydrogenation of I would lead to isoquinoline. Quebrachamine methiodide gives no easily volatile base on attempted Hofmann degradation, and the dehydrogenation of the free alkaloid does not lead to an isoquinoline.

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