THE ERGOT ALKALOIDS

IX. THE STRUCTURE OF LYSERGIC ACID

By WALTER A. JACOBS and LYMAN C. CRAIG

(*From the Laboratories of The Rockefeller Institute for Medical Research, New York*)

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The fact that lysergic acid gives a number of reactions usually attributed to certain indole derivatives led us to assume from the start a possible biogenetic relationship of this ergot acid to tryptophane. And, since the alkaloids of the indole group (with the exception of eserine) such as harmine, yohimbine, and evodiamine have been shown to be closely related as 4-carboline derivatives, it was natural to assume as a working hypothesis that lysergic acid is also such a carboline derivative.[1] This view seemed to be supported by some of its properties and by the interpretation of several substances formed on alkali fusion of dihydrolysergic acid. The attempt was then made to verify this conclusion by synthesis. The synthesis of tetrahydrocarboline carbonic acids was readily realized from both tryptophane and methyltryptophane (abrine), as reported elsewhere,[2] but the synthetic substances differed in certain respects from lysergic acid or its dihydro derivative. Contrary to the latter, the carboline acids and other carboline derivatives, such as harman and yohimbine, do not give promptly the characteristic color reaction with dimethylaminobenzaldehyde and hydrochloric acid usually given by α- or β-unsubstituted indoles. This fact and other observations which were accumulating have made it necessary to look less favorably on the carboline structure.

There is no question that the indole ring system is contained in the molecule. The ultra-violet absorption spectra curves of dihydrolysergic acid and dihydrolysergol have been recently shown

[1] Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, **111**, 455 (1935).

[2] Jacobs, W. A., and Craig, L. C., *Science*, **82**, 421 (1935); *J. Biol. Chem.*, **113**, 759 (1936).

by us[3] to be practically superimposable on those of α-β-dimethylindole, of one of the synthetic carboline acids, and of yohimbine, all of which possess the indole nucleus in common. Also, from among the products of the alkali fusion of dihydrolysergic acid a substance was obtained, the picrate of which gave figures suggesting a formula $C_{11}H_{13}N$ for the substance. In our previous communication,[1] we believed that this substance could be a methylethylindole. However, its preparation from dihydrolysergic acid has been recently repeated and has been found to be different from synthetic α-methyl-β-ethylindole, since the picrates gave a definite melting point depression when mixed. Further, it gave a strong test with dimethylaminobenzaldehyde, indicating, contrary to our first assumption, that either the α or the β position is unsubstituted. β-*n*-Propylindole has also been excluded by comparison with a substance which we prepared synthetically. The identity of this substance, which is unquestionably a simple indole derivative, is still a subject of inquiry. The very small yield which can be obtained makes this difficult.

Further significant information has been recently obtained by a study of the behavior of lysergic acid on catalytic hydrogenation. This reaction has been found to be somewhat involved. It appears that several reactions occur simultaneously. On shaking in acetic acid solution with Adams and Shriner's catalyst in an atmosphere of hydrogen, the solution rapidly develops a strong blue-violet fluorescence which persists until the absorption has slowed up and has almost reached the 2 mole stage. The nature of the material causing this fluorescence has not been determined. The only crystalline substance which could be isolated was dihydrolysergic acid in a yield of about 50 per cent. This appeared to be identical in all respects with the dihydrolysergic acid which has already been described as a product of the reduction of lysergic acid with sodium and amyl alcohol.[4] This identity was confirmed by comparison of the methyl esters from both sources. When the attempt was made to hydrogenate further, with dihydrolysergic acid itself, absorption was very slow and ultimately the characteristic indole reaction disappeared. No characteristic product of the reaction could be isolated from the reaction mixture.

[3] Jacobs, W. A., Craig, L. C., and Rothen, A., *Science*, **83**, 166 (1936).
[4] Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, **106**, 398 (1934).

Similarly, in an experiment where dihydrolysergic acid was reduced with palladium black and hydrogen in acetic acid solution, a second crystalline substance was isolated from the mother liquor of unchanged dihydrolysergic acid in very small yield, which gave practically no dimethylaminobenzaldehyde reaction and was apparently the product of further hydrogenation in the indole nucleus. Thus, no evidence could be obtained for the presence of a double bond other than those contained in the indole nucleus and the easily reduced double bond of lysergic acid. This behavior therefore strongly indicates a tetracyclic structure for the latter.

This conclusion fits well with the interpretation of the nature of two substances which we have already reported as products of the degradation of lysergic acid. The base $C_{11}H_{11}N$[1] previously obtained as a product of the alkali fusion of dihydrolysergic acid has now been definitely identified as 1-methyl-5-aminonaphthalene.[5] Its diazonium salt couples to give azo dyes, and likewise it acts as a coupler. The odor and physical properties as well as the comparison of its benzoyl derivative and picrate with the derivatives of the synthetic base confirmed this identity. The further oxidation of the methyl group to carboxyl with formation of an aminonaphthoic acid was apparently prevented by the rapid distillation of the base out of the melt before such secondary changes could occur. This fact strongly indicates the primary nature of this base. Although it is not excluded that opening of a ring and closing in another way, as has been noted on occasion with complex substances, is not excluded, the fact that this base is a naphthylamine makes this possibility appear remote. We are inclined to accept the formation of this substance as good evidence for the presence of two fused 6-membered rings in lysergic acid, which emerge under the conditions of alkali fusion as a naphthalene derivative. Since methylamine has been almost quantitatively collected from the gases evolved during the production of this substance and since there was no suspicion of contamination of the methylnaphthylamine with an N-methyl derivative, the source of the amino group must obviously be sought in the cyclic indole nitrogen which does not carry the methyl group. The formation of an α-naphthylamine derivative thus restricts the

[5] Veselý, V., Stursa, F., Olejnicek, H., and Rein, E., *Collect. Czechsl. Chem. Communicat.*, **1**, 506 (1929).

position which can be assigned to the fused pyrrole ring in relationship to the two rings liberated as the naphthalene nucleus.

The second substance, the nature of which we believe we have been able to interpret correctly, is the product of the nitric acid oxidation of ergotinine (lysergic acid) for which the formula $C_{14}H_9O_8N$[6] was derived. Titration of this substance had shown it to be tribasic and it was found to contain an N-methyl group. The remaining 2 oxygen atoms had not been determined. More recently, on distillation with soda-lime, this acid has been found to yield an oil, the properties of which immediately suggested quinoline. This was at once verified by its identification as the picrate. It is therefore very probable that the acid, $C_{14}H_9O_8N$, is an *N-methylquinolinebetaine tricarboxylic acid*. The formation of a quinoline acid from that portion of the lysergic acid molecule carrying the N-methyl group by direct nitric acid oxidation strongly indicates that such a ring system preexists in lysergic acid. Although quinoline derivatives are sometimes formed by rearrangement of substituted indoles, this appears scarcely likely under the conditions of the nitric acid oxidation. This would also be incompatible with the fact that the NCH_3 group is the strongly basic group of the molecule. The ring containing this basic group must therefore be the fourth ring of the molecule.

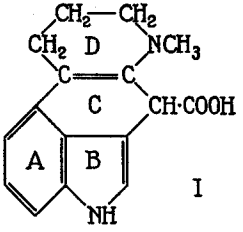
A tetracyclic ring system, although without precedent, may be constructed to satisfy these more recent observations, as given in Formula I.

In our recent discussion[3] of the ultra-violet absorption spectrum of lysergic acid as compared with that of its hydrogenated derivative, dihydrolysergic acid, it was pointed out that the displacement of the bands and maxima definitely indicates conjugation of this double bond with one of those of the indole ring system. Finally, the recent comparison of the behavior of lysergic acid and dihydrolysergic acid on titration against phenolphthalein has shown that whereas the former consumes almost 1 equivalent of alkali,[7] the latter behaves like a saturated substituted amino acid and cannot be titrated, thus indicating the influence of the double bond in lysergic acid on the carboxyl group or the cyclic NCH_3 group. It appears, therefore, that the latter must be contained in a substi-

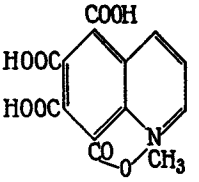
[6] Jacobs, W. A., *J. Biol. Chem.*, **97**, 739 (1932).
[7] Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, **104**, 550 (1934).

tuted hydroquinoline ring system. Although further data will have to be obtained to fix conclusively the exact position of the extra double bond of lysergic acid and also that of the carboxyl group in Ring C or D, we believe that the positions assigned in Formula I can account for the observations which we have thus far made.

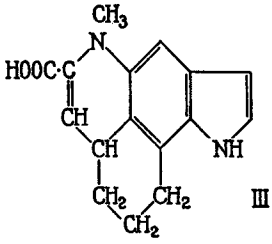
Thus, such a formula could explain the loss of CO_2 and methylamine on pyrolysis of lysergic acid (at 210–230°), whereas the dihydro acid is far more stable. The formation of 1-methyl-5-aminonaphthalene is also apparent from the cleavage of Ring B,



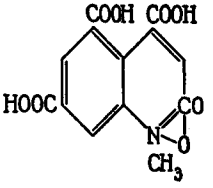
I



II



III



IV

giving the amino group, and Ring D, giving the methyl group. Finally, the formation of a methylquinolinebetaine tricarbonic acid, as given in Formula II, by oxidative cleavage of Rings A and B is a distinct possibility. (Its exact formula, however, is dependent upon the real position of the carboxyl group in lysergic acid.) And, conversely, the formation of such a quinoline derivative indicates that the hydroquinoline nucleus as it occurs in lysergic acid must be substituted in three positions (other than that occupied by the original carboxyl group of lysergic acid) and therefore requires such a condensed ring system as given, because of the restrictions imposed by the empirical formula.

There is an alternative structure, as given in Formula III, which also fits with the assumption of the primary nature of 1-methyl-5-aminonaphthalene and of a quinolinebetaine tricarboxylic acid (Formula IV). Such a substance could also give an indole derivative, $C_{11}H_{13}N$, unsubstituted in the α and β positions. However, our experience in the attempt to degrade lysergic acid by exhaustive methylation has given results which appear incompatible with such a structure. Although the substances encountered were mostly amorphous, their character and behavior were such as to justify conclusions.

β-Dihydrolysergol[8] yields a crystalline *quaternary salt*, $C_{16}H_{20}ON_2 \cdot CH_3I$. When this salt was decomposed with silver oxide, a base was obtained which on distillation gave an oily distillate which was formed apparently by loss of water and ring cleavage. No crystalline derivative was obtained, but the analysis was in agreement with the required formula of a des-base, *viz.* $C_{17}H_{22}ON_2$. On further methylation the latter gave an addition product (not crystalline) which when treated with silver oxide and then distilled gave trimethylamine. Unfortunately, the remainder of the molecule was changed to a hopeless resin. However, the formation of trimethylamine after such a series of steps is in conformity with the exhaustive methylation at that point in the molecule originally consisting of the cyclic NCH_3 group and not involving the indole nitrogen. Exhaustive methylation with ultimate cleavage of trimethylamine would not be expected from the structure given in Formula III in which the NCH_3 group is directly attached to a benzenoid ring. Such a substance would behave rather like a tetrahydroquinoline derivative in which rupture between the nitrogen atom and the aromatic nucleus does not usually occur. The observed behavior is consistent with the requirements of Formula I.

A final point which we have attempted to determine is whether lysergic acid occurs conjugated as such in the alkaloid molecule or is formed from a precursor on alkaline hydrolysis. The formula of the new alkaloid from ergot (ergobasine, ergometrine, etc.) is now established with certainty as $C_{19}H_{23}O_2N_3$.[9] In agreement

[8] Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, **108**, 601 (1935).

[9] Stoll, A., and Burckhardt, E., *Compt. rend. Acad.*, **200**, 1680 (1935). Jacobs, W. A., and Craig, L. C., *Science*, **82**, 16 (1935). Dudley, H. W., *J. Am. Chem. Soc.*, **57**, 2009 (1935).

with this formula is the fact established by us that it is the hydroxyisopropylamide of lysergic acid (or isomer).[9] We have therefore studied the hydrogenation of this alkaloid and its subsequent hydrolysis in order to determine whether the resulting dihydro acid would be identical or different from that obtained on direct hydrogenation of lysergic acid itself. The hydrogenation of the alkaloid proceeded as in the case of lysergic acid and became very slow after 2 moles were absorbed. However, the only crystalline substance which could be isolated was a *dihydro alkaloid*, $C_{19}H_{25}O_2N_3$. This alkaloid on hydrolysis gave a dihydrolysergic acid which proved to be identical in all respects with that obtained from lysergic acid. Although this result does not eliminate the possibility that a shift of the double bond may occur in the transition of these alkaloids to lysergic acid on hydrolysis with alkali, any deep seated rearrangements which involve obscure ring changes appear to be definitely excluded.

EXPERIMENTAL

1-Methyl-5-Aminonaphthalene—400 mg. of dihydrolysergic acid were fused with 2 gm. of potassium hydroxide as previously described,[1] and the heating was continued at 300° for 40 minutes. During this time a small amount of red-colored oil slowly distilled over into the condenser. The condenser was cut from the fusion chamber and extracted with about 1 cc. of ether. The ether extract was shaken with 1 cc. of 10 per cent hydrochloric acid and washed with fresh acid. A crystalline insoluble hydrochloride appeared in the acid layer.

Without filtering, the acid layer containing the crystals was made alkaline with potassium hydroxide and extracted with ether. The ether layer gave 12 mg. of a partly crystalline residue on evaporation. This was fractionated under 0.3 mm. pressure. 10 mg. of an oil which crystallized almost entirely on the condenser distilled up to an oil bath temperature of 160°. This proved to be somewhat impure methylaminonaphthalene. The yield was 4.7 per cent of the theoretical. For recrystallization, the base was dissolved in petroleum ether, concentrated to small volume, and the mixture was chilled. 2.8 mg. of colorless leaves were collected. It began to melt at 65° and was completely melted at 67°. Upon recrystallization from petroleum ether, the melting point was raised to 71.2–72°. A further recrystallization from petroleum

ether gave finally 0.7 mg. of colorless leaves which melted at 71.5–72.8°.

Synthetic 1-methyl-5-aminonaphthalene was prepared according to the direction of Veselý, Stursa, Olejnicek, and Rein.[5] This material melted at 74–74.5° (Veselý *et al.* reported 77–78°). Our substance had the same odor (almost identical with that of α-naphthylamine) and appeared identical in all respects with the synthetic amine. The mixed melting point was 71.5–74.5°. After diazotization, both bases coupled with β-naphthol to give identical colors and, conversely, gave indistinguishable dyes with diazobenzenesulfonic acid.

From the mother liquors of the last two recrystallizations of the base, 2 mg. of base were recovered. This was treated with 2.3 mg. of picric acid in a few drops of ethyl alcohol. Upon cooling, 3 mg. of a yellow picrate were obtained, which began to darken at 200° and melted with decomposition at 208–210°, depending somewhat on the rate of heating. The synthetic picrate was identical in crystalline form and decomposed at 210°. A mixture of the two substances showed no depression. The analysis of this picrate from a previous fusion has been reported.[1] We give these figures again as follows:

$C_{17}H_{14}O_7N_4$.	Calculated.	C 52.84, H 3.65, N 14.50
	Found. (*a*)	" 53.17, " 3.49, " 14.65
	(*b*)	" 53.30, " 3.55

6 mg. of crude base obtained from another fusion were treated with 4 cc. of 10 per cent sodium hydroxide solution followed by 15 mg. of benzoyl chloride. The mixture was thoroughly shaken and finally warmed until the odor of benzoyl chloride disappeared. The benzoylated product was extracted with ether and the extract was dried with potassium carbonate. The ether solution on concentration to a small volume deposited crystals which were collected with ether. It melted at 163–165°. After recrystallization from ether, the substance melted at 165–167°. A further recrystallization from ethyl alcohol raised the melting point to 168–170°. The mother liquors from the second and third recrystallizations were combined, evaporated to dryness, and recrystallized from ether. This material was used for analysis.

$C_{18}H_{15}ON$. Calculated, C 82.73, H 5.79; found, C 82.62, H 5.54

A benzoyl derivative prepared in exactly the same way from synthetic 1-methyl-5-aminonaphthalene melted at 170–172° (Veselý *et al.* reported 173–174°). The mixture of the substances from both sources melted at 168–170°, and in all other respects the properties of the synthetic substance and that obtained from dihydrolysergic acid were indistinguishable.

Soda-Lime Distillation of the Tribasic Acid, $C_{14}H_9O_8N$—0.15 gm. of the recrystallized acid, $C_{14}H_9O_8N$, obtained by oxidation of ergotinine with nitric acid was ground in a mortar with 0.8 gm. of soda-lime and the mixture was placed in a small apparatus similar to that used in the potassium hydroxide fusion.[1] A current of hydrogen was passed through the apparatus during the reaction. The material was slowly heated with a free flame until decomposition occurred with the distillation of an oil. Evaporation of the hydrochloric acid in the last trap gave no residue which showed the absence of methylamine. The brown-colored distillate in the first trap was washed out with a few drops of ether. The ether extract was dried over potassium carbonate and fractionated. 8 mg. of a colorless oil were collected up to an oil bath temperature of 150° under 25 mm. Most appeared to distil at approximately 140°. It had the odor of quinoline. The yield was thus 13 per cent of the theoretical. The oil was treated with 15 mg. of picric acid, dissolved in ethyl alcohol, and the crystalline picrate was collected with this solvent. 15 mg. of yellow needles were obtained, which melted at 195°. After recrystallization from ethyl alcohol, 9 mg. remained which melted at 197°. A further recrystallization gave a product melting at 198–200°. The crystalline form and properties were indistinguishable from the picrate of synthetic quinoline which melted at 200°. The mixed melting point was at 199–200°.

$C_{15}H_{10}N_4$. Calculated, C 50.28, H 2.81; found, C 50.67, H 2.72

Catalytic Hydrogenation of Lysergic Acid—0.1 gm. of lysergic acid dissolved in 2 cc. of glacial acetic acid was shaken with hydrogen and 25 mg. of Adams and Shriner's catalyst. Absorption of hydrogen occurred rapidly at first but became much slower when 1.5 to 2 moles of hydrogen had been absorbed. At the outset of the reaction a brilliant violet-pink fluorescence developed in the solution but disappeared as the reduction proceeded. The same

color appeared when ergotinine and the alkaloid, as described below, were hydrogenated in this manner. If the reaction was interrupted when 1 mole of hydrogen had been absorbed, the reaction products quickly turned to a deep bluish purple color upon exposure to the air. It was difficult to remove the color from the crystalline material which was isolated. When, however, reduction was allowed to proceed until absorption of hydrogen had become rather slow, the products were quite stable to the atmosphere although the yield of crystalline material was the same as when the reduction was interrupted at an earlier stage. The filtrate from the catalyst was evaporated to dryness under reduced pressure. The residue was heated with 5 cc. of water, and on cooling crystallized. 50 mg. of substance were collected, the properties of which corresponded with those of dihydrolysergic acid previously obtained by us on reduction of lysergic acid with butyl alcohol and sodium.

$[\alpha]_D^{25} = -99°$ ($c = 0.505$ in pyridine)

$C_{16}H_{18}O_2N_2$. Calculated, C 71.06, H 6.72; found, C 70.95, H 6.60

The methyl ester prepared from this dihydro derivative with methyl alcoholic hydrochloric acid melted after recrystallization at 181° after slight preliminary softening and gave no depression when mixed with the ester of the acid obtained by the older method.

Catalytic Hydrogenation of the Ergot Alkaloid, $C_{19}H_{23}O_2N_3$—For hydrogenation the alkaloid was carefully recrystallized by addition of chloroform to the concentrated methyl alcoholic solution. 0.1 gm. of this material, which contains 1 mole of chloroform, was placed in the reduction chamber and evaporated two successive times at low pressure with ethyl alcohol in order to remove the chloroform completely. The residue was dissolved in 2 cc. of glacial acetic acid and 50 mg. of Adams and Shriner's catalyst were added. The mixture was shaken under an excess pressure of approximately 1.3 atmospheres of hydrogen. Absorption of hydrogen had become rather slow and the operation was interrupted when 2 moles of hydrogen had been absorbed. The brilliant violet fluorescence which had first developed had become much fainter. The catalyst was filtered off and the filtrate evaporated to dryness. The residue was taken up in dilute sodium

hydroxide and the solution was extracted with hot chloroform. Upon drying the chloroform extract with potassium carbonate and cooling, crystalline needles separated. 50 mg. were collected. The substance sintered considerably at 110° and melted with decomposition at 225–230°, depending somewhat on the rate of heating. The analysis indicated a dihydro derivative. It was dried for analysis *in vacuo* at 140°.

$C_{19}H_{25}O_2N_3$. Calculated, C 69.68, H 7.64; found, C 69.37, H 7.46

Hydrolysis of the Dihydro Alkaloid, $C_{19}H_{25}O_2N_3$—40 mg. of the dihydro derivative were dissolved in 1 cc. of 14 per cent methyl alcoholic potassium hydroxide solution and refluxed for 1 hour. The solvent was evaporated *in vacuo* and the residue was taken up in water. This solution was saturated with carbon dioxide and evaporated again to dryness. The solid residue was extracted with hot ethyl alcohol and the alcoholic extract was then evaporated to dryness. The residue was dissolved in a small volume of water and first made acid to Congo red with sulfuric acid. An excess of ammonium hydroxide was then added and the solution was boiled down to a small volume. Crystals separated on cooling. 15 mg. were collected, which appeared identical in every respect with dihydrolysergic acid.

$[\alpha]_D^{25} = -96°$ ($c = 0.424$ in pyridine)
$C_{16}H_{18}O_2N_2$. Calculated, C 71.06, H 6.72; found, C 70.80, H 6.39

Exhaustive Methylation of β-Dihydrolysergol—0.13 gm. of β-dihydrolysergol[8] was dissolved in 5 cc. of methyl alcohol and 2 cc. of methyl iodide were added. After standing 3 hours at 30°, the solvent was evaporated under reduced pressure and the residue was recrystallized from a small volume of methyl alcohol. 0.16 gm. of rhombs or plates was collected. The substance sintered at 250° and melted at 253–254°.

For analysis it was necessary to dry the substance at 160° and 0.2 mm.

$C_{16}H_{20}ON_2 \cdot CH_3I$. Calculated, C 51.26, H 5.82; found, C 51.53, H 5.53

0.26 gm. of the above methiodide was dissolved in methyl alcohol and treated with silver oxide until the halogen test was negative. Likewise, the filtrate gave no test for silver. The clear, slightly

colored filtrate was evaporated to dryness in a sublimation apparatus. The residue was sublimed under 0.30 mm. The apparatus was attached to a carbon dioxide trap in order to condense any volatile amines. However, none could be detected. Samples for analysis were taken directly from the condenser of the sublimation apparatus.

$C_{17}H_{22}ON_2$.	Calculated.	C 75.56, H 8.21, CH_3 11.11
	Found.	" 75.46, " 7.96, " 10.30

The material on the condenser was removed with methyl alcohol and the solvent was evaporated to dryness. The residue weighed 0.131 gm. and could not be made to crystallize from any solvent. It was dissolved in 3 cc. of methyl alcohol and treated with 1.5 cc. of methyl iodide. After standing 3 hours at 30°, the solution which had become a deep blue was evaporated to dryness under reduced pressure. The residue weighed 0.2 gm. which is approximately the amount calculated for the addition of 1 mole of methyl iodide. The residue was colored and could not be made to crystallize. It was dissolved in methyl alcohol, and the solution was treated with silver oxide as above. Upon evaporation of the filtrate in the sublimation apparatus, a strong odor of trimethylamine became apparent. When an attempt was made to sublime the residue as above under 0.3 mm. pressure, it was converted almost entirely into a non-volatile tar which would not sublime up to 250°. The carbon dioxide trap leading from the sublimation apparatus, however, had a strong odor of trimethylamine and was washed out with dilute hydrochloric acid. The hydrochloric acid was evaporated to dryness, and the residue was dissolved in a minimal volume of 10 per cent hydrochloric acid. The solution was treated with excess gold chloride and 15 mg. of crystalline material were collected with dilute hydrochloric acid. It melted with decomposition at 245°, depending somewhat on the rate of heating.

$(CH_3)_3N \cdot HAuCl_4$.	Calculated.	C 9.02, H 2.51, Au 49.42
	Found.	" 9.50, " 2.51, " 48.98