THE ERGOT ALKALOIDS

VI. LYSERGIC ACID

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Each of the alkaloids of ergot which we have had opportunity to study has in turn been shown to be a derivative of lysergic acid, in which the latter is conjugated with α-amino acids or with substances derived from them. In our very recent communication[1] on the alkaloid, $C_{19}H_{23}O_2N_3$ (the ergobasine of Stoll and Burckhardt),[2] this has been shown to be the hydroxyisopropylamide of lysergic acid. Since this fact conforms so well with the above formulation first given by Stoll and Burckhardt, it at once appears fairly certain that lysergic acid occurs as such in the molecule of this alkaloid. However, since, as we shall see later, lysergic acid is unsaturated, it is not excluded that in other alkaloids it may occur as a precursor which contains an additional water molecule in its make-up.[3] But we shall return to this point on another occasion. Lysergic acid is unquestionably the component of the ergot alkaloids to which the latter owe their pharmacodynamic action. This is found influenced in the individual alkaloids by the other substances with which lysergic acid is conjugated. The determination of its structure has therefore become a major problem. This problem has naturally been complicated by the difficulty of obtaining sufficient material for studies in degradation. In the first place the amount of crude mixed alkaloids which can be obtained from crude ergot is at best not more than 0.1 per cent and the resulting mixture must be fractionated to separate the individual alkaloids, ergotinine,

[1] Jacobs, W. A., and Craig, L. C., *Science*, **82**, 16 (1935).

[2] Stoll, A., and Burckhardt, E., *Compt. rend. Acad.*, **200**, 1680 (1935).

[3] Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, **110**, 523 (1935).

ergotoxine, and ergoclavine. These alkaloids in turn must be hydrolyzed for lysergic acid. In later work, however, it has been found expeditious to hydrolyze the crude alkaloid mixture directly. Finally, for a portion of the work here reported the additional step of reduction to dihydrolysergic acid became necessary. Thus because of the comparative inaccessibility of the material needed it has been compulsory to work with small quantities of substance. And because of the very small yields obtained in degradation studies manipulations throughout have been on a micro scale, for which special methods have had to be employed.

As a result of many analyses of lysergic acid and its derivatives there appears to be no need to change the original formulation adopted by us,[4] *viz.* $C_{16}H_{16}O_2N_2$. It contains an N-methyl group and a carboxyl group and is therefore obviously of aromatic character. Both lysergic acid and dihydrolysergic acid resisted all attempts to acetylate them. They form stable salts with only 1 equivalent of acid. These facts indicate that both N atoms are tertiary or that perhaps one of them may be contained in a pyrrole ring. Since lysergic acid when heated to 210–230° decomposes with the copious evolution of CO_2 and methylamine it appears unlikely that the N-methyl group is situated on such a pyrrole nitrogen atom. The color reactions of lysergic acid suggest that it is an indole derivative and may be biogenetically related to tryptophane. This possibility appears to have been definitely supported by the information which we have obtained from the study of alkali fusion. In these experiments lysergic acid was first employed but gave rather unsatisfactory results, although the odor produced was definitely suggestive of indole derivatives. The use of dihydrolysergic acid, however, proved to be more encouraging.

For this purpose dihydrolysergic acid was fused at about 300° with potassium hydroxide in an atmosphere of hydrogen and the evolved gases were collected. Methylamine was almost quantitatively collected in acid and was identified among other ways as the picrate. The aqueous solution of the alkali fusion, which exhibited a pronounced fecal odor, was extracted directly with ether and finally, after acidification, with this solvent and so separated into acid and basic or neutral products. However,

[4] Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, **104**, 547 (1934).

since some of the products appeared to change in their behavior towards alkali as the separation progressed, we must refer the reader to the experimental part for further details. The individual substances were separated or purified by sublimation in a microsublimation apparatus.

One of the non-acid substances was characterized as a brownish red picrate, which melted at 148–150° and gave analytical figures which agreed with the picrate of a methylethylindole, $C_{11}H_{13}N \cdot C_6H_3O_7N_3$. On the theory that this could have been formed by rupture of a 4-carboline ring, which we shall later discuss, 2-methyl-3-ethylindole was synthesized from the phenylhydrazone of methyl-*n*-propyl ketone and converted into the picrate for comparison. The latter melted at 148–150° and appeared to be identical with the substance obtained from the alkali fusion. However, owing to the very small amount of the latter, which remained after the necessary recrystallization, analysis, etc., it was impossible to complete the comparison in proper fashion. The suggestion of identity must therefore be presented with necessary reservation.

As the separation of the products of the alkali fusion proceeded, other substances were isolated. One of these was a base which melted at 68° and gave a yellow picrate which melted at 195–200°. The analytical figures with the latter indicated the formula $C_{11}H_{11}N \cdot C_7H_3O_7N_3$ or that of the picrate, perhaps of a methylethyleneindole. The red picrate of the isomeric tricyclic[5] cyclopentindole which we prepared for comparison according to Perkin and Plant gave a definite melting point depression with the above picrate and is therefore excluded. The substance will require further study. Other substances were an apparent indole derivative which gave a red picrate melting at 165–170° and an acid which melted at 270°. The amounts of these substances, however, were too small to obtain a clue to their nature which must be left for later work.

Of special importance was the study of the lowest boiling acid fraction which resulted from the alkali fusion. An acid was obtained which was identified as propionic acid by its *p*-bromophenacyl ester. From the mother liquor of the latter other material was obtained, the preliminary study of which strongly

[5] Perkin, W. H., Jr., and Plant, S. G. P., *J. Chem. Soc.*, **123**, 3244 (1923).

suggested the corresponding derivative of acetic acid. The amount available was too small to make this conclusive. We believe the formation of propionic acid of special significance, since it indicates the presence in the molecule of a propyl or propylene side chain.

From the above observations a number of points are suggested. In the first place there seems now to be little doubt that the indole portion of tryptophane discussed above is present in the molecule in which, as in formula (I), the benzene ring is unsubstituted.

We have already reported[6] that lysergic acid can be reduced by sodium and amyl (or butyl) alcohol to dihydrolysergic acid and that similarly the corresponding alcohol, (α- and β-) dihydrolysergol,[7] is produced on reductive cleavage of ergotinine (or from lysergic methyl ester) with these reagents. Since according to general experience it is difficult to reduce the indole ring system, it appears certain that at least one double bond, which is reduced in the formation of the dihydro derivative, must be contained in another portion of the molecule.

These conclusions may perhaps be related to the facts already established in the cases of two other groups of alkaloids related to tryptophane, namely, the harmine alkaloids, studied by Perkin and Robinson[8] and their coworkers, and the yohimbine alkaloids, for which especially Barger and Scholz[9] have established a related formula. It appears probable that in the case of lysergic acid we are also dealing with a similar 4-carboline[10] structure. Since methylamine is practically quantitatively produced along with the other indole derivatives, it appears certain that the basic carboline nitrogen atom carries the N-methyl group and is the salt-forming portion of the molecule. This would permit a partial structure as given in (II), in which the numbering is in accordance with the proposal of Perkin and Robinson.[10] There remain a propyl or propylene side chain and a carboxyl group to complete the 16 carbon atoms of the derived formula and it appears that

[6] Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, **106**, 393 (1934).

[7] Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, **108**, 595 (1935).

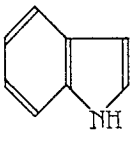
[8] Perkin, W. H., Jr., and Robinson, R., *J. Chem. Soc.*, **115**, 933 (1919). Kermack, W. O., Perkin, W. H., Jr., and Robinson, R., *J. Chem. Soc.*, **119**, 1602 (1921); **121**, 1872 (1922).

[9] Barger, G., and Scholz, C., *J. Chem. Soc.*, 614 (1933); *Helv. chim. acta*, **16**, 1343 (1933).

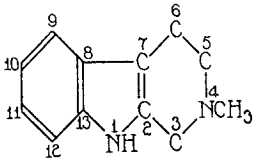
[10] Perkin, W. H., Jr., and Robinson, R., *J. Chem. Soc.*, **115**, 970 (1919).

these groups must be situated on the hydropyridine ring. In addition the formula would require that there must be two double bonds, one of which has been directly shown by the formation of dihydrolysergic acid. This double bond is presumably in the pyridine ring, apparently between carbon atoms (5) and (6), which would account for its ready reduction with sodium and butyl alcohol, just as harmine and harmaline can be reduced to tetrahydroharmine.

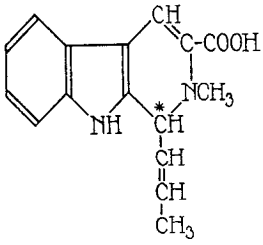
As regards the positions occupied by the carboxyl and propylene groups it is scarcely probable that position (6) can be in question, because of the inferred genetic relationship to tryptophane.



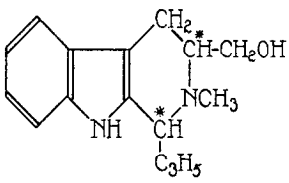
I



II



III



IV

There remain therefore only carbon atoms (3) and (5) as the most likely positions for such attachments and of several possibilities general considerations would seem to favor most the arrangement of groups as given in (III). Carbon atom (3) would thus become the center of asymmetry required, since lysergic acid itself is optically active. The formation of epimeric dihydrolysergols from either ergotinine or lysergic methyl ester would be accounted for by the formation of a new center of asymmetry at carbon atom (5) (IV) by reduction of the double bond between (5) and (6).

The presence of a second double bond in lysergic acid must be

inferred from its formulation and there appears to be no other place for it than in the assumed propyl side chain which would thus become propylene, as given in (III). This is still consistent with the production on alkali fusion of propionic acid and perhaps of acetic acid. It is not impossible that the side chain could be an allyl group and that the double bond shifts during alkali fusion. It is, of course, possible to place both carboxyl and propylene groups on carbon atom (3) but it would then be more difficult to explain the appearance of epimers when dihydrolysergol is produced. However, a study of the catalytic hydrogenation and oxidative cleavage of lysergic acid and dihydrolysergic acid, which is now in progress, will, we hope, give additional essential data on these points.

Finally, in the special case of α-dihydrolysergol we have found that a crystalline methiodide is produced with methyl iodide which has the properties of a true onium salt. Whether, as discussed in the case of harmane by Kermack, Perkin, and Robinson,[11] the alkyl group adds on the indole nitrogen atom to give an Ind-N-methyl derivative, since the Py-N is already substituted, must be left open for the present.

Owing to the difficulties of work with such limited material as is available, the above conclusions are presented with necessary reservations and it is hoped to supplement and confirm them by further evidence (synthesis) or if necessary to revise them in the light of any new conflicting data.

EXPERIMENTAL

Alkali Fusion of Dihydrolysergic Acid—400 mg. of dihydrolysergic acid[6] were ground in a mortar with 2 gm. of potassium hydroxide and placed in the fusion chamber of a small glass apparatus built for the purpose. The apparatus was made of Pyrex glass and consisted of a thick walled compartment of about 10 cc. capacity which contained the fusion mixture. At one end of the compartment was sealed a smaller glass tube for entrance of the hydrogen and at the other a rather narrow tube was bent in the shape of a U-tube in order to act as a condenser when immersed in ice water. The U-tube then led through a trap kept in acetone-

[11] Kermack, W. O., Perkin, W. H., Jr., and Robinson, R., *J. Chem. Soc.*, **121**, 1877 (1922).

carbon dioxide mixture and this trap then was connected to another trap containing dilute hydrochloric acid in order to collect any volatile base. A slow current of hydrogen was passed through the apparatus during the fusion. The fusion chamber was heated by a metal bath. After many experiments it was found that the optimum conditions for the decomposition were rapid heating to 295–300° (bath temperature) and maintaining at this point for ½ hour.

After fusion the ice water trap contained considerable water and a small amount of a reddish colored semisolid material that had a strong fecal odor. The fused caustic was dissolved out with water. It was colored bright bluish purple. Upon extraction with ether the ether layer became a brilliant fluorescent red. The alkaline layer was set aside to be treated as described below. The ether layer was then evaporated to dryness in a small sublimation outfit. 30 mg. of a dark colored residue remained. The oily materials in the ice trap and in the carbon dioxide trap were washed out with ether and the ether solution was added to the residue in the sublimation outfit. Upon evaporation a combined residue of 40 mg. remained. It was sublimed by slowly raising the oil bath temperature to 170° under 0.2 mm. pressure. 20 mg. of a reddish colored oil sublimed. It was washed off the condenser with ether and the ether evaporated. The oily residue was taken up in 1 cc. of 10 per cent hydrochloric acid and the mixture was shaken several times with 1 cc. portions of ether. The acid aqueous layer, which contained basic material, was set aside for treatment as described below. The ether extracts which contained neutral or very weakly basic products were combined and washed with 1 cc. of water. After evaporation of the ether 10 mg. of a dark colored semicrystalline mass remained. It was placed in a microstill and it was possible to collect a fraction at between 130–160° (oil bath temperature). This proved to be 4.9 mg. of a semicrystalline colorless material. It was removed from the condenser with ether. The oil bath temperature was then raised further to 200°. 1 mg. of additional solid material distilled. The lower boiling, semicrystalline fraction was treated with petroleum ether and concentrated to about 2 drops. After cooling, the crystalline material was sucked off and proved to be the same as the above 1 mg. of higher boiling fraction with which

it was then combined. This substance could be recrystallized nicely from ethyl ether and then melted at 192°. Owing to the small amount of material available, its further investigation has been deferred.

The petroleum ether filtrate from this substance was evaporated to dryness. The residue which was a mobile oil weighed 3.8 mg. and could not be made to crystallize as such or from any solvent. It was treated with 6 mg. of picric acid and 2 drops of ethyl alcohol. Upon heating, a dark brownish red solution was formed which upon cooling deposited brownish red crystals of the picrate. After collection this weighed 3 mg. and melted at 148–150°.

$C_{17}H_{16}O_7N_4$. Calculated, C 52.57, H 4.16; found, C 53.01, H 3.94

Since the melting point of this substance corresponded with that recorded for 2-methyl-3-ethylindole[12] of the same formula, the latter was prepared by the Fischer synthesis from the phenylhydrazone of methyl-*n*-propyl ketone. The synthetic indole proved to be a rather mobile oil which was soluble in petroleum ether and had the identical odor of the above indole from the alkali fusion. It distilled in the microdistillation apparatus at 145–150° under 0.2 mm. pressure (oil bath temperature). It formed a picrate which was indistinguishable in properties from our picrate.

$C_{17}H_{16}O_7N_4$. Calculated, C 52.57, H 4.16; found, C 52.56, H 4.53

Unfortunately we were not able to make a mixed melting point of our picrate with the synthetic picrate, since by the time we had prepared the latter the melting point of the former had lowered because of slow decomposition which occurred during long standing in a warm room. The mixed melting point must therefore be left until a later occasion with fresh material.

The above dilute hydrochloric acid solution containing the basic products of the reaction was treated with an excess of sodium hydroxide solution and the mixture was extracted with ether. After evaporation of the ether 9 mg. of oil remained. It was placed in the microstill under a pressure of 0.2 mm. and the oil

[12] Oddo, B., and Alberti, C., *Gazz. chim. ital.*, **63**, 236 (1933), abstracted in *Chem. Zentr.*, **2**, 1678 (1933). Arbusow, A. J., and Rotarnel, W. A., *Chem. Zentr.*, **1**, 2935 (1933).

bath temperature was gradually raised to 170°. Most of the material appeared to go over at about 145–150°. 8.3 mg. of colorless oil were collected, which entirely solidified on standing. Upon recrystallization from petroleum ether it melted at 68°. It was further characterized as the picrate which crystallized from benzene in yellowish green rosettes which decomposed at 195–200°.

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

The original caustic solution from the fusion which was set aside after extraction with ether was acidified to Congo red with sulfuric acid. It was extracted with ether and the ether extract was slowly concentrated, the ether distillate being carefully condensed. The last small drop of volatile material which would distil on raising the temperature up to 80° was removed along with the remaining ether and condensed on a microcondenser to be described in another communication. This volatile material which strongly suggested propionic acid was added to the ether distillate. The undistilled residue was set aside for treatment as described below. The ether distillate was treated with water and was carefully titrated with 0.1 N sodium hydroxide against phenolphthalein. 5.42 cc. were required. The titrated solution was evaporated to dryness and then treated with 2 cc. of 60 per cent alcohol. 120 mg. of *p*-bromophenacyl bromide were then added and the mixture was refluxed for 1 hour. The hot solution was filtered from a slight amount of solid material and cooled. 15 mg. of crystalline material were filtered off. The mother liquor was set aside for further treatment. The crystalline material melted at 53–54°. Upon recrystallization from dilute alcohol 4.5 mg. were obtained which melted at 58–59°, and thus agreed in melting point with that of *p*-bromophenacylpropionic ester recorded by Judefind and Reid.[13]

$C_{11}H_{11}O_3Br$. Calculated, C 48.71, H 4.11; found, C 48.51, H 4.27

[13] Judefind, W. L., and Reid, E. E., *J. Am. Chem. Soc.*, **42**, 1055 (1920).

The mother liquor from the crude ester was evaporated to dryness and the residue was extracted with ether. The ether extract on evaporation left material which was dissolved in a small volume of ethyl alcohol. Upon cooling, a very small crystalline fraction separated, the melting point of which was unsatisfactory since it dragged from about 50° up to 105°. *p*-Bromophenacyl acetate melts at 85°. The analysis of this suggested the acetic acid derivative, but, since the melting point obtained was so unsatisfactory, this result cannot be considered conclusive.

$C_{10}H_9O_3Br$. Calculated, C 46.68, H 3.56; found, C 46.34, H 3.45

The undistilled residue from the above volatile crude acid fraction was taken up in dilute sodium hydroxide and the mixture was extracted with ether. The ether extract contained a small amount of material with a fecal odor but was not studied further. The alkaline layer was made acid to Congo red with hydrochloric acid and then extracted with ether. The extract was washed with water and evaporated to dryness in the sublimation apparatus. A residue of 40 mg. remained. It was sublimed up to a bath temperature of 220° and under 0.2 mm. pressure. 26 mg. of partially crystalline material sublimed. Since some indole acids are known to decarboxylate readily, the sublimate was again separated into acid and basic fractions. For this purpose it was taken up in dilute sodium hydroxide and extracted with ether. The alkaline layer was set aside to be treated further. The ether extract upon evaporation gave 2 mg. of material. It was treated with an equivalent of picric acid in benzene. Dark red crystals separated which melted at 165–170° but could not be further characterized because of lack of material.

The remaining sodium hydroxide solution containing any indole acid was acidified with hydrochloric acid and the acid which separated was extracted with ether. The ether, after being washed with water, was dried with sodium sulfate and evaporated to dryness. The residue gave about 1 mg. of crystalline material upon concentrating the acetone solution to about 2 drops. This acid melted at 270°. The amount was too small for analysis. More crystalline material could not be obtained from the mother liquor. When, however, the oily mother liquor residue was sublimed in the sublimation apparatus, more of the indole which

gave a picrate melting at 165–170° described above could be obtained. This suggests decarboxylation.

Identification of Methylamine—The trap from the alkali fusion which contained the hydrochloric acid was washed out with water and filtered from a small amount of solid material. It was evaporated to dryness *in vacuo* on the steam bath. 90 mg. of crystalline hygroscopic material remained. The theoretical weight of methylamine hydrochloride for 1 equivalent is 93 mg. It was taken up in dilute sodium hydroxide solution which was covered by a layer of ether. The ether extract which smelled strongly of methylamine was treated with an ethereal solution of picric acid. Crystals separated which melted at 206–207° as reported in the literature for the methylamine salt. It was recrystallized from alcohol for analysis.

$C_7H_8O_7N_4$. Calculated, C 32.31, H 3.11; found, C 32.65, H 3.07

Methylation of α-Dihydrolysergol—40 mg. of α-dihydrolysergol[7] were dissolved in 10 cc. of methyl alcohol and 2 cc. of methyl iodide were added. After standing overnight at 30°, the solution was concentrated. The residue was dissolved in methyl alcohol and the filtrate was concentrated to small volume and cooled. Crystalline material separated in broad plates. It was collected with methyl alcohol and was recrystallized from this solvent. It melted at 237°.

$C_{17}H_{23}ON_2I$. Calculated, C 51.24, H 5.84; found, C 50.59, H 5.82