THE STRUCTURE OF THE ERGOT ALKALOIDS

By A. L. GLENN, PH.D.

(LECTURER IN PHARMACEUTICAL CHEMISTRY, SCHOOL OF PHARMACY, UNIVERSITY OF LONDON)

THE ergot alkaloids have been reviewed on a number of occasions.[1] Whilst some of these accounts have dealt in detail with small sections of the structural work, others, in view of their brevity and wide scope, have conveyed only the broadest outline. Further, when the earlier reviews were written, our knowledge of the structure of these alkaloids was deficient in a number of important respects. The present Review therefore attempts to set forth a balanced account of some twenty years of investigation, at a time when the alkaloidal structures appear to have been established beyond reasonable doubt. When we look for final proof on almost any aspect of the structure, we find it among the elegant researches of Stoll and his co-workers, who have rounded off the efforts of earlier investigators. However, the detailed structural speculations of the latter were very close to the structures now firmly established by the Stoll school. Therefore, despite the fact that on so many points, these earlier workers did not achieve final proof, their efforts merit considerable attention in a Review of this kind.

Nomenclature. One of the misfortunes attending the investigation of natural products is the necessity of naming certain compounds before their chemistry has been fully understood. In the case of the ergot alkaloids, this has led to much confusion and hence this Review follows the suggestions of Stoll,[2, 3] who has altered much of the early nomenclature.

Isolation of the Alkaloids.—Ergotinine was the first alkaloid to be isolated from ergot, being obtained by Tanret [4] in 1875. It was devoid of pharmacological activity, but in 1906 an active alkaloid, ergotoxine, was isolated independently by Barger and Carr [5] and by Kraft.[6] Ergotoxine and ergotinine were interconvertible by acid or alkali and appeared to differ by only one molecule of water. However, there were discrepancies between the individual preparations of both alkaloids, which were explained when Stoll and Hofmann [3] showed ergotoxine to be a mixture of the three alkaloids, ergokryptine, ergocristine, and ergocornine. In a similar way, ergotinine preparations were shown to contain ergokryptinine, ergocristinine, and ergocorninine. The previous failure to recognise the inherent impurity of

[1] Barger, *Analyst*, 1937, **62**, 340; Turner, *Ann. Reports*, 1935, **32**, 345; 1936, **33**, 374; Haworth, *ibid.*, 1939, **36**, 331; Openshaw, *ibid.*, 1944, **41**, 220; Stoll, *Experientia*, 1945, **1**, 250; Sharp, *Pharm. J.*, 1946, 156, 349, 367; Henry, "The Plant Alkaloids", Churchill, 4th edn., 1949, p. 517; Stoll, *Chem. Reviews*, 1950, **47**, 197; Manske and Holmes, "The Alkaloids", Vol. II, Academic Press, New York, 1952, p. 375.

[2] Stoll, Hofmann, and Schlientz, *Helv. Chim. Acta*, 1949, **32**, 1947.

[3] Stoll and Hofmann, *ibid.*, 1943, **26**, 1570.

[4] Tanret, *Compt. rend.*, 1875, **81**, 896.

[5] Barger and Carr, *Chem. News*, 1906, **94**, 89.

[6] Kraft, *Arch. Pharm. Berl.*, 1906, **244**, 336.

ergotoxine and ergotinine had been due to the strong tendency for the pure alkaloids to form intermolecular compounds with each other and with molecules of solvent. The names "ergotoxine" and "ergotinine" are now only used when referring to the pure alkaloids as a group.

As the above suggests, the ergot alkaloids occur as interconvertible isomeric pairs, for example, ergocornine and ergocorninine. In all cases, the lævorotatory "ine" alkaloids are pharmacologically active and are based on lysergic acid, whereas the dextrorotatory "inine" alkaloids are inactive and derived from *iso*lysergic acid. This most important question of the alkaloidal isomerism is considered in detail below. Another group of alkaloids is referred to as the "ergotamine group" and comprises the pairs, ergotamine and ergotaminine,[7] together with ergosine and ergosinine.[8] The alkaloids, ergometrine and ergometrinine,[9] cannot be classified in either of the above two groups for reasons which will later become apparent. The above facts are summarised in Table 1, in which only the lævorotatory alkaloids are shown.

TABLE 1. *The lævorotatory ergot alkaloids*

Polypeptide alkaloids		
Ergotoxine group	Ergotamine group	
Ergokryptine . . . Ergocristine . . . Ergocornine . . .	Ergotamine Ergosine	Ergometrine

The compounds, ergoclavine [10] and sensibamine,[11] isolated from ergot and originally thought to be new alkaloids, were shown to be intermolecular compounds of the pure alkaloids mentioned above. One other alkaloid, ergomonamine,[12] was isolated in 1936, but is of no interest here, since it is based on neither lysergic acid nor its isomer.

Determination of the Structure of the Ergot Alkaloids.—The attempts to determine the structure of these alkaloids may be conveniently divided into several phases. The first was noted for its drastic degradations and resulted in very little progress. It ended with Jacobs's oxidative work [13] and the second began with Smith and Timmis's hydrolytic studies [14] which led to most fruitful results. Largely owing to the efforts of Jacobs and his co-workers, such progress was made that by 1937, the main structural features of all the known ergot alkaloids had been worked out. However, the later more careful degradative work was difficult; in most experiments

[7] Stoll, *Verh. schweiz. naturf. Ges.*, 1920, 190.
[8] Smith and Timmis, *J.*, 1937, 396.
[9] Dudley and Moir, *Brit. Med. J.*, 1935, I, 520.
[10] Küssner, *Merck's Jahresber.*, 1933, **47**, 5, [11] B.P. 388,529.
[12] Holden and Diver, *J. Pharm.*, 1936, **9**, 230.
[13] Jacobs, *J. Biol. Chem.*, 1932, **97**, 739.
[14] Smith and Timmis, *J.*, 1932, **763**, 1543.

yields were poor owing to the instability of the alkaloids and their larger fragments.

Early investigations. Structural investigation began as long ago as 1910, when Barger and Ewins [15] obtained *iso*butyrylformamide by the action of heat on ergotinine and ergotoxine. Some twenty years elapsed before Soltys [16] carried the work a stage further by means of oxidation studies, which established the presence of a benzene nucleus. It was already known [17] that on treatment with alcoholic sodium hydroxide the alkaloids rapidly liberated ammonia; Zerewitinoff determinations indicated four active hydrogen atoms per molecule, and the presence of a *N*-methyl group was firmly established. No free hydroxyl groups were detectable whilst destructive distillation in a high vacuum gave a base with the odour of pyrrolidine. The colour produced in the Keller reaction was strongly suggestive of an indole nucleus.

We can now see that from the standpoint of elucidating the gross structural outlines of the alkaloids, the above indications were trivial apart from the conclusion of the Keller reaction. However, in 1932, Jacobs [13] repeated Soltys's oxidative work, isolating the same products with the addition of a tribasic acid, which later proved to be a key fragment. It will be convenient to refer to this compound as "tribasic acid Z".

In 1932, Smith and Timmis [14] were able to show that all the ergot alkaloids then known gave a basic degradation product on treatment with methanolic alkali. Although they named this compound "ergine" we shall call it "*iso*ergine" for reasons which will become apparent (see p. 200). *iso*Ergine represented a considerable part of the alkaloidal molecule and there was also a great similarity between its ultra-violet absorption and those of the alkaloids. Jacobs and Craig [18, 19] confirmed these findings in 1934 and by the use of aqueous alkali obtained an acid which they named "lysergic acid". *iso*Ergine was shown [19, 20] to be the amide of *iso*lysergic acid, a stereoisomer of lysergic acid.

Immediately lysergic acid had been isolated, it was recognised as an important fragment of the alkaloids, but it was not until a year later that this acid was seen to be the basis of the entire alkaloidal structure. Thus, in 1935, Jacobs and Craig [21] proved that ergometrine, which had just been isolated, was a hydroxypropylamide of lysergic acid. The fact that lysergic acid existed as such in the original alkaloid and was not a modification of some precursor was strongly indicated by experiments on the hydrogenation of ergometrine,[22] and in 1938, Stoll and Hofmann [23] proved this finally by partial synthesis of ergometrine from lysergic acid of natural origin and L-2-aminopropan-1-ol.

[15] Barger and Ewins, *J.*, 1910, **97**, 284. [16] Soltys, *Ber.*, 1932, **65**, 553.
[17] Barger, "Ergot and Ergotism", Gurney & Jackson, London, 1931.
[18] Jacobs and Craig, *J. Biol. Chem.*, 1934, **104**, 547.
[19] *Idem, ibid.*, 1934, **106**, 393. [20] Smith and Timmis, *J.*, 1934, 674.
[21] Jacobs and Craig, *Science*, 1935, **82**, 16.
[22] *Idem, J. Biol. Chem.*, 1936, **113**, 767.
[23] Stoll and Hofmann, *Z. physiol. Chem.*, 1938, **251**, 155.

From this point onwards, the structural problem is divisible into two parts : the structure of lysergic acid and that of the " non-lysergic acid portion ". Although the two aspects were pursued simultaneously and were closely connected in the earlier stages, they are reviewed separately for the sake of clarity. For the same reason, the isomerism of lysergic and *iso*lysergic acids will be discussed after the main structural features have been established.

The Determination of the Structure of Lysergic Acid

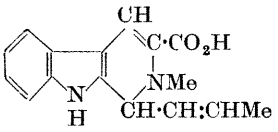
The following properties of lysergic acid were known soon after its isolation.[18, 19, 20] It was dextrorotatory and had the molecular formula, $C_{16}H_{16}O_2N_2$. Like the alkaloids, it gave a positive Keller reaction and contained a *N*-methyl group but no methoxyl groups. One carboxyl group was present, which seemed to be lost on dry distillation in a high vacuum. On reduction with sodium and amyl alcohol, dihydrolysergic acid was obtained. This acid and its derivatives possessed greater stability, and were thus more easily handled, than their unsaturated precursors. For this reason, many of the early degradative studies were carried out with the dihydro-compounds, especially dihydrolysergic acid. Reduction of ergotinine or methyl lysergate with sodium and butanol [24] gave two stereoisomeric bases, the dihydrolysergols, in which the carboxyl had been converted into a primary alcohol group. Evidently, reduction of lysergic acid had produced a new centre of asymmetry (see p. 205).

The Alkali Fusion of Dihydrolysergic Acid.—Fusion of dihydrolysergic acid with potassium hydroxide at 300° in an atmosphere of hydrogen [25] gave methylamine in almost quantitative yield together with three bases, that appeared to be indole derivatives, in addition to propionic and an unidentified acid. Jacobs and Craig then felt that they had enough evidence to suggest the β-carboline structure (I) tentatively for lysergic acid. Despite the early rejection of this formula, most of the conclusions upon which it rested were later shown to be justified. Thus, an indole nucleus was present, and the *N*-methyl group could not be attached to the indole nitrogen atom since methylamine was almost quantitatively produced along with indole derivatives. The easy reduction of lysergic acid indicated that at least one double bond must exist outside the indole nucleus. There were some important objections to this structure, which was abandoned after attempts to prove it by synthesis had broken down.[26] In the meantime, new degradative evidence had pointed to an entirely different structure.

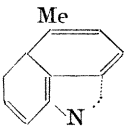
Investigations leading to the Tetracyclic Structure.—Further investigation of the alkaline fusion [22] established 1-amino-5-methyl naphthalene as one of the products. It followed that lysergic acid must contain two fused six-membered rings. Evidently the amino-group of this degradation product corresponded to the indole nitrogen atom of the original acid in view of the simultaneous formation of methylamine in good yield. This evidence

[24] Jacobs and Craig, *J. Biol. Chem.*, 1935, **108**, 595.
[25] *Idem, ibid.*, 1935, **111**, 455. [26] *Idem, ibid.*, 1936, **113**, 759.

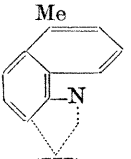
allowed only two possibilities, (II) and (III), for the arrangement of three of the rings of lysergic acid.



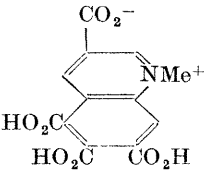
(I)



(II)



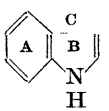
(III)



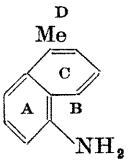
(IV)

Another vital piece of evidence concerned the tribasic acid " Z " obtained in early oxidation experiments (p. 193). This acid was now shown to give quinoline on distillation with soda-lime. It had the molecular formula, $C_{14}H_9O_8N$, contained a *N*-methyl group, and was not a lactone. Three carboxyl groups were detectable by titration, but a fourth would be expected to be masked on account of the positively charged quaternary nitrogen atom. Whilst the four carboxyl groups could be oriented in a number of ways, structure (IV) is that which may be inferred from later work.

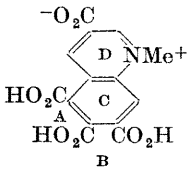
The great importance of acid " Z " lay in the fact that the 1-methylquinolinium nucleus in a more or less reduced form must exist in the lysergic acid molecule. Further, the *N*-methyl group of acid " Z " evidently resulted from the *N*-methyl group of the original molecule, which had been established as lying outside the indole nucleus. Structures (V), (VI), and (VII) of the important degradation products are seen to fit readily into the tetracyclic structure (VIII).



(V)



(VI)

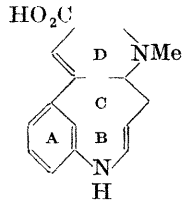


(VII)

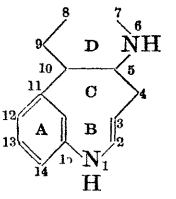
Catalytic hydrogenation studies indicated the presence of only one non-indole double bond and hence a tetracyclic structure was necessary to accommodate the molecular formula. Structure (VIII) is that now accepted for lysergic acid, but in 1936, it was only one of a number of possibilities which mainly differed in the position of the carboxyl group and the non-indole double bond. Jacobs and Craig [27] suggested the name " ergoline " for the tetracyclic base (IX) from which structure (VIII) could be considered to be derived.

The next step was to prove the ergoline structure by synthesis : it would suffice to synthesise dihydrolysergic acid or a suitable degradation product, thus avoiding the non-indole double bond, which could not be

[27] Jacobs and Gould, *J. Biol. Chem.*, 1937, **120**, 141.



(VIII)



(IX)

fixed with certainty at the time and even now constitutes an unsolved synthetic problem.

The non-indole double bond is involved in the isomerism of lysergic acid and of the alkaloids themselves, and is discussed on p. 200 ; in 1936 it was proved spectrophotometrically [28] that it could only occupy position 4 : 5, 5 : 10, or 9 : 10. However, although the synthesis of dihydrolysergic acid could be pursued without knowledge of the position of the double bond it was essential to know that of the carboxyl group.

Position of the Carboxyl Group in Lysergic Acid.[29]—The acid "Z" contained four carboxyl groups, three of which were formed by oxidation of those carbon atoms concerned in the linking of the quinoline fragment to the rest of the molecule. Therefore, the fourth carboxyl group must have been that originally present in lysergic acid. Because of this, the carboxyl group of lysergic acid was situated somewhere in ring C or D of ergoline. The angular positions, $C_{(5)}$ and $C_{(10)}$, were ruled out, for in either of these the carboxyl group could not have survived the formation of the quinoline structure possessed by acid "Z". Position 9 was unlikely for stereochemical and other reasons.

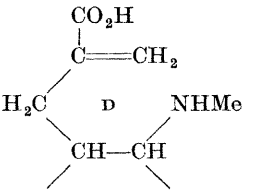
These considerations reduced the possible positions to 4, 7, and 8. If it were situated at position 4, dihydrolysergic acid would contain an indolylacetic acid fragment, which, if it behaved as the parent substance, should be readily decarboxylated : however, dihydrolysergic acid had high thermal stability. The group was finally placed at position 8, partly because dihydrolysergic acid behaved like a β-amino-acid on heating, but also in the light of pK measurements.

The following conclusions were derived from a study of the titration curves of lysergic acid derivatives and a number of related amines and amino-acid esters.[30] Presence of an ethoxycarbonyl group at the α-carbon atom of ethylamine increased the pK_b of the amino-group by 2·90 units : its presence at the β-carbon atom produced a smaller pK_b shift of 1·57 units. Now, the pK_b of dihydroergometrine exceeded that of 6-methylergoline by 1·49 units, and this was strongly indicative of β-substitution. It was of course reasonable to assume that the carbamyl group's inductive effect would closely resemble that of the ethoxycarbonyl group.

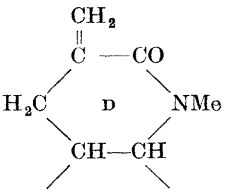
Although dihydrolysergic acid sublimed at 300°/25 mm. substantially

[28] Jacobs, Craig, and Rothen, *Science*, 1936, **83**, 166.
[29] Jacobs and Craig, *J. Biol. Chem.*, 1936, **115**, 227.
[30] Jacobs, Craig, Gould, and Shedlovsky, *ibid.*, 1938, **125**, 289.

unchanged it was shown [31] that during this treatment some decomposition occurred with the formation of a neutral substance. This contained one molecule of water less than the starting material, gave a positive Keller reaction, and contained one easily hydrogenated double bond. If dihydrolysergic acid were a β-amino-acid, one would expect heat to break ring D at the carbon–nitrogen bond, giving compound (X). This would then lactamise to give a neutral substance (XI), having the properties mentioned above. Hence, this reaction strongly favoured the assignment of the carboxyl group to position 8 and not to 7, for α-amino-acids do not undergo such decomposition.

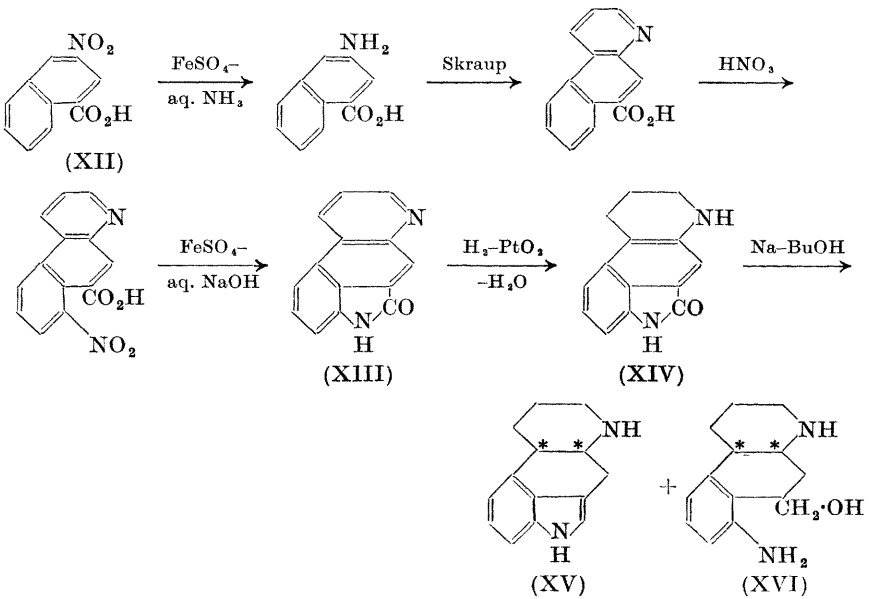


(X)



(XI)

Synthetic Attempts to confirm the Structure proposed for Dihydrolysergic Acid.—The ergoline ring system had no precedent and when, in 1936, Jacobs and Gould [27] turned their attention to its synthesis, it was first necessary to study model syntheses of simpler compounds, which embodied rings A,



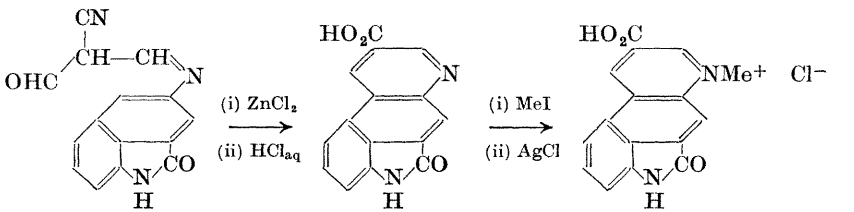
[31] Jacobs and Craig, *J. Amer. Chem. Soc.*, 1938, **60**, 1701.

B, and C of ergoline. The synthesis of ergoline from (XII) is of historical interest as well as illustrative of a number of routes to ergoline derivatives, including dihydrolysergic acid itself.

In 1938, the same authors [32] obtained 6-methylergoline by a similar route, in which the methiodide of (XIII) was catalytically reduced to the tetrahydro-*N*-methyl stage. If simple decarboxylation of dihydrolysergic acid could have been achieved, 6-methylergoline would have provided a suitable link for the synthetic establishment of the ergoline ring system for lysergic acid. Attempts [32] to prepare 6-methylergoline-7-carboxylic acid by a route analogous to that used for 6-methylergoline met with considerable difficulty, for the steric effect of the 7-ethoxycarbonyl group prevented methylation of the nitrogen atom in ring D. This provided further evidence against the 7-position for the carboxyl group, since methiodides of lysergic acid and its derivatives were easily prepared.

The first synthetic evidence for the ergoline structure and also for the 8-position of the carboxyl group was obtained by Jacobs and Gould [33] in 1939. They prepared 6 : 8-dimethylergoline by the usual route, introducing the 8-methyl group in the early part of the synthesis. The synthetic compound was then compared with 6 : 8-dimethylergoline of natural origin, obtained by catalytic hydrogenation of the non-indole double bond of the lactam (XIV), followed by sodium–butanol reduction of the lactam-carbonyl group to a methylene group. The base of natural origin strongly resembled synthetic 6 : 8-dimethylergoline, but was optically active and of lower melting point. However, these discrepancies were later eliminated [34] by preparing the " natural " base from racemic dihydrolysergic acid. Difficulties of this kind, which arose from the presence of three centres of asymmetry, also occurred in the later comparisons of natural and synthetic dihydrolysergic acids.

Synthesis of inactive dihydrolysergic acid was accomplished by Jacobs and Uhle [35] in 1945. The carboxyl group was introduced in the early part of the synthesis and, in contrast to its state in the ergoline synthesis given above, ring B already existed in the starting material as a lactam. Compound (XVII), prepared by condensing 4-aminonaphthostyril with cyanomalondialdehyde, was cyclised to a tetracyclic nitrile, which was subsequently



(XVII) (XVIII) (XIX)

[32] Jacobs and Gould, *J. Biol. Chem.*, 1938, **126**, 67. [33] *Idem, ibid.*, 1939, **130**, 399.
[34] Jacobs, Craig, and Gould, *ibid.*, 1942, **145**, 487.
[35] Jacobs and Uhle, *J. Org. Chem.*, 1945, **10**, 76.

hydrolysed to (XVIII). The methochloride (XIX) was then prepared in the usual way and catalytically hydrogenated to the tetrahydro-*N*-methyl compound as hitherto. Finally, inactive dihydrolysergic acid was obtained by sodium-butanol reduction, as in the original ergoline synthesis.

Although the synthetic product appeared to be identical with the natural acid, a completely satisfactory proof of identity required the separation of both natural and synthetic acids into uniform racemates. Owing to lack of material, Jacobs and Uhle could not achieve this and it was not until 1950 that Stoll and Rutschmann [36] were able to bring this work to a satisfactory completion. After synthesising dihydronorlysergic acid in good yield, they separated the mixture into three uniform racemates, which were then correlated with the natural dihydro-acid racemates.

The Isomerism of Lysergic Acid and its Derivatives

It has been known for a considerable time [6, 37] that the alkaloids of ergot exist in isomeric pairs, one member being lævorotatory and the other dextrorotatory. The pairs are interconvertible in hydroxylic solvents by the action of hydrogen and hydroxyl ions and so exhibit mutarotation. During the structural work discussed above, the isomerism received careful consideration and, in fact, theories [15, 38] as to its mechanism were propounded before even the vague structural outlines were known.

We now know that the isomerism is due to a change in the lysergic acid portion. The first indication of this was provided by Kleiderer's observation [39] that ergometrine underwent mutarotation in methanol. This could only be due to some change in the lysergic acid portion, in view of the simple amino-alcoholic nature of the amide substituent. In 1936, Smith and Timmis [40] observed that even the simple amide of *iso*lysergic acid ("*iso*ergine" according to the latest nomenclature *), could be converted into an isomer, "ergine", which was only slightly dextrorotatory. It followed that "lysergic acid" must exist in two forms, lysergic acid and *iso*lysergic acid, which thus explained the alkaloidal isomerism. *iso*Lysergic acid was obtained by the action of hot water on lysergic acid.[40] The relation between the three pairs of isomers is summarised in Table 2.

We thus see that all the lævorotatory, pharmacologically active alkaloids have the lysergic acid structure, whilst the dextrorotatory, pharmacologically inactive alkaloids possess the *iso*lysergic acid structure. Interpretation of

[36] Stoll and Rutschmann, *Helv. Chim. Acta*, 1950, **33**, 67.
[37] Barger and Carr, *J.*, 1907, **91**, 337.
[38] Jacobs and Craig, *J. Biol. Chem.*, 1935, **110**, 521.
[39] Kleiderer, *J. Amer. Chem. Soc.*, 1935, **57**, 2007.
[40] Smith and Timmis, *J.*, 1936, 1440.
[41] de Vries and Pepinsky, *Nature*, 1951, **168**, 431.

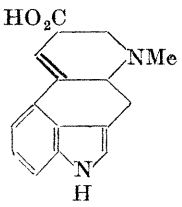
* In referring to ergine as the amide of lysergic acid, de Vries and Pepinsky [41] have reversed the nomenclature, which Smith and Timmis assigned to the amides of lysergic acid and its isomer. This reversal is logical and avoids the confusion of the original literature on this point. It has therefore been adopted throughout the Review. The relations expressed in Table 2 define the new nomenclature for the amides.

TABLE 2. *Relation between lysergic and* iso*lysergic acids and their typical alkaloidal derivatives*

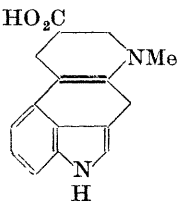
Type of compound	Lævorotatory series	Dextrorotatory series
Substituted amide (alkaloid)	Ergocornine	Ergocorninine
Simple amide	Ergine	*iso*Ergine
Corresponding acid	Lysergic acid	*iso*Lysergic acid

the literature is somewhat complicated because of the easy interconversion of the two acids and their derivatives. Thus, the conditions under which the alkaloid is split determine whether lysergic or *iso*lysergic acid will predominate. Starting with an alkaloid of either the lævo- or the dextro-series, lysergic acid is the main yield from alkaline hydrolysis,[20, 42] whereas fission by hydrazine [43, 44] gives *iso*lysergic hydrazide as the principal product.

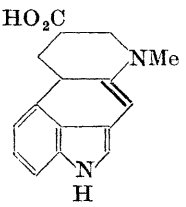
The Nature of the Isomerism.—This is closely associated with the position of the non-indole double bond. The ultra-violet absorption curves [28] show a definite shift towards shorter wave-lengths on hydrogenation of the non-indole double bond of either lysergic or *iso*lysergic acid. It follows that this double bond must be conjugated with the indole nucleus, leaving only three possible positions for it, indicated by (XX), (XXI), and (XXII).



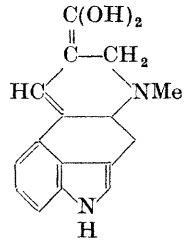
(XX)



(XXI)



(XXII)



(XXIII)

The further theories mostly concern a choice between two broad alternatives : the isomerism occurs either by a shift of the non-indole double bond or by inversion of the carboxyl group and the hydrogen atom at position 8. The latter possibility was considered by Jacobs and Craig [29] as long ago as 1936 : in the light of our present knowledge, it is ironical that it was rejected almost at the moment of conception, but the evidence against it appeared formidable. Thus, whilst methyl lysergate mutarotated in warm ethanol, its dihydro-compound did not : [29] similarly, dihydro-ergometrine could not be isomerised. At an early stage therefore, the above workers suggested that the double bond must be concerned in some way with the isomerism and, as we shall see, this conclusion was quite valid, when employed thus, though not in the way that Jacobs and Craig used it.

[42] Smith and Timmis, *J.*, 1936, 1166.
[43] Stoll and Hofmann, *Z. physiol. Chem.*, 1937, **250**, 7.
[44] *Idem, Helv. Chim. Acta*, 1943, **26**, 922.

The 'double-bond-shift' theory was immediately taken up by Smith and Timmis,[40] who suggested that the two acids were structural isomers. At that time, these workers regarded the carboxyl group as situated at position 4 of ergoline (cf. IX), and in view of the evidence from the rates of esterification with diazomethane, they assigned the double bond to position 5 : 10 in lysergic acid and 4 : 5 in *iso*lysergic acid. For simple unsaturated carboxylic acids, there was some evidence [45] that the proximity of a double bond to the carboxyl group reduced the rate of esterification of the latter by diazomethane. This was correlated with the fact that lysergic acid was esterified faster than *iso*lysergic acid.

Jacobs and Craig [29] also considered the possibility that the double bond occupied identical positions in the two acids, which differed only in the configuration at position 5 or 10, depending on the position assigned to the double bond [(XX) or (XXII)]. The mutarotation would then require a momentary shift of the double bond to the position 5 : 10 (cf. XXI) and back. If, for example, the double bond normally rested in the 9 : 10-position (XX), the momentary shift to position 5 : 10 would alter the configuration at position 5. However, the final form of the double-bond-shift theory was decided by pK evidence.[30] Ergometrine was found to have a lower basic strength than ergometrinine, as could well arise from different positions of the double bond. Positions 4 : 5 and 5 : 10, being equidistant from the tertiary nitrogen atom, could not explain the observed pK difference. However, the latter received reasonable explanation by assigning structure (XXI) to lysergic acid and (XX) to *iso*lysergic acid. The mutarotation therefore entailed a shift of the double bond between the two positions. This theory was accepted for more than a decade, although it underwent slight modification by Adams and Mahan [46] in 1942, who demonstrated that a double bond adjacent to a tertiary nitrogen atom actually increased the basic strength instead of decreasing it, as had been supposed previously. This proposal made no difference to the essentials of the theory but merely entailed a transposition of the structural assignments so that (XX) represented lysergic and (XXI) *iso*lysergic acid.

Although the double-bond-shift theory has now been abandoned as an explanation of the lysergic–*iso*lysergic acid transformation it should not be supposed that such a rearrangement is implausible. Indeed, it still appears necessary to rely on a momentary shift to account for the racemisation of the ergoline nucleus on the basis of Stoll's theory (see p. 208). A double bond in either the 5 : 10- or the 9 : 10-position together with the 3 : 4-bridging of the indole nucleus combine to make ergolene a somewhat strained structure. It is thus probable that neither the 5 : 10- nor the 9 : 10-position of ergolene is particularly stable and that a delicate balance exists between the two.

In 1949, Stoll, Hofmann, and Troxler [47] put an end to the above theory by publishing a large volume of evidence, which indicated that the two

[45] Sudborough and Thomas, *J.*, 1912, **101**, 317.
[46] Adams and Mahan, *J. Amer. Chem. Soc.*, 1942, **64**, 2588.
[47] Stoll, Hofmann, and Troxler, *Helv. Chim. Acta*, 1949, **32**, 506.

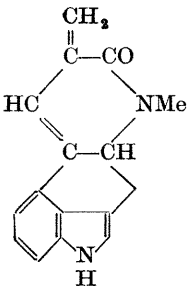
acids differed only in the configuration at $C_{(8)}$, the non-indole double bond occupying the same position in both, as shown in (XX). In other words, the two acids and their derivatives were epimers at $C_{(8)}$ and the mutarotation consisted of an epimerisation at this centre.

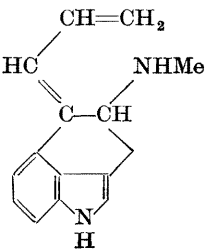
This theory was not new (see p. 201), having been rejected by the earlier workers because mutarotation appeared to be impossible in the absence of the double bond, but the objection could now be explained away. In the average case, the double bond was essential to the mutarotation, for in the 9 : 10-position it was in just the right place to stabilise the intermediate enol form (XXIII) in which the enolic double bond is conjugated with the 9 : 10-double bond and with the indole system. Jacobs and Craig's original contention was thus rounded off in a most satisfactory manner.

Investigation of the Stoll School into the Nature of the Isomerism.—For experimental establishment of the nature and mechanism of the isomerism, the first aim [48] was to remove the centre of asymmetry at position 8. Then, if the double-bond-shift theory were true, such treatment would lead to an optically inactive derivative in the case of (XXI), whilst (XX) would give an optically active compound, for a centre of asymmetry would remain at $C_{(5)}$. Whilst initial attempts in this direction failed, an accidental observation led to important evidence.

It was found [47] that neither lysergic nor *iso*lysergic acid was acetylated by acetic anhydride : the reaction took instead a different course, with the formation of an optically active compound. This was obtained in good yield from both acids and appeared to be a lactam (XXIV), the positions of the double bonds being assigned according to unequivocal ultra-violet absorption data. Compound (XXIV) is seen to be an unsaturated analogue of (XI) and is produced by an identical mechanism, namely, by ring closure of the carboxyl group with the secondary amino-group formed in the thermal splitting of ring D.

(XXIV)





(XXV)

It was concluded that the 9 : 10-double bond of the lactam had remained unchanged throughout the reaction and must therefore occupy the same position in the lactam precursors, lysergic and *iso*lysergic acid. It is important that the lactam was optically active. The only centre of asymmetry in the lactam was $C_{(5)}$, which meant that lysergic and *iso*lysergic acid possessed identical configurations at this position. It followed that

[48] Hofmann, *Helv. Chim. Acta*, 1947, **30**, 44.

the only possible explanation for the mutarotation rested with the asymmetric centre $C_{(8)}$.

The easy interconversion of the two acids and their derivatives constitutes an unfortunate uncertainty in most of the chemical evidence. Thus, the above lactam evidence, including the optical activity of the lactam, can be explained by the double-bond-shift theory in a way that is not unreasonable.[49] To a smaller extent, the same objection applies to the decarboxylation evidence.

Early attempts [19, 20] to decarboxylate lysergic acid and its isomer failed owing to substantial decomposition of the ergoline nucleus, involving the loss of the tertiary nitrogen atom. After this problem had been solved by special technique,[47] both acids were shown to give the same optically active decarboxylation product. The latter was assigned the structure (XXV) on grounds of analytical and ultra-violet absorption data and, as might have been expected, ring D had undergone fission during the process. This decarboxylation evidence led to the same conclusions as the lactam evidence.

In pointing out the virtual identity of the ultra-violet absorption curves of the two unsaturated acids and their derivatives, Stoll and his co-workers drew attention to a most important difficulty of the double-bond-shift theory and also gave powerful support for the conclusions drawn from the lactam and decarboxylation experiments. It is extremely improbable that the absorption curves of lysergic and *iso*lysergic acids would be so close unless the non-indole double bond occupied the same position in both. This is to be regarded as key evidence as there is no possibility of the isomerisation objection referred to above.

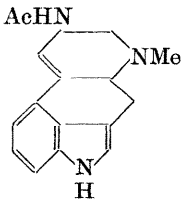
The fact that amides or esters of dihydro*iso*lysergic acid I (XXXIII) give dihydrolysergic acid I (XXXI) on alkaline hydrolysis constitutes sure proof for the Stoll theory of the isomerism. Thus, mutarotation proceeds, mainly in one direction, without the presence of the double bond, and must be due to a change of configuration at position 8, since a change of configuration at position 5 or 10 would require the rupture of carbon–carbon or carbon–nitrogen bonds. Another certain proof is given [47] by the isomeric pair (XXVI). Despite the presence of the double bond, neither isomer mutarotates, but decomposition of the quaternary hydroxide of either leads to the same optically active base (XXVII). This reaction is analogous to the lactam and decarboxylation experiments and identical conclusions can be drawn from it with the additional advantage that there is no uncertainty about the mutarotation.

The fact that (XXVI) will not mutarotate constitutes additional evidence that the carboxyl group is essential for the mutarotation. The same situation obtains with compounds of the type (XXVIII) in which R is NH_2, $NH{\cdot}CO_2Et$, or $CH_2{\cdot}OH$.[2, 48, 50]

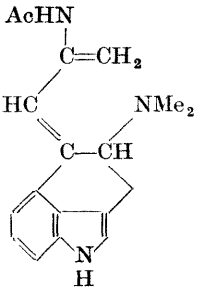
Having accepted the Stoll theory of the isomerism, one can no longer

[49] Glenn, "Synthetic compounds structurally related to the ergot alkaloids", Ph.D. Thesis, London, 1951.

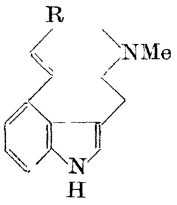
[50] Troxler, *Helv. Chim. Acta*, 1947, **30**, 163.



(XXVI)



(XXVII)



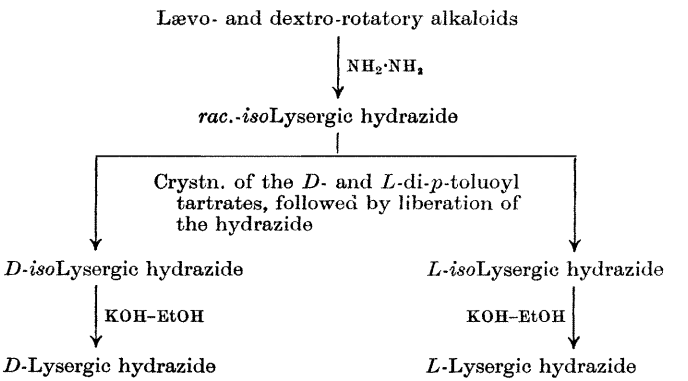
(XXVIII)

rely upon the non-indole double bond to explain the different basic strengths of the tertiary nitrogen atom in ring D of lysergic and *iso*lysergic acid. This must have a stereochemical explanation.

The Stereochemistry of Lysergic Acid and its Derivatives

Despite the very considerable difference in optical rotation, lysergic and *iso*lysergic acid as isolated by Smith and Timmis were both dextrorotatory. The lævorotatory isomers were expected to exist and indeed the same workers [40] obtained, by the action of barium hydroxide on either acid, an optically inactive compound, which was evidently the racemate. Subsequently, the lævorotatory isomers were isolated by Stoll and Hofmann,[43] who made a careful study of the stereochemistry of these acids in 1937. According to the Stoll nomenclature,[44] lysergic acid, which has a positive rotation in pyridine, is designated " *D*-lysergic acid " * and used as reference point for all configurational relationships of the ergot alkaloids. Scheme I shows how all four isomers required by Stoll's final structure (VIII) were isolated [44] in the form of their hydrazides.

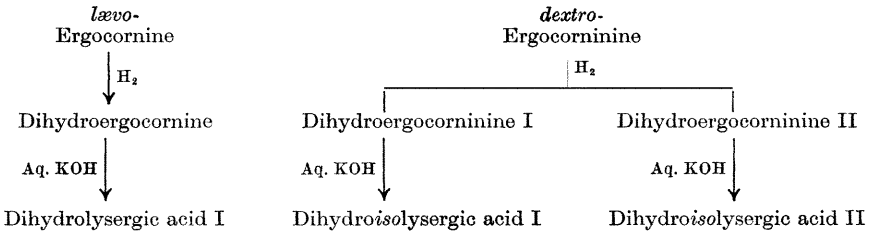
Scheme I.



* Italic capital letters are used in this Review, in contradistinction to the small capital roman D and L used to denote correlation with glyceraldehyde.

The early experiments of Jacobs and Craig [22, 29, 30] had shown that catalytic hydrogenation of lysergic acid and its derivatives to the dihydro-stage resulted in the production of a new asymmetric centre. However, for a complete picture of the stereochemistry of these derivatives one must refer to the work of the Stoll school. With dioxan as solvent and palladium black as catalyst, there was a marked difference in the rate of hydrogen uptake between the lævo- and the dextro-rotatory alkaloids.[51, 52] The latter took up hydrogen much less readily and required the use of palladium black or platinum oxide in glacial acetic acid. The results of these experiments are typified by those given in Scheme II for the case of the ergocornine pair of alkaloids.

Scheme II.



It will be noted that the lævorotatory alkaloid gives only one dihydro-acid, whereas the isomer gives two. The same pattern has been observed in numerous experiments with the other alkaloidal pairs. Thus, hydrogenation of *iso*lysergic acid and its derivatives leads to the two dihydro-*iso*lysergic acids I and II, in yields which depend upon the catalyst employed. On the other hand, hydrogenation of lysergic acid or its derivatives, gives dihydrolysergic acid I as the sole product. The corresponding isomer, dihydrolysergic acid II,* has been obtained only recently [53] from compounds of the dihydro*iso*lysergic acid II configuration by processes, which cause inversion at $C_{(8)}$. The fact that dihydrolysergic acid II is the least stable of the four isomers explains why it has not been obtained by direct homogeneous reduction of lysergic acid with such reagents as sodium and amyl alcohol. Its failure to result from the milder process of catalytic hydrogenation has been explained by Cookson (see p. 209) in terms of catalyst hindrance.

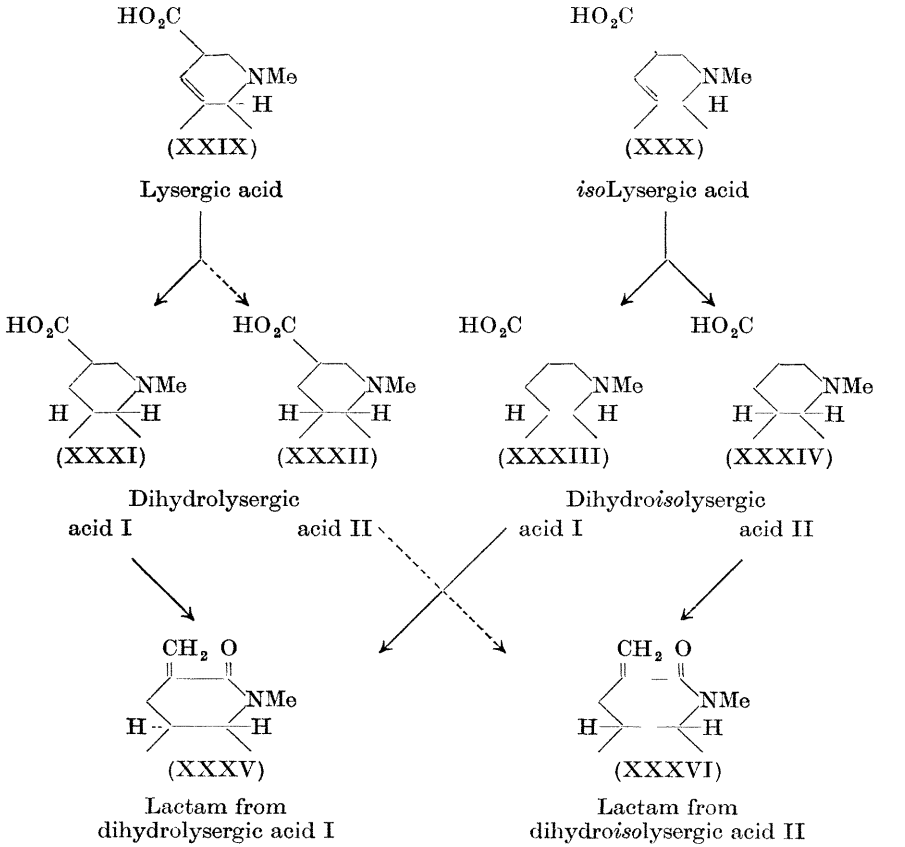
The stereochemistry of the acids and their derivatives is summarised

[51] Stoll and Hofmann, *Helv. Chim. Acta*, 1943, **26**, 2070.
[52] Stoll, Hofmann, and Petrzilka, *ibid.*, 1946, **29**, 635.
[53] Stoll and Rutschmann, *ibid.*, 1953, **36**, 1512.

* All but the latest literature refers to dihydrolysergic acid II as the "missing isomer". Its isolation makes the gap in earlier stereochemical investigations even more noticeable, in that a number of transformations carried out with the other dihydro-derivatives would have been applied to dihydrolysergic acid II and its derivatives, had they existed. In this Review, therefore, broken arrows have been used to show transformations which could probably occur, but which remain to be demonstrated.

in formulæ (XXIX)—(XXXVI), in which only ring D is shown. (A bond below the plane of the paper is represented by a broken line, and a bond above it by a full line.) The configurations indicated are those favoured by Stoll,[53] which in the case of the dihydro-acids (XXXI—XXXIV) are also supported by the conformational deductions of Cookson [54] and Stenlake.[55]

Now the existence of the stereoisomeric dihydro-acids must be explained on the basis of three centres of asymmetry, positions 5, 8, and 10. According to Stoll, the configuration at $C_{(5)}$ determines whether or not the acid or derivative belongs to the *D*- or the *L*-series. This discussion concerns the *D*-series only, that is, the natural alkaloids both dextro- and lævorotatory, so that the configuration at $C_{(5)}$ is constant. The configuration at $C_{(8)}$ determines whether the compound is related to lysergic or *iso*lysergic acid. The two pairs, (XXXI–XXXII) and (XXXIII–XXXIV) of dihydro-acids I and II must therefore owe their isomerism to the configuration at



[54] Cookson, *Chem. and Ind.*, 1953, 337. [55] Stenlake, *ibid.*, 1953, 1089.

$C_{(10)}$. This follows in any case, for the asymmetric centre at $C_{(10)}$ arises during the course of reduction.

Dihydrolysergic acid I and dihydro*iso*lysergic acid I are known to have the same configuration at $C_{(5)}$ and $C_{(10)}$. Thus, on treatment with aqueous potassium hydroxide,[52] the methyl ester of dihydro*iso*lysergic acid I is converted into dihydrolysergic acid I and, since this change concerns only the configuration at $C_{(8)}$, the two acids must be identical at the other two centres of asymmetry. In a similar way, vigorous hydrolysis [53] of the hydrazide of dihydro*iso*lysergic acid II leads to dihydrolysergic acid II, which indicates that this pair of acids also have identical configurations at $C_{(5)}$ and $C_{(10)}$.* Additional evidence [47] is provided by lactamisation experiments, in which the two related dihydro-acids, (XXXI) and (XXXIII), give the same lactam (XXXV), which differs from that, (XXXVI), obtained from dihydro*iso*lysergic acid II (XXXIV) and would be expected from dihydrolysergic acid II (XXXII).

A detailed study [2] of another series of ergoline derivatives, the dihydrolysergols, has given results which are entirely analogous to those from the dihydro-acids. The stereochemistry of these alcohols is exactly represented by formulæ (XXXI)—(XXXIV) if the carboxyl groups are all replaced by methanol residues. For example, reduction of dihydro*iso*lysergic acid I methyl ester with lithium aluminium hydride gives dihydro*iso*lysergol I, corresponding with (XXXIII). The close connection between the configurations of dihydrolysergic and dihydro*iso*lysergic acid I is again seen in the dihydrolysergols. Thus, sodium–butanol reduction of dihydroergocorninine I gives dihydrolysergol I, as well as dihydro*iso*lysergol I, whilst reduction of dihydroergocornine by the same method gives a mixture of the same alcohols. Hence under certain conditions the more stable dihydrolysergic acid I configuration can be transformed into the less stable dihydro-*iso*lysergic acid I configuration.

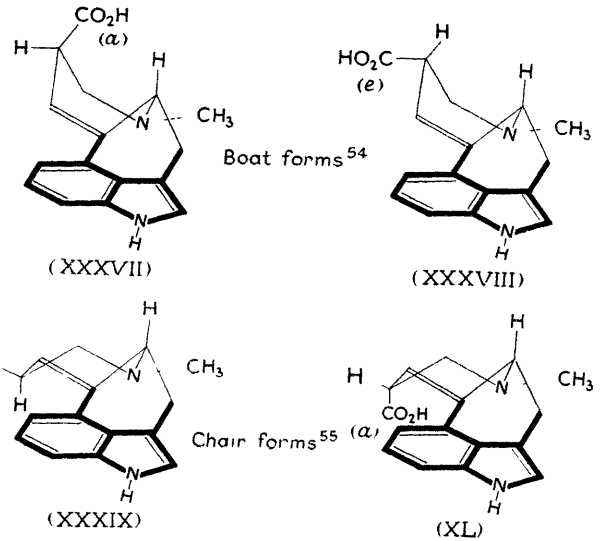
One aspect of the Stoll theory which does not appear to have received attention relates to the racemisation mechanism. When the alkaloids are subjected to fission by hydrazine,[43, 44] the lysergic acid residue appears in the form of *rac.-iso*lysergic acid. Jacobs's theory, as modified by Adams and Mahan, accounted for this, since racemisation would concern the asymmetric centre at position 8 and the mechanism would be obvious in view of the attached carboxyl group. According to Stoll's theory, however, racemisation concerns position 5 and it is difficult to see how this centre could be racemised without a momentary shift of the double bond from the 9 : 10- to the 5 : 10-position, as envisaged earlier by Jacobs and Craig.[29] An indication that the double bond is concerned in the racemisation is furnished by the absence of racemisation during the treatment of the dihydro-alkaloids with hydrazine.[52]

* In this connection, it is interesting that the same stereochemical transformation occurs in the conversion of the methyl ester of dihydronor*iso*lysergic acid II into dihydrolysergic acid II by transmethylation, during which the methyl group of an amino-acid methyl ester migrates from carboxyl to nitrogen. In producing epimerisation at position 8 of ergoline, transmethylation is thought to involve a racemisation mechanism previously unknown.

Conformational Analysis of Lysergic Acid Derivatives

By applying the methods of conformational analysis, various speculations regarding the finer points of the stereochemistry of lysergic acid and its derivatives have been independently advanced by Cookson [54] and Stenlake.[55] Whilst these authors agree concerning the dihydro-acids, they do not with regard to the unsaturated acids. The assignments for the latter are shown in (XXXVII)—(XL).

Lysergic acid *iso*Lysergic acid

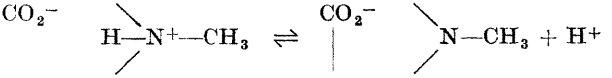


(XXXVII) (XXXVIII)

(XXXIX) (XL)

In arranging the above formulae so that they emphasize the variation in distance between the carboxyl group and the ring-D nitrogen, it has proved impossible to incorporate the latest views on the conformation of *cyclo*hexene (Barton, Cookson, Klyne, and Shoppee, *Chem. and Ind.*, 1954, 21; Raphael and Stenlake, *ibid.*, 1953, 1286).

In the form of their strong acid salts, lysergic acid and its isomer are dibasic acids, comprising a carboxyl group and a much weaker cation acid formed from the nitrogen atom in ring D. The present discussion concerns the relative strengths of the cation acids, so that the following equilibrium applies :



As mentioned on p. 205, the observed difference between the cation-acid strengths can only be explained by steric factors. In the zwitterion form therefore, the two isomers must differ in respect of the distance between the carboxyl group and the nitrogen atom in ring D. According to standard explanations [56] of the relative strengths of the two dissociating groups of

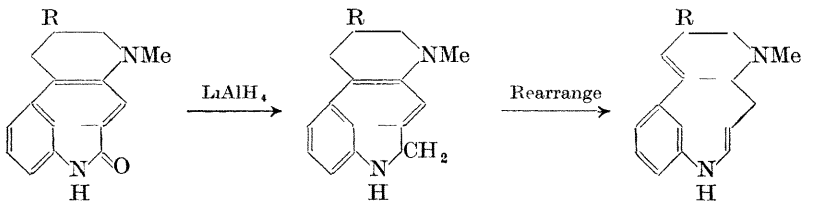
[56] Ingold, "Structure and Mechanism in Organic Chemistry", Bell & Sons, 1953, Chap. 13.

a dibasic acid, increased distance between the two groups strengthens the weaker group. Adoption of this view by one author,[55] together with the use of its converse by the other,[54] largely explains the divergent conclusions. Whilst these speculations explain some of the facts, they will probably need considerable elaboration before they give a truly satisfactory explanation. At present, however, further experimental evidence seems to be necessary.* Space does not permit a discussion of Cookson's views concerning the dihydro-acids, but the conclusions as to configuration may be seen in (XXXI)—(XXXIV).

Attempts to Synthesise Lysergic Acid and its Isomer

A number of attempts have been made to synthesise lysergic acid. However, apart from providing additional routes to the dihydro-acids, all have so far proved fruitless. All that can be done in this Review is to indicate the broad trends and to give a few examples. It is convenient to divide the various approaches into two groups according to the order in which rings B, C, and D of the ergoline nucleus are completed. In the "first approach", ring D is completed before B and C, this being reversed in the "second approach". Examples of the first may be found in the earlier syntheses [35, 36] of dihydrolysergic acid.

Two possible ways of completing rings B and C are illustrated by the conversion of (XIV) into (XV) and by the transformation of (XLII) into (XLIII). The latter has proved impossible to date, but even if it could be achieved, it would not provide rigid synthetic confirmation of the 9 : 10-double bond in lysergic acid. Whilst the conversion of (XIV) into (XV) has been carried out,[27] it has the disadvantage of sudden reduction of yield in the final stage of synthesis owing to the formation of the by-product (XVI). The following example [57] of the first approach is of interest, for its failure brings to light a general consideration that must apply to any synthesis of the unsaturated acid.

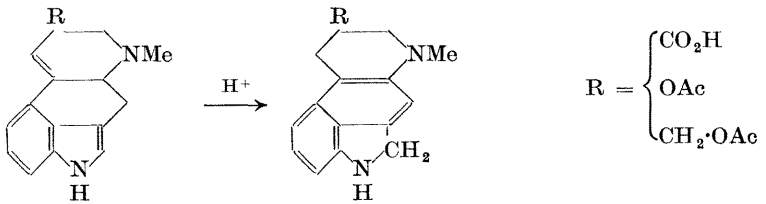


(XLI) (XLII) (XLIII)

[57] Stoll, Petrzilka, and Rutschmann, *Helv. Chim. Acta*, 1950, **33**, 2254.

* When considered with the aid of Fischer–Hirschfelder (Catalin) models, these theories are not entirely convincing, because of the uncertainties arising from the peculiar strains associated with the 9 : 10-unsaturated ergolene ring system and to some extent with ergoline itself. For example, it is difficult to see how the above decisions between boat and chair forms of the unsaturated acids were made. However, when considered in the light of the p*K* and other evidence offered by Stenlake, the models give a plausible indication that the carboxyl group of lysergic acid should be assigned an equatorial position, irrespective of whether one chooses the boat or the chair form.

Compound (XLI), prepared from 4-aminonaphthostyril,[58] was reduced to (XLII), which was an isomer of the two lysergols in the case when R was CH_2·OH. It was a tempting possibility that the corresponding lysergol (XLIII) could be obtained by rearrangement of the two double bonds in ring C of (XLII). However, numerous attempts [59, 60] to carry out this rearrangement have failed, as might be expected [49, 59] by consideration of the resonance energies of (XLII) and (XLIII). A striking demonstration of the improbability of the above rearrangement has been given by an investigation [59] of the decomposition of lysergic and *iso*lysergic acid in the presence of hydrogen ions : the typical indole reaction disappears and the decomposition involves transformation of (XLIV) into (XLV). Kinetic experiments have established that the equilibrium of this reaction favours the product to such an extent that after a time no trace of indole derivatives remains. It follows, first, that attempts to rearrange (XLII) to (XLIII) are almost certain to fail and, secondly, that in any synthesis of the unsaturated acids serious attention must be paid to the tendency represented by (XLIV) → (XLV).

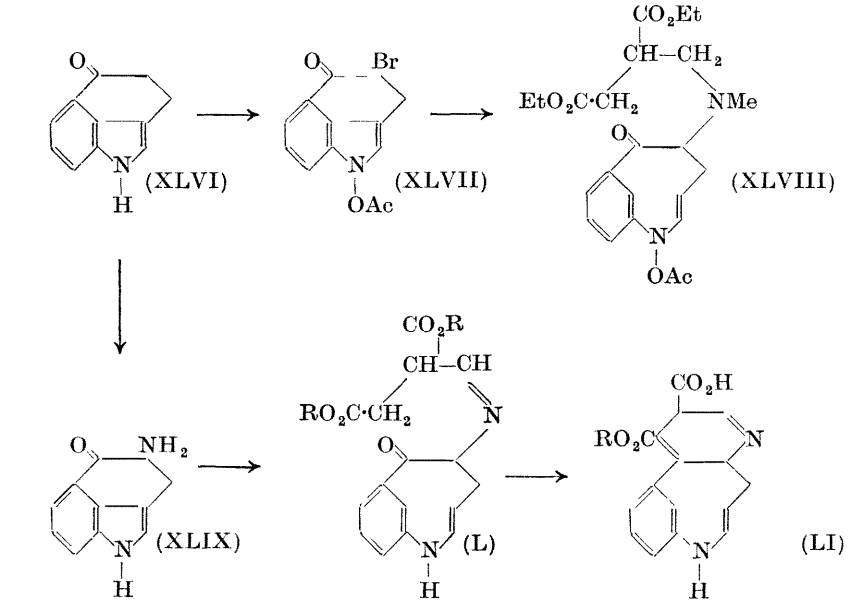


(XLIV) (XLV)

The second approach was suggested by Uhle [61] in order to overcome the disadvantage of by-product (XVI) in the conversion of (XIV) into (XV). He worked out the synthesis of the intermediate, 1 : 3 : 4 : 5-tetrahydro-5-oxobenz(*c*,*d*)indole (XLVI), before commencing the formation of ring D. Attempts to construct the latter are shown in the reaction sequences (XLVI)—(XLVIII) [62] and (XLVI)—(LI). [63] The general idea of condensing the keto-group of (XLVI) with an active methylene group of a compound that is later formed into ring D seems to constitute a rigid method of introducing the 9 : 10-double bond. In the examples cited, this takes the form of an intramolecular Stobbe condensation.

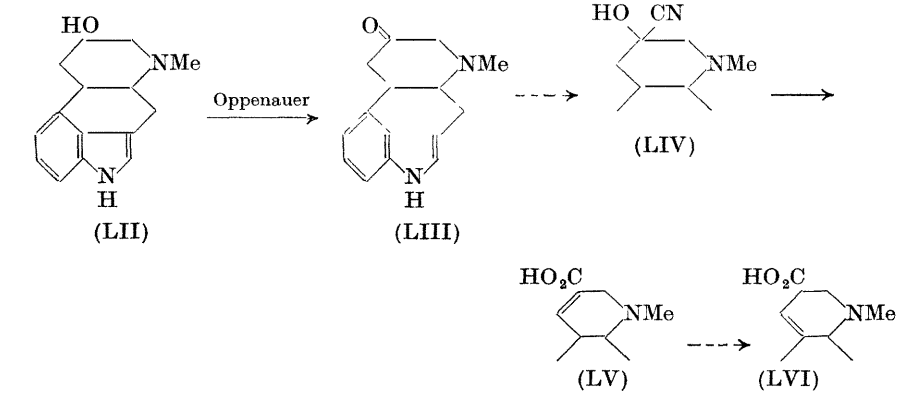
Ring closure of (XLVIII) has not been reported but, if it could be effected, there would still remain the difficulty of removing the 9-substituent. The same problem arises with (LI), in which ring D has been successfully closed. It seems that condensation of the keto-group of (XLVI) with an active methylene group is bound to leave the activating 9-substituent at

[58] Stoll and Rutschmann, *Helv. Chim. Acta*, 1951, **34**, 382.
[59] Stoll and Petrzilka, *ibid.*, 1953, **36**, 1125.
[60] Atherton, Bergel, Cohen, Heath-Brown, and Rees, *Chem. and Ind.*, 1953, 1151.
[61] Uhle, *J. Amer. Chem. Soc.*, 1949, **71**, 761.
[62] *Idem*, *ibid.*, 1951, **73**, 2402.
[63] Stoll, Rutschmann, and Petrzilka, *Helv. Chim. Acta*, 1950, **33**, 2257.



the end of the reaction, and it is not clear how this group could be removed without breaking ring D or shifting the double bond.

Finally, we may mention an attempt [64] more or less in between the two approaches. In this, rings B and C are completed about half-way through the synthesis, the precursor of ring D being formed at an early stage. The final stages of this proposed route are shown in (LII)—(LVI). A small yield of the ketone (LIII) has been obtained by Oppenauer oxidation of *rac.*-8-hydroxy-6-methylergonine (LII). It is then hoped to reach lysergic acid by forming the cyanohydrin (LIV), dehydrating and hydrolysing this



[64] Stoll, Petrzilka, and Rutschmann, *Helv. Chim. Acta*, 1952, **35**, 1249.

to (LV) and then rearranging the product to (LVI). However, the latter rearrangement would hardly be certain enough to confirm the position of the non-indolic double bond.

The Non-lysergic Acid Portion of the Alkaloids

Since lysergic acid is common to all the typical ergot alkaloids, differences between the latter are functions of the non-lysergic acid portion of the molecule. This subject received close attention along with the early work on lysergic acid.

Ergometrine.—This alkaloid is in a class of its own (cf. p. 193), the non-lysergic acid portion being a simple amino-alcohol. Within a few months of its isolation, the alkaloid was known to be the amide of *D*-lysergic acid and L-2-amino-propan-1-ol.[21, 42] This was later confirmed by its partial synthesis [23] from *D*-lysergic acid of natural origin and L-2-amino-propan-1-ol.

Alkaloids of the Ergotoxine and Ergotamine Groups.—Apart from ergometrine, all the typical ergot alkaloids belong to one or other of these groups and possess the same basic structure. The first useful conclusions as to the nature of this part of the alkaloid molecule came from a study of reductive hydrolysis of ergotinine with sodium and butanol. In this way, Jacobs and Craig [24] obtained seven bases, two of which (the dihydrolysergols) were reduction products of lysergic acid. The other five were evidently fragments of the non-lysergic acid portion and identification [38, 65, 66] of one of them as D-proline methyl ester suggested that the rest might also be amino-acid derivatives. Attention was therefore paid to the products of direct hydrolysis with alkali and concentrated hydrochloric acid.[38] The resultant information cleared up the structures of the remaining four products of reductive hydrolysis. Two of the latter were identified as 2-pyrrolidylmethanol and 2-amino-3-phenylpropanol, whilst the other two were piperazines. The isolation of the piperazines was subsequently important as an indication of the cyclol structure of the non-lysergic acid portion.

The products of acid hydrolysis of ergotinine were shown to be lysergic acid, ammonia, dimethylpyruvic acid, D-proline, and L-phenylalanine. (Evidently, ergocristinine was the main constituent of the ergotinine used in these experiments.) The acid hydrolysis products of all the other alkaloids of the ergotoxine and ergotamine groups were found to follow the same pattern.[8, 65, 67, 68] In every case, *D*-lysergic acid, ammonia, and D-proline were obtained and in addition there was a keto-acid and another amino-acid, which will be referred to as the " second amino-acid ". The alkaloids thus differed in regard to the exact structure of the last pair of hydrolysis products and the results are summarised in Table 3.

At this stage it was quite clear that the non-lysergic acid portion of the alkaloids possessed a polypeptide structure having the same basic layout

[65] Jacobs and Craig, *J. Amer. Chem. Soc.*, 1935, **57**, 960.
[66] *Idem, ibid.*, p. 383.
[67] *Idem, J. Org. Chem.*, 1936, **1**, 245.
[68] Stoll, Hofmann, and Becker, *Helv. Chim. Acta*, 1943, **26**, 1602.

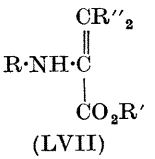
TABLE 3

	Alkaloid	Keto-acid fragment	Second amino-acid
Ergotoxine group	Ergocristine Ergokryptine Ergocornine	Dimethylpyruvic acid	L-Phenylalanine L-Leucine L-Valine
Ergotamine group	Ergotamine Ergosine	Pyruvic acid	L-Phenylalanine L-Leucine

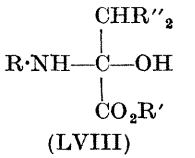
in all of them. In the later work, Stoll *et al.*[68] successfully exploited high-vacuum fission of the alkaloids, isolating a keto-amide, together with a dipeptide which could be hydrolysed to D-proline and the second amino-acid.

Determination of the Structures of the Polypeptide Side chains.—Having identified the units from the side chain, the next problem was to determine their arrangement and modes of combination, which from the hydrolytic studies seemed to entail only amide and ester linkages. Whilst there was every reason to suppose that proline and the second amino-acid existed as such in the original molecule, this view did not appear to hold for the keto-acid. Furthermore, catalytic hydrogenation [69] confirmed the view that the latter came from some precursor. For example, hydrolysis of dihydroergotinine gave dimethylpyruvic acid but, if this had existed as such in the original molecule, it would have been expected to appear in the form of its reduction product, α-hydroxy*iso*valeric acid. It was improbable that participation in the polypeptide side chain would protect the keto-group from hydrogenation. Investigation of ergotamine and its dihydro-derivative led to a similar conclusion.

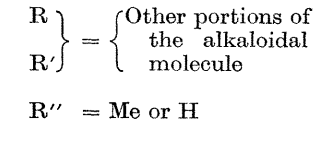
In 1935, Jacobs and Craig [38] suggested that the precursor of the keto-acid might be either an unsaturated alanine derivative (LVII) or a hydroxyvaline derivative (LVIII). Of these, (LVIII) was later preferred, so that α-hydroxyvaline was regarded as the precursor of dimethylpyruvic acid.



(LVII)



(LVIII)

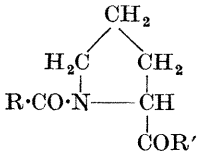


It was not improbable that acylation in the polypeptide chain would confer stability on an otherwise unstable structure.

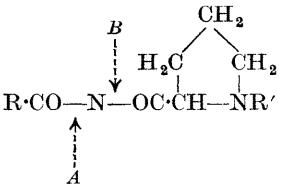
There were several indications [69] concerning the gross structure of the polypeptide side chain. It was possible to rule out such structures as (LIX) and (LX), in which lysergic acid was linked through a common nitrogen atom to proline or the second amino-acid. Thus, the appearance

[69] Jacobs and Craig, *J. Biol. Chem.*, 1938, **122**, 419.

of both ammonia and ergine among the hydrolysis products eliminated structures of the type (LIX). In imide structures such as (LX), both linkages *A* and *B* would possess about the same strength ; however, the formation of ergine under mild hydrolytic conditions indicated that linkage *B* was much weaker than *A*, so linkage *B* was not a typical peptide bond. On the positive side, it appeared that proline was directly linked to the second amino-acid on account of the appearance of these two amino-acids as dioxopiperazines. Further, the alkaloids gave no evidence of a carboxyl group or a salt-forming basic centre, apart from the *N*-methyl group of lysergic acid. This demanded an assembly of the structural units such that no amino- or carboxyl group remained uncombined. A cyclic structure was the obvious answer.



(LIX)

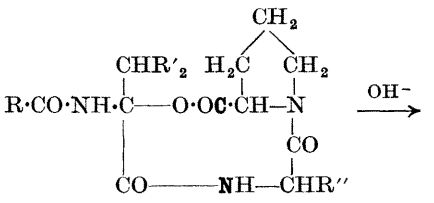


(LX)

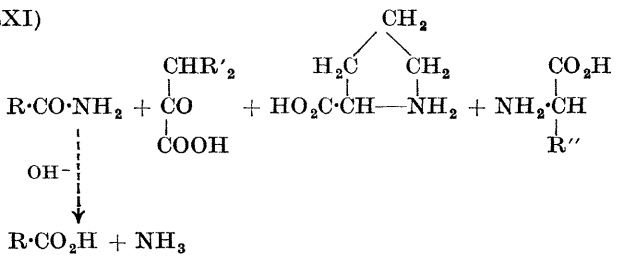
R = Lysergic acid ring system

R′ = Rest of the polypeptide side chain

Structure (LXI) was advanced [70] in the light of the above arguments. It incorporates all the features considered necessary and accounts for the hydrolysis products. In particular, the placing of the keto-acid precursor accords with the evidence that the ergine-nitrogen atom does not participate in an imide structure. In an alternative formula,[69] which was also supported by the above evidence, the proline and the second amino-acid were



(LXI)



R = Lysergic acid ring system ; R′ = Me or H ; R″ according to the structure of the second amino-acid

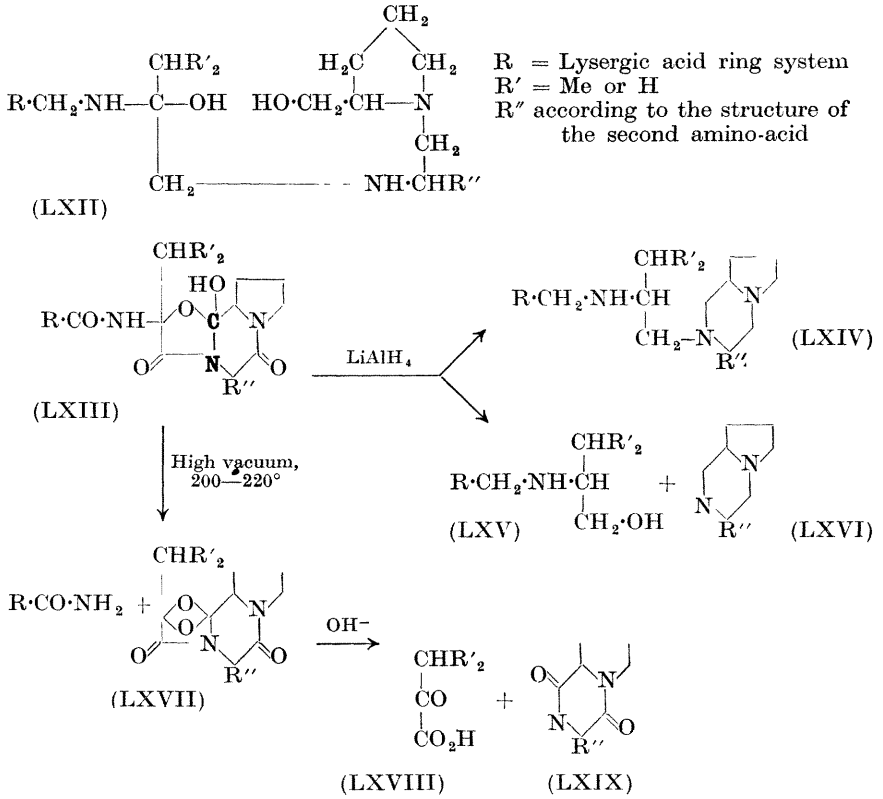
[70] Barger, " Handbuch der experimentellen Pharmakologie ", Suppl. Vol. VI, 1938, pp. 84, 221.

transposed. However, the precise amino-acid arrangement in (LXI) was confirmed by Stoll and his co-workers [71] in 1949. When either the alkaloids or their dihydro-derivatives were heated with hydrazine, the molecule was split into two parts, yielding an acyldipeptide and the appropriate lysergic acid derivative. Although dipeptide components were also isolated, the main interest centred around the acyldipeptides, for these must be closely related to the complete polypeptide side chain ; their structures were worked out by hydrolysis and confirmed by synthesis. As example, the acyldipeptide from dihydroergocristine was found to be *iso*valeryl-L-phenylalanyl-L-proline, in which the keto-acid normally obtained appeared in its reduced form as a result of the hydrazine treatment. It will also be noted that, contrary to earlier results, the proline appeared in the L-configuration. Subsequent work [72] has shown that the alkaloidal molecule actually contains L-proline and that the D-form arises as an artefact under certain hydrolytic conditions. Acyldipeptides were also obtained [73] by the careful hydrolysis of the alkaloids with one equivalent of potassium hydroxide in aqueous alcohol. Under these conditions, dihydroergocristine gave dimethylpyruvoyl-L-phenylalanyl-L-proline, in which the keto-acid was obtained unreduced, as in the early experiments.

The Cyclol Structure of the Polypeptide Side Chains.—The nine-membered lactone ring probably constitutes the least likely feature of structure (LXI), and the original alkaloid might well contain two smaller rings in its place. An early indication of this was afforded by the isolation of dioxopiperazines as hydrolysis products, for it was most unlikely that these six-membered ring structures could be formed from free amino-acids during hydrolysis. In accordance with this view, the alkaloids were reduced with lithium aluminium hydride [72] in the hope that the expected reduction of amide linkages to the amine stage might show up structural subtleties which had not been revealed in the hydrolytic experiments. This has led to what now appears to be the final structure for the polypeptide side chains.

On treatment with lithium aluminium hydride, the structure (LXI) would be expected to give a poly-amino-alcohol of type (LXII). However, the actual products of such treatment were identified as (LXIV), (LXV), and (LXVI), their structures being proved by syntheses of a kind well known in polypeptide work. The structures of compounds (LXIV) and (LXVI) provided strong evidence for the existence within the original molecule of bicyclic precursors of the dioxopiperazines which appear on hydrolysis. It was therefore necessary to divide the nine-membered lactone ring of (LXI) into a five- and a six-membered ring. The resultant structure is shown in (LXIII), in which an additional bond will be noted between the proline-carboxyl carbon atom and the nitrogen atom of the second amino-acid (both shown in heavy type). This additional bond required the formation of a tertiary alcoholic group from the carbonyl-oxygen atom of the proline-carboxyl group.

[71] Stoll, Petrzilka, and Becker, *Helv. Chim. Acta*, 1950, **33**, 57.
[72] Stoll, Hofmann, and Petrzilka, *ibid.*, 1951, **34**, 1544.
[73] Stoll and Hofmann, *ibid.*, 1950, **33**, 1705.

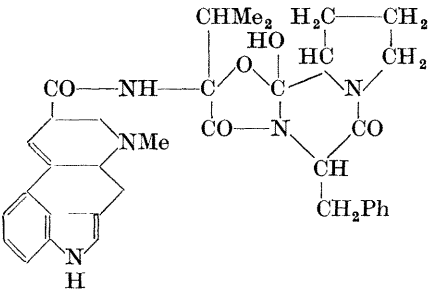


The fact that the primary alcoholic group of (LXV) must have arisen during the lithium aluminium hydride treatment furnished good evidence for the tertiary nature of the nitrogen of the second amino-acid in (LXIII) : for, under lithium aluminium hydride reduction, aldehydes result from tertiary but not from secondary amides. Further evidence in favour of (LXIII) was provided by the high-vacuum thermal fission of the alkaloidal molecule into ergine and the peptide derivative (LXVII). The latter contained all the carbon atoms of the polypeptide side chain, and its appearance could scarcely have been explained by (LXI) though it is readily intelligible on the basis of (LXIII) ; in particular, it was evident that the removal of the lysergic acid portion in the form of ergine was facilitated by the tertiary hydroxyl group of (LXIII), which was converted into an ether linkage during the thermal fission.

Another structural feature to be established by (LXIV) and (LXV) is the amide linkage between lysergic acid and the amino-group of the α-amino-α-hydroxy-acid. Final proof of the configuration of the proline in the original alkaloid is thought to have been offered by the fact that the residue of this amino-acid in the polyamines (LXIV) and (LXVI) possesses the L-configuration. In view of the mild reduction employed, it is most unlikely

that inversion of the proline could have occurred, so that in the alkaloid molecule this acid must also exist in the same L-configuration. It is possible that production of D-proline by more vigorous hydrolysis may be explained by the guidance of the asymmetric carbon atom in (LXIII) bearing the tertiary hydroxyl group. Structure (LXIII) is of special interest to protein chemistry, for it seems to constitute the first proved example of a polypeptide having the cyclol structure proposed by Wrinch.

At this point, it is safe to conclude that the structure of all the typical ergot alkaloids has been established beyond reasonable doubt. By way of example, the complete structural formula of ergocristine is annexed.



In conclusion, the author thanks Dr. D. W. Mathieson for reading the manuscript and offering many valuable suggestions, also Drs. A. H. Beckett and J. B. Stenlake for helpful discussions.