THE ERGOT ALKALOIDS

XIV. THE POSITIONS OF THE DOUBLE BOND AND THE CARBOXYL GROUP IN LYSERGIC ACID AND ITS ISOMER. THE STRUCTURE OF THE ALKALOIDS

By LYMAN C. CRAIG, THEODORE SHEDLOVSKY, R. GORDON GOULD, Jr., and WALTER A. JACOBS

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

(Received for publication, June 3, 1938)

In our previous studies (1) it has been shown that lysergic acid is a cleavage product formed on alkaline hydrolysis of all of the alkaloids which are characteristic of ergot. Since this acid is unique and occurs in these alkaloids conjugated with amino acids (or with hydroxyisopropylamine in the case of ergometrine), it was concluded that lysergic acid itself or perhaps an isomer must be responsible for the unique pharmacodynamic properties of these alkaloids. It was then shown (2, 3) that the double bond of lysergic acid functions in some way in the isomerism of the levorotatory biologically active alkaloids and the dextrorotatory inactive alkaloids, since the dihydro alkaloids lost this property. In conformity with this, it was also noted that the hydrogenated levorotatory alkaloids of the ergotoxine series gave α-dihydrolysergic acid on hydrolysis, whereas the isomeric dextrorotatory alkaloids yielded the isomeric γ-dihydrolysergic acid (2, 3). From the first, we were inclined to regard lysergic acid itself as the component of the active alkaloids, since lysergic methyl ester mutarotated in solution, becoming more strongly dextrorotatory. However, an uncertainty persisted, since lysergic acid on hydrogenation was found to yield not only α-dihydrolysergic acid, but also (although in smaller amount) γ-dihydrolysergic acid. In our subsequent, unpublished attempts to clarify this point it was soon found that lysergic methyl ester, unlike lysergic acid itself, behaved on hydrogenation like the levorotatory

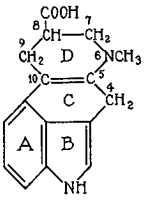
alkaloids of the ergotoxine series. From the reaction mixture only α-dihydrolysergic acid could be isolated and no γ-dihydrolysergic acid. Thus direct evidence was obtained that the active alkaloids of the ergotoxine-ergometrine series must be derivatives of lysergic acid itself. The simultaneous formation of γ-dihydrolysergic acid with the α acid from lysergic acid must be explained apparently by the more ready partial isomerization of the latter under the conditions of the hydrogenation.

In the meantime, additional evidence that lysergic acid occurs as such in the active alkaloids has been presented from another angle in the recent reports of Stoll and Hofmann (4). Also the report of the isolation by Smith and Timmis (5) (which anticipated work that was naturally already in progress in our laboratory) of a very strongly dextrorotatory acid, isolysergic acid, by the isomerization of lysergic acid made it appear probable that this isomer is the form which occurs in the dextrorotatory alkaloids of the ergotinine type.

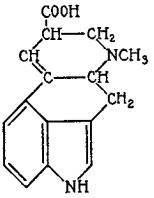
On the basis of degradation studies a tetracyclic structure for lysergic acid has been derived, as given in Formula I (2, 3). Such a structure has been supported by the properties of synthetic substances possessing this ring structure ((6) and unpublished work). In the formula given, the position of the carboxyl group, with certain limitations, remained tentative. Similarly, questions concerning the manner in which the double bond functions in the isomerism of the two series of alkaloids remained to be determined. Comparison of the ultraviolet absorption spectra (7) of lysergic acid and the parent alkaloids with those of the hydrogenated substances indicated quite conclusively that the double bond must be conjugated with the benzene (or pyrrole) ring of the indole nucleus and therefore must be present in the hydroquinoline portion. It was still uncertain whether its position remains the same in the two series of alkaloids and the isomerism is due to different configurations on an asymmetric carbon atom, or whether the double bond occupies different positions in each series of alkaloids—in other words, whether the isomerism is purely stereochemical or structural. In either case it must be concluded that the double bond can undergo change of position quite easily. Certain evidence will be presented in the present report bearing on the question of the exact position of the double

bond in each series of alkaloids as well as that of the carboxyl group in lysergic acid.

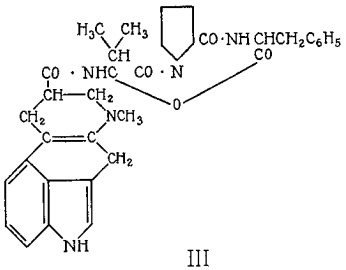
It is well known that the proximity of a double bond influences the basicity of a basic group and the acidity of an acid group (8). With this in mind we have made a study of the dissociation constants of a number of derivatives of lysergic acid in the hope that such data would throw light on the remaining uncertainties of lysergic acid.



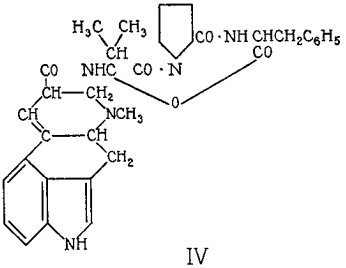
I



II



III



IV

Since the interpretation of effects is somewhat more difficult when both a basic and an acid group are present, substances were first studied in which the acid group is covered. The three alkaloids ergometrine, ergometrinine, and dihydroergometrine proved to be suitable for this purpose because of their sufficient solubility in water. In each of these substances the carboxyl group is joined to hydroxyisopropylamine in amide linkage and therefore cannot act as an acid. Conversely, basic properties of hydroxyisopropylamine are essentially removed by acylation. The $CH_3N=$ group is the only basic group present in the molecule, since the indole nitrogen, as is well known, lacks basic properties. The basic apparent dissociation constants obtained from pH

measurements made at the mid-points of the titration curves are given in Table I.

TABLE I

Dissociation Constants of Lysergic Acid and Its Derivatives

	pK'_2 (pH half neutralized with NaOH)	Temperature	pK'_1 (pH half neutralized with HCl)	Temperature
		°C.		°C.
Lysergic acid	7.96	24	3.19	24
	7.68	24	3.44	24
	7.70*	38	3.20*	38
	7.75	38	3.46	38
Isolysergic acid	8.31	24	3.21	24
	8.61	24	3.44	24
	8.40	38	3.50	38
α-Dihydrolysergic acid	8.45	24	3.57	24
	8.10	38	3.70	38
γ-Dihydrolysergic "	8.57	24	3.60	24
	8.64	24		
	pK' (pH half neutralized with HCl)			
Ergometrine	6.68	24		
	6.91	24		
	6.60	24		
Ergometrinine	7.26	24		
	7.38	24		
Dihydroergometrinine	7.30	24		
	7.46	24		
α-Dihydrolysergol	8.30	24		
β-Dihydrolysergol	8.23	24		
6-Methyl ergoline	8.84	24		
	8.89	24		
	8.87	24		

* Measured at twice the concentration.

These figures show ergometrine to be a weaker base than ergometrinine. The difference appears to be essentially due to the effect of the position of the double bond in relation to the NCH_3 group. This interpretation is supported, as later discussed, by

the measurements made on lysergic acid, isolysergic acid, and their dihydro derivatives.

Examination of Formula I will show that the only positions possible for the double bond are (4–5), (5–10), (10–9), (9–8), and (8–7). Regardless of where the carboxyl group is placed, positions (9–8) and (8–7) are incompatible with absorption spectra studies (7) which have shown that the double bond must be conjugated with the indole nucleus in some way.

If we turn to positions (4–5) and (5–10) in which the double bonds are equidistant from the NCH_3 group, to explain the difference between the two isomers, the experimental evidence appears to be against such a view. The fact that ergometrine is the weaker base and that there is little difference between ergometrinine and dihydroergometrine[1] makes it necessary to conclude that the double bond in ergometrinine must be further removed from the NCH_3 group than in ergometrine. Therefore position (4–5) or (5–10) appears excluded in the case of this alkaloid. The only position remaining to meet the requirements for this alkaloid is (10–9). Conversely, the double bond of the ergometrine series (*i.e.* lysergic acid) must be located at position (5–10).[2]

Similar conclusions are possible from measurements which have been made with lysergic acid, isolysergic acid, and the dihydrolysergic acids. In these cases since both acid and basic groups are free, conditions are met similar to those noted with amino acids, which can therefore best be interpreted in terms of the zwitter ion theory. If according to this concept we take as the basic apparent dissociation constant the pH measured when the substance is half neutralized with sodium hydroxide (pK'_2), we obtain the values given in Table I.

Again as in the case of the alkaloids it is found that the basic group of lysergic acid is weaker than that of isolysergic acid. The average difference at 24° is seen to be about 0.64 of a unit in both series of determinations. Also the strengths of the basic

[1] Dihydroergometrinine was not available for these studies.

[2] Although this appears to leave open position (4–5) for the double bond of lysergic acid, position (5–10) remains definitely to be preferred for a number of reasons. We hope to obtain conclusive evidence of this by work which is now in progress.

group in the dihydro acids and isolysergic acid show a close approximation. Since we can now accept that ergometrine (the ergotoxine series) is derived from lysergic acid while ergometrinine (the ergotinine series) is derived from isolysergic acid, the values found are consistent with those found for the alkaloids themselves.

In the case of the acid dissociation constants, pK'_1 (the pH measured when the substances were half neutralized with HCl), no significant difference was noted between lysergic and isolysergic acids. On passing to the dihydro acids in each case a slight weakening became apparent.

Certain inferences in regard to the position of the carboxyl group can now be drawn. In order to permit the formation of the quinoline betaine tricarbonic acid, $C_{14}H_9O_8N$ (9, 2), and also in order to furnish a necessary asymmetric carbon atom in lysergic acid, the only positions of the carboxyl group which are possible are positions (4), (9), (8), and (7) (Formula I). Of these, position (9) appears eliminated, since migration of the double bond between positions (5–10) and (10–9) should cause ready racemization.

Position (4) appears unlikely on biogenetic grounds because of the inferred relationship to tryptophane. It is still less likely on chemical grounds. Although when heated at low pressure above 200°, lysergic acid loses carbon dioxide and methylamine, dihydrolysergic acid, on the contrary, can be sublimed at 300°. Position (4) for the carboxyl group would make the latter a substituted indoleacetic acid which should lose carbon dioxide readily on pyrolysis. There remain for consideration only positions (7) and (8). Of the two, position (8) appears to be indicated by a comparison of the relative magnitudes of the basic dissociation constants of α-dihydrolysergic acid and synthetic 6-methyl ergoline (decarboxydihydrolysergic acid) (6). It should be mentioned in passing that the values found for the dihydrolysergols are in agreement with what should be expected from such a hydroxy derivative of 6-methyl ergoline.

From the data given in Table II it can be seen that substitution of a COOH group in an aliphatic amine in a position α to the NH_2 group reduces the basic dissociation constant by about 1.0 unit, while substitution in the β position diminishes it by about 0.5 unit. The average value of the dihydrolysergic acids (8.55 at 24°) was

found to be 0.32 unit lower than that of 6-methyl ergoline which is in best agreement with a carboxyl group in the β position or position (8). Similarly the magnitude of the dissociation constant of dihydroergometrine likewise indicates β substitution.

If we consider the dissociation constants of the series ethylamine (10), β-alanine ethyl ester (11), and alanine ethyl ester (11) (Table II), the relative effect of the carbethoxyl group on the basicity of the amino group can be seen. The COOR group is very negative in character, having approximately the same influence as a CON= group, as can be seen from the fact that glycine ethyl ester has the same dissociation constant as glycylglycine ethyl ester (11). From this series it is evident that a COOR group

TABLE II

	pK′ (pH half neutralized with HCl)	
Ethylamine	10.7	
Alanine ethyl ester	7.80	
β-Alanine " "	9.13	
6-Methyl ergoline	8.87	
Dihydroergometrine	7.30, 7.46	
	pK′$_2$ (pH half neutralized with NaOH)	pK′$_1$ (pH half neutralized with HCl)
Alanine	9.72	2.39
β-Alanine	10.19	3.60
α-Dihydrolysergic acid	8.45	3.57, 3.70
γ-Dihydrolysergic "	8.57, 8.64	3.60

in the α position diminishes the value of pK′ for ethylamine by 2.9 units, while in the β position the decrease is 1.57 units. When the average value of dihydroergometrine, 7.38, is compared with that of 6-methyl ergoline, 8.87, it is found that the introduction of the CONHR group has reduced the pK′ by 1.49 units. This is in good agreement with the effect noted for such a substituent in the β position.

A further indication of the β position is to be seen in the magnitude of the acid dissociation constant (pK′$_1$) of the dihydrolysergic acids, *viz.* 3.6 (average), which is in good agreement with that of β-alanine (3.6) (11), while that of alanine and other α-amino acids is roughly 2.3 (11).

It is therefore provisionally concluded that lysergic acid is represented by Formula I and isolysergic acid by Formula II. The pharmacologically active alkaloids of the ergotoxine group are derivatives of lysergic acid, while the very strongly dextrorotatory isomeric alkaloids of the ergotinine group are derived from an isomeric lysergic acid. The completed formula of ergotoxine would therefore be most satisfactorily represented by Formula III and that of ergotinine by Formula IV. By replacement of the α-hydroxyvaline by α-hydroxyalanine in these formulas, the formulas of ergotamine and ergotaminine are represented respectively. Finally, by replacing the terminal phenylalanine in the latter by *l*-leucine, the formulas of ergosine and ergosinine are obtained.

Further work is naturally in progress to ascertain the validity of these conclusions regarding the structure of lysergic acid, its derivatives, and the parent alkaloids.

EXPERIMENTAL

Preparation of Isolysergic Acid—The method of preparation of the crude acid was essentially that reported by Smith and Timmis (5). However, since it was found difficult to repeat the purification of the product by their method, the following procedure was found to give sharply and rapidly the desired result. Although in accordance with this the acid can be sharply separated from lysergic acid by crystallization from dilute ammonium hydroxide, it was found impossible to effect similar separation by crystallization from equivalent amounts of dilute acetic acid or by fractional precipitation from the hydrochloride.

1.630 gm. of lysergic acid were boiled in an atmosphere of hydrogen for 6 hours. The solution was treated with a little bone-black to remove the color and the filtrate was cooled in ice. 0.9 gm. of crystals was collected which showed a rotation of $[\alpha]_D^{25} = +155°$ ($c = 0.6$ in pyridine). The filtrate was quickly evaporated under reduced pressure to about 40 cc. and the crystalline material which separated was collected after cooling. 0.7 gm. of additional material was collected which had a rotation of $[\alpha]_D^{25} = +183°$ ($c = 0.57$).

The two fractions were combined and treated with 90 cc. of water to which were added 7 cc. (1 equivalent) of N ammonium

hydroxide. The mixture was warmed sufficiently for complete solution and was then quickly cooled in ice. 0.67 gm. of crystalline material was collected after cooling for several hours in ice. The rotation was $[\alpha] = +282°$ ($c = 0.5$ in pyridine for the anhydrous substance), which is in good agreement with that reported by Smith and Timmis ($[\alpha]_D = 281°$). A repetition of the above treatment with dilute ammonia did not raise the rotation of the recovered substance.

Determination of Dissociation Constants—The materials used in obtaining the data given in Table I were prepared according to directions given by us in previous publications, with the exception of isolysergic acid. All gave correct analyses, rotations, and melting points.

The pH values were obtained with the glass electrode (12) with solutions half neutralized and then made up to volume. In the case of comparisons between isomers, equal weights of substances were treated with the calculated 0.5 equivalent of standard acid or base from a microburette. On check runs, a full equivalent of acid or base was employed and the solution was brought back to the half neutralization point by addition of 0.5 equivalent of standard base or acid. Such a procedure insured that all would be in solution when the concentration was just above saturation. The solutions were then diluted to equal volumes. The solutions were roughly 0.002 N with respect to the substance being measured.

For example, 3.0 mg. of α-dihydrolysergic acid were treated with 0.108 cc. of 0.1028 N HCl. After solution by addition of a fractional volume of water, 0.055 cc. of 0.1016 N NaOH was added and the volume was made up to 5 cc.

Measurements of pH were made at 24° and also at 38° and in a number of cases checked after dilution.

BIBLIOGRAPHY

1. Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, **104**, 547 (1934); *J. Org. Chem.*, **1**, 245 (1936); *Science*, **82**, 16 (1935).
2. Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, **113**, 767 (1936).
3. Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, **115**, 227 (1936).
4. Stoll, A., and Hofmann, A., *Z. physiol. Chem.*, **251**, 155 (1937).
5. Smith, S., and Timmis, G. M., *J. Chem. Soc.*, 1440 (1936).
6. Jacobs, W. A., and Gould, R. G., Jr., *J. Biol. Chem.*, **120**, 141 (1937).

7. Jacobs, W. A., Craig, L. C., and Rothen, A., *Science*, **83**, 166 (1936).
8. Hixon, R. M., and Johns, I. B., *J. Am. Chem. Soc.*, **49**, 1786 (1927).
9. Jacobs, W. A., *J. Biol. Chem.*, **97**, 739 (1932).
10. Roth, W. A., and Scheel, K., Landolt-Börnstein physikalisch-chemische Tabellen, Berlin, 5th edition, **2**, 1157 (1923).
11. Edsall, J. T., and Blanchard, M. H., *J. Am. Chem. Soc.*, **55**, 2337 (1933).
12. MacInnes, D. A., and Longsworth, L. S., *Tr. Electrochem. Soc.*, **71**, 73 (1937).