THE ERGOT ALKALOIDS

V. THE HYDROLYSIS OF ERGOTININE*

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The reductive cleavage of ergotinine with sodium and butyl alcohol has produced a number of substances which have been described in a previous communication.[1] More recently, in the series of operations employed for the separation of these substances an additional base has been obtained as the gold double salt. The analysis of this salt has given figures which agree with a formula $C_6H_{11}O_2N \cdot HAuCl_4$. The odor of the free base indicated an amino acid methyl ester, and it became probable that we were dealing with proline methyl ester. This was then substantiated by comparison with the ester and gold double salt produced from proline itself.

This result, as well as a number of other observations which were accumulating, has given clues to the identities of the other substances which were isolated from the above reaction mixture, and therefore also to their precursors in the original alkaloid molecule. Aside from lysergic acid and isobutyrylformic acid which we had already shown[2] to result from the alkaline hydrolysis of ergotinine, the possibility appeared that these precursors were phenylalanine and proline. This has been demonstrated by a further study of the hydrolysis of ergotinine by both alkali and acid.

When ergotinine was hydrolyzed by alkali as previously described,[2] in addition to lysergic and isobutyrylformic acids a crude

* Preliminary reports of this work have appeared in the *J. Am. Chem. Soc.*, **57**, 383, 960 (1935).

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

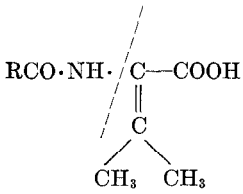
[2] Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, **104**, 547 (1934).

fraction was obtained which appeared to be of peptide character. Our attempts to crystallize it have thus far been unsuccessful. However, on further hydrolysis by strong acid it yielded proline and phenylalanine which were practically inactive. Phenylalanine was isolated as the amino acid itself, while the proline fraction was converted into the methyl ester which after distillation was identified as the gold double salt.

Since the initial alkaline hydrolysis of ergotinine caused almost complete racemization of the amino acids, a logical attempt was then made to hydrolyze this alkaloid directly with strong hydrochloric acid. Under the conditions given in the experimental part, the lysergic acid portion of the molecule was changed to dark colored, amorphous material of obscure character, and no isobutyrylformic acid appeared to survive the treatment. However, after decolorization and removal of chlorine ions, *l*-phenylalanine ($[\alpha]_D^{20} = -28°$ ($c = 0.39$ in H_2O)) was isolated without difficulty but was apparently not quite optically pure. The limited amount of material at our disposal made a repeated recrystallization impossible. The mother liquor of this amino acid on concentration yielded a relatively very small amount of compact crystals of another substance which on analysis gave figures agreeing with a formula $C_{14}H_{18}O_3N_2$ or those required by a peptide of proline and phenylalanine. Owing to the very small amount of this material at present available, its further investigation has been deferred for the moment. From the resulting mother liquor which gave a strong pyrrole test, the methyl ester was prepared. This on distillation was obtained as *d-proline methyl ester*, which exhibited a rotation of $[\alpha]_D^{25} = +34°$ ($c = 0.65$ in methyl alcohol). For comparison, the methyl ester was prepared from *l*-proline (Hoffmann-La Roche) and the rotation of this ester was found to be $[\alpha]_D^{20} = -35.5°$ ($c = 1.07$ in methyl alcohol). Thus, while the phenylalanine obtained from ergotinine is the expected natural form, we are confronted by a most unique occurrence in the appearance of the unnatural proline as a simultaneous cleavage product. This will require further investigation since it is a most unusual observation. It appears scarcely probable that a preliminary optical inversion (Walden rearrangement) of either of the amino acids could have occurred under the conditions of acid hydrolysis.

Thus, ergotinine and therefore ergotoxine are composed of

proline and phenylalanine combined in peptide linkage with lysergic acid, isobutyrylformic acid, and perhaps ammonia. If these five products of hydrolysis could be added together with loss of 4 moles of water, a substance $C_{35}H_{39}O_5N_5$ would result, which is the formula now accepted for ergotinine. However, the ammonia molecule has been shown to be directly attached to lysergic acid by the production of ergine (the amide of lysergic acid)[3] with alcoholic alkali. It is not excluded that this amide group may originally have the form of a linkage of lysergic acid on the amino group of an unsaturated valine (or hydroxyvaline) as shown in the formula



Such a substance on alkaline hydrolysis could give isobutyrylformic acid and lysergic amide, which then would go further to ammonia and lysergic acid.[4] Such a structure could account for the union of the above three components with loss of 2 moles of water. A difficulty, however, persists in the conciliation of the fact that the further addition of the two components proline and phenylalanine with the loss of 2 more moles of water would give a substance $C_{35}H_{39}O_5N_5$ which would still contain a free carboxyl group instead of a substance, such as ergotinine, which does not appear to combine as such with alkali. It is possible that an additional mole of water enters in some way in the make-up of lysergic acid itself, which is unsaturated in the remainder of the molecule, so that the apparent absence of a free carboxyl group in ergotinine may be explained by lactam or lactone formation. The opening of such a group may explain the conversion of ergotinine into ergotoxine. However, further studies which are now in progress on the partial cleavage of the ergot alkaloids with hydrolytic agents

[3] Smith, S., and Timmis, G. M., *J. Chem. Soc.*, 763, 1543 (1932); 674 (1934); *Nature*, **133**, 579 (1934).

[4] Bergmann, M., Miekeley, A., and Kann, E., *Z. physiol. Chem.*, **146**, 247 (1925).

and enzymes will, we hope, throw light on the exact nature and order of linkage of the structural units of these alkaloids.

We shall leave to a later paper the detailed discussion of the cleavage of ergotamine and ergoclavine which we have already presented elsewhere in preliminary form.[5]

The presence of phenylalanine and proline in ergotinine now explains a number of previous observations. Both benzoic and *p*-nitrobenzoic acids which were found to result on oxidation of ergotinine with nitric acid and permanganate must be products of phenylalanine. The pyrrolidine-like odor noted on destructive distillation of ergotinine must be formed from proline. The series of bases previously described as products of the reductive cleavage of ergotinine with sodium and butyl alcohol[1] can now be properly interpreted.

The substance designated as Base II, isolated as the picrate and assigned the formula $C_{14}H_{20}N_2$, must be a piperazine resulting from the reduction of prolylphenylalanine anhydride. Base IV, also isolated as a picrate but assigned the formula $C_{10}H_{20}N_2$, must be a piperazine, $C_{10}H_{18}N_2$, resulting from the reduction of proline anhydride. Base V, isolated as a di(*p*-bromobenzoyl) derivative, must be the alkamine, $C_5H_{11}ON$, or α-pyrrolidylcarbinol resulting from the reductive cleavage of the prolyl residue. Finally, Base VI, also isolated as a di(*p*-bromobenzoate), must be phenylpropanolamine, $C_9H_{13}ON$, resulting from the reductive cleavage of the phenylalanyl residue. This amine also has since been found to yield, like phenylalanine, *p*-nitrobenzoic acid on oxidation with nitric acid.[6] Since aliphatic carboxyl groups as such cannot be reduced as a rule to alcohols, the formation of the dihydrolysergols, α-pyrrolidylcarbinol, and phenylpropanolamine is to be expected from the fact that their precursors in the molecule do not occur as free acids but are conjugated.

In conclusion, mention must be made of the recent reports of the isolation of an apparently identical new alkaloid from ergot by two separate groups of workers — Dudley and Moir[7] (ergome-

[5] Jacobs, W. A., and Craig, L. C., *Science*, **81**, 256 (1935); *J. Am. Chem. Soc.*, **57**, 960 (1935).

[6] Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, **108**, 606 (1935).

[7] Dudley, H. W., and Moir, C., *Brit. Med. J.*, **1**, 520 (1935).

trine), and Kharasch and Legault[8] (ergotocin). This is unquestionably a most important contribution to the pharmacology and chemistry of ergot. The former workers have described more fully the isolation of their new alkaloid, ergometrine, which from its color reactions appeared obviously to be related to the known ergot alkaloids. We have convinced ourselves of this fact since it has yielded to the same methods which we have employed in the case of the other alkaloids. It yields lysergic acid without difficulty on alkaline hydrolysis. Also as already reported by Kharasch and Legault,[8] we have failed to detect the liberation of ammonia as in the case of the older ergot alkaloids, and in addition we could obtain no evidence of the production of isobutyrylformic or pyruvic acids. This latter point, taken in connection with the absence of ammonia formation, is perhaps significant in the light of our previous discussion.

Thus, it is evident that the new alkaloid differs from the old in respect to the nature of the other substances conjugated with lysergic acid (or its precursor in the molecule) which appears to be directly responsible for the characteristic pharmacodynamic action inherent in this group of alkaloids. It is the character of the molecule as a whole created by its conjugation with other substances which must determine the solubility, absorption, etc., which in turn determine the character and intensity of the pharmacodynamic action. The difference already noted between ergotinine and ergotoxine is but a more exaggerated instance of the contrast in action of such closely related substances.

We are therefore actively continuing the study of lysergic acid, the isolation and study of which was first described by us.[2] It appears without question to be related biogenetically to tryptophane since in still unpublished work we have found that degradation by a number of procedures has given among other substances simple indole derivatives. The benzene portion of the indole group is unsubstituted and the N-methyl group appears definitely to be attached to the non-indole nitrogen. The results of this portion of the work as well as possible structures for lysergic acid will be presented shortly in a separate communication.

[8] Kharasch, M. S., and Legault, R. R., *Science*, **81**, 388 (1935); *J. Am. Chem. Soc.*, **57**, 956 (1935).

EXPERIMENTAL

Alkaline Hydrolysis of Ergotinine—1 gm. of ergotinine was hydrolyzed in potassium hydroxide solution as previously described. The slightly dark colored solution was cooled and made acid to Congo red with sulfuric acid. After extraction of the mixture with ether to remove isobutyrylformic acid, the aqueous solution was treated with sodium carbonate until just neutral to Congo red, and the suspension allowed to stand in the refrigerator in order to complete the separation of lysergic acid. The almost colorless filtrate was evaporated to dryness under reduced pressure and extracted with hot alcohol. Evaporation of the alcoholic extract gave 500 mg. of residue which was soluble in water. Free amino acids could not be isolated from this material as such. It was taken up in 10 cc. of concentrated hydrochloric acid and the solution was heated on the steam bath overnight. The dark colored solution was evaporated to dryness under reduced pressure, and the residue was dissolved in water and made slightly alkaline with sodium carbonate. A bluish black precipitate was filtered off. The filtrate was treated with 200 mg. of ammonium chloride and then evaporated to dryness. The residue was taken up in water and the hot solution was treated with bone-black to remove color.

Phenylalanine—The filtrate was concentrated to about 1.5 cc. and cooled. Lustrous leaflets separated. After collection with a few drops of water, .110 mg. of phenylalanine were obtained. The filtrate was set aside for further treatment as given below for the separation of proline. The phenylalanine was recrystallized from water for analysis.

$C_9H_{11}O_2N$.	Calculated.	C 65.45, H 6.72, N 8.48
	Found.	" 65.34, " 6.41, " 8.33

It melted with decomposition at 260–265° depending on the rate of heating, exactly as phenylalanine, and showed identical properties with the naturally occurring amino acid, with the exception of optical rotation. It showed only slight optical activity.

Proline—The filtrate from the crude phenylalanine was evaporated to dryness and the residue was treated with 10 cc. of 17 per cent methyl alcoholic hydrogen chloride. The solution was

allowed to stand overnight at room temperature and was then evaporated to dryness *in vacuo*, with every precaution to exclude moisture. The residue was dissolved in 1 cc. of methyl alcohol and filtered. The filtrate was treated with an excess of powdered anhydrous potassium carbonate and again filtered. The filtrate was further treated with powdered calcium oxide and filtered. The filtrate was then carefully evaporated to a syrup and taken up in 5 cc. of dry ether. The ether solution after filtration from solid insoluble material was fractionated in a microstill. 60 mg. of an oil distilled at 60–85° under 5 mm. pressure and 15 mg. of an oil distilled at a higher temperature, under 140°, which was probably the methyl ester of phenylalanine. The lower boiling fraction was shown to be the methyl ester of proline as follows:

When dissolved in dilute hydrochloric acid and treated with gold chloride, leaflets separated. These were sparingly soluble in water but quite soluble in acidified methyl alcohol. It was recrystallized from dilute methyl alcohol acidified with hydrochloric acid. It melted at 146–147° and showed no depression in a mixed melting point with the synthetic material described below, which was prepared from the naturally occurring *l*-proline.

$C_6H_{11}O_2N \cdot HAuCl_4$.	Calculated.	C 15.35, H 2.57, Au 42.04
	Found.	" 15.60, " 2.73, " 42.21

The ester proved to have only slight optical activity. It reacted readily with phenyl isothiocyanate in ether solution, and the resulting thiourea after recrystallization from methyl alcohol-ether melted at 151° and gave no depression with the analogous derivative from *l*-proline ester.

Acid Hydrolysis of Ergotinine—1 gm. of ergotinine was heated with 50 cc. of HCl (1.19). Solution occurred rapidly and the heating was continued on the steam bath for about 18 hours. The almost black solution was diluted and without filtering the precipitated material, the mixture was repeatedly extracted with ether. The ether residue, while smelling somewhat suggestively of an acid like isobutyrylformic acid, did not yield to further investigation.

The extracted aqueous layer was concentrated as such at 12 mm. to dryness to remove excess HCl. The residue was extracted with water and left undissolved the almost black amorphous decomposition products of the lysergic acid portion of the molecule.

After filtration through bone-black, the still colored filtrate was treated with excess silver sulfate to remove Cl ions. The excess silver was removed from the filtrate with H_2S. The filtrate from this, after removal of excess H_2S, was neutralized with $BaCO_3$.

l-Phenylalanine—The colorless filtrate was concentrated to small bulk. After being filtered from a small amount of $BaCO_3$ which separated, the filtrate was again concentrated to about 2 cc. Voluminous needles of phenylalanine separated. This was collected after standing with a few drops of water. This fraction was recrystallized by the addition of alcohol to the solution in about 1 cc. of H_2O. It formed characteristic leaflets and melted at 260° with decomposition.

$[\alpha]_D^{20} = -28°$ ($c = 0.39$ in H_2O)

$C_9H_{11}O_2N$.	Calculated.	C 65.41, H 6.72
	Found.	" 65.30, " 6.74

The Dipeptide, $C_{14}H_{18}O_3N_2$ (?)—The mother liquor from the first phenylalanine crystallization was allowed to evaporate almost to dryness. Two types of crystals were observed: voluminous needles apparently of phenylalanine, and some hard, stout aggregates of heavier crystals which absorbed some pigment. On addition of a small amount of water, the needles dissolved more rapidly and gave opportunity for the collection of the heavier crystals with a few drops of water. This substance was also quite soluble in water and because of the small amount available at the time it was analyzed directly. It gave a strong pyrrole test and melted with effervescence at 252° after preliminary softening above 200°.

For analysis it was dried at 110° and 20 mm.

$C_{14}H_{18}O_3N_2$.	Calculated.	C 64.08, H 6.92
	Found.	" 64.43, " 7.08

d-Proline—The mother liquor from the peptide was evaporated to dryness. On treating the residue with dry methyl alcohol preparatory to esterification, a sparingly soluble substance remained which was collected with methyl alcohol. It gave no pyrrole test and proved to be additional phenylalanine.

The mother liquor from this material was again concentrated to dryness and the residue was dissolved in a small volume of dry methyl alcohol. The solution was saturated with HCl gas for

esterification. The ester was isolated as described above and was fractionated in the microstill. On redistillation, material was collected at 80–85° (bath temperature) and 15 mm.

$[\alpha]_D^{25} = +34°$ ($c = 0.65$ in methyl alcohol)

$C_6H_{11}O_2N$.	Calculated.	C 55.77, H 8.59
	Found.	" 55.70, " 8.81

l-Proline Methyl Ester—*l*-Proline (Hoffmann-La Roche) was esterified as usual in dry methyl alcohol with HCl gas. After removal of excess solvent and acid, the remaining salt was dissolved in methyl alcohol and the free ester was liberated by successive treatments with dry potassium carbonate and calcium oxide. The refractionated ester was collected at 85° (bath temperature) and 15 mm.

$[\alpha]_D^{20} = -36°$ ($c = 1.07$ in methyl alcohol)

$C_6H_{11}O_2N$.	Calculated.	C 55.77, H 8.59
	Found.	" 55.42, " 8.59

The thiourea obtained from this ester melted at 151°.

The gold double salt was prepared from this ester as described above. It melted at 146–147°.

$C_6H_{11}O_2N \cdot HAuCl_4$.	Calculated.	C 15.35, H 2.57, Au 42.04
	Found.	" 15.40, " 2.40, " 42.11

Hydrolysis of Ergometrine—0.1 gm. of ergometrine was heated on the steam bath in 10 cc. of a 12.5 per cent solution of KOH in 50 per cent methyl alcohol. During the heating, a stream of nitrogen was passed through the mixture and then into 0.1 N HCl. After 1 hour and 10 minutes the reaction was interrupted. No ammonia was found to have been collected by the acid. The alkaline mixture was diluted with a small volume of water and carefully treated with dilute H_2SO_4 until acid to Congo red, which caused copious precipitation of salts. The mixture as such was repeatedly extracted with ether. The washed and dried ether extract gave no appreciable residue and no crystalline material when treated with phenylhydrazine in dilute acetic acid.

The aqueous suspension was just neutralized to Congo red with Na_2CO_3 and treated with absolute alcohol. The deposit which was mostly a mixture of Na_2SO_4 and K_2SO_4 was collected with alcohol. This precipitate was digested with a small volume

of methyl alcohol containing an excess of ammonia and filtered from undissolved salts. The filtrate and washings on concentration gave a small residue which crystallized under water. This was dissolved by the addition of a slight excess of ammonia, and, while hot, the solution was slightly acidified with acetic acid and treated with bone-black. The hot filtrate was practically colorless and on concentration gave practically colorless leaflets which corresponded in all properties with lysergic acid, and gave the characteristic Keller test. It melted at 228–230°. The limited amount of material prevented further recrystallization.

For analysis the substance was dried at 140° and 2 mm.

$C_{16}H_{16}O_2N_2$.	Calculated.	C 71.69, H 6.00
	Found.	" 71.49, " 5.60

The original alcoholic mother liquor of the above inorganic salts on concentration and further manipulation gave a second small fraction which contained lysergic acid. The mother liquor of this fraction was then treated with barium carbonate to remove excess SO_4 ions. With the resulting solution no pyrrole test could be obtained, thus indicating absence of proline and hydroxyproline. A crystalline substance was obtained, however, which was too small in amount for proper identification.

Addendum—While this paper has been in press, certain discrepancies have appeared in the rotation and analyses obtained with our "ergometrine" and the published data for ergometrine and ergotocin. The relationship of our "ergometrine" to these substances is therefore not certain. However, our substance appears to be identical with the recently reported *Ergobasine* of Stoll and Burckhardt (Stoll, A., and Burckhardt, E., *Compt. rend. Acad.*, **200**, 1680 (1935)), and, as we are presenting elsewhere, it has proved to be the hydroxyisopropylamide of lysergic acid, $C_{19}H_{23}O_2N_3$.