THE ERGOT ALKALOIDS

IV. THE CLEAVAGE OF ERGOTININE WITH SODIUM AND BUTYL ALCOHOL

By WALTER A. JACOBS and LYMAN C. CRAIG

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

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The successful investigation of the structure of the ergot alkaloids will depend upon the development of proper methods for their degradation. Thus far degradation products have been obtained by oxidation, alkaline hydrolysis, and in smaller measure by pyrolysis. We have already described the formation of lysergic acid, $C_{16}H_{16}O_2N_2$,[1] as a characteristic alkaline cleavage product of ergotinine, of which ergine obtained by Smith and Timmis[2] was later shown by them to be the amide. Thus, in the latter substance 3 of the N atoms of ergotinine have been generally located. A suggestion of the nature of the remaining 2 has been obtained from the occurrence on pyrolysis of a piperidine-like odor which also occurs in the basic fraction of the products of acid hydrolysis of ergotinine.

During the alkaline hydrolysis of ergotinine and also in most other attempts to transform it, a considerable amount of colored, tarry decomposition products is formed. Lysergic acid itself exhibits also a measure of lability. This instability has made isolation of the cleavage products more difficult.

There is reason to believe that we are dealing with unsaturated nitrogen heterocyclic derivatives which as a class are less stable than their reduction products. Accordingly we have studied the reductive cleavage of ergotinine with sodium and amyl alcohol in

[1] Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, **104**, 547 (1934); **106**, 393 (1934).

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

the hope that more stable cleavage products would result. Such a study was further suggested by the apparently greater stability towards light and air of dihydrolysergic acid[3] which we have already described as a product of the reduction of lysergic acid with these reagents. With ergotinine a study of conditions led finally to the adoption of normal butyl alcohol as the solvent to be preferred. The new procedure has given a reaction mixture from which a number of substances have been isolated.

During the reduction of ergotinine approximately 1 mole of ammonia was evolved, thus substantiating the earlier evidence of the presence of one primary or secondary amide group in the alkaloid. By steam distillation of the alkaline reaction mixture a number of steam-volatile bases were detected which will be discussed below. The non-volatile residue after saturation with carbon dioxide, concentration to dryness, and subsequent extraction with absolute alcohol gave material from which a number of substances were isolated.

One substance crystallized directly from a solution in methyl alcohol of the above desiccated alcoholic extract. This proved to be a base from the analysis of which the formula $C_{16}H_{20}ON_2$ was derived. This formulation was confirmed by the analysis of the *sulfate*, $(C_{16}H_{20}ON_2)_2H_2SO_4$. The base contained an N-methyl group and gave the Keller reaction of ergotinine. It is therefore a derivative of the lysergic acid portion of the molecule. It formed readily a *monoacetyl derivative*, indicating that lysergic acid while being reduced to a dihydro derivative has also suffered reduction of its carboxyl group to the carbinol. This substance, as we shall see later, was also obtained directly by the reduction of lysergic acid itself. We have adopted therefore the designation *α-dihydrolysergol* for this cleavage product.

The possibility that the above reduction product could be a lactam due to reduction of a tertiary cyclic N atom to NH with subsequent lactamization with the carboxyl group is excluded, among other things, by the formation of the acetyl derivative. The methyl-carrying N atom cannot participate in this reaction, since its character as a cyclic tertiary nitrogen atom has been shown in our previous work.

The crude mother liquor from the above base gave additional

[3] Jacobs, W. A., and Craig, L. C., *J. Biol. Chem.*, **106**, 398 (1934).

basic material which could be extracted from an aqueous suspension with ether. This ether extract yielded on fractional sublimation at low pressure two additional bases. The less volatile base proved to be isomeric with α-dihydrolysergol, since the analytical figures agreed well for a formula, $C_{16}H_{20}ON_2$. It gave the same color reactions and formed a crystalline *hydrochloride*, *sulfate*, and *monoacetyl* derivative. It is possible that reduction in lysergic acid gives rise to a new center of asymmetry and that epimeric dihydro derivatives are formed. The new base has been called *β-dihydrolysergol*.

The more volatile base which sublimed at a lower temperature proved to belong in a different category. The base itself has not been obtained crystalline but was isolated as a picrate, the analysis of which indicated the formula $C_{14}H_{20}N_2(C_6H_3O_7N_3)_2$. This base does not give the color reactions of ergotinine and contains no N-methyl group. It appears possible that it is a dipyrrole or bipyridine derivative and has its source in the basic portion of the molecule other than lysergic acid. This base we have provisionally called *Base II*.

An acid was isolated from the aqueous solution which remained after ether extraction of Base II and β-dihydrolysergol. This acid proved to be α-hydroxyisovaleric acid. This substance unquestionably had its origin in isobutyrylformic acid which, as we have shown, is a product of the alkaline hydrolysis of ergotinine.[1]

The mixture of steam-volatile bases which had been collected and concentrated as mixed hydrochlorides was likewise submitted to sublimation for preliminary isolation as described in the experimental part. The mixture of bases so obtained was then fractionated in a microstill.[4] The fraction which distilled at an oil bath temperature of 220–230° at 760 mm. proved to be of interest. This fraction was found to be a mixture from which two crystalline derivatives could be isolated. An ether solution of the mixed bases gave a crystalline picrate (*Base IV*). Because of the very small amount of material at our disposal, its homogeneity may still be in question. The analytical figures suggested a formula $C_{10}H_{20}N_2(C_6H_3O_7N_3)_2$ for the picrate of Base IV. It contained no N-methyl group and gave no color with Keller's reagent. This substance will be made a subject for further study.

[4] The microdistilling apparatus which was used will be described elsewhere.

On acylation of the basic material recovered from the mother liquor of the above picrate, a crystalline *p*-bromobenzoyl derivative was obtained which proved to be a dibromobenzoyl derivative of a hydroxyamine (*Base V*). The analytical figures indicated a formulation $C_{20}H_{19}O_3NBr_2$ for this diacyl derivative or $C_6H_{13}ON$ for the base. The consumption of 1 equivalent of alkali on saponification was in harmony with the presence of a bromobenzoyl hydroxy group.

Finally, from one of the fractions of the steam-volatile bases, as described in the experimental part, a crystalline bromobenzoate of another base, tentatively called *Base VI*, was obtained but any attempt to interpret its nature with the data available is premature. The analytical figures suggest that the substance was possibly a dibromobenzoate of an aromatic hydroxyamine with a formula $C_9H_{13}ON$. The possibility is not excluded that this fragment is the portion of the molecule responsible for the production of benzoic and *p*-nitrobenzoic acids on oxidation of the original alkaloid.

As a check on the origin of the above degradation products of ergotinine we have repeated the study of the reduction of lysergic methyl ester under similar conditions. In addition to the formation of dihydrolysergic acid, which has been described in our previous communication, both α- and β-dihydrolysergol were now obtained. However, none of the steam-volatile bases or Base II obtained from ergotinine and no hydroxyisovaleric acid could be detected in the reaction mixture. It is apparent, therefore, that Bases II, IV, V, and VI have their origin in that portion of the ergotinine molecule apart from lysergic acid and which contains the remaining 2 nitrogen atoms. It appears to be joined to lysergic acid (ergine) by means of isobutyrylformic acid or its precursor.

Because of the limitations of our experimental procedures with the amount of the costly material available, we present the above observations with necessary reservation and are attempting to confirm and amplify them by further work.

EXPERIMENTAL

1 gm. of ergotinine was dissolved in 40 cc. of carefully dried butyl alcohol, 2 gm. of sodium were added to the boiling solution, and the mixture was shaken vigorously to emulsify the molten sodium.

The gases coming from the reduction were passed through dilute hydrochloric acid. After all sodium was dissolved, 40 cc. more of butyl alcohol and 2 gm. of sodium were added. The mixture was again heated to boiling and shaken vigorously.

The acid solution through which the issuing gases were passed gave upon evaporation 60 mg. of hydrochloride. Only ammonia and no organic bases could be detected in this salt. Calculated for 1 mole of ammonium chloride, 89 mg.

The colorless butyl alcohol solution after addition of 20 cc. of water was distilled with steam until 400 cc. of distillate had passed. The latter was acidified with hydrochloric acid and evaporated to dryness. 140 mg. of a viscous, syrupy residue of hydrochlorides of the steam-volatile bases remained.

The aqueous alkaline residue from the steam distillation was saturated with carbon dioxide and the mixture was evaporated to dryness at 80° and 20 mm. pressure. A little alcohol was added and the evaporation was repeated in order to insure removal of water. The dry solid residue was then exhaustively extracted with hot ethyl alcohol which was filtered from the solid insoluble carbonates. The combined filtrates after evaporation to dryness gave 870 mg. of residue.

This material was reduced again with sodium and butyl alcohol exactly as above. The acid solution through which the issuing gases were passed gave no appreciable residue upon evaporation. Steam distillation of the reaction mixture gave a distillate from which 70 mg. of a syrupy residue of hydrochlorides were obtained on evaporation to dryness. This material was combined with the similar fraction from the first steam distillation. The investigation of these steam-volatile bases will be described below. The aqueous alkaline residue from the steam distillation was again saturated with carbon dioxide and evaporated to dryness at 80° and 20 mm. pressure. Exhaustive extraction of the dry carbonates with alcohol and concentration yielded 820 mg. of alcohol-soluble residue.

α-Dihydrolysergol, $C_{16}H_{20}ON_2$—The above residue on treatment with hot methyl alcohol was completely dissolved. The resulting solution was concentrated on the steam bath until crystallization began. At this point the volume of solution was about 2.5 cc. After cooling overnight at 0°, the crystals were collected with a

small volume of methyl alcohol. The filtrate was set aside and treated further as described below. The crystalline material, 160 mg., contained still a small amount of sodium carbonate. It was purified by boiling with water, filtering, and recrystallizing the undissolved base from methyl alcohol. It crystallized in stout prisms which melted at 282°, depending somewhat on the rate of heating, as it darkened slightly a few degrees below the melting point. It gives the characteristic Keller test for the ergot alkaloids. It is optically active. $[\alpha]_D^{25} = -92°$ ($c = 0.5$ in pyridine).

The base sublimes readily at 210–220° and 0.15 mm. pressure without decomposition. It is sparingly soluble in water and ether and somewhat more soluble in methyl alcohol.

$C_{16}H_{20}ON_2$.	Calculated.	C 74.96, H 7.87, N 10.93, NCH_3 11.32
	Found.	" 74.95, " 7.47, " 10.97, " 11.84
		" 75.00, " 7.72, " 10.83

The base is soluble in dilute acids but forms a crystalline hydrochloride and sulfate which are rather sparingly soluble in cold water. The sulfate melts at 302° with decomposition. It forms a hydrate which loses its water only at 150° and 0.2 mm. The analyses of the hydrate and anhydrous substance follow.

$(C_{16}H_{20}ON_2)_2H_2SO_4 \cdot H_2O$.	Calculated.	C 61.11, H 7.06, N 8.91
	Found.	" 60.84, " 7.17, " 9.03
Anhydrous Substance		
$(C_{16}H_{20}ON_2)_2H_2SO_4$.	Calculated.	C 62.91, H 6.94, N 9.17
	Found.	" 62.94, " 6.96, " 9.16

Acetyl-α-Dihydrolysergol—30 mg. of the base were refluxed for 2 hours with 1 cc. of acetic anhydride. After removal of the excess anhydride under diminished pressure the residue was recrystallized from ethyl alcohol. It formed irregular leaves which melted at 200°.

$C_{18}H_{22}O_2N_2$.	Calculated.	C 72.54, H 7.43, N 9.39
	Found.	" 72.46, " 7.53, " 9.60

The acetyl derivative was insoluble in water but dissolved in dilute sulfuric acid. From more concentrated solutions the sulfate crystallizes readily.

13.103 mg. of the acetyl derivative were refluxed with 3 cc. of 0.1 N alkali and 2 cc. of alcohol for 4 hours and then titrated back

against phenolphthalein. Found, 0.328 cc. of NaOH. Calculated for 1 mole of acetic acid, 0.440 cc.

Base II, $C_{14}H_{20}N_2(?)$—The methyl alcoholic filtrate from the above base was evaporated to dryness and the residue was dissolved in 2 cc. of water. A viscous oil remained undissolved, which slowly but entirely dissolved on extraction with ether. The remaining aqueous solution was set aside to be further treated as described below for the isolation of hydroxyisovaleric acid. The ethereal extract after concentration was placed in a small sublimation outfit. This consisted of a Pyrex pear-shaped test-tube with a wide flat bottom and fitted with a small side tube for connection with the vacuum pump. The substance was placed on the bottom of this. In the mouth of the tube a smaller tube for condensation was fitted with a ground joint. In this smaller tube water could be circulated or chopped ice or a dry ice-acetone mixture could be introduced for chilling. After introduction of the concentrated ether solution, the remaining ether was removed under reduced pressure. A residue of 400 mg. remained. This was then fractionally sublimed. A fraction was first taken at an oil bath temperature of 160° and 5 mm. pressure. 50 mg. of an oil had sublimed. It could not be induced to crystallize and was finally converted into the picrate which crystallized readily from ethyl alcohol. It melted upon repeated recrystallization from alcohol at 246°. It does not contain an N-methyl group and does not give the Keller color test for the ergot alkaloids.

$C_{14}H_{20}N_2(C_6H_3O_7N_3)_2$.	Calculated.	C 46.28, H 3.88, N 16.61
	Found.	" 46.28, " 3.96, " 16.44
		" 46.99, " 3.89, " 16.70

β-Dihydrolysergol, $C_{16}H_{20}ON_2$—After sublimation of Base II the pressure of the sublimation apparatus was then reduced to 0.15 mm. and the temperature of the oil bath was slowly raised to 220°. The resulting sublimate was removed from the condenser with methyl alcohol and the solution was evaporated to dryness. A viscous residue of 280 mg. remained. This was dissolved in 1 cc. of methyl alcohol. After standing for some time in the refrigerator, a crystalline mass separated. After collection with a few drops of methyl alcohol it weighed 50 mg.

$[\alpha]_D^{25} = -64°$ ($c = 0.5$ in pyridine)

$C_{16}H_{20}ON_2$.	Calculated.	C 74.96, H 7.87, N 10.93
	Found.	" 75.08, " 7.47, " 11.09
		" 75.01, " 7.78, " 10.84

The base crystallized in leaflets which melted at 190°. It was quite soluble in methyl alcohol but insoluble in water. It dissolved in hot dilute acids as did the α-isomer and formed a crystalline hydrochloride and sulfate upon cooling. It contains one N-methyl group, as is shown by analysis of the sulfate. The latter melted at 305°. For analysis the substance was dried at 120° and 0.2 mm.

$(C_{16}H_{20}ON_2)_2H_2SO_4$.	Calculated.	C 62.91, H 6.94, N 9.17, NCH_3 9.50
	Found.	" 62.91, " 6.53, " 9.39, " 8.76
		" 62.67, " 6.85, " 9.56

Acetyl-β-Dihydrolysergol—The above base reacted readily with acetic anhydride. 30 mg. were refluxed for 2 hours with 1 cc. of acetic anhydride. The acetic anhydride was removed under reduced pressure and the residue was dissolved in dilute sulfuric acid. Excess sodium carbonate was then added and the precipitated oil was extracted with ether. After evaporation of the ether the residue was recrystallized from benzene. It crystallized in needles which melted at 129°.

$C_{18}H_{22}O_2N_2$.	Calculated.	C 72.54, H 7.43, N 9.39
	Found.	" 71.99, " 7.31, " 9.51

α-Hydroxyisovaleric Acid—The aqueous layer from the ether extraction of Base II and β-dihydrolysergol described above was acidified to Congo red with dilute sulfuric acid and extracted with ether. The aqueous layer contained a viscous tar which could not be crystallized. Excess sodium carbonate solution was added and the mixture was evaporated to dryness. It was then extracted with ethyl alcohol and the insoluble mixture of sodium sulfate and carbonate was removed by filtration. Evaporation of the alcoholic filtrate yielded 300 mg. of residue which was amphoteric. Investigation of this material must be left for future work.

The ether layer was dried with Na_2SO_4 and evaporated to dryness. 100 mg. of an oily acid remained. Upon sublimation from the steam bath at 20 mm. pressure colorless crystals were obtained

which were recrystallized from petroleum ether. The substance melted at 82°.

$C_5H_{10}O_3$.	Calculated.	C 50.81, H 8.55
	Found.	" 51.47, " 8.53
		" 51.19, " 8.73

Isobutyrylformic acid has been previously isolated by us from ergotinine on alkaline hydrolysis. Accordingly α-hydroxyisovaleric acid should be expected as a product of the above sodium reduction. The melting point recorded in the literature for this acid is 82°.

Base IV—The combined hydrochlorides from the steam-volatile bases were placed in a sublimation apparatus and carefully decomposed in the cold with 5 drops of 25 per cent sodium hydroxide. The condenser of the sublimation apparatus was cooled with a carbon dioxide and acetone mixture. The temperature of the water bath in which the apparatus was immersed was slowly raised to 100°. The sublimate obtained up to this point at 760 mm. was removed and washed off with dilute hydrochloric acid. The pressure in the apparatus was then reduced to 20 mm. and the temperature was again slowly raised to 100°. These conditions gave additional sublimate which was removed from the condenser with dilute hydrochloric acid. This was combined with the first and evaporated to dryness. 110 mg. of a viscous, syrupy hydrochloride remained. This material was removed to a small test-tube with a few drops of water and then decomposed with a few drops of strong sodium hydroxide solution. The resulting oil was dissolved in a minimal volume of ether and the ether layer was removed with a capillary pipette. The alkaline solution was washed again with a minimal amount of ether and the combined ether solutions were dried overnight with solid potassium hydroxide. On fractionation in a microstill[4] a very small amount of a volatile base distilled with the ether up to a temperature of 80° (oil bath temperature). On further distillation at 760 mm. approximately 50 mg. of a clear viscous base distilled at an oil bath temperature of 220–230°. 20 mg. of base were then collected at 185° and 18 mm. pressure.

Crystalline derivatives could not be isolated from the first and third fractions but the second fraction gave two crystalline prod-

ucts. It was taken up in ether and from this was precipitated an oily mixture of picrates by addition of an ether solution of picric acid. This mixture crystallized under alcohol.

After recrystallization from ethyl alcohol 11 mg. of picrate of Base IV were obtained which melted at 226°. Although further recrystallization did not raise the melting point, the homogeneity of the substance is in question, since the analytical results from different preparations did not show good agreement. Because of lack of material the following tentative results will be reported. The compound did not contain an NCH_3 grouping and did not give the characteristic blue color with Keller's reagent.

$C_{10}H_{20}N_2(C_6H_3O_7N_3)_2$.	Calculated.	C 42.17, H 4.18, N 17.89
	Found.	" 42.86, " 4.28, " 17.29
		" 42.29, " 4.09, " 17.43

Di(p-Bromobenzoyl) Derivative of Base V, $C_6H_{13}ON(?)$—The mother liquors from the above picrate were evaporated to dryness and after addition of an excess of dilute hydrochloric acid the picric acid was removed by extraction with ether. The hydrochloric acid solution was then evaporated to dryness. The residue was treated with 200 mg. of *p*-bromobenzoyl chloride and 4 cc. of 10 per cent sodium hydroxide. It was warmed gently at first and finally on the steam bath until the odor of the acid chloride disappeared. The cooled mixture was extracted with ether, and the ether solution was dried with potassium carbonate and evaporated to dryness. The residue was heated with a small volume (about 1 cc.) of ethyl alcohol, and undissolved *p*-bromobenzoic anhydride was filtered off. The filtrate on standing yielded rather stout needles, which were collected with alcohol (20 mg.). The substance melted at 149°.

$C_{20}H_{19}O_3NBr_2$.	Calculated.	C 49.90, H 3.98, N 2.91, Br 33.23
	Found.	" 49.46, " 4.05, " 3.21, " 33.12
		" 49.36, " 4.09, " 2.83, " 33.43

The substance did not contain the NCH_3 grouping and did not give the characteristic blue color in the Keller test for the ergot alkaloids.

The molecular weight determination was made by the Rast method. 2.910 mg. of substance: 29.19 mg. of camphor, Δ = 7.6°. Molecular weight found, 526; calculated, 480.9.

On boiling with 0.1 N alkali, approximately 1 equivalent was consumed, indicating the saponification of an ester linkage.

11.470 mg. of substance were refluxed for 4 hours with 1.5 cc. of alcohol and 3 cc. of 0.1 N sodium hydroxide and then titrated against phenolphthalein. Found, 0.268 cc.; calculated for 1 equivalent, 0.238 cc.

From this hydrolysis solution *p*-bromobenzoic acid melting at 150° was isolated and also an oil which, however, could not be crystallized.

Base VI—The residue which remained in the sublimation apparatus after sublimation of the mixture of Bases IV and V was treated with a few drops of water and then extracted with ether. The ether extract after being dried with potassium carbonate and evaporated to dryness gave 50 mg. of an oil. This was distilled in a microstill under 16 mm. pressure and appeared to distil when the temperature of the oil bath was approximately 180°. 40 mg. of a clear viscous distillate were so obtained. This material failed to give a crystalline picrate. It was insoluble in water but was completely soluble in dilute hydrochloric acid.

This fraction was acylated by treating with 100 mg. of *p*-bromobenzoyl chloride and 2 cc. of 10 per cent sodium hydroxide. The mixture was heated gently at first and finally on the steam bath with vigorous shaking until all the free acid chloride had disappeared. The resulting reaction product was extracted with ether, and the ether solution was dried with K_2CO_3 and then concentrated to dryness. The residue was refluxed for 2 hours with ethyl alcohol to destroy any acid anhydride. After concentrating somewhat and chilling, a substance crystallized as long needles. 15 mg. were collected with alcohol. The substance melted at 204°.

$C_{23}H_{19}O_3NBr_2$.	Calculated.	C 53.39, H 3.71, N 2.70, Br 31.01
	Found.	" 53.16, " 3.55, " 2.93, " 31.05

Reductive Cleavage of Lysergic Acid Methyl Ester—240 mg. of lysergic acid methyl ester were reduced exactly as in the case of ergotinine with 1 gm. of sodium and 20 cc. of butyl alcohol. The substance was steam-distilled until 200 cc. of distillate were collected. The distillate after acidification with dilute hydrochloric acid and evaporation to dryness yielded no residue corresponding to the volatile bases obtained in the case of ergotinine. The aque-

ous alkaline residue from the steam distillation was saturated with carbon dioxide, and after concentration to dryness gave a residue from which the organic bases were extracted with alcohol. After removal of alcohol from the extract the residue was dissolved in methyl alcohol. After concentration to about 1 cc. and standing in the ice box, crystals of α-dihydrolysergol separated. After collection with a few drops of methyl alcohol, the substance weighed 25 mg. It proved to be identical with the base obtained from ergotinine. It melted at 282°.

$C_{16}H_{20}ON_2$.	Calculated.	C 74.96, H 7.87
	Found.	" 74.88, " 7.55

The methyl alcoholic mother liquor was concentrated to dryness and the residue was treated with 1 cc. of water. The viscous oil which remained was extracted with ether and the aqueous layer set aside. The ether extract after being dried with K_2CO_3 gave on evaporation to dryness a residue of 70 mg. When dissolved in a few drops of methyl alcohol and seeded with a crystal of β-dihydrolysergol, crystallization immediately began. 12 mg. of crystals were collected with a few drops of methyl alcohol. The base showed all the properties of the β base and melted at 190°.

$C_{16}H_{20}ON_2$.	Calculated.	C 74.96, H 7.87
	Found.	" 75.02, " 7.46

When the aqueous layer from the ether extraction was worked up, 58 mg. of dihydrolysergic acid were obtained and about 90 mg. of an amorphous, much more soluble, amphoteric material.

Addendum—Since this paper was sent to press we have had opportunity to check directly the suggestion given in the introduction that Base VI might be that portion of the alkaloid molecule responsible for the production of benzoic and *p*-nitrobenzoic acids on oxidation. On hydrolysis of its diacyl derivative, the base itself has now been obtained. This base, apparently a phenylpropanolamine, has given *p*-nitrobenzoic acid on oxidation with nitric acid.

This fact together with other as yet unpublished observations offers the suggestion that certain fragments of the alkaloid molecule may have as their precursors amino acids or substances related to them. The investigation is being continued from this standpoint.