

threonine could be obtained from the original virus or virus protein; and that only after enzymatic release of the threonine was it possible to obtain the new terminal amino acid and peptide. The apparent homogeneity of the sub-units comprising the virus, at least as far as the C-terminal sequence is concerned, is noteworthy, since it is a necessary requirement for further structural elucidation. It is also of interest that the masked strain which is closely related to TMV has the same C-terminal sequence. However, the dethreoninated H.R. strain failed to yield the prolylalanine peptide. Since all of the other dethreoninated TMV proteins gave rise to the peptide on partial hydrazinolysis, the failure of the H.R. strain to do so represents indirect but definite evidence for a structural difference. This difference may be at the fourth amino acid since the resistance of this as all other dethreoninated TMV proteins to carboxypeptidase suggests the presence of the same pro-ala- sequence.

The results with ovalbumin already have been mentioned. The same results were obtained with four different samples, prepared by two procedures.²⁷ Since no N-terminal amino acid has ever been detected in ovalbumin, a six-shaped structure appears probable. The attack of subtilisin²⁸ (and contaminated carboxypeptidase) appears to be on a particularly labile site in the ring, while the actual C-terminal proline is disregarded by the enzyme for specificity reasons.

Our finding of C-terminal alanine in bovine serum albumin has since been anticipated in the

(27) Akabori⁸ had found C-terminal alanine, and this question is being further investigated through an exchange of samples. We have recently found the same C-terminal sequence in a preparation kindly sent to us by Prof. Akabori and have been informed by Dr. Ohno that they have now also indentified the C-terminal amino acid as proline.

(28) D. Steinberg, *THIS JOURNAL*, **74**, 4217 (1952).

literature.²⁹ A peptide was obtained in too low yield for identification. With ribonuclease valine was found C-terminal, in accord with results obtained with carboxypeptidase²¹; however, the next amino acid appears to be serine, contradictory to that study. For chymotrypsin we have confirmed Neurath's conclusion^{30,31} that this represents a two-chain protein. β -Lactoglobulin also appears to be a two-chain protein, but both chains seem to terminate in isoleucine and the histidine obtained after carboxypeptidase treatment³¹ must represent a penultimate amino acid.³²

No terminal amino acids were found in chymotrypsinogen and ovomucoid. In the latter, phenylalanine appeared C-terminal by the reduction³³ and thiocyanate³⁴ methods, and nothing is released by carboxypeptidase. From crystalline soybean trypsin inhibitor serine and leucine were obtained in non-stoichiometric amounts, as well as traces of other amino acids. Carboxypeptidase released only leucine stoichiometrically.³⁵ Several preparations of pepsin, as well as of denatured and dialyzed pepsin, contained free amino acids when tested by dinitrophenylation. Therefore the complex results of hydrazinolysis are of no significance; however, the absence of stoichiometric amounts of alanine is in contrast to the conclusions of others.³⁶

(29) W. F. White, J. Shields and K. C. Robbins, *ibid.*, **77**, 1267 (1955).

(30) J. A. Gladner and H. Neurath, *J. Biol. Chem.*, **206**, 911 (1954).

(31) H. Neurath, J. Gladner and E. Davie, in "The Mechanism of Enzyme Action," Ed. McElroy and Glass, Baltimore, Md., 1954, p. 51.

(32) The absence of histidine was also demonstrated, after direct chromatography of the hydrazinolysis residue, by spraying with the very sensitive diazobenzenesulfonic acid reagent.

(33) L. M. Penasse, M. Jutisz, C. Fromageot and H. Fraenkel-Conrat, *Biochim. Biophys. Acta*, **9**, 551 (1952).

(34) R. A. Turner and G. Schmerzler, *ibid.*, **11**, 586 (1953).

(35) E. W. Davie and H. Neurath, *J. Biol. Chem.*, **212**, 507 (1955).

(36) M. B. Williamson and J. M. Passmann, *Biochim. Biophys. Acta*, **15**, 246 (1954).

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[CONTRIBUTION FROM THE LABORATORY OF CHEMISTRY OF NATURAL PRODUCTS, NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, U. S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE]

Alkaloids of the Amaryllidaceae. VI. The Action of Oxidizing Agents on Lycorine and Caranine¹

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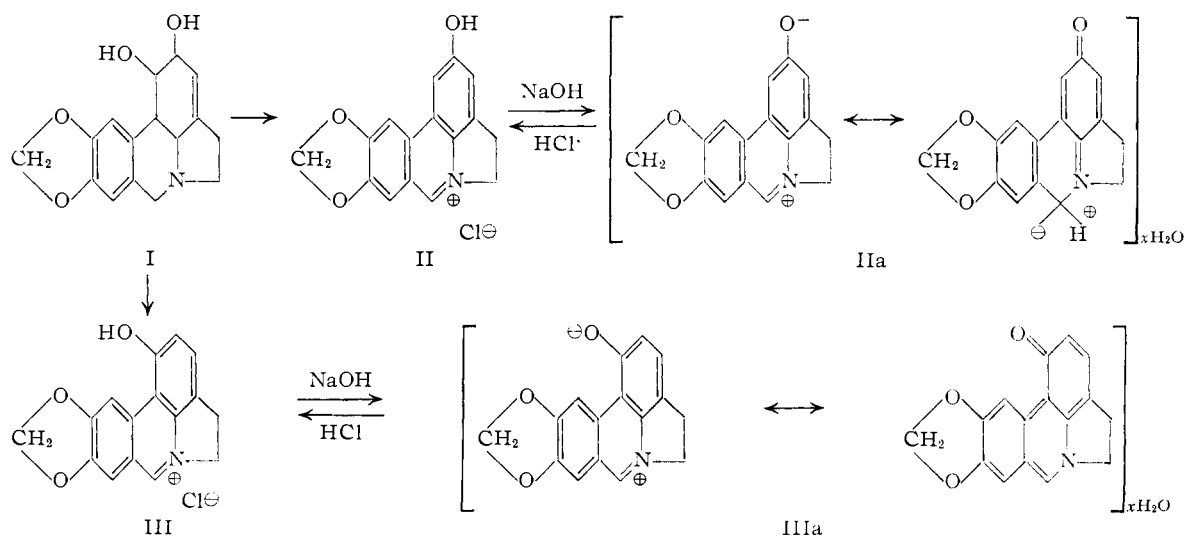
Oxidation of lycorine with selenium dioxide, *t*-butyl hypochlorite, pyridine perbromide hydrobromide or N-bromosuccinimide has been shown to yield a compound C₁₆H₁₁NO₃ for which structure IIa is suggested. A modified Oppenauer oxidation of caranine affords an isomeric compound for which structure IIIa is proposed. Oxidation of lycorine with mercuric acetate has been shown to yield a mixture of IIa and IIIa. The structures of IIa and IIIa are supported by the ultraviolet spectra of synthetic model compounds. A partial formula for caranine is proposed.

Although the principal alkaloid of the Amaryllidaceae, lycorine, has been known for sixty years, the most valuable evidence for its structure was published last year through the joint efforts of research groups in Japan and New Brunswick.² In the currently accepted formula for lycorine (I), one of the

(1) Previous paper, W. C. Wildman and C. J. Kaufman, *THIS JOURNAL*, **77**, 4807 (1955).

(2) L. G. Huinber, *et al.*, *J. Chem. Soc.*, 4622 (1954).

two alcoholic groups is placed in an allylic position. With the advent of manganese dioxide as a selective oxidizing agent for allylic alcohols, it seemed desirable to study the action of this reagent upon lycorine and other alkaloids of this family. Unfortunately, the insolubility of lycorine in the common solvents under the normal reaction conditions made impossible a direct comparison with other allylic alcohols. However, when lycorine was extracted



by the Soxhlet technique into a boiling suspension of manganese dioxide in chloroform, a 47% recovery of starting material was obtained.

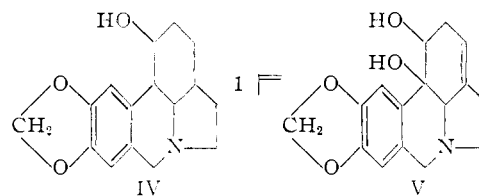
From a study of other types of mild oxidants it was found that selenium dioxide, *t*-butyl hypochlorite, pyridine perbromide hydrobromide and *N*-bromosuccinimide each easily oxidized lycorine to a compound $C_{16}H_{11}NO_3$. From the ultraviolet spectrum and previous work on the known carbon skeleton of lycorine, as well as subsequent synthetic work, the compound was assigned structure IIa. Compound IIa is an amorphous, dark red solid, m.p. 260–270° dec., which on exposure to moisture in the air turns to an orange solid within a few seconds. The betaine IIa may be described by several resonance forms whose relative contributions to the molecule probably vary with the state of hydration. We propose that this action is responsible for the observed color changes. The hygroscopicity of IIa made analytical results for the parent base less satisfactory than those of its salts. Compound IIa formed a hydrated hydrochloride II and an unsolvated hydronitrate that afforded satisfactory analyses.

Compound IIa absorbed one mole of hydrogen upon catalytic reduction to give a pale yellow, fluorescent dihydro derivative which also could be obtained by sodium borohydride reduction of II. In common with other dihydrophenanthridines,^{2,3} isolation and recrystallization techniques caused it to revert to starting material. A pure sample finally was obtained by vacuum sublimation. The picrate of the dihydro derivative of IIa was relatively more stable and gave satisfactory analyses.

When oxidized with mercuric acetate,⁴ lycorine gave a mixture of IIa and a second compound which was isomeric with IIa. On the basis of the currently accepted formula for lycorine, this material can only be IIIa. Whereas II is a very pale yellow, III is a brilliant yellow. The free base IIIa

is a bright red even when hydrated. Upon drying at 160° under reduced pressure, the compound turns to a black powder which neither melts nor decomposes below 400°. Upon exposure to moist air, the material reverts to its original red color.

Compound IIIa was formed as the sole product of a modified Oppenauer oxidation of caranine. Caranine, a minor alkaloid of the Amaryllidaceae, was shown in a previous paper⁵ to be a pentacyclic base of molecular formula $C_{16}H_{17}NO_3$. The oxygen atoms are contained in a methylenedioxy group and an aliphatic hydroxyl group. Although caranine contains one double bond and two hydrogen atoms are lost in the oxidation, four additional hydrogen atoms must be removed to convert caranine to IIIa. There is some evidence that air oxidation plays a part in this. The colorless chloroform extracts of the basic aqueous solution gradually deposited violet crystals of IIIa at the chloroform–water interface on exposure to air. This oxidation suggests partial formula IV for caranine.

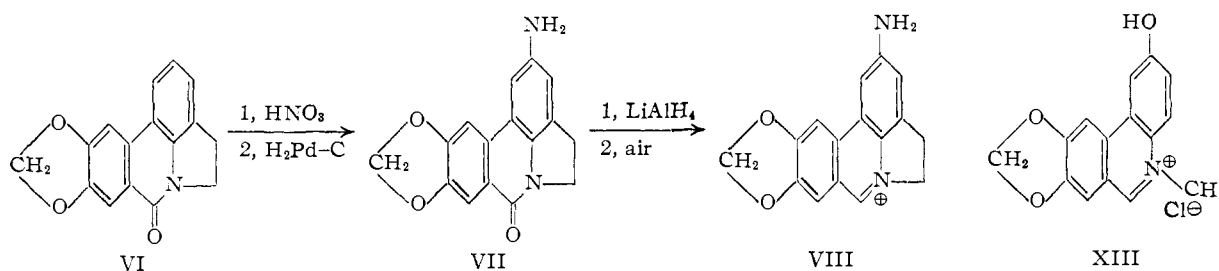


The validity of our assignments of formulas IIa and IIIa is supported by the ultraviolet spectra of synthetic hydroxyphenanthridinium compounds. Synthesis of II was undertaken starting with the lactam VI which had been prepared previously by Humber, *et al.*² The extreme difficulty in obtaining VI, coupled with the onus of five additional steps, precluded full characterization of VIII. Upon treatment with nitrous acid, VIII gave an extremely low yield of a compound which showed identical fluorescence and R_f values in acid and base as II. Since conclusions drawn from the mere identity of R_f values are questionable, compound XIII and its betaine were synthesized as spectral models by methods analogous to those used

(3) (a) P. Karrer, L. Szabo, H. J. V. Krishna and R. Schwyzer, *Helv. Chim. Acta*, **33**, 294 (1950); (b) H. Kondo, K. Takeda and K. Kodera, *Ann. Rept. IITSUU Lab.*, **5**, 66 (1954); (c) J. W. Cook, J. D. Loudon and P. McCloskey, *J. Chem. Soc.*, 4176 (1954).

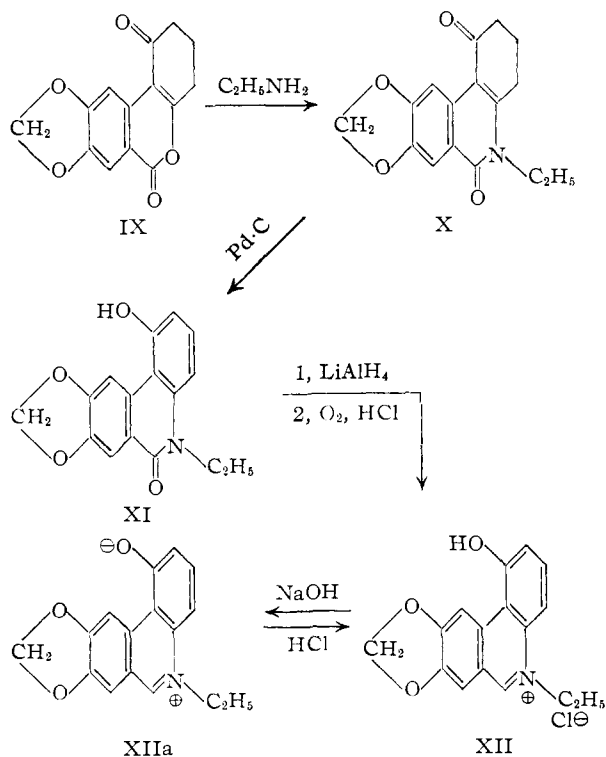
(4) The reaction with mercuric acetate probably proceeds through an intermediate enamine followed by elimination of either hydroxyl group to form the completely aromatic system II or III.

(5) L. H. Mason, E. R. Puschett and W. C. Wildman, *THIS JOURNAL*, **77**, 1253 (1955).



in the preparation of VI. Metol was converted to the 6-nitropiperonylamine. The phenol was protected as the benzoate. Catalytic reduction of the nitro group and Pschorr cyclization gave the phenanthridone. Lithium aluminum hydride served both to reduce the phenanthridone and to cleave the benzoate protecting group. Oxidation with air in the presence of acid furnished a good yield of the phenanthridinium chloride (XIII) whose properties closely resembled those of II.

Although we have not yet synthesized III, a new synthesis of 1-hydroxyphenanthridones and phenanthridinium salts has led to model compounds, the properties and spectra of which closely parallel those of III. Condensation of 6-bromopiperonylic acid with dihydroresorcinol under the conditions used so successfully by Adams and co-workers⁶ gave the pyrone IX. Conversion of IX to the phenanthridone XI was achieved in good yield by standard procedures. Compound XI was reduced with lithium aluminum hydride; acid hydrolysis followed by air oxidation gave the product XII.



The ultraviolet spectra of the oxidation products and the synthetic models show a strong dependence on *p*H. The spectrum of III in ethanol shifts con-

siderably toward that of IIIa upon dilution. To minimize the effects of hydrolysis, all spectra were run in aqueous buffered solutions. The heavier lines of Fig. 1 record the spectra of II in acid and base, while the corresponding spectra of the model XIII are shown by the dots or small dashes. These spectra are uniquely different from those of III and its model XII, which are shown in Fig. 2. The spectra of the models show a hypsochromic shift of a few millimicrons from those of II and III but essentially are identical even in the small points of

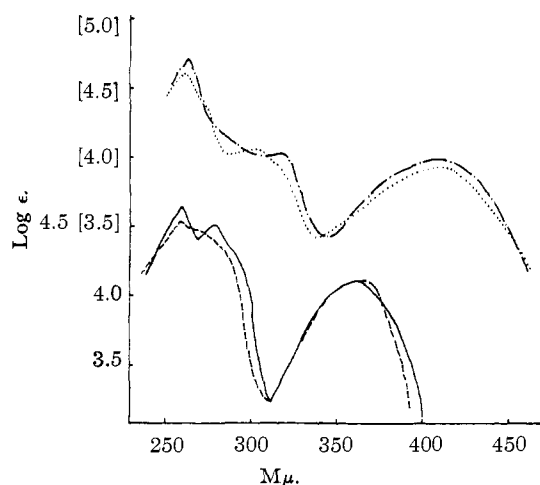


Fig. 1.—Ultraviolet absorption spectra in 0.1 *N* hydrochloric acid of: II, —; XIII, - - - -; in 0.1 *N* sodium hydroxide: II, ·····; XIII, - · - · - ·.

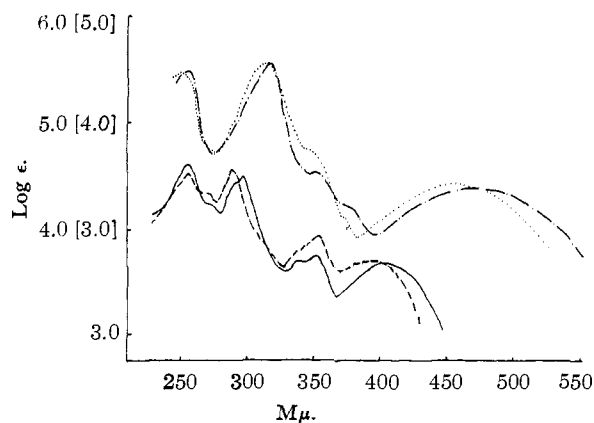


Fig. 2.—Ultraviolet absorption spectra in 0.1 *N* hydrochloric acid of: III, —; XII, - - - -; in *p*H 10.0 buffer of III, ·····; XII, - · - · - ·.

(6) R. Adams and co-workers, *THIS JOURNAL*, **62**, 2204, 2208 (1940).

inflection. While it would be expected that II and III would differ spectrally, the spectra of 1- and 3-oxyphenanthridinium compounds might be quite similar. Unfortunately, synthetic routes to 3-oxygenated phenanthridines are not available. Thus the oxidation products of lycorine are compatible with the accepted formula but do not offer unequivocal proof of the hydroxyl positions. Structure V, suggested by Wenkert⁷ for lycorine, may be eliminated since two phenolic products would not be derived from this formula without an unlikely rearrangement.

Experimental⁸

Oxidation of Lycorine with Selenium Dioxide.—A suspension of 0.370 g. of lycorine and 0.25 g. of sublimed selenium dioxide in 20 ml. of ethanol was warmed on a steam-bath to effect solution. After several minutes, selenium metal precipitated. The solution was heated under reflux for 4 hours and filtered while hot. Partial evaporation of the ethanol under a stream of nitrogen left a yellow solid IIa which was converted to the insoluble hydronitrate. The collected hydronitrate was suspended in water and 0.200 g. of the free base was precipitated by ammonia. The compound was very slightly soluble in hot water from which it precipitated in bright yellow flocculi. In hot dimethylformamide, a small amount of material dissolved and imparted a red hue to the solution. The compound dissolved readily in the lower alcohols forming an orange solution but was insoluble in the hydrocarbon solvents. The free base changed from yellow to bright red when dried over phosphorus pentoxide in a vacuum. When heated to 177° under the same conditions, it became nearly black. Admission of moist air to the sample caused a reversal of the above color changes. The compound possessed no definite crystalline form or melting point but reddened at 120° and decomposed slowly from 260 to 270°. Several analyses were performed on samples which had been dried under various conditions.

Anal. Calcd. for $C_{16}H_{11}NO_3$: C, 72.44; H, 4.18. Found (dried 9 hours, 177°, 0.1 mm.): C, 72.00; H, 4.44. Calcd. for $C_{16}H_{11}NO_3 \cdot 3/5H_2O$: C, 69.61; H, 4.45; N, 5.07. Found (dried 4 hours, 79°, 0.1 mm.): C, 69.69; H, 4.39; N, 5.08. Calcd. for $C_{16}H_{11}NO_3 \cdot 2\frac{1}{2}H_2O$: C, 61.93; H, 5.20. Found (dried 4 hours, 79°, 0.1 mm. and then equilibrated with air): C, 61.96; H, 5.49.

The hydrochloride II was precipitated from an aqueous suspension of the base by the addition of 10% hydrochloric acid. Reprecipitation from water gave pale yellow curds which were dried at 137° (0.1 mm.). No decomposition was noted when the sample was heated to 300°.

Anal. Calcd. for $C_{16}H_{11}NO_3 \cdot HCl \cdot H_2O$: C, 60.09; H, 4.41. Found: C, 59.69; H, 4.33.

The picrate of IIa was precipitated from an aqueous solution of II by the addition of lithium picrate. Recrystallization from dimethylformamide gave yellow prisms, m.p. 275–280° dec.

Anal. Calcd. for $C_{16}H_{11}NO_3 \cdot C_6H_3N_3O_7 \cdot H_2O$: C, 51.57; H, 3.15. Found: C, 51.82; H, 3.14.

The hydronitrate of IIa was formed from the free base by the addition of dilute nitric acid. Reprecipitation from water gave a pale yellow amorphous solid which was collected by centrifugation and dried at 150° (0.1 mm.), m.p. 270–280° dec.

Anal. Calcd. for $C_{16}H_{11}NO_3 \cdot HNO_3$: C, 58.54; H, 3.69. Found: C, 58.53; H, 3.90.

Oxidation of Lycorine with Pyridine Perbromide Hydrobromide.—To a solution of 0.260 g. of lycorine in 3 ml. of

freshly distilled dimethylformamide, 0.30 g. of pyridine perbromide hydrobromide was added in portions with swirling. The reaction mixture was warmed on a steam-bath for 3 minutes and allowed to stand at room temperature for 30 minutes. A brown precipitate formed which contained some fine yellow flakes.⁹ Sodium hydroxide was added to the entire product and the mixture was evaporated under an air jet. A dense yellow-brown precipitate was collected, redissolved in warm hydrochloric acid and filtered. This solution slowly precipitated the pale yellow hydrochloride II which was identified by infrared and ultraviolet spectra. The pure free base, also spectrally identical with IIa, formed when a solution of the hydrochloride was treated with ammonia.

Oxidation of Lycorine with N-Bromosuccinimide.—A solution of 0.3 g. of lycorine in 10 ml. of 10% acetic acid was treated with 0.3 g. of N-bromosuccinimide and heated on a steam-bath for 2 hours. The fluorescent product was filtered while hot and treated with an excess of 10% sodium hydroxide solution. Compound IIa, 0.200 g., precipitated as the free base and was collected by centrifugation. The product was spectrally identical with IIa formed in the oxidation of lycorine with selenium dioxide.

Oxidation of Lycorine with *t*-Butyl Hypochlorite.—A solution of 0.15 ml. of *t*-butyl hypochlorite and 0.2 ml. of pyridine in 3 ml. of ice-cold dimethylformamide was mixed with 0.100 g. of lycorine and allowed to stand overnight. The resulting dark mixture was poured into water and the precipitate was collected. Paper strip chromatography indicated the presence of compound IIa. The R_f of compound IIa was 0.56 when it was developed with a mixture of *n*-butyl alcohol, ethanol, water and acetic acid (9:2:1.5:1) on Whatman #1 filter paper. The R_f of IIa was 0.22 when developed on the same paper with a solution of *n*-butyl alcohol, ammonia and ethanol (8:1:3).

Oxidation of Lycorine with Mercuric Acetate.—To a solution of 0.5 g. of lycorine in 10 ml. of warm 10% acetic acid was added 1.3 g. of mercuric acetate. The solids dissolved completely, but after the solution was heated on a steam-bath for 3 minutes a dense precipitate of mercurous acetate formed. After 45 minutes the solid was removed by centrifugation and hydrogen sulfide was admitted to the centrifugate to precipitate excess mercury ions. The mercuric sulfide was removed by filtration and 2 g. of salt was added to precipitate 0.152 g. of the hydrochloride II which was identified by ultraviolet and infrared spectra. Addition of hydrochloric acid to the filtrates from this precipitate caused the formation of 0.090 g. of bright yellow flakes having the same infrared and ultraviolet spectra as III obtained from the oxidation of caranine.

Reduction of Compound II. (a).—A suspension of 0.060 g. of II in 3 ml. of water was treated with a slight excess of sodium borohydride. Effervescence occurred immediately and a voluminous orange precipitate formed. The precipitate was extracted into chloroform and the brightly fluorescent extracts were dried over sodium sulfate under an atmosphere of nitrogen. The chloroform was removed under reduced pressure with a stream of nitrogen admitted through the capillary tube. The yellow, semi-crystalline mass which remained was oxidized easily in air even in the dry state. Decomposition of the compound began at 70° on the hot stage, but under a nitrogen atmosphere it melted with decomposition from 180 to 190°. It was soluble in both acid and base, but in basic solution it very rapidly formed a dense yellow precipitate of IIa. A sample was sublimed at 0.01 mm. to form a very pale yellow solid which was analyzed immediately.

Anal. Calcd. for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90. Found: C, 71.50; H, 5.03.

The picrate of the dihydro derivative of IIa was found to be considerably more stable than the free base and crystallized as long needles from ethanolic dimethylformamide. A sample was dried at 100° (0.1 mm.), m.p. (under nitrogen) 267–270° dec.

Anal. Calcd. for $C_{16}H_{13}NO_3 \cdot C_6H_3N_3O_7$: C, 53.23; H, 3.25. Found: C, 53.07; H, 3.58.

(b).—A sample of 40.4 mg. of II took up slightly more than one equivalent of hydrogen at 25° over pre-reduced

(7) E. Wenkert and J. H. Hansen, *Chemistry & Industry*, 1262 (1954).

(8) All melting points are corrected and were observed on a Kofler microscope hot-stage equipped with polarizer. Analyses were performed by Dr. W. C. Alford and staff, National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland, and the Clark Microanalytical Laboratory, Urbana, Illinois. Ultraviolet spectra were recorded with a Cary model 11MS spectrophotometer. The spectral work was performed by Miss F. C. Bateman. The technical assistance of Mrs. Laura Guiffrida is gratefully acknowledged.

(9) The yellow compound probably was the isomeric phenanthridinium bromide of III the chloride of which was isolated later from an oxidation of lycorine with mercuric acetate.

platinum oxide in glacial acetic acid. The mixture became strongly fluorescent, indicating the presence of a dihydrophenanthridine. When the filtered solution was acidified with hydrochloric acid, the fluorescence disappeared and 23 mg. of II slowly precipitated.

Oxidation of Caranine.—To a solution of 0.20 g. of potassium metal in 15 ml. of *t*-butyl alcohol (distilled from sodium) was added 500 mg. of caranine, m.p. 179–181°, and 1.60 g. of dry benzophenone. The reaction mixture was refluxed under nitrogen for 10 hours. After most of the *t*-butyl alcohol had been blown off in a stream of nitrogen, the residue was diluted with water and chloroform. On attempted extraction of the aqueous layer with chloroform, a purple solid separated at the interface. Acidification of the aqueous solution did not dissolve the precipitate but caused it to turn bright yellow. Centrifugation gave 361 mg. (65%) of yellow, insoluble phenanthridinium chloride (III) which did not melt below 400°. Paper chromatography indicated this to be a single substance. The material was appreciably soluble only in water and methanol. A sample was recrystallized three times from ethanol–methanol to give mustard-yellow prisms.

Anal. Calcd. for $C_{16}H_{11}NO_3 \cdot HCl$: C, 63.68; H, 4.01; N, 4.64; Cl, 11.75. Found: C, 63.60; H, 3.97; N, 4.71; Cl, 11.50.

The aqueous acid solution from the reaction mixture was basified and extracted with chloroform to give 124 mg. (25%) of recovered caranine which after filtration through alumina in ethyl acetate and recrystallization from ethyl acetate had m.p. 177.5–181°; the mixture melting point with caranine was not depressed.

The phenanthridinium chloride (100 mg.) was dissolved in aqueous methanol and 10% ammonium hydroxide solution was added, whereupon a deep red precipitate formed. This was centrifuged and washed with water to give 63 mg. of dark red powder which was recrystallized four times from absolute ethanol to give deep red needles which did not melt below 400°. A solution in pyridine or dimethylformamide was a blue-green.

Anal. Calcd. for $C_{16}H_{11}NO_3 \cdot \frac{1}{2}C_2H_6O$: C, 71.59; H, 4.55; N, 5.06. Found: C, 71.58; H, 4.46; N, 5.49.

The picrate of IIIa was prepared in quantitative yield by the addition of lithium picrate to an aqueous solution of III. Recrystallization from dimethylformamide gave yellow-green prisms, m.p. 260–280° dec.

Anal. Calcd. for $C_{16}H_{11}NO_3 \cdot C_6H_3N_3O_7$: C, 53.45; H, 2.85. Found: C, 53.57; H, 3.12.

1-(2'-Amino-4',5'-methylenedioxybenzoyl)-2,3-dihydroindole.—This compound was prepared by catalytic reduction of 1-(2'-nitro-4',5'-methylenedioxybenzoyl)-2,3-dihydroindole in ethanol over 10% palladium-on-charcoal. The product initially isolated melted at 123–125° instead of the reported² 142–143°. However, later preparations melted at the higher temperature, and fusions of the lower melting form resolidified when seeded with the higher melting form and melted at 143–144°. The infrared spectra of Nujol mulls of the two polymorphic forms differed at several frequencies, but solution spectra were identical.

4,5-Dihydro-9,10-methylenedioxy-2-nitro-7-oxo-7H-dibenzo[f,h,i]pyrrocoline.—A solution of 0.888 g. of 4,5-dihydro-9,10-methylenedioxy-7-oxo-7H-dibenzo[f,h,i]pyrrocoline (VI)² in 12 ml. of glacial acetic acid was treated with 3 ml. of concentrated nitric acid. The nitro compound precipitated in clumps when the mixture was warmed on a steam-bath. The filtered product was washed with water and ethanol and recrystallized from 500 ml. of dimethylformamide. The product (0.80 g., 77%) did not decompose up to 300° under nitrogen.

Anal. Calcd. for $C_{18}H_{10}N_2O_5$: C, 61.94; H, 3.25; N, 9.03. Found: C, 62.07; H, 3.51; N, 8.89.

2-Amino-4,5-dihydro-9,10-methylenedioxy-7-oxo-7H-dibenzo[f,h,i]pyrrocoline (VII).—A suspension of 0.800 g. of the nitro compound mentioned above and 0.50 g. of 10% palladium-on-charcoal in 50 ml. of absolute ethanol was shaken overnight at 70° in an atmosphere of hydrogen. The catalyst was filtered from the warm solution and a flocculent precipitate of the amino compound (0.700 g., 97%) formed on cooling. A sample was recrystallized from toluene and then sublimed; m.p. 247–250° dec.

Anal. Calcd. for $C_{16}H_{12}N_2O_3$: C, 68.56; H, 4.32. Found: C, 68.67; H, 4.49.

The benzal derivative of VII formed when the free amine was combined with an equal weight of benzaldehyde in ethanol. A sample was recrystallized from toluene and formed small prisms, m.p. 268–269°.

Anal. Calcd. for $C_{23}H_{16}N_2O_3$: C, 74.99; H, 4.38. Found: C, 75.01; H, 4.46.

Reduction of VII by Lithium Aluminum Hydride.—A sample of 0.59 g. of VII was added to a slurry of 0.70 g. of lithium aluminum hydride in 25 ml. of dry tetrahydrofuran. The mixture was heated under reflux in a nitrogen atmosphere overnight and then decomposed with water and sulfuric acid. The chilled solution was treated immediately with 0.20 g. of sodium nitrite, stirred for 5 minutes, and 0.20 g. of urea was added to decompose excess nitrous acid. The solution was boiled gently under reflux until a negative test with β -naphthol was obtained. The dark solution was treated with alkali and extracted with a mixture of ethanol and butanol. The extracts were filtered from much dark material which collected at the interface and dried over anhydrous sodium sulfate. In the systems described under oxidation of lycorine with *t*-butyl hypochlorite, a component in the solution had R_f values that were identical with those found for authentic IIa. The compound exhibited a blue-green fluorescence in acid and a yellow fluorescence in base.

6-Bromopiperonylic Acid.—The existing methods for the preparation of this acid based on potassium permanganate oxidation of 6-bromopiperonal were found to be unsatisfactory with respect to yield. Excellent yields were obtained from the Cannizzaro reaction of 6-bromopiperonal.¹⁰ A suspension of 280 g. of 6-bromopiperonal in 1.5 l. of 50% sodium hydroxide solution was agitated with a Vibro-mixer and warmed to 70°. The mixture changed abruptly from a paste to a fluid mass. The product was cooled and the 6-bromopiperonyl alcohol was removed by filtration. The aqueous solutions were extracted twice with ether to remove traces of 6-bromopiperonyl alcohol and acidified with hydrochloric acid. The precipitated 6-bromopiperonylic acid was removed by filtration and washed with water, m.p. 200–204°, yield 146 g. (97%). The 6-bromopiperonyl alcohol was recovered from the extractions and combined with that initially precipitated, m.p. 90°, yield 125 g. (89%).

1-Keto-8,9-methylenedioxy-1,2,3,4-tetrahydrodibenzo[b,d]6-pyrone (IX).—The yields in the following reaction varied for no apparent reason. One run, conducted in the absence of copper powder, gave none of the desired product. The following method was found to give the most reproducible results. To a solution of 2.0 g. of sodium in 50 ml. of absolute ethanol was added 0.3 g. of anhydrous cupric acetate, 0.1 g. of precipitated copper metal, 5.5 g. of 1,3-cyclohexanedione and 10.9 g. of 6-bromopiperonylic acid. The mixture was stirred under reflux in an atmosphere of nitrogen for 24 hours, cooled and poured into an excess of 5% hydrochloric acid. The mixture was allowed to stand 4 hours to ensure complete lactone formation, then filtered and washed with warm sodium bicarbonate solution to redissolve residual bromoacid. The precipitated pyrone (7.25 g., 64%), m.p. 211°, was pure enough for the next step. An analytical sample was prepared by recrystallization from ethanol; m.p. 213°.

Anal. Calcd. for $C_{14}H_{10}O_5$: C, 65.12; H, 3.90. Found: C, 64.95; H, 4.23.

The 3,3-dimethyl analog of IX was prepared from the reaction between methone and 6-bromopiperonylic acid by the same procedure as described for IX. A sample was recrystallized from benzene forming long needles, m.p. 184–185°.

Anal. Calcd. for $C_{16}H_{14}O_5$: C, 67.12; H, 4.93. Found: C, 67.23; H, 5.23.

5-Ethyl-1-keto-8,9-methylenedioxy-1,2,3,4-tetrahydro-6-phenanthridone (X).—A suspension of 2.0 g. of IX in 25 ml. of absolute ethanol was treated with 3 ml. of a 33% solution of ethylamine in water. An additional 4 ml. of ethylamine solution was added after the mixture had been heated under reflux for 5 minutes. The solution then was completely homogeneous and was boiled for 3 hours, decolorized with charcoal and allowed to cool. The phenanthridone precipitated in long needles (1.08 g., 49%) which were recrystallized from ethanol; m.p. 214.5–215°.

Anal. Calcd. for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30. Found: C, 67.18; H, 5.41.

(10) R. G. Naik and T. S. Wheeler, *J. Chem. Soc.*, 1780 (1938).

5-Ethyl-1-hydroxy-8,9-methylenedioxy-6-phenanthridone (XI).—A suspension of 0.738 g. of X and 1.83 g. of 10% palladium-on-charcoal in 25 ml. of *p*-cymene was boiled under a nitrogen atmosphere for 5 hours. The catalyst, which retained the phenol, was removed by filtration, washed with benzene and dried. Repeated washing with hot 10% sodium hydroxide solution was necessary to remove the phenol from the catalyst. The basic washings were treated with acetic acid to reprecipitate the phenol. The filtered and dried phenol (0.37 g., 51%) was sublimed at 0.07 mm., m.p. 303–310° dec. (in a nitrogen atmosphere).

Anal. Calcd. for $C_{18}H_{13}NO_4$: C, 67.84; H, 4.63. Found: C, 67.98; H, 4.58.

5-Ethyl-1-hydroxy-8,9-methylenedioxyphenanthridinium Chloride (XII).—To a slurry of 0.10 g. of lithium aluminum hydride in 25 ml. of dry tetrahydrofuran was added 0.150 g. of XI in one portion. The mixture was heated overnight under reflux in a nitrogen atmosphere. Successive portions of 2 ml. of ethanol and 10 ml. of 10% hydrochloric acid were added, and the mixture was heated to drive off the tetrahydrofuran. Air was passed through the hot solution for 1 hour causing the formation of a fine yellow precipitate of XII. The suspension was cooled and the product (0.080 g., 50%) was collected and recrystallized from aqueous ethanol, m.p. 285–292° dec. (in a nitrogen atmosphere).

Anal. Calcd. for $C_{18}H_{13}NO_3 \cdot HCl \cdot H_2O$: C, 59.72; H, 5.01. Found: C, 59.74; H, 5.32.

The free base XIIa was obtained by adding dilute sodium hydroxide to an aqueous alcoholic solution of XII. The dark red needles that formed became gray-black when dried over phosphorus pentoxide in a vacuum but regained their color when moist air was admitted to the chamber. Alcoholic solutions of the base were deep red; dimethylformamide or pyridine solutions were deep blue-violet. The product became opaque on the hot-stage at 180° and decomposed from 280 to 290°. The extremely hygroscopic nature of the free base made analysis difficult.

Anal. Calcd. for $C_{18}H_{13}NO_3 \cdot \frac{1}{2}H_2O$: C, 70.94; H, 4.98. Found: C, 71.08; H, 5.08.

N-(4,5-Methylenedioxy-2-nitrobenzoyl)-*p*-methylaminophenol.—4,5-Methylenedioxy-2-nitrobenzoyl chloride prepared from 38 g. of the corresponding acid was dissolved in 75 ml. of dioxane and added alternately with 60 g. of sodium bicarbonate to a solution of 73 g. of *p*-methylaminophenol sulfate in 1 l. of water. The mixture darkened rapidly and the black precipitate which formed was removed by filtration and washed with dilute hydrochloric acid. The residue was dissolved in 10% sodium hydroxide and filtered to remove non-phenolic compounds. The alkaline solutions were reacidified with acetic acid. The brown, crystalline product was collected, dried and recrystallized from ethanol. Several recrystallizations gave 20 g. (34%) of yellow needles, m.p. 224–225°.

Anal. Calcd. for $C_{15}H_{12}N_2O_5$: C, 56.96; H, 3.82; N, 8.86. Found: C, 57.28; H, 3.81; N, 8.79.

The *O*-benzoyl derivative was prepared by the Schotten-Baumann technique in aqueous alkali with benzoyl chloride.

The crude product was recrystallized first from xylene, then from butyl acetate, as short tablets, m.p. 179–180°, yield 68%.

Anal. Calcd. for $C_{22}H_{16}N_2O_7$: C, 62.86; H, 3.84. Found: C, 62.67; H, 3.95.

N-(6-Amino-3,4-methylenedioxybenzoyl)-*p*-methylaminophenyl Benzoate.—A suspension of 4.55 g. of the nitrobenzoylamide and 0.5 g. of 10% palladium-on-charcoal in 50 ml. of ethanol was shaken overnight under hydrogen at 50 p.s.i. and 70°. The catalyst was separated by filtration and the solvents partially evaporated in a current of nitrogen causing the free amine to precipitate, 3.83 g. (91%), m.p. 130–133°. After several recrystallizations from ethanol, the amine formed colorless plates, m.p. 132–135°.

Anal. Calcd. for $C_{22}H_{18}N_2O_5$: C, 67.68; H, 4.65. Found: C, 67.43; H, 4.46.

The benzal derivative crystallized easily when benzaldehyde was added to an ethanolic solution of the amine. A sample was recrystallized from aqueous ethanol, forming hydrated tablets, m.p. 188–189°.

Anal. Calcd. for $C_{20}H_{22}N_2O_6 \cdot H_2O$: C, 70.15; H, 4.87. Found: C, 70.08; H, 4.77.

2-Benzoyloxy-5-methyl-8,9-methylenedioxy-6-phenanthridone.—A solution of 2.14 g. of the hydrochloride of N-(6-amino-3,4-methylenedioxybenzoyl)-*p*-methylaminophenyl benzoate in 5 ml. of ethanol, 2.5 ml. of water and 2.5 ml. of concentrated hydrochloric acid was treated at 10° with 2 ml. of 20% sodium nitrite solution. After 5 minutes, 0.25 g. of urea and 0.1 g. of precipitated copper were added, and the mixture was warmed on a steam-bath. Effervescence occurred and a white precipitate formed slowly. The product (0.80 g., 43%) was recrystallized from xylene and formed short, columnar prisms, m.p. 265–267°, after recrystallization at 225° in a nitrogen atmosphere.

Anal. Calcd. for $C_{22}H_{15}NO_5$: C, 70.77; H, 4.05. Found: C, 70.93; H, 4.26.

The filtrates from this reaction slowly deposited a white solid which was recrystallized from butyl acetate forming short, columnar prisms, m.p. 242–245°, which were not investigated further.

2-Hydroxy-5-methyl-8,9-methylenedioxyphenanthridinium Chloride (XIII).—By the technique described for the reduction of XI, 0.40 g. of 2-benzoyloxy-5-methyl-8,9-methylenedioxy-6-phenanthridone gave 0.253 g. (93%) of XIII. Recrystallization from aqueous ethanol gave long yellow needles, m.p. 282–284° dec. (in a nitrogen atmosphere).

Anal. Calcd. for $C_{15}H_{11}NO_3 \cdot HCl$: C, 62.18; H, 4.18. Found: C, 62.00; H, 4.37.

The free base was precipitated from an aqueous solution of XIII with ammonia and recrystallized several times from ethanol; m.p. 260–270° dec. (in a nitrogen atmosphere). Thorough drying at 170° and 0.1 mm. caused a color change from orange to dark red in the same manner as II.

Anal. Calcd. for $C_{15}H_{11}NO_3 \cdot H_2O$: N, 5.16. Found: N, 5.26.

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