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

Alkaloids of the Amaryllidaceae. XIX. On the Structures of Crinamidine, Flexinine, and Nerbowdine[1]

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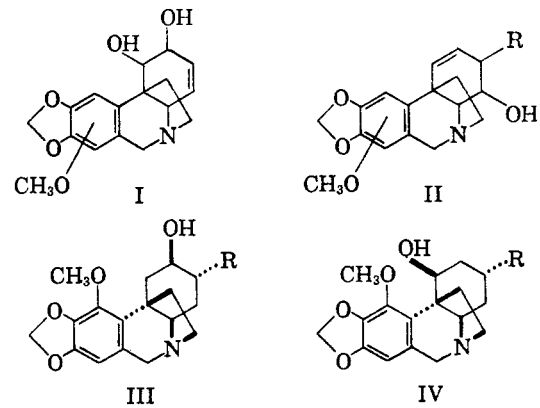
Received May 16, 1960

From degradative evidence and partial syntheses, crinamidine and flexinine have been assigned structures Va and Va (no OCH_3), respectively. A key intermediate (XVIa) in the proof of these structures has been identified as the naturally occurring alkaloid nerbowdine.

Crinamidine, a relatively rare alkaloid of the Amaryllidaceae, occurs in small amounts in *Crinum moorei* Hook. f.,[2,3] *Nerine bowdenii* W. Wats.,[2,4] *N. corusca* Herb.[5] and *N. flexuosa* Herb.[5] It was characterized by the German workers as a tertiary base, $C_{17}H_{19}NO_5$, which contains one methylenedioxy and one methoxyl group but no *N*-methyl function.[3-5] The two remaining oxygen atoms were considered to be present in hydroxyl groups because crinamidine was reported to form both a mono- and a di-*O*-acetyl derivative. As the infrared spectrum of crinamidine is nearly identical with that of powelline, these authors proposed that the alkaloid was a hydroxypowelline. Based on the structure of powelline considered most likely at that time,[6] crinamidine was formulated as I.[5] With the determination of the correct structure of powelline (XIV),[7,8] Boit and Döpke[9] amended the structure of crinamidine to IIa. Additional support for this structure was claimed in the observation that crinamidine gave a positive test for vicinal glycol with periodic acid.[9]

In previous papers of this series the structure of undulatine has been elucidated to the complete stereostructure Vb.[8,10] Conformational assignments cited for the substituents of ring C of Vb are valid when this ring is in the half-chair form. As *O*-methylation of crinamidine afforded undulatine,[8] and it has been demonstrated that this methylation takes place with retention of configuration at C_3, we consider Va the only tenable structure for

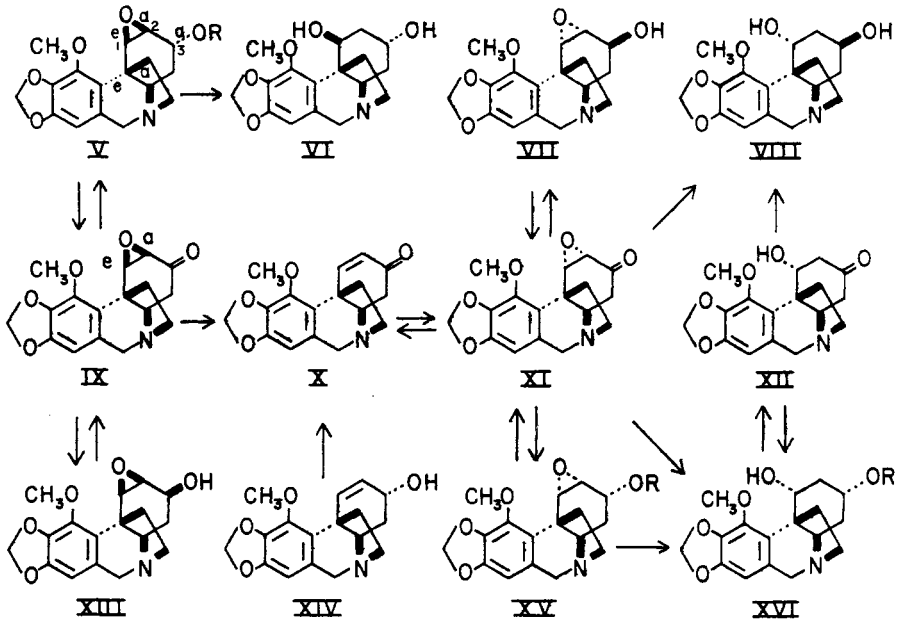


Series a. R = OH
b. R = OCH_3
c. R = 2-tetrahydropyranyloxy

crinamidine. The additional degradations described below provide complete substantiation for this structure.

In agreement with structure Va, crinamidine was found to contain only one active hydrogen and was not reduced by catalytic methods in neutral solution. It gave no test for a 1,2-glycol with periodic acid[11] in the cold but gave a positive test when the crinamidine, periodic acid, and dilute nitric acid solution were warmed briefly. This behavior is consistent with a hydrolytic cleavage of the epoxide group in the warm, acidic reaction mixture. Acetylation of crinamidine in the presence of acetic anhydride and pyridine at room temperature gave, in our hands, a single, crystalline mono-*O*-acetyl derivative in good yield. The infrared spectrum of the product, either in chloroform solution or as a Nujol mull, showed no hydroxyl absorption from 5000–3000 cm.$^{-1}$ It could be converted to an *O*-acetylcrinamidine hydroperchlorate which proved to be dimorphic. The two melting points of this salt, 157–160° and 200–206°, agree well with those reported by Boit and Ehmke[5] for "diacetylcrinamidine perchlorate" (m.p. 160–161°) and "monoacetylcrinamidine perchlorate" (m.p. 205–206° dec.), respectively. We were not able to obtain any

(1) Previous paper, Y. Inubushi, H. M. Fales, E. W. Warnhoff, and W. C. Wildman, *J. Org. Chem.*, **25**, 2153 (1960).
(2) R. E. Lyle, E. A. Kielar, J. R. Crowder, and W. C. Wildman, *J. Am. Chem. Soc.*, 82, 2620 (1960).
(3) H.-G. Boit, *Chem. Ber.*, 87, 1704 (1954).
(4) H.-G. Boit and H. Ehmke, *Chem. Ber.*, 89, 2093 (1956).
(5) H.-G. Boit and H. Ehmke, *Chem. Ber.*, 90, 369 (1957).
(6) W. C. Wildman, *Chem. & Ind.*, 1090 (1956).
(7) W. C. Wildman, *J. Am. Chem. Soc.*, 80, 2567 (1958).
(8) H. M. Fales and W. C. Wildman, *J. Am. Chem. Soc.*, 82, 3368 (1960).
(9) H.-G. Boit and H. Döpke, *Chem. Ber.*, 92, 2582 (1959).
(10) E. W. Warnhoff and W. C. Wildman, *Chem. & Ind.*, 1293 (1958); *J. Am. Chem. Soc.*, 82, 1472 (1960).
(11) R. L. Shriner and R. C. Fuson, *Systematic Identification of Organic Compounds*, p. 115, John Wiley and Sons, Inc., New York (1948).



Series a, R=H; b, R=CH_3; c, R=2 TETRAHYDROPYRANYL

evidence that crinamidine formed an *O,O*-diacetyl derivative under these acetylating conditions, and the nature of the *O,O*-diacetate reported by Boit and Ehmke[5] remains uncertain.

A second degradative sequence supporting structure Va for crinamidine was found in the oxidation of crinamidine with manganese dioxide. It has been our experience that usually one gram of activated manganese dioxide will oxidize completely one millimole of an alkaloid containing the allylic alcohol group within three hours. In contrast, saturated alcohols, *e.g.*, α-dihydroundulatine[10] and nerbowdine,[2] require several days for even partial oxidation. With crinamidine, oxidation was found to proceed as rapidly as it had for powelline. While this observation might be considered to support the Boit and Ehmke structure (IIa), the spectral and analytical data derived from the oxidation product were consistent with structure IX, rather than II (R = O) which would result from the oxidation of IIa. The oxidation product, oxocrinamidine (IX), showed carbonyl absorption at 1724 cm.$^{-1}$ The α,β-unsaturated ketone derived from the oxidation of IIa would be expected to absorb near 1681 cm.$^{-1}$, the frequency found earlier for X. No hydroxyl absorption was found in the 5000–3000 cm.$^{-1}$ range. The ultraviolet absorption spectrum was nearly identical with that of the starting material. At 230 mμ, where intense absorption due to an α,β-unsaturated ketone group would be expected if IIa were the structure of crinamidine, oxocrinamidine, and crinamidine differed in ε values by only 800.[12] These data are incompatible with formulation of oxocrinamidine as II (R = O). Reduction of oxocrinamidine with sodium borohydride gave largely (85%) an epimeric alcohol (XIII), along with a trace of crinamidine. The epimeric nature of the C_3 hydroxyl in XIII was demonstrated by the facile reoxidation of XIII to IX by manganese dioxide. Although the oxidation of cyclopropylcarbinols to ketones by manganese dioxide has been reported,[13,14] the utility of this reagent in the oxidation of α,β-epoxyalcohols has not been appreciated. It would appear that a combination of the steric strain due to the epoxide group and the electronegative nature of the heterocyclic oxygen atom provides activation comparable to that of a double bond.

In the presence of zinc and acetic acid at room temperature, oxocrinamidine was converted to oxopowelline (X). When the reduction was carried out at reflux temperature, dihydroöxopowelline was the predominant product. Presumably reduction occurs first at the C_2—O bond to form a β-hydroxy ketone which is dehydrated to X in the acidic reaction medium. Further reduction of X to dihydroöxopowelline at reflux temperature is feasible, as pure X afforded dihydroöxopowelline under these conditions.[15] The isolation of X from this degradative sequence may be taken as an alternate proof for the (+)-powellane nucleus of the alkaloid and the C_3 location of the hydroxyl group of crinamidine.

(12) 1-Acetoxy-2-lycorinone possesses a similar arrangement of functional groups to II (R = O) and shows $\lambda_{max.}^{C_2H_5OH}$ 235 mμ (ε 15,800) and 290 mμ (ε 5000); the unsaturated carbonyl band is at 1675 cm.$^{-1}$. Y. Nakagawa and S. Uyeo, *J. Chem. Soc.*, 3736 (1959).

(13) R. M. Evans, *Quart. Revs.*, 13, 61 (1959).

(14) G. Ohloff, H. Farnow, W. Philip, and G. Schade, *Ann.*, 625, 206 (1959).

With oxocrinamidine established as IX, it seemed possible that some oxocrinamidine might be formed when oxopowelline (X) was treated with hydrogen peroxide and alkali. The reaction product proved to be a single, crystalline epoxide. Analytical data showed that one atom of oxygen had been introduced, and the infrared and ultraviolet spectra were in agreement with the change of an α,β-unsaturated ketone to an α,β-epoxycyclohexanone. However, the product was not identical with oxocrinamidine as shown by differences in melting point, optical rotation, and infrared spectrum. Like oxocrinamidine, this product was converted to oxopowelline with zinc and acetic acid. Because it can differ from oxocrinamidine only in the configuration of the epoxide group, this epoxy ketone is assigned structure XI. The stereochemistry of the epoxy group of epoxyoxopowelline (XI) is in accord with an initial axial attack of HOO^- at C_1 of X on that side of ring C which is unhindered by the 5,10b-ethano bridge.

It has been reported that lithium aluminum hydride reduces undulatine (Vb) to a mixture of IIIb and IVb.[10] In a comparable manner, the free hydroxyl of cinamidine was protected by conversion to a mixture of diastereomeric 2-tetrahydropyranyl ethers (Vc). Subsequent reduction of the mixture with lithium aluminum hydride afforded two alcohols (IIIc). These alcohols differed in the configuration at C_2 of the tetrahydropyranyl protecting group, as both isomers gave the same diol (IIIa) upon mild acid hydrolysis. The vicinal nature of the diol was apparent from the consumption of 1.05 equivalents of periodic acid. The relative slowness of the reaction ($t_{1/2}$ = 86 min.) may be indicative of the *trans* arrangement of the hydroxyl groups, but the failure to obtain a *cis* isomer from analogous reactions with either XIII or XV for comparison makes any conclusion based on this observation of dubious value. It is unlikely that the configuration of the hydroxyl of crinamidine has been altered during the reaction sequence, and it is still *cis* to the phenyl and axial in IIIa and IIIc. The hydroxyl at C_2 would be expected to retain the configuration (axial) present in the epoxide group before reduction. Proof that the hydroxyls at C_2 and C_3 are *trans* diaxial is obtained from the infrared spectra of IIIa and IIIc, neither of which showed any evidence of intramolecular hydrogen bonding.[16]

Crinamidine, possessing an unprotected hydroxyl at C_3, behaved in a different manner when reduced with lithium aluminum hydride. The product, dihydrocrinamidine (VI), was a nonvicinal diol as determined by periodate titration. It formed an amorphous *O,O'*-diacetyl derivative which was stable to distillation at 130° (1 μ). The infrared spectrum of VI in the 5000–3000 cm.$^{-1}$ range showed only one stretching frequency at 3625 cm.$^{-1}$, characteristic of an unbonded hydroxyl group. The positions and stereochemistry of the hydroxyl groups of VI are in accord with the supporting data cited above and are derived from the precursor (Va) by attack of hydride ion at C_2. Analogous reductions have been cited in the steroid series.[17] No reduction occurred when either epicrinamidine (XIII), epicrinamidine perchlorate, or the 3-(2-tetrahydropyranyl) ether of epicrinamidine was treated with lithium aluminum hydride under conditions comparable to those which were successful in the reduction of crinamidine.

As might be expected, two diols were formed when XI was reduced with lithium aluminum hydride. Neither diol (VIII or XVIa) was vicinal as shown by lack of oxidation by periodic acid. Prolonged stirring of a chloroform solution of XVIa with manganese dioxide resulted in a 50% yield of a hydroxy ketone (XII). The structure of XII was proved through a facile conversion of XII to X by aluminum *t*-butoxide in refluxing toluene. An attempt to prepare the 2,4-dinitrophenylhydrazone of XII resulted in the formation of the 2,4-dinitrophenylhydrazone of X. Barring an unlikely epimerization at C_1 during the reduction of XI, the configuration of the C_1 hydroxyls of XVIa and VIII would be *cis* to the phenyl. When XII was reduced with sodium borohydride, both XVIa and VIII were produced. This conversion demonstrates that XVIa and VIII have the same hydroxyl configuration at C_1 and are epimeric at C_3. Finally, XVIa was established as the *cis* diol because it formed a monomeric *O,O'*-carbonate. The infrared spectrum of the carbonate showed carbonyl absorption at 1748 cm.$^{-1}$, characteristic of the carbonate of a 1,3-glycol.[18] With regard to ring C, the hydroxyls of XVIa should be *cis* to the phenyl if the lithium aluminum hydride reduction of XI proceeds with no inversion of the C_1 oxygen function. If ring C of XVIa is in the chair form, both hydroxyls are axial.

Reduction of XI with the milder reagent sodium borohydride gave two epimeric epoxy alcohols, epoxypowelline (XVa) and epiepoxypowelline (VII). Both could be reoxidized by manganese dioxide to XI, indicating that the compounds were epimeric at C_3. The hydroxyl configurations at C_3 of VII and XVa are assigned from the reduction of each with lithium aluminum hydride; epoxypowel-

(15) This reduction is in contrast to a recent report on the action of zinc and acetic acid on several 4,5-unsaturated 3-oxo steroids, where loss of the oxygen atom and double bond migration were observed. *Cf.* J. McKenna, J. K. Norymberski, and R. D. Stubbs, *J. Chem. Soc.*, 2502 (1959).

(16) A critical analysis of the 3650–3400 cm.$^{-1}$ region of the infrared spectra for oxygenated derivatives of crinane and powellane will be reported in the near future.

(17) Pl. A. Plattner, H. Heuser, and A. B. Kulkarni, *Helv. Chim. Acta*, **31**, 1885 (1948).

(18) Carbonates of 1,2-glycols show carbonyl absorption near 1817 cm.$^{-1}$; J. L. Holes, J. I. Jones, and W. Kynaston, *J. Chem. Soc.*, 618 (1957).

line (XVa) afforded a 65% yield of XVIa, while VII gave a 77% yield of VIII.

Structure XVIa is significant in another respect. From the bulbs of *Nerine bowdenii* W. Wats. and one unidentified *Brunsvigia* species we have isolated an alkaloid, $C_{17}H_{21}NO_5$, which was named nerbowdine.[2] From deductions provided by the analytical and spectral data, the alkaloid was tentatively considered to be a dihydroxypowellane. A comparison of XVIa prepared from oxopowelline with nerbowdine isolated from *N. bowdenii* showed the two substances to be identical.[19]

Studies on the structure of flexinine were limited in scope because of the scant supply of *Nerine flexuosa*. This alkaloid, $C_{16}H_{17}NO_4$, was isolated first from this source by Boit and Ehmke.[5] It was reported by them to contain one methylenedioxy and one hydroxyl group but no methoxyl. Acetylation of flexinine afforded a monoacetylflexinine, the infrared spectrum of which showed no hydroxyl absorption. A solution of flexinine in dilute hydrochloric acid absorbed no hydrogen in the presence of a platinum catalyst. We have confirmed these observations and found that flexinine was oxidized by manganese dioxide to a ketone, oxoflexinine, which, like oxocrinamidine, showed infrared carbonyl absorption at 1724 cm.$^{-1}$ and no band attributable to a hydroxyl group. The ultraviolet spectrum showed only the normal methylenedioxyphenyl absorption which was present in the parent alkaloid. In the presence of zinc and acetic acid, oxoflexinine was converted to oxocrinine (X, no OCH_3). This conversion establishes the basic ring system, (−)-crinane,[8] of flexinine and locates the hydroxyl group at C_3. To accommodate the molecular formula $C_{16}H_{17}NO_4$, flexinine must contain either a double bond or a sixth ring. As flexinine is stable to catalytic reduction and oxoflexinine is a saturated cyclohexanone containing no free hydroxyl, a 1,2-epoxide is in accord with the experimental facts. Although these data do not provide complete proof of the stereochemistry of the 1,2-epoxide of flexinine, the evidence that flexinine and crinamidine occur together in *N. flexuosa*, possess strikingly similar infrared spectra, and undergo identical reactions indicates a close relationship between them. Also, epoxyoxocrinine (XI, no OCH_3) prepared by the alkaline epoxidation of oxocrinine is isomeric but not identical with oxoflexinine, and the epimeric nature of the epoxide group in the two derivatives thereby is established. By analogy with this relationship in the powelline-crinamidine series, the epoxide group of flexinine is established as *trans* to the phenyl, and flexinine is *ar*-demethoxycrinamidine (Va, no OCH_3).

EXPERIMENTAL[27]

Isolation. Isolations of crinamidine from the seeds of *Crinum moorei* J. D. Hook. and the bulbs of *Nerine bowdenii* W. Wats. have been reported previously.[2] Flexinine, ambelline, crinamidine, lycorine, and undulatine were obtained from *Nerine flexuosa* Herb. The same major alkaloids were reported from this source by Boit and Ehmke.[5]

Flexinine (V, no OCH_3). The alkaloid was recrystallized twice from ethanol to give long prisms, m.p. 230–231°, and finally sublimed in vacuum for analysis, m.p. 232–234°, $[\alpha]^{25}_{589}$ −12.7°, $[\alpha]^{25}_{436}$ −38.8° (*c* 0.82); reported[5]: m.p. 221–222°, $[\alpha]^{25}_{589}$ −14° (*c* 0.85); λ_{max} 238 mμ (ε 3900) and 295 mμ (ε 5550).

Anal. Calcd. for $C_{16}H_{17}NO_4$: C, 66.88; H, 5.96; N, 4.88; OCH_3, 0.00. Found: C, 66.92; H, 5.93; N, 4.85; OCH_3, 0.00.

Crinamidine (Va). The physical constants of crinamidine have been reported in an earlier paper.[2] In addition, 0.27% active hydrogen was found (calcd. for one hydroxyl, 0.32%).

O-Acetylcrinamidine. A solution of 207 mg. of crinamidine in 5 ml. of anhydrous pyridine was treated with 1 ml. of acetic anhydride and allowed to stand 5 days at 0°. The mixture was poured into water. Sodium bicarbonate was added and the mixture was extracted with chloroform. Evaporation of the chloroform left 255 mg. of oil which was chromatographed over ethyl acetate–washed alumina and eluted with 5% ethyl acetate in benzene. The *O-acetylcrinamidine* was crystallized from ether-cyclohexane, 210 mg. (89%), m.p. 133–136°. One recrystallization from ether raised the m.p. to 137–139°, $[\alpha]^{23}_{589}$ +15°, $[\alpha]^{23}_{436}$ +32° (*c* 1.0). The compound exhibited no absorption from 5000–3000 cm.$^{-1}$ but showed an *O*-acetyl band at 1733 cm.$^{-1}$ and λ_{max} 287 mμ (ε 1600).

Anal. Calcd. for $C_{19}H_{21}NO_6$: C, 63.50; H, 5.89; (1) $OCOCH_3$, 11.98. Found: C, 63.72; H, 6.06; $OCOCH_3$, 12.08.

(19) In a recent paper [A. Goosen and F. L. Warren, *J. Chem. Soc.*, 1097 (1960)] an alkaloid "buphanitine," possessing the same melting point behavior, optical rotation, and empirical formula as nerbowdine, has been reported to be a 1,3-dihydroxy derivative of 5,10b-ethanophenanthridine in which the B:C ring fusion is *cis* rather than *trans* as in XVIa. As nerbowdine can be synthesized from oxopowelline and degraded *via* XII to oxopowelline, it is impossible for XVIa to have a B:C ring fusion other than that of oxopowelline. Overwhelming evidence from our laboratory that oxopowelline (as well as powelline and crinine) has a B:C *trans* ring fusion has been cited in earlier papers.[8,10,20–24] The same degradations, reasoning and conclusions as those presented in these papers have been published in recent articles by Warren and his associates.[25,26]

(20) P. F. Highet and W. C. Wildman, *J. Org. Chem.*, **25**, 287 (1960).

(21) H. M. Fales and W. C. Wildman, *J. Am. Chem. Soc.*, **82**, 197 (1960).

(22) S. Uyeo, H. M. Fales, R. J. Highet, and W. C. Wildman, *J. Am. Chem. Soc.*, **80**, 2590 (1958).

(23) H. M. Fales, D. H. S. Horn, and W. C. Wildman, *Chem. & Ind.*, 1415 (1959).

(24) W. C. Wildman and H. M. Fales, *J. Am. Chem. Soc.*, **80**, 6465 (1958).

(25) J. Goosen, P. W. Jeffs, J. Graham, F. L. Warren, and W. G. Wright, *J. Chem. Soc.*, 1088 (1960).

(26) P. W. Jeffs, F. L. Warren, and W. G. Wright, *J. Chem. Soc.*, 1090 (1960).

(27) All melting points were observed on a Kofler microscope hot-stage and are corrected. The boiling points are uncorrected. Unless otherwise noted, rotations were measured in chloroform solution on a Rudolph photoelectric polarimeter, using a 2-dm. tube, and ultraviolet spectra were obtained in absolute ethanol solution on a Cary model 11 MS recording spectrophotometer. Infrared spectra were recorded on a Perkin-Elmer model 21 double-beam spectrophotometer, in chloroform solution unless noted to the contrary. Analyses were performed by Mr. J. F. Alicino, Metuchen, N. J.

A sample was converted to the *perchlorate* with aqueous perchloric acid and recrystallized from water to form small prisms, m.p. 157–160°. Another sample, prepared in the same way, melted at 200–206°. The two polymorphs exhibited identical infrared spectra in chloroform although their spectra in potassium bromide were dissimilar. A mixture of the two forms melted at 200–208°. The compounds exhibited maxima at 3508 cm.$^{-1}$ (N^+—H) and 1739 cm.$^{-1}$ (*O*-acetate) in chloroform.

Anal. Calcd. for $C_{19}H_{21}NO_6.HClO_4$: C, 49.62; H, 4.82; (1) $OCOCH_3$, 9.36. Found, polymorph m.p. 157–160°: C, 49.32; H, 4.99; $OCOCH_3$, 10.11. Found, polymorph m.p. 200–206°: C, 49.47; H, 5.00; $OCOCH_3$, 9.75.

Oxocrinamidine (IX). A solution of 500 mg. of crinamidine in 50 ml. of chloroform was combined with 3.0 g. of activated manganese dioxide and allowed to stir for 6 hr. The oxidant was removed by filtration, and the solvents were evaporated, leaving 500 mg. of crude ketone which was crystallized from ethyl acetate to yield 327 mg. (65%) of prisms, m.p. 202–212°. An additional 85 mg., m.p. 190–195°, was obtained from the filtrates. A sample was recrystallized from ethanol, m.p. 208–211°, and sublimed at 200° (0.1 mm.), m.p. 210–212°, $[\alpha]^{25}_{589}$ −64.8°, $[\alpha]^{25}_{436}$ −141° (*c* 0.88), λ_{max} 287 mμ (ε 1540). The compound lacked absorption from 5000–3000 cm.$^{-1}$ (Nujol) but showed a strong band at 1724 cm.$^{-1}$

Anal. Calcd. for $C_{17}H_{17}NO_5$: C, 64.75; H, 5.43; N, 4.44. Found: C, 64.86; H, 5.29; N, 4.53.

Sodium borohydride reduction of oxocrinamidine. An ice cold solution of 252 mg. of oxocrinamidine and 500 mg. of boric acid in 25 ml. of methanol was treated with 500 mg. of sodium borohydride. The mixture was allowed to warm to room temperature, acidified with acetic acid, then made basic with sodium hydroxide and extracted with chloroform. On evaporation, the extracts yielded 257 mg. which was chromatographed over Florisil and eluted with 1–10% methanol in chloroform. Early fractions consisted of a 1:1 mixture of crinamidine and epicrinamidine which was separated into its components by preparative-scale paper chromatography on Whatman No. 3 filter paper using a solvent system consisting of *n*-amyl alcohol:water:acetic acid (8:1:1). The section containing crinamidine (R_f 0.51) was separated from that containing epicrinamidine (R_f 0.39), and the crinamidine was eluted with ethanol. The solvent was evaporated; the residue was made basic with alkali and extracted with chloroform. Evaporation left crystals of *crinamidine* which were recrystallized from ethyl acetate and found identical in infrared spectrum (potassium bromide), m.p. (236°) and mixture m.p. with authentic material.

Further elution of the original column provided 215 mg. (85%) of *epicrinamidine hydrate* (XIII), m.p. 114–118°, which was strenuously dried at 100° (0.1 mm.) before analysis, $[\alpha]^{25}_{589}$ −35°, $[\alpha]^{25}_{436}$ −86° (*c* 1.0), λ_{max} 287 mμ (ε 1488).

Anal. Calcd. for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.04; neut. equiv., 317. Found: C, 64.27; H, 6.01; neut. equiv., 316.

Epicrinamidine did not sublime at 140° (10 μ). At higher temperatures, it formed an insoluble glass.

A solution of 68 mg. of epicrinamidine in 5 ml. of chloroform was oxidized with 500 mg. of activated manganese dioxide by the procedure described for the oxidation of crinamidine. Evaporation of the solvent gave amorphous *oxocinamidine* which was crystallized from ethyl acetate, m.p. 208–211° alone or when mixed with authentic material. An infrared spectrum (potassium bromide) confirmed the identity of the product.

O-3,5-Dinitrobenzoylepicrinamidine. The free base (XIII) was mixed with an equal weight of 3,5-dinitrobenzoyl chloride in pyridine. The mixture was allowed to stand overnight at room temperature and then was poured into aqueous sodium bicarbonate and extracted with benzene. Recrystallization from ethanol produced yellow prisms, m.p. 246–249° dec.

Anal. Calcd. for $C_{24}H_{21}N_3O_{10}$: C, 56.36; H, 4.14. Found: C, 56.16; H, 4.10.

Dihydrocrinamidine (VI). A solution of 247 mg. of crinamidine in tetrahydrofuran was refluxed with an excess of lithium aluminum hydride for 3 hr. After decomposition with ethyl acetate and water, the mixture was extracted with chloroform and the chloroform was evaporated. The residue was crystallized from ethanol to yield 177 mg. (72%) of fine prisms, m.p. 255–260°, $[\alpha]^{24}_{589}$ +22.7°, $[\alpha]^{24}_{436}$ +52.6° (*c* 0.5, ethanol), λ_{max} 286 mμ (ε 1479).

Anal. Calcd. for $C_{17}H_{21}NO_5$: C, 63.93; H, 6.63; N, 4.39; vic. glycol, 0.00. Found: C, 64.02; H, 6.62; N, 4.34; vic. glycol, 0.00.

O,O'-Diacetyldihydrocrinamidine. Preparation in the usual manner gave an oil which refused to crystallize. A sample was distilled at 130° (1 μ) for analysis, $\lambda^{C_2H_5OH}_{max}$ 287 mμ (ε 1550).

Anal. Calcd. for $C_{21}H_{25}NO_7$: C, 62.52; H, 6.25; $OCOCH_3$, 21.34. Found: C, 62.53; H, 6.39; $OCOCH_3$, 21.09.

2-Tetrahydropyranyloxycrinamidine (Vc). A solution of 630 mg. of crinamidine in 6 ml. of freshly distilled dihydropyran and 5 ml. of chloroform was cooled to 5° and treated with sufficient hydrogen chloride to render the mixture distinctly acid to congo red paper. The solution stood 4 hr. at room temperature and was poured into dilute sodium hydroxide. The basic and neutral compounds were extracted with chloroform. The oil remaining after evaporation of solvents was chromatographed over alumina. Neutral compounds were removed with 5% ethyl acetate in benzene while 25% ethyl acetate in benzene eluted a basic, amorphous solid which could not be induced to crystallize. This oil possessed no absorption in the hydroxyl region from 3400–3800 cm.$^{-1}$ but exhibited a profusion of intense bands in the 1030–1124 cm.$^{-1}$ region characteristic of cyclic ethers.[28]

Anal. Calcd. for $C_{22}H_{27}NO_6$: C, 65.82; H, 6.78. Found: C, 65.87; H, 6.82.

α- and β-2-Tetrahydropyranyloxyisodihydrocrinamidine (IIIc). A solution of 850 mg. of Vc in 15 ml. of tetrahydrofuran was refluxed overnight with 2.0 g. of lithium aluminum hydride. The mixture was decomposed with ethyl acetate and sodium hydroxide and extracted with chloroform. Evaporation of the extracts left 792 mg. of an oil which yielded 351 mg. of crystals on trituration in acetone, m.p. 235–250°. Chromatography over alumina or Florisil did not change the melting point, but after many recrystallizations from ethanol and ethyl acetate, a pure sample of the α-isomer was obtained, m.p. 252–254°, $[\alpha]^{22}_{589}$ +11°, $[\alpha]^{22}_{436}$ +28° (*c* 0.62), $\lambda^{C_2H_5OH}_{max}$ 287 mμ (ε 1790). The infrared spectrum (chloroform) of the pure material was nearly identical with that of the initial product, indicating that the initial product contained only traces of a persistent impurity.

Anal. Calcd. for $C_{22}H_{29}NO_6$: C, 65.49; H, 7.25; neut. equiv., 403. Found: C, 65.49; H, 7.34; neut. equiv., 409.

The *β-isomer* was obtained from the filtrates of the α-isomer by recrystallization from ethyl acetate and chromatography over alumina. The yield was 119 mg. (14%), m.p. 225–227°, $[\alpha]^{24}_{589}$ −75.7°, $[\alpha]^{24}_{436}$ −142° (*c* 0.53), $\lambda^{C_2H_5OH}_{max}$ 287 mμ (ε 1820). The infrared spectra (potassium bromide) of the two isomers were different although in chloroform they were identical. The β-compound depressed the melting point of the α-isomer.

Isodihydrocrinamidine (IIIa). A solution of 20 mg. of either isomer of IIIc was warmed briefly with 0.5 ml. of 4*N* hydrochloric acid. The mixture was made basic with sodium hydroxide and extracted with chloroform. The extracts were evaporated, and the residue was crystallized from acetone as fine needles, m.p. 245–247° after recrystallizing as short prisms at 210°, $[\alpha]^{24}_{589}$ −25°, $[\alpha]^{24}_{436}$ −46° (*c* 0.58, C_2H_5OH), $\lambda^{C_2H_5OH}_{max}$ 287 mμ (ε 1690). In excess 0.4% aqueous potassium

(28) L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, 2nd ed., John Wiley and Sons, Inc., New York (1958), p. 119.

periodate (*p*H 6.5) at 25°, the diol exhibited a pseudo first-order rate constant of 1.34×10^{-4} sec.$^{-1}$

Anal. Calcd. for $C_{17}H_{21}NO_5$: C, 63.93; H, 6.63; neut. equiv., 319; glycol, 1.0 mole. Found: C, 63.75, 63.71; H, 6.73, 6.67; neut. equiv., 316; glycol, 1.05 mole.

Conversion of oxocrinamidine to oxopowelline (X). A stirred solution of 200 mg. of oxocrinamidine in 15 ml. of 50% acetic acid was treated with 3 g. of zinc dust in small portions over 30 min. at room temperature; 3 ml. of ethanol was added and stirring continued for 10 min. The solution was filtered, made strongly basic with sodium hydroxide, and extracted with chloroform. Evaporation of the extract left 180 mg. of an oil which was dissolved in ethyl acetate and passed through a small column of alumina to produce 166 mg. of crystalline *oxopowelline*, m.p. 165–166°. This proved to be a lower melting polymorph of oxopowelline since it recrystallized when seeded with authentic oxopowelline[7] (m.p. 179–180°) and remelted at 179–180°. A mixture with authentic oxopowelline showed no depression of m.p., and the infrared spectra (chloroform) of the two materials were identical, $[\alpha]^{24}_{589}$ −273°, $[\alpha]^{24}_{436}$ −727° (*c* 0.5); reported[7]: $[\alpha]^{25}_{589}$ −258°, $[\alpha]^{25}_{436}$ −697°.

A solution of 27 mg. of oxopowelline and 27 mg. of 2,4-dinitrophenylhydrazine was refluxed in 4 ml. of ethanol with the addition of 3 drops of 12*N* hydrochloric acid. After 5 min., water and ammonia were added; the orange precipitate was collected, washed with water, and dried. The *oxopowelline 2,4-dinitrophenylhydrazone* was recrystallized from chloroform-ethanol to form bright red prisms, m.p. 246–247°.

Anal. Calcd. for $C_{22}H_{21}N_5O_7$: C, 57.62; H, 4.42. Found: C, 57.32; H, 4.68.

Epoxyoxopowelline (XI). A solution of 0.5 ml. of 30% hydrogen peroxide was added dropwise to a stirred solution of 540 mg. of oxopowelline and 400 mg. of potassium carbonate in 50 ml. of 80% ethanol at 0°. After 15 min. a small amount of palladium-on-charcoal was added to destroy excess hydrogen peroxide, and the mixture was extracted with chloroform. Evaporation of the solvents left 515 mg. of residue which spontaneously crystallized, m.p. 190–200°. One recrystallization from ethanol produced fine prisms, m.p. 199–200°, $[\alpha]^{23}_{589}$ −147°, $[\alpha]^{23}_{436}$ −382° (*c* 0.4), λ_{max} 287 mμ (ε 1820). The infrared spectrum in chloroform differed from that of oxocrinamidine and showed absorption at 1709 cm.$^{-1}$ (potassium bromide).

Anal. Calcd. for $C_{17}H_{17}NO_5$: C, 64.75; H, 5.43; neut. equiv., 315. Found: C, 64.77; H, 5.50; neut. equiv., 310.

Nerbowdine (XVIa) *and/or epinerbowdine* (VIII). (a) *From oxonerbowdine* (XII). A solution of 175 mg. of oxonerbowdine (XII) was treated with an equal weight of sodium borohydride in 10 ml. of methanol at 0°. After standing 10 min. at 0° and 2 min. at reflux temperature, the mixture was acidified with acetic acid and subsequently made basic with ammonia. The solution was extracted with chloroform, and the extracts were evaporated to leave 167 mg. of an oil which was chromatographed on Florisil. Elution with 2–5% methanol in chloroform produced 88 mg. (50%) of *nerbowdine* (XVIa), identified by infrared spectrum. Finally, 10–20% methanol in chloroform eluted 54 mg. (31%) of *epinerbowdine* (VIII).

Epinerbowdine recrystallized from wet benzene as an ill-defined hydrate, m.p. 150–165°, $[\alpha]^{23}_{589}$ −77°, $[\alpha]^{23}_{436}$ −166° (*c* 0.74), $\lambda^{C_2H_5OH}_{max}$ 287 mμ (ε 1210). The hygroscopic nature of the product prevented satisfactory analytical results; however, from one strenuously dried sample, a neut. equiv. of 321 (calcd. 319) was obtained.

Epinerbowdine picrate crystallized as fine prisms from water, m.p. 162–169°.

Anal. Calcd. for $C_{23}H_{24}N_4O_{12}$: C, 50.36; H, 4.41. Found: C, 50.15; H, 4.49.

A sample of epinerbowdine, recovered from the picrate by passage over IRA-400 resin in ethanol, showed properties identical with those of recrystallized material. Epinerbowdine (15 mg.) was irreversibly adsorbed from a chloroform solution on 200 mg. of activated manganese dioxide.

(b) *By reduction of epoxyoxopowelline* (XI). A solution of 208 mg. of epoxyoxopowelline in 10 ml. of distilled tetrahydrofuran was refluxed 4 hr. with a large excess of lithium aluminum hydride. After decomposition of excess hydride with ethyl acetate and water, the bases were extracted with chloroform and the extracts dried over sodium sulfate. Evaporation of the chloroform left a residue which was chromatographed on alumina with 0–5% ethanol in chloroform. The first product eluted, 72 mg. (34%), proved to be identical in melting point, mixture melting point and infrared spectrum (potassium bromide) with *nerbowdine* (XVIa). Further elution gave 97 mg. (41%) of epinerbowdine (VIII). This was confirmed by the preparation of *epinerbowdine picrate*, m.p. 162–169° alone or on admixture with authentic material. Infrared spectra (potassium bromide) also confirmed the identity of these picrates.

(c) *From epoxypowelline* (XVa). A solution of 20 mg. of epoxypowelline in 5 ml. of tetrahydrofuran was refluxed overnight with 200 mg. of lithium aluminum hydride. The hydride was decomposed with ethyl acetate, acidified with dilute hydrochloric acid, and extracted with benzene. The acidic layer was made basic with sodium hydroxide, extracted with chloroform, and the extracts dried with sodium sulfate. Evaporation of the solvent left 13 mg. of nerbowdine, m.p. 233–238° alone or on admixture with an authentic sample. Infrared spectra (potassium bromide) confirmed their identity.

Under identical conditions, 100 mg. of epiepoxypowelline hydroperchlorate monohydrate was recovered unchanged.

(d) *From epiepoxypowelline* (VII). A solution of 48 mg. of VII was refluxed with excess lithium aluminum hydride in tetrahydrofuran for 4 hr. and processed as above to yield 37 mg. (77%) of an oil possessing an infrared spectrum identical with that of *epinerbowdine*. A picrate prepared from the oil melted at 162–169° alone or on admixture with authentic *epinerbowdine picrate*.

Nerbowdine O,O′-carbonate. Nerbowdine (200 mg.) was dissolved in 5 ml. of pyridine and cooled to 0°. A large excess of phosgene in benzene was added to the pyridine solution which was allowed to stand overnight at 0°. The mixture was then poured onto ice, neutralized with sodium bicarbonate, and extracted with chloroform. The dried solutions were evaporated, leaving 210 mg. of a partially crystalline residue which was chromatographed over ethyl acetate–deactivated alumina. Elution with 15% ethyl acetate in benzene produced a crystalline carbonate (82 mg., 38%) which was recrystallized from ethyl acetate as small tablets, m.p. 249–250°, $[\alpha]^{25}_{589}$ −146°, $[\alpha]^{25}_{460}$ −263° (*c* 0.66), λ_{max} 287 mμ (ε 1510). A maximum was observed at 1748 cm.$^{-1}$ (chloroform).

Anal. Calcd. for $C_{18}H_{19}NO_6$: C, 62.60; H, 5.55; mol. wt., 345. Found: C, 62.75; H, 5.46; mol. wt. (Rast), 352.

Oxonerbowdine (XII). A solution of 400 mg. of nerbowdine in 20 ml. of chloroform was allowed to stir for 36 hr. with 4.0 g. of activated manganese dioxide. On filtration and evaporation, 380 mg. of a glass was obtained which exhibited only one band at 1720 cm.$^{-1}$ in the carbonyl region. This was chromatographed over activated, basic alumina with chloroform; 37 mg. of *oxopowelline*, m.p. and mixture m.p. 176–178°, was obtained, presumably by β-elimination of the hydroxyl group during chromatography. Subsequent elution with 2–4% methanol in chloroform produced 199 mg. (50%) of pure *oxonerbowdine*. One recrystallization from ethyl acetate gave fine prisms, m.p. 208–212°, $[\alpha]^{23}_{589}$ −104°, $[\alpha]^{23}_{436}$ −226° (*c* 0.52), λ_{max} 287 mμ (ε 1590).

Anal. Calcd. for $C_{17}H_{15}NO_5$: C, 64.34; H, 6.04; neut. equiv., 317. Found: C, 64.31; H, 6.29; neut. equiv., 316.

An attempt to prepare the 2,4-dinitrophenylhydrazone of oxonerbowdine in ethanolic hydrochloric acid gave the *2,4-dinitrophenylhydrazone* of *oxopowelline*, m.p. 246–247°, presumably by acid-catalyzed β-elimination of the hydroxyl group. The derivative was identical in melting point, mix-

ture melting point and infrared spectrum (potassium bromide) with an authentic sample.

Conversion of oxonerbowdine to oxopowelline. A solution of 80 mg. of oxonerbowdine and 80 mg. of aluminum *t*-butoxide in 5 ml. of toluene was refluxed 4 hr. The toluene layer was washed with water and evaporated, leaving 46 mg. of oil which was chromatographed over alumina. Elution with 50% benzene-ethyl acetate produced 20 mg. of crystals, m.p. 176–178° alone or on admixture with authentic *oxopowelline*.

Sodium borohydride reduction of epoxyoxopowelline. A solution of 670 mg. of epoxyoxopowelline (XI) in 30 ml. of methanol at 0° was reduced with 500 mg. of sodium borohydride in the usual manner. The oil was chromatographed twice over ethyl acetate–deactivated alumina. Elution with 2% methanol in ethyl acetate furnished a total of 231 mg. (35%) of crude *epoxypowelline* (XVa) which was crystallized from ethyl acetate to form bladed prisms, m.p. 195–195.5°, $[\alpha]_{589}^{23}$ −30.8°, $[\alpha]_{436}^{23}$ −65.5° (*c* 0.36), λ_{max} 287 mμ (ε 1620).

Anal. Calcd. for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.04. Found: C, 64.48; H, 6.17.

Further elution with 2–3% methanol in ethyl acetate produced a total of 220 mg. (33%) of *epiepoxypowelline* (VII). Rechromatography of this material over Florisil and examination of the infrared spectra (chloroform) of the various fractions suggested that the compound was pure, but it resisted crystallization as did its picrate, perchlorate,[29] and 3,5-dinitrobenzoate. It could not be distilled at 170° (1 μ) and at higher temperatures formed an insoluble glass. The infrared spectrum differed in many respects from that of epoxypowelline, and there was ample evidence for the absence of epoxypowelline in the product. A sample that had been subjected to 100° (1 μ) for one day showed $[\alpha]_{589}^{24}$ −32.5°, $[\alpha]_{436}^{24}$ −64° (*c* 0.6).

When solutions of either epoxypowelline or epiepoxypowelline were allowed to stir for 5 hr. with activated manganese dioxide in chloroform, epoxyoxopowelline was formed in yields of 67% and 76%, respectively. The product was identified by infrared spectrum (potassium bromide) and m.p. 198–199° alone or on admixture with authentic material.

2-Tetrahydropyranyloxyepoxypowelline (XVc). By the same method used in the preparation of 2-tetrahydropyranyloxycrinamidine, 763 mg. of epoxypowelline (XVa) was converted to its 2-tetrahydropyranyl ether, an amorphous solid purified by chromatography on alumina and distilled at 160° (1 μ) for analysis.

Anal. Calcd. for $C_{22}H_{27}NO_6$: C, 65.82; H, 6.78. Found: C, 65.62; H, 6.88.

3-(2-Tetrahydropyranyloxy)nerbowdine (XVIc). A solution of 981 mg. of XVc was allowed to reflux overnight with 2.0 g. of lithium aluminum hydride in tetrahydrofuran. The mixture was decomposed with sodium hydroxide and extracted with chloroform. On evaporation, the residue was purified by chromatography over basic alumina with benzene–ethyl acetate to yield 705 mg. (72%) of a product which could not be crystallized. A sample was distilled at 130° (<1 μ) for analysis.

(29) A compound previously referred to as *epiepoxypowelline hydroperchlorate monohydrate* [W. C. Wildman in R. H. F. Manske, *The Alkaloids*, Vol. VI, p. 407, Academic Press, New York (1960)], m.p. 153–155°, $[\alpha]_{589}^{25}$ −33°, $[\alpha]_{436}^{25}$ −69° (*c* 0.4, ethanol), has been found to be the hydroperchlorate of another substance. This material could be formed by allowing the methanol-borohydride reduction mixture to evaporate to dryness on the steam bath before isolation of the basic materials as described above. It neither was oxidized by manganese dioxide to epoxyoxopowelline nor was affected by lithium aluminum hydride in tetrahydrofuran. It may be a dimeric dioxane caused by condensation of the epoxy alcohol functions.

Anal. Calcd. for $(C_{17}H_{19}NO_5.HClO_4.H_2O)_x$: C, 46.85; H, 5.09. Found: C, 47.02; H, 5.18; vic. glycol, 0.0.

Anal. Calcd. for $C_{22}H_{29}NO_6$: C, 65.49; H, 7.25; neut. equiv., 403. Found: C, 65.38; H, 7.17; neut. equiv., 402.

A sample of the ether was hydrolyzed in 2*N* hydrochloric acid to produce a high yield of *nerbowdine* as the only product.

Oxoflexinine (IX, no OCH_3). By the method described for oxocrinamidine, 225 mg. of flexinine was oxidized by 2.0 g. of activated manganese dioxide to give 220 mg. of oxoflexinine which showed carbonyl absorption at 1724 $cm.^{-1}$ and no absorption due to a hydroxyl group between 4000–3000 $cm.^{-1}$ Because the product resisted crystallization, it was converted to the *hydroperchlorate monohydrate* and recrystallized from water to yield prisms which gradually decomposed from 200–260°.

Anal. Calcd. for $C_{16}H_{15}NO_4.HClO_4.H_2O$: C, 47.59; H, 4.49. Found: C, 47.83; H, 4.59.

Another sample was dried at 137° (vac.) before analysis.

Anal. Calcd. for $C_{16}H_{15}NO_4.HClO_4$: C, 49.81; H, 4.18. Found: C, 49.70; H, 4.30.

The free base, recovered from the hydroperchlorate, possessed an infrared spectrum (chloroform) identical with that of the crude oxidation product.

Oxocrinine (X, no OCH_3). A solution of 100 mg. of oxoflexinine in 8 ml. of 50% acetic acid was treated with 1.5 g. of zinc dust and worked up according to the procedure outlined for oxocrinamidine. A yield of 93 mg. of product showing an infrared spectrum (chloroform) identical with that of oxocrinine was obtained. Two recrystallizations from ether provided prisms, m.p. 186–187° alone or on admixture with authentic oxocrinine, $[\alpha]_{589}^{24}$ −321°, $[\alpha]_{436}^{24}$ −885° (*c* 1.3); reported[7]: m.p. 184–186°, $[\alpha]_{589}^{24}$ −307°, $[\alpha]_{436}^{24}$ −848°.

Epoxyoxocrinine (XI, no OCH_3). By the method used in the conversion of oxopowelline to epoxyoxopowelline, 390 mg. of oxocrinine was converted to 320 mg. of crude epoxyoxocrinine. This was chromatographed over deactivated alumina to yield 210 mg. (51%) of epoxyoxocrinine which crystallized from ethanol as a solvate, irregular prisms, m.p. 62–75°, $[\alpha]_{589}^{24}$ −156°, $[\alpha]_{436}^{24}$ −402° (*c* 0.50), λ_{max} 237 mμ (ε 3590) and 294 mμ (ε 4920). The unsolvated base was an oil exhibiting maxima due to the unconjugated carbonyl group at 1709 $cm.^{-1}$ (chloroform) and no absorption in the hydroxyl region.

Anal. Calcd. for $C_{16}H_{15}NO_4.C_2H_5OH$: C, 65.24; H, 6.39; neut. equiv., 331. Found: C, 65.02; H, 6.33; neut. equiv., 329.

A sample was dried at 100° in vacuum, forming a glass.

Anal. Calcd. for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30. Found: C, 67.17; H, 5.37.

cis-1,3-Dihydroxycrinane (XVIa, no OCH_3). A solution of 103 mg. of XI (no OCH_3) in 10 ml. of tetrahydrofuran was refluxed overnight with 100 mg. of lithium aluminum hydride. After decomposition with sodium hydroxide and extraction with chloroform, 89 mg. of semicrystalline product was obtained. Three recrystallizations from ethanol furnished bladed prisms which recrystallized on the hot-stage at 250° and finally melted at 266–267°, $[\alpha]_{589}^{24}$ −88°, $[\alpha]_{436}^{24}$ −198° (*c* 0.67, ethanol), λ_{max} 235 mμ (ε 3460) and 292 mμ (ε 4820).

Anal. Calcd. for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.62; neut. equiv., 289. Found: C, 66.57; H, 6.54; neut. equiv., 287.

cis-1,3-Dihydroxycrinane O,O'-carbonate. A solution of 7 mg. of XVIa (no OCH_3) was treated with phosgene in benzene and pyridine under the conditions used in the formation of nerbowdine *O,O'*-carbonate. The product crystallized on evaporation of the solvents, m.p. 252–256°. The total sample was sublimed at 160° (1 μ), forming rectangular plates, m.p. 254–256°. A maximum was observed at 1748 $cm.^{-1}$ due to the cyclic carbonate.

Anal. Calcd. for $C_{17}H_{17}NO_5$: C, 64.75; H, 5.37; mol. wt., 315. Found: C, 64.85; H, 5.37; mol. wt. (Rast), 329.

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