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THE CONFIGURATION OF CEVINE*†

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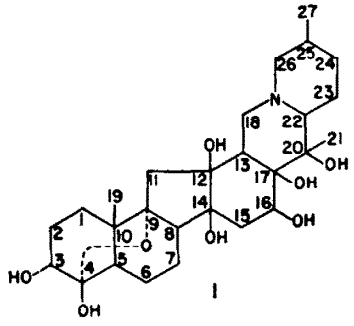
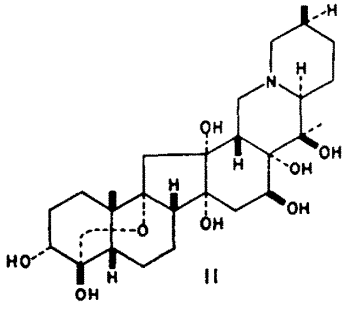
Abstract—The alkaloid cevine has been shown to have configuration II. The configurations of nine (C_3, C_4, C_5, C_9, C_{10}, C_{12}, C_{14}, C_{17}, C_{25}) of the fourteen asymmetric centers were established by earlier work. Hydrogenation of 16-dehydrocevine 3,4-diacetate (XIII) over platinum proceedeb stereoselectively to cevine 3,4-diacetate (XIV). Sodium borohydride reduction of 16-dehydrocevadine-D-orthoacetate-4-acetate (XVII) afforded a mixture of cevadine-D-orthoacetate 4-acetate (XVIII) and 16-epicevadine-D-orthoacetate 4-acetate (XVI). Both XVIII and XVI were submitted to alkaline hydrolysis followed by acid treatment to give cevagenine C-orthoacetate (XX) and 16-epicevagenine-C-orthoacetate (XIX). XIX consumed lead tetraacetate faster than XX, thereby indicating β-orientation for the C_{16} hydroxyl of cevine. Arguments are presented for assignment of configurations at C_8, C_{13} and C_{20}. Oxidation of veracevine-D-orthoacetate triacetate (XXII) with N-bromosuccinimide gave a bridged oxide (XXIII) which, in turn, afforded a formamido ketone (XXIV) on chromic anhydride-pyridine oxidation. The bridged β-oriented oxide structure of XXIII requires that the hydrogen at C_{22} be α-oriented.

THE currently accepted structure (I) for the extensively studied alkaloid cevine was first proposed in a classic collaborative paper in 1954.[1] Cevine has fourteen asymmetric centers: C_3, C_4, C_5, C_8, C_9, C_{10}, C_{12}, C_{13}, C_{14}, C_{16}, C_{17}, C_{20}, C_{22} and C_{25}. Previous studies in several laboratories have contributed to the partial elucidation of the configuration of cevine.[2–5] It is the purpose of this paper to present, in detail,



I



II

* This is Part XXXI of a series entitled "Veratrum Alkaloids"; Part XXX, S. M. Kupchan, *J. Amer. Chem. Soc.* **81**, 1925 (1959).

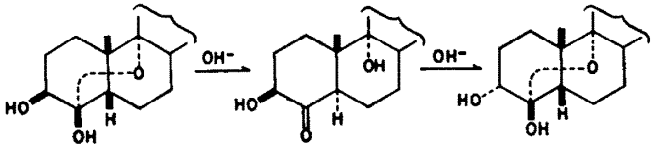
† The investigations which form the subject of this paper were first outlined in part in two preliminary communications. *J. Amer. Chem. Soc.* **78**, 3864 (1956); **80**, 1769 (1958). This work was supported in part by grants (H-2275, H-2952, A-2134) from the National Institute of Health, U.S. Public Health Service, from the National Science Foundation, and from the Sterling-Winthorp Research Institute.

‡ Deceased.

[1] D. H. R. Barton, O. Jeger, V. Prelog and R. B. Woodward, *Experientia* **10**, 81 (1954).
[2] D. H. R. Barton, C. J. W. Brooks and P. De Mayo, *J. Chem. Soc.* 3950 (1954).
[3] O. Jeger, V. Prelog, E. Sundt and R. B. Woodward, *Helv. Chim. Acta* **37**, 2302 (1954).
[4] F. Gautschi, O. Jeger, V. Prelog and R. B. Woodward, *Helv. Chim. Acta* **38**, 296 (1955).
[5] S. M. Kupchan, *J. Amer. Chem. Soc.* 77, 686 (1955).

evidence for assignment of configuration at the remaining asymmetric centers of cevine which now can be represented completely by formula II.

The absolute configuration at C_{10} was shown to be identical with that (β-orientation) of C_{10} of the steroids through a masterful interrelationship of degradation products.[4] The orientations at C_3, C_4, C_5 and C_8 are related to C_{10} as shown in formula II by the steric requirements of the peculiar cage-like structure of the A/B system and the veracevine–cevagenine–cevine isomerizations,[1] depicted by formulas III–IV–V in the accompanying flow sheet. The configurations at C_{12}, C_{14} and C_{17}



III IV V

were related to C_9 *via* the cevagenine-D- and C-orthoacetates.[5] The structure of cevagenine-C-orthoacetate (XX) requires that the 12- and 14-hydroxyl groups have the same configuration as that at position 9, which is α-oriented (see above). The structure of the D-orthoacetates (e.g. XVIII and XXI) indicates the α-orientation of the 17-hydroxyl group as well, because of the common hinge (attached at C_{12} and C_{14}) of the orthoacetate residue in both the C and D orthoacetates. These conclusions received additional support from the demonstration that dihydrocevine-D-orthoacetate, which has a free α-hydroxyl group at C_9, is isomerized by acid to dihydrocevine-C-orthoacetate.[2] The configuration at C_{25} was established by partial synthesis of the piperidone VI from D-(+)-citronellal. This product was shown to be the antipode of the optically-active piperidone (VII) obtained by oxidation of cevine.[3]

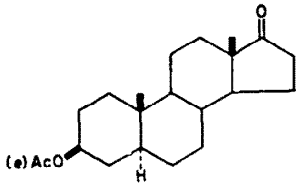
VI VII

Configuration at C_{16}. Prior to the present study the hydroxyl group at this position was assigned the α-*equatorial*-orientation. This assignment was made on the basis of the ready methanolysis of compounds acetylated at this position.[1,2]

The susceptibility of the C_{16} acetate esters to base catalyzed[6] methanolysis appeared to us to be abnormally high even for an equatorial ester. This rate of methanolysis, indeed, was found to exceed that of an *unhindered* equatorial ester. Wheras cevadine-D-orthoacetate 4,16-diacetate[7] (XV) underwent methanolysis in 75 per cent yield after 20 hr in dilute methanol, epiandrosterone-3-*equatorial-acetate* (VIII) was recovered largely (75 per cent) unchanged after similar treatment in the presence of cevine to serve as the basic catalyst. In the germine series the C_7 acetate, which is

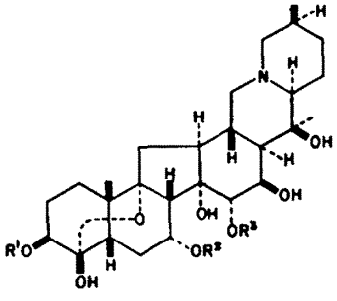
[6] W. J. Rosenfelder, *J. Chem. Soc.* 2638 (1954).
[7] A. Stoll and E. Seebeck, *Helv. Chim. Acta* **35**, 1942 (1952).

undoubtedly α-*axially*-oriented[8] exhibits reactivity comparable with the C_{16} acetate in the cevine derivatives. The facile methanolysis of germitrine [IX:R^1 = (l)-2-methylbutyric acid (MB); R^2 = Ac; R^3 = (d)-2-hydroxyl-2-methyl butyric acid (HMB)] to germerine (IX: R^1 = MB; R^2 = H; R^3 = HMB)[9,10] and of neogermitrine (IX: $R^1 = R^2$ = Ac; R^3 = MB) to germidine[10,11] (IX: R^1 = Ac; R^2 = H; R^3 = MB) are noteworthy examples of this phenomenon. These two cases were discovered in the search for the cause of the remarkable loss in hypotensive activity which accompanies the methanolysis of the C_7 acetate group of the naturally

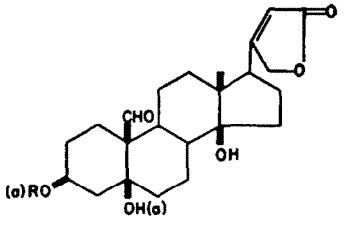


VIII

occurring germine esters.[9,11] This unusual behavior prompted us to postulate a facilitation of methanolysis by a neighboring hydroxyl group (at C_{14} in these two cases) bearing a *cis*-1,3-di*axial* relationship to the ester group and thus juxtaposed for participation. This hypothesis was tested by treatment of strophanthidin 3-acetate (X: R = Ac) with dilute methanol and cevine for twenty hours; strophanthidin (X: R = H) was isolated in 66 per cent yield. Henbest and Lovell have also independently presented convincing demonstrations of this effect in the sterol series and have discussed mechanistic interpretations.[12]



IX



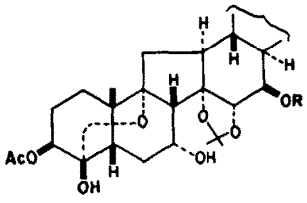
X

The C_{16} acetate in the germine series also exhibits abnormal susceptibility to methanolysis. Germine 14,15-acetonide 3,16-diacetate (XI: R = Ac) is readily methanolyzed to germine 14,15-acetonide 3-acetate (XI: R = H),[8] and germine 3,4,7,15,16-pentaacetate (XII: R = Ac) is rapidly transformed by methanol to the 3,4,7,15-tetraacetate (XII: R = H).[10] Now the C_{16}-acetoxy group is evidently

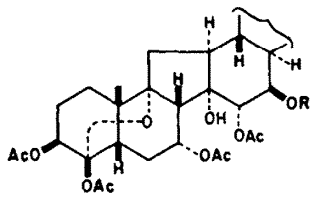
[8] S. M. Kupchan and C. R. Narayanan, *J. Amer. Chem. Soc.* **81**, 1913 (1959).
[9] J. Fried, H. L. White and O. Wintersteiner, *J. Amer. Chem. Soc.* **72**, 4621 (1950).
[10] S. M. Kupchan, *J. Amer. Chem. Soc.* **81**, 1921 (1959).
[11] J. Fried, P. Numerof and N. H. Coy, *J. Amer. Chem. Soc.* **74**, 3041 (1952).
[12] H. B. Henbest and B. J. Lovell, *Chem. & Ind.* 278 (1956); *J. Chem. Soc.* 1965 (1957).

β-axially-oriented,[8] which requires, on the basis of a 1,3-di*axial* facilitation, a *β-axial*-orientation of the C_{20} hydroxyl. It is therefore attractive to postulate identical disposition of the C_{16} and C_{20} hydroxyl groups in cevine. The following experiments establish this point.

Hydrogenation of 16-dehydrocevine 3,4-diacetate[2] (XIII) over platinum proceeded stereoselectively to give cevine 3,4-diacetate (XIV). The molecular model of the ketone shows that the α- is much less hindered than the β-face for approach to the catalyst, which suggests that reaction would proceed to give a β-oriented hydroxyl.

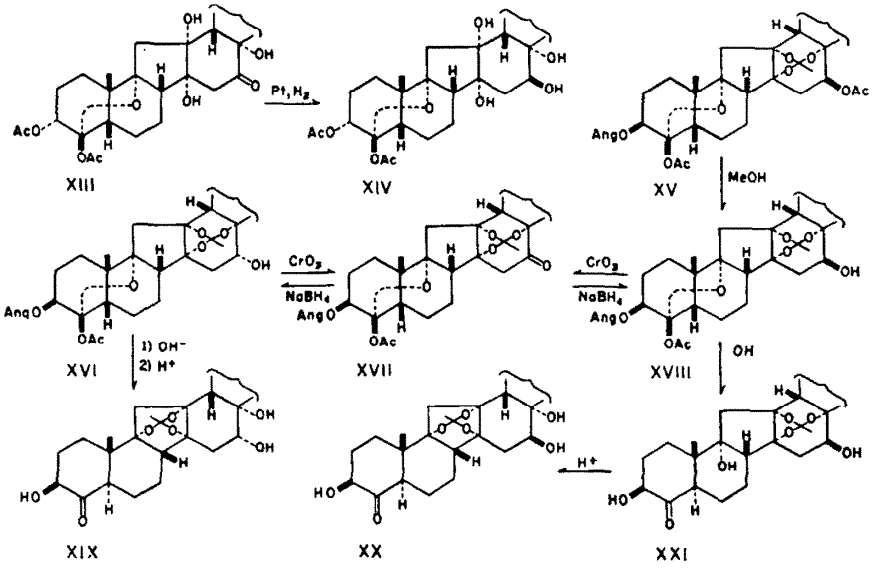


XI



XII

Sodium borohydride reduction of 16-dehydrocevadine-D-orthoacetate-4-acetate[2] (XVII) afforded a mixture of cevadine-D-orthoacetate-4-acetate (XVIII)[7] and 16-epicevadine-D-orthoacetate-4-acetate (XVI). The structure of XVI was confirmed by oxidation with chromic acid to regenerate the original ketone (XVII). In order to liberate the C_{17}-hydroxyl group, both epimeric D-orthoacetates were submitted to alkaline hydrolysis followed by treatment with mineral acid to effect rearrangement to the C-orthoacetates.[5] 16-Epicevagenine-C-orthoacetate (XIX) was found to react



with lead tetraacetate at a strikingly faster rate than cevagenine-C-orthoacetate (XX), which suggests that the hydroxyl groups at C_{16} and C_{17} are *cis*-disposed in the former and *trans*- in the latter. Since the C_{17} hydroxyl group is undoubtedly α-oriented (see above), the configurations are established as XIX and XX, respectively.

Configuration at C_8, C_{13} *and* C_{20}. Now with the establishment of the β-configuration of the C_{16} hydroxyl group in cevine, the β-configuration may be assigned to the C_{20} hydroxyl group—a point concerning which there has been considerable doubt.[2,4,13] The *cis*-1,3-di*axial* disposition of the C_{16} and C_{20} hydroxyl groups requires, in turn, that rings D and E be *trans*-fused, which, in view of the established α-orientation of the hydroxyl group at C_{17} (see above) requires β-orientation of the hydrogen at C_{13}. A similar argument has been advanced with regard to the orientation at C_8 in germine, namely that the *cis*-1,3-di*axial* relationship of the substituents at C_7 and C_{14} requires that the hydrogen at C_8 be β-oriented.[8] At each of the asymmetric carbon atoms common to both veracevine and germine which have been discussed up to this point (C_3, C_4, C_5, C_9, C_{10}, C_{13}, C_{14}, C_{16}, C_{17}, C_{20}) the configurations at the common asymmetric carbon atoms are identical. It is considered reasonable to assume that the configuration at C_8 in cevine is the same as that in germine and all other naturally occurring steroids.

We have noted that the C_{16}-hydroxyl group of 16-epicevadine-D-orthoacetate 4-acetate (XVI) is relatively resistant to acetylation. This fact is consistent with the α-*equatorial*-configuration for the methyl group at C_{20} which is held rigidly eclipsed with the C_{16} α-hydroxyl group and accordingly exerts serious crowding.

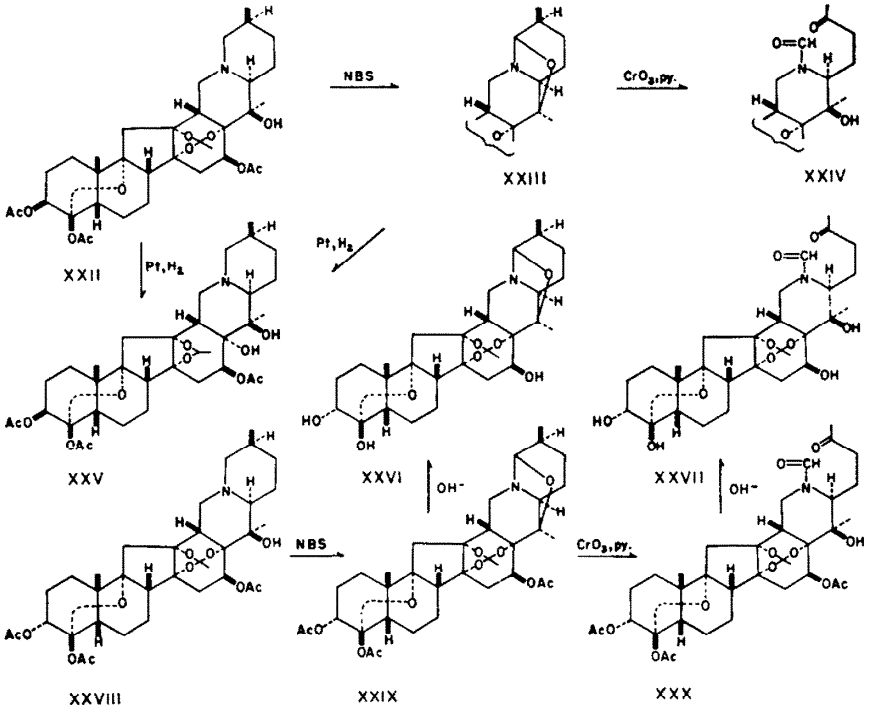
Configuration at C_{22}. The foregoing considerations provide a basis for assigning configurations to thirteen of the fourteen asymmetric centers of cevine. Only the assignment of the configuration at C_{22} remained, and this proved to be our most difficult task.

In the hope of obtaining a carbinolamine for hydrogenation studies (of the iminium salt), veracevine D-orthoacetate triacetate[14] (XXII) was oxidized with N-bromosuccinimide.[15] A dehydro compound was obtained which evidently has the bridged oxide structure XXIII for the following reasons. (1) The infrared spectrum of the dehydro compound in Nujol showed no absorption in the hydroxyl region. (2) The 16-acetate group survived prolonged treatment with methanol and triethylamine, an indication of the absence of an *axial* hydroxyl at C_{20} to facilitate methanolysis (see above). (3) That the N-bromo-succinimide reaction did not lead to introduction of a carbonyl group was demonstrated as follows. Because XXIII would be expected to isomerize to the 4-keto compound (see formulas III — IV), it was considered necessary to operate in the alkali-stable cevine series. Cevine D-orthoacetate triacetate (XXVIII) was oxidized with N-bromo-succinimide to give an entirely analogous dehydrocevine D-orthoacetate triacetate (XXIX). Alkaline hydrolysis of XXIX afforded dehydrocevine D-orthoacetate (XXVI) the infrared spectrum of which exhibited no absorption in the carbonyl region. (4) Chromium trioxide–pyridine oxidation of XXIII afforded a neutral product in 35 per cent yield, evidently the formamido ketone XXIV (see below). The same formamido ketone was obtained directly in 12 per cent yield by similar oxidation of XXII.

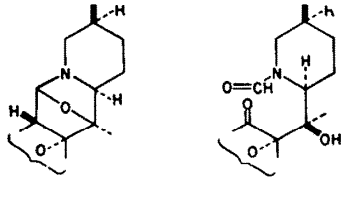
The structure of the formamido ketone was shown to be XXIV as follows. Upon exhaustive acid-catalysed hydrolysis XXIV yielded five mole-equivalents of volatile acid; one mole-equivalent of formic acid was found among the acids formed. The

[13] K. Macek and Z. J. Vejdelek, *Nature, Lond.* **176,** 1173 (1955); *Coll. Czech. Chem. Comm.* **22,** 253 (1957).
[14] S. M. Kupchan, D. Lavie, C. V. Deliwala and B. Y. A. Andoh, *J. Amer. Chem. Soc.* **75,** 5519 (1953).
[15] O. E. Edwards, F. H. Clarke and B. Douglas, *Canad. J. Chem.* **32,** 235 (1954).

infrared spectrum of XXIV showed a band at 6·07 μ attributable to the formamido grouping. In order to detect whether a carbonyl group was introduced in the course of the chromium trioxide oxidation, we elected to study the alkali stable (see above) cevine analog. Oxidation of XXIX with chromium trioxide–pyridine gave the corresponding formamido ketone (XXX). This substance gave a monosemicarbazone. Alkaline hydrolysis of XXX gave the desacetyl-formamido-ketone XXVII. In addition to the amide band at 6·07 μ, the infrared spectrum of XXVII exhibited normal ketone absorption at 5·85 μ.

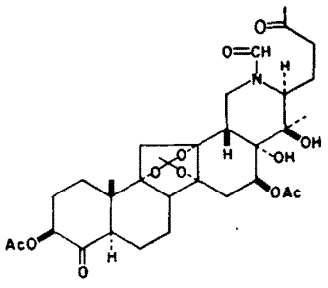


The evidence presented thus far supports an oxide structure for the products of N-bromosuccinimide oxidation and a formamido ketone structure for the products of chromium trioxide–pyridine oxidation. The alternative partial formulations XXXI and XXXII for the respective products may be excluded on the basis of the following

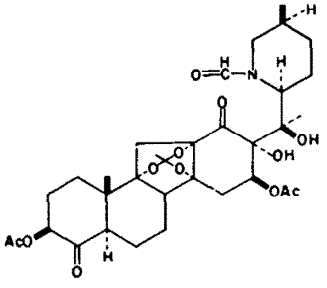


XXXI XXXII

evidence.* (1) The formamido ketone derived from cevine *readily* formed a semicarbazone. This behavior is consistent with structure XXX which is expected to show normal ketone reactivity, but not with tetrasubstituted ketone structure XXXII which would be expected to react very slowly, if at all. (2) The formamido-ketone showed active methylene group reactivity in the Zimmermann test.[16] (3) Upon treatment in alkaline solution with furfural the formamido-ketone gave an amorphous product, λ_{max} 318 mμ, characteristic of a furfurylidene ketone.[17] (4) The formamido-ketone derived from cevagenine-C-orthoacetate diacetate[14] was, like the parent alkaloid, stable to lead tetraacetate. The compound with structure XXXIII is expected to show this stability because the integrity of the rigid *trans* di*axial* glycol system at C_{17}, C_{20} is maintained during cleavage of Ring F. A product with structure XXXIV, on the other hand, would be expected to cleave readily with glycol-splitting reagents.



XXXIII



XXXIV

(5) Nuclear magnetic resonance spectroscopy† studies afforded direct evidence that the formamido ketone was a methyl ketone. The substances examined were only sparingly soluble in chloroform of carbon disulfide; hence pyridine was used as the solvent and water (contained in a separate capillary tube) was employed for reference.‡ The spectra of cevine D-orthoacetate[18] and of the corresponding dehydro compound XXVI exhibited a family of appropriate sharp signals above +217 c.p.s. (relative to benzene, i.e., water = +70 c.p.s.) corresponding to the four methyl groups. A detailed analysis of these spectra will be recorded in a future publication. Noteworthy to the case at hand is the fact that the spectrum of the corresponding formamido ketone XXVII exhibited a sharp signal, characteristic of methyl proton resonance, at +200 c.p.s. (+170 c.p.s. corrected for chloroform solution) which corresponds to

* The iodoform test on the formamido ketone was negative in our hands. This evidence was inconclusive, particularly in view of the fact that the test failed also with 3α-acetoxypregnane-11,20-dione.

† The spectra were determined at 40 megacycles/second with a Varian Associates V-4300-B High Resolution NMR Spectrometer having a 12-in. Magnet System equipped with a V-K 3506 Flux Stabilizer. We wish to thank K. L. Williamson for determining these spectra.

‡ Control experiments with related substances of known constitution showed that, under the conditions employed here, NMR signals obtained from pyridine solutions of the compounds in question could be corrected to chloroform or carbon disulfide solutions by subtracting ca. 30 c.p.s. It is to be noted that pyridine has not been considered to be a reliable standard solvent for NMR spectroscopy, N. F. Chamberlain, *Analyt. Chem.* **31**, 56 (1959).

[16] W. Zimmermann, *Z. Phys. Chem.* **233**, 257 (1935); D. H. R. Barton and P. De Mayo, *J. Chem. Soc.* 887 (1954).

[17] W. S. Johnson, B. Bannister and R. Pappo, *J. Amer. Chem. Soc.* **78**, 6331 (1956).

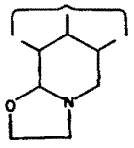
[18] D. H. R. Barton, C. J. W. Brooks and J. S. Fawcett, *J. Chem. Soc.* 2137 (1954).

the methyl group of a methyl ketone.[19] The spectra of the precursor compounds showed no significant absorption in the +200 c.p.s. region.

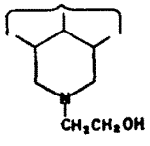
The bridged β-oriented oxide structure of the dehydro bases requires that the hydrogen at C_{22} be α-oriented (see formula XXXVIII). That the stereochemical integrity of the molecule was preserved during the oxide formation was demonstrated by catalytic hydrogenation of XXIII over platinum in acetic acid. Two mole-equivalents of hydrogen was absorbed to give a substance identical with the product of hydro-genation (one mole equivalent uptake) of XXII, namely veracevine-D-dihydro-orthoacetate triacetate.*

It is of interest to note that the orthoacetate cage structure is not required for production of the stable bridged carbinolamine ether. Thus cevine triacetate[18] was oxidized with N-bromosuccinimide to a dehydrocevine triacetate entirely analogous to the substance XXIII in the orthoacetate series. The further conversion with chromium trioxide–pyridine to a formamido ketone (cf. formula XXIV) was also effected. The properties of these products closely resembled their counterparts in the orthoacetate series.

On the basicity of the carbinolamine ether. Carbinolamine ethers of the type $ROCNR_2$, like the free carbinolamines, would be expected *a priori* to exhibit basicity significantly stronger than that of a tertiary amine, because protonation on oxygen leads to a structure, $ROC^{\oplus}NR_2$, that can cleave to give the alcohol (ROH) plus the stable ternary iminium cation, $>C=NR_2\oplus$. Thus the carbinolamine ether system (shown, in part, by formula XXXV) of veatchine (p*K*a′ 11·5) and of its isomer garryine (p*K*a′ 8·7) is responsible for enhanced basicity in these alkaloids as compared with the corresponding amino alcohol XXXVI (p*K*a′ 6·85).[20]



XXXV



XXXVI

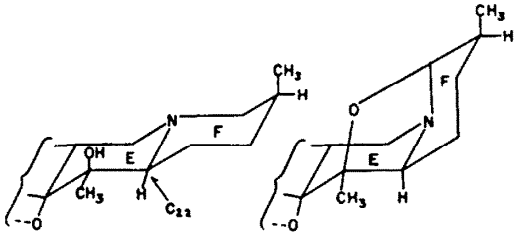
If, however, cleavage to the ternary iminium salt were inhibited, the carbinolamine ether would be expected to exhibit relatively weak basic properties because of the electron withdrawing inductive effect of the ether–oxygen atom on the neighboring nitrogen atom. The bridged-oxido dehydro bases (XXIII, XXIX) of the present work appear to be the first examples of such carbinolamine ethers that are resistant to cleavage to iminium salts by acid. Thus the perchlorate of dehydroveracevine-D-orthoacetate triacetate (XXIII) failed to show the infrared absorption characteristic

* This product is analogous to that produced by hydrogenation of cevine-D-orthoacetate triacetate (cf. 21).

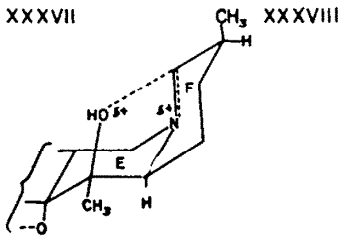
[19] Cf. the value of +171 ± 1 c.p.s. in chloroform, J. N. Shoolery and M. T. Roberts, *J. Amer. Chem. Soc.* **80**, 5121 (1958).

[20] K. Wiesner, S. K. Figdor, M. F. Bartlett and D. R. Henderson, *Canad. J. Chem.* **30**, 608 (1952); K. Wiesner, R. Armstrong, M. F. Bartlett and J. A. Edwards, *J. Amer. Chem. Soc.* **76**, 6068 (1954).

of ternary iminium salts and failed to undergo substitution by cyanide ion.[21] In accordance with expectation, the basicity of XXIII (cf. XXXVIII) (p*Ka*′ 3·8) was strikingly weak compared with that of the alkaloid XXII (cf. XXXVII) (p*Ka*′ 7·4).* It is not known whether the proton prefers residence on the oxygen or the nitrogen atom in the salts of these bridged ring oxido-bases, but their unusual stability may be attributed to unfavorable bond angles (in violation of Bredt's rule) required in the transition state for formation of the iminium cation (see formula XXXIX). The foregoing is not meant to imply preclusion of the possibility that the barrier of this high energy transition state can be surmounted under appropriate conditions.



XXXVII XXXVIII



XXXIX

It should be noted that carbinolamine ethers with basicities slightly diminished from those of closely related amines are known among the solanum[22] and the aconite[23] alkaloids. The small diminution in basicity in each of the latter cases is evidently not due to extreme stability of the carbinolamine ether systems, for infrared spectroscopy has shown that corresponding salts in each case have iminium structures.[23,24]

EXPERIMENTAL†

Methanolysis of epiandrosterone acetate

Epiandrosterone acetate [25] (1 g). m.p. 110–112°, was dissolved in methanol (25 ml) and water (5 ml), and cevine (1 g) was added to the solution. After 21 hr at room temp, acetic acid (4 ml) and water (5 ml) were added and the methanol was removed by distillation at reduced pressure. The aqueous suspension was extracted thoroughly with chloroform. The chloroform extract was washed

* The p*Ka*′ values were determined by potentiometric titration with 0·1 N $HClO_4$ in 80% methylcellosolve by Drs. T. Higuchi and K. Connors to whom we extend our thanks.

† Melting points are corrected for stem exposure. Values of $[\alpha]_D$ have been approximated to the nearest degree. Ultraviolet absorption spectra were determined on a model 11 MS Cary recording spectrophotometer and 95% ethanol was used as solvent. Infrared spectra were determined on a Baird Model B double beam infrared recording spectrophotometer, and unless otherwise specified chloroform was used as solvent. Microanalyses were carried out by Dr. S. M. Nagy and his associates at Massachusetts Institute of Technology on samples dried at reduced pressure at 110°.

[21] Cf. N. J. Leonard and F. P. Hauck, Jr., *J. Amer. Chem. Soc.* **79**, 5279 (1957).
[22] H. Bloom and L. H. Briggs, *J. Chem. Soc.* 3591 (1952).
[23] K. Wiesner, S. Ito and Z. Valenta, *Experientia* **14**, 167 (1958).
[24] E. Schlittler and H. Uehlinger, *Helv. Chim. Acta* **35**, 2034 (1952).
[25] Footnote 29 on ref. 17.

with dilute acetic acid, then with water, and evaporated to dryness. Crystallization of the residue from methanol–water yielded starting material (750 mg), m.p. 107–109°. The infrared spectrum was identical with that of authentic epiandrosterone 3-acetate.

Methanolysis of strophanthidin 3-acetate

Strophanthidin 3-acetate[26]* (400 mg) m.p. 248–250°, was dissolved in methanol (15 ml) and water (5 ml), and cevine (400 mg) was added. After 21 hr at room temp acetic acid (3 ml) and water (5 ml) were added, and the methanol was evaporated at reduced pressure. The aqueous suspension was extracted thoroughly with chloroform. The chloroform extract was washed with dilute acetic acid, then with water and evaporated to dryness. The residue was crystallized from methanol–water to give 242 mg of strophanthidin, m.p. 170–175° after softening at 160°, undepressed on admixture with authentic strophanthidin, m.p. 171–177° after softening at 163°. The infrared spectra of the two samples were identical.

Hydrogenation of 16-dehydrocevine 3,4-diacetate

16-Dehydrocevine 3,4-diacetate[2] (100 mg), m.p. 275–277° dec. in glacial acetic acid (15 ml) was hydrogenated over 30 mg of platinum oxide catalyst at room temp and atmospheric pressure. One mole-equivalent of hydrogen was absorbed in 15 min and no further uptake was observed during the next 30 min. The mixture was filtered, dilute ammonia was added to pH 9, and the solution was extracted thoroughly with chloroform. The residue obtained on evaporation was crystallized from ether–petroleum ether (b.p. 40–60°) to yield needles (68 mg), m.p. 274–277° dec. undepressed on admixture with authentic cevine 3,4,-dicaetate,[2] m.p. 275–278° dec. The infrared spectra of the two samples were identical.

Sodium borohydride reduction of 16-dehydrocevadine-D-orthoacetate 4-acetate

A solution of 16-dehydrocevadine-D-orthoacetate 4-acetate[3] (3·2 g), m.p. 268–270° dec. in hot methanol (800 ml) was treated with sodium borohydride (1·6 g) and allowed to stand for 45 min. Additional sodium borohydride (1·6 g) was added and the solution was allowed to stand for 1 hr. Acetic acid (8 ml) was added and the solution was concentrated at reduced pressure to a volume of 50 ml. Water (30 ml) was added and the remaining methanol evaporated at reduced pressure. The aqueous solution was adjusted to pH 9 with dil ammonia and extracted with chloroform. The chloroform solution was concentrated (approximately 10 ml), 95% ethanol (20 ml) was added, and concentration continued until crystallization began. Filtration afforded needle clusters (1·15 g), m.p. 305–307° dec. Recrystallization of a 400 mg sample by dissolution in a large volume of boiling 95% ethanol, filtering, and then concentrating the filtrate, gave 230 mg of 16-epicevadine-D-ortho-acetate 4-acetate as colorless needles, m.p. 314–316° dec. $[\alpha]_D^{21}$ +65° (*c*, 1·17, chf.). (Found: C, 66·21; H, 7·96. Calcd. for $C_{34}H_{49}O_9N$: C, 66·31, H, 8·02%).

The mother liquor of the 1·15 g fraction, after concentration, gave 370 mg (second crop), m.p. 280–283° dec. Evaporation of the mother liquor to dryness and crystallization of the residue from acetone–water gave 180 mg (third crop), m.p. 276–279° dec. The infrared spectra of the second and third crops showed that these fractions consisted largely of cevadine-D-orthoacetate 4-acetate.

16-*Epicevagenine-C-orthoacetate* (XIX)

16-Epicevadine-D-orthoacetate 4-acetate (XVI, 1·15 g), m.p. 305–307° dec. in 15 ml of 5% methanolic KOH was heated under reflux for 30 min. Water (15 ml) was added, the methanol was evaporated at reduced pressure, and the aqueous suspension was extracted with chloroform. The chloroform was evaporated and the residue dissolved in 5% H_2SO_4. After 15 min at room temp the pH was adjusted to 9 with dilute ammonia and the solution was extracted with chloroform. The residue obtained on concentration was crystallized from benzene–ether to yield needles (370 mg), m.p. 255–263° dec. after melting initially at 220–230° and resolidifying at 230–235°. This product

* We thank Dr. M. Ehrenstein of the University of Pennsylvania and Dr. W. G. Bywater of S. B. Penick and Co. for samples of strophanthin from which strophanthidin was obtained for this work. We also thank S. B. Penick and Co. for a generous gift of veratrine.

[26] T. Reichstein and H. Rosenmund, *Pharm. Acta Helv.*, **15**, 150 (1940).

was dissolved in chloroform and chromatographed on Woelm "neutral" alumina (8 g). The fractions from elution with 90 ml of chloroform were combined and crystallized from benzene–95% ethanol to give 120 mg of 16-epicevagenine-C-orthoacetate as colorless needles containing one mole-equivalent of benzene of crystallization, m.p. 276–279° dec. $[\alpha]_D^{22}$ −10° (*c*, 1·22, chf.); λ_{max} 239 mμ, 249 mμ (ε 250), 261 mμ. (Found: C, 69·09; H, 7·81. Calcd. for $C_{29}H_{43}O_8N \cdot C_6H_6$: C, 68·66; H, 8·07%).

*Lead tetraacetate titrations**

Fifteen to twenty mg samples of compounds to be examined were dissolved in acetic acid (1 ml), 2 ml of approximately 0·06 N lead tetraacetate in acetic acid was added and then the solution was diluted to 10 ml with acetic acid. At noted intervals 1 ml aliquots were drawn out and titrated according to the procedure of Hockett and McClenahan.[27] The results obtained with the epimeric cevagenine-C-orthoacetates are summarized in Table 1.

TABLE 1. LEAD TETRAACETATE TITRATIONS

Time (hr)	Mole-equivalents consumed by cevagenine-C-orthoacetate	Mole-equivalents consumed by 16-epicevagenine-C-orthoacetate
0·15	0·0	0·9
0·5	0·0	1·0
0·65	0·0	1·1
5	0·4	1·4
10	0·8	1·6
15	0·9	1·7
24	1·4	1·8

Dehydroveracevine-D-orthoacetate triacetate (XXIII)

Veracevine-D-orthoacetate triacetate[14] (4 g), m.p. 254–256° dec. in chloroform (50 ml) was treated with a solution of N-bromosuccinimide (1·5 g) in chloroform (125 ml) and the mixture was allowed to stand at room temp for 5 min. The solution was washed thoroughly with cold, dil ammonium hydroxide, then with water and evaporated to dryness under reduced pressure. Crystallization of the residue from aqueous acetone afforded colorless needles (1·39 g), m.p. 267–268° dec. Purification was effected by adsorption of the product (2·3 g) in chloroform on Woelm "neutral" alumina (50 g) and elution with chloroform (ten 50 ml fractions). Fractions 2–7 afforded oils which crystallized from ether (m.p. 275–277° dec.). Recrystallization from aqueous acetone gave colorless prismatic needles (1·8 g), m.p. 279–280° dec.

A preparation of comparable purity was obtained by oxidative purification of the crude product, m.p. 267–268°. A solution of the product (300 mg) in acetic acid (5 ml) was treated with 0·65 N chromium trioxide in 98·5% acetic acid (5 ml) and the mixture was allowed to stand at room temp for 1 hr. Cold water, excess dilute sodium bisulfite solution and ammonium hydroxide were added to adjust the pH to 9 and the solution was extracted with chloroform. The residue obtained on evaporation at reduced pressure was crystallized from aqueous acetone to yield needles (240 mg), m.p. 279–281° dec. Recrystallization from the same solvents gave colorless prismatic needles, m.p. 280–282° dec. $[\alpha]_D^{25}$ +33° (*c*, 1·88 diox). (Found: C, 63·51; H, 7·35; acetyl, 25·52. Calcd. for $C_{36}H_{47}O_{11}N$: C, 63·90; H, 7·20; acetyl, 26·17%).

The perchlorate was prepared in methanol and crystallized upon dilution with water as colorless prisms, m.p. 222–224° dec. The salt decomposed upon attempted recrystallization. It showed no infrared absorption characteristic of ternary iminium salts and dehydroveracevine-D-orthoacetate triacetate was recovered unchanged after treatment with aqueous potassium cyanide solution.[21]

* Experiment by Miss Arlene G. Gardner.

[27] R. C. Hockett and W. S. McClenahan, *J. Amer. Chem. Soc.* **61**, 1670 (1939).

The methiodide was prepared by treatment of the dehydro base (400 mg) in methanol (10 ml) with methyl iodide (2 ml) at room temp. After 12 hr the solution was evaporated at reduced pressure and the residue was crystallized from aqueous acetone giving 150 mg of starting material. The acetone was evaporated, water and ammonium hydroxide were added, and the suspension was extracted with chloroform. The residue obtained by evaporation of the chloroform was crystallized from aqueous acetone to give colorless needles (180 mg), m.p. 236–239° dec. Recrystallization from the same solvents gave the methiodide, m.p. 244° dec. $[\alpha]_D^{25}$ +62° (*c*, 2·00, py.). (Found: C, 52·67; H, 6·48; N-methyl, 2·08. Calcd. for $C_{36}H_{50}O_{11}NI \cdot H_2O$: C, 52·88; H, 6·41; N-methyl, 1·84%).

*Attempted methanolysis of dehydroveracevine-*D*-orthoacetate triacetate*

A solution of the dehydro base XXIII (250 mg) in methanol (300 ml) and triethylamine (0·1 ml, ca. two mole-equivalents) was left at room temp for 20 hr. Evaporation to dryness under reduced pressure and crystallization from aqueous acetone gave 225 mg of starting material, m.p. 277–279° dec. undepressed on admixture with a sample of starting material. The infrared spectra (potassium bromide pellet) of the two samples were identical.

*Dehydrocevine-*D*-orthoacetate triacetate* XXIX

A solution of cevine-D-orthoacetate triacetate[28] (6 g), m.p. 300–303° dec. in chloroform (100 ml) was treated with a solution of N-bromosuccinimide (2 g) in chloroform (200 ml) and the mixture was allowed to stand at room temp for 30 min. The product was isolated as described above for XXIII and the residue from chloroform crystallized readily from acetone giving plates (3·1 g), m.p. 256–257° dec. Recrystallization from acetone afforded glistening plates, m.p. 256–257° dec. $[\alpha]_D^{25}$ +58° (*c*, 2·00, py.). (Found: C, 63·62; H, 7·44; acetyl, 25·36. Calcd. for $C_{35}H_{47}O_{11}N$: C, 63·90; H, 7·20; acetyl, 26·17%).

*Dehydrocevine-*D*-orthoacetate* XXVI

Dehydrocevine orthoacetate triacetate (XXIX, 2 g), m.p. 256–257° dec. was dissolved in 20% alcoholic KOH (20 ml) and the solution was heated under reflux for 30 min. Water (20 ml) and acetic acid (4 ml) were added, whereupon a colorless crystalline solid separated. Recrystallization of the solid from methanol afforded: first crop, 400 mg, m.p. 283–284° dec. second crop, 110 mg, m.p. 271–274° dec. third crop, 106 mg, m.p. 245–260° dec. An additional 61 mg, m.p. 266–272° dec. was obtained by chloroform extraction of the mother liquors of the first crude crystalline solid described above. All of the crystalline crops were combined and recrystallized from methanol to yield 525 mg of colorless needles, m.p. 283–284° dec. $[\alpha]_D^{25}$ +3° (*c*, 2·00, py.). (Found: C, 65·41; H, 7·93; acetyl, 6·97. Calcd. for $C_{29}H_{41}O_8N$: C, 65·52; H, 7·78; acetyl, 8·10%).

Formamido ketone (XXIV) *derived from veracevine-*D*-orthoacetate triacetate*

(a) *From veracevine-*D*-orthoacetate triacetate.* Veracevine-D-orthoacetate triacetate[14] (7 g), m.p. 254–256° dec. in pyridine (75 ml) was added to the complex prepared from chromium trioxide (14 g) and pyridine (140 ml). The mixture was allowed to stand for 40 hr at room temp with occasional shaking. Water (300 ml), chloroform (400 ml) and dilute ammonia (75 ml of 1 : 1) were added and the mixture was shaken and filtered through a bed of Filtercel The chloroform layer was washed with water and evaporated on the steam bath at reduced pressure The highly colored residue was dissolved in acetone–petroleum ether (b.p. 40–60°), filtered to remove some red-brown insoluble solid material, and cooled. The crystalline product thus obtained was recrystallized from benzene to give colorless needles (1·06 g), m.p. 263–264° dec. $[\alpha]_D^{27}$ +54° (*c*, 2·02, diox.); λ_{max} 6·07 μ

(N—C=O). (Found: C, 60·59; H, 7·17. Calcd. for $C_{35}H_{47}O_{13}N$: C, 60·94; H, 6·87%).

In a volatile acid determination[29] 19·95 mg of the formamido ketone yielded an amount of acid equivalent to 37·15 ml of 0·003999 N sodium thiosulfate; calculated for four mole-equivalents of acetic acid and one mole-equivalent of formic acid, as expected for structure XXIV, 36·16 ml. Formic acid formed during acid hydrolysis of the formamido ketone was determined by reduction of

[28] A. Stoll and E. Seebeck, *Helv. Chim. Acta* **36**, 189 (1953).

[29] J. B. Niederl and V. Niederl, *Micromethods of Quantitative Organic Analysis* pp. 257–262. John Wiley, New York (1942).

mercuric chloride and gravimetric determination of the calomel formed.[30] (Found: 0·88 mole-equivalent).

Tests for active methylene group reactivity were run as follows: (1) A Zimmermann test[16] was run by treating the formamido ketone (ca. 1 mg) in 2 N alcoholic potassium hydroxide (1 ml) with 1% alcoholic *m*-dinitrobenzene (1 ml). A strong purple color developed within 10 min. Veracevine-D-orthoacetate triacetate (XXII) and its dehydro derivative (XXIII) gave no color when treated as above. (2) Furfural (0·1 ml) was added to a solution of the formamido ketone (10 mg) in alcohol (2 ml) and 15% sodium hydroxide solution (0·1 ml). After 30 min at room temp, the mixture was extracted with chloroform and the chloroform solution was washed with dilute sodium bisulfite solution and water and evaporated to dryness at reduced pressure giving a yellow amorphous residue, λ_{max} 318 mμ, attributable to a furfurylidene grouping.[17] Treatment of XXII in a similar manner afforded an amorphous product with no significant absorption in the ultraviolet.

(b) *From dehydroveracevine-D-orthoacetate triacetate.* A solution of the dehydro compound (XXIII, 350 mg), 277–279° dec. was added to the complex from chromium trioxide (700 mg) and pyridine (15 ml), and the mixture was treated as in the previous experiment. The formamido ketone (XXIV) obtained by crystallization from acetone–petroleum–ether (b.p. 40–60°), amounted to 140 mg, m.p. 262–264° dec.

Formamido ketone (XXX) *derived from cevine-D-orthoacetate triacetate*

(a) *From cevine-D-orthoacetate triacetate.* Cevine-D-orthoacetate triacetate[28] (18 g), m.p. 300–303° dec. in pyridine (100 ml) was added to the complex from chromium trioxide (36 g) and pyridine (250 ml), and the mixture was treated as in the previous experiment. The formamido ketone (XXX) crystallized from acetone–petroleum–ether (b.p. 40–60°) in the form of clusters of prismatic needles (1·21 g), m.p. 286–288° dec. Recrystallization from benzene afforded prismatic plates, (1·09 g), m.p. 287–288° dec. $[\alpha]_D^{25}$ +52° (*c*, 2·00, py.); λ_{max} 6·07 μ. (Found: C, 60·98; H, 7·08. Calcd. for $C_{35}H_{47}O_{13}N$: C, 60·94; H, 6·87%).

In a volatile acid determination[29] 30·00 mg of the formamido ketone yielded an amount of acid equivalent to 26·01 ml of 0·007547 N sodium thiosulfate; calculated for four mole-equivalents of acetic acid and one mole-equivalent of formic acid, as expected for structure XXX, 28·82 ml. The compound gave a strong positive test for active methylene group reactivity in the Zimmermann test.

The semicarbazone, prepared by treatment of XXX in dilute ethanol with semicarbazide hydrochloride and sodium acetate at room temp, crystallized from the reaction mixture on concentration under a stream of air. Recrystallization from aqueous ethanol afforded colorless needles, m.p. 273–274° dec. (Found: C, 57·91; H, 6·80; N, 6·93. Calcd. for $C_{36}H_{50}O_{13}N_4$: C, 57·90; H, 6·75; N, 6·93%).

(b) *From dehydrocevine-D-orthoacetate triacetate.* Oxidation of dehydrocevine-D-othoacetate (9·4 g), m.p. 256–257° dec. in pyridine (80 ml) with the complex from chromium trioxide (19 g) and pyridine (120 ml) as above afforded 7·0 g of twice recrystallized formamido ketone XXX, m.p. 287–288° dec.

Alkaline hydrolysis of the formamido ketone derived from cevine-D-orthoacetate triacetate

To a solution of the formamido ketone XXX (5 g), m.p. 286–288° dec. in methanol (40 ml) was added 20% sodium hydroxide solution (5 ml) and the mixture was allowed to stand at room temp overnight. Water (25 ml) was added and the solution was extracted with chloroform. The chloroform extract was washed with water (10 ml) and the residue obtained on evaporation at reduced pressure was crystallized from acetone. Recrystallization from acetone gave colorless needles (2·3 g) m.p. 259–260° dec. $[\alpha]_D^{25}$ +22° (*c*, 2·00, py.); λ_{max} 5·85 μ, 6·07 μ. (Found: C, 61·66; H, 7·24. Calcd for $C_{29}H_{41}O_{10}N$: C, 61·80; H, 7·34%).

Formamido ketone (XXXIII) *derived from cevagenine-C-orthoacetate diacetate*

Cevagenine-C-orthoacetate diacetate[14] (18 g), m.p. 283–285° dec. in pyridine (100 ml) was added to the complex from chromium trioxide (36 g) and pyridine (250 ml), and the mixture was treated as described above for XXIV. Crystallization of the crude product from acetone–petroleum ether (b.p. 40–60°) gave prisms (4·0 g), m.p. 252–254° dec. A further recrystallization from 95% ethanol

[30] J. R. Dyer in David Glick, *Methods of Biochemical Analysis* Vol. III, p. 131. Interscience, New York (1956).

gave colorless prisms (2·5 g), m.p. 253–255° dec. $[\alpha]_D^{27}$ −83° (*c*, 2·26, diox.). (Found: C, 60·96; H, 7·10. Calcd. for $C_{33}H_{45}O_{12}N$: C, 61·17; H, 7·00%).

In a volatile acid determination[29] 22·23 mg of product yielded an amount of acid equivalent to 13·75 ml of 0·009675 N sodium thiosulfate; calculated for three mole-equivalents of acetic acid and one mole-equivalent of formic acid, as expected for structure XXXIII, 14·19 ml.

Lithium aluminum hydride reduction was effected as follows. The formamido ketone (500 mg), m.p. 253–255° dec. in tetrahydrofuran (25 ml) was treated with lithium aluminum hydride (1 g) in tetrahydrofuran (50 ml) and the mixture was shaken for 15 min. The solution was then heated under reflux for 4 hr. After cooling in ice, water (2·7 ml) was added cautiously. Chloroform (50 ml) was added and the mixture was shaken and passed through Filtercel. The filter cake was washed with chloroform (150 ml) and the combined chloroform extracts were washed with water (15 ml) and evapoated under reduced pressure. The amorphous residue was purified by fractional precipitation from ether with petroleum ether (b.p. 40–60°) and rejection of precipitated colored solid until the filtrate was colorless. Evaporation to dryness afforded a colorless amorphous solid, m.p. 140–210°, λ_{max} 2·85–3·00 μ (OH). The infrared spectrum showed no significant absorption in the 5·75–6·10 μ (C=O) region. (Found: N-methyl, 2·38. Calcd. for $C_{29}H_{43}O_9N$: N-methyl, 2·75%).

Veracevine-D-dihydroorthoacetate triacetate (XXV)

(a) *From dehydroveracevine-D-orthoacetate triacetate.* Dehydroveracevine-D-orthoacetate triacetate (XXIII, 500 mg), m.p. 277–279° dec. in acetic acid (25 ml) and conc HCl (0·1 ml) was hydrogenated over platinum oxide (100 mg) at room temp and atmospheric pressure. In 2¼ hr two mole-equivalents of hydrogen was absorbed and hydrogen uptake ceased. The reaction mixture was filtered, diluted with water, adjusted with ammonium hydroxide to pH 9 and extracted with chloroform. The residue obtained on evaporation of the chloroform was crystallized from ether to yield needles (310 mg), m.p. 299–300° dec. $[\alpha]_D^{25}$ +21° (*c*, 2·09, diox.). (Found: C, 63·53; H, 7·85; acetyl, 19·98. Calcd. for $C_{35}H_{51}O_{11}N$: C, 63·52; H, 7·77; acetyl, 19·53%).

(b) *From veracevine-D-orthoacetate triacetate.* Hydrogenation of veracevine-D-orthoacetate triacetate (150 mg), m.p. 254–256° dec. in acetic acid (15 ml) and conc HCl (0·1 ml) with platinum oxide (30 ml) as described above resulted in uptake of one mole-equivalent of hydrogen in 1 hr. Isolation as described above afforded veracevine-D-dihydroorthoacetate triacetate (105 mg) in the form of colorless needles, m.p. 299–300° dec. undepressed on admixture with authentic XXV obtained as above. The infrared spectra of the two samples were identical.

Dehydrocevine triacetate

Cevine triacetate[18] (1·5 g), m.p. 303–304° dec. in chloroform (50 ml) was treated with N-bromosuccinimide (0·6 g) in chloroform (60 ml) and the mixture was allowed to stand at room temp for 30 min. The product was treated as described above for XXIII and the residue from chloroform was crystallized from water–acetone–methanol to give 816 mg, m.p. 234–239° dec. Partial purification was effected by dissolving this material in dil HCl, adding excess sodium acetate, and extracting the mixture with ether. The residue obtained on evaporation of the ether was crystallized from aqueous acetone to give 390 mg, m.p. 255–260° dec. This product was dissolved in benzene and chromatographed on Merck acid-washed alumina (10 g), using benzene, benzene–chloroform (50 : 50) and chloroform as eluants. The residues obtained from the benzene–chloroform and chloroform fractions were combined and crystallized from aqueous acetone to yield 135 mg, m.p. 292–294° dec. Recrystallization from the same solvents gave colorless needles, m.p. 294–295° dec. $[\alpha]_D^{25}$ +5° (*c*, 2·00, py.) The ultraviolet spectrum exhibited no maximum other than end-absorption. (Found: C, 60·98; H, 7·31. Calcd. for $C_{33}H_{47}O_{11}N \cdot H_2O$: C, 60·89; H, 7·59%).

Catalytic hydrogenation of dehydrocevine triacetate

Dehydrocevine triacetate (250 mg), m.p. 293–295° dec. in acetic acid (20 ml) and conc HCl (0·1 ml) was hydrogenated over 50 mg of platinum oxide at room temp and atmospheric pressure. Uptake of hydrogen ceased after one mole-equivalent had been absorbed. Treatment as described above for XXV gave a crude product which crystallized from benzene–ether in the form of colorless needles (218 mg), m.p. 304–305° dec. undepressed on admixture with an authentic sample of cevine triacetate. The infrared spectra of the two samples were identical.

Formamido ketone derived from cevine triacetate

(a) *From cevine triacetate.* Chromium trioxide–pyridine oxidation of cevine triacetate (5 g), m.p. 303–304° dec. by the procedure described above for XXIV afforded a crude product which was crystallized from acetone to give 1·04 g of product. Recrystallization from acetone gave colorless prismatic plates (774 mg), m.p. 210–220° dec. after sintering from 162°; $[\alpha]_D^{27}$ −31° (*c*, 2·16, diox.). (Found: C, 59·81; H, 7·49. Calcd. for $C_{33}H_{47}O_{13}N\cdot(CH_3)_2CO$: C, 59·70; H, 7·38%).

(b) *From dehydrocevine triacetate.* Chromium trioxide–pyridine oxidation of dehydrocevine triacetate (250 mg), m.p. 293–295° dec. as described above gave 85 mg of formamido ketone, m.p. 210–220° dec. undepressed on admixture with the specimen described above under part (a). The infrared spectra of the two specimens were identical.