product was recrystallized three times from benzene–ligroin; m.p. 151–153°, $[\alpha]^{25}D$ −161° (lit.[7] m.p. 153°, $[\alpha]$ −168°). Upon admixture with an authentic sample there was no depression of m.p.

Anal. Calcd. for $C_{15}H_{20}O_3$ (248.31): C, 72.55; H, 8.12. Found: C, 72.77; H, 8.15.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

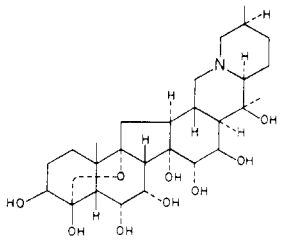
Veratrum Alkaloids. XXXVIII.[1] The Structure and Configuration of Protoverine[2,3]

BY S. MORRIS KUPCHAN, C. IAN AYRES, MOSHE NEEMAN,[4] RUPRECHT H. HENSLER, TADASHI MASAMUNE AND S. RAJAGOPALAN[5]

RECEIVED AUGUST 31, 1959

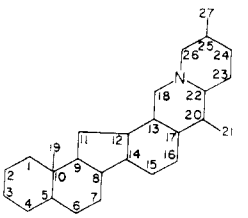
The alkaloid protoverine has been shown to have structure and configuration I. Alkaline isomerization of I leads to isoprotoverine (X) and thence to pseudoprotoverine (XI). Acetic anhydride–pyridine acetylation of I affords a pentaacetate (XII); acetic anhydride–perchloric acid acetylation yields a hexaacetate (XIII). Methanolysis of XIII affords an isopentaacetate (XV) which is oxidized by chromic acid to dehydroprotoverine isopentaacetate (XIV). Alkaline treatment of XIV affords a cross-conjugated diosphenol derivative (VII). Compound I yields an acetonide (XVI) which is acetylated to a triacetate (XVIII). Acid hydrolysis of XVIII yields protoverine triacetate (XXII) which is oxidized by periodate to a cyclopentenone aldehyde (XXIII). Chromic acid oxidation of XVIII affords a dehydroprotoverine acetonide triacetate (XXV) which yields a dehydroprotoverine triacetate (XXVI) on acid hydrolysis. Sodium borohydride reduction of XXV gives a protoverine acetonide diacetate (XXIV). Treatment of isoprotoverine with acetone and hydriodic acid yields a diacetonide (XXVIII). Acetylation of XXVIII gives a diacetate (XXIX). Tosylation of XVI affords a protoverine acetonide tosylate (XXX) which is acetylated to a diacetate (XXXI). Acid hydrolysis of XXXI followed by acetylation yields a tosylate tetraacetate (XXXIII). Proof that protoverine is 6-α-hydroxygermine was obtained by calcium–liquid ammonia reduction of XXV to the known 7-dehydrogermine 14,15-acetonide 3,16-diacetate (XXXIV).

Protoverine, $C_{27}H_{43}O_9N$, is the alkamine present in several polyester alkaloids which occur in *Veratrum* species.[6–10] The structure of protoverine is of particular interest in view of the potent hypotensive action of its ester derivatives[11] and of the use of this antihypertensive action in clinical conditions associated with high blood pressure.[12] In this paper evidence is presented for assignment of structure and configuration I to protoverine.



I

Protoverine was first obtained in amorphous form by Poethke, in 1937, from alkaline hydrolysis of protoveratrine.[6,7] A $C_{28}H_{45}O_{10}N$ formulation was proposed. Jacobs and Craig isolated the alkamine in crystalline form and prepared several crystalline derivatives.[8] On the basis of their analytical study of the base and its derivatives, they assigned the correct $C_{27}H_{43}O_9N$ formulation to protoverine. The latter authors also demonstrated a close structural analogy to cevine and germine by isolating 2-ethyl-5-methylpyridine, cevanthrol and cevanthridine from the products of selenium dehydrogenation of protoveratrine.[13] These results led Jacobs and Pelletier to propose skeletal structure II for protoverine as well as cevine and germine.[14]



II

Protoverine undergoes a series of isomerizations (protoverine → isoprotoverine → pseudoprotoverine)[8,15] which parallels those of zygadenine,[16] veracevine[17,18] and germine.[17,19] The close analogy of the isomerizations to the veracevine-cevagenine-cevine[20] and the germine–isogermine–pseudoger-

(1) Part XXXVII, S. M. Kupchan and A. Afonso, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 731 (1959).

(2) The investigations which form the subject of the present paper were first outlined in part in two preliminary communications: *Chemistry & Industry*, 1626 (1958), and THIS JOURNAL, **81**, 4753 (1959).

(3) This investigation was supported by research grants from the National Institutes of Health (H-2275, C_1–C_3), Pitman–Moore Co., and the Wisconsin Alumni Research Foundation.

(4) On leave from the Technion–Israel Institute of Technology, Haifa, Israel.

(5) Deceased.

(6) W. Poethke, *Arch. Pharm.*, **275**, 357 (1937).

(7) W. Poethke, *ibid.*, **275**, 571 (1937).

(8) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **149**, 271 (1943).

(9) M. W. Klohs, M. Draper, F. Keller, S. Koster, W. Malesh and F. J. Petracek, THIS JOURNAL, **76**, 1152 (1954).

(10) G. S. Myers, P. Morozovitch, W. L. Glen, R. Barber, G. Papineau-Couture and G. A. Grant, *ibid.*, **77**, 3348 (1955).

(11) O. Krayer and G. A. Acheson, *Physiol. Rev.*, **26**, 383 (1946).

(12) L. S. Goodman and A. Gilman, "The Pharmacological Basis of Therapeutics," The Macmillan Co., New York, N. Y., second edition, 1955, pp. 747–754; O. Krayer in V. A. Drill, "Pharmacology in Medicine," McGraw-Hill Book Co., Inc., New York, N. Y., second edition, 1958, pp. 515–524.

(13) L. C. Craig and W. A. Jacobs, *J. Biol. Chem.*, **143**, 427 (1942).

(14) W. A. Jacobs and S. W. Pelletier, *J. Org. Chem.*, **18**, 765 (1953).

(15) H. Auterhoff and F. Gunther, *Arch. Pharm.*, **288**, 455 (1955).

(16) S. M. Kupchan and C. V. Deliwala, THIS JOURNAL, **75**, 1025 (1953).

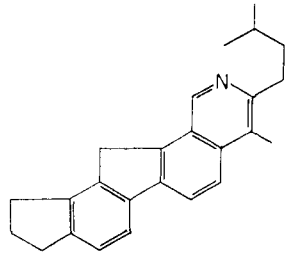
(17) S. W. Pelletier and W. A. Jacobs, *ibid.*, **75**, 3248 (1953).

(18) S. M. Kupchan, D. Lavie, C. V. Deliwala and B. Y. A. Andoh, *ibid.*, **75**, 5519 (1953).

(19) S. M. Kupchan, M. Fieser, C. R. Narayanan, L. F. Fieser and J. Fried, *ibid.*, **77**, 5896 (1955).

(20) D. H. R. Barton, O. Jeger, V. Prelog and R. B. Woodward, *Experientia*, **10**, 81 (1954).

mine[21] isomerizations suggested that protoverine possesses a 3-hydroxy-4-hemiketal system similar to that found in veracevine and germine. At least two earlier experiments support this view concerning the ring A system of protoverine. First, the presence of the masked keto system in ring A, with its potentiality for ring contraction, accounts for the appearance of the cyclopentanofluorene derivative cevanthridine (III) among the dehydrogenation products of protoveratrine.

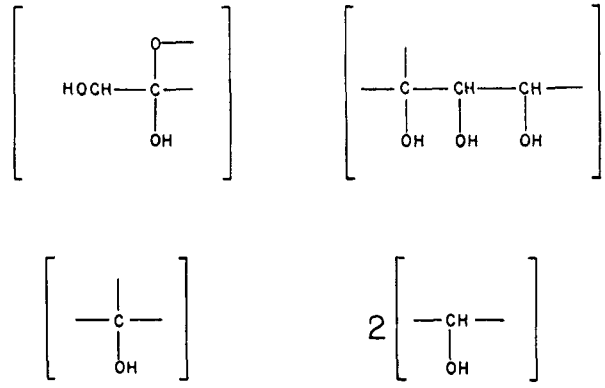


III

Second, the formation of a diosphenol ("dehydroisoprotoverine") upon oxidation of isoprotoverine with triphenyltetrazolium chloride[15] closely parallels the reactions of cevagenine and isogermine. Confirmation for the α-ketol hemiketal system involving the oxygen functions at C_3, C_4 and C_9 has been obtained by periodate oxidation (uptake: 1 mole equivalent) of protoverine acetonide monoisobutyrate. The amorphous oxidation product showed infrared absorption at 3.65, 5.62 and 5.80 μ in agreement with the expected [19,21] aldehydo-γ-lactone. A crystalline aldehydo-γ-lactone with identical absorption at these significant wave lengths is described in the accompanying paper.[22]

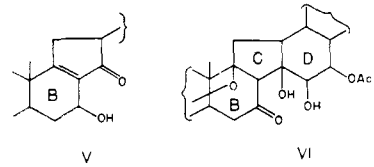
Protoverine readily formed a pentaacetate which was stable to chromic acid; hence five non-tertiary hydroxyl groups are present in the molecule. Under more vigorous acetylating conditions, protoverine formed a hexaacetate, and, by analogy with the behavior of germine,[21] it may be assumed that the difficultly-acylable hydroxyl is the C_4-hemiketal hydroxyl. Furthermore, isoprotoverine (periodic acid consumption, 3.9 mole equivalents) yielded isoprotoverine pentaacetate on acetylation with acetic anhydride–pyridine.

Protoverine consumed 4.0 mole equivalents of periodic acid and yielded 1.3–1.5 mole equivalents of formic acid; protoverine acetonide[8] consumed 2.0 mole equivalents and gave 0.1 mole equivalent of formic acid. Hence the acetonide blocks the uptake of periodic acid by a 1,2,3-triol system with a secondary hydroxyl group on the middle carbon atom. Protoverine acetonide was readily acetylated to a triacetate which consumed one oxygen equivalent of chromic acid and yielded a crystalline ketone (dehydroprotoverine acetonide triacetate). Mineral acid hydrolysis of protoverine acetonide triacetate afforded protoverine triacetate, which consumed one mole equivalent of sodium periodate. The foregoing facts show that: (1) the acetonide is tertiary–secondary, for three of protoverine's original five non-tertiary groups are still available for acetylation, and a fourth subsequently available for oxidation to a ketone; (2) the triol system is tertiary–secondary–secondary, for one of the acetate groups in protoverine acetonide triacetate clearly occupies a terminal secondary hydroxyl of a triol system; and (3) three oxygen functions are bound in the α-ketol hemiketal system and three in the triol; the remaining oxygen functions consist of two secondary and one tertiary hydroxyl groups. One of these three remaining hydroxyl groups is vicinal to another hydroxyl group in the molecule. The arguments presented thus far lead to tentative disposition IV of the functional groups of protoverine.



IV

The problem of locating these functional groups in the skeletal framework (II) is considerably simplified by the ready exclusion of all locations outside of ring D for the triol system. Rings A and B cannot accommodate both the α-ketol hemiketal and a tertiary–secondary–secondary triol system. Rings C and E will not accommodate the triol. Ring F may be excluded on the bases: (1) that protoverine shows no carbinolamine properties, hence ruling out sites alpha to nitrogen for hydroxyl location and eliminating a C_{22}, C_{23}, C_{24}-triol; and (2) that if the triol system were located at C_{23}, C_{24}, C_{25}, consumption of periodic acid by protoverine would not stop at four moles. Specific location at C_{14}, C_{15}, C_{16} is made possible by the following results. When the crude product of periodate oxidation of protoverine triacetate was exposed to dilute ammonia, an unsaturated ketone was obtained, λ_{max} 234 mμ (ϵ 9200); λ_{max} 2.90(s), 5.78–5.85(s), 5.92(s), 6.05(m)μ. This unsaturated ketone is assigned partial structure V by analogy with the corresponding derivative in the germine series.[21]



V VI

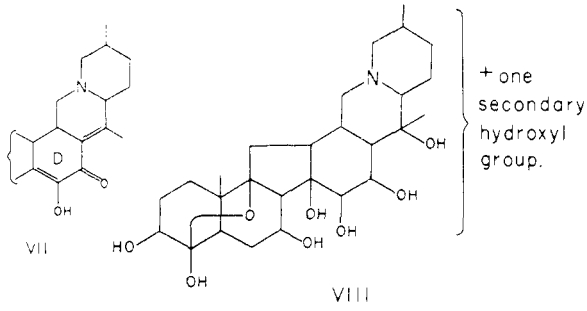
Treatment of dehydroprotoverine acetonide triacetate with dilute mineral acid afforded dehydro-

(21) S. M. Kupchan and C. R. Narayanan, THIS JOURNAL, **81**, 1913 (1959).

(22) S. M. Kupchan and C. I. Ayres, *ibid.*, **82**, 2252 (1960).

protoverine triacetate. The latter compound consumed five mole equivalents of sodium periodate in twenty-four hours. The result is readily compatible with partial structure VI for dehydroprotoverine triacetate. Oxidation apparently proceeds *via* initial cleavage of the C_{14}, C_{15}-glycol system to generate a cyclic 1,3-diketone (*cf.* reference 21).

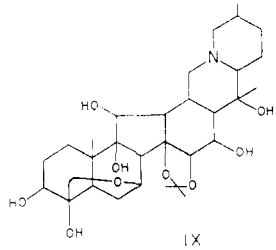
Protoverine hexaacetate was methanolyzed to an isopentaacetate, which, when oxidized with chromic acid, afforded a dehydroprotoverine pentaacetate. The latter compound, on alkaline treatment, yielded an amorphous diosphenol, λ_{max} 330 mμ (ϵ 17,700), $\lambda_{max}^{0.01\ N\ NaOH}$ 384 mμ (ϵ 11,600). Analogy with the diosphenol derived from 16-dehydrogermine 3,4,7,15-tetraacetate[21] supports assignment of partial structure VII.



VII VIII

The latter sequence and the characteristically facile methanolysis of the 16-acetate support assignment of hydroxyl groups to C_{16} and C_{20} in protoverine. Expression VIII, which incorporates eight of the nine oxygen atoms of protoverine, summarizes the conclusions drawn to this point.

Assignment of the remaining secondary hydroxyl group to C_6 is made on the following grounds. (1) Periodic acid oxidations gave the following results: Protoverine (uptake 4.0 mole equivalents) yielded 1.3–1.5 mole equivalents of formic acid.[23] Protoverine 14,15-acetonide (uptake 2.0 mole equivalents) yielded 0.1 mole equivalent of formic acid. Protoverine 6-isobutyrate, obtained by acid hydrolysis of the acetonide monoisobutyrate, consumed 2.8 mole equivalents of periodic acid and gave 0.9 mole equivalent of formic acid. These periodic acid values indicate that the last secondary hydroxyl group is vicinal to another hydroxyl group and limit possibilities to C_2, C_6 or C_{11}. However, the formic acid determinations show that only one triol system is present in protoverine; thus C_2 is eliminated from consideration. (2) An argument for exclusion of C_{11} as a possible site for attachment of the last hydroxyl group can be made on the basis of the uptake of two mole equivalents of periodic acid by protoverine acetonide. However, this argument is subject to the objection that a 4,7-hemiketal structure is possible (though unlikely) for those compounds which have a free hydroxyl group at C_7 (*cf.* reference 21, footnote 27). The alternative 4,7-hemiketal 14,15-acetonide structure IX for protoverine acetonide would account for the facts presented to this point. This



IX

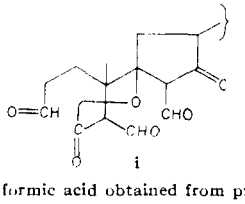
alternative was excluded on the basis of the following evidence. Treatment of isoprotoverine with acetone and hydriodic acid afforded isoprotoverine 6,7;14,15-diacetonide, which consumed 1.0 mole equivalent of periodate but only a negligible amount of lead tetraacetate. On acetylation with acetic anhydride–pyridine, the diacetonide gave a diacetate which was completely stable toward chromic acid. If the ninth oxygen atom were attached to C_{11}, the resulting isoprotoverine 9,11;14,15-diacetonide 3,16-diacetate would have contained a free chromic acid-sensitive secondary hydroxyl group at C_7.

The foregoing preliminary considerations served as a basis for the development of the *structure* (apart from configurational relationships) of protoverine represented by formulation I. Additional corroborative evidence for this structure as well as for the configuration indicated will now be discussed in the light of the proposed formulation.

The protoverine–isoprotoverine–pseudoprotoverine isomerizations are now to be formulated as I → X → XI. The pentaacetates formed by acetic anhydride–pyridine acetylation of protoverine and isoprotoverine are 3,6,7,15,16-pentaacetates (XII, XXVII). The hexaacetate formed by perchloric acid-catalyzed acetylation of protoverine is to be represented as the 3,4,6,7,15,16-hexaacetate XIII. The isopentaacetate derived by methanolysis is most reasonably formulated as the 16-alcohol XV (*cf.* reference 24), and the related ketone as XIV. The diosphenol derived from XIV clearly contains partial structure VII on the basis of its ultraviolet absorption characteristics.[24] Unfortunately, the product resisted all attempts at purification and, in the absence of proper analytical data, a full structural assignment is not possible.

The acetonide derivative of protoverine, as indicated earlier, is formulated as the 14,15-acetonide XVI. Protoverine acetonide monoisobutyrate is the 6-monoester XVII, and the product obtained by acid hydrolysis of XVII is protoverine 6-isobutyrate (XX). The product obtained by periodate oxidation of XVII is the aldehydo-γ-lactone XIX. The triacetate formed from the acetonide is formulated as XVIII. Hydrolysis of XVIII with dilute hydrochloric acid gave protoverine 3,6,16-

(23) The high value for formic acid over that expected from cleavage of the ring D triol is regarded as an artifact which arises during distillation of the volatile acid in the presence of mineral acid. The initial periodic acid product would have partial structure i. Partial acid cleavage of either β-carbonyl-aldehyde system (4,5,6, or 7,8,14) could readily yield formic acid. The normal value for formic acid obtained by oxidation of the 6-isobutyrate supports the view that the extra formic acid obtained from protoverine arises as a secondary result of cleavage of the 6,7-glycol system.



i

(24) S. M. Kupchan, THIS JOURNAL, **81**, 1921 (1959).

triacetate (XXII). Periodate oxidation of XXII followed by exposure of the initial product to dilute base gave the cyclopentenone aldehyde XXIII. Methanolysis of XVIII led to loss of the 16-acetate and afforded XXI. The oxidation of protoverine 14,15-acetonide 3,6,16-triacetate to 7-dehydroprotoverine 14,15-acetonide 3,6,16-triacetate is represented by XVIII → XXV. The hydrolysis product of XXV is 7-dehydroprotoverine 3,6,16-triacetate (XXVI).

Sodium borohydride reduction of XXV gave protoverine 14,15-acetonide 6,16-diacetate (XXIV) in good yield.[25] Acetylation of XXIV afforded XVIII. Periodate oxidation of XXIV afforded a crude product which showed aldehydo-γ-lactone absorption. To evaluate the degree of stereoselectivity of the borohydride reduction of the 7-ketone grouping, the residue after removal of XXIV was acetylated and a high yield of protoverine 14,15-acetonide 3,6,16-triacetate (XVIII) was obtained. Some stereochemical implications of the stereoselective reduction are discussed below.

The diacetonide formed by treatment of isoprotoverine with acetone and concentrated hydriodic acid is formulated as XXVIII. Acetylation of XXVIII afforded a diacetate stable to chromic acid; formulation XXIX represents isoprotoverine diacetonide diacetate.

Protoverine has seventeen asymmetric carbon atoms: C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{20}, C_{22} and C_{25}. The similarity of the optical rotatory dispersion curves of 7-dehydroprotoverine 14,15-acetonide 3,6,16-triacetate (XXV) and 7-dehydrogermine 14,15-acetonide 3,16-diacetate (XXXIV)[21] first indicated that the absolute configuration at C_{10} is the same in both compounds.[26] Consequently the absolute configuration at C_{10} in protoverine is the same as that in the naturally occurring steroids. The configurations at C_3, C_4, C_5 and C_9 are related to C_{10} as shown in formula I by the steric requirements of the isomerization sequence I → X → XI.

A tentative assignment of α-orientation to the hydroxyl group at C_7 follows from the behavior of

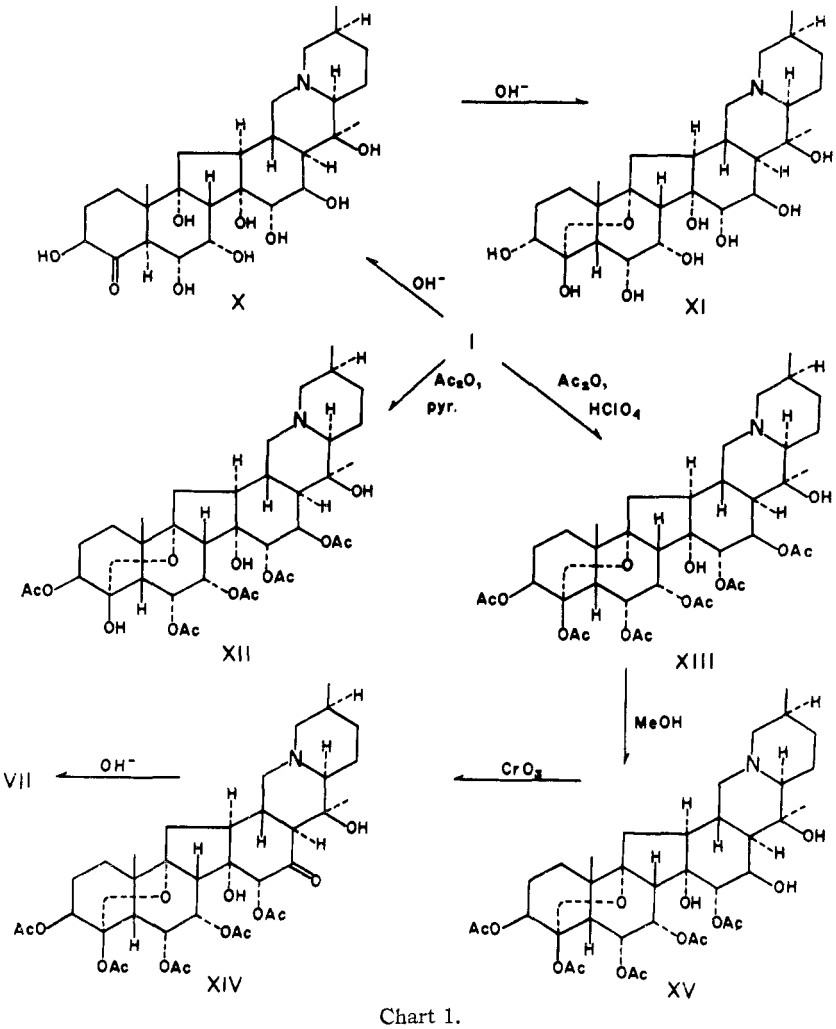


Chart 1.

TABLE I

PERIODATE OXIDATIONS

Substrate	Mole equivalents of sodium periodate consumed[a] (hr.)
Protoverine (I)	4.7(2.5)
Isoprotoverine (X)	4.1(2)
Protoverine 14,15-acetonide (XVI)	2.1(3.5)
Protoverine 14,15-acetonide 6-isobutyrate (XVII)	1.1(2)
Protoverine 6-isobutyrate (XX)	2.8(2.5)
Protoverine 3,6,16-triacetate (XXII)	0.9(1.5)
Protoverine 14,15-acetonide 6,16-diacetate (XXIV)	0.9(1.2)
7-Dehydroprotoverine 3,6,16-triacetate (XXVI)	1.8(1.5), 2.7(5), 4.8(24)
Isoprotoverine 6,7;14,15-diacetonide (XXVIII)	1.3(5)
Protoverine 14,15-acetonide 6-tosylate (XXX)	1.1(2)

[a] The last uptake recorded in each case is the one beyond which no significant change occurred on further standing.

(25) The selective cleavage of the acetyl group at C_3 during reduction with sodium borohydride is noteworthy. Earlier work has suggested a facilitation of attack of sodium borohydride upon a C_3-ester of an α,β-dihydroxy acid and the facilitation was attributed to an hydroxyl group of the acid moiety (S. M. Kupchan and C. I. Ayres, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 440 (1959)). The present observation suggests that attack of sodium borohydride upon an ester may be facilitated by a hydroxyl group (the β-oriented C_4-hemiketal hydroxyl in this case) bearing a 1,2-relationship to the ether oxygen of the ester.

(26) We thank Professor Carl Djerassi and Dr. E. J. Eisenbraun (Wayne State University) for the optical rotatory dispersion measurements and their interpretation.

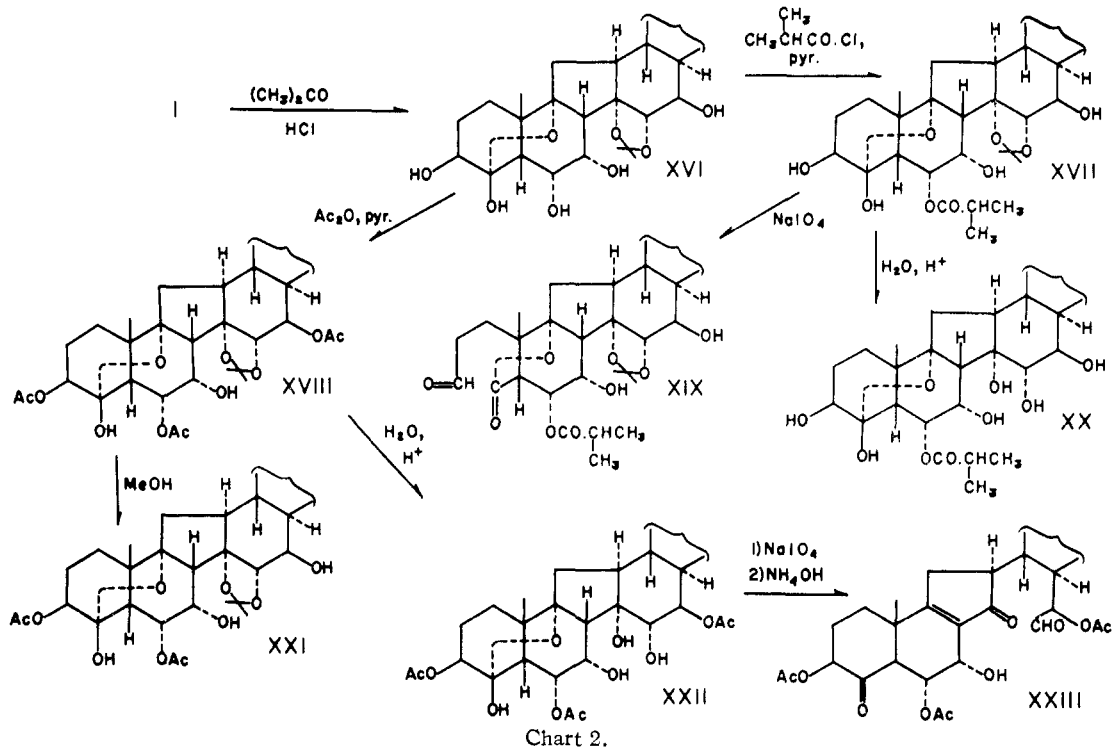


Chart 2.

protoverine toward acetylation. Acetylation of protoverine with acetic anhydride–pyridine (conditions which lead to acetylation of the C_4-hemiketal hydroxyl in veracevine[18,20]) afforded protoverine 3,6,7,15,16-pentaacetate (XII). The sluggishness of acetylation of the C_4-hydroxyl is consistent with the view that acetylation of the C_7-α-hydroxyl group proceeds rapidly, and that the resulting 7-α-acetoxy group hinders reaction of the C_4-hydroxyl group (as in germine[21]). Acetylation in the presence of perchloric acid apparently overcomes this hindrance and yields protoverine 3,4,6,7,15,16-hexaacetate (XIII) (*cf.* reference 21). Formation of a 6,7-acetonide derivative of isoprotoverine (XXVIII) requires that the C_6-hydroxyl group be oriented *cis* to the C_7-hydroxyl group, and the α-configuration is therefore assigned to the C_6-hydroxyl group.

TABLE II

PERIODIC ACID OXIDATIONS

Substrate	Mole equivalents of HIO_4 consumed[a] (hr.)
Protoverine (I)	4.0(3)
Isoprotoverine (X)	3.9(3)
Protoverine 14,15-acetonide (XVI)	1.8(2)
Protoverine 6-isobutyrate (XX)	2.8(2.5)

[a] The last uptake recorded in each case is the one beyond which no significant change occurred on further standing.

TABLE III

CHROMIC ACID TITRATIONS

Substrate	Oxygen equivalents of chromic acid consumed[a] (hr.)
Protoverine 3,6,7,15,16-pentaacetate (XII)	0.1(2)
Protoverine 14,15-acetonide 3,6,16-triacetate (XVIII)	0.7(1), 0.9(2.5)
Isoprotoverine 6,7;14,15-diacetonide 3,16-diacetate (XXIX)	0.0(1), 0.1(2), 0.3(24)

[a] The last uptake recorded in each case is the one beyond which no significant change occurred on further standing.

Assignment of the α-orientation to the hydroxyl groups at C_{14} and C_{15} can be made on the basis of the inertness of the C_7-hydroxyl in protoverine 14,15-acetonide to acetylation (see Chart 2, XVI → XVIII). Examination of the molecular model indicates that the hindrance to acetylation of the C_7-α-hydroxyl group is explicable on the basis of an α-oriented 14,15-isopropylidene grouping in a B/C *trans* (*i.e.*, C_8-β-H) system. The failure of the disecondary glycol system at C_{15},C_{16} to form an acetonide indicates that these groups are *trans*, *i.e.*, that the hydroxyl at C_{16} is β-oriented. The C_{16}-acetate in the protoverine series is readily methanolized (*e.g.*, XVIII → XXI) and this is attributed to facilitation by a hydroxyl group bearing a *cis*-1,3-diaxial relationship to the ester groups (*cf.* references 21 and 27). This requires a β-(axial)-orientation of the C_{20}-hydroxyl. Furthermore, the *cis*-1,3-diaxial disposition of the C_{16}- and C_{20}-hydroxyl groups requires, in turn, that rings D and E be *trans* fused, *i.e.*, that the hydrogen at C_{13} be β-oriented and the hydrogen at C_{17} α-oriented.

Of the fourteen asymmetric carbon atoms of protoverine discussed up to this point, thirteen (C_3, C_4, C_5, C_7, C_8, C_9, C_{10}, C_{13}, C_{14}, C_{15}, C_{17}, C_{20}) are present in the germine molecule. *In every case*, the configurations at the common asymmetric carbon atoms are identical. It was deemed likely indeed that the configurations at C_{12}, C_{22} and C_{25}, the remaining common asymmetric carbon atoms, are also alike. Nevertheless it was considered

(27) S. M. Kupchan, W. S. Johnson and S. Rajagopalan, *Tetrahedron*, **7**, 47 (1959).

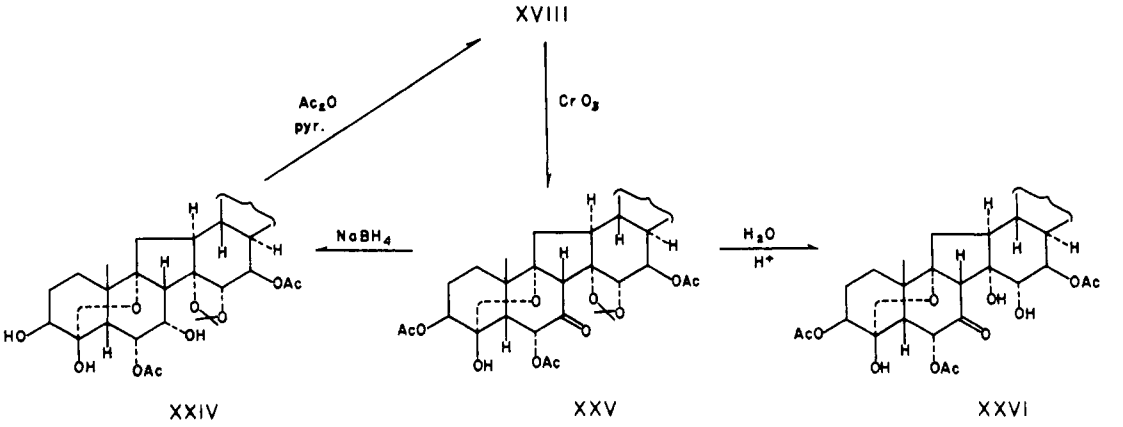


Chart 3.

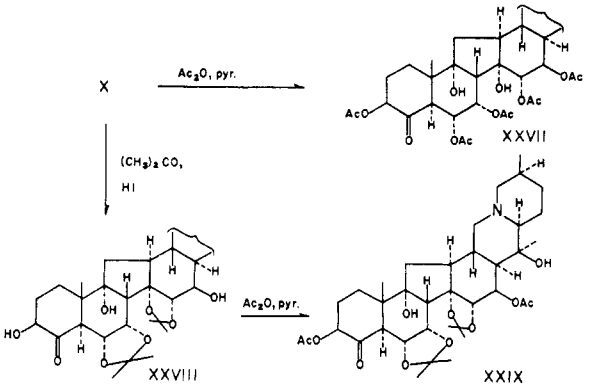


Chart 4.

desirable to seek conclusive evidence for the strongly-supported thesis that protoverine possesses structure and configuration I.

Proof that protoverine is 6-α-hydroxygermine was sought by attempted interrelation of protoverine with germine. Attempted elimination of the C_6-hydroxyl group by mild alkaline treatment of isoprotoverine or its derivatives was unsuccessful; more vigorous alkaline treatment gave intractable mixtures. The readiness with which sulfonic esters undergo fission of the C–O bond suggested their use in this degradation. Tosylation of protoverine 14,15-acetonide (XVI) afforded protoverine 14,15-acetonide 6-tosylate (XXX) in good yield. This structure was assigned on the basis that the tosylate consumed 1.1 mole equivalents of sodium periodate and that the oxidation product showed aldehydo-γ-lactone absorption. The tosyl group proved to be extraordinarily unreactive; treatment with sodium iodide in acetonitrile, sodium methoxide in methanol, or Raney nickel and hydrogen left the tosylate unchanged. Reactions with lithium aluminum hydride and sodium acetate in hot acetic acid afforded intractable mixtures. Protoverine 3,7,-15,16-tetraacetate 6-tosylate (XXXIII), prepared by acid hydrolysis of XXXI followed by acetylation, likewise was inert toward sodium iodide in acetonitrile or to Raney nickel in alcohol; an intractable mixture was obtained upon reaction with sodium acetate in acetic acid. The 6-tosylate 14,15-acetonide 3,16-diacetate (XXXI) showed similar behavior. The 6-tosylate 7-ketone XXXII, prepared by chromic acid oxidation of XXXI, was largely unaffected by zinc and hot acetic acid, zinc and hot toluene, zinc and hot acetic anhydride, or sodium iodide in acetonitrile at room temperature. Intractable tars were obtained upon treatment with sodium iodide in hot acetonitrile or with sodium acetate in hot acetic acid. The stability of the tosyl group is more readily compatible with a 6-α-(equatorial)-tosylate structure than the alternative 6-β-epimer formulation.

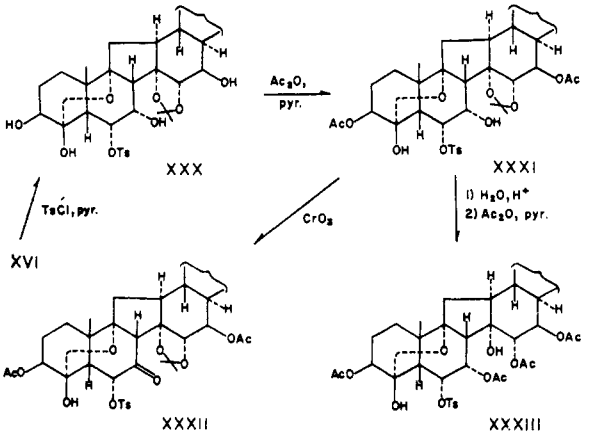
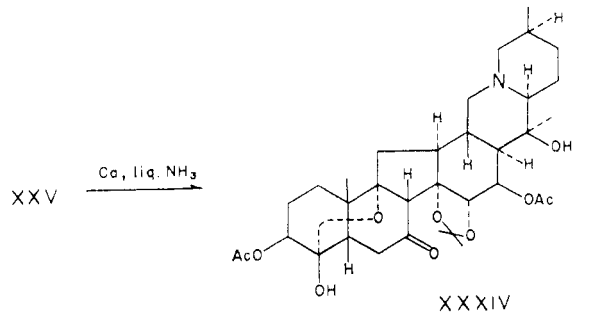


Chart 5.

Successful interrelation of protoverine with germine finally was accomplished by treatment of 7-dehydroprotoverine 14,15-acetonide 3,6,16-triacetate (XXV) in tetrahydrofuran with calcium in liquid ammonia (*cf.* reference 28) whereby the known[21] 7-dehydrogermine 14,15-acetonide 3,16-diacetate (XXXIV) was obtained. The yield of desired product apparently was limited by elimination reactions of the β-hydroxyketones as well as de-acetylation. Re-acetylation of the crude reaction mixture was precluded by the instability of the ketones toward acylation conditions. The configurations at C_3, C_4, C_5, C_9, C_{10}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{20}, C_{22} and C_{25} were thereby proved to be the same as those at the corresponding carbon atoms in germine. In the light of this interrelation, an elaboration of the configuration of protoverine may now be completed. The hindrance toward acetylation of the C_7-hydroxyl group by the

(28) J. H. Chapman, J. Elks, G. H. Phillipps and L. H. Wyman, *J. Chem. Soc.*, 4344 (1956).



now definitely assigned α-oriented 14,15-isopropylidene grouping (XVI → XVIII) is uniquely explicable on the basis of a C_8-β-hydrogen (as in all natural steroids), C_7-α-hydroxyl configuration. Strong support for this assignment at C_7 follows from the stereoselective sodium borohydride reduction of 7-dehydroprotoverine 14,15-acetonide 3,6,-16-triacetate (XXV). An inspection of the molecular model of this ketone clearly shows that the β-is much less hindered than the α-face for the approach of the borohydride, which suggests that reaction would proceed to give an α-oriented hydroxyl group.[29] It has been shown already that the C_6- and C_7-hydroxyls bear a *cis* relationship and consequently protoverine is assigned the 6-α-hydroxygermine structure and configuration (I).

Acknowledgment.—We gratefully acknowledge the generous assistance of Dr. Harold A. Nash, Pitman–Moore Co., Indianapolis 6, Ind., in supplying protoveratrine for the preparation of protoverine used in this work.

Experimental[30]

Protoverine (I).—Protoveratrine (A and B,[22] 20 g.) was suspended in absolute methanol (35 ml.) and cooled to 0°. A previously cooled (0–4°) methanolic sodium hydroxide solution (1 *N*, 160 ml.) then was added portionwise under continuous stirring. During the addition, the temperature was maintained at 0–3°. The pale yellow solution was allowed to stand at 0° for 20 hours.

Aqueous sulfuric acid (10%) then was added to bring the *p*H to 7, most of the methanol was removed under reduced pressure, the residue was diluted with water to a volume of *ca.* 120 ml. and the mixture was made alkaline (*p*H 8–8.5) with 10% aqueous sodium carbonate. The mixture was continuously extracted with chloroform as rapidly as possible. At intervals of 30 minutes, the *p*H was restored to 8–8.5 by addition of 10% aqueous sodium hydroxide. Protoverine (10–11 g.) separated from the cooled chloroform extract as long white needles, m.p. 190–195° dec. Recrystallization from methanol afforded needles (9–10 g.), m.p. 195–200° dec., $[\alpha]^{25}D$ −12° (*c* 1.00, pyr.).

Sodium Periodate Titrations.—Between 25 and 30 mg. of substrate was dissolved in 5% acetic acid (2 ml.) and 0.08 *M* aqueous sodium periodate (5 ml.) and water (3 ml.) were added. Two-milliliter aliquots were withdrawn at appropriate time intervals and titrated by the procedure described by Jackson ("Organic Reactions," Vol. II, p. 361). Several compounds also were titrated with periodic acid using the procedure described in an earlier paper.[21]

Chromic Acid Titrations.—Between 25 and 30 mg. of substrate was dissolved in acetic acid (5 ml.) and treated with 0.065 *N* chromium trioxide in 99.8% acetic acid. Two-milliliter aliquots were withdrawn at appropriate time intervals, treated with 10% potassium iodide solution (5 ml.) and the liberated iodine was titrated with 0.01 *N* sodium thiosulfate solution in the usual manner.

Protoverine 14,15-Acetonide 6-Isobutyrate (XVII).—A solution of protoverine 14,15-acetonide hydrochloride[8] (7.25 g.), m.p. 259–260° dec., in pyridine (90 ml.) was cooled to −10°, treated with isobutyryl chloride (2.2 ml.), allowed to stand at room temperature for 12 hours and finally warmed on a water-bath at 50° for 2 hours. The cooled solution was basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated until a volume of 50 ml. was obtained. On cooling, starting material (4.9 g.) crystallized out and was removed by filtration. The filtrate was evaporated to yield a resin. A paper chromatogram[31a] indicated the presence of three new compounds. The resin was chromatographed on Merck acid-washed alumina (50 g.). The column yielded to 80% chloroform–benzene a solid which was crystallized from acetone–petroleum ether as large prisms (50 mg.), m.p. 231–233° dec., $[\alpha]^{26}D$ +24° (*c* 1.20, pyr.).

Anal. Calcd. for protoverine 14,15-acetonide 3,6-diisobutyrate, $C_{38}H_{59}O_{11}N$: C, 64.65; H, 8.42; isobutyryl, 22.95. Found: C, 64.58; H, 8.52; isobutyryl, 19.86.

The column yielded to chloroform and to 1% methanol–chloroform a solid which was crystallized from acetone–petroleum ether as fine needles (110 mg.), m.p. 270–271° dec. Recrystallization from acetone–petroleum ether gave needles (100 mg.), m.p. 270–271° dec., $[\alpha]^{21}D$ −2° (*c* 1.00, pyr.).

Anal. Calcd. for protoverine 14,15-acetonide 6-isobutyrate, $C_{34}H_{53}O_{10}N$: C, 64.23; H, 8.41; isobutyryl, 12.74. Found: C, 64.91; H, 8.45; isobutyryl, 12.75.

A 10-mg. sample of this compound was oxidized with sodium periodate under conditions described for a sodium periodate titration. After 2 hours, the solution was basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform solution was dried over anhydrous sodium sulfate and evaporated. The amorphous residue (5 mg.) showed absorption at 3.65, 5.62 and 5.80μ.

Protoverine 3,6,7,15-Pentaacetate (XII).[32]—Protoverine (3 g.), m.p. 195–200° dec., was treated with pyridine (19 ml.) and acetic anhydride (68 ml.) and warmed on a steam-bath for 2.5 hours. After cooling, the excess of the anhydride was decomposed by the cautious addition of methanol (30 ml.). The solution was evaporated to dryness *in vacuo*, the residue was dissolved in water, basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was crystallized from acetone–petroleum ether as discolored prisms (2.4 g.), m.p. 259–260° dec., $[\alpha]^{25}D$ −52° (*c* 1.50, pyr.). The prisms were dissolved in chloroform and chromatographically filtered through a column of Merck acid-washed alumina (1 g.). The chloroform solution was evaporated and the residue was crystallized from acetone–petroleum ether as colorless fine needles (1.5 g.), m.p. 257–258° dec., $[\alpha]^{27}D$ −53° (*c* 1.60, pyr.).

Anal. Calcd. for $C_{37}H_{53}O_{14}N$: C, 60.39; H, 7.26; acetyl, 29.25. Found: C, 60.64; H, 7.36; acetyl, 29.08.

Protoverine 3,4,6,7,15,16-Hexaacetate (XIII).[32]—Protoverine (5 g.), m.p. 195–200° dec., was treated at −10° with acetic anhydride (50 ml.) and 60% perchloric acid (1.5 ml.). After standing for 1 hour, a clear solution was obtained. After a further 30 minutes, the excess of the anhydride was decomposed by the cautious addition of methanol (50 ml.) and the solution was evaporated to dryness under reduced pressure. The residue was dissolved in water, basified with dilute ammonium hydroxide and extracted with chloroform.

(29) *Cf.* W. G. Dauben, G. F. Fonken and D. S. Noyce, THIS JOURNAL, **78**, 2579 (1956).

(30) 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 unless otherwise specified. Infrared spectra were determined on a Baird model B double beam infrared recording spectrophotometer and chloroform was used as a solvent. Microanalyses were carried out by Dr. S. M. Nagy and his associates at the Massachusetts Institute of Technology on samples dried at reduced pressure at 110°. "Petroleum ether" refers to the fraction of boiling point 60–80°.

(31) The solvent systems used in the paper chromatographic work were those of J. Levine and H. Fischbach, *J. Am. Pharm. Assoc., Sci. Ed.*, **44**, 543 (1955); (a) *n*-butyl acetate–1-butanol–formic acid (25:5:1 by volume); (b) the solution prepared by adding 1 ml. of formic acid to the separated solvent layer of the system *n*-butyl acetate–1-butanol–water (10:25:10 ml.).

(32) We thank Dr. C. R. Narayanan for the first preparation of this compound.

The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was crystallized from ether as prisms (2.1 g.), m.p. 275–276° dec. Two recrystallizations from acetone–petroleum ether gave clusters of colorless hexagonal prisms (1.14 g.), m.p. 281–282° dec., $[\alpha]^{25}D$ −72° (*c* 1.10, pyr.).

Anal. Calcd. for $C_{39}H_{55}O_{15}N$: C, 60.21; H, 7.13; acetyl, 33.20. Found: C, 60.17; H, 7.31; acetyl, 32.67.

Isoprotoverine 3,6,7,15,16-Pentaacetate (XXVII).—Isoprotoverine, 1 g., m.p. 247–248° dec., was treated with pyridine (20 ml.) and acetic anhydride (25 ml.) and warmed on a steam-bath for 2 hours. Methanol (25 ml.) was added to the cooled solution to decompose the excess of the anhydride and the solution was evaporated under reduced pressure nearly to dryness. The residue was dissolved in water, basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was crystallized from acetone–ether as needles, 0.75 g., m.p. 187–192° dec. Recrystallization from acetone–ether gave needles, 0.45 g., m.p. 191° dec. The compound was recrystallized for analysis from acetone–ether as long colorless needles, 0.30 g., m.p. 191° dec., $[\alpha]^{24}D$ −67° (*c* 1.53, pyr.).

Anal. Calcd. for $C_{37}H_{53}O_{14}N$: C, 60.39; H, 7.26; acetyl, 29.25. Found: C, 60.25; H, 7.63; acetyl, 29.25.

Protoverine 6-Isobutyrate (XX).—Protoverine 14,15-acetonide 6-isobutyrate, 300 mg., m.p. 270–271° dec., was dissolved in 2% hydrochloric acid (20 ml.) and allowed to stand at room temperature for 16 hours. The cooled solution was basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was crystallized from acetone–petroleum ether as needles, 243 mg., m.p. 219–221° dec. A paper chromatogram[31b] indicated that the compound was homogeneous. The compound was recrystallized from acetone–petroleum ether as long, fine needles, 200 mg., m.p. 219–221° dec., $[\alpha]^{23}D$ −37° (*c* 1.00, pyr.).

Anal. Calcd. for $C_{31}H_{49}O_{10}N \cdot 2H_2O$: C, 58.93; H, 8.45; isobutyryl, 11.25. Found: C, 59.04; H, 8.29; isobutyryl, 12.26.

Formic Acid Determinations.[33]—The alkaloid (250 mg.) was dissolved in 0.05 *M* aqueous periodic acid (60 ml.) and allowed to stand at room temperature for 3 hours. An excess of 5% aqueous barium hydroxide solution was added and the precipitate was removed by filtration and washed well with water. The combined filtrates (*ca.* 200 ml). were evaporated until a volume of 100 ml. was obtained. The solution was acidified with 10% phosphoric acid, using methyl blue as an indicator, and distilled almost to dryness *in vacuo*. Water (40 ml.) was added and the solution was again distilled almost to dryness *in vacuo*. This operation was repeated until the distillate virtually did not consume 0.1 *N* sodium hydroxide. The number of mole equivalents of formic acid was calculated from the total titration value of the combined distillates.

The combined distillates were made alkaline by addition of 0.1 *N* sodium hydroxide (10 ml.) and the volume was reduced to *ca.* 75 ml. by evaporation under reduced pressure. The solution was treated with saturated aqueous sodium acetate (5 ml.), 4 *N* hydrochloric acid (1 ml.) and saturated aqueous mercuric chloride (15 ml.) and warmed on a steam-bath, in the dark, for 3 hours. After cooling, the calomel precipitate was collected.

The results are shown in Table IV.

TABLE IV

Substrate	Mole equivalents of formic acid (a) from titration	(b) from calomel ppt.
Protoverine (I)	1.3, 1.3, 1.4	1.0, 1.2, 1.3
Protoverine 14,15-acetonide (XVI)	0.1, 0.1	0.0, 0.0
Protoverine 6-isobutyrate (XX)	0.9	0.9

Protoverine 14,15-Acetonide 3,6,16-Triacetate (XVIII).[32]—Protoverine 14,15-acetonide hydrochloride,[8] 5 g., m.p. 259–263° dec., was treated with pyridine (30 ml.) and acetic anhydride (50 ml.) and the solution was warmed on a water-bath at 85° for 3 hours. Methanol (60 ml.) was added to the cooled solution to decompose the excess of the anhydride, and the solution was evaporated nearly to dryness under reduced pressure, basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was crystallized from acetone–petroleum ether as prisms, 3.3 g., m.p. 261–262° dec., $[\alpha]^{25}D$ +21° (*c* 1.97, pyr.). A second crop of prisms, 400 mg., m.p. 260–262° dec., also was obtained.

Anal. Calcd. for $C_{36}H_{53}O_{12}N$: C, 62.51; H, 7.72; acetyl, 18.67. Found: C, 62.27; H, 7.89; acetyl, 18.84.

7-Dehydroprotoverine 14,15-Acetonide 3,6,16-Triacetate (XXV).—A solution of protoverine 14,15-acetonide 3,6,16-triacetate (3.5 g.), m.p. 261–262° dec., in acetic acid (40 ml.) was treated with 0.66 *N* chromium trioxide in 98.5% acetic acid (65 ml.). The reaction mixture was allowed to stand at room temperature for 2.5 hours, cooled in ice, aqueous sodium bisulfite added to destroy the excess of the oxidizing agent, basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin. A paper chromatogram[34] indicated the formation of a new material in a yield of approximately 60%. The resin was chromatographed on Merck acid-washed alumina (70 g.). The column yielded to 75% chloroform–benzene, a yellow oil; to chloroform, a resin which was crystallized from acetone–petroleum ether as colorless rods (1.65 g.), m.p. 256–257° dec. A second crop of rods, 200 mg., m.p. 256–258° dec., was also obtained. A paper chromatogram[34] indicated that the material was homogeneous. A sample was recrystallized for analysis from acetone–petroleum ether as rods, m.p. 261–262° dec., $[\alpha]^{25}D$ −32° (*c* 1.89, pyr.).

Anal. Calcd. for $C_{36}H_{51}O_{12}N$: C, 62.68; H, 7.45; acetyl, 18.72. Found: C, 62.19; H, 7.41; acetyl, 17.39.

Protoverine 3,6,16-Triacetate (XXII).—Protoverine 14,15-acetonide 3,6,16-triacetate, 8.5 g., m.p. 261–262° dec., was dissolved in a mixture of concentrated hydrochloric acid (6 ml.) and water (200 ml.). The solution was allowed to stand at room temperature for 15 hours, basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was chromatographed on Merck acid washed alumina (100 g.). The column yielded to 75% chloroform–benzene a small amount of resin; to chloroform and to 1% methanol–chloroform, a resin which was crystallized from acetone–petroleum ether as needles, 4.5 g., m.p. 236–238° dec. A paper chromatogram[31b] indicated that the material was homogeneous. A sample was recrystallized for analysis from acetone–petroleum ether as needles, m.p. 236–238° dec., $[\alpha]^{25}D$ −4° (*c* 1.00, pyr.).

Anal. Calcd. for $C_{33}H_{49}O_{12}N$: C, 60.72; H, 7.57; acetyl, 19.78. Found: C, 60.67; H, 7.70; acetyl, 20.60.

Sodium Periodate Oxidation of Protoverine 3,6,16-Triacetate.—A solution of protoverine 3,6,16-triacetate, 100 mg., m.p. 236–238° dec., in 5% acetic acid (5 ml.) was treated with 0.08 *M* aqueous sodium periodate solution. The clear solution was allowed to stand at room temperature for 1.5 hours, was basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a white solid. The solid was dissolved in ethanol (10 ml.), 10 drops of concentrated ammonia was added and the solution allowed to stand at room temperature for 20 minutes. The solution was diluted with water and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield an amorphous material (88 mg., XXIII) which showed λ_{max} 234 mμ (ε 9200); λ_{max} 2.90(s), 5.78–5.85(s), 5.92(s), 6.05(m) μ.

7-Dehydroprotoverine 3,6,16-Triacetate (XXVI).—7-Dehydroprotoverine 14,15-acetonide 3,6,16-triacetate, 1 g., m.p. 261–262° dec., was suspended in a mixture of acetic acid (2 ml.), water (20 ml.) and concentrated hydrochloric acid (4 ml.). After shaking vigorously for 45 minutes, a clear solution was obtained which was allowed to stand at room

(33) J. R. Dyer in David Glick, "Methods of Biochemical Analysis," Vol. III, Interscience Publishers, Inc., New York, N. Y., 1956, pp. 130–131.

(34) The paper chromatographic system used was that of J. Levine and H. Fischbach, *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 191 (1957); ethylene chloride–Cellosolve acetate–pyridine (15:10:1 by volume).

temperature for 3 hours. The solution was cooled in ice, carefully brought to *p*H 8 with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was chromatographed on Merck acid-washed alumina (20 g.). The column yielded to benzene and to ether, a series of oils; to chloroform, a trace of white solid. The column yielded to 3% methanol–chloroform, a solid which was crystallized from ether as needles, 170 mg., m.p. 215–216° dec. A sample was recrystallized for analysis from ether as colorless, long needles, m.p. 217° dec., $[\alpha]^{25}D$ −46° (*c* 1.02, pyr.). A paper chromatogram[31b] indicated that the compound was homogeneous.

Anal. Calcd. for $C_{33}H_{47}O_{12}N$: C, 61.01; H, 7.29; acetyl, 19.41. Found: C, 60.53; H, 7.39; acetyl, 19.63.

A further quantity, 125 mg., m.p. 215–216° dec., of this compound was obtained when the column was eluted with 10% methanol–chloroform.

Protoverine 3,4,6,7,15-Pentaacetate (XV).—Protoverine 3,4,6,7,15,16-hexaacetate, 5 g., m.p. 275–276° dec., was dissolved in a mixture of methanol (200 ml.) and water (20 ml.) and allowed to stand at room temperature for 20 hours. The methanol was removed by evaporation under reduced pressure, the residue basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was crystallized from acetone as needles, 1.9 g., m.p. 253–254° dec. A second crop of needles, 0.6 g., m.p. 250–254° dec., was also obtained. The crystalline material was combined and chromatographed on Merck acid-washed alumina (50 g.). The main fraction of the material was eluted with 2% methanol–chloroform and crystallized from acetone as needles, 1.5 g., m.p. 257–258° dec. Recrystallization from acetone gave colorless needles, 0.8 g., m.p. 259–260° dec., $[\alpha]^{25}D$ −65° (*c* 2.01, pyr.).

Anal. Calcd. for $C_{37}H_{53}O_{14}N$: C, 60.39; H, 7.26; acetyl, 29.25. Found: C, 60.35; H, 7.33; acetyl, 28.90.

16-Dehydroprotoverine 3,4,6,7,15-Pentaacetate (XIV).—Protoverine 3,4,6,7,15-pentaacetate (2 g.), m.p. 256–257° dec., was dissolved in a mixture of acetic acid (2.5 ml.) and carbon tetrachloride (47.5 ml.) and treated with 0.66 *N* chromium trioxide in 98.5% acetic acid (50 ml.). The reaction mixture was allowed to stand at room temperature for 17 hours, cooled in ice, the excess of the oxidizing agent was destroyed by addition of aqueous sodium bisulfite, basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was crystallized from ethanol–water as needles, 0.9 g., m.p. 187° dec. The material was recrystallized from methanol as silky needles, 250 mg., m.p. 194–195° dec., $[\alpha]^{25}D$ −128° (*c* 1.64, pyr.).

Anal. Calcd. for $C_{37}H_{51}O_{14}N$: C, 60.56; H, 7.01; acetyl, 29.32. Found: C, 60.13; H, 7.05; acetyl, 29.04.

Alkaline Treatment of 16-Dehydroprotoverine 3,4,6,7,15-Pentaacetate.—A solution of 16-dehydroprotoverine 3,4,6,7,-15-pentaacetate (200 mg., m.p. 194–195° dec.) in methanol (25 ml.) was treated with 50% aqueous sodium hydroxide solution (0.4 ml.) and heated under reflux for 10 minutes. The cooled solution was acidified with acetic acid and evaporated nearly to dryness under reduced pressure. The residue was basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated. The residue was taken up in ether (50 ml.) and a small amount of dark impurity was removed by filtration. The ether solution was evaporated to yield a tan product (45 mg.) which showed λ_{max} 330 mμ (ε 17700), $\lambda_{max}^{0.01\ N\ NaOH}$ 384 mμ (ε 11600).

Isoprotoverine 6,7;14,15-Diacetonide (XXVIII).—Isoprotoverine (5 g., m.p. 252–253° dec.) was treated with ethanol (50 ml.), acetone (250 ml.) and hydriodic acid (5 ml.). The reaction mixture was vigorously shaken for 3 hours and a clear solution was obtained which was allowed to stand overnight at room temperature. The solution was evaporated under reduced pressure until a volume of *ca.* 10 ml. was obtained. This residue was treated with crushed ice, basified with dilute ammonium hydroxide, decolorized with aqueous sodium thiosulfate solution and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was crystallized from methanol as colorless prismatic needles, 3.6 g., m.p. 246–247° dec. A paper chromatogram[31a] indicated that the compound was homogeneous. A sample was recrystallized for analysis from methanol as long needles, m.p. 246–247° dec., $[\alpha]^{22}D$ −20° (*c* 0.99, pyr.).

Anal. Calcd. for $C_{33}H_{51}O_9N \cdot {}^1/_2CH_3OH$: C, 64.69; H, 8.59. Found: C, 64.65; H, 8.80.

This compound was titrated[35] with lead tetraacetate and consumed, after 90 minutes, 0.18 mole equivalent of the oxidizing agent.

Isoprotoverine 6,7;14,15-Diacetonide 3,16-Diacetate (XXIX).—A solution of isoprotoverine 6,7;14,15-diacetonide (1.6 g. m.p. 246–247° dec.) in pyridine (3 ml.) was treated with acetic anhydride (6 ml.) and warmed on a steam-bath for 90 minutes. The solution was cooled in ice, basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was crystallized from benzene as colorless needles, 810 mg., m.p. 299–300° dec. A second crop of needles, 496 mg., m.p. 299–300° dec., was also obtained. A sample was recrystallized for analysis from benzene as needles, m.p. 300–301° dec., $[\alpha]^{22}D$ −31° (*c* 1.25, pyr.).

Anal. Calcd. for $C_{37}H_{55}O_{11}N$: C, 64.43; H, 8.04; acetyl, 12.19. Found: C, 64.74; H, 8.42; acetyl, 11.61.

Attempted Chromic Acid Oxidation of Isoprotoverine 6,7;14,15-Diacetonide 3,16-Diacetate.—A solution of isoprotoverine 6,7;14,15-diacetonide 3,16-diacetate (400 mg., m.p. 299–300° dec.) in acetic acid (3 ml.) was treated with 0.66 *N* chromium trioxide in 98.5% acetic acid (7 ml.) and was allowed to stand at room temperature for 2 hours. The reaction mixture was cooled in ice, the excess of the oxidizing agent destroyed by addition of aqueous sodium bisulfite solution, basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin. A paper chromatogram[31a] showed only the presence of starting material. The resin was crystallized from benzene–petroleum ether as needles, 252 mg., m.p. 299–301° dec. The infrared spectrum was identical with that of starting material.

In a repeat experiment where the time of oxidation was increased to 20 hours, a paper chromatogram[31a] of the crude reaction product again showed only the presence of starting material. The recovery of crystalline starting material was 55%.

Sodium Borohydride Reduction of 7-Dehydroprotoverine 14,15-Acetonide 3,6,16-Triacetate.—Sodium borohydride (45 mg.) was added to a solution of 7-dehydroprotoverine 14,15-acetonide 3,6,16-triacetate (150 mg., m.p. 261–262° dec.) in *t*-butyl alcohol (45 ml.). The mixture was allowed to stand, with occasional shaking, at room temperature for 90 minutes during which time most of the borohydride dissolved. Water (1.5 ml.) was added to obtain a homogeneous solution which was allowed to stand at room temperature for 10 minutes. The solution was acidified with acetic acid and evaporated nearly to dryness under reduced pressure. The residue was dissolved in water, basified with dilute ammonium hydroxide and extracted thoroughly with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was chromatographed on Merck acid-washed alumina (3 g.). The column yielded to chloroform an oil (15 mg.); to 0.5% methanol–chloroform and to 1% methanol–chloroform, a white solid (98 mg.). The solid material, which appeared to be homogeneous on a paper chromatogram,[34] was crystallized from acetone–petroleum ether as needles, 75 mg., m.p. 229–230° dec., $[\alpha]^{25}D$ +4° (*c* 0.95, pyr.).

Anal. Calcd. for protoverine 14,15-acetonide 6,16-diacetate (XXIV) monohydrate $C_{31}H_{51}O_{11}N \cdot H_2O$: C, 61.15; H, 8.00; acetyl, 12.85. Found: C, 61.54; H, 8.16; acetyl, 12.62.

This compound on oxidation with sodium periodate, as described for the oxidation of XVII, gave an amorphous product which showed absorption at 3.65, 5.62 and 5.80 μ.

Further solution of the column with 2% methanol–chloroform and with 5% methanol–chloroform, afforded mixtures (total yield 30 mg.) as shown by paper chromatography.[34]

All the non-crystalline material (64 mg.) was combined and treated with pyridine (1 ml.) and acetic anhydride (1 ml.) and heated on a water-bath at 85° for 90 minutes. The solution was cooled, basified with dilute ammonium hydroxide

(35) The titration was performed as described in ref. 27.

and extracted with chloroform. The chloroform solution was dried over anhydrous sodium sulfate and evaporated to yield a resin (60 mg.). The resin was chromatographed on Merck acid-washed alumina (2 g.). The column yielded to 75% chloroform–benzene, a trace of yellow oil; to chloroform, a white solid (49 mg.) which was crystallized from acetone–petroleum ether as colorless prisms, 41 mg., m.p. 256–258° dec. The melting point was not depressed on admixture with a sample of protoverine 14,15-acetonide 3,6,16-triacetate. The infrared spectra and paper chromatographic[34] behavior of the respective samples were identical.

Acetylation of Protoverine 14,15-Acetonide 6,16-Diacetate.—Protoverine 14,15-acetonide 6,16-diacetate (150 mg., m.p. 229–230° dec.) was treated with pyridine (1 ml.) and acetic anhydride (1 ml.) and warmed on a water-bath at 80° for 2 hours. The cooled solution was basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was chromatographed on Merck acid-washed alumina (7 g.). The column yielded to 75% chloroform–benzene, a yellow oil; to chloroform, a white solid (30 mg.); to 1% methanol–chloroform, a white solid (100 mg.). The solid material was combined and crystallized from acetone–petroleum ether as colorless prisms, 111 mg., m.p. 258–260° dec. The melting point was not depressed on admixture with a sample of protoverine 14,15-acetonide 3,6,16-triacetate. The infrared spectra and paper chromatographic[34] behavior of the respective samples were identical.

Optical Rotatory Dispersion Studies.[26]—(a) 7-Dehydroprotoverine 14,15-acetonide 3,6,16-triacetate (XXV) showed R.D. in dioxane (*c* 0.125): $[\alpha]_{700}$ +3°, $[\alpha]_{550}$ −18°, $[\alpha]_{425}$ −13°, $[\alpha]_{350}$ −10°, $[\alpha]_{340}$ +3°, $[\alpha]_{310}$ +85°, $[\alpha]_{285}$ −200°. (b) 7-Dehydrogermine 14,15-acetonide 3,16-diacetate (XXXIV) showed R.D. in dioxane (*c* 0.090); $[\alpha]_{700}$ −16°, $[\alpha]_{525}$ −44°, $[\alpha]_{450}$ −35°, $[\alpha]_{425}$ −20°, $[\alpha]_{350}$ −22°, $[\alpha]_{340}$ −13°, $[\alpha]_{294}$ +18°, $[\alpha]_{284}$ −8°.

Protoverine 14,15-Acetonide 3,6-Diacetate (XXI).—A solution of protoverine 14,15-acetonide 3,6,16-triacetate (3 g., m.p. 261–262° dec.) in methanol (75 ml). was allowed to stand at room temperature for 10 hours. The methanol was evaporated under reduced pressure and the residue was crystallized from ether as prisms, 1.8 g., m.p. 240–241° dec. A second crop of prisms, 212 mg., m.p. 246–248° dec., was also obtained. The crystalline material was combined, dissolved in chloroforom and chromatographically filtered through Merck acid-washed alumina (2 g.). The chloroform solution was evaporated and the residue was crystallized from acetone–ether as colorless prisms, 640 mg., m.p. 257–259° dec., $[\alpha]^{27}D$ +26° (*c* 1.20, pyr.).

Anal. Calcd. for $C_{34}H_{57}O_{11}N$: C, 62.84; H, 7.91; acetyl, 13.25. Found: C, 62.99; H, 8.05; acetyl, 12.89.

Protoverine 14,15-Acetonide 6-Tosylate (XXX).—Protoverine 14,15-acetonide (8 g., m.p. 263–266° dec.) was dissolved in pyridine (40 ml.), cooled in ice and treated portionwise with tosyl chloride (12 g.). The yellow solution was allowed to stand at room temperature for 14 hours, cooled in ice, basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was crystallized from acetone as rods, 4.2 g., m.p. 229–231° dec. A second crop of rods, 0.5 g., m.p. 230–231° dec., was also obtained. A sample was recrystallized for analysis from acetone as rods, m.p. 230–231° dec., $[\alpha]^{26}D$ +10° (*c* 0.80, pyr.).

Anal. Calcd. for $C_{37}H_{53}O_{11}NS \cdot {}^1/_2H_2O$: C, 60.98; H, 7.46; S, 4.39. Found: C, 61.02; H, 7.48; S, 2.42.

This compound, when oxidized with sodium periodate and worked up as described for the oxidation of XVII, gave an amorphous residue which showed absorption at 3.65, 5.62 and 5.80 μ.

Protoverine 14,15-Acetonide 6-Tosylate 3,16-Diacetate (XXXI).—A solution of protoverine 14,15-acetonide 6-tosylate (2.5 g.) in pyridine (5 ml.) was treated with acetic anhydride (10 ml.) and warmed on a steam-bath for 90 minutes. The solution was cooled, basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resinous oil. Although a paper chromatogram[31a] indicated that the product was homogeneous, all attempts at crystallization were unsuccessful. On trituration with water, a white powder (2.7 g.), which decomposed above 220°, was obtained.

Protoverine 6-Tosylate 3,7,15,16-Tetraacetate (XXXIII).—Protoverine 14,15-acetonide 6-tosylate (10 g.) was dissolved in a mixture of concentrated hydrochloric acid (6 ml.) and water (200 ml.) and allowed to stand at room temperature for 24 hours. The cooled solution was basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin (9 g.). The resin was dissolved in pyridine (30 ml.) and treated with acetic anhydride (60 ml.) and warmed on a steam-bath for 2 hours. The cooled solution was basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was crystallized from ether–petroleum ether as needles (5 g.). This compound melted at 175–180°, resolidified and then decomposed at 225–230°. The compound was recrystallized from acetone–petroleum ether as needles (4 g.), and exhibited identical behavior when the m.p. was determined; $[\alpha]^{26}D$ −57° (*c* 0.94, pyr.).

Anal. Calcd. for $C_{42}H_{57}O_{15}NS$: C, 59.48; H, 6.76; S, 3.77; acetyl, 20.36. Found: C, 59.92; H, 6.86; S, 3.45; acetyl, 19.49.

7-Dehydroprotoverine 14,15-Acetonide 6-Tosylate 3,16-Diacetate (XXXII).—A solution of protoverine 14,15-acetonide 6-tosylate 3,16-diacetate (2.5 g.) in acetic acid (15 ml.) was treated with 0.66 *N* chromium trioxide in 98.5% acetic acid (35 ml.) and allowed to stand overnight at room temperature. The reaction mixture was cooled in ice, the excess of the oxidizing agent was decomposed with aqueous sodium bisulfite solution, basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin which was chromatographed on Merck acid-washed alumina (40 g.). The column yielded to benzene and to mixtures of chloroform–benzene, a series of yellow oils; to chloroform, a resin which was crystallized from ether as colorless prisms (618 mg.), m.p. 215–216° dec. A sample was recrystallized for analysis from chloroform–ether as colorless prisms, m.p. 215–216° dec., $[\alpha]^{26}D$ −69° (*c* 0.86, pyr.).

Anal. Calcd. for $C_{41}H_{55}O_{13}NS \cdot H_2O$: C, 60.05; H, 7.01; acetyl, 10.50. Found: C, 60.01; H, 7.20; acetyl, 11.20.

Calcium–Liquid Ammonia Reduction of 7-Dehydroprotoverine 14,15-Acetonide 3,6,16-Triacetate.—Calcium (500 mg.) was added to dry liquid ammonia (50 ml.) at −70°. A solution of 7-dehydroprotoverine 14,15-acetonide 3,6,16-triacetate (100 mg., m.p. 261–262° dec.) in tetrahydrofuran (2 ml.) was quickly added: addition time < 5 seconds. After standing for 2 minutes at −70°, the reaction was terminated by addition of bromobenzene (3 ml.), ammonium chloride (3 g.), tetrahydrofuran (2 ml.) and water (2 ml.). The ammonia was evaporated at room temperature and chloroform and iced water added to the residue. The chloroform layer was separated and the aqueous layer was thoroughly extracted with chloroform. The combined chloroform solutions were dried over anhydrous sodium sulfate and evaporated. The residue was dissolved in ether and the basic material extracted with 0.5 *N* hydrochloric acid. The acid solution was basified with cold ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield a resin (60 mg.). A paper chromatogram[34] indicated that the resin contained 20–25% of 7-dehydrogermine 14,15-acetonide 3,16-diacetate (XXXIV).

An identical repetition of the experiment yielded a resin (70 mg.) which exhibited a very similar paper chromatographic behavior.

The combined reaction products (130 mg.) were chromatographed on Merck acid-washed alumina (3.9 g.). The column separation was checked by paper chromatography.[34] Fractions eluted with 50% benzene–chloroform and with chloroform which appeared to be homogeneous and identical with XXXIV on a paper chromatogram were combined to yield a resin (25 mg.). The resin was crystallized from ether as prisms, 15 mg., m.p. 260–263° dec. The prisms were recrystallized from acetone–petroleum ether as colorless prisms, 5 mg., m.p. 268–270° dec. The melting point was not depressed on admixture with a sample of XXXIV. The infrared spectra and paper chromatographic behavior of the respective samples were identical.

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