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Some Hypotensive Alkaloids from *Veratrum album*

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The isolation from commercial *Veratrum album* of the six hypotensive ester-alkaloids protoveratrine, germitetrine, veratetrine (neoprotoveratrine), desacetylneoprotoveratrine, neogermbudine and desacetylprotoveratrine is described. Desacetylprotoveratrine is a new hypotensive triester of the alkamine protoverine in which the esterifying acids are one mole each of acetic, α-methylbutyric and methylethylglycolic. It can also be obtained by methanolysis of protoveratrine. Methanolysis of germitetrine has resulted in the loss of a labile acetyl group to give a new hypotensive triester which has been named desacetylgermitetrine. Under these methanolysis conditions, a minor portion of the germitetrine loses both acetyl groups to give an alkaloid identical to the naturally occurring diester neogermbudine. The infrared spectra of germitetrine and the new alkaloids desacetylgermitetrine and desacetylprotoveratrine are recorded for identification purposes.

In a preliminary note[1] we described briefly the examination of the crude crystalline "protoveratrine fraction" obtained from *Veratrum album* and its resolution by means of counter-current distributions and fractional crystallizations into the hypotensive ester-alkaloids protoveratrine, germitetrine and veratetrine. Germitetrine was described as a tetraester of the alkamine germine, the esterifying acids being α-methylbutyric, two moles of acetic and an unidentified non-volatile acid. Veratetrine was reported to be an ester which gave, on alkaline hydrolysis, the alkaline isoprotoverine, α-methylbutyric acid, acetic acid and an unidentified non-volatile acid.

We now wish to elucidate further the isolation and characterization of these alkaloids and to correlate these findings with those of other investigators in the veratrum field,[2-4] whose publications have appeared more recently. In addition, the amorphous alkaloidal fraction from *Veratrum album*[5] which remains after the removal of the ether-crystallizable "protoveratrine" fraction, has been examined and found to contain in addition to further amounts of germitetrine, the triester desacetylneoprotoveratrine, the diester neogermbudine (both isolated previously from *Veratrum viride*[6,7]) and a new triester which has been called desacetylprotoveratrine.

The basic benzene extract of the ground roots and rhizomes of commercial *Veratrum album*[8] was crystallized from ether to give crude crystalline "protoveratrine." This material was subjected to a 24-plate counter-current distribution between benzene and 2 *M* acetate buffer of *p*H 5.5. Protoveratrine ($C_{41}H_{63}O_{14}N$) was isolated[1] from tubes 17–24 of this distribution. Germitetrine was obtained from tubes 10–15; m.p. 229–230°, $[\alpha]^{25}D$ −74° (*c* 1 in pyridine). Hydrolysis of the latter gave the alkamine germine and an acid fraction. The acids were converted to their *p*-phenylphenacyl esters and these have been identified as the esters of α-methylbutyric acid, acetic acid (2 moles) and the levorotatory low-melting diastereoisomer of α,β-dihydroxy-α-methylbutyric acid. The structure of this dihydroxy acid was confirmed by synthesis. It is identical (infrared spectra and *p*-phenylphenacyl ester derivatives) to the synthetic *l*-low-melting isomer of α,β-dihydroxy-α-methylbutyric acid (made by hydroxylation of tiglic acid), the preparation of which has been described in a preceding paper.[6]

The formula for germitetrine, based on carbon–hydrogen analyses, hydrolysis products and equivalent weight determination is therefore $C_{41}H_{63}O_{14}N$, which is the same as that suggested from preliminary data.[1] This alkaloid is undoubtedly identical to the germitetrine B isolated more recently by Nash and Brooker[2] and by Kupchan and Deliwala,[3] from the amorphous alkaloidal fraction of *Veratrum album*, to which the former assigned the same structure.

Germitetrine possesses a labile acetate group which can be cleaved readily with methanol. Thus, if a solution of germitetrine in methanol is allowed to stand a day or so (a procedure used by Fried, White and Wintersteiner[9] for the conversion of germitrine to germerine), a new crystalline triester is formed together with a minor amount of a diester alkaloidal fraction. The hypotensive crystalline triester has been named desacetylgermitetrine, m.p. 143–149° dec., $[\alpha]^{28}D$ −8° (*c* 1 in pyridine). Its formula, $C_{39}H_{61}O_{13}N$, is based on its hydrolysis products (one mole each of germine, acetic acid, α-methylbutyric acid and the low-melting isomer of α,β-dihydroxy-α-methylbutyric acid) and C and H analyses.

The diester fraction was crystallized from benzene to give silky white needles melting at 149–152°, $[\alpha]^{24}D$ −7° (*c* 1 in pyridine). Its hydrolysis products indicated that it resulted from the loss of the 2 acetate groups of germitetrine and suggested that it might be the alkaloid neogermbudine. Accordingly, the two were compared. They were found to be identical (infrared spectra).

Veratetrine was isolated from tubes 0–9; $[\alpha]^{26}D$ −32° (*c* 1 in pyridine), m.p. 269–270° dec. Its alkaline hydrolysis products were disclosed in our preliminary notes[1,10] to be the alkamine isoproto-

(1) W. L. Glen, G. S. Myers, R. Barber, P. Morozovitch and G. A. Grant, *Nature*, **170**, 932 (1952).

(2) H. A. Nash and R. M. Brooker, THIS JOURNAL, **75**, 1942 (1953).

(3) S. M. Kupchan and C. V. Deliwala, *ibid.*, **75**, 4671 (1953).

(4) M. W. Klohs, R. Arons, M. D. Draper, F. Keller, S. Koster, W. Malesh and F. J. Petracek, *ibid.*, **74**, 5107 (1952).

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

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

(7) M. W. Klohs, M. D. Draper, F. Keller, W. Malesh and F. J. Petracek, *ibid.*, **75**, 3595 (1953).

(8) The crude drug was obtained from S. B. Penick and Co. in 1950.

(9) J. Fried, H. L. White and O. Wintersteiner, THIS JOURNAL, **72**, 4621 (1950).

(10) G. S. Myers, W. L. Glen, P. Morozovitch, R. Barber and G. A. Grant, *ibid.*, **74**, 3198 (1952).

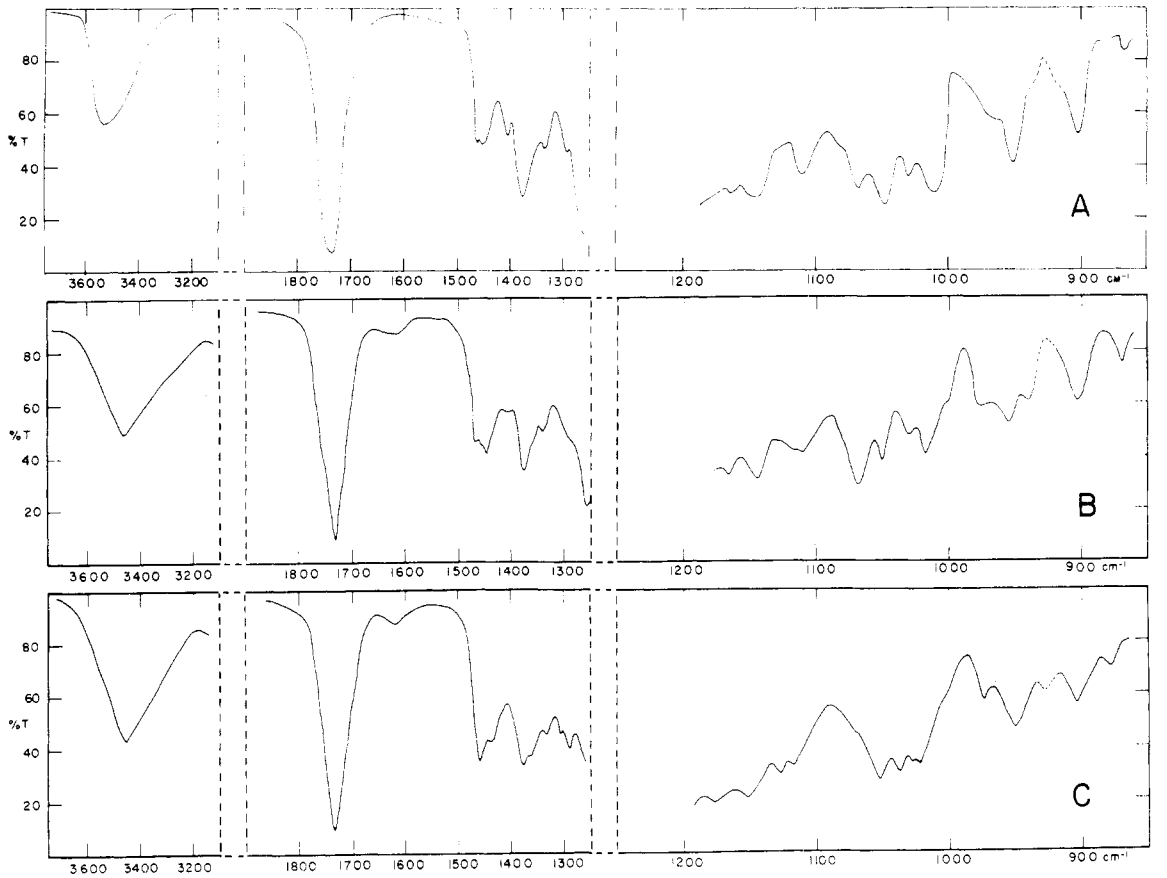

Fig. 1.—Infrared spectra: A, germitetrine; B, desacetylgermitetrine; and C, desacetylprotoveratrine; all in chloroform.

verine, α-methylbutyric acid, acetic acid and an unidentified acid. It is identical to the veratetrine (neoprotoveratrine) isolated from *V. viride* which has been shown to be a tetraester ($C_{41}H_{63}O_{15}N$) that gives on hydrolysis isoprotoverine (or its isomer protoverine), two moles of acetic acid and one mole each of α-methylbutyric acid and the high-melting α,β-dihydroxy-α-methylbutyric acid.[2,4,6] The crystalline "crude protoveratrine" fraction has been found, therefore, to be a mixture of the three tetraester alkaloids protoveratrine, germitetrine and veratetrine (neoprotoveratrine or protoveratrine B).

The amorphous alkaloidal residue remaining after the removal of the ether-crystallizable "protoveratrine" fraction also had high hypotensive activity. To isolate some of the alkaloids responsible for its activity, the material was subjected to an 18-plate counter-current distribution between benzene and 2 *M* acetate buffer of *p*H 5.5. Crystallization of the more organophilic material (tubes 7–18) from acetone gave additional amounts of germitetrine. The hydrophilic fraction (tubes 0–6) was a mixture of several alkaloids which was resolved by further counter-current distributions, as follows. The Craig distribution of the material in tubes 0–6 was continued for another 30-plates using buffer of *p*H 6.5 and benzene as the immiscible phases. Crystallization of the more organophilic material (tubes 10–20) gave a new crystalline hypotensive ester which melted at 191–192°, $[\alpha]^{25}D$ −15° (*c* 1 in pyridine). This ester on hydrolysis gave protoverine and one mole each of acetic acid, α-methylbutyric acid and methylethylglycolic acid. These hydrolysis products suggested the possibility of obtaining the alkaloid from partial hydrolysis of the tetraester protoveratrine. Accordingly, protoveratrine was treated with methanol and indeed a compound was obtained which proved to be identical with this triester. It has been named desacetylprotoveratrine ($C_{39}H_{61}O_{13}N$).

The more hydrophilic material (tubes 0–7) was dissolved in benzene and the crystals which separated were subjected to a prolonged Craig distribution between chloroform and 2 *M* acetate buffer of *p*H 4.4.[11] Crystallization of the more hydrophilic fraction of this distribution from benzene gave a triester which was found to be identical to desacetylneoprotoveratrine (infrared spectrum and hydrolysis products). Although this alkaloid has been isolated previously from *V. viride*,[6,7] this is the first time it has been reported to be present in *V. album*.

The more organophilic material after several crystallizations from benzene melted at 149–152°, $[\alpha]^{25}D$ −12° (*c* 1 in pyridine). This diester gave on hydrolysis the alkamine germine and one mole each of α-methylbutyric acid and the low-melting

(11) A 100-tube, automatic, glass, Craig counter-current distribution apparatus, made by the Post Scientific Instrument Company, Maspeth, N. Y., was used. Each tube had a capacity of 200 cc.—100 cc. of each phase.

isomer of α,β-dihydroxy-α-methylbutyric acid and was found to be identical (infrared) to the alkaloid neogermbudine ($C_{37}H_{59}O_{12}N$) which has been isolated both from *V. viride*[6] and from hydrolysis of germitetrine.

Although the following relationships have been shown to exist

desacetylgermitetrine $\xleftarrow[1\text{ acetate}]{\text{loss of}}$ germitetrine $\xrightarrow[2\text{ acetates}]{\text{loss of}}$

neogermbudine and protoveratrine $\xrightarrow[1\text{ acetate}]{\text{loss of}}$ desacetylprotoveratrine

one cannot conclude that the neogermbudine and desacetylprotoveratrine isolated directly from the alkaloidal extracts of the plants are of secondary rather than primary origin since, although we have isolated neogermbudine in the extracts of both *V. viride* and *V. album* and have found germitetrine to be one of the main alkaloidal constituents of *V. album*, we have been unable to find the latter in *V. viride*. Moreover, desacetylgermitetrine, the other possible precursor of the neogermbudine of plant origin, has not been found in either *V. album* or *V. viride*.

Pharmacology.—All seven ester-alkaloids are powerful antihypertensive agents. The pharmacology of protoveratrine, neoprotoveratrine (veratetrine) and desacetylneoprotoveratrine has been discussed previously.[1,12–14] The hypotensive activity of germitetrine, desacetylgermitetrine, neogermbudine and desacetylprotoveratrine have been determined in the anesthetized dog.[15] In comparison with a mixed alkaloidal ester preparation from *Veratrum viride* (Deravine), which produced a 30% fall in the mean arterial blood pressure of the anesthetized dog at a dose of 2 γ per kg., administered intravenously over a 10-minute period, their relative activities are 2.1, 1.8, 1.0 and 0.3 to 0.5, respectively.

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Experimental

All melting points are corrected and all evaporations were carried out under reduced pressure unless stated otherwise.

Fractionation of the Ether-crystallizable Alkaloids.—Commercial *Veratrum album* (100 lb.) was extracted with ammoniacal benzene and the alkaloids so obtained (460 g.) were crystallized from ether (1200 cc.)[5] to give an ether-crystallizable alkaloidal fraction (51.5 g., m.p. 248° dec.). The ethereal mother liquors on evaporation gave an amorphous "ether-soluble alkaloidal residue."

A portion of the ether-crystallizable fraction (39.2 g.) was subjected to a 24-plate Craig counter-current distribution between benzene and 2 *M* acetate buffer of *p*H 5.5, using 800-cc. portions of each phase.

Isolation of Veratetrine (Neoprotoveratrine).—The material recovered from the more hydrophilic region (tubes 0–9, 17.3 g.) was crystallized from benzene to give 11.2 g., m.p. 257–260° dec., which after further recrystallization melted at 264–265° dec., $[\alpha]^{26}D$ −32° (*c* 1 in pyridine).

Anal. Calcd. for $C_{41}H_{63}O_{15}N$: C, 60.8; H, 7.84; N, 1.73. Found (after drying at 110° *in vacuo*): C, 61.1; H, 7.72; N, 1.4.

This alkaloid was identical (infrared spectrum and hydrolysis products) to the veratetrine (neoprotoveratrine) isolated from *Veratrum viride*[4,6,10] and to the protoveratrine B from *Veratrum album*.[2]

Isolation of Protoveratrine.—The alkaloidal material in tubes 17–24 of the above distribution (13.6 g.) was combined and digested several times with warm benzene. The insoluble, white crystalline residue of protoveratrine so obtained (5.8 g.) melted at 270–271° with dec., $[\alpha]^{25}D$ −36° (*c* 1 in pyridine).

Anal. Calcd. for $C_{41}H_{63}O_{14}N$: C, 62.0; H, 8.00. Found (after drying at 110° *in vacuo*): C, 61.7; H, 7.78.

This alkaloid was further identified as protoveratrine[5] (protoveratrine A)[2] by its saponification in the usual manner to give the alkamine protoverine and the acids acetic, α-methylbutyric and methylethylglycolic.

Isolation of Germitetrine.—The alkaloids in tubes 10–16 of the above distribution (7.1 g.) were combined, digested with benzene and the insoluble protoveratrine which remained was removed by filtration. The benzene filtrate on concentration deposited crystalline germitetrine (2.4 g.) which after several recrystallizations from the same solvent melted at 229–230°, $[\alpha]^{25}D$ −74° (*c* 1 in pyridine), $[\alpha]^{25}D$ −12° (*c* 1 in chloroform).

Anal. Calcd. for $C_{41}H_{63}O_{14}N$: C, 62.0; H, 7.92; N, 1.8; equiv. wt., 794. Found (after drying at 110° *in vacuo*): C, 62.0; H, 7.83; N, 1.4; equiv. wt., 784.

In a volatile acid determination 32.8 mg. of substance consumed 12.4 cc. of 0.01 *N* sodium hydroxide; calcd. for 3 equivs. of volatile acid is 12.4 cc.

Hydrolysis of Germitetrine.—A solution of germitetrine (557 mg.) in methanol (18 cc.) containing 1 *N* alkali (5.9 cc.) was warmed to reflux for 30 minutes. Extraction of the hydrolyzate with chloroform (5 × 40 cc.) gave 0.28 g. of crude germine which after crystallization from methanol and drying at 110°, melted at 221–222° dec. It was identical to authentic germine (infrared).

Anal. Calcd. for $C_{27}H_{43}O_8N$: C, 63.6; H, 8.50. Found (after drying at 110° *in vacuo*): C, 63.3; H, 8.41.

The alkaline aqueous phase from the above extraction was adjusted to *p*H 6.7 with hydrochloric acid and esterified in the usual manner[9] with *p*-phenylphenacyl bromide (0.88 g.). The product so obtained (0.84 g.) was dissolved in 3:1 hexane–benzene and chromatographed on a column of silicic acid–Celite (3:1, 20 g.). Elution with the same solvent mixture removed excess *p*-phenylphenacyl bromide (191 mg.), followed by *p*-phenylphenacyl α-methylbutyrate (186 mg., 89% yield) which after purification from hexane melted at 71–72° (identified by mixed melting point and infrared spectral comparisons with an authentic specimen). Continued elution with benzene gave crystals (373 mg.) which were rechromatographed on sulfuric acid-washed alumina using benzene as the eluting solvent, to give 194 mg. of *p*-phenylphenacyl acetate (54% yield), melting at 111.5° after crystallization from benzene–hexane (identified by C and H analysis, mixed melting point and infrared comparisons with authentic acetate ester of m.p. 111°). Final elution of the silicic acid–Celite column with methanol gave an ester (119 mg., 51% yield) which was crystallized from ethanol to a constant m.p. of 164–165°. It was identified as the *p*-phenylphenacyl ester of the *l*-low-melting diastereoisomer of α,β-dihydroxy-α-methylbutyric acid by infrared spectral and mixed melting point comparisons with an authentic synthetic sample.[6]

Anal. Calcd. for $C_{19}H_{20}O_5$: C, 69.5; H, 6.16. Found: C, 69.2; H, 6.10.

Fractionation of the Ether-soluble Alkaloidal Residue. A portion (51 g.) of this fraction (obtained as described above) was given an 18-plate counter-current distribution between benzene and 2 *M* acetate buffer of *p*H 5.5, using 900-cc. portions of each phase in each tube. The more organophilic alkaloids (tubes 7–18, 28.3 g.) on fractional crystallization from acetone gave 8.9 g. of germitetrine,

(12) G. L. Maison, Eleanor Gotz and J. W. Stutzman, *J. Pharmacol. Expll. Therap.*, **103**, 74 (1951).

(13) E. D. Swiss, *ibid.*, **104**, 76 (1952).

(14) Lois Mosey and A. Kaplan, *ibid.*, **104**, 67 (1952).

(15) The pharmacological data were supplied by Dr. C. Chappel of the Ayerst pharmacology section.

m.p. 229–230°. The counter-current distribution of the material in tubes 0–6 was continued for another 30-plates using benzene and 2 *M* acetate of *p*H 6.5 as the immiscible phases (900-cc. portions of each phase were used in each funnel). The alkaloids were then recovered in the usual manner with the bulk of the material occurring in the more hydrophilic region (tubes 0–24).

Isolation of Desacetylprotoveratrine.—The alkaloidal material in tubes 10–20 of this distribution (3.1 g.) was crystallized several times from acetone and from chloroform–ether to give spherical clumps of white, crystalline desacetylprotoveratrine, m.p. 191–192°, $[\alpha]^{25}D$ −15° (*c*, 1 in pyridine). The alkaloid became tacky on drying at 110° in vacuum so it was analyzed as its crystalline methiodide derivative.

Desacetylprotoveratrine Methiodide.—A solution of desacetylprotoveratrine (92 mg.) and methyl iodide (4 drops) in acetone was allowed to evaporate to dryness over a period of 2 days. The crystalline residue of the methiodide which remained (112 mg.) was washed with warm acetone and dried at 110°, m.p. 231–233° with dec.

Anal. Calcd. for $C_{39}H_{61}O_{13}N\cdot CH_3I$: C, 53.7; H, 7.22. Found: C, 53.7; H, 7.12.

Hydrolysis of Desacetylprotoveratrine.—A solution of this substance (159 mg.) in methanol (16 cc.) containing 4.5 cc. of 0.3 *N* sodium hydroxide was allowed to stand for 29 hours. Continuous extraction of the hydrolyzate with chloroform (18 hours) in the usual manner[4] gave an alkamine (66 mg.) which after 2 crystallizations from methanol melted at 196–199° dec. It was identified as protoverine by comparison of its infrared spectrum with that of authentic protoverine.

Anal. Calcd. for $C_{27}H_{43}O_9N$: C, 61.7; H, 8.25. Found: C, 61.3; H, 8.42.

The alkaline aqueous phase from the extraction was adjusted to *p*H 2.7 with sulfuric acid, diluted to 100 cc. with water and concentrated under reduced pressure to remove the volatile acids. Titration of this distillate to *p*H 8.2 with 0.0206 *N* sodium hydroxide required 19.5 cc.; calcd. for 2 moles of volatile acid: 20.4 cc. Esterification of the volatile acids with *p*-phenylphenacyl bromide (143 mg.), as described above in the hydrolysis of germitetrine, gave material which was separated chromatographically into *p*-phenylphenacyl α-methylbutyrate (36 mg., melting at 70.5–71° after crystallization from hexane) and *p*-phenylphenacyl acetate (30 mg., melting at 108–109° after crystallization from benzene–hexane). Both esters were identical to authentic specimens by mixed melting point and infrared spectral comparisons.

The non-volatile residue from the distillation of the volatile acids was extracted continuously with ether for 18 hours. The crystalline acid which remained on evaporation of the ether extract (13 mg.) was converted to its *p*-phenylphenacyl ester (13 mg., melting at 119.5–120° after crystallization from benzene). It was identified as *p*-phenylphenacyl methylethylglycolate by mixed melting point and infrared spectral comparisons with an authentic sample.

Isolation of Neogermbudine.—The more hydrophilic alkaloids from the above 30-plate counter-current distribution (tubes 0–7, 12.7 g.) were crystallized from benzene which had been wetted with a drop of water, to give 3.7 g. of white plates, m.p. 160–165°. This crystalline material was subjected to a counter-current distribution between chloroform and 2 *M* acetate buffer of *p*H 4.4 as follows. The alkaloidal material was moved through the 100 tubes of the apparatus[11] (each tube of which contained 100 cc. of chloroform) with 100-cc. portions of buffer, and into the collector (140 portions of buffer were required). Two crystallizations of the more organophilic material (in buffer effluents 85–130, 0.84 g.) from benzene gave silky threads of neogermbudine, m.p. 149–152°, $[\alpha]^{25}D$ −12° (*c* 1 in pyridine), yield 435 mg. A mixed melting point with neogermbudine isolated from *V. viride*[6] gave no depression. The infrared spectra of the two were identical.

Anal. Calcd. for $C_{37}H_{59}O_{12}N$: C, 62.6; H, 8.38. Found (after drying at 110° *in vacuo*): C, 62.0; H, 8.18.

Hydrolysis of Neogermbudine.—This substance (158 mg.) was hydrolyzed with methanolic sodium hydroxide using the procedure described for the hydrolysis of the neogermbudine isolated from *V. viride*.[6] The hydrolyzate was found to contain the same hydrolysis products as the latter (the alkamine germine and one mole each of α-methylbutyric acid and the *l*-low-melting diastereoisomer of α,β-dihydroxy-α-methylbutyric acid).

Isolation of Desacetylneoprotoveratrine.—The more hydrophilic material contained in the 140 buffer effluents of the above counter-current distribution (tubes 36–60, 1.35 g.) was recovered and crystallized several times from benzene to give small white crystals (265 mg.), m.p. 181–183°, $[\alpha]^{25}D$ −9° (*c* 1 in pyridine). A mixed melting point with desacetylneoprotoveratrine isolated from *V. viride* was not depressed. The infrared spectra of the two compounds were identical.

Anal. Calcd. for $C_{39}H_{61}O_{14}N$: C, 61.0; H, 8.01. Found (after drying at 110° *in vacuo*): C, 60.5; H, 7.96.

Conversion of Germitetrine to Desacetylgermitetrine and Neogermbudine.—Germitetrine (1.12 g.) was allowed to stand for 30 hours in methanol (90 cc.). The methanol was then evaporated and the resinous residue so obtained was subjected to a 24-plate counter-current distribution using 100-cc. portions of benzene and 2 *M* acetate buffer of *p*H 5.5. The material recovered from tubes 3–9 (0.51 g.) was crystallized from benzene, and then from acetone–ether, yielding white, crystalline plates of desacetylgermitetrine (260 mg.), m.p. 143–149° dec., $[\alpha]^{28}D$ −8° (*c* 1 in pyridine).

Anal. Calcd. for $C_{39}H_{61}O_{13}N$: C, 62.3; H, 8.19. Found (after drying at 80° *in vacuo*): C, 61.9; H, 8.37.

The most hydrophilic material recovered from this methanolysis (tubes 0–2, 145 mg.) was pooled with the corresponding material recovered from the methanolysis of several batches of germitetrine. This pooled fraction (30 g.) was crystallized from benzene to give silky threads (7.0 g.), m.p. 149–152°, $[\alpha]^{24}D$ −7° (*c* 1 in pyridine). A mixed melting point with the neogermbudine isolated directly from the roots of *V. album* gave no depression. The infrared spectra and the hydrolysis products of the two compounds were identical.

Anal. Calcd. for $C_{37}H_{59}O_{12}N$: C, 62.6; H, 8.37. Found (after drying at 110° *in vacuo*): C, 62.1; H, 8.26.

Hydrolysis of Desacetylgermitetrine.—This substance (118 mg.) was hydrolyzed using the method described above for the hydrolysis of desacetylprotoveratrine. Extraction of the hydrolyzate with chloroform gave crystalline germine (45 mg., m.p. 227–228° dec.). The volatile acids in the hydrolyzate consumed 14.2 cc. of 0.0206 *N* sodium hydroxide (calcd. for 2 moles of volatile acid, 15.2 cc.) and were identified by conversion to their *p*-phenylphenacyl esters, as acetic and α-methylbutyric acids. The non-volatile residue was extracted continuously with ether and the extract was concentrated to give the low-melting isomer of α,β-dihydroxy-α-methylbutyric acid (17 mg.) which was identified by conversion to its dicyclohexylamine salt, m.p. 177–178°.

Anal. Calcd. for $C_5H_{10}O_4\cdot C_{12}H_{23}N$: C, 64.6; H, 10.5. Found: C, 64.3; H, 10.5.

Conversion of Protoveratrine to Desacetylprotoveratrine.—A solution of protoveratrine (576 mg.) in methanol (100 cc.) was allowed to stand for 16 hours. The solvent was then removed and the product so obtained was dissolved in warm acetone (5 cc.). The crystalline protoveratrine which separated (257 mg.) was remethanolized for 64 hours. The two methanolized materials were combined (560 mg.) and subjected to a 24-plate counter-current distribution between benzene and 2 *M* acetate buffer of *p*H 5.5 (100 cc. of each phase was used in each tube). The material recovered from tubes 2–7 (200 mg.) was crystallized several times from acetone and from chloroform–ether, yielding white crystals (36 mg.), m.p. 190–191°, $[\alpha]^{25}D$ −12° (*c* 1 in pyridine). A mixed melting point with desacetylprotoveratrine isolated directly gave no depression. The infrared spectra of the two compounds were identical.

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