Oxidation of Desazatetrahydro-α-erythroidinol (XI) to *o*-Ethylbenzoic Acid (X).—A suspension of 283 mg. of desazatetrahydro-α-erythroidinol (XI) in 12 ml. of water was treated with a solution of 1.17 g. of potassium permanganate in 35 ml. of water. The mixture was warmed until the permanganate color disappeared, after which it was acidified with hydrochloric acid and clarified by bubbling sulfur dioxide through the solution. The resulting solution was extracted several times with ether and the combined ether extracts were then extracted in turn with two 5-ml. portions of 10% aqueous sodium bicarbonate solution. Acidification of the alkaline extract precipitated 14 mg. (8%) of a white solid, m.p. 58–62.5°. Treatment of an ethanol solution of this solid with Norite followed by recrystallization of the product from an ethanol–water mixture gave white crystals, m.p. 63.5–65°. A mixture of this sample and an authentic sample of *o*-ethylbenzoic acid (X) showed no depression of melting point. Also, the infrared spectra of the naturally-derived and synthetic samples of *o*-ethylbenzoic acid were identical.

Oxidation of Desazatetrahydro-α-erythroidinol to *o*-Ethylphenyl 3-Tetrahydrofuranyl Ketone (XII).—To a solution of 398 mg. of desazatetrahydro-α-erythroidinol (XI) in 30 ml. of acetone there was added 500 mg. of magnesium sulfate and 110 ml. of a 1% solution of potassium permanganate in acetone. The oxidation was allowed to proceed at 0° for 8.5 hours before it was stopped by adding a solution of 6.0 g. of sodium thiosulfate in 17 ml. of water. After the precipitated manganese dioxide had been removed by filtration, the acetone solution was concentrated to a small volume and 1.0 g. of potassium hydroxide was added. The resulting solution was extracted with ether; the combined ether extracts were washed with base, dried over magnesium sulfate, and concentrated *in vacuo*. This gave 215 mg. of a residual yellow oil which was taken up in benzene and chromatographed over Florisil. From the first 30 ml. of eluate there was obtained 164 mg. of an oil which was then purified by distillation using a short-path still. There was obtained 70 mg. of a colorless, mobile oil; b.p. (pot temperature) 45° at 0.001 mm., $[\alpha]^{26}D$ +7.2 (*c* 0.013, ethanol).

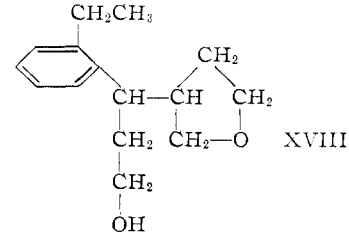
Anal. Calcd. for $C_{13}H_{16}O_2$: C, 76.44; H, 7.90. Found: C, 76.31, 76.41; H, 8.07, 8.10.

Although several attempts were made to obtain solid derivatives of XII, these were unsuccessful. This is not too surprising in view of the hindered nature of the carbonyl group. However, as discussed in the previous section, the ultraviolet and infrared absorption spectra of this material are so characteristic that no alternative structure appears suitable.

Desazahexahydro-α-erythroidinol (XVIII).—A solution of 162 mg. of des-N,N-dimethylhexahydro-α-erythroidinol (**XIII**) in 5 ml. of absolute ethanol containing 5 ml. of methyl iodide was boiled under reflux for 1.5 hours. After removal of the solvent, the resulting methiodide was obtained as a gum which failed to crystallize and so it was converted directly to the corresponding methohydroxide derivative by passage of an aqueous solution of the gum over an ion exchange column (Amberlite I.R. A-400-OH). Concentration of the combined eluate and washings from the column gave an oil which was distilled using a short-path still. There was obtained 83.6 mg. of a colorless, mobile oil; b.p. (pot temperature) 150° at 0.02 mm., $[\alpha]^{25}D$ +13.2° (*c* 0.009, ethanol).

Anal. Calcd. for $C_{15}H_{22}O_2$: C, 76.88; H, 9.47. Found: C, 76.83, 76.22; H, 10.15, 9.58.

The ultraviolet absorption spectrum of this compound was almost identical with that of XIII and was very similar to that of *o*-xylene. Its infrared absorption spectrum does not show the expected peaks for a terminal vinyl group but instead has absorption peaks at 6.91, 7.33, 8.29, 9.41 and 11.06 μ, indicating the presence of a tetrahydrofuran ring. For these reasons this product has been assigned structure XVIII. Thus, this is a second instance in which the Hofmann elimination proceeds by internal displacement of the trimethylamino group with formation of a tetrohydrofuran ring and shows that this type of elimination is not unusual or peculiar to the ring system present in IX.



XVIII

ROCHESTER, NEW YORK

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Some New Hypotensive Ester Alkaloids from *Veratrum viride*

BY GORDON S. MYERS, PAUL MOROZOVITCH, WILLIAM L. GLEN, RICHARD BARBER, GILLES PAPINEAU-COUTURE AND GORDON A. GRANT

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The isolation from commercial *Veratrum viride* of the five hypotensive ester alkaloids isogermidine, germbudine, neogermbudine, desacetylneoprotoveratrine and veratetrine (neoprotoveratrine) is described. Isogermidine is the diester germine monoacetate-mono-α-methylbutyrate. Germbudine is a diester of the alkamine germine which gives on hydrolysis germine, α-methylbutyric acid and the high melting diastereoisomer of α,β-dihydroxy-α-methylbutyric acid (m.p. 99–100°). Neogermbudine is a new diester of germine in which the esterifying groups are α-methylbutyric acid and the low-melting diastereoisomer of α,β-dihydroxy-α-methylbutyric acid. Desacetylneoprotoveratrine is a known triester of protoverine which gives on hydrolysis one mole each of acetic acid, α-methylbutyric acid and the high melting isomer of α,β-dihydroxy-α-methylbutyric acid. Veratetrine has been shown to be a tetraester of protoverine in which the esterifying acids are two moles of acetic acid and one mole each of α-methylbutyric acid and the high melting α,β-dihydroxy-α-methylbutyric acid. It has been shown to be identical to the alkaloid neoprotoveratrine. The structures of the naturally occurring high and low melting isomeric α,β-dihydroxy-α-methylbutyric acids obtained by hydrolysis of some of these ester alkaloids, have been confirmed by the synthesis of the racemic form of the former and both the racemic and resolved optical isomers of the latter, from hydroxylation of tiglic acid. The infrared absorption spectra of these hypotensive alkaloids are recorded for identification purposes.

Previous communications have disclosed the isolation of the hypotensive ester alkaloids germbudine, isogermidine, veratetrine[1] and desacetylneoprotoveratrine[2] from commercial *Veratrum viride*. This report presents further findings on their constitution together with the isolation of the new hypotensive ester neogermbudine.

The benzene-extractable alkaloids obtained from the ground roots and rhizomes of commercial *Veratrum viride* using the procedure of Jacobs and Craig[3]

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

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

(3) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **160**, 555 (1945).

were fractionated using methods similar to those employed by Fried, White and Wintersteiner[4] to give an amorphous alkaloidal concentrate of tertiary bases which contained the bulk of the hypotensive activity. This concentrate (0.3 to 0.5% of the crude drug) was subjected to a 24-plate Craig countercurrent distribution between benzene and 2 *N* acetate buffer of *p*H 5.5. The more organophilic alkaloidal material (tubes 4–24) from this distribution of a batch of drug received in 1952[5] was further fractionated and yielded the known esters germitrine,[4] neogermitrine,[6] protoveratrine[7] (from tubes 12–18) and germerine[1,6] (from tubes 4–6).

The Craig distribution curve of the total amorphous alkaloidal ester concentrate, together with the "ester number"[8] of the alkaloids present in each tube suggested that approximately 35% of the hypotensive ester alkaloids occurred in the hydrophilic fraction (tubes 0–3). It is this fraction which has received most of our attention since the more organophilic fraction has been studied quite considerably by others.

The hydrophilic fraction (tubes 0–3) was subjected first to a 72-plate Craig distribution between benzene and 2 *M* acetate of *p*H 6.5. Crystallization of the material in tubes 12–18 of this distribution from benzene gave the diester isogermidine which has been shown[1] to give on hydrolysis the alkamine germine and one mole each of α-methylbutyric acid and acetic acid. Isogermidine ($C_{34}H_{53}O_{10}N$) melted at either 221–222° or 229–230°, depending on which form separated $[\alpha]^{28}D$ −63° (*c* 1, in pyridine). Recently, Kupchan and Deliwala[9] have described the isolation of the ester neogermidine from *Zygadenus venenosus* and have shown it gives the same hydrolysis products. The two alkaloids have been compared and found to be identical (infrared spectrum).

Crystallization of the material in tubes 19–36 from benzene gave crystalline veratetrine, m.p. 269–270° dec., $[\alpha]^{26}D$ −33° (*c* 1, in pyridine). The same alkaloid has been isolated in much larger amounts from *Veratrum album.*[1,10]

Preliminary data[1] on the nature of this compound indicated it to be an ester of protoverine in which the esterifying acids were acetic, α-methylbutyric and an unidentified acid. The latter has now been isolated as a crystalline acid, m.p. 99–100°; $[\alpha]^{26}D$ +2° (water), which has been identified as the high melting isomer of α,β-dihydroxy-α-methylbutyric acid. The presence of two moles of acetic acid in the hydrolyzate also has been demonstrated. This tetraester ($C_{41}H_{63}O_{15}N$) has been described independently and almost simultaneously by two other groups of investigators. One group has named it neoprotoveratrine[7] and the other, protoveratrine B.[11] An exchange of samples has now served to demonstrate the identity of the three.

Crystallization of the alkaloids in tubes 0–2 of this distribution from benzene yielded a crystalline triester, melting at 185–186°; $[\alpha]^{25}D$ −8° (pyridine). It gave on hydrolysis one mole each of acetic acid, α-methylbutyric acid and the high melting isomer of α,β-dihydroxy-α-methylbutyric acid (m.p. 99–100°) and was found to be identical to desacetylneoprotoveratrine[12] (infrared, mixed melting point) which was prepared by methanolysis of neoprotoveratrine using the procedure described by Klohs and his associates.[2]

The alkaloids in tubes 0–9 which remain after the removal of the crystalline desacetylneoprotoveratrine, were separated by prolonged countercurrent distributions between chloroform and 2 *M* acetate buffer of *p*H 4.4, into fractions from which the crystalline diesters germbudine and neogermbudine were isolated.

A preliminary communication[1] disclosed that germbudine (m.p. 160–164°, $[\alpha]^{24}D$ −7° (*c* 1, in pyridine)) on hydrolysis gave germine and an acid fraction and that the acids had been converted to their *p*-phenylphenacyl esters which were separated into the ester of α-methylbutyric acid and an unidentified ester. The latter has now been shown to be the ester of the high melting isomer of α,β-dihydroxy-α-methylbutyric acid (m.p. 99–100°). Germbudine is therefore germine mono-α-methylbutyrate-mono-high melting α,β-dihydroxy-α-methyl-butyrate.

A new hypotensive alkaloid, slightly more organophilic than germbudine has been isolated. It has been named neogermbudine. This alkaloid (m.p. 149–152°, $[\alpha]^{25}D$ −12° (*c* 1, in pyridine)) has the same empirical formula ($C_{37}H_{59}O_{12}N$) as the latter. It has been shown to be a diester of germine which gives on hydrolysis the alkamine germine and one mole each of α-methylbutyric acid and the low-melting diastereoisomer of α,β-dihydroxy-α-methylbutyric acid.

The structures of the naturally occurring low and high melting isomeric α,β-dihydroxy-α-methylbutyric acids (*erythro–threo* relationship) obtained by the hydrolysis of these alkaloids, were substantiated by synthesis. Thus, the racemic low melting (m.p. 88°) and the racemic high melting (m.p. 110°) diastereoisomers of α,β-dihydroxy-α-methylbutyric acid were made by hydroxylating tiglic acid with alkaline permanganate[13] and with peracetic acid, respectively. The racemic acid of melting point 88° was resolved into its optical iso-

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

(5) Ground roots and rhizomes were supplied by S. B. Penick Company.

(6) J. Fried, P. Numerof and Nettie H. Coy, THIS JOURNAL, **74**, 3041 (1952).

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

(8) The ester number is the relative degree of esterification of the alkaloids determined by measurement of the intensity of the carbonyl band (at 1738 cm.$^{-1}$) of the infrared spectrum; G. Papineau-Couture and R. A. Burley, *Anal. Chem.*, **24**, 1918 (1952).

(9) S. M. Kupchan and C. W. Deliwala, THIS JOURNAL, **74**, 3202 (1952). Dr. Kupchan has kindly furnished us with a sample of neogermidine for comparison with isogermidine.

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

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

(12) The authors, in a paper presented at the 123rd Meeting of the Am. Chem. Soc., Los Angeles, March 15–19, 1953, described the isolation of this triester alkaloid from *Veratrum viride* and named it germbutrine. Since then, an article by Klohs, *et al.*,[2] has appeared in print describing an alkaloid which they called desacetylneoprotoveratrine. The data presented here indicate the two are identical and the name desacetylneoprotoveratrine has been adopted for germbutrine.

(13) R. Fittig and M. Penschuck, *Ann.*, **283**, 109 (1894).

mers by way of the brucine salt using methods similar to those employed by Adams and Van Duuren[14] for the resolution of racemic α-isopropyl-α,β-dihydroxybutyric acid. The (*levo*)-α,β-dihydroxy-α-methylbutyric acid so obtained melted at 63–65°, $[\alpha]^{22}D$ −3° (water), and gave a *p*-phenylphenacyl ester (m.p. 165°) identical in melting point and infrared spectrum with the corresponding ester of the natural non-volatile acid isolated from hydrolysis of neogermbudine. The melting point of a mixture (m.p. 164°) of the *p*-phenylphenacyl esters of the synthetic (*levo*)-acid and the natural acid (m.p. 164–165°) was not depressed while that of a mixture of the esters of the (*dextro*)-acid and the natural acid was 185–190°, which, as expected, was about the same as that of the corresponding ester of the synthetic racemic acid (192°). We were unable to resolve the synthetic racemic acid of melting point 110° with brucine. The following observations, however, in addition to C and H analysis and equivalent weight determination serve to establish the structure of the naturally occurring acid of melting point 99–100°, which is derived from hydrolysis of germbudine, germbutrine and veratetrine, as the optically active (*dextro*)-high-melting isomer of α,β-dihydroxy-α-methylbutyric acid. The infrared spectra (carbon disulfide solution) of both the *p*-phenylphenacyl ester (m.p. 90°) of the natural acid and the ester (m.p. 119°) of the synthetic racemic high melting isomer of α,β-dihydroxy-α-methylbutyric acid were identical. The dicyclohexylamine salts of both these acids melted at 170° and had identical infrared spectra. A mixed melting point of the two was not depressed.

The infrared spectra of these hypotensive alkaloids are recorded for identification purposes in Fig. 1.

Pharmacology.—All five ester alkaloids are powerful antihypertensive agents.[15] The hypotensive activity of germbudine, neogermbudine, isogermidine, veratetrine (neoprotoveratrine) and deacetylneoprotoveratrine have been determined in the anesthetized dog. 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 2γ per kg., administered intravenously over a 10-minute period, their relative activities are 0.8, 1.0, 0.1, 1.5 and 0.4, respectively.

Acknowledgment.—The authors are indebted to Mr. W. J. Turnbull for the microanalyses, optical rotations and equivalent weight determinations and to Mrs. J. Jachner and Mr. R. Burley for their infrared spectral determinations. They also wish to thank Dr. C. Chappel and his associates for the pharmacological data reported above.

Experimental[16]

Preparation of the Hypotensive Concentrate from *Veratrum viride*.—Dried and ground roots and rhizomes of commercial *Veratrum viride* (600 lb.; received in 1950[5]), were extracted with benzene and dilute ammonia (Jacobs and Craig[3]), the benzene extract concentrated (60 l.) and the crystals which separated (largely jervine) removed by filtration (826 g.) The filtrate was diluted with benzene to 72 l. and extracted with 2 *M* acetate buffer of *p*H 4 (1 × 48 l. and 4 × 24 l.). The buffer extracts were cooled, adjusted to *p*H 9–10 with ammonia and extracted with chloroform (4 × 30 l.). The chloroform extracts were washed with water, dried with anhydrous sodium sulfate and concentrated. The residue was dissolved in 5% acetic acid (36 l.) treated with saturated aqueous ammonium sulfate (4.8 l.) and the mixture cooled overnight. The insoluble sulfates of jervine and veratramine, which separated, were removed by filtration and the filtrate was cooled, adjusted to *p*H 9–10 and extracted with chloroform (4 × 12 l.). The chloroform extracts were dried, concentrated and the residue so obtained was dissolved in warm acetone (2 l.) and the solution was cooled to 0° for several hours. The crystals which separated (largely rubijervine and isorubijervine) were removed by filtration (149 g.) and the filtrate was concentrated to dryness to give 844 g. (0.3% of the crude drug) of amorphous, hypotensive, alkaloidal concentrate.

Fractionation of the Amorphous Alkaloidal Concentrate.—A portion of the concentrate obtained above (63.3 g.) was freed from residual amounts of the secondary base jervine by treating it with acetic anhydride in methanol as in (4). The jervine-free alkaloidal concentrate (49.3 g.) was then subjected to a 24-plate Craig countercurrent distribution between equal portions of benzene (2 l.) and 2 *M* acetate buffer (2 l.) of *p*H 5.5, in 24 separatory funnels. Alkaloidal fractions were then isolated from each funnel by alkalizing with ammonia and extracting into chloroform in the usual manner.

Neogermitrine and protoveratrine were obtained from funnels 12–20 while crystallization of the esters in funnels 4–11 from benzene gave germerine.[1] Alkaloids from funnels 0–3 (23.8 g.) were next subjected to a 72-plate Craig countercurrent distribution (72 separatory funnels) using 900-cc. portions of both benzene and 2 *M* acetate of *p*H 6.5. The material in funnels 0–36 contained essentially all the hypotensive potency.

Isolation of Veratetrine (Neoprotoveratrine).—The alkaloids from funnels 19–36 of the above distribution (weight 2.38 g.) were crystallized several times from benzene to give 0.37 g. of veratetrine; m.p. 269–270° dec., $[\alpha]^{28}D$ −2° (*c* 1, in chloroform), $[\alpha]^{26}D$ −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, 60.8; H, 7.56; N, 1.4.

Hydrolysis of Veratetrine (Neoprotoveratrine).—This alkaloid was hydrolyzed using methods similar to those employed by Klohs, *et al.*,[7] for the saponification of neoprotoveratrine. It was found to give the same hydrolysis products as the latter (the alkamine protoverine (or its alkaline isomerization product isoprotoverine), two moles of acetic acid and one mole each of α-methylbutyric acid and the high melting isomer of α,β-dihydroxy-α-methylbutyric acid). A sample of neoprotoveratrine, furnished by the above investigators, was then compared with veratetrine and the two were found to be identical (infrared and mixed melting point comparisons).

Isolation of Isogermidine.—Crystallization of the alkaloids in funnels 12–18 of the second distribution (wt. 5.40 g.) from benzene gave 1.3 g.; m.p. 195–205°. On recrystallization from benzene it melted at either 221–222° or 229–230° (dec.); depending on which form separated; $[\alpha]^{28}D$ −63° (*c* 1, in pyridine), −26° (*c* 1, in chloroform).

Anal. Calcd. for $C_{34}H_{53}O_{10}N$: C, 64.2; H, 8.40; equiv. wt., 635. Found (after drying at 110° *in vacuo*): C, 64.0; H, 8.33; equiv. wt., 650.

The volatile acid liberated by 8.28 mg. of isogermidine consumed 2.19 cc. of 0.01 *N* sodium hydroxide; calcd. for germine monoacetate-mono-α-methylbutyrate, 2.61 cc.

Hydrolysis of Isogermidine.—Isogermidine (100 mg.) was treated (20 hours) with 0.3 *N* aqueous sodium hydroxide (2.4 cc.) in methanol (3.3 cc.). The crude germine (80 mg.) was extracted from the alkaline hydrolyzate with chloroform (4 portions of 10 cc.) and crystallized from methanol to melt at 226–228° dec. Its infrared spectrum was identical to that of an authentic sample of germine.

Anal. Calcd. for $C_{27}H_{43}O_8N$: C, 63.6; H, 8.50. Found (after drying at 110° *in vacuo*): C, 63.0; H, 8.20.

(14) R. Adams and B. L. Van Duuren, THIS JOURNAL, **74**, 5349 (1952).

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

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

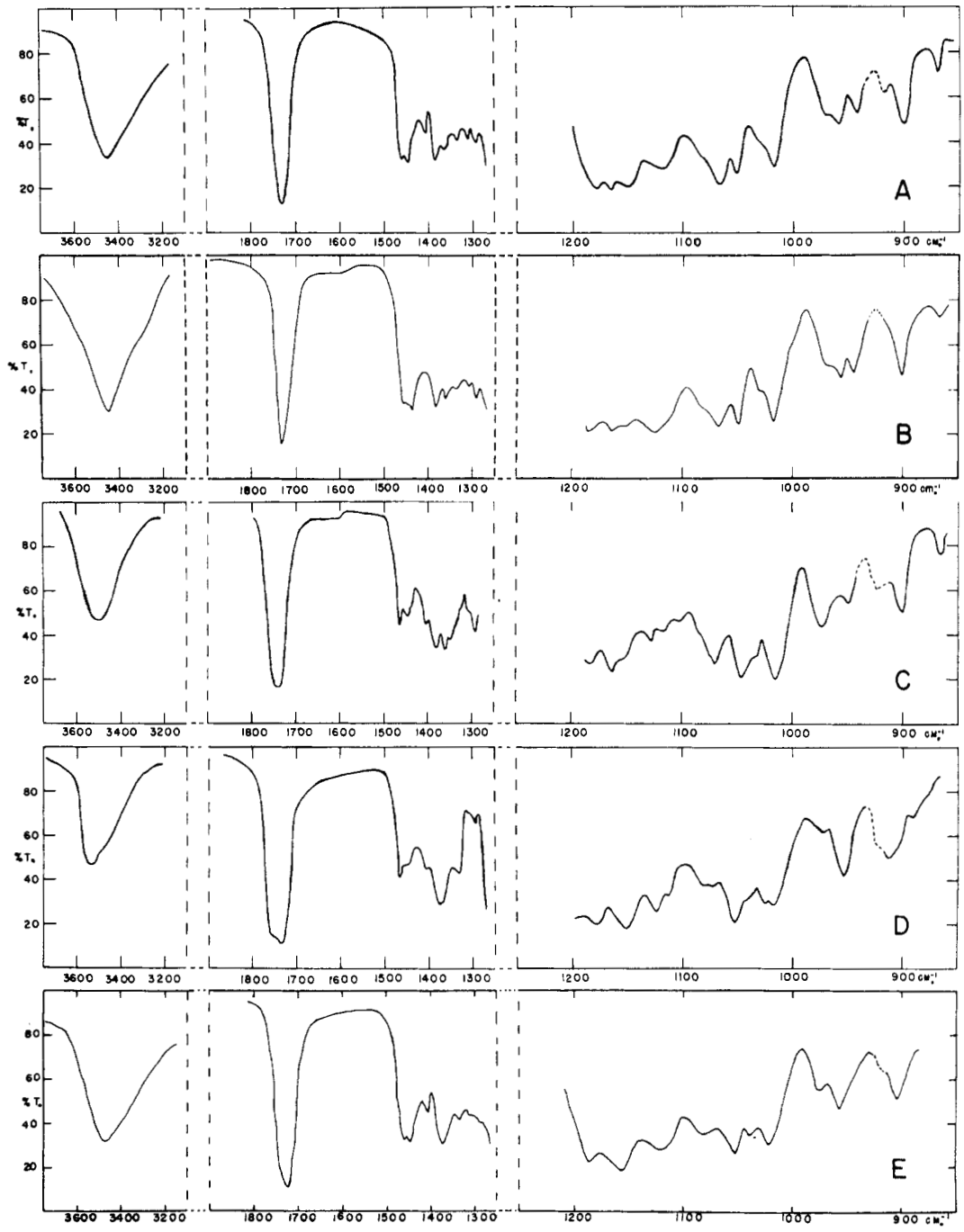

Fig. 1.—Infrared spectra: A, germbudine; B, neogermbudine; C, isogermidine; D, veratetrine (neoprotoveratrine); E, desacetylneoprotoveratrine; all in chloroform.

The chloroform-extracted aqueous phase was concentrated to 2 cc., adjusted to *p*H 6.5 with a few drops of hydrochloric acid, and refluxed with *p*-phenylphenacyl bromide (200 mg.) and 3 vol. of ethanol for 2 hours. The product (175 mg.) was extracted with benzene and chromatographed on a column of 3:1 silicic acid–Celite. Elution with a 3:1 mixture of hexane–benzene gave *p*-phenylphenacyl bromide (118 mg.), followed by *p*-phenylphenacyl α-methylbutyrate (22 mg.), m.p. 71.5–72° after crystallization from hexane.

Calcd. for $C_{19}H_{20}O_3$: C, 77.0; H, 6.80. Found: C, 76.9; H, 6.78.

Subsequent elution with 1:1 hexane–benzene gave a small amount of *p*-phenylphenacyl alcohol followed by *p*-phenylphenacyl acetate (13 mg.), m.p. 109.5–110.5° after crystallization from benzene–hexane mixture (identified as the acetate ester by mixed melting point and infrared spectral comparisons with an authentic specimen).

Isolation of Desacetylneoprotoveratrine.—The "hypotensive alkaloidal concentrate" (500 g.) from a batch of *Veratrum viride* received in 1952[5] was fractionated as above and the alkaloids corresponding to those contained in tubes 0–9 of the above 72-plate countercurrent distribution were separated into desacetylneoprotoveratrine, germbudine and neogermbudine, as follows:

The alkaloids from tubes 0–2 (23 g.) were dissolved in warm benzene (200 cc.), and the benzene solution was shaken with a drop of water and allowed to stand for several hours. The crystals which separated (10.7 g.) were recrys-

tallized from wet benzene to give 4.8 g., m.p. 177–181°. Further crystallization of this material from dry benzene gave pure desacetylneoprotoveratrine; m.p. 185–186°, $[\alpha]^{25}D$ −8° (*c* 1, in pyridine), 14° (*c* 1, in chloroform).

Anal. Calcd. for $C_{39}H_{61}O_{14}N$: C, 61.0; H, 8.01. Found (after drying at 110° *in vacuo*): C, 61.0; H, 7.92.

It was identical (hydrolysis products, infrared spectrum, mixed melting point) to an authentic sample of desacetylneoprotoveratrine which was prepared by methanolysis of neoprotoveratrine using the procedure described by Klohs, *et al.*[2]

Isolation of Neogermbudine.—The alkaloids in tubes 0–9 of the above 72-plate countercurrent distribution, except for the crystalline crude desacetylneoprotoveratrine (10.7 g.) which was removed as above, were combined (45 g.). A portion (35.6 g.) of this was countercurrently distributed between chloroform and 2 *M* acetate buffer of *p*H 4.4, as follows.[17] The 35.6 g. of alkaloids was added to tubes 0–4 of the apparatus, each tube of which had been loaded with a 100-cc. portion of chloroform. Acetate buffer in 100-cc. portions was then passed countercurrently through the apparatus until the alkaloids were moved through the 100 chloroform phases and into the collector (294 portions of buffer were used). Buffer effluents 35–94 were combined and processed for germbudine (see below).

Buffer effluents 95–124 were combined, made slightly alkaline with ammonium hydroxide and extracted with chloroform. Evaporation of the chloroform gave 2.6 g. of resinous residue which after several crystallizations from benzene gave 1.06 g. of neogermbudine; m.p. 149–152°, $[\alpha]^{25}D$ −12° (*c* 1, in pyridine).

Anal. Calcd. for $C_{37}H_{59}O_{12}N$: C, 62.6; H, 8.38. Found (after drying at 110° *in vacuo*): C, 62.5; H, 8.27.

Hydrolysis of Neogermbudine.—Neogermbudine (211 mg.) was hydrolyzed with dilute methanolic sodium hydroxide as described above for isogermidine. The crystalline residue from the chloroform extract (149 mg.) after two recrystallizations from methanol melted at 221–222° dec. after drying at 110°. Its melting point was not depressed on admixture with authentic germine and its infrared spectrum was identical to that of the latter.

The alkaline, chloroform-extracted, aqueous hydrolyzate was adjusted to *p*H 2.4 with sulfuric acid, diluted with water (100 cc.) and concentrated under reduced pressure to remove the volatile acid. Titration of the distillate to *p*H 8.2 required 16 cc. of 0.0206 *N* sodium hydroxide (theory for one mole of volatile acid in 15 cc.). This titrated distillate was concentrated to 3 cc., adjusted to *p*H 6.5 and treated with *p*-phenylphenacyl bromide as described above in the hydrolysis of isogermidine. The *p*-phenylphenacyl α-methylbutyrate so obtained (63 mg.) melted at 72–72.5° after crystallization from hexane and was identified by mixed melting point and infrared spectral comparisons with an authentic sample.

The non-volatile residue from the distillation (3 cc.) was diluted with water (40 cc.) and extracted continuously with ether for several hours. Evaporation of the ether extract gave a sirupy non-volatile acid (40 mg.) which was esterified with *p*-phenylphenacyl bromide to give 41 mg. of crude ester. This, after several crystallizations from methanol, melted at 165° and was identified as the *p*-phenylphenacyl ester of the (*levo*)-low-melting diastereoisomer of α,β-dihydroxy-α-methylbutyric acid by mixed melting point and infrared spectral comparisons with the corresponding ester of the synthetic (*levo*)-low-melting α,β-dihydroxy-α-methylbutyric acid described below.

Isolation of Germbudine.—Buffer effluents 35–94 of the above countercurrent distribution were combined, made slightly alkaline, extracted with chloroform and the chloroform evaporated to give 14.6 g. of alkaloidal residue. This residue was countercurrently distributed between chloroform and 2 *M* acetate buffer of *p*H 4.4 whereby it was moved through 365 tubes of chloroform (100 cc. in each tube) and into the collector using 280 portions of buffer (100-cc. portions). The bulk of the alkaloids was contained in buffer effluents numbered 80–249. The alkaloids in buffer effluents 180–224 were recovered, combined (2.2 g.) and crystallized several times from benzene to give 0.82 g. of crystalline germbudine; m.p. 160–164°, $[\alpha]^{24}D$ −7° (*c* 1, in pyridine).

Anal. Calcd. for $C_{37}H_{59}O_{12}N$: C, 62.6; H, 8.38; equiv. wt., 709. Found (after drying at 110° *in vacuo*): C, 62.0; H, 8.34; equiv. wt., 722.

Hydrolysis of Germbudine.—Germbudine (202 mg.) was hydrolyzed with aqueous methanolic sodium hydroxide as described above for neogermbudine. The residue from the chloroform extract (126 mg.) was crystallized three times from methanol. The crystalline alkamine so obtained (44 mg.) melted at 218–219° (after drying at 110°) and was identified as germine by comparison with an authentic specimen (mixed melting point and infrared spectrum).

Anal. Calcd. for $C_{27}H_{43}O_8N$: C, 63.6; H, 8.50. Found (after drying at 110° *in vacuo*): C, 63.1; H, 8.35.

The alkaline, chloroform-extracted, aqueous hydrolyzate was adjusted to *p*H 2.5 with sulfuric acid and the volatile acid was distilled and titrated as described above for neogermbudine. It required 27 cc. of 0.01 *N* sodium hydroxide (theory for 1 mole is 28.5 cc.). This acid was identified as α-methylbutyric acid by conversion to its *p*-phenylphenacyl ester which melted at 72.5°. This ester gave no melting point depression on admixture with an authentic specimen and had an infrared spectrum identical to that of the latter.

The non-volatile residue was diluted with water (40 cc.) and extracted continuously with ether (17 hours). Evaporation of the ether extract gave 36 mg. of non-volatile acid which after two crystallizations from ether melted at 99–100°.

Anal. Calcd. for $C_5H_{10}O_4$: C, 44.8; H, 7.51; equiv. wt., 134. Found: C, 45.1; H, 7.55; equiv. wt., 133.

It was identical (infrared spectrum and mixed melting point) to the dihydroxy acid isolated from veratetrine (neoprotoveratrine) and desacetylneoprotoveratrine and although optically active ($[\alpha]^{26}D$ +2° (water)) it had an infrared spectrum (mull) similar to that of synthetic (*racemic*)-high-melting isomer of α,β-dihydroxy-α-methylbutyric acid (m.p. 110–111°) described below.

(*racemic*)-Low-melting Isomer of α,β-Dihydroxy-α-methylbutyric Acid (m.p. 88°) by Hydroxylation of Tiglic Acid.—An alkaline solution of tiglic acid (100 g.) was hydroxylated with potassium permanganate (158 g.) using the method described by Fittig and Penschuck[18] to give 83 g. of crude dihydroxy acid (m.p. 66–79°) which after two recrystallizations from cold ether gave the (*racemic*)-low-melting isomer of α,β-dihydroxy-α-methylbutyric acid melting at 87.5–88.5° (47 g., 35% yield). The dicyclohexylamine salt and the *p*-phenylphenacyl ester of this racemic dihydroxy acid were prepared. They melted at 178–179° and 192–193°, respectively.

Resolution of the (*racemic*)-Low-melting α,β-Dihydroxy-α-methylbutyric Acid (m.p. 87.5–88.5°).—A solution of the racemic dihydroxy acid (20.1 g.) in warm ethanol (250 cc.) was treated with a warm solution of brucine (69.9 g.) in ethanol (200 cc.) in a manner similar to that described by Adams and Van Duuren for the resolution of (*racemic*)-α-isopropyl-α,β-dihydroxybutyric acid.[14] On cooling, a white crystalline solid separated; m.p. 233–234°, yield 41 g. After two recrystallizations from ethanol a salt of constant melting point and rotation was obtained; m.p. 239–240°, yield 23.9 g., $[\alpha]^{25}D$ −20° (*c* 1, in chloroform).

The combined mother liquors from the crystallization of the less soluble salt were diluted with a little ether. On standing, a white crystalline solid separated; m.p. 227–229°, yield 33.7 g., $[\alpha]^{25}D$ −29° (*c* 1, in chloroform). The melting point and rotation remained unchanged on recrystallization from ethanol.

(*dextro*)-Acid.—A solution of the less soluble brucine salt (15 g.) in 10% aqueous sulfuric acid (75 cc.) was extracted continuously with ether for 21 hours. The extract was dried over sodium sulfate. Filtration and removal of the ether left a sirup which was precipitated twice from ether with hexane to give a sirupy acid which crystallized on drying *in vacuo*, yield 3.2 g. (84%), $[\alpha]^{25}D$ 4° (*c* 5, in water), m.p. 64–66°.

(*levo*)-Acid.—The (levo)-acid was obtained from the more soluble brucine salt in the same manner; yield was 82%, m.p. 63–65°, $[\alpha]^{25}D$ −3° (*c* 6, in water).

Anal. Calcd. for $C_5H_{10}O_4$: C, 44.8; H, 7.51. Found: C, 44.9; H, 7.53.

(17) A 100-tube, automatic, glass Craig countercurrent distribution apparatus, made by the Post Scientific Instrument Co., Maspeth, N. Y., was used. Each tube had a capacity of 200 cc.–100 cc. of each phase.

p-Phenylphenacyl Ester of the (*levo*)-Acid.—The ester, prepared by the procedures described above, was crystallized from methanol to give colorless needles, m.p. 165°. It was identical (mixed melting point and infrared spectrum) to the *p*-phenylphenacyl ester (m.p. 165°) of the dihydroxy acid isolated above from neogermbudine.

Anal. Calcd. for $C_{19}H_{20}O_5$: C, 69.5; H, 6.16. Found: C, 69.3; H, 6.32.

(*racemic*)-High-melting Isomer of α,β-Dihydroxy-α-methylbutyric Acid (m.p. 110°) by Hydroxylation of Tiglic Acid.—A solution of tiglic acid (50 g.) in acetic acid (600 cc.) containing 1.5 cc. of concentrated sulfuric acid was treated on the steam-bath with 30% hydrogen peroxide (200 cc. added portionwise over a period of 4 hours). After an additional hour of heating the warm mixture was made alkaline with sodium hydroxide and digested for a half hour at 100° to ensure saponification of any acetylated hydroxy acid. The resulting solution was adjusted to *p*H 2 with hydrochloric acid, evaporated to dryness and the residue extracted with acetone. The acetone extract was filtered from the inorganic salts and evaporated to give 62 g. of crude acid, m.p. 72–88°, which after several recrystallizations from acetone–ether gave colorless crystalline (*racemic*)-high-melting α,β-dihydroxy-α-methylbutyric acid; yield 40 g. (60%), m.p. 110–111°.

Anal. Calcd. for $C_5H_{10}O_4$: C, 44.8; H, 7.54. Found: C, 45.3; H, 7.63.

Attempts to resolve this racemic acid, by way of the brucine salt, were unsuccessful.

Dicyclohexylamine Salt.—Treatment of this racemic dihydroxy acid in ether with dicyclohexylamine gave the crystalline salt, m.p. 170–171°, which was identical (mixed melting point and infrared spectrum) to the dicyclohexylamine salt (m.p. 170–171°) of the non-volatile acid isolated above from germbudine, veratetrine and desacetylneoprotoveratrine.

***p*-Phenylphenacyl Ester.**—The ester of this synthetic dihydroxy acid, prepared in the usual way, was recrystallized several times from methanol. It melted at 119–120° and had an infrared spectrum identical to that of the *p*-phenylphenacyl ester (m.p. 90–91°) of the non-volatile acid isolated from germbudine, veratetrine and desacetylneoprotoveratrine.

MONTREAL, CANADA

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF CHAS. PFIZER AND CO., INC.]

Magnamycin. IV. Mycaminose, an Aminosugar from Magnamycin

BY F. A. HOCHSTEIN AND PETER P. REGNA

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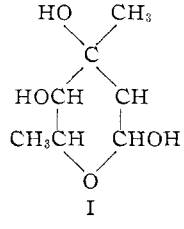
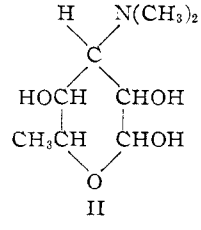
Mycaminose, $C_8H_{17}NO_4$, is a dimethylamino sugar isolated from the acid hydrolysis products of the Magnamycin antibiotics. Mycaminose is a reducing sugar, which contains one C-methyl group, and yields two triacetates on acetylation. Periodate oxidation has yielded formic acid and a new sugar $C_7H_{15}NO_3$. Further oxidation forms acetaldehyde, but no formaldehyde or acetic acid. Mycaminose liberates dimethylamine very rapidly with base, indicating it to be a β-dimethylaminoaldehyde. The seven-carbon sugar, by contrast, loses dimethylamine only slowly. These observations are compatible with only one structure, II, for mycaminose.

When the antibiotics Magnamycin[1] or Magnamycin B are subjected to acid hydrolysis,[2,3] two unusual sugars may be isolated. One of these, mycarose, has been shown to have the structure I.[4] The second, mycaminose, is here shown to have the structure II.



I



II

Mycaminose, $C_8H_{17}NO_4$, contains a dimethylamino group, and one C-methyl group.[3] It is therefore, a dimethylaminomethylpentose. Mycaminose reduces Fehling solution readily, loses dimethylamine rapidly in alkaline solution, and gives a slow positive iodoform test. Methylation in methanol–hydrochloric acid yields a methyl derivative, which no longer reduces Fehling solution, nor does it readily lose dimethylamine in alkaline solution. The presence of an aldehyde group in mycaminose is evident from the fact that periodate oxidation yields formic acid and a seven-carbon sugar. This aldehyde function must be present as a cyclic hemiacetal, since the infrared spectrum (Fig. 1) shows no carbonyl absorption. Further, the acetylation of mycaminose has yielded two isomeric triacetates,[3] as would be expected of a cyclic hemiacetal, and the hydrogenation of mycaminose to the alcohol can be effected only under forcing conditions.

The oxidation of mycaminose with one equivalent of periodate results in the formation of formic acid and of a new dimethylamino sugar, $C_7H_{15}NO_3$, both in excellent yield. The new sugar differs from mycaminose in that it reduces Fehling solution only slowly. Like mycaminose, it contains one C-methyl group, and the infrared spectrum shows no free carbonyl group. Further periodate oxidation of mycaminose, or of the derived seven carbon sugar proceeds more slowly, as would be expected for cleavage of a $HOC–CN(CH_3)_2$ bond.[5] Mycaminose eventually consumes up to four equivalents of periodate, and both it and the derived methyltetrose yield acetaldehyde, but no formaldehyde, and no acetic acid.

The formation of *formic acid*, and of a dimethylaminomethyltetrose on partial periodate oxidation, together with the qualitative tests, indicates that mycaminose must have the terminal group CHOH-CHO common to C.1 and C.2 of formulas III and IV.

(1) Magnamycin is Chas. Pfizer & Co.'s registered trade name for the antibiotic carbomycin.

(2) R. L. Wagner, F. A. Hochstein, K. Murai, N. Messina and P. P. Regna, THIS JOURNAL, **75**, 4684 (1953).

(3) F. A. Hochstein and K. Murai, *ibid.*, **76**, 5080 (1954).

(4) P. P. Regna, F. A. Hochstein, R. L. Wagner and R. B. Woodward, *ibid.*, **75**, 4625 (1953).

(5) E. H. Flynn, M. V. Sigal, P. F. Wiley and K. Gerzon, *ibid.*, **76**, 3121 (1954). See especially p. 3124.