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

Veratrum Alkaloids. XXXIX.[1] The Structures of Protoveratrine A and Protoveratrine B[2,3]

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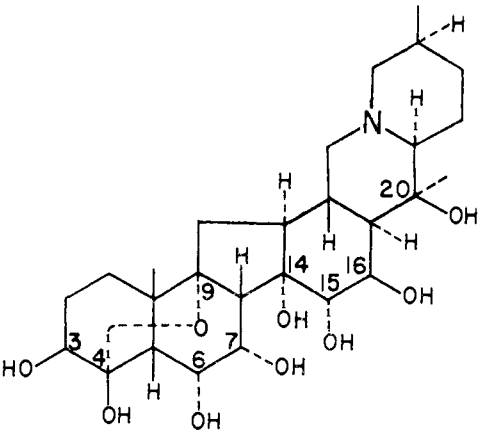
The structures of protoveratrine A and protoveratrine B have been elucidated. Protoveratrine A is protoverine 3-(*d*)-2'-hydroxy-2'-methylbutyrate 6,7-diacetate 15-(*l*)-2'-methylbutyrate (II). Protoveratrine B is protoverine 3-(*d*)-*threo*-2',3'-dihydroxy-2'-methylbutyrate 6,7-diacetate 15-(*l*)-2'-methylbutyrate (XV).

Protoveratrine was the name assigned by Salzberger, in 1890,[4] to an alkaloid isolated from *Veratrum album*. The pharmacological properties of the highly toxic preparation were described two years later by Edens.[5] An attempt at purification of protoveratrine was reported by Bredemann, in 1906,[6] and the extraction and purification procedures were improved by Poethke, in 1937,[7] and Craig and Jacobs, in 1942.[8] Krayer and his associates did extensive investigation on the pharmacology of protoveratrine and advanced the material as a suitable drug for clinical trials.[9,10] These trials were followed by introduction of protoveratrine into clinical use in the treatment of hypertension.[11]

In 1952–1953, four groups reported almost simultaneously that protoveratrine was not a homogeneous entity but a mixture of two closely related ester alkaloids, protoveratrine A and protoveratrine B.[12–15] Alkaline hydrolysis of protoveratrine A was shown to yield the known alkamine protoverine (I),[1] two molecular equivalents of acetic acid, one of (*l*)-2-methylbutyric acid and one of (*d*)-2-hydroxy-2-methylbutyric acid. Similar hydrolysis of protoveratrine B gave protoverine, two molecular equivalents of acetic acid, one of (*l*)-2-methylbutyric acid and one of (*d*)-*threo*-2,3-dihydroxy-2-methylbutyric acid. The present report presents evidence for assignment of structure II to protoveratrine A and structure XV to protoveratrine B.

Protoveratrine A consumed 0.9 oxygen equivalent of chromic acid, an indication that the C_4-hydroxyl group is not acylated. The oxidation product, protoveratrone A (III), m. p. 221–223° dec., $[\alpha]^{25}D$ −97° (pyr.), on alkaline hydrolysis afforded an amorphous diosphenol with spectral properties identical with those of the diosphenol (partial structure V) obtained from alkaline hydrolysis of 16-dehydroprotoverine 3,4,6,7,15-pentaacetate (*cf.* ref. 1). Thus the C_{16}-hydroxyl group is not acylated in protoveratrine A. In keeping with only a single secondary hydroxyl group, protoveratrine A readily formed a monoacetate (XI), m. p. 249–250° dec., $[\alpha]^{22}D$ −52° (pyr.), and a monoisobutyrate (IV), m.p. 245–246° dec., $[\alpha]^{21}D$ −41° (pyr.). The fact that protoveratrine A could be obtained from methanolysis of the latter compound provided supporting evidence for a free C_{16}-hydroxyl group in the naturally occurring tetraester (*cf.* ref. 1 for the facile methanolysis of C_{16}-esters).



I

Vigorous methanolysis of protoveratrine A afforded a protoverine mono-(*l*)-2'-methylbutyrate, m.p. 218–220° dec., $[\alpha]^{23}D$ −18° (pyr.). This compound consumed 1.9 mole equivalents of sodium periodate, an indication that the (*l*)-2-methylbutyrate residue was attached to the C_{15}-hydroxyl group (see formula VI). This was confirmed by acetylation to a tetraacetate, m.p. 262–263° dec., $[\alpha]^{23}D$ −46° (pyr.), shown to be protoverine 15-(*l*)-2'-methylbutyrate 3,6,7,16-tetraacetate (VII) in the following manner.

Protoverine 3,6,16-triacetate (VIII),[1] on treatment with a limited amount of (*l*)-2-methylbutyryl chloride,[16] afforded protoverine 15-(*l*)-2'-methylbutyrate 3,6,16-triacetate (IX), m.p. 234–235° dec., $[\alpha]^{23}D$ −4° (pyr.), which was stable toward sodium periodate but consumed 1.0 oxygen equiva-

(1) Part XXXVIII, preceding paper, p. 2242.

(2) The investigations which form the subject of the present paper were first outlined in part in a preliminary communication: THIS JOURNAL, **81**, 1009 (1959).

(3) This investigation was supported in part by research grants from the National Institutes of Health (H-2275, C_2 and C_3) and the Wisconsin Alumni Research Foundation.

(4) G. Salzberger, *Arch. Pharm.*, **228**, 462 (1890).

(5) T. W. Edens, *Arch. Exper. Path. Pharmakol.*, **29**, 440 (1892).

(6) E. Bredemann, *Apoth. Ztg.*, **21**, 41 (1906).

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

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

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

(10) E. Meilman and O. Krayer, *Circulation*, **1**, 204 (1950).

(11) 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.

(12) W. L. Glen, G. S. Myers, R. Barber, P. Morozovitch and G. A. Grant, *Nature*, **170**, 932 (1952). These authors used the terms protoveratrine for protoveratrine A and veratetrine for protoveratrine B.

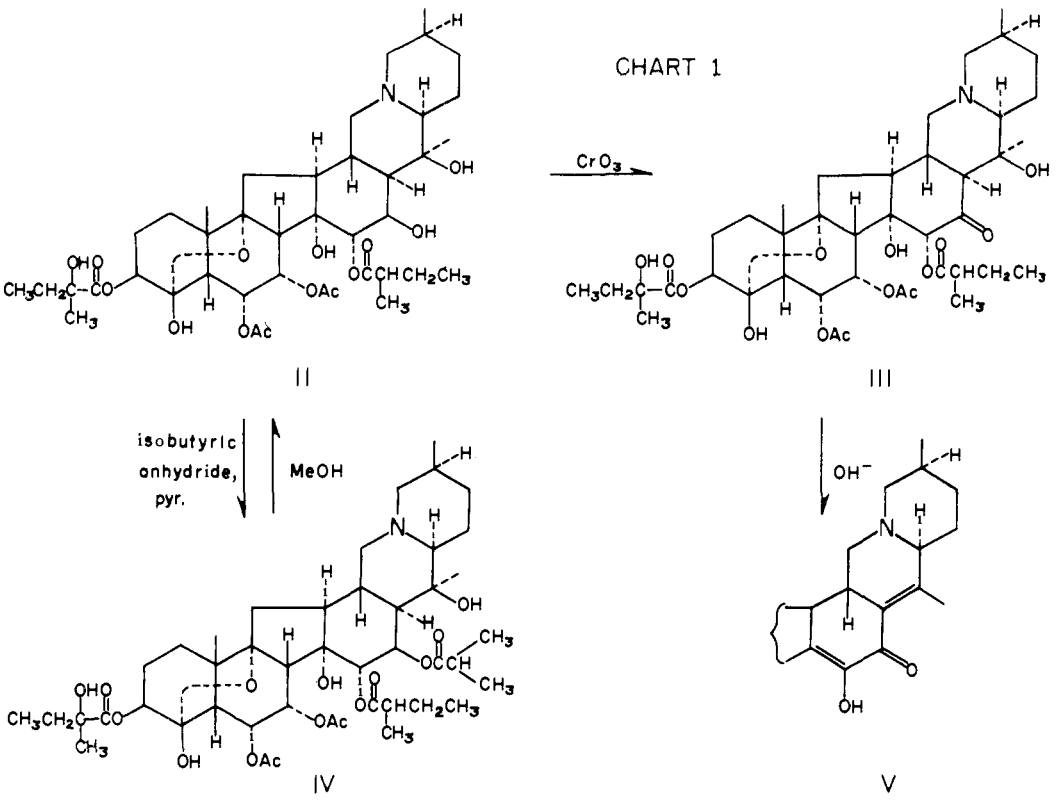
(13) M. W. Klohs, R. Arons, M. D. Draper, F. Keller, S. Koster, W. Malesh and F. J. Petracek, THIS JOURNAL, **74**, 5107 (1952). These authors used the terms protoveratrine for protoveratrine A and neoprotoveratrine for protoveratrine B.

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

(15) A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **36**, 718 (1953).

(16) F. L. Weisenborn, J. W. Bolger, D. B. Rosen, L. T. Mann, Jr., L. Johnson and H. L. Holmes, THIS JOURNAL, **76**, 1792 (1954).

CHART 1



II III IV V

lent of chromic acid. Acetylation of the latter compound gave protoverine 15-(*l*)-2′-methylbutyrate 3,6,7,16-tetraacetate (VII) which was identical with the product of acetylation of the protoverine mono-(*l*)-2′-methylbutyrate obtained by methanolysis.

TABLE I

PERIODATE OXIDATIONS

Substrate	Mole equivalents of sodium periodate consumed[a] (hr.)
Protoveratrine A (II)	0.1(2), 0.2(24)
Protoverine 15-(*l*)-2′-methylbutyrate (VI)	1.8(2.5), 1.9(6)
Protoverine 15-(*l*)-2′-methylbutyrate 3,6,16-triacetate (IX)	0.03(6)
Protoverine 3-(*d*)-2′-hydroxy-2′-methylbutyrate 15-(*l*)-2′-methylbutyrate (X)	0.8(2.25), 0.9(4.5)
Protoverine 6,15-diisobutyrate[18]	1.1(1.75), 1.4(4.0)
Protoverine 3-(*d*)-2′-hydroxy-2′-methylbutyrate 4-isobutyrate 15-(*l*)-2′-methylbutyrate (XIV)	1.1(2.75), 1.2(4.75)
Protoveratrine B (XV)	0.9(2.3)
Protoveratrine B tosylate (XVI)	0.0(5)
Protoverine 6,7-diacetate 15-(*l*)-2′-methylbutyrate (XVIII)	1.0(1)

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

A diester of protoverine, resulting from loss of both acetate groups, was also isolated from the methanolysis of protoveratrine A. Acetylation of this protoverine 15-(*l*)-2′-methylbutyrate (*d*)-2′-hydroxy-2′-methylbutyrate, m.p. 203–205° dec., $[\alpha]^{23}D$ −19° (pyr.), afforded protoveratrine A monoacetate (XI). The diester consumed 0.9 mole equivalent of sodium periodate and the infrared spectrum of the amorphous oxidation product did *not* show the absorption characteristic of the γ-lactone formed by periodate cleavage of ring A of protoverine derivatives (*cf.* ref. 1). Thus the diester is protoverine 3-(*d*)-2′-hydroxy-2′-methylbutyrate 15-(*l*)-2′-methylbutyrate (X). This assignment was supported by a comparison of periodate–cyanometric titrations[17] on protoverine 6,15-diisobuyrate[18] and the diester obtained by methanolysis of protoveratrine A. The former compound consumed one mole of potassium cyanide in keeping with the single aldehyde produced from cleavage in ring A, while the latter compound consumed 1.6 mole equivalents of potassium cyanide, suggesting the presence of a dialdehyde arising from scission between C_6 and C_7 (X → XIII).

TABLE II

CHROMIC ACID TITRATIONS

Substrate	Oxygen equivalents of chromic acid consumed[a] (hr.)
Protoveratrine A (II)	0.9(1.2), 1.2(3.5)
Protoveratrine A 16-isobutyrate (IV)	0.1(3.5)
Protoverine 15-(*l*)-2′-methylbutyrate 3,6,16-triacetate (IX)	1.1(1.5), 1.2(2.5)
Protoveratrine B (XV)	2.1(1.0), 2.1(3.0)
Protoveratrine B tosylate (XVI)	1.0(1.0), 1.1(4.0)

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

Confirmation of the position of the 2-hydroxy-2-methylbutyric acid came from the following sequence. Protoveratrine A was converted to proto-

(17) J. R. Dyer in David Glick, "Methods of Biochemical Analysis," Interscience Publishers, Inc., New York, N. Y., Vol. III, 1956, p. 132.

(18) S. M. Kupchan and R. H. Hensler, unpublished results.

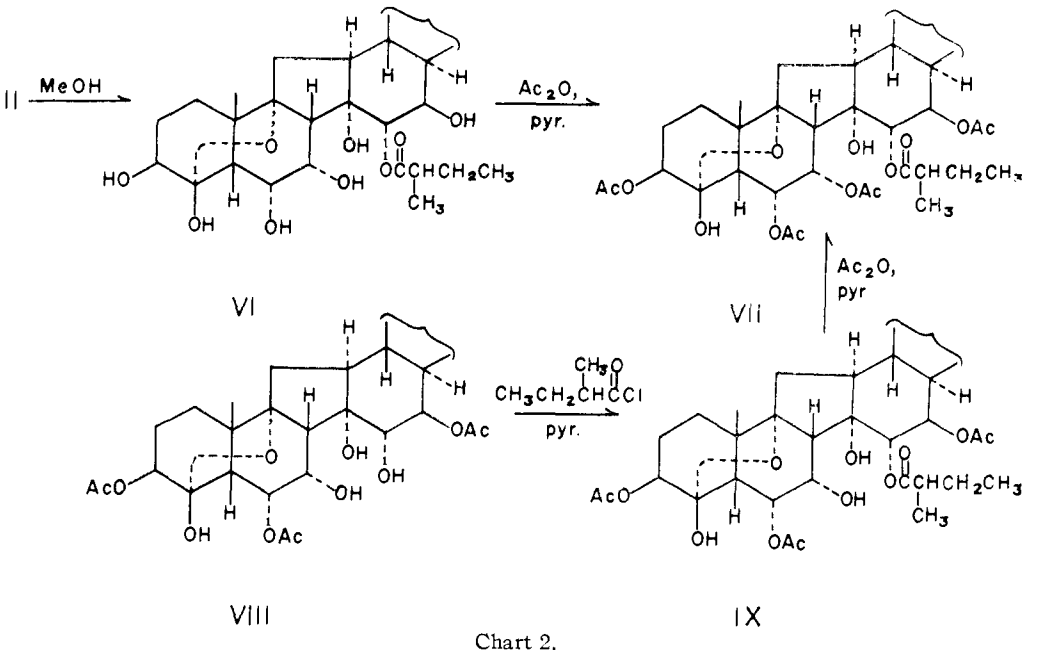


Chart 2.

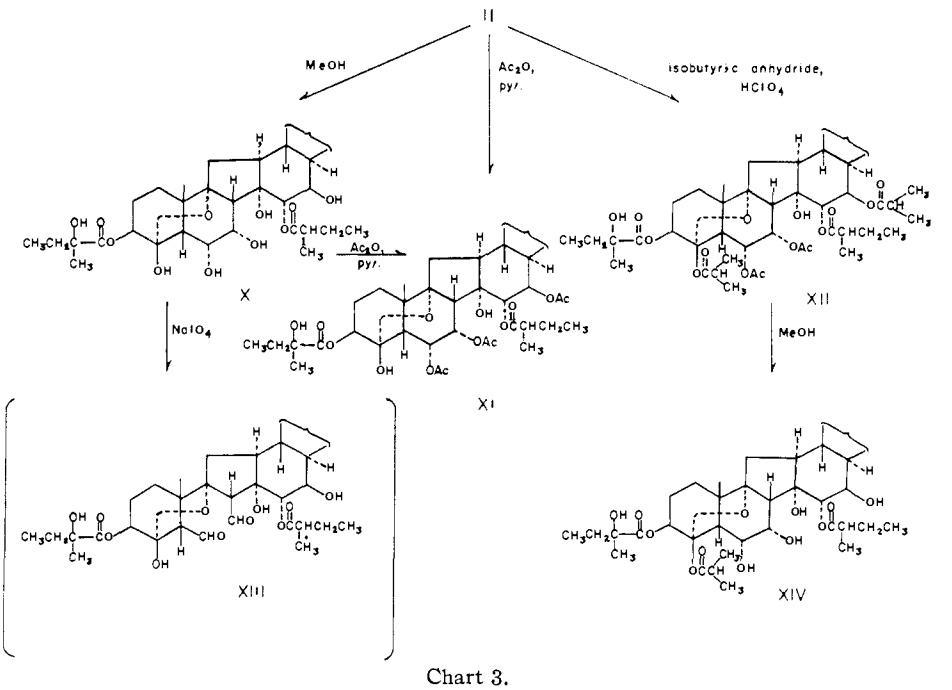


Chart 3.

veratrine A 4,16-diisobutyrate (XII), m.p. 140°, $[\alpha]^{26}D$ −29° (pyr.), which on methanolysis (of the labile 16-isobutyrate and acetates) afforded protoverine 4-isobutyrate 15-(*l*)-2′-methylbutyrate 2′-hydroxy-2′-methylbutyrate (XIV), m.p. 140°, $[\alpha]^{25}D$ −8° (pyr.). If the hydroxybutyric acid were attached to either C_6 or C_7, the triester would be stable toward sodium periodate. In fact, the triester consumed 1.1 mole equivalents of sodium periodate, thereby demonstrating that the hydroxybutyric acid was attached to the C_3-hydroxyl group.

Thus protoveratrine A is protoverine 3-(*d*)-2′-hydroxy-2′-methylbutyrate 6,7-diacetate 15-(*l*)-2′-methylbutyrate (II).

A chromic acid titration of protoveratrine B showed the presence of two secondary hydroxyl groups, an indication that the C_4-hydroxyl group is not acylated. Protoveratrine B readily formed a monotosylate (XVI), m.p. 214–217° dec., $[\alpha]^{22}D$ −22° (pyr.). which was stable toward sodium periodate but consumed 1.1 oxygen equivalents of chromic acid. The oxidation product, dehydroprotoveratrine B monotosylate (XVII), m.p. 194–197° dec., $[\alpha]^{22}D$ −66° (pyr.), on alkaline hydrolysis afforded a diosphenol whose spectral properties were identical with those of the diosphenol from protoveratrine A. Thus in protoveratrine B, the C_{16}-hydroxyl group is not acylated.

Vigorous methanolysis of protoveratrine B af-

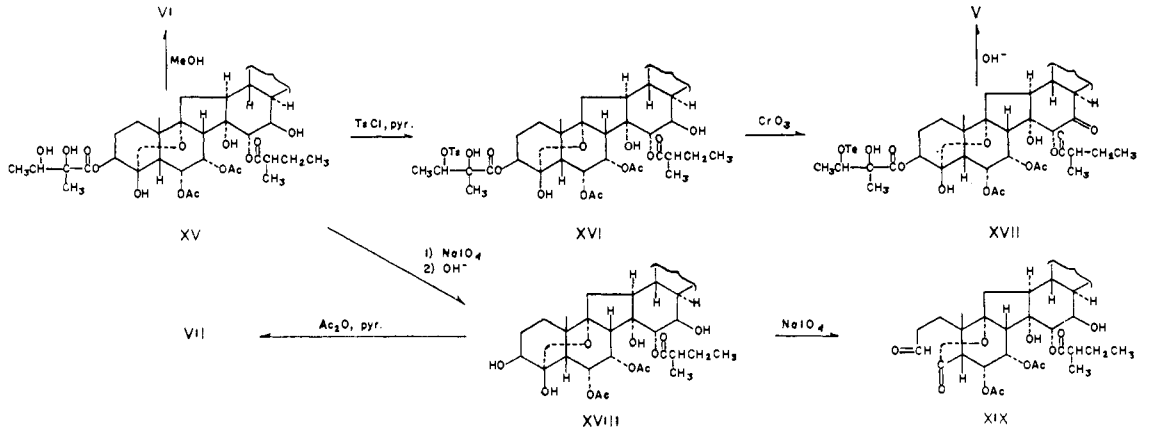


Chart 4.

forded a protoverine mono-(*l*)-2′-methylbutyrate which was found to be identical with protoverine 15-(*l*)-2′-methylbutyrate (VI) previously isolated from the methanolysis of protoveratrine A.

It was anticipated that in a molecule where both acetate groups are attached to the protoverine nucleus, the dihydroxybutyric acid residue would be subject to attack by sodium periodate and that cleavage of the dihydroxybutyric acid residue would result in the formation of a pyruvate ester. The extremely facile alkaline hydrolysis of pyruvate esters[19] suggested the possibility of selective removal of this ester grouping. In fact, protoveratrine B consumed 0.9 mole equivalent of sodium periodate and the normal dilute ammonia workup of the oxidation led to the formation of a protoverine 15-(*l*)-2′-methylbutyrate diacetate, m.p. 232–233° dec., $[\alpha]^{26}D$ −46° (pyr.). Acetylation of this compound gave protoverine 15-(*l*)-2′-methylbutyrate 3,6,7,16-tetraacetate (VII). On sodium periodate oxidation, the protoverine 15-(*l*)-2′-methylbutyrate diacetate (XVIII) consumed 1.0 mole equivalent. The oxidation product XIX, m.p. 241–242° dec., $[\alpha]^{26}D$ +17° (pyr.), showed absorption at 3.65, 5.62 and 5.80 μ, characteristic of the aldehydo-γ-lactone produced by periodate cleavage in ring A of protoverine derivatives (*cf.* ref. 1).

Thus protoveratrine B is protoverine 3-(*d*)-*threo*-2′,3′-dihydroxy-2′-methylbutyrate 6,7-diacetate 15-(*l*)-2′-methylbutyrate (XV).

Experimental[20]

Protoveratrone A (III).—Protoveratrine A (II, 4 g., m.p. 273–275° dec.) was treated with carbon tetrachloride (95 ml.), acetic acid (5 ml.) and 0.66 *N* chromium trioxide in 98.5% acetic acid (50 ml.). The mixture was allowed to stand at room temperature for 19 hours and then was cooled in an ice-bath. Aqueous sodium bisulfite solution was added to destroy the excess of the oxidizing agent, the mixture was made alkaline 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 aqueous ethanol as needles, 1.4 g., m.p. 217–219° dec. Recrystallization from aqueous ethanol afforded colorless needles, 1.2 g., m.p. 221–223° dec., $[\alpha]^{25}D$ −97° (*c* 1.18, pyr.).

Anal. Calcd. for $C_{41}H_{61}O_{14}N$: C, 62.18; H, 7.76. Found: C, 62.24; H, 8.04.

Alkaline Treatment of Protoveratrone A.—A solution of protoveratrone A (III, 200 mg., m.p. 217–219° dec.) in methanol (25 ml.) was treated with 50% aqueous sodium hydroxide (0.4 ml.) and heated under reflux for 10 minutes. The deep red solution was acidified with acetic acid and evaporated under reduced pressure. The oily residue was treated with water (5 ml.) and dilute ammonium hydroxide to *p*H 8.5 and extracted with chloroform. The powdery residue obtained by evaporation of the chloroform showed λ_{max} 328 mμ (ϵ 15,000), $\lambda_{max}^{0.1\ N\ NaOH}$ 380 mμ (ϵ 12,000).

Protoveratrine A Monoacetate (XI).—A solution of protoveratrine A (II, 1 g., m.p. 270–271° dec.) in pyridine (5 ml.) was treated with acetic anhydride (5 ml.) and warmed on the steam-bath for 90 minutes. The excess of acetic anhydride was decomposed by cautious addition of methanol (5 ml.) and the solution was evaporated to dryness under reduced pressure. The residue was treated with water (3 ml.) and ammonium hydroxide (to *p*H 8.5) and the solution was 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 colorless prisms, 540 mg., m.p. 244–245° dec. A sample was recrystallized from acetone–petroleum ether; m.p. 249–250° dec., $[\alpha]^{22}D$ −52° (*c* 1.07, pyr.).

Anal. Calcd. for $C_{43}H_{65}O_{15}N$: C, 61.77; H, 7.84. Found: C, 61.82; H, 7.78.

In a volatile acid determination[21] 30.90 mg. of the compound yielded an amount of acid equivalent to 23.95 ml. of 0.006452 *N* sodium thiosulfate; calcd. for 3 mole equivalents of acetic acid and 1 mole equivalent of (*l*)-2-methylbutyric acid as expected for structure XI, 22.91 ml.[22]

Protoveratrine A 16-Isobutyrate (IV).—A solution of protoveratrine A (II, 2 g., m.p. 270–271° dec.) in pyridine (9 ml.) was treated with isobutyric anhydride (9 ml.), warmed on the steam-bath for 3 hours, and allowed to stand overnight at room temperature. Methanol (25 ml.) was added to decompose the excess of the anhydride and the solution was evaporated nearly to dryness under reduced pressure. The residue was dissolved in water, made alkaline with dilute ammonium hydroxide, and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate

(19) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 212.

(20) 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°. The sodium periodate and chromic acid titrations were performed as in reference 1.

(21) J. B. Niederl and V. Niederl, "Micromethods of Quantitative Organic Analysis," John Wiley and Sons, Inc., New York, N. Y., 1942, pp. 257–262.

(22) In a volatile acid determination using the same procedure, 20.15 mg. of protoveratrine A yielded an amount of acid equivalent to 14.05 ml. of 0.005053 *N* sodium thiosulfate; calcd. for 2 mole equivalents of acetic acid and 1 mole equivalent of (*l*)-2-methylbutyric acid, 15.04 ml. It is apparent that (*d*)-2-hydroxy-2-methylbutyric acid is non-volatile under the conditions used in this determination.

and evaporated to yield a resin. A paper chromatogram[23a] indicated the formation of a new material in a yield of about 50%. The resin was chromatographed on Merck acid-washed alumina (50 g.), and 100-ml. fractions were collected. Fraction 1, eluted with 10% chloroform in benzene, yielded a brown oil. Fractions 2–4, eluted with 10% chloroform in benzene, yielded a residue which was crystallized from acetone–petroleum ether as colorless prisms, 470 mg., m.p. 230–233° dec. A sample was recrystallized for analysis from acetone–petroleum ether; m.p. 245–246° dec., $[\alpha]^{21}D$ −41° (*c* 1.36, pyr.).

Anal. Calcd. for $C_{45}H_{69}O_{15}N$: C, 62.55; H, 8.04. Found: C, 62.98; H, 8.03.

In a volatile acid determination[21] 26.00 mg. of the compound yielded an amount of acid equivalent to 13.32 ml. of 0.008480 *N* sodium thiosulfate; calcd. for 2 mole equivalents of acetic acid, 1 mole equivalent of (*l*)-2-methylbutyric acid and 1 mole equivalent of isobutyric acid as expected for structure IV, 14.20 ml.

Further elution of the column afforded fractions which were shown by paper chromatography[23a] to contain starting material.

Methanolysis of Protoveratrine A 16-Isobutyrate.—Protoveratrine A 16-isobutyrate (IV, 4 g., m.p. 230–233° dec.) was dissolved in methanol (100 ml.) and allowed to stand at room temperature for 20 hours. The solution was evaporated under reduced pressure to yield a resin which was crystallized from acetone–petroleum ether to yield starting material (740 mg.). The mother liquor was evaporated to dryness and chromatographed on Merck acid-washed alumina (60 g.). The column yielded to benzene, 10% chloroform–benzene, 20% chloroform–benzene, 30% chloroform–benzene and 40% chloroform–benzene, a series of orange oils. To 50% chloroform–benzene and 75% chloroform–benzene, the column yielded starting material (1.2 g.). Further development of the column with chloroform afforded fractions which were shown by paper chromatography[23a] to be mixtures of protoveratrine A and a compound which was probably a desacetylprotoveratrine A 16-isobutyrate. From the mixtures, protoveratrine A (400 mg., m.p. 260–262° dec.) was obtained by fractional crystallization from acetone–petroleum ether.

Methanolysis of Protoveratrine A.—Protoveratrine A (II, 3 g., m.p. 270–271° dec.) was dissolved in methanol (250 ml.) and the solution was heated under reflux for 26 hours. The solution was evaporated to dryness to yield a resin which was chromatographed on Merck acid-washed alumina (100 g.). The column yielded to chloroform, a yellow oil; to 1% methanol in chloroform, a colorless resin (660 mg.) which was crystallized from ether as colorless prisms, 350 mg., m.p. 203–204° dec. A sample was recrystallized from ether for analysis; m.p. 203–205° dec., $[\alpha]^{23}D$ −19° (*c* 1.07, pyr.).

Anal. Calcd. for protoverine 3-(*d*)-2′-hydroxy-2′-methylbutyrate 15-(*l*)-2′-methylbutyrate (X) monohydrate, $C_{37}H_{59}O_{12}N \cdot H_2O$: C, 61.04; H, 8.43; (*l*)-2-methylbutyryl, 11.65. Found: C, 60.97; H, 8.49; (*l*)-2-methylbutyryl, 11.32.

A further quantity (400 mg.) of this diester was obtained from the fraction eluted with 2% methanol–chloroform.

The column yielded to 5% methanol–chloroform a series of mixtures, but with 10% methanol–chloroform a homogeneous (by paper chromatography[23b]) compound (300 mg.) was obtained which was crystallized from acetone–petroleum ether as needles, 170 mg., m.p. 214–216° dec. A sample was recrystallized from acetone–petroleum ether, m.p. 218–220° dec., $[\alpha]^{23}D$ −18° (*c* 0.97, pyr.).

Anal. Calcd. for protoverine 15-(*l*)-2′-methylbutyrate (VI), $C_{32}H_{51}O_{10}N$: C, 63.02; H, 8.43; (*l*)-2-methylbutyryl, 13.9. Found: C, 62.64; H, 8.41; (*l*)-2-methylbutyryl, 13.1.

Acetylation of Protoverine 15-(*l*)-2′-Methylbutyrate.—Protoverine 15-(*l*)-2′-methylbutyrate (VI, 300 mg., m.p. 214–216° dec.) was treated in pyridine (2 ml.) and acetic anhydride (2 ml.) and warmed on a steam-bath for 90 minutes. The solution was cooled in ice, made alkaline 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 (10 g.). The column yielded to benzene, 20% chloroform–benzene and 40% chloroform–benzene, yellow oils. The 80% chloroform–benzene and 100% chloroform fractions afforded a white solid which was crystallized from acetone–petroleum ether as needles, 250 mg., m.p. 261–262° dec. The melting point was not depressed on admixture with protoverine 15-(*l*)-2′-methylbutyrate 3,6,7,16-tetraacetate (VII) prepared from protoverine 3,6,16-triacetate (VIII). The infrared spectra and paper chromatographic[23a] behavior of the respective samples were identical.

Protoverine 15-(*l*)-2′-Methylbutyrate 3,6,16-Triacetate.—A solution of protoverine 3,6,16-triacetate[1] (VIII, 2 g., m.p. 235–238° dec.) in pyridine (10 ml.) was treated at ice temperature with (*l*)-2-methylbutyryl chloride[24] (0.5 ml.). The solution was allowed to stand at room temperature for 20 hours and was then cooled in ice, made alkaline 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 25% chloroform–benzene, a yellow oil; to 50% chloroform–benzene, a resin (560 mg.). A paper chromatogram[23a] indicated that the mixture probably consisted principally of a di-(*l*)-2′-methylbutyrate and a mono-(*l*)-2′-methylbutyrate. The column yielded to 75% chloroform–benzene and to chloroform a resin (1.05 g.) which was crystallized from acetone–petroleum ether as clusters of needles 800 mg., m.p. 230–232° dec. A paper chromatogram[23a] indicated that the crystalline material was homogeneous. A sample was recrystallized for analysis from acetone–petroleum ether as needles, m.p. 234–235° dec., $[\alpha]^{23}D$ −4° (*c* 0.98, pyr.).

Anal. Calcd. for $C_{38}H_{57}O_{13}N$: C, 62.02; H, 7.82. Found: C, 61.85; H, 7.91.

In a volatile acid determination[21] 21.60 mg. of the compound yielded an amount of acid equivalent to 20.60 ml. of 0.005345 *N* sodium thiosulfate; calcd. for 3 mole equivalents of acetic acid and 1 mole equivalent of (*l*)-2-methylbutyric acid as expected for structure IX, 21.98 ml.

Protoverine 15-(*l*)-2′-Methylbutyrate 3,6,7,16-Tetraacetate (VII).—A solution of protoverine 15-(*l*)-2′-methylbutyrate 3,6,16-triacetate (IX, 225 mg., m.p. 230–232° dec.) in pyridine (1 ml.) was treated with acetic anhydride (1 ml.) and warmed on the steam-bath for 90 minutes. The solution was cooled in ice, made alkaline with dilute ammonium hydroxide, and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and chromatographically filtered through a short column of Merck acid-washed alumina (0.5 g.) to remove a colored impurity. The chloroform solution was evaporated to yield a resin which was crystallized from acetone–petroleum ether as needles, 150 mg., m.p. 262–263° dec., $[\alpha]^{23}D$ −46° (*c* 1.10, pyr.).

Anal. Calcd. for $C_{40}H_{59}O_{14}N$: C, 61.75; H, 7.65. Found: C, 61.80, H, 7.74.

In a volatile acid determination[21] 17.46 mg. of compound yielded an amount of acid equivalent to 19.65 ml. of 0.005345 *N* sodium thiosulfate; calcd. for 4 mole equivalents of acetic acid and 1 mole equivalent of (*l*)-2-methylbutyric acid as required for structure VII, 20.99 ml.

Acetylation of Protoverine 3-(*d*)-2′-Hydroxy-2′-methylbutyrate 15-(*l*)-2′-Methylbutyrate.—A solution of protoverine 3-(*d*)-2′-hydroxy-2′-methylbutyrate 15-(*l*)-2′-methylbutyrate (X, 130 mg., m.p. 203–206° dec.) in pyridine (1 ml.) was treated with acetic anhydride (1 ml.) and heated on the steam-bath for 90 minutes. The solution was cooled in ice, made alkaline 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 colorless prisms, 43 mg., m.p. 244–246° dec. The melting point was not depressed on admixture of protoveratrine A monoacetate (XI). The paper chromatographic behavior[23a] and infrared spectra of the respective samples were identical.

(23) 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.).

(24) F. L. Weisenborn, J. W. Bolger, D. B. Rosen, L. T. Mann, L. Johnson and H. L. Holmes, THIS JOURNAL, **76**, 1792 (1954).

Periodate–Cyanometric Titrations.—The method used was essentially that described by Dyer.[25] The alkaloid (30 mg.) was dissolved in 5% acetic acid (2 ml.) and treated with 0.08 *M* sodium periodate (2 ml.). After 2.5 hours at room temperature, the solution was treated with 0.1 *N* sodium arsenite (4 ml.), saturated aqueous sodium bicarbonate (5 ml.) and a crystal of potassium iodide. After 10 minutes, the solution was titrated with 0.1 *N* iodine solution to determine the amount of sodium periodate consumed. To the reaction mixture, 0.1 *N* potassium cyanide solution (2 ml.) was added and the tightly stoppered flask allowed to stand at ice temperature for 24 hours. The mixture was titrated with 0.1 *N* iodine solution to determine the amount of cyanide consumed. In every case, a blank containing 30 mg. of protoverine 3,6,7,15,16-pentaacetate[1] was used. The results are shown in Table III (the values represent three separate runs).

TABLE III

Substrate	Mole equivalents of sodium periodate consumed	Mole equivalents of cyanide consumed
Protoverine 6,15-diisobutyrate	1.1, 1.1, 0.9	1.0, 1.1, 0.9
Protoverine 3-(*d*)-2′-hydroxy-2′-methylbutyrate 15-(*l*)-2′-methylbutyrate	1.0, 0.9, 0.9	1.6, 1.6, 1.6

Protoveratrine A Diisobutyrate (XII).[26]—A suspension of protoveratrine A (II, 2 g., m.p. 270–271° dec.) in isobutyric anhydride (20 ml.) was treated at ice temperature with 60% perchloric acid (0.5 ml.). The resulting solution, which rapidly discolored, was allowed to stand at room temperature for 14 hours. The dark brown solution was cautiously treated with methanol (100 ml.) to destroy the excess of anhydride and evaporated nearly to dryness under reduced pressure. The oily residue was dissolved in water, made alkaline with dilute ammonium hydroxide, and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and passed through a short column of Merck acid-washed alumina (1 g.) to remove some of the colored impurities. The chloroform was evaporated to yield a light brown sirup which was crystallized from petroleum ether as needles. Recrystallization from acetone and a large volume of petroleum ether afforded needles (360 mg.) which sintered at 140–143° and decomposed at 210–215°, $[\alpha]^{26}D$ −39° (*c* 1.10, pyr.).

Anal. Calcd. for $C_{49}H_{75}O_8N$: C, 63.00; H, 8.09. Found: C, 62.47; H, 8.31.

In a volatile acid determination[21] 12.26 mg. of the compound yielded an amount of acid equivalent to 13.85 ml. of 0.004908 *N* sodium thiosulfate; calcd. for 2 mole equivalents of acetic acid, 1 mole equivalent of (*l*)-2-methylbutyric acid and 2 mole equivalents of isobutyric acid, as expected for structure XII, 13.40 ml.

Methanolysis of Protoveratrine A 4,16-Diisobutyrate: Protoverine 3-(*d*)-2′-Hydroxy-2′-methylbutyrate 4-Isobutyrate 15-(*l*)′-Methylbutyrate (XIV).[26]—Protoveratrine A 4,16-diisobutyrate (XII, 3.3 g., m.p. 140–143°) was dissolved in methanol (30 ml.) and allowed to stand at room temperature for 50 days. The methanol was evaporated and the residue was chromatographed on Merck acid-washed alumina (70 g.). The column yielded to benzene and to mixtures of benzene–chloroform a series of resins which were shown to be non-homogeneous by paper chromatography.[23a] The column yielded to chloroform a resin which was crystallized from ether as long, colorless needles, 970 mg., m.p. fused at 140–150° to colorless melt which decomposed at 220–230°, $[\alpha]^{25}D$ −8° (*c* 1.20, pyr.). A paper chromatogram[23a] indicated that the product was homogeneous.

Anal. Calcd. for $C_{41}H_{65}O_{13}N$: C, 63.13; H, 8.40. Found: C, 62.89; H, 8.56.

In a volatile acid determination[21] 15.10 mg. of the compound yielded an amount of acid equivalent to 8.08 ml. of 0.004908 *N* sodium thiosulfate; calcd. for 1 mole equivalent of isobutyric acid and 1 mole equivalent of (*l*)-2-methylbutyric acid as expected for structure XIV, 7.95 ml.

Protoveratrine B Monotosylate (XVI).—Protoveratrine B (XV, 2 g.), m.p. 267–269° dec.), was dissolved in pyridine (20 ml.), cooled to 0° and tosyl chloride (2 g.) was added portionwise. The light yellow solution was allowed to stand overnight at room temperature, made alkaline with ice-cold ammonium hydroxide, and extracted with chloroform. The chloroform solution was dried over anhydrous sodium sulfate and evaporated to yield a resin which was crystallized from acetone–petroleum ether as colorless prisms (1.35 g.), m.p. 214–219° dec. A second crop of prisms, 350 mg., m.p. 213–219° dec., was also obtained. A sample of the first crop was recrystallized for analysis from acetone–petroleum ether, m.p. 214–217° dec., $[\alpha]^{22}D$ −22° (*c* 1.13, pyr.).

Anal. Calcd. for $C_{48}H_{71}O_{17}NS$: C, 59.68; H, 7.39; S, 3.31. Found: C, 59.74; H, 7.31.; S, 3.32.

In a volatile acid determination[21] 18.28 mg. of the tosylate yielded an amount of acid equivalent to 10.00 ml. of 0.005053 *N* sodium thiosulfate; calcd. for 2 mole equivalents of acetic acid and 1 mole equivalent of (*l*)-2-methylbutyric acid as expected for structure XVI, 9.81 ml.

Chromic Acid Oxidation of Protoveratrine B Monotosylate.—Protoveratrine B monotosylate (XVI, 400 mg., m.p. 215–218° dec.) was dissolved in glacial acetic acid (5 ml.), treated with 0.66 *N* chromium trioxide in 98.5% acetic acid (10 ml.), and allowed to stand at room temperature for 75 minutes. The mixture was cooled in ice and aqueous sodium bisulfite was added to destroy the excess of oxidizing agent. Dilute ammonium hydroxide was added until the solution was alkaline, and the alkaloid was extracted with chloroform. The chloroform solution was dried over anhydrous sodium sulfate and evaporated to yield a resin which was crystallized from 95% ethanol to give colorless needles, 300 mg., m.p. 190–192° dec. A paper chromatogram[23a] indicated the compound was homogeneous. A sample was crystallized from 95% ethanol for analysis; m.p. 194–197° dec., $[\alpha]^{22}D$ −66° (*c* 1.13, pyr.).

Anal. Calcd. for $C_{48}H_{69}O_{17}NS$: C, 59.80; H, 7.21; S, 3.31. Found: C, 59.87; H, 7.01; S, 3.32.

In a volatile acid determination[21] 14.25 mg. of the compound yielded an amount of acid equivalent to 9.00 ml. of 0.005053 *N* sodium thiosulfate; calcd. for 2 mole equivalents of acetic acid and 1 mole equivalent of (*l*)-2-methylbutyric acid as expected for structure XVII, 8.77 ml.

Alkaline Treatment of Dehydroprotoveratrine B Monotosylate.—A solution of dehydroprotoveratrine B monotosylate (XVII, 50 mg., m.p. 190–192° dec.) in methanol (5 ml.) was treated with 40% aqueous sodium hydroxide (0.1 ml.) and heated under reflux for 12 minutes. The resulting red solution was acidified with acetic acid and evaporated under reduced pressure. The oily residue was treated with water (2 ml.) and dilute ammonium hydroxide to *p*H 8.5 and extracted with chloroform. The residue obtained by evaporation of the chloroform extract was taken up in ether (50 ml.) and filtered from a small amount of highly colored insoluble material. The clear ethereal filtrate was evaporated to give a brown powder, 13 mg., λ_{max} 328 mμ (ε 13,400), $\lambda_{max}^{0.1N\ NaOH}$ 380 mμ (ε 8,300).

Methanolysis of Protoveratrine B.—A solution of protoveratrine B (XV, 2 g., m.p. 267–269° dec.) in methanol (150 ml.) was heated under reflux for 24 hours. The methanol was evaporated and the residue was chromatographed on Merck acid-washed alumina (50 g.). The column yielded to chloroform, 1% methanol–chloroform, 2% methanol–chloroform and 10% methanol–chloroform a series of resins which were combined and crystallized from acetone–petroleum ether; yield 388 mg., m.p. 218–220° dec. The melting point was not depressed on admixture with protoverine 15-(*l*)-2′-methylbutyrate (VI). The infrared spectra and paper chromatographic behavior[23b] of the respective samples were identical.

Sodium Periodate Oxidation of Protoveratrine B. Protoverine 6,7-Diacetate 15-(*l*)-2′-Methylbutyrate (XVIII).—A solution of protoveratrine B (XV, 700 mg., m.p. 267–269° dec.) in 5% acetic acid (15 ml.) was treated with 0.08 *M* sodium periodate (38 ml.) and allowed to stand at room temperature for 5 hours. The solution was cooled in ice, made alkaline 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 (15 g.). The column yielded to benzene a yellow oil and to 50% chloroform–benzene a resin. Elution with chloroform gave a white solid which was crystallized from acetone–petroleum ether as needles, 300 mg., m.p. 233–236° dec.

(25) J. R. Dyer in David Glick, "Methods of Biochemical Analysis," Vol. III, Interscience Publishers, Inc., New York, N. Y., 1956, p. 132.

(26) Experiment by Dr. R. H. Hensler.

A sample was recrystallized for analysis from acetone–petroleum ether as needles, m.p. 232–233° dec., $[\alpha]^{26}D$ −46° (*c* 0.95, pyr.).

Anal. Calcd. for $C_{36}H_{55}O_{12}N$: C, 62.32; H, 7.99. Found: C, 62.05; H, 8.15.

In a volatile acid determination[21] 26.84 mg. of the compound gave an amount of acid equivalent to 21.70 ml. of 0.005172 *N* sodium thiosulfate; calcd. for 2 mole equivalents of acetic acid and 1 mole of (*l*)-2-methylbutyric acid as expected for structure XVIII, 22.50 ml.

Acetylation of Protoverine 6,7-Diacetate 15-(*l*)-2′-Methylbutyrate.—A solution of protoverine 6,7-diacetate 15-(*l*)-2′-methylbutyrate (XVIII, 85 mg., m.p. 223–225° dec.) in pyridine (2 ml.) was treated with acetic anhydride (2 ml.) and the solution was heated on the steam-bath for 90 minutes. The solution was cooled in ice, made alkaline 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 (45 mg.), m.p. 261–262° dec. The melting point was not depressed on admixture with protoverine 15-(*l*)-2′-methylbutyrate 3,6,7,16-tetraacetate (VII). The infrared spectra and paper chromatographic behavior[23a] of the respective samples were identical.

Sodium Periodate Oxidation of Protoverine 6,7-Diacetate 15-(*l*)-2′-Methylbutyrate.—A solution of protoverine 6,7-diacetate 15-(*l*)-2′-methylbutyrate (XVIII, 1.45 g.), m.p. 232–233° dec., in 5% acetic acid (50 ml.) was treated with 0.08 *M* sodium periodate (150 ml.) and allowed to stand at room temperature for 150 minutes. The solution was cooled in ice, made alkaline with 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 as rods (800 mg.), m.p. 240–241° dec. A sample was recrystallized for analysis from acetone–petroleum ether as rods, m.p. 241–242° dec., $[\alpha]^{26}D$ +17° (*c* 1.12, pyr.); λ_{max} 3.65, 5.62, 5.80 μ.

Anal. Calcd. for $C_{36}H_{53}O_{12}N$: C, 62.49; H, 7.72. Found: C, 62.38; H, 7.89.

In a volatile acid determination[21] 24.12 mg. of the compound yielded an amount of acid equivalent to 10.50 ml. of 0.009388 *N* sodium thiosulfate; calcd. for 2 mole equivalents of acetic acid and 1 mole equivalent of *l*-(2)-methylbutyric acid as expected for structure XIX, 11.10 ml.

MADISON 6, WIS.

[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

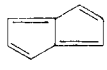
The Synthesis of a Pyrrole Acrylic Lactam

BY WILLIAM C. AGOSTA[1]

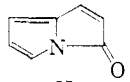
RECEIVED AUGUST 27, 1959

The synthesis of the lactam VI is described. Properties of this compound are discussed in connection with the possibility of an "azapentalene" type structure (*cf.* II ⟷ III).

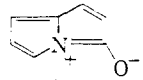
In recent years numerous efforts have been made to prepare the unknown bicyclo[3.3.0]-octatetraene (I), commonly known as pentalene, or simple derivatives of this system.[2] The compounds which have been synthesized seem to indicate that no special aromatic stability is associated with the pentalene system of unsaturation.[3–5]



I



II



III

We considered it interesting to investigate the properties of a compound containing the system of II, which is the lactam of pyrrole-2-(β-acrylic acid). In this lactam three of the four pentalene (or in this case "azapentalene") double bonds are present in the well-known vinylpyrrole moiety; the fourth should be formed, should the azapentalene structure be energetically favored, by interaction of the unshared pair of electrons on nitrogen with the adjacent carbonyl group, giving, as the extreme structure, III. Such interaction is of course normal in amides and accounts for the resonance stabilization and planar structure of this functional group.[6]

To synthesize a molecule incorporating the desired features[7] we started with the readily accessible 2,4-dimethyl-3-carbethoxy-5-formylpyrrole (IV).[8] This was condensed with malonic acid in a Knoevenagel reaction to give the α,β-unsaturated diacid V, as reported by Küster.[9] We found that this substance could be cyclized and decarboxylated in rather low yield (20–37%) by treatment with re-

(1) Department of Chemistry, University of California, Berkeley 4, Calif.

(2) For a review of this work see W. Baker and J. F. W. McOmie, in *Prog. in Org. Chem.*, **3**, 68 (1955).

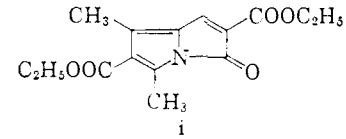
(3) C. T. Blood and R. P. Linstead, *J. Chem. Soc.*, 2263 (1952).

(4) H. J. Dauben, V. R. Ben and S. H.-K. Chiang, Abstracts of Papers, 123rd Meeting, Am. Chem. Soc., Los Angeles, Calif., March, 1953, p. 9-M.

(5) Since the completion of this work the syntheses of a benzazapentalene, a dibenzazapentalene and two naphthbenzazapentalenes have been announced by W. Treibs (*Naturwiss.*, **46**, 170 (1959)). These interesting compounds are briefly reported to possess "all properties of non-benzenoid aromatics,"

(6) (a) L. Pauling, "The Nature of the Chemical Bond," 2nd edition, Cornell University Press, Ithaca, N. Y., 1948, p. 133; (b) R. B. Woodward, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 443; (c) S. Mizushima, T. Simanouti, S. Nagakura, K. Kuratani, M. Tsuboi, H. Baba and H. Fujioka, THIS JOURNAL, **72**, 3490 (1950); (d) R. J. Kurland, *J. Chem. Phys.*, **23**, 2202 (1955).

(7) Over thirty years ago Küster described the preparation of a pyrrole acrylic lactam closely related to the one presented here (W. Küster, E. Brudi and G. Koppenhöfer, *Ber.*, **58**, 1014 (1925)). A compound obtained by refluxing an absolute methanolic solution of the diacid V for 60 hours was assigned structure i on the basis of carbon and hydrogen analysis, molecular weight determination and its lack of acidic properties. It was isolated as yellow platelets on cooling the reaction mixture. We have attempted unsuccessfully to repeat this reaction both under the original conditions and with organic acid catalysis. Infrared examination of the reaction mixtures gave no evidence of the reported product. Further it seems to us unlikely that the crystalline material reported by Küster could have this structure (i). We would not expect lactamization and esterification of the malonic acid to proceed in absolute methanol to give the 60% yield recorded.



i

(8) See Experimental.

(9) See reference in footnote 7.