ALKALOIDS OF *Veratrum lobelianum* VERDININE AND 3,15-DI-O-(2-METHYLBUTYROYL)GERMINE

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The results are given of an investigation of the alkaloid composition of the epigeal part of Veratrum lobelianum *Bernh. Veralomidine, rubijervine, germinaline, and the new bases verdinine (1) and 3,15-di-O-(2-methylbutyroyl)germine (2) were isolated. The structures of (1) and (2) have been established on the basis of their physicochemical properties and transformations. This is the first time that veralomidine has been isolated from a plant.*

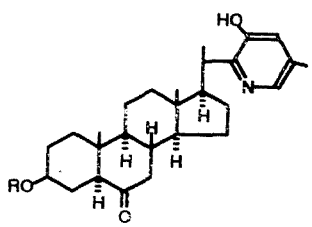
Continuing a study of the alkaloid composition of the epigeal part of the plant *Veratrum lobelianum* [1], from the total alkaloids we have isolated veralomidine, rubijervine, and germinaline [2-4], and the new bases verdinine (**1**) and 3,15-di-O-(2-methylbutyroyl)germine (**2**). The known alkaloids were identified from their physicochemical constants and spectral characteristics, and also by comparison with authentic specimens. This is the first time that veralomidine has been isolated from the plant.

Verdinine (**1**) has the composition $C_{29}H_{41}NO_4$ (M^+ 467). The IR spectrum (KBr, ν, cm^{-1}) of the alkaloid contained absorption bands at 3515 (OH), 1715-1740, 1265 (carbonyl and ester groupings), 3045, 1610, 1585, 750 (pyridine ring), 2865, 2970 (—CH_2, —CH_3—) [5].

The PMR spectrum (100 MHz, $CDCl_3$:CD_3OD, ppm, J, Hz) contained singlets at δ 0.66 (CH_3-18), 0.80 (CH_3-19), 2.20 (—CH_3 in an aromatic system), 6.94 (2H, aromatic protons), a doublet at 1.11 (J = 7 Hz, CH_3-21), and a three-proton singlet from the protons of an acetoxy group at 2.00.

The mass-spectrometric fragmentation of (**1**) was similar to that of petisidinine (**3**) [6]. For both bases, the maximum peak was that of an ion with *m/z* 137, formed by the cleavage of the C17—C20 bond and the migration of hydrogen from the C-17 position [7]. The peaks of ions with *m/z* $M^+ - 42$ (8%) and $M^+ - 60$ (42%) were also observed. The molecular mass of (**1**) was 42 a.u. greater than that of (**3**) [6], which again confirmed the presence of an acetic acid residue in (**1**). An intense peak of an ion with *m/z* and the presence of the signal of the protons of the CH_3-27 group in the form of a singlet (2.20 ppm) in place of the usual doublet ($\sim$1.0 ppm) permitted the conclusion that in verdinine ring *F* is aromatic and one tertiary hydroxy group is present in this ring.

On the basis of the facts presented above, it may be assumed that verdinine is a monoacetyl derivative of petisidinine (**3**). In actual fact, when verdinine (**1**) was saponified an amino alcohol identical with petisidine (**3**) was obtained. Consequently, verdinine has the structure of 3β-O-acetylpetisidinine (**1**)



1. R=CO-CH_3
3. R=H

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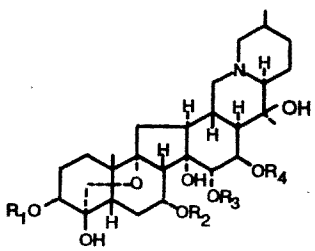
Alkaloid (**2**) had the elementary composition $C_{37}H_{59}NO_{10}$ (M^+ 677.41358), which was determined by the methods of ordinary and high-resolution mass-spectrometric measurements.

The IR spectrum (KBr, ν, cm^{-1}) of (**2**) had absorption bands at 3290-3490 (OH), 2900-2960 (—CH_2—, —CH_3), 2790 2830 (*trans*-quinolizidine) [8], and 1190, 1735 (ester grouping).

The acetylation of (**2**) formed the diacetyl derivative (**4**). The saponification of (**2**) with a methanolic solution of caustic soda gave an amino alcohol identical with germine (**5**) [9, 10], while 2-methylbutyric acid was detected with the aid of paper chromatography.

The PMR spectrum (100 MHz, $CDCl_3$, ppm, J, Hz) of (**2**) showed the signals of the protons of secondary and tertiary methyl groups at δ 0.88 (3H, s, CH_3-19), 1.13 (3H, s, CH_3-21), 1.01 (3H, d, J = 7, CH_3-27) and of gem-protons to acyl groups at 5.27 (1H, d, J=3, H-15) and 5.03 (1H, m, $W_{1/2}$ = 8, H-3), a broadened singlet from a hydroxyl proton at 6.28 (1H, s, OH-4), and signals from protons of hydroxyls and of gem-protons to hydroxy groups in the 3.70-4.60 region. There were the signals of the methyl protons of 2-methylbutyroyl groups at δ 0.84 (6H, t, J = 7, CH_3-4′ and CH_3-4″), 1.07 (6H, d, J = 7, CH_3-5′ and CH_3-5″) [11].

Consequently, alkaloid (**2**) could be an ester alkaloid of the amino alcohol germine and 2-methylbutyric acid. Alkaloid (**2**) is not identical with 15-O-(2-methylbutyroyl)germine (**6**) or 16-O-(2-methylbutyroyl) germine (**7**) [11, 12], since its molecular mass is greater than that of either of these alkaloids by 84 m.u., i.e., by a 2-methylbutyric acid residue.



2. R_1=R_3=CO–CH(CH_3)–C_2H_5, R_2=R_4=H
4. R_1=R_3=CO–CH(CH_3)–C_2H_5, R_2=R_4=$COCH_3$
5. R_1=R_2=R_3=R_4=H
6. R_1=R_2=R_4=H, R_3=CO–CH(CH_3)–C_2H_5
7. R_1=R_2=R_3=H, R_4=CO–CH(CH_3)–C_2H_5

The results given above showed that alkaloid (**2**) is composed of two molecules of 2-methylbutyric acid and the aminoalcohol germine (**5**).

Base (**2**) was not oxidized by periodic acid, which showed the absence of vicinal hydroxy groups. The negative reaction with periodic acid, a doublet at 5.27 ppm (J = 3 Hz), and a multiplet at 5.03 ppm with a half-width $W_{1/2}$ = 8 Hz [13] from protons geminal to alkyl groups showed that in the molecule of this base one 2-methylbutyric acid residue is present at C-3 of germine and the other at C-15.

Thus, alkaloid (**2**) is 3,15-di-O-(2-methylbutyroyl)germine.

EXPERIMENTAL

IR spectra were taken on a UR-20 spectrometer, mass spectra on an MKh-1310 spectrometer fitted with a system for direct injection into the ion source, and PMR spectra on a Tesla BS-567 A spectrometer. Chemical shifts are given relative to the internal standard HMDS on the δ scale.

For TLC and column chromatography we used type KSK silica gel with particle sizes of 40-80 and 125-160 μm, respectively.

Separation of the Total Alkaloids. For the isolation of the total alkaloids from the epigeal part of *Veratrum lobelianum*, see [14]. The mixture of alkaloids was extracted first with 80% alcohol (A) and then with ammoniacal chloroform (B).

The total alkaloids from the alcoholic extract (A) (89.25 g) were dissolved in 5% aqueous acetic acid. The acetic acid solution was treated with a solution of metaphosphoric acid to pH 6 and was separated into sparingly soluble (C) and readily soluble (D) metaphosphate fractions.

The total alkaloids from the ammoniacal chloroform extract (9 g) were chromatographed on a column of silica gel, and eluted with benzene—acetone (4:2). Verdinine (0.03 g) was isolated from the initial fractions of the eluate.

Verdinine (1). mp 265-267°C (acetone).

Mass spectrum (EI, 50 eV) *m/z* (I_{rel}, %): M^+ 467 (25), 452 (4), 451 (8), 450 (17), 425 (M^+ — CH_3CO_2H — H_2O; 8), 424 (21), 407 (M^+ — CH_3CO_2H, 42), 393 (21), 392 (63), 271 (12), 175 (17), 174 (24), 160 (24), 136 (63), 137 (100), 123 (24), 119 (8), 111 (8), 110 (8), 97 (12).

Saponification of Verdinine. A solution of 20 mg of (**1**) in 5 ml of 5% methanolic caustic potash was heated for 3 h. Then it was cooled and diluted with water, and the reaction product was extracted with chloroform. The residue (15 mg) after the chloroform had been distilled off was recrystallized from methanol, giving an amino alcohol identical with petisidinine (**3**).

Veralomidine and Rubijervine. The sparingly soluble metaphosphate fraction (35 g) of the total alkaloids was chromatographed on a column of silica gel, with elution by benzene—acetone (95:5), (80:20), (1:1), and (1:2). The (80:20) eluate yielded veralomidine (0.08 g) with mp 170-172°C (acetone), and the (1:1) eluate yielded rubijervine (0.05 g) with mp 235—237°C (acetone).

Germinaline and 3,15-Di-O-(2-methylbutyroyl)germine (2). The total alkaloids (11 g) from the readily soluble metaphosphate fraction were dissolved in benzene and extracted with 2% acetic acid. The acid solution was made alkaline with ammonia and was extracted with chloroform (6.3 g), and the extracted material was chromatographed on a column of silica gel. Elution was performed with benzene—acetone (4:1) and (3:2), benzene—methanol (5:1), and chloroform—methanol (9:1). The initial fractions from the benzene—acetone (4:1) eluate were rechromatographed on a column of silica gel with elution by benzene—acetone (5:1). This gave 18 fractions. Germinaline (0.031 g), mp 132-134°C (benzene) was isolated from fractions 1-6, and 3,15-di-O-(2-methylbutyroyl)germine (**2**) (0.07 g) from fractions 7-18.

3,15-Di-O-(2-methylbutyroyl)germine (2). mp 214-216°C (benzene), $[\alpha]_D$ −25.15° (*c* 0.676; pyridine).

Mass spectrum (EI, 50 eV), *m/z* (I_{rel}, %): 677 (M^+, 2), 593 ($M^+ - C_2H_5 - CH(CH_3) - CO_2H - H_2O$, 37), 576 (9), 575 ($M^+ - C_2H_5 - CH(CH_3) - CO_2H$, 8), 558 (3), 557 (3), 550 (4), 548 (2), 535 (5), 509 ($M^+ - 2C_2H_5 - CH(CH_3) - CO_2H - 2H_2O$, 2), 492 (7), 491 (6), 473 ($M^+ - 2C_2H_5 - CH(CH_3) - CO_2H$, 8), 471 (4), 456 (3), 454 (2), 448 (2), 182 (2), 180 (2), 164 (2), 162 (2), 154 (4), 150 (2), 138 (3), 136 (2), 125 (2), 112 (100), 111 (37), 98 (29).

HRMS: found 677.41358; calculated for $C_{37}H_{59}NO_{10}$ (M^+) 677.41390.

3,15-Di-O-(2-methylbutyroyl)germine Diacetate (4). Acetic anhydride (2 ml) was added to a solution of 3,15-di-O-(2-methylbutyroyl)germine (**2**) (0.068 g) in pyridine (2 ml), and the mixture was heated in the water bath for 1.5 h. After evaporation of the solvent in vacuum, the residue was treated with ammoniacal chloroform. The residue after the chloroform had been distilled off was chromatographed on a column of silica gel and eluted with benzene—acetone (4:1), 15-20 ml fractions being collected. The initial fractions yielded 0.032 g of 3,15-di-O-(2-methylbutyroyl)germine diacetate (**4**) with mp 231-233°C (acetone—hexane) M^+ 761 (HRMS), LSIMS(+), glycerol being used as the liquid matrix.

Saponification of 3,15-Di-O-(2-methylbutyroyl)germine (2). A solution of 0.04 g of 3,15-di-O-(2-methylbutyroyl)germine (**2**) in 25 ml of a 0.1 N methanolic solution of caustic soda was left at room temperature for 24 h. Then it was diluted with water and extracted with chloroform. When the chloroform solution was concentrated, it deposited crystals with mp 220-222°C, identical with germine (**5**), M^+ 509 (MS). The alkaline solution after the separation of the germine was acidified with 5% sulfuric acid and extracted with ether. The residue from the distillation of the ether was chromatographed on paper (Filtrac N3) with organic acid markers. This showed the presence of 2-methybutyric acid with R_f 0.54 in the *n*-butanol—1.5 N ammonia (1:1) system. Exposure time 18 h. Revealing agent: Bromophenol Blue, 0.025 g; citric acid, 0.1 g; acetone, 90 ml; water 10 ml.

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