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NEW C-NOR,D-HOMOSTEROID ALKALOID GERMININE FROM *Veratrum lobelianum*

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The results are given of an investigation of the alkaloid composition of the hypogeal part of Veratrum lobelianum *Bernh. The new alkaloid germinine has been isolated and its structure has been established on the basis of physicochemical properties and chemical transformations.*

Steroid alkaloids are of interest, in the first place, as steroid raw material for the synthesis of drugs and, in the second place, thanks to their broad spectrum of biological activity, they have independent value in medicinal practice. Continuing a study of the alkaloid composition of the hypogeal part of *Veratrum lobelianum* growing in the Karkara valley, Alma-Ata oblast, we have isolated isorubijervine and the new alkaloid germinine (1) with mp 195-197°C, composition $C_{39}H_{61}NO_{11}$ (M^+ 719.43235). Its composition was determined by ordinary highly resolved mass-spectrometric measurements.

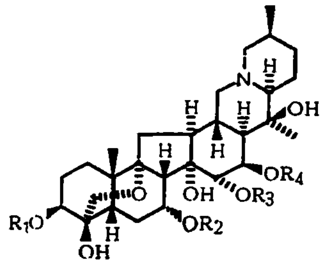
In the IR spectrum (KBr, ν, cm^{-1}) of germinine there were absorption bands at 3290-3510 (OH), 2860-2995 ($-CH_2-$, $-CH_3$), 2780-2810 (*trans*-quinolizidine) [1], 1250 and 1748 (ester grouping). The alkaline hydrolysis of (1) led to germine (2) [2, 3] and acetic and 2-methylbutyric acids (paper chromatography). Consequently, germinine is an ester of the amino alcohol alkaloid germine.

Analysis of the PMR spectrum of (1) also showed that the germine molecule contains residues of 2-methylbutyric and acetic acids.

The molecular mass of (1) was 42 a.u. greater than that of 3,15-di-O-(2-methylbutyroyl)germine (3) [4]. Consequently, germinine could be a monoacetyl derivative of (3). With acetic anhydride in pyridine, germinine formed a monoacetyl derivative. This product was identical with the 7,16-diacetate (4) obtained under analogous conditions from the alkaloid (3).

The formation of one and the same product (4) from alkaloids (1) and (3) showed that the 2-methylbutyric acid residues in the germinine molecule were present at C-3 and C-15, while the acetyl group could be located at C-7 or C-16. In compounds of the germine series, a 16β-acetyl group undergoes methanolysis far more readily than a 7α-acetyl group thanks to the participation in the reaction of the axial hydroxy group at C-20 and the tertiary nitrogen atom as a result of bifunctional general acid-base catalysis [2, 5, 6]. In view of this, the position at C-7 remained for the acetyl group in (1). The appearance of the signal of the protons of 19-CH_3 group in the PMR spectrum of (1) in a stronger field by 3 Hz than that in the PMR spectrum of alkaloid (3) [4] also showed the location of the acetyl group in (1) at C-7.

On the basis of the facts given above, germinine has the structure of 7-acetyl-3,15-di-O-(2-methylbutyroyl)germine.



1. $R_1=R_3=CO\text{-}CH(CH_3)\text{-}CH_2\text{-}CH_3$, $R_2=CO\text{-}CH_3$, $R_4=H$
2. $R_1=R_2=R_3=R_4=H$
3. $R_1=R_3=CO\text{-}CH(CH_3)\text{-}CH_2\text{-}CH_3$, $R_2=R_4=H$
4. $R_1=R_3=CO\text{-}CH(CH_3)\text{-}CH_2\text{-}CH_3$, $R_2=R_4=CO\text{-}CH_3$

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EXPERIMENTAL

General observations are given in [4]. For the isolation of the total alkaloids from the hypogeal part of *Veratrum lobelianum*, see [7].

Germinine and Isorubijervine. Germinaline has previously been isolated from 11 g of the readily soluble metaphosphate part of the total alkaloids [4]. Continuing the separation of the mixture of bases, the benzene mother solution after the removal of the germinaline was rechromatographed on a column of silica gel (KSK with a particle size of 125-160 μm) with elution by benzene–acetone mixtures in ratios of 10:2, 9:1, 9:2, and 3:2. The 9:1 eluate yielded germinine with mp 195-197°C (acetone–hexane) (0.068 g).

Mass spectrum (EI, 50 eV), *m/z* (I_{rel}, %) 98(4), 111(8), 112(100), 124(0.5), 154(3), 514(1), 515(0.3), 540(0.5), 574(1), 575(0.5), 598(0.5), 600(2), 616(1), 617(1), 618(1), 635(1.6), 642(0.5), 658(0.7), 659(1), 677(0.5), 676(1), 701(0.3), 704(0.3), 718(1), 719(M^+, 4).

PMR spectrum (100 MHz, $CDCl_3$, δ, ppm, J, Hz): 0.91 (3H, s, CH_3-19), 1.13 (3H, s, CH_3-21), 1.01 (3H, d, CH_3-27), 2.01 (3H, s, $-OCOCH_3$), 3.26-6.14 (OH, HC–O–acyl) and signals of the methyl protons of 2-methylbutyroyl residues at 0.84 (6H, t, J =7, CH_3-4′ and CH3-4″) and 1.08 (6H, J = 7, CH_3-5′ and CH_3-5″) [8].

The 10:2 eluate yielded 0.005 g of isorubijervine [9], M^+ 413 (MS). IR spectrum (KBr, ν cm^{-1}): 3450 (OH), 3035 (C=CH).

Alkaline Hydrolysis of Germinine (1). The alkaloid (1) (0.018 g) was subjected to alkaline hydrolysis with 0.1 N caustic soda solution in a similar way to the saponification of alkaloid (3) (see [4]): from the chloroform solution we isolated an amino alcohol with mp 220-222°C identical with germine, M^+ 509 (MS), and from the alkaline solution after the separation of the germine, 2-methylbutyric and acetic acids (see [4]).

Germinine Monoacetate (4). A solution of 0.03 g of germinine in 2 ml of pyridine was treated with acetic anhydride (2 ml), and the mixture was left at room temperature for 72 h. After elimination of the solvent in vacuum, the residue was treated with ammoniacal chloroform. The new residue after the chloroform had been distilled off was chromatographed on a column of silica gel with elution by benzene–acetone (8:2). Twelve 5- to 7-ml fractions of eluate were collected. The combined fractions 2-4 deposited crystals with mp 235-237°C (acetone), M^+ 761 (MS), identical with the diacetate of alkaloid (3).

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