ALKALOIDS OF *Veratrum lobelianum*
THE STRUCTURE OF GERMINALINE

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We are studying for the first time the alkaloids of the epigeal part of *Veratrum lobelianum* growing in Dzhergalan. The combined bases from this plant collected at the beginning of the vegetation period were extracted by the usual method with chloroform (Table 1).

From the ethereal fraction of the combined alkaloids on the epigeal part of *V. lobelianum* (collected on May 5, 1968) veralosine [1] and veratroylzygadenine [2] have been isolated by separation on a column of silica gel. After the separation of the veralosine, the mother liquor was fractionated with citrate–phosphate buffer solutions having pH 8-3.8. The fraction with pH 4.2–3.8 gave veralosinine [1-3].

The fraction with pH 8.0–6.6 was chromatographed on a column of silica gel. The benzene–ethanol eluate gave a new base, $C_{39}H_{61}O_{12}N$, mp 156-158°C, $[\alpha]_D$ +3.7°. The substance was identical with the base having mp 156-158°C isolated from the epigeal part of *V. lobelianum* of different origin (collected in the Karkara valley) [3]. The alkaloid formed a picrate. The IR spectrum of the base showed absorption bands at (cm^{-1}) 3450 (OH), 2950, 1450, 1380 (CH_3 and $-CH_2-$), and 1738, 1250 (ester C = O). In the NMR spectrum there was a three-proton singlet at 1.79 ppm from a $OCOCH_3$ group. The alkaloid proved to be new, and we have called it germinaline (I). On alkaline hydrolysis, germinaline yielded an amino alcohol with the composition $C_{27}H_{43}O_8N$, mp 218-220°C, $[\alpha]_D$ +16.1°, identical with germine (II) obtained from the products of the saponification of germerin (III) [2, 4]. By paper chromatography, *l*-2-methylbutyric acid (MB), d-2-hydroxy-2-methylbutyric acid (HMB), and acetic acid (Ac) were detected.

The acetylation of germinaline gave an acetyl derivative with mp 192-194°C, identical (from its IR spectrum and melting point) with the acetyl derivative that we isolated from germerine.

The facts presented confirm once again the presence of *l*-2-methylbutyric and d-2-hydroxy-2-methylbutyric acid residues in the germinaline molecule.

Germinaline differs sharply from the known alkaloid germitrine (IV) [4, 5] in its melting point and the sign of its specific rotation; it does not form a crystalline thiocyanate. Germitrine contains the heterocyclic skeleton of germine [6] and three acyl groups: a *l*-2-methylbutyric acid residue at C-3, a d-2-

TABLE 1

Date of collection	Height of the plant, cm	Amount of combined alkaloids on the weight of the dry plant, %
5. V 1968	5–12	2,08
11. V 1970	7–15	1,66
11. V 1970	7–15 (hypogeal part)	1,26

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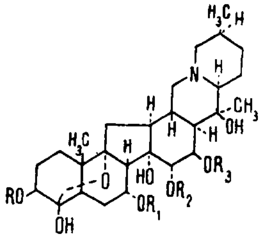
hydroxy-2-methylbutyric acid residue at C-15, and an acetic acid residue at C-7. Consequently, germinaline is an isomer of germitrine, and the acetic acid residue in the germinaline molecule can be only at C-16. On the basis of the facts given above, germinaline has the structure and configuration of 16β-acetyl-15α-(d-2-hydroxy-2-methylbutyryl)-3β-(*l*-2-methylbutyryl)germine (I).



I. $R{=}MB$, $R_1{=}H$, $R_2{=}HMB$, $R_3{=}Ac$, II $R{=}R_1{=}R_2{=}R_3{=}H$,
III $R{=}MB$, $R_1{=}H$, $R_2{=}HMB$, $R_3{=}H$, IV $R{=}MB$, $R_1{=}Ac$, $R_2{=}HMB$. $R_3{=}H$.

EXPERIMENTAL

Thin-layer chromatography (TLC) was performed with KSK silica gel (10 μ) and the following solvent systems: 1) benzene–ethanol (9 : 2.5) and 2) ethanol–benzene (35 : 5). The spots were revealed with Dragendorff's reagent. The IR spectra were recorded on a UR-10 double-beam spectrophotometer (molded tablets with KBr).

The epigeal part of V. lobelianum (various samples) was moistened with 10% ammonia, and the alkaloids were extracted with chloroform in a continuous apparatus (see Table 1).

Veralosine, Veratroylzygadenine, and Veralosinine. The epigeal part of the plant (30 kg) was moistened with 10% ammonia, and was percolated with chloroform until the bases had been extracted completely. From a sulfuric acid solution made alkaline with ammonia, the alkaloids were extracted with ether (103.5 g) and chloroform (482 g). The total yield of combined alkaloids was 585.5 g (1.95% of the weight of the dry plant).

The ethereal solution of combined alkaloids, on concentration, yielded 31.5 g of a mixture of crystals (A), mp 178-180°C, which were separated on a column of alumina. The eluates [benzene–ethanol (9 : 2.5)] yielded a base with mp 213-215°C [from methanol–acetone (1 : 3)], R_f in system 1, identified as veralosine (IR spectrum, melting point).

After the separation of A, 72 g of the residue from the mother liquor was dissolved in 5% sulfuric acid, and the solution was made alkaline with ammonia and extracted with benzene and chloroform. The benzene extract was treated with 20% acetic acid, the acetic acid solution was made alkaline, and the alkaloids were extracted with ether – 33.7 g (9.2 g of a precipitate sparingly soluble in ether) – and then with chloroform – 8 g.

The residue (9.2 g) was separated on a column of silica gel. The chloroform–ethanol (9 : 1) eluate deposited a base with mp 259-261°C (from acetone), R_f 0.18 in system 1. A mixture with veratroylzygadenine gave no depression of the melting point. Their IR spectra were identical [2].

The combined ethereal fraction (33.7 g) was dissolved in benzene and separated by means of citrate–phosphate buffer solutions with pH 8-3.8. Treatment of the pH 4.2–3.8 fraction with acetone gave an alkaloid with mp 163-165°C, identical with veralosinine (UV and IR spectra and melting point).

Germinaline. The fractions with pH 8.0–6.6 were combined and chromatographed on a column of silica gel. By treatment with ether, from the first 100 ml of benzene–ethanol (20 : 1) eluate we isolated germinaline, $C_{39}H_{61}O_{12}N$, mp 156-158°C (from benzene, mp 136-138°C), $[\alpha]_D$ +3.7° (c 1.06; chloroform), R_f 0.51 in system 1.

Germinaline Picrate. The substance was obtained from acetic acid solution by the addition of a saturated solution of picric acid, mp 170-172°C (from aqueous acetone). A mixture with germinaline melted at 150°C.

Saponification of Germinaline. A solution of 0.32 g of germinaline in 16 ml of aqueous methanolic caustic soda was left at room temperature for 48 h. Then it was diluted with water and extracted with chloroform. When the chloroform extract was concentrated, crystals (0.165 g) deposited with mp 218-220°C (from chloroform), $[\alpha]_D$ +16.1° (c 0.372; 5% acetic acid), R_f 0.18 in system 2, identical with an authentic sample of germine. The IR spectrum [3350 cm^{-1} (OH), 2940 and 1450 cm^{-1} (CH_3 and CH_2)] lacked the absorption band of an ester carbonyl. M^+ 509 (mass spectrometrically).

Found %: C 63.30; H 8.60; N 2.78. $C_{27}H_{43}O_8N$. Calculated %: C 63.70; H 8.51; N 2.75.

The alkaline solution after the separation of the germine was acidified with sulfuric acid and was extracted repeatedly with ether. The combined ethereal extract was treated with a few drops of concentrated ammonia, and the ether was distilled off to a volume of 5 ml. The residue was chromatographed on paper in the butan-1-ol–1.5 N aqueous ammonia (1 : 1) system for 20 h. The spots were revealed with Bromphenol Blue in ethanol. Three spots were shown up: with R_f 0.13 (acetic acid), 0.32 (d-2-hydroxy-2-methylbutyric acid), and 0.54 (*l*-2-methylbutyric acid).

Acetylation of Germinaline. A mixture of 0.53 g of germinaline, 2 ml of pyridine, and 4 ml of acetic acid was kept at room temperature for three days. After the appropriate working up, the acetyl derivative isolated melted at 192-194°C [from acetone–petroleum ether (1 : 3)], R_f 0.69 in system 1; it was identical with the acetyl derivative of germine obtained by the acetylation of germine with acetic anhydride under similar conditions to the formation of acetylgerminaline. IR spectrum (cm^{-1}): 3350 (OH), 2945, 1470, 1380 (CH_3 and $-CH_2-$), 1745, 1245 (ester C = O).

CONCLUSIONS

1. From the epigeal part of *Veratrum lobelianum* collected in Dzhergalan we have isolated the formerly unknown alkaloids obtained previously from the epigeal part of *V. lobelianum* collected in the Karkara valley: veralosine, veralosinine, veratroylzygadenine, and the new alkaloid germinaline.

2. The structure of germinaline has been established as 16β-acetyl-15α-(d-2-hydroxy-2-methylbutyryl)-3β-(*l*-2-methylbutyryl)germine.

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