A STUDY OF THE ALKALOIDS OF VERATRUM LOBELIANUM

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At the present time, about 60 alkaloids have been isolated from various species of the genus Veratrum [1–7]. Among the species of this genus, V. lobelianum has been studied very little, while the V. lobelianum growing in the valley of the Kar-Kara has not been studied at all. We give the results of an initial study of the epigeal part of this plant. The content of alkaloids in the plant was determined according to the vegetation period (see table).

Date of collection (day, month, year)	Height of the plant, cm	Vegetation period	Total alkaloids (% of the weight of the raw material)
2. May 1968	3— 5	Beginning of vegetation	2.50
13. May 1968	5— 10	Beginning of vegetation	2.07
5. June 1967	30— 40	Before budding	0.30
16. June 1967	50— 60	Budding	0.17
29. Sept. 1967	90—100	Dying off	0.036

When the combined ethereal alkaloids isolated from the epigeal part of the plant (collected on May 13, 1968) were separated by their solubilities and basicities, the following new alkaloids were isolated: veralosine $C_{35}H_{55}O_8N$, veralosinine, and veralosidine $C_{27}H_{43}O_2N$ (I).

The IR spectrum of veralosine has absorption bands at (cm^{-1}) 3450 (OH), 2900, 1460 (CH_3), 1725 (C=O of an ester), 1660 (C=N—), and 1000–1100 (broad absorption band characteristic for glucoalkaloids); UV spectrum: λ_{max} 245 mμ ($\log\varepsilon$ 2.20).

The IR spectrum of veralosinine has absorption bands at (cm^{-1}) 3470, 1040 (OH), 2940, 1460 (CH_3), 3080, 1650 (C=C), 1730, and 1260 (C=O of an ester). The IR spectrum of veralosidine has absorption bands at (cm^{-1}) 3330, 1060 (OH), 2930, 1460 (CH_3), 1650 (C=N), 3035, and 1650 (C=CH); UV spectrum: λ_{max} 242 mμ ($\log\varepsilon$ 2.45), similar to that for verazine [8]. The absorption at about 1060 cm^{-1} in the IR spectrum of veralosidine shows the presence of a Δ^5-3β-OH group [6, 9].

The acetylation of veralosidine with acetic anhydride in pyridine gave N,O,O-triacetylveralosidine (II) with mp 193–195° C, $[\alpha]_D^{27}$ +13.3°, as a result of a shift of the C=N double bond to $\Delta^{22(23)}$ and the migration of hydrogen from C-23 to the nitrogen, as in the case of the other alkaloids [8, 10].

The IR spectrum of triacetylveralosidine had absorption bands at (cm^{-1}) 2590, 1450 (CH_3), 1720, 1250 ($OCOCH_3$), and 1640 ($NCOCH_3$); UV spectrum: λ_{max} 235 mμ ($\log\varepsilon$ 4.0) [8]. The saponification of triacetylveralosidine with ethanolic alkali led to veralosidine.

The hydrogenation of veralosidine forms a tetrahydro derivative whose IR spectrum has absorption bands at (cm^{-1}) 3420, 1060 (OH), 2930, and 1443 (CH_3), but lacks the absorption band of a double bond. The NMR spectrum of veralosidine has the following signals: singlets (δ scale) at 0.68 (3H, C-18 CH_3), 0.94 (3H, C-19 CH_3); doublets at 0.91 (3H, C-21 CH_3), 1.09 (3H, C-27 CH_3); and multiplets with centers at 5.25 (H, C-6) and 3.67 (H, C-3).

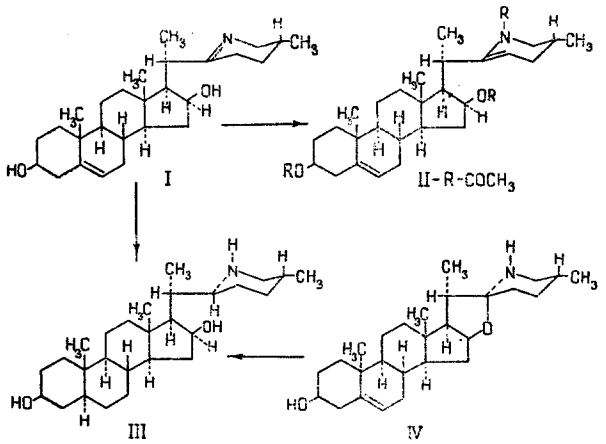
The NMR spectrum of N,O,O-triacetylveralosidine has the following resonance signals: singlets at 0.67 (3H, C-18 CH_3), 0.95 (3H, C-19 CH_3), 1.93 (3H, $COOCH_3$), 1.98 (3H, $COOCH_3$), 2.07 (3H, N—$COCH_3$); and doublets at 0.88 (3H, C-21 CH_3) and 1.18 (3H, C-27 CH_3).

Veralosidine is a typical steroid alkaloid: in ethanolic solution with digitonin it gives a digitonide, which shows the presence of a 3β-OH group in it [8, 11].

The mass spectrum of veralosidine, like that of the alkaloid verazine [8], has peaks for ions with m/e 98, 111, 125 (100%), 138, 162, and 413 (M^+).

The catalytic reduction of veralosidine (I) in acetic acid by Adams' method gave a mixture of two isomeric tetrahydroveralosidines whose separation gave a tetrahydroveralosidine with mp 287–289° C (from methanol), identical (mixed melting point and mass spectrum) with tetrahydrosolasodine (III) [12], obtained by the hydrogenation of solasodine (IV) [13].

On the basis of what has been said above, structure and configuration I has been established as the most probable for veralosidine.



EXPERIMENTAL

Thin-layer chromatography (TLC) was carried out with KSK silica gel having a grain size of 10 mμ and the following solvent systems: 1) butyl acetate–chloroform–ethanol (1 : 2 : 3), 2) benzene–ethanol (9 : 1.5), 3) chloroform–ethanol (9 : 1); and 4) chloroform–ethyl acetate–methanol (4 : 4 : 3). The developer was Dragendorff's solution. The IR spectra were taken on a UR-10 double-beam spectrometer (molded tablets with KBr), the UV spectra on an SF-4 spectrometer (ethanolic solutions), the mass spectra on a MKh-1303 mass spectrometer, and the NMR spectra on a JNM-4H-100 MHz instrument in deuterochloroform.

The air-dried comminuted epigeal part of V. lobelianum (various samples) was moistened with 10% ammonia and the alkaloids were extracted with chloroform in a continuous apparatus. The results of quantitative determinations are given in the table.

The epigeal part of the plant (47 kg) was moistened with 10% ammonia and percolated with chloroform until the bases had been extracted completely. An H_2SO_4 solution was made alkaline with ammonia and the alkaloids were extracted with ether and chloroform. The yield of total chloroformic alkaloids was 729.3 g. On concentration, the ethereal extract deposited 51.42 g of technical veralosine with mp 184–186° C, and the mother liquor contained 126.43 g. The total yield of combined alkaloids was 907.1 g (1.93% of the weight of the dry plant).

Veralosine. Technical veralosine (10 g) was passed through a column filled with silica gel (261 g, 63-mμ sieve). Elution was carried out with a chloroform–benzene–methanol mixture (4 : 1 : 3). The first 650 ml of eluate gave veralosine (4.01 g) with mp 213–215° C (from methanol–acetone (1 : 5)), $[\alpha]_D^{20}$ −147.7° (c 0.406, methanol), R_f 0.78 on GLC in system 1.

Found, %: C 68.2; H 9.4; N 2.35. Calculated for $C_{35}H_{55}O_8N$, %: C 68.12; H 8.91; N 2.26.

Veralosinine and Veralosidine The product from the mother liquor from veralosine, 97 g, was dissolved in 5% H_2SO_4 and the solution was made alkaline with ammonia and extracted successively with petroleum ether (3 *l*), benzene (10 *l*), ether (4 *l*), and chloroform (4 *l*).

The benzene fraction was separated by means of acetate buffer solutions with pH 5.8–3.6 at intervals of 0.2 unit.

From the fraction with pH 4.0–3.8, further treatment with acetone yielded veralosinine, mp 161–163° C (from acetone), $[\alpha]_D^{25}$ −186.2° (c 0.918, chloroform), R_f 0.61 on TLC in system 2. The fraction with pH 5.4–5.2 was treated

with chloroform. The crystals that deposited from the chloroform were dissolved in 5% H_2SO_4, and the solution was made alkaline with ammonia and extracted with ether. The concentrated ethereal solution yielded veralosidine, $C_{27}H_{43}O_2N$ (I), with mp 153–155° C [methanol–acetone (1 : 3)], $[\alpha]_D^{26}$ –92.2° (c 0.466, ethanol), R_f 0.25 on TLC in system 3, mol wt 413 (mass spectrometry).

N, O, O-Triacetylveralosidine. A mixture of 0.308 g of veralosidine, 3 ml of pyridine and 3 ml of acetic anhydride was kept at room temperature for 2 days. After the elimination of the pyridine and the addition of a 5% solution of H_2SO_4, the acetylation product was extracted with ether. The ethereal extract was shaken with ammonia and then repeatedly washed with water. The residue after the distillation of the ether yielded acetylveralosidine (0.3805 g) with mp 193–195° C (from acetone), $[\alpha]_D^{27}$ +13.3° (c 0.516, ethanol), R_f 0.82 in system 4. The number of acetyl groups was three, mol wt 539 (from the mass spectrum and a comparison of the amount of products obtained with the amount of starting material).

Saponification of N, O, O-triacetylveralosidine. A solution of 0.1053 g of acetylveralosidine in 10 ml of 5% ethanolic caustic potash was boiled for 3 hr. The solvent was driven off, and the residue was diluted with water and extracted with chloroform. The saponification product was treated with an ethanol–acetone mixture (1 : 3). Crystals deposited with mp 153–155° C, identical with veralosidine (according to a mixed melting point and R_f values).

Hydrogenation of veralosidine. Veralosidine, 0.4 g, was hydrogenated in glacial acetic acid by Adams' method (0.4 g of PtO_2). The acetic acid solution, after removal of the platinum black, was diluted with water, made alkaline with ammonia, and extracted with chloroform. On TLC (alumina), the product of the hydrogenation of veralosidine showed the presence of two substances with R_f 0.31 and 0.62 in system 2. This mixture was passed through a column of silica gel (20 g) and eluted with butyl acetate–methanol–chloroform (5 : 40 : 25), in 50 2-ml fractions. Then fractions 12–40 were combined and were refractionated preparatively on a plate with a fixed layer of silica gel in the butyl acetate–methanol–chloroform (5 : 40 : 25) system. A substance with R_f 0.62, mp 287–289° C, corresponding to tetrahydrosolasodine was isolated. A mixture with an authentic sample showed no depression of the melting point.

In the mass spectrum of tetrahydroveralosidine, the molecular ion (M^+ 417) is heavier than the molecular ion of veralosidine (M^+ 413) by four units.

CONCLUSIONS

1) At the beginning of vegetation, the total alkaloids in the epigeal part of Veratrum lobelianum is 2.5% of the weight of the dry raw material, and at the end of vegetation, 0.036%.

2) The new alkaloids veralosine, veralosinine, and veralosidine have been isolated from the epigeal part of the plant.

3) On the basis of a study of chemical properties, IR, UV, NMR, and mass spectra, and also its conversion into tetrahydrosolasodine, structure and configuration I has been proposed as the most probable for veralosidine.

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