A STUDY OF THE ALKALOIDS OF THE EPIGEAL PART OF *Veratrum lobelianum*

THE STRUCTURE OF VERALOSININE

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When the benzene fraction of the combined alkaloids of Veratrum lobelianum was separated by means of acetate buffer solutions with pH 5.8-3.6 (in steps of 0.2 of a unit), the fraction with pH 4.0-3.8 yielded new alkaloids – veralosidine (I) and veralosinine (II) [1, 2].

The fraction with pH 5.4-5.2 yielded a base with mp 263-265°C identical with veratroylzygadenine, which has been obtained previously from the epigeal part of V. lobelianum [3], and the fraction with pH 5.8-5.6 gave alkaloids with mp 156-158°C and 180-183°C.

Veralosinine, $C_{29}H_{45}O_3N$, contains > C = N–, OH, and ester C = O groups [1]. The UV spectrum of veralosinine is similar to those of veralosidine, veralosine, and verasine [1, 2, 4]. The NMR spectrum of veralosinine, unlike that of veralosidine, has a three-proton singlet at 1.93 ppm ($-OCOCH_3$) and a one-proton multiplet at 4.87 ppm ($-CHOCOCH_3$) (Table 1). To determine the nature of the esterified acid, veralosinine was saponified. The alkaline fraction yielded an amino alcohol with mp 153-155°C, identified as veralosidine (I), and the acid fraction was found to contain acetic acid (paper chromatography). The acetylation of veralosinine with acetic anhydride in pyridine gave an acetyl derivative with mp 193-195°C identical with O,O',N-triacetylveralosidine (III) [1] (according to its IR and UV spectra and a mixed melting point). The formation of O,O',N-triacetylveralosidine and the presence of a singlet in the NMR spectrum at 1.93 ppm and of a molecular ion with m/e 455 in the mass spectrum of veralosinine show that its molecule contains one acetic acid residue. This may be located at either C_3 or C_{16}. Position C_3 is excluded on the basis of the NMR spectrum since the chemical shifts (CSs) of the protons from the C-19 CH_3 group are the same as for veralosidine [5]. Furthermore, with an ethanolic solution of digitonin, veralosinine gives a digitonide, which shows the presence of a free 3β-OH group in it. Thus, veralosinine is 16α-O-acetylveralosidine.

TABLE 1. Chemical Shifts (δ scale)

Substance	C–19CH_3, s	C–18CH_3, s	C–21CH_3, d	C–27CH_3, d	$COOCH_3$, s	$COOCH_3$, s	N–$COCH_3$, s	H, C–3, m	H, C–6, m	H, C–16, m	H, C–4, m	H, C–23, m
I	0,94	0,68	1,06	0,88	—	—	—	—	5,26	—	—	—
II	0,94	0,71	1,03	0,81	1,93	—	—	—	5,27	4,87	—	—
IV	1,12	0,68	1,03	0,83	—	—	—	—	—	4,38	5,65	—
IX	0,94	0,68	1,02	0,78	1,87	1,90	—	4,62	5,29	4,96	—	—
XII	0,96	0,82	1,02	0,85	1,87	1,90	—	4,60	5,29	5,13	—	—
III	0,95	0,64	1,18	0,87	1,93	1,98	2,07	4,50	5,27	4,72	—	5,11
XIII	0,99	0,84	1,23	0,88	1,94	1,98	2,11	4,54	5,28	5,07	—	5,10
X	0,91	0,64	0,95	0,73	—	—	—	3,44	5,27	3,99	—	—
XIV	0,93	0,84	0,96	0,74	—	—	—	3,40	5,25	4,33	—	—

Note. s – singlet, d – doublet, m – multiplet.

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Fig. 1. IR spectra of the diketone (VI) from a mixture of tetrahydroveralosidines (a) and of the diketone (VII) from a mixture of tetrahydrosolasodines (b).

A study of the IR, NMR, and mass spectra of veralosidine and the formation of the digitonide showed that the base veralosidine includes the heterocyclic skeleton of verasine and petiline [4, 6]. The position of the second hydroxy group and the configuration of veralosidine were determined from the absence of a depression of the melting point of a mixture of tetrahydroveralosidine with tetrahydrosolasodine and their identical Rf values on chromatography [1].

However, a repeat preparation and a comparison of the IR spectra of tetrahydroveralosidine and tetrahydrosolasodine showed their nonidentity. Consequently, we have revised the structure of veralosidine (I).

The Oppenauer oxidation of (I) gave an α,β-unsaturated ketone – Δ^4-veralosidin-3-ol (IV) [UV spectrum: λ_{max} 242 nm (log ε 4.16)], with a shift in the double bond from the Δ^5 to the Δ^4 position, as for other typical steroid alkaloids [4, 7]. The oxidation of a mixture of the isomeric tetrahydroveralosidines (V) with chromium trioxide gave a mixture of isomeric diketones (VI), the IR spectrum of which was almost identical with that of the mixture of isomeric diketones (VII) obtained by the oxidation of a mixture of isomeric tetrahydrosolasodines (VIII) (Fig. 1).

The IR spectra of both products (VI and VII) showed the absorption bands of five-membered and six-membered carbonyls in the 1710 cm^{-1} region. The absorption of the five-membered carbonyl in compounds (VI and VII) in the low-frequency region is explained by the formation of an intramolecular hydrogen bond. The structures of (VI and VII), considered as models, were confirmed by what has been said above.

In the presence of zinc chloride, veralosidine gave an O,O'-diacetyl derivative (IX) and in the presence of pyridine an O,O'-N-triacetyl derivative (III), while with sodium tetrahydroborate it gave dihydroveralosidine (X) (at the C_{22}–N bond).

The acetolysis of solasodine (XI) with subsequent acetylation gave O,O'-diacetylpseudosolasodine (XII) [8] and O,O',N-triacetylsolasodine (XIII) [9], and its reduction with sodium tetrahydroborate yielded dihydrosolasodenol (XIV). The UV spectrum of (IX) is similar to that of (XII), and that of (III) to that of (XIII).

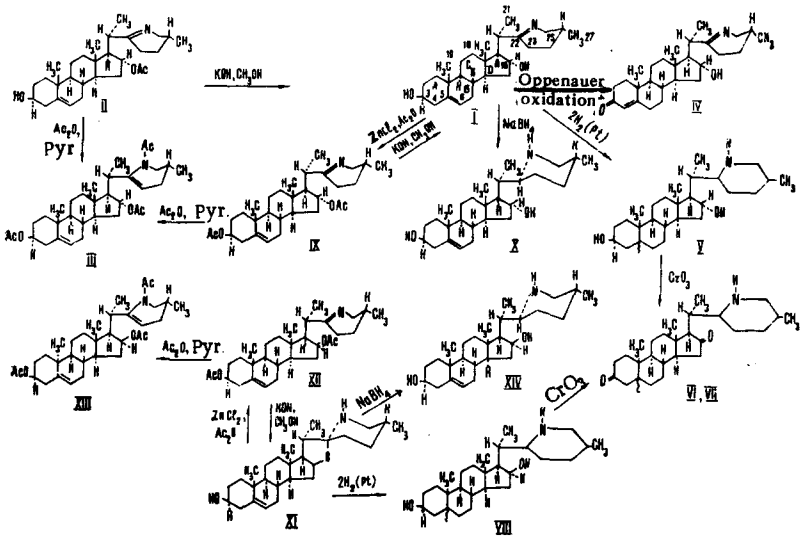
The UV spectra of (III, IX, XII, and XIII) show that the structures of these substances are similar. To confirm what has been said above, we have studied the NMR spectra of (III, IX, and X), in comparison with the NMR spectra of compounds (XII, XIII, and XIV) (see Table 1).

It can be seen from Table 1 that in compounds (IX, III, and X) the C-18 protons of the methyl group resonate at 0.68, 0.64, and 0.64 ppm, while in compounds (XII, XIII, and XIV) they do so at 0.82, 0.84, and 0.84 ppm, respectively, i.e., downfield by 14-20 Hz [as compared with (III, IX, and X)]. The protons of the methine groups at the 16-OH and 16-$OCOCH_3$ groups in substances (IX, III, and X) are likewise shifted upfield by 17-34 Hz as compared with the protons of the methine groups at the 16-OH and 16-$OCOCH_3$ groups in compounds (XII, XIII, and XIV). The chemical shifts of the other methyl, methine, and olefinic protons in (IX, III, X, and XII-XIV) are similar.

In steroids, the chemical shifts from the C-18 methyl group are shifted upfield (by 25 Hz) in those cases where the molecule has a C_{16} α-hydroxy group [10]. Consequently, in veralosidine the second hydroxy group is located at C_{16} and has the α orientation.

The saponification of O,O'-N-triacetylpseudosolasodine (XIII) gave solasodine (XI). When (III) was saponified, because of steric hindrance no $C_{16}-O-C_{22}$ bond was formed, and veralosidine was obtained (as can be seen from the model (III)].

On the basis of the information given, in veralosidine (I) the B/C and the C/D rings are trans-linked, while the hydroxy group is located at C_{16} and has the α orientation. Thus, veralosinine (II) has the most probable structure and configuration of 16α-acetylveralosidine.



EXPERIMENTAL

Thin-layer chromatography (TLC) was performed with silica gel (10 μ) and the following solvent systems: 1) chloroform–ethyl acetate–methanol (4 : 4 : 3); 2) benzene–ethanol (9 : 1); and 3) benzene–ethanol (9 : 1.5). The spots were revealed with Dragendorff's reagent.

The UV spectra were taken on a Hitachi spectrophotometer, the IR spectra (KBr) on a UR-10 spectrophotometer, the mass spectra on an MKh-1303 mass spectrometer, and the NMR spectra on a JNM-4H-100 MHz instrument (deuterochloroform) with hexamethyldisiloxane as internal standard.

Veratroylzygadenine. A solution of 7.4 g of the fraction with pH 5.4–5.2 in 5% sulfuric acid and made alkaline with ammonia and was extracted with ether and chloroform. The concentrated ethereal solution deposited 2.1 g of crystals with mp 263-265°C [methanol–acetone (1 : 1)], R_f 0.15 (system 1). A mixed melting point with veratroylzygadenine gave no depression. Their IR spectra were also identical [3].

The Base with mp 180-183°C. A solution of 29.67 g of the fraction with pH 5.8–5.6 in 5% sulfuric acid made alkaline with ammonia and was extracted first with ether and then with chloroform. The concentrated ethereal extract deposited 2.01 g of a mixture of crystals (A) which was separated off and passed through a column of alumina, elution being performed with benzene–ethanol (9 : 1). The first 75 ml of eluate yielded 0.36 g of veralosidine, and the next 175 ml of eluate yielded 0.12 g of the base with mp 180-183°C [benzene–ethanol (9 : 1)], $[\alpha]_D$ +5.1° (c 0.613; chloroform), R_f 0.22 (system 1).

The Base with mp 156-158°C. The mother liquor from (A) (11.84 g) was passed through a column of alumina, elution being performed with benzene–ethanol (9 : 1). The first 110 ml of eluate yielded 0.51 g of crystals with mp 156-158°C (ether). R_f 0.56 (system 1).

Veralosinine (II) $C_{29}H_{45}O_3N$. The isolation of this alkaloid has been described in a previous paper [1]. UV spectrum: λ_{max} 242 nm (log ε 2.39).

Saponification of Veralosinine. A solution of 4.19 g of veralosinine in 420 ml of 5% caustic potash solution was heated for 3 h. Then it was diluted with water and extracted with ether. After the distillation of the ether, the residue (3.5 g) was recrystallized from methanol–acetone (1 : 3). mp 153-155°C, $C_{27}H_{43}O_2N$, M^+ 413 (mass spectrometry), R_f 0.30 (system 1). The melting point of a mixture with veralosidine showed no depression. IR spectrum, cm^{-1}: 3300, 1060 (OH), 2930, 1460 (CH_3), 1650 (>C=N–), 3035, 1650 (C = CH). λ_{max} 242 nm (log ε 2.45), these being identical with the IR and UV spectra of veralosidine. The alkaline solution after the separation of the crystals was acidified with 1% sulfuric acid, extracted with ether, and made alkaline with ammonia. The ethereal residue was chromatographed on paper (Leningrad slow) with a marker (acetic acid) by the ascending method using butanol saturated with 1.5 N aqueous ammonia solution for 15 h. The R_f values obtained were the same for the ethereal residue and for acetic acid (0.13). The spots were revealed with an ethanolic solution of Bromphenol Blue.

Acetylation of Veralosinine. The acetylation of 0.61 g of veralosinine was performed in 6 ml of acetic anhydride in the presence of 6 ml of pyridine in a similar manner to the acetylation of veralosinine (I). This gave 0.72 g of an acetyl derivative, $C_{33}H_{49}O_5N$, mp 193-195°C (acetone), M^+ 539 (mass spectrometry), R_f 0.82 (system 1). IR spectrum, cm^{-1}: 2950, 1450 (CH_3), 1720, 1250 ($OCOCH_3$), 1640 (N–$COCH_3$); UV spectrum: λ_{max} 235 nm (log ε 4.0), these being identical with the IR and UV spectra of O,O',N-triacetylveralosidine [1]. The melting point of a mixture likewise showed no depression.

O,O'-Diacetylveralosidine (IX). A mixture of 0.2 g of veralosidine, 0.56 g of zinc chloride dissolved in 5 ml of acetic anhydride, and 2 ml of glacial acetic acid was left overnight at room temperature. Then it was cooled with ice and, in the cold, a concentrated solution of ammonia was gradually added to give a strongly alkaline reaction. The precipitate that deposited was separated off and washed with water. On treatment with methanol, the precipitate crystallized, mp 166-168°C (methanol), $C_{31}H_{47}O_4N$, M^+ 497 (mass spectrometry), R_f 0.29 (system 2). IR spectrum (cm^{-1}): 2950, 1450 (CH_3) 1730, 1250 ($OCOCH_3$), 1660 (C = N–); UV spectrum: λ_{max} 240 (log ε 2.39).

O,O'-Diacetylpseudosolasodine (XII). By the method described by Sato et al. [8], 1 g of solasodine gave 1.16 g of O,O'-diacetylpseudosolasodine, $C_{31}H_{47}O_4N$, mp 197-199°C (methanol), M^+ 497 (mass spectrometry), R_f 0.49 (system 2). A mixture of O,O'-diacetylveralosidine with O,O'-diacetylpseudosolasodine melted at 159-161°C, IR spectrum (cm^{-1}): 2970-2830, 1465 (CH_3), 1725, 1255 ($OCOCH_3$), 1655 (>C=N–), UV spectrum; λ_{max} 243 nm (log ε 2.43).

O,O',N-Triacetylpseudosolasodine (XIII). By the method of Sato and Ikekawa [9], 0.5 g of O,O'-diacetylpseudosolasodine yielded 0.53 g of O,O'N-triacetylpseudosolasodine, $C_{33}H_{49}O_5N$, mp 164-165°C, $[\alpha]_D$ + 88.03° (c 0.886; chloroform), M^+ 539 (mass spectrometry), R_f 0.82 (system 1). IR spectrum (cm^{-1}): 2980-2850, 1450 (CH_3), 1745, 1260 ($OCOCH_3$), 1650 (N–$COCH_3$); UV spectrum: λ_m 238 nm (log ε 3.89).

The melting point of a mixture of O,O',N-triacetylveralosidine with O,O',N-triacetylpseudosolasodine showed a depression.

Dihydroveralosidine (X). A solution of 0.3 g of veralosidine in 20 ml of 90% aqueous methanol was treated with 1.5 g of sodium tetrahydroborate for 1.5 h; then it was diluted with water and extracted with ether. After the ether had been distilled off, dihydroveralosidine, $C_{27}H_{45}O_2N$, was isolated with mp 214-216°C (ether), $[\alpha]_D$ –43.5° (c 1.26; chloroform), M^+ 415 (mass spectrometry), R_f 0.25 (system 2 on TLC with alumina). IR spectrum (cm^{-1}): 3400, 1070 (OH), 2960, 1470 (CH_3); there was no absorption band of a >C=N bond.

Dihydrosolasodenol (XIV). Solasodine (1 g) was hydrogenated with sodium tetrahydroborate in a similar manner to the hydrogenation of veralosidine. This gave dihydrosolasodine, $C_{27}H_{45}O_2N$ with mp 255-257°C (ether); $[\alpha]_D$ –50.3° (c 1.37; chloroform), M^+ 415 (mass spectrometry), R_f 0.42 (system 2, on TLC with alumina). IR spectrum (cm^{-1}): 3420, 1050 (OH), 2940, 1450 (CH_3).

Δ^4-Veralosidin-3-one (IV). A solution of 0.4 g of veralosidine in 30 ml of absolute benzene was concentrated to 15 ml, and 1.5 g of aluminum tertiary butoxide dissolved in 20 ml of absolute benzene was added, together with 10 ml of absolute acetone. The mixture was boiled for 8 h. The reaction product was left overnight at room temperature. Then the reaction mixture was treated with 50 ml of a saturated solution of sodium carbonate and extracted with chloroform. After the chloroform had been distilled off, Δ^4-veralosidin-3-one, $C_{37}H_{41}O_2N$, was isolated with mp 194-196°C (acetone), M^+ 411 (mass spectrometry).

IR spectrum of Δ^4-veralosidin-3-one (cm^{-1}): 3320, 1070 (OH), 2940, 1460 (CH_3), 1690 (C = O in a six-membered ring), 1630 (C = N–); UV spectrum: λ_{max} 242 nm (log ε 4.16).

Oxidation of the Mixture of Isomeric Tetrahydroveralosidines [1]. A mixture of 0.66 g of the isomeric tetrahydroveralosidines (R_f 0.31 and 0.62, system 3, on TLC with alumina), 12 ml of glacial acetic acid, and 350 mg of chromium trioxide dissolved in 6 ml of 80% acetic acid was heated in the water bath for 30 min. The solvent was evaporated in vacuum, the residue was dissolved in water, and the solution was made alkaline with sodium carbonate solution and was extracted with chloroform. The residue after the evaporation of the chloroform was passed through a column of silica gel and was eluted with acetone. The acetonic eluate was again passed through a column of alumina. It was eluted with a mixture of benzene and ethanol (9.5 : 0.5).

The eluate was concentrated to about 15 ml and separated preparatively on a plate of alumina in the benzene–ethanol (9.5 : 0.5) system. The top fraction, which contained a mixture of two amorphous isomeric diketones with R_f 0.95 and 0.83 was separated off. IR spectrum (cm^{-1}): 2950-2880, 1455 (CH_3), 1710 (C = O in six-membered and five-membered rings).

Oxidation of the Mixture of Isomeric Tetrahydrosolasodines [1]. The mixture of isomeric tetrahydrosolasodines (2 g) was oxidized with chromium trioxide in a similar manner to the oxidation of the isomeric tetrahydroveralosidines. This gave a mixture of two isomeric amorphous diketones with R_f 0.95 and 0.83 (system 2, TLC with alumina). IR spectrum (cm^{-1}): 2950-2800, 1455 (CH_3), 1710 (C = O in five- and six-membered rings).

CONCLUSIONS

1. Bases with mp 156-158°C and 180-183°C and the known alkaloid veratroylzygadenine have been isolated from the combined alkaloids extracted from the epigeal part of Veratrum lobelianum.

2. The orientation of the C_{16}–OH group in veralosidine has been corrected. It has the α orientation.

3. The structure of veralosinine has been established as α-acetylveralosidine.

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