THE STRUCTURE OF VERALODININE

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Continuing an investigation of the alkaloids of the epigeal part of Veratrum lobelianum collected in Dzhergalan and the Caucasus [1, 2], from the chloroform-soluble fraction of the combined bases by separation according to basic strength and by column chromatography we have isolated a new alkaloid veralodinine with the composition $C_{35}H_{53}NO_9$ (I). This is a tertiary unsaturated base the IR spectrum of which shows absorption bands at (cm^{-1}) 3500-3260 (hydroxy groups), 3040, 1630 (CH=C), 1728, 1250 (ester C=O), 1710 (carbonyl in a six-membered ring), 1690 (C=N), and 1100-1000 (broad absorption band characteristic for glycoalkaloids) [3]. The UV spectrum [λ_{max} 268 nm (log ε 2.43)] is similar to that of the typical steroid alkaloids tomatilidine and veralodisine [4, 5]. The NMR spectrum has singlets at 0.79 ppm (3 H, 18-CH_3), 0.95 ppm (3 H, 19-CH_3), and 1.87 ppm (3H, $OCOCH_3$), doublets at 0.99 and 1.06 ppm (two secondary methyl groups), and multiplets at 3.12-4.62 ppm (signals of the protons of the sugar component), 4.97 ppm (H, CH−$OCOCH_3$), and 5.28 ppm (H, C=CH).

With acetic anhydride in pyridine, veralodinine forms tetraacetylveralodinine (II). In the IR spectrum of (II) the absorption band of the hydroxy groups had disappeared, and in the NMR spectrum signals have appeared in the form of singlets at 1.90, 1.95, 1.97, 1.99, and 2.03 ppm (15 H, $OCOCH_3$), and in veralodinine itself the signals from the protons of a CH_3CO group resonate at 1.87 ppm.

When compound (I) was hydrogenated by the Adams method in acetic acid, tetrahydroveralodinine (III) was obtained. Its IR spectrum lacked the absorption bands of C=O (1710 cm^{-1}) and C=N (1690 cm^{-1}) but the CH=C bond had not been hydrogenated since the NMR spectrum of (III) retained the multiplet with its center at 5.22 pp from one olefinic proton.

Veralodinine was saponified with a methanolic solution of caustic potash. From the products of the saponification of (I) we isolated deacetylveralodinine (IV) with mp 236-239°C and acetic acid (paper chromatography) [6]. In the IR spectrum of (IV), the broad band characteristic for glycoalkaloids was retained, and the absorption bands of C=O (1687 cm^{-1}) and C=N (1630 cm^{-1}) had shifted in the low-frequency direction.

Consequently, veralodinine is an ester-glycoalkaloid and has the following expanded formula: $C_{19}H_{26}(CH_3)_4(CH=C)$ (C=N) (−O−$C_6H_{11}O_5$) (−O−$COCH_3$) (C=O).

When compound (I) was reduced by the Huang-Minlon method [7], deoxodeacetylveralodinine (V) with mp 229-231°C was formed, the IR spectrum of which lacked an absorption band characteristic for carbonyl and ester groups. Deoxodeacetylveralodinine was identical with deacetylveralosine (VI) [6] (mixed melting point, IR spectrum).

Thus, veralodinine is based on the heterocyclic skeleton of veralosine [6]. However, the position of the carbonyl group remained obscure. The weakly basic nature of the veralodinine indicates that the carbonyl group is present in the nitrogen-containing part of the molecule. From the products of the hydrolysis of tetrahydroveralodinine we isolated an amino alcohol (VII) with mp 247-249°C, M^+ 431, identical with tetrahydroveralodisinol (VIII) [5] (mixed melting point, IR and mass spectra), D-glucose, and acetic acid (paper chromatography). The identity of the amino alcohol (VII) with tetrahydroveralodisinol (VIII), obtained by the reduction of veralodisine (IX) with lithium tetrahydroaluminate [5], shows the position of the carbonyl group at C_{24} in the veralodinine molecule.

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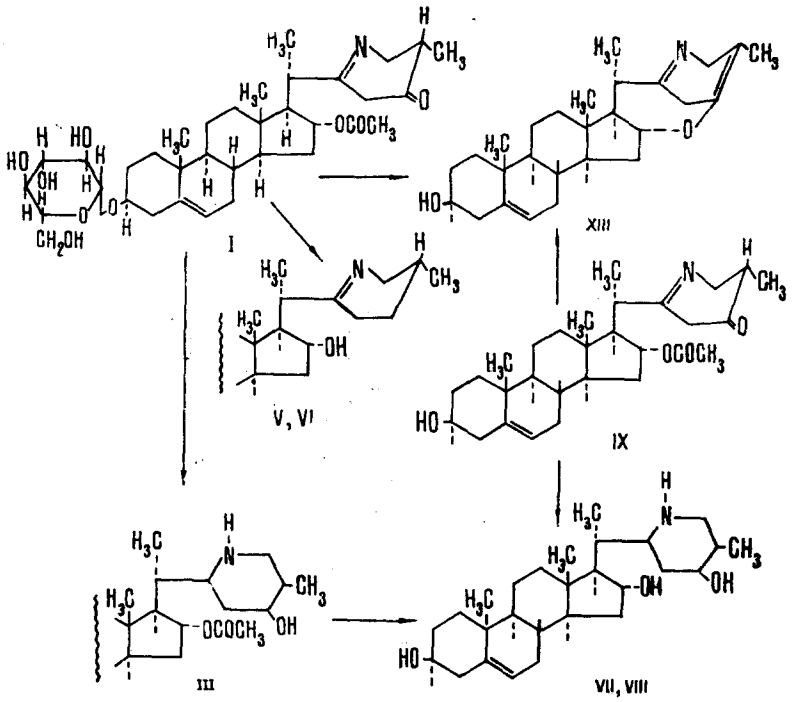

On the basis of the facts presented, veralodinine (I) has the structure and configuration of 16α-acetyl-3β-D-glucopyranosido-22,26-iminocholesta-5,22(N)-dien-24-one (see Scheme).

The hydrolysis of veralodinine in hydrochloric acid in the presence of ethanol gave the aglycone with mp 256-257°C, composition $C_{27}H_{39}NO_2$, M^+ 409, containing one molecule of water less than the expected composition. D-Glucose was found in the neutral fraction of the hydrolyzate after the separation of the aglycone by paper chromatography. Furthermore, a gas-chromatographic investigation of veralodinine [8] also confirmed the presence of D-glucose in its molecule.

The IR spectrum of the aglycone lacked the absorption band of a carbonyl group and had a strong band at 1640 cm^{-1} (C = N bond) and one at 1050 cm^{-1} (Δ^5-3β-OH) [9, 10]. The NMR spectrum showed a signal at 1.77 ppm from the protons of a methyl group on a double bond, and the signal in the form of a doublet from the protons of a secondary methyl group observed in the NMR spectrum of veralodinine was absent.

Acetylation of the aglycone formed the O,N-diacetyl derivative (X) with M^+ 493. Reduction of the aglycone with sodium tetrahydroborate gave the dihydro derivative (XI) with M^+ 411, and further hydrogenation in acetic acid in the presence of platinum gave the tetrahydro derivative (XII) with M^+ 413. The IR spectra of the dihydro derivative and of compound (XII) lacked the absorption band at 1640 cm^{-1} (C = N bond). Substance (XII) thus differs from the composition of tetrahydroveralodisinol (VIII) [5] by one molecule of water.

When veralodisine (IX) was heated in ethanolic hydrochloric acid, the reaction products yielded an amino alcohol with mp 256-257°C identical with the aglycone isolated in the hydrolysis of veralodinine (mixed melting point and IR spectrum), while the expected product, veralodisinol [5], was not formed. This confirms once more that the elimination of a molecule of water does in fact take place on the hydrolysis of veralodinine.

The chemical and physical facts presented permit formula (XIII) (see Scheme) to be put forward for the aglycone.

EXPERIMENTAL METHOD

Thin-layer chromatography (TLC) was performed with KSK silica gel (100 nm) and the following solvent systems: 1) benzene–ethanol (9 : 1); 2) benzene–ethanol (9 : 2.5); 3) benzene–ethanol (9 : 3); 4) benzene–ethanol (9 : 4); 5) benzene–ethanol (9 : 5); 6) chloroform–methanol (40 : 1); 7) chloroform–methanol (5 : 1); and 8) chloroform–methanol (1 : 1). The spots were revealed with Dragendorff's solution. For column chromatography we used KSK silica gel (250 nm). The UV spectra were taken on a Hitachi spectrophotometer, the IR spectra on a UR-10 double-beam spectrometer (molded tablets with KBr), the NMR

spectra on a JNM-4H-100 instrument (deuterochloroform) with hexamethyldisiloxane as the internal standard (δ scale), and the mass spectra on an MKh-1303 mass spectrometer.

Veralodinine (I). The chloroform and chloroform–methanol (98 : 2) fractions [2] were evaporated, and treatment of the residue with methanol led to the isolation of 5.1 g of veralodinine with mp 226-228°C (from methanol), $[\alpha]_D$ −95.4° (c 0.524; chloroform), R_f 0.47 in system 2. Veralodinine was also isolated by the method described above [2] from the chloroform-soluble alkaloids of the epigeal part of Veratrum lobelianum collected in the Caucasus.

Tetraacetylveralodinine (II). A mixture of 0.1 g of veralodinine, 2 ml of pyridine, and 4 ml of acetic anhydride was kept at room temperature for 72 h. After the elimination of the pyridine, a 5% solution of sulfuric acid was added to the residue and it was extracted with chloroform. The chloroform extract was made alkaline with ammonia and washed with water. The residue after the distillation of the chloroform was chromatographed on a column of silica gel (3 g). Elution was performed with a mixture of benzene and ethanol (9 : 1). Fractions with a volume of 5-7 ml were collected. Fractions 15-21 yielded amorphous tetraacetylveralodinine with R_f 0.80 (system 2).

IR spectrum, ν_{max}, cm^{-1}: 1760, 1240 (ester C=O), 1710 (C=O), 1670 (C=N).

Deacetylveralodinine (IV). A solution of 1.03 g of veralodinine in 30 ml of 5% methanolic caustic potash was heated for 4 h. After the solution had been cooled it was diluted with water and was then extracted with chloroform. The saponified product was passed through a column filled with silica gel (20 g), being eluted with benzene–ethanol (9 : 3) in 20-ml fractions. The first 100 ml of eluate yielded deacetylveralodinine (IV) with mp 236-239°C, R_f 0.27 (system 1).

The alkaline solution after the isolation of the deacetylveralodinine was acidified with 5% acid and extracted with ether, and the presence of acetic acid with R_f 0.13 was shown by the method of Khashimov et al. [6].

Hydrolysis of Veralodinine. A mixture of 0.7 g of veralodinine, 35 ml of ethanol, and 35 ml of a 10% solution of hydrochloric acid was boiled for 4 h. Then the ethanol was driven off, and the acid solution was made alkaline with ammonia and extracted with chloroform. The concentrated chloroform solution yielded the aglycone (XIII) with mp 256-257°C [ethanol–acetone (1 : 3)], $[\alpha]_D$ −226.8° (c 0.873; ethanol), R_f 0.70 (system 3).

IR spectrum, ν_{max}, cm^{-1}: 3430-3170 (associated hydroxy groups), 3030, 1640 (CH=C, C=N). Mass spectrum: m/e 97, 177 (100%), 253, 376, 391, 394, 409 M^+. UV spectrum: λ_{max} 245 nm (log ε 4.15); NMR spectrum: singlets at (ppm) 0.96 (3H, CH_3), 1.01 (3H, CH_3), 1.77 (3H, C=C−CH_3), doublet at 0.92 (from the protons of a secondary methyl group), multiplet at 5.28 (H, C=CH) (deuteroethanol).

The alkaline solution after the isolation of the aglycone was neutralized with 5% sulfuric acid and evaporated to dryness. The dry residue was treated with ethanol. The ethanolic residue was shown by chromatography on paper (Leningrad slow) to contain D-glucose [3]. The time of chromatography was 17 h.

Tetrahydroveralodinine (III). Veralodinine (0.268 g) was hydrogenated in glacial acetic acid (20 ml) by the Adams method (0.063 g of PtO_2). The acetic acid solution after separation from the platinum black was diluted with water, made alkaline with ammonia, and extracted with chloroform. The residue from the distillation of the chloroform was chromatographed on a column of silica gel and eluted with a mixture of benzene and ethanol (9 : 1). Fractions with a volume of 5-10 ml were collected. Fractions 12-17 yielded tetrahydroveralodinine with mp 227-229°C (from acetone). R_f 0.38 (system 7).

Hydrolysis of Tetrahydroveralodine. Tetrahydroveralodinine (0.08 g) was hydrolyzed in 3.5% ethanolic hydrochloric acid (5 ml) in a similar manner to the hydrolysis of veralodinine. The hydrolysis product was chromatographed on a column of silica gel and eluted with a mixture of benzene and ethanol (9 : 5). Fractions with a volume of 5-7 ml were collected. Fractions 6-11 yielded crystals of (VII) with mp 247-249°C (from methanol) identical with tetrahydroveralodisinol (VIII) according to a mixed melting point and IR spectrum; M^+ 431.

Deoxodeacetylveralodinine (VI). A mixture of 0.12 g of veralodinine, 4 ml of ethanol, 4 ml of diethyleneglycol, and 1.5 ml of 85% hydrazine hydrate was heated in the water bath for 30 min. Then 0.39 g of caustic potash was added and the mixture was heated for another 30 min. It was heated at 190-200°C for 150 min. After cooling, the reaction mixture was diluted with water (50 ml) and extracted with chloroform. The chloroform was distilled off and the residue was chromatographed on a column of silica gel (3 g). Elu-

tion was performed with a mixture of benzene and ethanol (9 : 3). Fractions with a volume of 8-10 ml were collected. Fractions 11-18 yielded deoxodeacetylveralodinine with mp 229-231°C [methanol–acetone (1 : 3)], R_f 0.17 (system 5), identical with deacetylveralosine [6] according to mixed melting point and IR spectrum.

IR spectrum, ν_{max}, cm^{-1}: 3400 (OH), 2935, 1450 (CH_2, CH_3), 1650 (C = N), 1100-1000 (characteristic for glycoalkaloids).

The O,N-Diacetylaglycone (X). A mixture of 0.03 g of the aglycone, 1.5 ml of pyridine, and 1.5 ml of acetic anhydride was acetylated in a similar manner to the acetylation of veralodinine. The amorphous diacetylaglycone with Rf 0.36 (system 6), M^+ 493, was isolated.

IR spectrum, ν_{max}, cm^{-1}: 1730, 1250 (O-acetyl), 1650 (N-acetyl). NMR spectrum, ppm: singlets at 0.94 (3 H, CH_3), 1.00 (3 H, CH_3), 1.48 (3 H, $>CH_3$), 1.98 (3 H, $OCOCH_3$), 2.02 (3 H, $N-COCH_3$), doublet (3 H, $>CH-CH_3$), and multiplets at 4.51 (H, $CH-OCOCH_3$) and 5.32 (H, C = CH).

The Dihydroaglycone (XI). Over 1.5 h, 0.43 g of sodium tetrahydroborate was added to a solution of 0.087 g of the aglycone in 15 ml of 90% aqueous methanol, and then the mixture was diluted with water and extracted with chloroform. Distillation of the chloroform yielded the dihydroaglycone with mp 276-278°C (acetone), R_f 0.33 (system 4), M^+ 411. IR spectrum, ν_{max}, cm^{-1}: 3500-3210 (OH, NH), 3030, 1650 (C = CH).

The Tetrahydroaglycone (XII). The dihydroaglycone (0.077 g) was hydrogenated in glacial acetic acid (10 ml) by the Adams method (0.071 g of PtO_2) as for the hydrogenation of tetrahydrovalerodinine. After the chloroform had been distilled off, a mixture of crystals was obtained with mp 269-272°C (from acetone), R_f 0.28, 0.47 (system 8), which was separated preparatively on a plate with a fixed layer of silica gel in the chloroform–methanol (1 : 1) system. This yielded the tetrahydroaglycone with mp 232-234°C (from acetone), R_f 0.28 (system 8). M^+ 413.

The Aglycone (XIII). A mixture of 0.04 g of veralodisine [5] and 4 ml of 3.5% ethanolic hydrogen chloride was heated for 180 min. After cooling, the reaction mixture was diluted with water and treated with chloroform. The concentrated chloroformic solution deposited crystals with mp 256-257°C [ethanol–acetone (1 : 3)], R_f 0.70 (system 3), identical with the aglycone (XIII) obtained by the hydrolysis of veralodinine.

SUMMARY

1. A new alkaloid, veralodinine, has been obtained by the separation of the combined alkaloids from the epigeal part of Veratrum lobelianum.

2. On the basis of the IR, UV, and NMR spectra of veralodinine and the products of its transformation, and also its conversion to known alkaloids – veralosine and tetrahydroveralodisinol – its structure and configuration has been established as 16α-acetyl-3β-D-glucopyranosido-22,26-iminocholesta-5,22(N)-dien-24-one.

LITERATURE CITED

1. A. M. Khashimov, R. Shakirov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 779 (1971).
2. K. Samikov, R. Shakirov, and S. Yunusov, Khim. Prirodn. Soedin., 770 (1972).
3. R. Shakirov, R. N. Nuriddinov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 384 (1965).
4. E. Bianchi, C. Djerassi, H. Budzikiewicz, and J. Sato, J. Org. Chem., 30, 754 (1965).
5. R. Shakirov, A. M. Kashimov, K. Samikov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 44 (1974).
6. A. M. Khashimov, R. Shakirov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 343 (1970).
7. Huang-Minlon, J. Amer. Chem. Soc., 71, 3301 (1949).
8. T. T. Gorovits, Khim. Prirodn. Soedin., 49 (1969).
9. T. Masamune, J. Mori, M. Takasugi, A. Mura, S. Obuchi, N. Sato, and N. Katsui, Bull. Chem. Soc. Japan, 38, 1374 (1965).
10. I. Tomko, Z. Voticky, H. Budzikiewicz, and F. I. Darkan, Collection Czech Chem. Commun., 3320 (1965).