STRUCTURE OF VERALOSIDININE

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UDC 547.944/945

In a preceding paper, we gave the results of the isolation from the epigeal part of Veratrum lobelianum of a base with mp 220 221°C, $[\alpha]_D$ −173.17° [1]. A study of its physicochemical properties has shown that the alkaloid is a new one, and we have called it veralosidinine.

Veralosidinine, $C_{29}H_{45}O_4N$ (I) is a tertiary unsaturated base. Its IR spectrum (ν_{max}, cm^{-1}): 3420, 1065, (OH), 3030 (C=CH), 2930, 1445 (CH_3-, $-CH_2-$), 1650 (C=N−), 1725, 1250 (ester C=O). The fingerprint region is similar to that of veralosinine (Fig. 1) [2]. The UV spectrum [λ_{max} 248 nm (log ε 2.44)] is identical with the UV spectra of verasine, veralosinine, and veralosidine, which have cholestane skeletons [2-4]. The mass spectrum of veralosidinine has as its main peaks those of ions with m/e 98, 99, 110, 111, 124, 125 (100%), 126, 138, 148, 162, 163, $(M-60)^+$, 412, 413, $(M-42)^+$, $(M-15)^+$, 471 (M^+), as in the fragmentation of the alkaloids veralosidine and veralosinine [2, 4]. In the NMR spectrum of (I) there are the following resonance signals (ppm): singlets at 0.58 (3H, 18−CH_3), 0.89 (3H, 19−CH_3), 1.95 (3H, $OCOCH_3$); doublets at 0.86 (3H, 21−CH_3), 0.92 (3H, 27−CH_3), and multiplets at 4.85 (H, H−C−$OCOCH_3$) and 5.23 (H, olefinic proton).

The hydrogenation of veralosidinine in ethanol formed dihydroveralosidinine (II), in the IR spectrum of which the absorption band characteristic for a C=N bond had disappeared. Acetylation in pyridine with acetic anhydride led to the amorphous O,O',N-triacetylveralosidinine (III) with R_f 0.91. IR spectrum of the triacetyl derivative (ν_{max}, cm^{-1}): 1730 (O-acetyl), 1670 (N-acetyl), and 1650 (CH=C bond). The formation of a N-acetyl group is explained by the migration of hydrogen from position 23 to the nitrogen atom with the formation of a C=C double bond between atoms 22 and 23. The NMR spectrum of (III) showed the following signals (ppm): singlets at 0.67 (3H, 18−CH_3), 0.93(3H, 19−CH_3), 1.95 (9H, −$OCOCH_3$), 2.11 (3H, N−$COCH_3$), doublets at 0.91 (3H, 21−CH_3), 0.86 (3H, 27−CH_3); and multiplets at 5.02; 4.69; 4.46 (3H, H−C−$OCOCH_3$), and 5.27 (2H, olefinic protons). The appearance of a second signal of an olefinic proton at 5.32 ppm in (III) confirms once again the migration of hydrogen and the displacement of the double bond in O,O',N-triacetylveralosidinine and recalls the N-acetylation of tomatilidine and of verasine [3, 5]. Consequently, of the four oxygen atoms in the molecule of veralosidinine, two are present in the form of secondary hydroxy groups and two are in the form of an ester grouping. Veralosidinine is saponified by ethanolic alkali. From the products of alkaline hydrolysis have been isolated an amino alcohol – veralosidininol – $C_{27}H_{43}O_3N$ (IV) – and acetic acid (paper chromatography).

The IR spectrum of (IV) lacks the absorption band of an ester carbonyl. Its NMR spectrum has the signals (ppm): singlets at 0.56 (3H, 18−CH_3), 0.91 (3H, 19−CH_3); doublets at 0.87 (3H, 27−CH_3), 1.13, (3H, 21−CH_3); and a multiplet at 5.26 (H, olefinic proton). In its mass spectrum the main peaks are those of the ions with m/e 98, 100, 111, 112, 125 (100%), 126, 138, 150, 151, 162, 291, $(M-18)^+$, $(M-CH_3)^+$, 429 (M^+). This fragmentation pathway resembles those of the typical steroid alkaloids verasine, petiline, and veralosidine [3, 4, 6], in which the maximum peak of the ion with m/e 125 comprises the nitrogen-containing moiety of the molecule; it is formed as a result of the cleavage of the $C_{17}-C_{20}$ bond.

Thus, the results of a comparative study of the physicochemical properties of veralosidinine and its transformation products with those of veralosidine and of other steroid alkaloids [2-7] shows that veralosidinine (I) contains the heterocyclic skeleton of veralosidine (V).

Absorption at about 3030 and 1065 cm^{-1} in the IR spectrum of veralosidinine shows the presence of a Δ^5-3β-OH group in it [8, 9]. Differences in the chemical shifts (CSs) of the protons of the 18−CH_3 and 21−CH_3 groups between veralosidinine and veralosidininol show that the acetyl of the secondary hydroxy groups

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 501-504, July-August, 1973. Original article submitted July 20, 1972.

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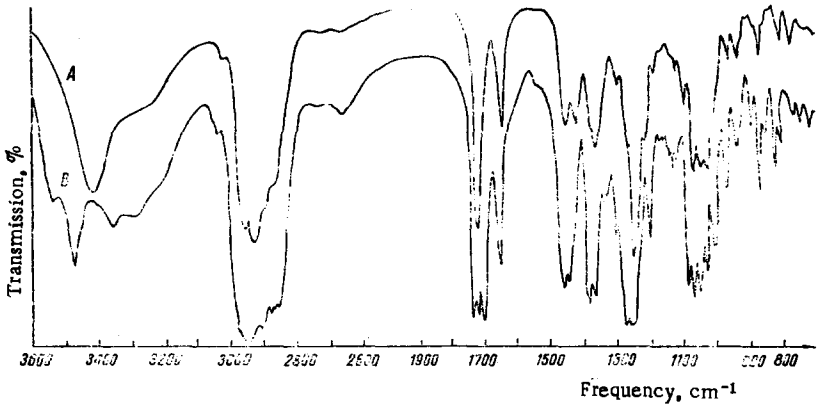
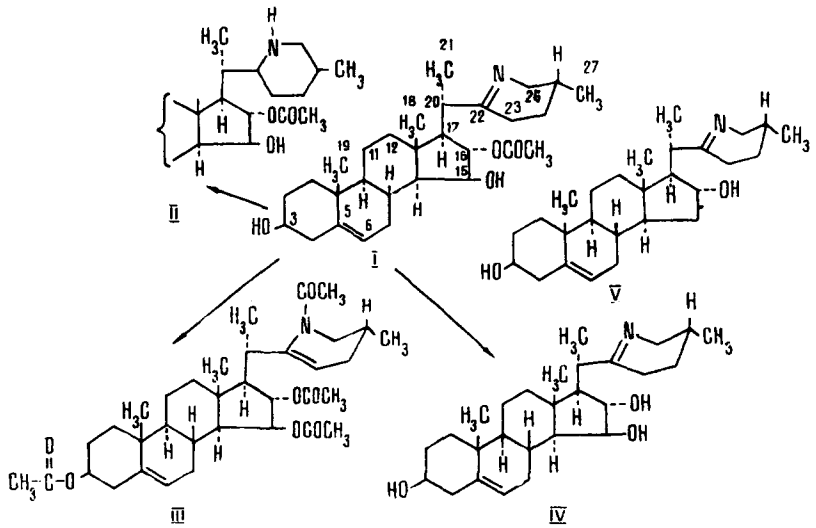


Fig. 1. IR spectra of veralosidinine (a) and veralosinine (b).

in veralosidinine can be located only on the carbon atoms of rings C and D in the 11, 12 or 15, 16 positions. The change in the CSs of the protons of the $18-CH_3$ group in the NMR spectrum of veralosidininol in the upfield direction by 3 Hz in comparison with the CSs of the protons of the $18-CH_3$ group of veralosidinine excludes the 11, 12 positions of the acetyl group, which may be present in ring D on carbon C_{16}; it has the α orientation [2, 10]. This is confirmed by the difference in the molecular rotations ($\Delta[M]_D - 301.56°$) of veralosidinine and veralosidininol [$-815.63°$ for (I) and $-514.07°$ for (IV)], which is close to the increment that has been found for C_{16}-α-$OCOCH_3$ groups [11].

In the NMR spectrum of O,O',N-triacetylveralosidinine the signal from the protons of the $18-CH_3$ group resonates in a stronger field by 9 Hz than the protons of the $18-CH_3$ group of veralosidinine, which excludes positions 11 or 12 for the secondary hydroxy group and shows that it is located at C_{15}; it has the β orientation [10]. The CSs of the protons of the $18-CH_3$, $19-CH_3$ and $27-CH_3$ groups in the NMR spectrum of O,O',N-triacetylveralosidinine agree well with the CSs of the protons of the corresponding methyl groups in O,O',N-triacetylveralosidine. This shows that the B/C and C/D rings in the molecule of veralosidine are trans-linked and the remaining asymmetric centers have the same configuration as the asymmetric centers of veralosidine [2, 4]. The large difference in the CSs of the protons of the $21-CH_3$ group in the compounds O,O',N-triacetylveralosidine and O,O',N-triacetylveralosidinine is apparently due to the presence in the molecule of O,O',N-triacetylveralosidinine of a 15β-$OCOCH_3$ group. On the basis of what has been said, veralosidinine (I) has the most probable structure and configuration of 16α-acetoxy-22,26-nitrilocholesta-5,22(N)-diene-3β, 15β-diol.



EXPERIMENTAL

Thin-layer chromatography (TLC) was performed with KSK silica gel with a size of 100 nm containing 10% of $CaSO_4$ and the following solvent systems: 1) butyl acetate–chloroform–ethanol (1:2:3) and 2) ben-

zene–acetone (1:1). The spots were revealed with Dragendorff's solution. The UV spectrum was 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 (I and III in $CDCl_3$; IV in $CDCl_3+CD_3OD$), δ scale, with hexamethyldisiloxane as internal standard. In the NMR spectra of (I, III, and IV) there are doublets with J = 6 Hz.

Veralosidinine $C_{27}H_{45}O_4N$ was isolated [1] from the total ethereal extract of the epigeal part of the plant Veratrum lobelianum by separation with an acetate buffer solution. mp 220-221°C (from acetone), $[\alpha]_D$ −173.17° (c 0.589; chloroform), R_f 0.82 (on TLC in system 1), mol. wt. 471 (mass spectrum).

O,O',N-Triacetylveralosidinine. A mixture of 0.05 g of veralosidinine, 2 ml of acetic anhydride, and 2 ml of pyridine was kept at room temperature for 24 h. After the solvent had been distilled off in vacuum, the residue was treated with 5% sulfuric acid and was extracted with ether. The ethereal extract was made alkaline with 1% ammonia solution and was washed with water. The ether was distilled off and the residue was dried in vacuum. The O,O',N-triacetylveralosidinine obtained could not be crystallized; it was amorphous and homogeneous with R_f 0.91 (on TLC in system 1), mol. wt. 597 (mass spectrum).

Dihydroveralosidinine. Veralosidinine (0.06 g) was hydrogenated in 10 ml of ethanol by Adams' method (0.05 g of PtO_2) until the absorption of hydrogen ceased. After separation from the platinum black, the ethanol was distilled off in vacuum. On TLC in system 2, the reaction product showed the presence of a mixture of two substances with R_f 0.06 and 0.18.

This mixture (0.05 g) was dissolved in benzene–acetone (4:1) and chromatographed on a column of silica gel with elution by the same mixture; 10 5-ml fractions were collected and then five 20-ml fractions. The first nine fractions yielded dihydroveralosidinine with mp 222-223°C (from acetone), $[\alpha]_D$ −147.41° (c 0.251; chloroform), R_f 0.18 (on TLC in system 2), mol. wt. 473 (mass spectrum).

Saponification of Veralosidinine. A solution of 0.3 g of veralosidinine in 20 ml of 5% methanolic caustic potash was boiled for 3 h. Then the reaction mixture was cooled, diluted with water, and extracted with chloroform. The chloroform residue (0.27 g) was treated with acetone, giving veralosidininol, $C_{27}H_{43}O_3N$, with mp 204-206°C (from acetone), $[\alpha]_D$ −117.5° (c 0.4; methanol), R_f 0.56 (on TLC in system 1), mol. wt. 429 (mass spectrum). The alkaline solution after the separation of the veralosidininol was found by a method described previously [2] to contain acetic acid with R_f 0.13.

SUMMARY

On the basis of the IR, UV, NMR, and mass spectra of veralosidinine and its conversion products its most probable structure and configuration have been established as 16α-acetyl-22,26-nitrilocholesta-5,22 (N)-diene-3β, 15β-diol.

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