STRUCTURE OF VERALODISINE

R. Shakirov, A. M. Khashimov,
K. Samikov, and S. Yu. Yunusov

UDC 547.944/945

Continuing the separation of the mixture of bases from the epigeal part of Veratrum lobelianum [1, 2] we have isolated a new alkaloid veralodisine $C_{29}H_{34}O_4N$ (I). This is a tertiary base which forms with digitonin a sparingly soluble digitonide showing that substance (I) has a 3β-OH group and belongs to the typical steroid alkaloids. The IR spectrum of veralodisine shows absorption bands at (cm^{-1}) 3475 (OH), 2940, 1460, 1435 ($-CH_3-$, $-CH_2-$), 3030, 1680 (CH = C), 1680 (C = N), 1730, 1270 (ester carbonyl), 1700 (carbonyl in a six membered ring). The UV spectrum of (I) [λ_{max} 275 nm (log ε 3.63)] is similar to that of tomatillidine [3]. In the NMR spectrum of substance (I) there are signals at (ppm) 0.64 (3 H, 18-CH_3), 0.92 (3 H, 19-CH_3), and 1.99 (3 H, $OCOCH_3$); doublets at 0.93 and 1.00 (two secondary methyl groups, J = 6 Hz); and multiplets at 3.71 (H, CH−OH), 4.96 (H, CH−OCO−CH_3), and 5.26 (one olefinic proton).

Consequently, veralodisine contains four C-methyl groups. The nitrogen is present in a C = N system, two oxygen atoms in the residue of an ester grouping and the other two oxygen atoms in the form of hydroxy and carbonyl groups. The mass spectrum of (I) (Fig. 1) has the main peaks of ions with m/e 83, 84, 110, 111, 139, 140, 164, 177, 272, 281, 298, 299, 300, 314, 326, 352, 366, 381, 382, 394, 398, 409 (100%) (M−CH_3 COOH) +, 426 $[M-(CH_3 + CO)]^+$, 427 $(M-42)^+$, 441 $(M-CO)^+$, 454 $(M-CH_3)^+$, 469 (M^+).

A similar pattern is given in the mass-spectrometric decomposition of tomatillidine [3].

Veralodisine is saponified in an aqueous methanolic solution of potassium carbonate. From the products of its saponification we isolated the amino alcohol veralodisinol $C_{27}H_{41}O_3N$ (II) and acetic acid (paper chromatography). The IR spectrum of (II) lacks the absorption band of an ester carbonyl, and the absorption band of the C = O group (1690 cm^{-1}) and the C = N group (1652 cm^{-1}) are shifted in the low-frequency direction. In the NMR spectrum there are singlets at (ppm) 0.61 (3 H, 18-CH_3) and 0.91 (3 H, 19-CH_3), and a six-proton doublet at 0.98 ppm from two secondary methyl groups, and multiplets at 3.60 and 4.10 (2 H, 2CH−OH) and 5.23 (olefinic proton), the signal of the protons of an acetyl group being absent.

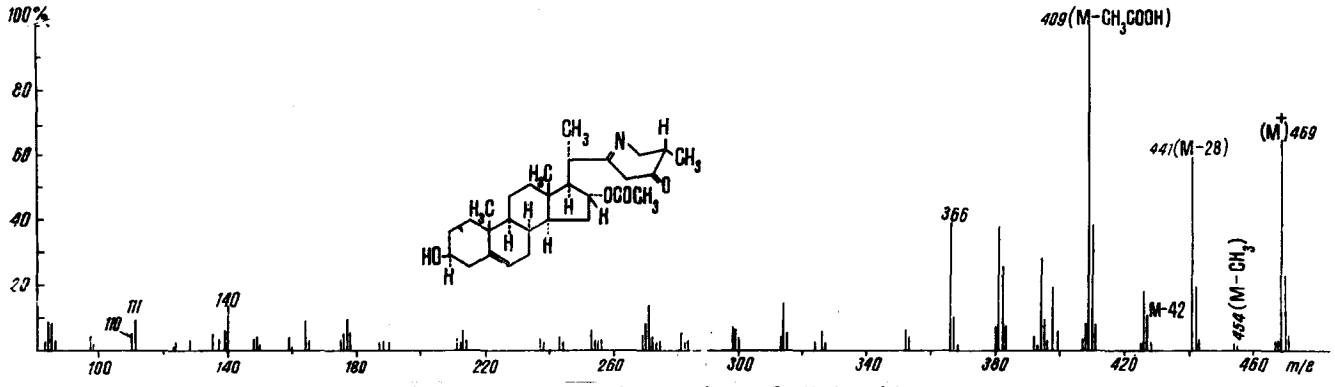
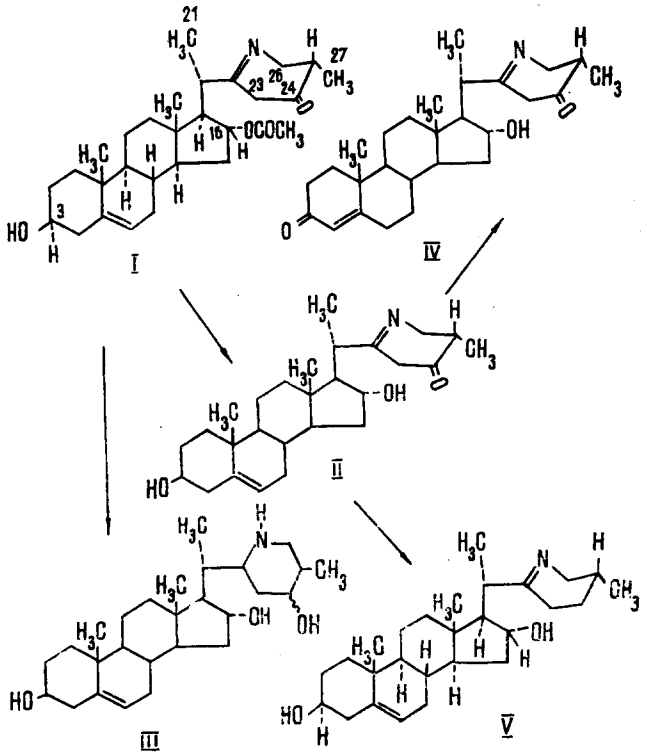


Fig. 1. Mass spectrum of veralodisine (I).

Order of the Red Banner of Labor Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 44-48, January-February, 1974. Original article submitted November 21, 1972.

The reduction of (I) with lithium tetrahydroaluminate gave a tetrahydro derivative $C_{27}H_{45}O_3N$ (III) with mol. wt. 431 (mass spectrum), in the IR spectrum of which the absorption bands of C = N and C = O groups had disappeared. The Oppenauer oxidation of (II) yielded an α, β-unsaturated ketone (IV). Its UV spectrum [λ_{max} 241 nm (log ε 4.15)] is similar to those of the unsaturated oxo derivatives of tomatillidine, verasine, and veralosidine [3-5]. The facts given above permit the assumption that compound (I) is based on the heterocyclic skeleton of tomatillidine and veralosidine [3-5].

From the products of the Huang-Minlon [6] reduction of (II) we isolated deoxoveralodisinol with mp 153-155°C, the IR spectrum of which lacked the absorption band characteristic for a carbonyl group but clearly showed the absorption band of the C = N system. Deoxoveralodisinol proved to be identical with veralosidine (V) [5] (Scheme) from the melting point of a mixture and from the IR spectrum. Thus, veralodisine is a carbonyl derivative of veralosidine acetate [5].



In the mass spectrum of tetrahydroveralodisinol, the maximum peak is that of the ion with m/e 114 due to the fragment of the side chain at C_{20} of the compounds hexahydrotomatillidine, veratramine, isojervine, and hexahydrokorsevinine [3, 7, 8], which is formed as a result of the cleavage of the $C_{20}-C_{22}$ bond.

The facts given confirm that the carbonyl group in veralodisine is located in the heterocyclic part of the molecule. It may be positioned at C_{23}, C_{24}, or C_{26}. Veralodisine does not possess the properties of an amide or of an α, β-unsaturated ketone. These facts exclude the C_{23} and C_{26} positions for the carbonyl group, and only the C_{24} position remains for it.

The formation of a sparingly soluble digitonide by veralodisine shows that the acetate group in (I) is located at C_{16}. This is shown by the fact that the signal of the proton geminal to the acetate group in veralodisine resonates at 4.96 ppm and in veralodisinol the signal of this proton is shifted upfield by 0.86 ppm. Such a shift of the signal of the proton geminal to an acetate group on passing from (I) to (II) shows that the acetate group in veralodisine is located in the five-membered ring at C_{16} [9]. The configurations of the asymmetric centers of veralodisine follow from the structure of veralosidine [5].

Consequently, veralodisine has the most probable structure and configuration of 16α-acetyl-3β-hydroxy-22,26-iminocholesta-5,22(N)-dien-24-one.

EXPERIMENTAL

Thin-layer chromatography (TLC) was performed with KSK silica gel (100 nm) and the following solvent system: 1) butyl acetate–ethanol–chloroform (3 : 2 : 20); 2) benzene–ethanol (9 : 1.5); 3) benzene–

ethanol (9 : 4); 4) chloroform–methanol (10 : 1); and 5) chloroform–ethanol (9 : 1). The chromogenic agent was Dragendorff's solution. For column chromatography we used alumina (activity grade II) and KSK silica gel (250 nm). The UV spectra were taken on a Hitachi spectrophotometer (in ethanol); the UV spectra on a UR-10 double-beam spectrophotometer (molded tablets with KBr); the NMR spectra on a JNM-4H-100 MHz instrument (deuterochloroform), with hexamethyldisiloxane as internal standard (δ scale); and the mass spectra on an MKh-1303 mass spectrometer.

Veralodisine (I). The residue (20 g) from the mother liquor from the isolation of veralodine [2] was dissolved in benzene and chromatographed on a column of alumina. Elution was performed with benzene (four liters), benzene–ethanol (30 : 1), and chloroform–methanol (25 : 8). When the benzene eluate was concentrated and allowed to stand, it deposited veralodisine $C_{29}H_{43}O_4N$ with mp 172-174°C $[\alpha]_D$ −92.79° (c 0.486; chloroform), R_f 0.67 on TLC in system 1; mol. wt. 469 (mass spectrometrically). Veralodisine has also been isolated from the mother liquors from the isolation of veralosinine [10] by the above-described method.

Veralodisinol (II). A solution of 0.25 g of (I) in 10 ml of methanol was treated with 3 ml of a 10% aqueous methanolic solution of potassium carbonate and the mixture was left at room temperature for 72 h. Then it was diluted with water, and the alkaloids were extracted with chloroform. The chloroform was distilled off and the residue was crystallized from acetone–petroleum ether (1 : 4), mp 188-190°C (acetone –petroleum ether); R_f 0.54 on TLC in system 2. Composition $C_{27}H_{41}O_3N$, mol. wt. 427 (mass spectrometrically). In an alkaline solution after the separation of the veralodisinol by a method described previously [10], acetic acid was detected by paper chromatography.

Tetrahydroveralodisinol (III). In drops, 0.13 g of a suspension of lithium tetrahydroaluminate in 30 ml of absolute ether was added over 12 min to a solution of 0.12 g of (I) in 40 ml of absolute ether. After standing for 20 min, the reaction mixture was heated in the water bath for 4 h. Then it was cooled, 5 ml of water was added, and it was extracted with ether. The concentrated ethereal solution deposited a mixture of crystals with R_f 0.08, 0.15, 0.33, and 0.65 on TLC in system 3. The mixture of crystals was dissolved in chloroform, chromatographed on a column of alumina (activity grade II), and eluted with 15-ml portions of chloroform and also with 15-ml portions of chloroform–methanol (50 : 2). On treatment with acetone, the third fraction of the chloroform eluate deposited crystals with mp 247-249°C [from methanol–acetone (1 : 3)]; R_f 0.13 on TLC in system 4; composition $C_{27}H_{45}O_3N$; mol. wt. 431 (mass spectrometrically).

Oppenauer Oxidation of (II). A solution of 1.5 g of aluminum tert-butoxide in 20 ml of absolute benzene was added together with 10 ml of absolute acetone to 0.4 g of (II) in 15 ml of absolute benzene. The mixture was boiled for 8 h and was then allowed to stand at room temperature for 24 h, after which 50 ml of a saturated solution of sodium carbonate was added and the alkaloids were extracted with chloroform. When the chloroform had been distilled off, the residue was chromatographed on a column of Al_2O_3. The benzene –ethanol (9 : 1) eluate yielded the α, β-unsaturated ketone (IV) with mp 167-169°C (from benzene), R_f 0.32 in system 5.

Deoxoveralodisinol (V). A mixture of 0.14 g of (II), 4.6 ml of ethanol, 4.6 ml of diethyleneglycol, and 1.2 ml of 85% hydrazine hydrate was heated at 100°C in an atmosphere of nitrogen for 30 min. Then 0.52 g of caustic potash was added and the mixture was heated for another 35 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, giving a mixture of products with R_f 0.00, 0.30, and 0.47 in system 4. This mixture was separated preparatively in a thin layer of silica gel–gypsum (10 : 1) in the chloroform–methanol (10 : 1) system. Deoxoveralosidinol was isolated with mp 153-155°C (from acetone), R_f 0.30 in system 4; it was identical with veralosidine according to a mixed melting point and its IR spectrum. Composition $C_{27}H_{43}O_2N$, mol wt. 413 (mass spectrometrically).

CONCLUSIONS

1. The new alkaloid veralodisine has been isolated by separating the combined alkaloids from the epigeal part of Veratrum lobelianum.

2. On the basis of the results of a study of the IR, UV, NMR, and mass spectra of veralodisine and its transformation products, and also passage to the known alkaloid veralosidine, the most probable structure and configuration of veralodisine has been found to be 16α-acetyl-3β-hydroxy-22,26-iminocholesta-5,22 (N)-dien-24-one.

LITERATURE CITED

1. K. Samikov, R. Shakirov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 790 (1971).
2. K. Samikov, R. Shakirov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 770 (1972).
3. E. Bianchi, C. Djerassi, H. Budzikiewicz, and Y. Sato, J. Org. Chem., 30, 754 (1965).
4. G. Adam, K. Schreiber, and A. Vassova, Tetrahedron, 23, 167 (1967).
5. A. M. Khashimov, R. Shakirov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 779 (1971).
6. Huang-Minlon, J. Amer. Chem. Soc., 71, 3301 (1949).
7. H. Budzikiewicz, Tetrahedron, 20, 2267 (1964).
8. R. N. Nuriddinov and S. Yu. Yunusov, Khim. Prirodn. Soedin., 600 (1969).
9. Y. Kawazoa, Y. Sato, T. Okamato, and K. Tsuda, Chem. Pharm. Bull. (Tokyo), 11, 328 (1963).
10. A. M. Khashimov, R. Shakirov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 339, 343 (1970).