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STRUCTURE OF PANICULAMINE, A NEW ALKALOID FROM *Aconitum paniculatum*

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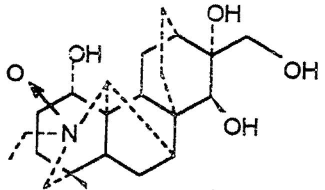
From the roots of the cultivated plant Aconitum paniculatum *Lam. we have isolated a new diterpene alkaloid of the denudatine type, which we have called paniculamine and have established its structure by x-ray structural analysis (diffractometer, CuK_α radiation, 1729 reflections, direct method, R = 0.055).*

In the Polar-Alpine Botanical Garden (PABS) work has been carried on for more than 50 years on the introduction of plants of various species of the *Aconitum* L. genus into the Subarctic [1]. Continuing a study of the alkaloids of cultivated species of this plant [2], we have analyzed for their alkaloid content the hypogeal organs (rhizomes with roots) of the cultivated plant *A. paniculatum* (panicled monkshood), which belongs to the section *Napellus* DC. The initial material for cultivation consisted of seeds gathered in the wild and introduced into the Sarajevo Botanical Garden (Yugoslavia).

From the roots of *A. paniculatum* gathered at the moment of harvesting the green fruit (end of vegetation, 1991) we obtained a mixture of alkaloids amounting to 0.5% of the weight of the air-dry raw material. Column chromatography of the total alkaloids permitted the isolation of a new polar crystalline base with mp 222-223°C (from pyridine). The substance was readily soluble in water, sparingly in ethanol and methanol, and insoluble in ether and acetone. It crystallized from methanol and pyridine.

The structure of the compound isolated was first studied by IR, PMR, and mass spectrometries, which permitted its composition to be determined as $C_{22}H_{35}NO_5$ (M^+, mass spectrometry) and showed the presence of hydroxy and 18-methyl and N-ethyl groups and the absence of methoxy, carbonyl, and exomethylene groups. These facts, and also the presence in the PMR spectrum of the base of signals at 3.90 and 3.67 ppm in the form of an *AB* quartet with the SSCC 12 Hz enabled the substance to be assigned to the C_{20}-diterpenoid alkaloids with a hydroxymethyl group at C-16 in place of an ethylene group.

However, the spectral characteristics proved to be insufficient to establish the structure of the alkaloid isolated, which we have called paniculamine. We therefore performed an x-ray structural investigation (XSI), which showed that paniculamine has the structure (**1**).



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The XSI results established that the compound isolated was an N-oxide (from the electron density and the N—O distance of 1.39 Å) of a diterpene alkaloid of the denudatine type not yet described in the literature. The mass and PMR spectra agreed well with an N-oxide structure for paniculamine. Its mass spectrum (M^+ 393) contained the triplet of peaks at *m/z* 377 (M − 16, 84%), 376 (M − 17, 36%) and 375 (M − 18, 27%) that is characteristic for N-oxides. In the PMR spectrum, the signal of protons at the carbon atoms present in the α-positions to the N-oxide group were observed in a weaker field: H-20 in the form of a sharp signal at 4.01, and 2H-19 in the form of an *AB* quartet at 3.36 and 3.11 with a *gem* coupling constant of 13

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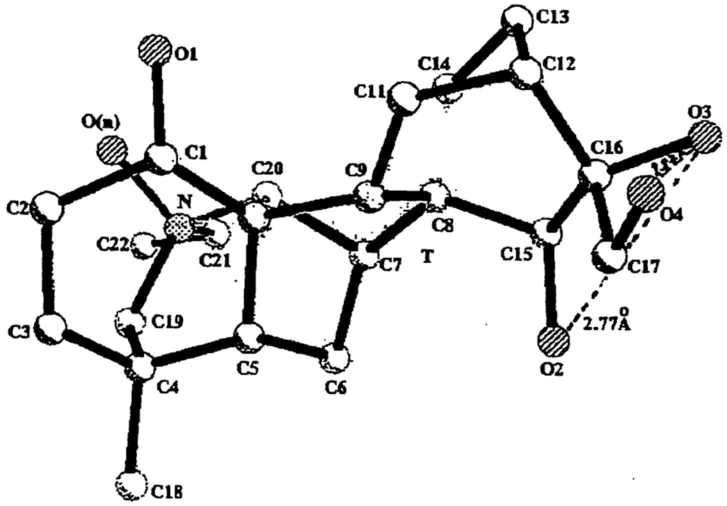

Fig. 1. Spatial structure of paniculamine.

Hz. The signals of the methylene protons of an N-ethyl group, being nonequivalent, as in the cases of the N-oxides of the alkaloids flavamine and flavadine [3], resonated at 2.78 (quartet J = 7.5 Hz) and 3.40-3.00 ppm (signal unresolved because superposition on the signals of the protons at C-19).

The spatial structure of paniculamine is shown in Fig. 1, from which it can be seen that the molecule has a rigid skeleton of the denudatine type, consisting of six rings, where the hydroxy groups at C1 and C16 have the α-orientation and that at C-16 the β-orientation, while the hydroxymethyl group at C-16 is β-oriented. The ethyl group at the nitrogen atom is positioned α-axially, and the oxygen atom β-equatorially.

The six-membered ring *A* (the C1-C5 and C10 atoms) is in the chair conformation with C_S symmetry, the asymmetry parameters according to [4] being as follows: $\Delta C_S(1) = 2.1°$, the C1 atom deviates by 0.43 Å and the C4 atom by 0.72 Å from the plane of the other four atoms. Ring *B* has a distorted boat form with C_S symmetry and the degree of asymmetry $\Delta C_S(5\text{-}6) = 26.1°$. Rings *C, D,* and *E,* making a bicylo[2.2.2]octane system, have the form of a distorted boat with C_S symmetry and the degrees of asymmetry $\Delta C_S(8\text{-}9) = 19.2°$, $\Delta C_S(12) = 22.2°$, and $\Delta C_S(13\text{-}14) = 21.1°$, respectively. Heterocycle *H* has the form of a *twist*-chair with C_2 symmetry ($\Delta C_2(5\text{-}10) = 4.3°$), the N, C4, C5, and C20 atoms lying in one plane (±0.08 Å) and C10 and C19 deviating from it by −0.88 and 0.33 Å, respectively. The five-membered ring *F* has a half-chair conformation with C_2 symmetry ($\Delta C_S(9) = 6.5°$), and ring *G* an envelope form with C_S symmetry ($\Delta C_S(20) = 12.3°$).

Rings *A/B* are *trans*-linked (torsional angle C20C10C5H5 160.0°) and *B/C* are *cis*-linked (C15C8C9H9 38.7°).

Bond lengths and valence angles are given in Table 1. The lengths of the ordinary $C_{sp^3}\text{-}C_{sp^3}$ bonds range between 1.51 and 1.57 Å; however, within the 3σ limits of error they agree with the generally adopted value of 1.54 Å. The scatter in the lengths of the heterobonds is slight, and they agree with the standard lengths [5], while no anomalies are observed in the valence angles.

The mutual positions of the O2-H and O3-H and also of the O3-H and O4-H hydroxy groups correspond to the formation of intramolecular hydrogens bond of the O—H···O type, as is shown by the relevant distances and angles: O2···O3 2.77 Å, angle O2—H···O 149.9°, O3···O4 2.87 Å, angle O3···H—O4 151.2°.

It must be mentioned that in the independent part of the crystal structure of paniculamine there are also two molecules of methanol of crystallization and one water molecule that participate in H-bonds. In the crystal, intermolecular H-bonds of the O—H···O type are observed between the O4 atom of the hydroxy group and the O(N) atom (distance 2.66 Å) and also between O(N) and O(M2) (distance 2.74 Å) and between O(M1)* and O(M2) (distance 2.78 Å). In addition, a weak hydrogen bond has been detected between O(w)** and O(M1) (distance 3.07 Å) and between O(w) and O(M2) (distance 3.04 Å).

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* O(M1) and O(M2) are oxygen atoms of the methanol of crystallization.

** O(w) is a water oxygen atom.

TABLE 1. Interatomic Distances r (Å) and Valence Angles ω (degrees) in the Paniculamine Molecule

Bond	r	Angle	ω	Angle	ω
N–On	1.398(6)	On–N–C19	111.2(4)	C8–C9–C10	105.1(4)
N–C19	1.518(7)	On–N–C20	104.3(4)	C8–C9–C11	109.3(4)
N–C20	1.539(6)	C19–N–C20	116.0(4)	C10–C9–C11	124.4(4)
N–C21	1.517(7)	On–N–C21	108.1(4)	C1–C10–C5	117.3(4)
O1–C1	1.426(7)	C19–N–C21	109.2(4)	C1–C10–C9	114.4(4)
O2–C15	1.402(6)	C20–N–C21	107.7(4)	C5–C10–C9	103.5(4)
O3–C16	1.458(5)	O1–C1–C2	108.5(5)	C1–C10–C20	117.8(4)
O4–C17	1.425(6)	O1–C1–C10	111.7(4)	C5–C10–C20	98.6(4)
C1–C2	1.541(8)	C2–C1–C10	114.5(4)	C9–C10–C20	102.8(4)
C1–C10	1.530(7)	C1–C2–C3	113.8(5)	C9–C11–C12	107.0(4)
C2–C3	1.497(9)	C2–C3–C4	114.4(5)	C11–C12–C13	108.1(4)
C3–C4	1.533(8)	C3–C4–C5	108.3(4)	C11–C12–C16	112.3(4)
C4–C5	1.526(7)	C3–C4–C18	108.7(4)	C13–C12–C16	106.9(4)
C4–C18	1.541(8)	C5–C4–C18	109.9(4)	C12–C13–C14	107.8(4)
C4–C19	1.535(7)	C3–C4–C19	113.5(5)	C8–C14–C13	110.1(4)
C5–C6	1.541(7)	C5–C4–C19	109.2(4)	O2–C15–C8	112.5(4)
C5–C10	1.545(7)	C18–C4–C19	107.3(5)	O2–C15–C16	111.9(4)
C6–C7	1.545(7)	C4–C5–C6	114.7(4)	C8–C15–C16	107.0(4)
C7–C8	1.542(7)	C4–C5–C10	109.1(4)	O3–C16–C12	105.6(4)
C7–C20	1.548(7)	C6–C5–C10	102.0(4)	O3–C16–C15	110.0(4)
C8–C9	1.529(6)	C5–C6–C7	104.9(4)	C12–C16–C15	108.9(4)
C8–C14	1.553(7)	C6–C7–C8	112.0(4)	O3–C16–C17	105.8(4)
C8–C15	1.528(7)	C6–C7–C20	100.8(4)	C12–C16–C17	114.5(4)
C9–C10	1.570(7)	C8–C7–C20	98.8(4)	C15–C16–C17	111.8(4)
C9–C11	1.543(7)	C7–C8–C9	99.8(3)	O4–C17–C16	110.4(4)
C10–C20	1.549(7)	C7–C8–C14	111.8(4)	N–C19–C4	117.6(4)
C11–C12	1.548(6)	C9–C8–C14	110.1(4)	N–C20–C7	118.1(4)
C12–C13	1.501(7)	C7–C8–C15	120.5(4)	N–C20–C10	113.7(4)
C12–C16	1.540(7)	C9–C8–C15	110.8(4)	C7–C20–C10	94.6(4)
C13–C14	1.556(7)	C14–C8–C15	103.9(4)	N–C21–C22	114.0(5)
C15–C16	1.582(7)				
C16–C17	1.540(7)				
C21–C22	1.484(9)				
OM1–CM1	1.29(3)				
OM2–CM2	1.12(6)				

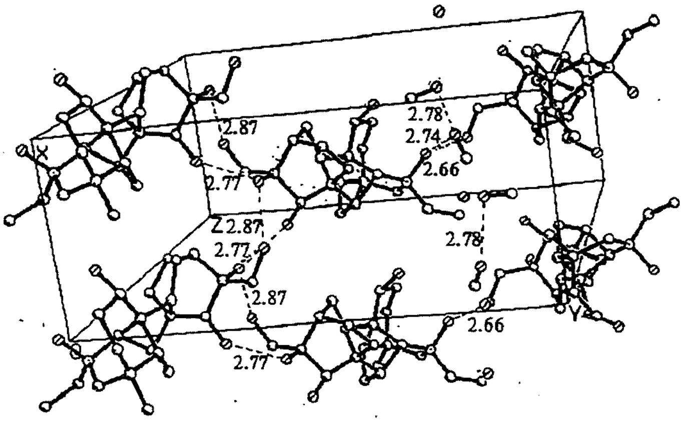

Fig. 2. Packing of the (**1**) molecules.

EXPERIMENTAL

For general observations, see [6]. The mass spectrum was taken on an MS 25 RF (Kratos) chromato-mass spectrometer (ionizing energy 70 eV, source temperature 200°C, direct injection temperature 120-150°C, collector current 100 μA). Chromatographic monitoring was done by TLC (alumina LSL 5/40, neutral, and Silufol) in the solvent systems chloroform—methanol (10:4) and ethyl acetate—ethanol—ammonia (10:3:5 drops).

TABLE 2. Coordinates ($\times 10^4$) of the C, O, and N Atoms and Temperature Factors (Å$^2 \times 10^3$) in the Structure of Paniculamine

Atom	*x/a*	*y/b*	*z/c*	*U*eq
N	2770(6)	4346(2)	12757(2)	39(1)
O1	−1925(5)	4824(3)	12669(2)	56(1)
O2	3676(5)	6896(2)	11044(2)	41(1)
O3	454(5)	7051(2)	9816(2)	36(1)
O4	−1240(5)	8035(2)	10850(2)	43(1)
O5	1350(5)	3857(2)	12955(2)	52(1)
C1	−806(7)	5404(3)	12978(3)	43(2)
C2	−376(8)	5173(4)	13752(3)	58(2)
C3	1258(8)	5569(4)	14057(3)	58(2)
C4	2952(8)	5551(3)	13579(3)	43(2)
C5	2503(6)	5972(3)	12888(2)	35(1)
C6	3993(7)	5922(3)	12311(3)	37(2)
C7	3201(7)	5381(3)	11739(3)	33(1)
C8	1757(6)	5783(3)	11273(2)	29(1)
C9	492(6)	6089(3)	11855(2)	30(1)
C10	876(6)	5571(3)	12522(3)	34(1)
C11	−1418(7)	6233(3)	11537(3)	35(1)
C12	−1154(7)	6381(3)	10731(2)	29(1)
C13	−819(7)	5625(3)	10374(3)	40(2)
C14	716(7)	5205(3)	10793(3)	37(1)
C15	2310(7)	6423(3)	10762(3)	32(1)
C16	520(7)	6889(3)	10577(2)	31(1)
C17	486(7)	7675(3)	10950(3)	35(1)
C18	4529(8)	5959(4)	13966(3)	53(2)
C19	3604(7)	4737(3)	13401(3)	41(2)
C20	1911(7)	4880(3)	12197(3)	35(1)
C21	4220(8)	3871(3)	12386(3)	50(2)
C22	4966(11)	3240(4)	12828(4)	69(2)
OM1	6832(28)	3033(10)	1078(9)	275(9)
CM1	7083(43)	3542(13)	592(9)	280(16)
OM2	−44(15)	2919(6)	1918(6)	149(4)
OW	2796(60)	2702(14)	747(34)	702(41)
CM2	1143(64)	2683(33)	1605(40)	597(49)

Isolation of the Alkaloids. The air-dry comminuted roots of panicled monkshood (985 g), sent by A. P. Gorelova from PABS, were wetted with a 5% solution of sodium carbonate, the alkaloids were extracted with chloroform, and the extract was shaken with 5% sulfuric acid. With cooling, the acid solution was alkalinized with sodium carbonate, and the alkaloids were exhaustively extracted with chloroform (5 g). The total alkaloids (5 g) were chromatographed on alumina (Al_2O_3, Brockmann activity grade II, neutral, deactivated, 1:60). The alkaloids were eluted with chloroform, 200-ml fractions being collected, to a total number of 40. When fractions 22-24 were treated with ethyl acetate, crystals of technical paniculamine separated out, with mp 217-218°C (MeOH).

Paniculamine. mp 222-224°C (Py). IR spectrum (ν, cm^{-1}); 3600-3025(OH), 1460, 1338, 1255, 1190, 1150, 1106, 1020, 950, 920. PMR (D_2O, ppm, J, Hz): 0.82 (3H, s, Me−18), 1.33 (3H, t, J = 7.5 Hz, $N-CH_2-CH_3$), 2.78 (1H, q, J = 7.5 Hz, H−21), 3.00-3.40 (1H, m, H−21), 3.11 and 3.36 (d, 1H each, J = 13 Hz, 2H−19), 3.67 and 3.90 (d, 1H each, J = 12 Hz, 2H−17), 3.97-4.10 (3H, narrow signal, H−1, H−15, H−20).

Mass spectrum, *m/z* (I_{rel}, %): 393 (M$^+$, 7), 377 (84), 376 (36), 375 (27), 360 (37), 349 (20), 348 (33), 347 (34), 346 (44), 330 (29), 319 (31), 318 (73), 302 (12), 286 (44), 258 (16), 242 (15), 186 (100), 158 (25), 143 (21), 131 (23), 122 (62), 117 (30), 105 (35), 91 (65), 79 (41), 67 (33), 58 (13), 55 (62). HRMS: Calculated for $C_{22}H_{25}NO_3$ (M^+-16) 377.25661. Found: 377.25524.

X-Ray Structural Analysis of Paniculamine. Crystals of paniculamine were grown from its solution in methanol. They were unstable and were converted to a white powder in the course of a few days, which was apparently connected with the presence of molecules of the solvent and water in the crystals. To perform the experiment, therefore, thew crystals were well coated with epoxide resin. The space group and the parameters of the unit cell were determined on a Syntex $P2_1$ diffractometer using CuK_α radiation: a = 7.331(10), b = 17.447(10), and c = 18.781(20) Å, d_{calc} = 1.199 g/cm^3. Space group $P2_12_12_1$, Z = 4.

In the calculations we used 1729 reflections. The structure was interpreted by the direct method using the SHELXS-86 program [7] and was refined by the SHELX-76 program in the anisotropic approximation. The theoretically calculated positions of the hydrogen atoms were included in the calculation, the hydrogen atoms for the hydroxy groups being found experimentally.

The final values of the R factors were R = 0.055 and R_w = 0.064. All the calculations were performed on a personal computer of the IBM PC/AT type. The coordinates of the nonhydrogen atoms are given in Table 2.

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