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SEPTERINE AND SEPTEFINE — NEW ALKALOIDS OF *Aconitum septentrionale*

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From the roots of Aconitum septentrionale *Koelle we have isolated the new nor- and bisnorditerpenoid alkaloids septerine (1) and septefine (2), each containing a N-methylanthraniloyl fragment. Their structures have been established from IR-, mass-, and PMR-spectral results. Structure (1) for septerine has been confirmed by the formation of lycoctonine on its hydrolysis.*

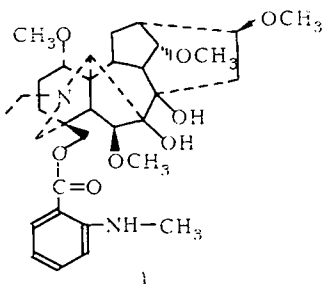
Lappaconitine from the roots of *Aconitum septentrionale* Koelle is the basis for the production of the drug allapinin. In order to isolate the alkaloids accompanying lappaconitine, we have investigted the alkaloid composition of the mother solutions from lappaconitine [1, 2] and have isolated two new diterpenoid alkaloids — septerine (**1**) amd septefine (**2**).

The amorphous base septerine has the composition $C_{33}H_{48}N_2O_8$. It forms a crystalline perchlorate. The IR spectrum of (**1**) contained absorption bands of hydroxy groups (3530-3460, 3383 cm^{-1}), of a carbonyl group (1685 cm^{-1}), and of a 1,2-disubstituted benzene ring (752 cm^{-1}).

The PMR spectrum (Table 1) contained signals from the protons of N-ethyl, N-methyl, and four methoxy groups, and, in the weak-field region, the signals of four aromatic protons and a one-proton signal from a NH group. The maximum peak of the $(M - 31)^+$ ion in the mass spectrum of (**1**) and also one-proton signals in the form of a triplet at 3.55 ppm with a SSCC of 5 Hz and a singlet at 4.05 ppm showed the positions of methoxy groups at C-1, C-6, and C-14 [3, 4]. In the PMR spectrum of (**1**) there were the signals of the geminal protons of an 18-methylene group in the form of a pair of doublets at 3.99 and 3.88 ppm (J = 11 Hz).

All these characteristics are close to those of anthranoyllycoctonine (**3**) [5]. The PMR spectrum of (**1**) differed from that of (**3**) (see Table 1) only by the absence of the signals of the protons of a NH_2 group, in place of which in the spectrum of (**1**) there was a three-proton doublet at 2.85 ppm and a one-proton quartet at 7.57 ppm with a SSCC of 5 Hz from the protons of a $NHCH_3$ group. This gave grounds for considering that septerine is an ester of lycoctonine, the hydroxy group at C-18 of which is esterified by methylanthranilic acid.

On the alkaline hydrolysis of (**1**) in aqueous methanolic solution, an aminoalcohol was obtained which, from its IR and mass spectra, and also by direct comparison with an authentic specimen (mixed melting point and TLC), was identified as lycoctonine (**4**). Consequently, septerine is a norditerpenoid alkaloid and has the structure (**1**).



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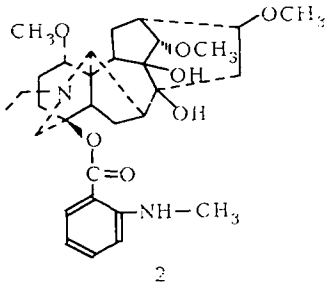

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TABLE 1. PMR Spectral Characteristics of the Alkaloids (1-3) and (5) (100 MHz, $CDCl_3$, 0 — HMDS, δ, ppm, J, Hz)

Protons		1	3	2	5
N-CH_2-CH_3		1.01(3H, t, 7.5)	1.00(3H, t, 7.5)	1.02(3H, t, 7.5)	1.04(3H, t, 7.5)
N-H		7.57(1H, q, 5)	–	7.50(1H, q, 5)	–
CH_3		2.85(3H, d, 5)	–	2.80(3H, d, 5)	–
OCH_3		3.19, 3.27, 3.35 (s, 3H, 6H, 3H)	3.17, 3.26, 3.28 3.32(s, each 3H)	3.19, 3.20, 3.31 (s, each 3H)	3.24, 3.26, 3.36 (s, each 3H)
14-βH		3.55(1H, t, 5)	3.51(1H, t, 5)	3.45(1H)	3.50(1H, d, 5)
17-H		2.88(1H, s)	2.84(1H, s)	2.90(1H, s)	2.95(1H, s)
18-2H		3.99(1H, d, 11) 3.88(1H, d, 11)	3.97(1H, d, 11) 3.86(1H, d, 11)	–	–
19-αH				3.53(1H, d, 11)	3.56(1H, d, 11)
NH_2		–	5.64(2H, br.s)	–	5.60(2H, br.s)
6-αH		4.05(1H, s)	4.03(1H, s)	–	–
Ar-H	3	6.60(d, 7)	6.58(dd, 7 and 2)	6.49(d, 7)	6.55(d, 7)
	4	7.35(t, 7)	7.19(td, 7 and 2)	7.22(t, 7)	7.15(t, 7)
	5	6.56(t, 7)	6.54(td, 7 and 2)	6.42(t, 7)	6.52(t, 7)
	6	7.81(d, 7)	7.70(dd, 7 and 2)	7.67(d, 7)	7.68(d, 7)

The structure of septerine agrees with its mass spectrum which had a series of ion peaks characteristic for the norditerpenoid bases anthranoyllycoctonine and delectine. Its main direction of fragmentation was the splitting out of the methoxy group at C-1, giving the maximum peak of an $(M - 31)^+$ ion. The presence of a 6-OMe-7,8-diol chain led to an increase in the contribution of the $(M - 15)^+$ ion with *m/z* 585. Another direction of fragmentation, competing with that described above, was the elimination of either the acyl residue or of a molecule of methylanthranilic acid, giving ions with *m/z* 134 and 151, respectively.

Septefine (**2**) has the composition $C_{31}H_{44}N_2O_7$, mp 194-195°C. In the IR spectrum of (**2**) absorption bands were observed at (cm^{-1}) 3525-3460, 3388 (OH, NH), 1690 (−OCO−) and 755 (1,2-disubstituted benzene ring).

The PMR spectrum of (**2**) (see Table 1) indicated the presence in septefine of a N-ethyl group, three methoxy groups, and a NH−CH_3 group, appearing, as in the case of septerine, in the form of a three-proton doublet at 2.80 ppm and a one-proton quartet at 7.50 ppm with a SSCC of 5 Hz. The signals of four adjacent aromatic protons were observed in the weak-field region. The above facts showed the presence of a N-methylanthranilic acid residue in the septefine molecule. The absence from the PMR spectrum of (**2**) of the signals of the protons of an 18-methylene group permitted the assumption that the methylanthranilic acid residue in septefine was attached at C-4. Consequently septefine belongs to the bisnorditerpenoid alkaloids.

A comparative analysis of the PMR spectra of septefine and of N-deacetyllappaconitine (**5**) [6, 7] (see Table 1), showed that they were very close. As in the case of alkaloids (**1**) and (**3**), their difference consisted in the presence of the signals of the protons of a $NHCH_3$ group in the spectrum of septefine and those of a NH_2 group in the spectrum of N-deacetyllappaconitine.

Thus, septefine is an ester of lappaconine esterified at the C-4 tertiary hydroxyl with methylanthranilic acid, and has the structure (**2**).

The behavior of septefine in the electron-impact regime fully confirmed the proposed structure. In the molecular-ion region, the peak of the $(M - 31)^+$ ion with *m/z* 525 had the highest intensity, unambiguously showing the presence of a methoxy group at C-1.

However, in contrast to alkaloids (**1**) and (**3**), in the spectra of which the peak of this ion had the maximum intensity, in the spectra of (**2**) and (**5**) the maximum peaks were those of ions arising by the elimination from the molecular ion of methylanthranilic and anthranilic acids, respectively. Such behavior under mass-spectrometric conditions is characteristic for bisnorditerpenoid alkaloids esterified at the C-4 hydroxy group [7, 8]. The further fragmentation of the $(M - 151)^+$ ion formed, having *m/z* 405 ($C_{23}H_{35}NO_5$), coincided completely with that of lappaconitine and N-deacetyllappaconitine (puberanidine) [7]. As in the case of septerine, the spectrum of septefine contained the peaks of ions with *m/z* 151 and 134 corresponding to methylanthranilic acid ($C_6H_4NHCH_3CO_2H$) and its acyl residue ($C_6H_4NHCH_3CO$).

It is interesting to note that this is the first time that septefine has been isolated from a plant, although this compound has been synthesized previously from N-deacetyllappaconitine [9].

EXPERIMENTAL

IR and PMR spectra were obtained, respectively, on UR-20 (KBr) and BS-567 A (100 MHz, $CDCl_3$, 0 — HMDS, δ) instruments. Mass spectra were taken on MKh-1310 and MS-3301 instruments.

Deactivated alumina was used for chromatography. The individuality of the alkaloids was checked by TLC on plates with alumina "for chromatography" (63 mesh) in ether and benzene–acetone (5:1).

Isolation of Septerine. After the total alkaloids (43 g) had been rechromatographed with hexane–ether (5:2) [1], fractions 14-27 (10.14 g) were again rechomatographed on alumina (300 g). Fractions with a volume of 50 ml were collected, elution being performed with hexane–acetone (98:2) (fractions 1-6), (96:4) (fractions 7-22), and (94:6) (fractions 23-35).

The septerine-containing fractions 12-25 were dissolved in methanol, and the solution was acidified to pH 2 with perchloric acid. The perchlorate that precipitated was separated off and crystallized from methanol (20:1). The perchlorate decomposed at temperatures above 300°C. A suspension of crystals of the perchlorate (0.5 g) in aqueous sodium carbonate solution was extracted with chloroform, and evaporation of the extract yielded septerine in the form of an amorphous powder.

IR spectrum (ν, cm^{-1}): 3530-3460, 3383, 2970, 2930, 2860, 2820, 1685, 1610, 1582, 1523, 1467, 1452, 1430, 1385, 1325, 1298, 1260, 1240, 1195, 1175, 1162, 1129, 1090, 1010, 990, 959, 860, 752.

Mass spectrum, *m/z* (%): 600 (M^+, 6.6), 585 (17), 582 (3.3), 570 (40), 569 (100), 567 (40), 551 (17), 537 (5.0), 521 (13), 434 (20), 151 (8.3), 134 (50).

Hydrolysis of Septerine. A solution of 0.06 g of septerine in 18 ml of methanol was treated with 2 ml of a 20% aqueous solution of NaOH, and the mixture was boiled for 2 h. The methanol was distilled off, and the residue was dissolved in water and extracted with ether. The residue obtained after evaporation of the ether was treated with acetone, giving lycoctonine (0.02 g), mp 135-137°C, M^+ 467 [10].

Isolation of Septefine. Part of fraction B (11.7 g) [2] was chromatographed on alumina (800 g) with elution by benzene. The fractions (7.55 g) were rechromatographed on alumina (230 g) with elution by hexane–ether (5:2) and the collection of 100-ml fractions. The treatment of fractions 3-11 with methanol led to the separation of 0.31 g of technical septefine with mp 189-190°C. Crystallization from methanol yielded septefine with mp 194-195°C.

IR spectrum (ν, cm^{-1}): 3525-3460, 3388, 2970, 2923, 2877, 2830, 1690, 1613, 1589, 1530, 1460, 1440, 1390, 1370, 1340, 1268, 1240, 1180, 1140, 1120, 1092, 1040, 1025, 1000, 970, 950, 905, 880, 850, 799, 755.

Mass spectrum: *m/z* (%): 556 (M^+, 2.7), 525 (5.0), 495 (2.2), 422 (2.2), 406 (30), 405 (100), 390 (56), 374 (27), 360 (13), 345 (30), 262.5^{++} (8.0), 151 (4.0), 134 (20).

HRMS: Calculated for $C_{30}H_{41}N_2O_6$ $(M-31)^+$ 525.29647, found: 525.29495; calculated for $(-OC-C_6H_4-NHCH_3)^+$ 134.06059, found: 134.06037.

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