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ALKALOIDS OF *Aconitum septentrionale*

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Together with the known alkaloids acetylsepaconitine and delvestidine, the roots of Aconitum septentrionale *Koelle have yielded the diethyl ester of succinylanthranilic acid and the new C_{20}-diterpene alkaloid septedinine, the structure of which has been established on the basis of spectral characteristics.*

Continuing an investigation of the alkaloid composition of the roots of *Aconitum septentriale* Koelle [1], we have isolated from the lappaconitine mother solution alkaloids with the compositions $C_{22}H_{31}NO_3$, mp 124-126°C (**1**), $C_{33}H_{48}N_2O_8$ in the form of an amorphous powder (**2**), $C_{15}H_{19}NO_5$, mp 57-58°C (**3**), and $C_{32}H_{44}N_2O_9$ (**4**).

Base (**1**) was readily soluble in chloroform, and less readily in methanol and acetone. Its IR spectrum contained a broad absorption band at (cm^{-1}) 3400 (OH) and a weak maximum at 1662 (exomethylene group). There was no absorption band of a carbonyl group. The functions of the oxygen atoms were determined from a study of the PMR spectrum of (**1**), in which there were two signals of protons geminal to hydroxy groups at (ppm) 4.18 (1H, triplet, J = 9 Hz) and 4.47 (1H, triplet, J = 2 Hz) and also two two-proton multiplets in the 2.46-3.13 and 3.40-3.88 ppm regions characteristic of the methylene protons of an oxazolidine ring [2]. Consequently, the alkaloid contained two secondary hydroxy groups and an oxazolidine ring. The presence of two hydroxy groups was also shown by the fact that when the alkaloid was deuterated under the usual conditions the peak of the molecular ion shifted by two units. The absence of the signal of a H-20 proton and the presence in the spectrum of an AB quartet from the protons of a 19-methylene group gave grounds for concluding that the C-20 carbon atom was linked to the oxygen atom of the oxazolidine ring and the C-14 carbon atom.

The protons of an exomethylene group were observed in the form of two doublets of doublets (Table 1) with SSCCs of 2 and 1 Hz, which was due to an allyl interaction of these protons with H-15 and H-12. Consequently, one hydroxy group was located at C-15. The CS and SSCC of the signal of the H-15 proton were close to those of the signal of the gem-hydroxylic proton at C-15 of talassamine (δ 4.53, t, J = 1.5 Hz) [3], which has a secondary hydroxy group at C-7.

A comparative analysis of the PMR spectra of (**1**) and septedine (**5**) [1], having the same heterocyclic skeleton, showed that they were close (see Table 1) and differed only by the presence in the spectrum of (**1**) of the signals of the protons of an exomethylene group and of the H-15 gem-hydroxylic proton in place of a doublet signal of the 17-methyl group in the spectrum of septedine.

As in the case of septedine, in the spectrum of (**1**) the signal of the H-7 proton was observed in the form of a triplet with a SSCC of 9 Hz. Consequently the base isolated had structure (**1**) with an α-oriented hydroxy group at C-7. Base (**1**) was new, and we have called it septedinine. The simultaneous presence in *Aconitum septentrionale* of the alkaloids septedinine and septedine, the structure of which represents an isomerization product of septedinine, permitted the assumption with high degree of probability that the hydroxy goup at C-15 had the β-orientation [3, 4].

The mass spectra of (**1**) and (**5**) showed the same set of ion peaks with *m/z* 357 (M^+), 340 ($M-17^+$), 329 ($M-28^+$), 314 ($M-43^+$), 312 ($M-45^+$), 300 ($M-57^+$), 286 ($M-71^+$) and 274 ($M-83^+$) but having different intensities. The strongest peaks in the mass spectrum of (**1**) were M^+ (86%) and $M-17^+$ (100%). The latter was formed as the result of the detachment of a a hydroxy radical from the molecular ion. In the septedine spectrum, however, these peaks were very weak, and the maximum

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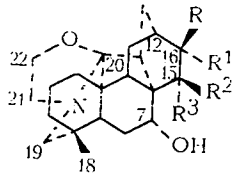
TABLE 1. PMR Spectra of Septedinine (**1**) and Septedine (**5**) (100 MHz, $CDCl_3$, 0-HMDS, δ, ppm, J, Hz)

Base	18-Me	17-Me	2H-17	2H-21	2H-22	H-19α H-19β	H-7	H-15
1	0.95 s	–	4.80 dd 4.97 dd (2; 1)	3.64 m	2.88 m	2.56 d 2.30 d (12)	4.18 t (9)	4.46 t (2)
5	0.97 s	1.04 d (7.5)	–	3.65 m	2.87 m	2.60 d 2.32 d (12)	4.07 t (9)	–

peak was that of the M-28$^+$ ion arising on the elimination of carbon monoxide at the expense of the carbonyl group at C-15 [1]. These results confirmed differences in the structures of (**1**) and (**5**).



1. $R-R^1=CH_2$
   $R^2=OH$
   $R^3=H$
5. $R=H$, $R^1=Me$
   $R^2+R^3=O$

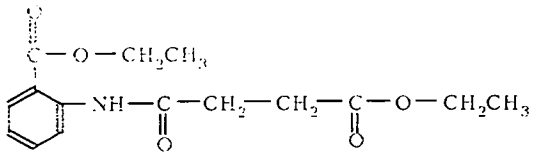
The chromatographic separation of the weakly basic fraction of the alkaloids gave base (**2**), the spectral characteristics of which were close to those of delvestidine [5]. The absence of an authentic specimen of delvestidine and the complexity of its identification on the basis of spectra impelled us to carry out a saponification of (**2**), which gave an aminoalcohol identical with a specimen of anthranoyllycococtine [6]. The structure of (**2**) was also confirmed by a comparison of its C^{13} NMR spectrum with that published for delvestidine [5].

The crystalline base (**3**) isolated from the weakly basic alkaloid fraction [1] was readily soluble in chloroform and less readily in acetone and methanol and gave no reaction for alkaloids with tungstosilicic acid.

The IR spectrum of (**3**) included absorption bands at (cm^{-1}) 3265 and 1685 (−NHCO), 1730 (RO−C=O), 1702 (−O−CO−Ar), 1540 (amide II), 1095 and 1085 (−C−O−C−) and 765 (1,2-substituted aromatic ring). Its PM spectrum contained the combinations of signals characteristic for ethyl groups. The presence in its PMR spectrum of signals of the methyl protons of O-ethyl groups at (ppm) 1.02 and 1.36 (t, 3H each, J = 7.5 Hz, 2 × $OCH_2\underline{CH_3}$) and of methylene protons at 4.1 and 4.31 (q, 2H each, J = 7.5 Hz, 2 × OCH_2CH_3) showed the presence of two O-ethyl groups in the (**3**) molecule. A singlet signal at 2.70 ppm (4H) related to two equivalent methylene protons ($CO-CH_2-CH_2-CO$). In the region of aromatic protons, signals characteristic for an *ortho*-substituted benzene ring with dissimilar substituents were detected. One-proton triplet-doublet signals at (ppm) 7.01 and 7.47 with J = 8 and 2 Hz were due to protons at C-4 and C-5, and doublet-doublet signals at 7.99 and 8.63 with J = 8 and 2 Hz were assigned to protons at C-3 and C-6, respectively. A one-proton broadened signal of a NH group appeared in the weakest field at 11.13 ppm.

In the mass spectrum of (**3**) there were the peak of the molecular ion with *m/z* 293 and the peaks of ions with *m/z* 248 and 202, due to the successive ejection of an ethoxy radical and an ethanol molecule from the molecular ion, as was confirmed by HRMS. The strongest peak in the mass spectrum of (**3**) was that of an ion with *m/z* 165 formed by the elimination of a $C_6H_7O_3$ fragment.

The above facts showed that the compound isolated had structure (**3**), i.e., it was the diethyl ester of succinylanthranilic acid.



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This the first time that this compound has been isolated from a plant. It was possibly formed in the process of treating the ethanolic extract with acid as a result of the ethanolysis of diterpene alklaoids containing a residue of the succinimide of anthranilic acid.

In order to isolate the accompanying alkaloids, lappaconitine with mp 217-218°C was chromatographed on alumina. The polar fraction yielded an amorphous substance identified by a direct comparison with a specimen of N-acetylsepaconitine isolated from *A. leucostomum* [7].

EXPERIMENTAL

IR, mass, and PMR spectra were taken on UR-20 (KBr), MKh-1321 and MS-3301, and BS-567 A instruments.

Chromatographic monitoring was conducted by TLC (Silufol UV-254 and silica gel "for chromatography", particle size 0.063 mm) in the solvent systems: 1) benzene–chloroform–diethylamine (40:10:3); 2) ether; 3) chloroform–methanol (40:1). After the separation of lappaconitine hydrobromide, the total alkaloids from the roots of wolfbane monkshood *Aconitum septentrionale* were dissolved in 2.5% sulfuric acid (2.6 liters), and the solution was filtered and extracted with chloroform, the distillation of which then yielded a fraction containing weakly basic alkaloids (160.1 g) (A).

The acid solution was fractionally alkalinized with sodium carbonate to pH 6, 7, and 10, with extraction of the alkaloids by ether at each stage; distillation of the extracts gave 25.70 g (B), 27.86 g (C) and 66.56 g (D) of mixtures of alkaloids.

Alkaloids were extracted from the alkaline mother solution with chloroform, which gave 66 g of mixture (E) of alkaloids.

Isolation of Septedinine (1). Alkaloid mixture (E) (66 g) was chromatographed on deactivated alumina (2400 g) with elution by hexane–acetone (10:1 and 5:1). When the first hexane–acetone (5:1) fractions were rechromatographed, 30 mg of septedinine was obtained, with mp 124-126°C (from hexane–acetone (5:1)).

IR spectrum (ν, cm^{-1}): 3600-3200, 2970, 2935, 2875, 1662, 1455, 1415, 1285, 1245, 1220, 1205, 1160, 1120, 1095, 1052, 1028, 1000, 925, 920, 885, 865.

Mass spectrum, *m/z* (%): 357 (M^+, 86), 340 (100), 329 (34), 314 (32), 312 (12), 300 (8), 286 (20), 274 (58), 256 (10), 202 (6), 192 (6), 149 (26), 129 (10), 101 (16).

For $C_{22}H_{31}NO_3$ calculated 357.22769, found 357.23038.

Part (20 g) of fraction A was chromatographed on a column of deactivated alumina (660 g), with elution by benzene and by benzene–alcohol (10:1). A total of 52 100-ml fractions was collected. The benzene fractions 8-12 (1.34 g) were rechromatographed on alumina (70 g). Hexane–ether (4:1) fractions yielded 1.9 g of delvestidine in the form of an amorphous powder.

Mass spectrum, *m/z* (%): 600 (M^+, 9.5), 585 (1.0), 569 (100), 553 (38), 537 (17), 523 (5), 507 (10), 137 (5), 120 (50).

Delvestidine (**2**) was demethylated by the method of [5], which led to anthranoyllycoctonine (0.04 g), mp 158-160°C.

IR spectrum (ν, cm^{-1}): 3578, 3520, 3450, 3335, 1690, 1625, 1592, 1579, 1490, 1460, 1390, 1302, 1252, 1250, 1169, 1112, 1090, 760.

Mass spectrum, *m/z* (%): 586 (M^+ 10), 571 (20), 568 (20), 555 (100), 537 (18), 523 (10), 507 (10), 434 (10), 418 (9), 404 (11), 137 (19), 120 (49).

Isolation of the Diethyl Ester of Succinoylanthranilic Acid (3). Part (4 g) of the alkaloid fraction A [1] was chromatographed on KSK silica gel (120 g). Fractions with a volume of 100 ml were eluted with hexane–ether (1:1). Fractions 3 and 4, on treatment with acetone, yielded 0.11 g of compound (**3**), mp 57-58°C (from acetone).

IR spectrum (ν, cm^{-1}): 3265, 2988, 1730, 1702, 1685, 1602, 1590, 1535, 1475, 1450, 1440, 1370, 1318, 1257, 1202, 1170, 1160, 1150, 1095, 1085, 1025, 765

HRMS: Calculated for $C_{15}H_{19}NO_5$ 293.12633, found 293.12547. For $C_{13}H_{14}NO_4$ calculated 248.09228, found 248.09222. For $C_{11}H_8NO_3$ calculated 202.05042, found 202.05012. For $C_9H_{11}NO_2$ calculated 165.07898, found 165.0770.

Isolation of N-Acetylsepaconitine. Lappaconitine (27 g) with mp 217-218°C (from methanol–chloroform (4:1)) was chromatographed on Al_2O_3 (Brockmann activity grade II, neutral, deactivated, 1:10). Benzene eluates were collected as 300-ml fractions.

By preparative TLC (Silufol UV-254, 15 × 15 cm plates) in the benzene–chloroform–diethylamine (40:10:3) system, fraction 120 yielded an amorphous substance identical with N-acetylsepaconitine (TLC and mass spectra).

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