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Norditerpenoid alkaloids from the roots of *Aconitum leave* Royle

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From the roots of *Aconitum leave* Royle growing wild in Northern Pakistan, Swat district, three new alkaloids, 8-methyllycaconitine (**1**), 14-demethyllycaconitine (**2**), *N*-deethyllycaconitine-*N*-aldehyde (**3**) have been isolated along with four known compounds, lappaconitine (**4**), lycaconitine (**5**), lapaconidine (**6**) and lycoctonine (**7**).

1. Introduction

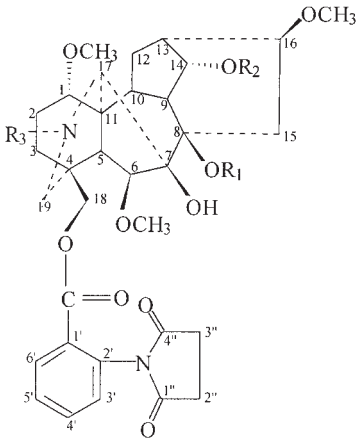
Aconitum as well as *Delphinium* species have been used as medicinal agents for centuries, especially as cardiotonic, febrifuges and sedatives [1, 2]. Lycaconitine (**5**) obtained from several *Aconitum* species was found to overcome multidrug resistance in cancer chemotherapy [3]. Intrategmental administration of methyllycaconitine was found to decrease both nicotine induced and food induced dopamine release in the Nucleus accumbens [4]. The affinity of lycaconitine for two neuronal nicotinic acetylcholine receptor subtypes was determined [5]. Like *Delphinium* species, in the old days *Aconitum* species were also used against lice and scorpions [6]. Alkaloids, although highly poisinous, were tested for cardiovascular (hypotensive and bradycardic) action [7, 8].

2. Investigations, results and discussion

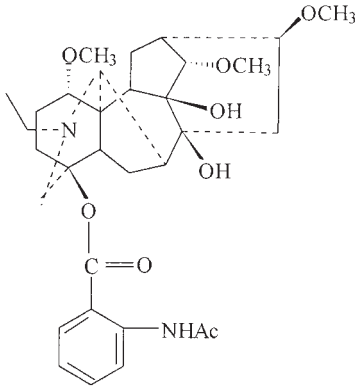
Besides of our studies on *Delphinium* species [9–12], we now studied an *Aconitum* species, *A. leave* Royle also from Pakistan. In an earlier study, the presence of lappaconitine (**4**) was established in the plant and no other alkaloid was found [13].

In the present study, the dried and powdered roots of the plant (1 kg) were extracted with n-hexane, and 8 g of an oily residue was obtained. The remaining plant material was extracted with 90% EtOH. The ethanol extract was concentrated and acidified with 5% HCl and extracted with $CHCl_3$ to yield 30 g of non-alkaloidal residue. The acidic aqueous solution was basified with 10% NaOH solution and extracted with $CHCl_3$ to yield 26 g of a crude alkaloidal mixture. A part of the alkaloidal mixture (5 g) was separated by VLC (Vacuum Liquid Chromatography) eluting with petroleum ether, a gradient of CH_2Cl_2 was added up to 100% followed by EtOH up to 10%. When the CH_2Cl_2 reached to 50% a crystalline compound started to separate (frac. 9 to frac. 14), about 2 g of a single crystalline compound was obtained. The structure of the crystalline compound was established as lappaconitine (**4**) using spectral data, m.p., optical rotation and TLC comparison with authentic sample [14, 15]. Frac. 15–21, frac. 22–29, frac. 30–36 were alkaloidal mixtures. They were separated by a Chromatotron apparatus [16]. Fractions 15–21 yielded lycaconitine (**5**), 8-methyllycaconitine (**1**), 14-demethyllycaconitine (**2**), fractions 22–29 contained *N*-deethyllycaconitine-*N*-aldehyde (**3**), fractions 30–36 yielded lapaconidine (**6**) and lycoctonine (**7**). Compounds **1**, **2** and **3** were new, while **4**–**7** were known.

8-Methyllycaconitine (**1**) is an amorphous compound with an optical rotation $[\alpha]_D^{30} = +19.5°$ ($c = 0.1$, $CHCl_3$). The molecular formula $C_{37}H_{50}N_2O_{10}$ was calculated from



	R_1	R_2	R_3
1	CH_3	CH_3	C_2H_5
2	H	H	C_2H_5
3	H	CH_3	CHO
5	H	CH_3	C_2H_5



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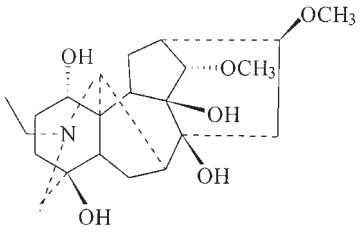

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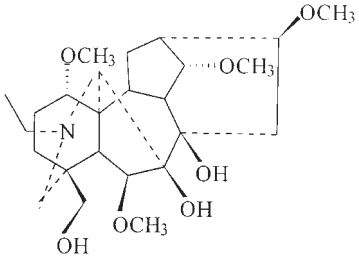

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HRMS (m/z 682.3464) and confirmed by ^{13}C NMR and APT (Attached Proton Test) spectral data. The ^{1}H NMR spectrum of **1** showed signals for aromatic protons at δ 8.02 (1 H, dd, J = 8 and 1.5 Hz, H-6′), 7.68 (1 H, dt, J = 1.5, 8 and 8 Hz), 7.54 (1 H, dt, J = 1.5, 8 and 8 Hz) (H-5′ and H-4′), 7.27 (1 H, dd, J = 1.5, 8 Hz, H-3′), five methoxy groups were at δ 3.42, 3.40, 3.35, 3.32 and 3.24 (each 3 H, s). A typical signal for succinimid CH_2-CH_2 group was at δ 2.90 as a broad singlet (4 H, s, 2″, 3″). A signal at δ 3.65 (1 H, t, J = 4.5 Hz, H-14) typical for methoxyl carrying C-14, a broad singlet signal at δ 3.92 indicated H-6α in a methoxyl carrying C-6 position. Other signals were at δ 2.92 (1 H, s, H-17), 1.12 (3 H, t, J = 7 Hz) (N−CH_2CH_3). The ^{13}C NMR spectrum of **1** (Table) is in agreement with the suggested structure.

The UV and IR spectra of 14-demethyllycaconitine (**2**) were similar to those of compound (**1**). The HRMS of **2** indicated the molecular formula $C_{35}H_{46}N_2O_{10}$ (m/z 654.3155) which was correlated by its ^{13}C NMR and APT spectra. The ^{1}H NMR spectra of compounds **1** and **2** were quite similar except for the chemical shift of H-14 at δ 4.09 (1 H, t, J = 4.5 Hz, H-14) which indicated a hydroxy group at C-14 instead of a methoxyl. The only other difference was the presence of three methoxy groups in compound **2** at δ 3.36 (6 H, s, 2 × OMe), 3.26 (3 H, s, OMe) versus five methoxyls of compound **1**. The aromatic protons and all the other signals were in their respective places. The ^{13}C NMR (Table) clearly showed that compound **2** is 14-demethyllycaconitine.

The ^{1}H NMR spectrum of *N*-deethyllycaconitine-*N*-aldehyde (**3**) showed the presence of an aldehyde group at δ 9.85 (1 H, s), and the lack of the methyl triplet at around δ 1.10 suggested that the aldehyde group should be situated at N as N-CHO instead of an ethyl group as N-CH_2CH_3. Four methoxy groups and all other signals were similar to those of compounds **1** and **2**. The ^{13}C NMR (Table) correlated to the given structure. The molecular formula $C_{35}H_{44}N_2O_{11}$ was calculated from its HRMS (m/z 668.2947).

Table: ^{13}C NMR assignments of 8-methyllycaconitine (1), 14-demethyllycaconitine (2) and N-deethyl-lycaconitine-N-aldehyde (3)

C	1	2	3
1	83.8 d	83.4 d	83.4 d
2	25.9 t	25.9 t	25.8 t
3	33.5 t	33.6 t	33.5 t
4	37.4 s	37.4 s	37.3 s
5	43.2 d	43.6 d	43.3 d
6	90.7 d	90.6 d	90.7 d
7	88.4 s	87.9 s	88.2 s
8	80.1 s	77.4 s	76.8 s
9	49.9 d	49.8 d	49.8 d
10	38.0 d	37.9 d	38.0 d
11	50.9 s	50.6 s	50.8 s
12	28.6 t	28.6 t	28.8 t
13	46.0 d	45.9 d	46.2 d
14	83.8 d	75.8 d	83.6 d
15	33.1 t	33.8 t	32.8 t
16	82.5 d	83.0 d	82.8 d
17	64.4 d	65.0 d	64.6 d
18	69.5 t	69.5 t	69.4 t
19	52.3 t	52.4 t	52.0 t
20	50.6 t	51.0 t	−
21	13.9 q	14.0 q	182.3 d
Ar-CO	164.1 s	164.1 s	164.2 s
1′	126.8 s	126.7 s	126.8 s
2′	132.8 s	132.8 s	132.6 s
3′	129.4 d	129.4 d	130.0 d
4′	133.6 d	133.6 d	134.0 d
5′	131.1 d	131.3 d	131.4 d
6′	130.0 d	129.9 d	130.2 d
1″	176.5 s	175.8 s	175.6 s
2″	31.9 t	32.0 t	32.8 t
3″	33.5 t	33.6 t	34.0 t
4″	175.0 s	174.8 s	175.6 s
OMe-1	57.7 q	58.0 q	58.0 q
OMe-6	55.7 q	56.0 q	56.2 q
OMe-8	58.0 q	−	58.3 q
OMe-14	58.1 q	−	58.3 q
OMe-16	56.2 q	56.3 q	56.0 q

3. Experimental

3.1. Equipment

UV spectra were recorded in MeOH on a Shimadzu UV 1601 spectrophotometer. IR spectra were recorded in $CHCl_3$ on a Perkin-Elmer 983 spectrophotometer. Optical rotations were determined in Opt. Act. Ltd. AA-5 polarimeter. ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra were recorded on a Bruker AC-200 instrument. EIMS and HRMS were recorded on a ZapSpec instrument.

3.2. Plant material

The roots (1 kg dry wt) of *Aconitum leave* Royle were collected and identified by one of us (H.A.) in Swat district of Northern Pakistan, at an of elevation 1200 m, in August 2000. A voucher specimen is deposited in the Herbarium of Department of Botany, Jahan Zeb College, Saidu Sharif, Swat, Pakistan (RA-01).

3.3. Extraction and isolation

The powdered plant material was first extracted with n-hexane yielding 8 g of an oily residue, then extracted with 90% EtOH. The concentrated EtOH extract was acidified with 5% HCl and extracted with $CHCl_3$. The remaining aqueous EtOH was basified with 10% NaOH and extracted with $CHCl_3$ to yield 26 g of a crude alkaloidal mixture. A part of this mixture (5 g) was fractionated by VLC using a basic alumina column (100 g, Al_2O_3 EM 1085) eluting with petroleum ether, and gradients CH_2Cl_2 up to 100% and EtOH 10%. Fractions 1–8 were oily and discarded, fractions 9–14 (2 g) (petroleum ether-dichloromethane, 1 : 1) yielded lappaconitine (**4**) (2 g). Other fractions were mixtures of alkaloids and could be separated on Si gel plates of a Chromatotron apparatus. Eluting with CH_2Cl_2 and a gradient of EtOH starting from 1% and by a slow increase up to 10%. Fractions 15–21 (1.3 g) yielded lycaconitine (**5**, 50 mg), 8-methyllycaconitine (**1**, 12 mg), 14-demethyllycaconitine (**2**, 8 mg), fractions 22–29 (270 mg) yielded *N*-deethyllycaconitine-*N*-aldehyde (**3**, 7 mg), fractions 30–36 (1.6 g) yielded lapaconidine (**6**, 17.3 mg) and lycoctonine (**7**, 12 mg). The known compounds were identified by comparing their ^{1}H and ^{13}C NMR data to those of authentic compounds and co-TLC behavior with standards.

3.3.1. 8-Methyllycaconitine (**1**)

$\alpha_D^{25} = +19.5°$ (c = 0.1); UV $\lambda_{max.}^{MeOH}$ (log ε) nm: 278 (2.10), 230 (4.00); IR $\nu_{max.}^{CHCl_3}$ cm^{-1}: 3460 (OH), 1710 (C=O), 1600, 1580, 1515 (arom. C−H), 1070 (C−O); ^{1}H NMR (see text); ^{13}C NMR (Table); EIMS (rel. int) at m/z 682 $[M]^+$ (2), 667 $[M\text{-}Me]^+$ (7), 637 $[M\text{-}Me\text{-}OMe]^+$ (50), 623 (18), 584 $[M-C_4H_4NO_2]^+$ (8), 480 $[584\text{-}C_7H_4O]^+$ (7), 464 $[584\text{-}C_7H_4O_2]^+$ (35), 406 (12), 358 (15), 202 $[C_{11}H_8NO_3]^+$ (100), 174 (30), 101 (35), 84 (65), 71 (53). HRMS: 682.3467 $C_{37}H_{50}N_2O_{10}$ (calcd. 682.3464).

3.3.2. 14-Demethyllycaconitine (**2**)

$\alpha_D^{25} = +20.0°$ (c = 0.1); UV $\lambda_{max.}^{MeOH}$ (logε) nm: 278 (2.05), 225 (4.10); IR $\nu_{max.}^{CHCl_3}$ cm^{-1}: 3450 (OH), 1715 (C=O), 1605, 1580, 1510 (arom C−H), 1075 (C−O), 880, 790, 657; ^{1}H NMR ($CDCl_3$) δ: 8.08 (1 H, dd, J = 1.5 and 8.0 Hz, H-6′), 7.70 (1 H, dt, J = 1.5, 8, 8 Hz) 7.56 (1 H, dt, J = 1.5, 8, 8 Hz) (H-4′ and H-5′), 7.28 (1 H, dd, J = 1.5 and 8 Hz, H-3′). The ^{13}C NMR spectrum is in agreement with the suggested structure; EIMS (rel. int) at m/z: 654 $[M]^+$ (12), 623 $[M\text{-}OMe]^+$ (7), 452 $[M\text{-}C_{11}H_8NO_3]^+$ (55), 436 $[M\text{-}C_{11}H_8NO_4]^+$ (20), 284 (15), 256 (20), 202 $[C_{11}H_8NO_3]^+$ (60), 97 (33), 83 (40), 71 (72). HRMS: 654.3152 $C_{35}H_{46}N_2O_{10}$ (calcd. 654.3155).

3.3.3. N-Deethyllycoctonine-N-aldehyde (**3**)

$\alpha_D^{25} = +22.5°$ (c = 0.1); UV $\lambda_{max.}^{MeOH}$ (log ε) nm: 280 (2.10), 230 (4.10); IR $\nu_{max.}^{CHCl_3}$ cm^{-1}: 3450 (OH), 2850, 1730 (CHO), 1710 (C=O), 1605, 1580, 1520 (arom C−H), 1080 (C−O); ^{1}H NMR ($CDCl_3$) δ: 8.05 (1 H, dd,

J = 1.8, 8 Hz), 7.68 (1 H, dt, J = 1.8, 8, 8 Hz), 7.54 (1 H, dt, J = 1.8, 8, 8 Hz), 7.28 (1 H, dd, 1.8, 8 Hz) for H-6′, H-5′, H-4′, H-3′ respectively, four methoxy groups at δ 3.44, 3.42 (each 3 H, s), 3.36 (6 H, s, 2 × OMe). The ^{13}C NMR signals (Table) are in agreement with the suggested structure; EIMS (rel. int) at m/z 668 $[M]^+$ (3), 653 $[M\text{-}Me]^+$ (5), 622 $[M\text{-}MeOMe]^+$ (5), 593 $[622\text{-}CHO]^+$ (12), 535 (18), 466 $[M\text{-}C_{11}H_8NO_3]^+$ (30), 401 (65), 355 (80), 341 (92), 202 (100), 97 (28), 84 (45), 71 (80). HRMS: 668.2946 $C_{35}H_{44}N_2O_{11}$ (calcd. 668.2947).

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