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SHORT COMMUNICATION

Norditerpenoid alkaloids from the roots of *Aconitum heterophyllum* Wall with antibacterial activity

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Abstract

Two new aconitine-type norditerpenoid alkaloids 6-dehydroacetylsepaconitine (**1**) and 13-hydroxylappaconitine (**2**), along with three known norditerpenoid alkaloids lycoctonine, delphatine and lappaconitine were isolated from the roots of the *Aconitum heterophyllum* Wall. These compounds exhibited significant antibacterial activity. The structure of compound **1** and **2** were deduced on the basis of their spectral data.

Keywords: *Norditerpenoid alkaloids,* Aconitum heterophyllum *Wall, antibacteria*

Introduction

Genus *Aconitum* is a rich source of diterpenoid alkaloids, many of which exhibited a broad spectrum of biological activities. Lappaconitine hydrobromide has been used as an antiarrhythmic drug [1]. The methyllycaconitine perchlorate is used in curaremimetic preparation [2]. Some aconitine and mesaconitine derivatives possess potent analgesic and anti-inflammatory activities [3]. The methyllycaconitine and lycaconitine exhibited neuronal nicotinic acetylcholine receptor affinity [4]. Lycaconitine, obtained from several *Aconitum* species, was found to be effective against multi-drug resistance cancers. *Aconitum* plants are widely used in Chinese and Indian traditional systems of medicine [5–6]. Turkish *Aconitum* species are used externally in the treatment of rheumatic pain and sciatica and also against body lice [7].

Previously, heterophyllisine, heterophylline, heterophyllidine, heteratisine, atisine, atidine, F-dihydroatisine, hetisine, benzoylheteratisine and atisenol were reported from *A. heterophyllum* [8–11]. In the present paper, we describe the isolation and structure elucidation of two new antibacterial norditerpenoid alkaloids 6-Dehydroacetylsepaconitine (**1**) and 13-hydroxylappaconitine (**2**), along with three known alkaloids lycoctonine (**3**), delphatine (**4**) and lappaconitine (**5**).

Experimental

General experimental

Optical rotations were measured on a JASCO DIP 360 polarimeter. IR spectra were recorded on a JASCO 302-A spectrophotometer. EI-MS and HREI-MS were recorded on JMS HX 110 with data system and on JMS-DA 500 mass spectrometers. The ^{1}H- and ^{13}C-NMR spectrums were recorded on Bruker NMR spectrometers operating at 400 MHz, (100 and 125 MHz for ^{13}C). The chemical shifts values are reported in ppm (δ) units and the coupling constants (J) are given in Hz.

Chromatographic conditions

For TLC, precoated aluminium sheets (silica gel 60F-254, E. Merck) were used. Visualization of the TLC

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plates was achieved under UV at 254 and 366 nm and by spraying with Dragendorff's reagent. Solvent system; "*n*-hexane–acetone–diethylamine 8:2:10 drops", was used.

Plant material

The roots (5 kg dry wt) of *Aconitum heterophyllum* Wall, were collected from District Swat of Pakistan at an elevation of 2000 m in August 2005 and identified by Mr Mehboob ur Rahman (Assistant Professor) Department of Botany, Jahanzeb Post Graduate College, Saidu Sharif, Swat, NWFP, Pakistan. The voucher specimen (HA-014) is deposited in the herbarium of the botany department.

Extraction and isolation

Dried and powdered roots (5 Kg) of the plant were extracted exhaustively with *n*-hexane which solvent extract *n*-hexane (3 × 8 L) followed by 80% EtOH (3 × 10 L) extraction at room temperature for 7 days (3-times). The filtrate was evaporated in *vacuo* to yield 60 g of residue. The residue was acidified to pH 1.5 by 0.5 N H_2SO_4 and extracted with CH_2Cl_2 (3 × 2 L) collected alkaloidal mixture (18 g). The acidic aqueous solution was basified (pH 8–10) by using 10% KOH and extracted with CH_2Cl_2 (5 × 2 L) to yield 13.8 g of crude acidic compounds fraction. The crude acidic compounds fraction was fractionated on silica gel column (260 g), five combined fractions were obtained. On repeated flash column chromatography using solvent system *n*-hexane–acetone–diethylamine (9:1:10 drops per 100 ml) 6-dehydroacetylsepaconitine (**1**), 13-hydroxylappaconitine **(2),** along with lycoctonine, delphatine and lappaconitine known alkaloids were obtained.

6-dehydroacetylsepaconitine (**1**), amorphous powdered (13 mg). mp 122–125°C; $[\alpha]_D^{30}$ + 23.33° (*c* 0.8, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 310 (3.75), 254 (4.22), and 225 (4.46) nm; IR $\nu_{max}CHCl_3$, 3492 (OH groups), 1700 (ester carbonyl, amide carbonyl), 1600, 1280, 1250, and 750 cm^{-1} (1,2-substituted aromatic ring), 1083 (simple ether bonds); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.17 (3H, t, $\mathcal{J}$ = 7.0 Hz, N-CH_2CH_3), δ 2.82 (1H, br s, C-17), δ 3.85 (1H, t, $\mathcal{J}$ = 7.9 Hz, C-1), δ 3.71 (1H, d, $\mathcal{J}$ = 4.8 Hz, C-14), δ 2.45 (1H, br s, C-5) δ 2.11 (1H, br s, C-7), δ 3.24 (1H, t, $\mathcal{J}$ = 8.4 Hz, C-16), δ 11.0 (NH). ^{13}C-NMR (Table I). HREI-MS (M^+*m/z*, 614.6828)

13-hydroxylappaconitine **(2),** amorphous powdered (10 mg). mp 145–147°C; $[\alpha]_D^{30}$ + 10.33° (*c* 1.0, $CHCl_3$); IR $\nu_{max}CHCl_3$, 3492 (OH groups), 1720 (ester carbonyl, amide carbonyl), 1608, 1270, 1255, and 790 cm^{-1} (1,2-substituted aromatic ring), 1089 (simple ether bonds); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.03 (3H, t, $\mathcal{J}$ = 7.0 Hz, N-CH_2CH_3), δ 3.41 (1H, br s, H-17), δ 3.20 (2H, dd, $\mathcal{J}$ = 9.0 and 6.0 Hz, H-1 and H-16), δ 4.32 (1H, s, H-14), δ 2.45 (1H, br s, H-5), δ 2.35 (1H, br s, H-7), δ 8.68 and 7.92 (each 1H, dd, Ar-H) 7.50 and 7.04 (each 1H, t, Ar-H). ^{13}C-NMR (Table I). FAB (+ ve and − ve) (M^+*m/z*, 601.0 and 599.0).

Table I. ^{13}C–NMR data of compounds 1 and 2 ($CDCl_3$).

	6-dehydroacetylsepaconitine (1)		13-hydroxylappaconitine (2)	
C. No	**δ_C**	**Multiplicity**	**δ_C**	**Multiplicity**
1	77.7	CH	84.8	CH
2	26.5	CH_2	26.7	CH_2
3	31.6	CH_2	33.1	CH_2
4	84.6	C	84.6	C
5	44.4	CH	47.9	C
6	206	C	27.2	CH_2
7	46.8	CH	47.8	CH
8	79.5	C	68.4	C
9	79.6	C	79.2	C)
10	78.9	C	43.8	CH
11	55.7	C	53.1	C
12	37.4	CH_2	35	CH_2
13	34.5	CH	78.1	CH
14	87.7	CH	87.4	CH
15	44.7	CH_2	32.4	CH_2
16	82.7	CH	82.2	CH
17	61.5	CH	58	CH
19	55.5	CH_2	55.8	CH_2
1′	115.7	C	115.7	C
2′	141.6	C	140.1	C
3′	120.2	CH	120.3	CH
4′	134.4	CH	134.4	CH
5′	122.3	CH	122.3	CH
6′	130.9	CH	131	CH
N-CH_2	48.9	CH_2	49	CH_2
CH_3	13.4	CH_3	13.5	CH_3
C=O	167	C	159.7	C
N–C=O	169.3	C	168	C
CH_3	21.4	CH_3	25.2	CH_3

Antibacterial activity

All of the isolated compounds were screened against strains of *Escherichia coli, Bacillus subtilis, Shigella flexneri, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Salmonella typhi*. For antibacterial screening, 3 mg of sample was taken and dissolved in 3 ml of DMSO. Molten nutrient agar (45 mL) was poured on sterile petri plates, where it was allowed to solidify. Bacterial spread were made on these nutrient agar plates by dispensing 7 mL of sterile soft agar containing 100 μL of test-organism culture. Wells were dug with the help of a 6-mm sterile metallic borer at appropriate distance. Then, 100 μL of sample was poured into each well, and the plates were incubated at 37°C for 24 h. The results, in terms of inhibition zone, were noted. The drug Imipenem, a broad-spectrum β-lactam antibiotic, was used as a positive control.

Table II. Table-2 Antibacterial activities of compounds **1-5.** Relative to the standard drug Imipenem. For details, see Experimental.

Compound	EC	BS	SF	SA	PA	ST
Imipenem	30	33	27	33	24	25
1	–	15	12	24	–	15
2	10	15	10	–	13	17
3	11	16	15	15	17	17
4	–	12	–	15	16	18
5	–	15	15	–	17	17

Inhibition zones are given in mm. Abbreviations: EC, Escherichia coli; BS, Bacillus subtilis; SF, Shigella flexneri; SA, Staphylococcus aureus; PA, Pseudomonas aeruginosa; ST, Salmonella typhi.

As a negative control, DMSO was used. The results are summarized in Table II.

Results and discussion

6-dehydroacetylsepaconitine (**1**) was obtained as a white amorphous powdered, and was assigned the molecular formula $C_{32}H_{42}N_2O_{10}$, on the basis of HREI-MS (*m/z* 614.6828, calcd. 614.6834). The mass spectrum of **1** was characteristic for diterpene bases of the C-18 series esterified through hydroxyl group at C-4 (lappaconitine, sepacnitine, *N*-deacetyllappacinitine, etc), where the maximum peak corresponds to the ejection of a molecule of acid from the molecular ion. In the mass spectrum of compound **1**, the peak of the $(M - 179)^+$ ion was the maximum peak and corresponded, as in the mass spectrum of *N*-acetylsepaconitine, to the ejection of acetylanthranilic acid [12]. The IR spectrum showed of compound **1** showed absorption bands at 3492 (OH groups), 1700 broadened band, ester carbonyl, amide carbonyl), and bands at 1600, 1280, 1250, and 750 cm^{-1} (1,2-substituted aromatic ring), 1083 (simple ether bonds). The ^{1}H- and ^{13}C-NMR spectra of 6-dehydroacetylsepaconitine (**1**) exhibited a close resemblance to that of the known compound *N*-acetylsepaconitine (**3**) [12] except the presence of carbonyl group at C-6, instead of methylene group in compound **1**.

The ^{1}H-NMR spectrum of 6-dehydroacetylsepaconitine (**1**) exhibited signals for *N*-ethyl, three methoxy groups and several methine protons with geminal oxygen substituents. In the down field region of the spectrum a doublet of one proton at δ 3.71 ($\mathcal{J} = 4.8$ Hz), characteristic for H-14. Triplet of three protons integration at δ 1.17 ($\mathcal{J} = 7.0$ Hz), was due to the methyl of *N*-ethyl group. Similarly, in the down field region a triplet of one proton at δ 3.85 ($\mathcal{J} = 7.9$ Hz), was assigned to the H-1, geminal to methoxy group. A broad singlet of one proton at δ 2.82 was assigned to the H-17, whereas, a broad singlet of one proton at δ 2.45, was assigned to the H-5, while, another singlet of one proton at δ 2.11 was assigned to H-7. The ^{13}C-NMR spectrum (BB, DEPT) (Table I), showed thirty two signals, including five methyls, six methylene, eleven methine, and ten quaternary carbons. Comparing the ^{13}C-NMR data of compound **1** with those of the reported compound *N*-acetylsepaconitine (**3**), (Table I), the appearance of a new quaternary carbon signal at δ 206.9, and the disappearance of CH_2 signal at δ 24.5, indicated the presence of carbonyl group at C-6. The ^{1}H- ^{13}C correlation was determined by the HMQC spectrum,

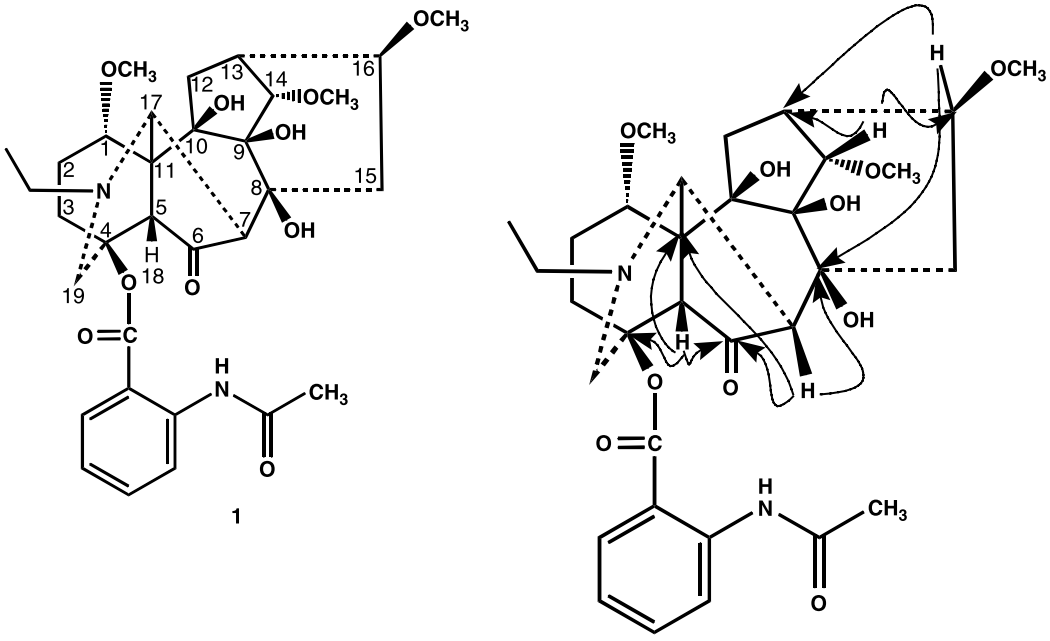

Figure 1. Selective HMBC interactions in compound 1.

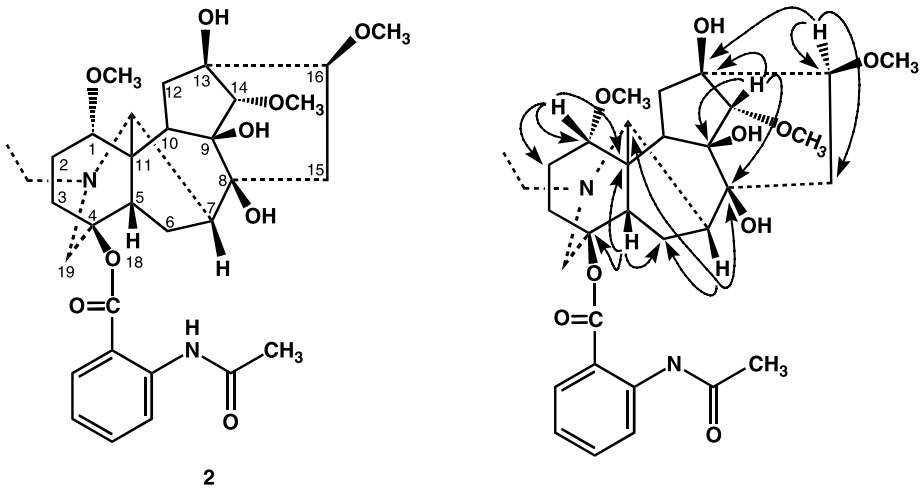

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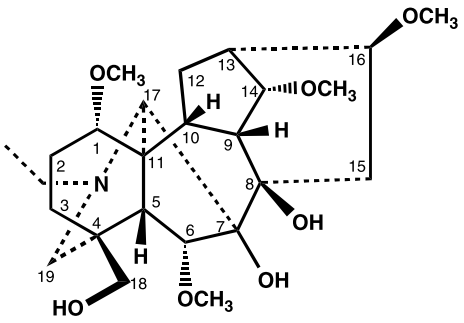
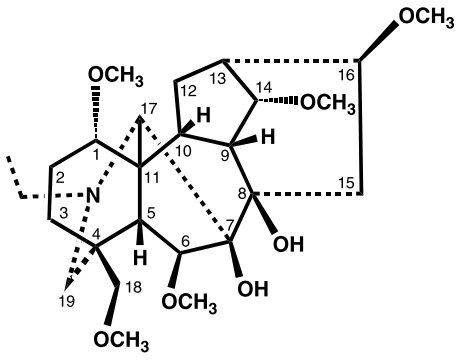
Figure 2. Selective HMBC interactions in compound 2.

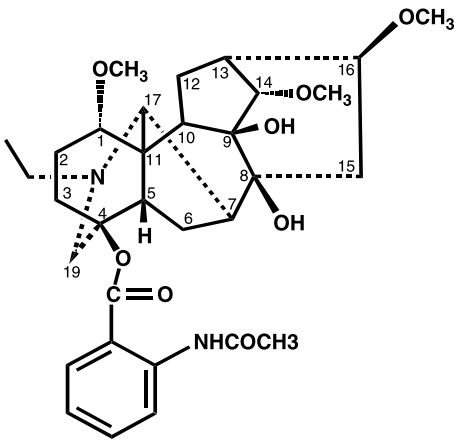
while the long range ^{1}H- ^{13}C connectivities were obtained through HMBC technique (Figure 1). The H-5 (δ 2.45) showed ^{2}J and ^{3}J correlation with C-4 (δ 84.6), C-11 (δ 55.7), C-6 (δ 206.9) and C-17 (δ 61.5), whereas H-7 (δ 2.11), exhibited ^{1}J and ^{2}J correlation with C-6 (δ 206.9), C-7 (δ 46.8), C-8 (δ 74.5), and C-11 (δ 55.7), while, H-14 (δ 3.71), showed correlation with C-13 (δ 34.5), C-16 (δ 82.7). Thus on the basis of above spectral data, the structure of compound **1** was deduced as 6-dehydroacetylsepaconitine.



Lycoctonine (**3**)



Delphatine(**4**)



Lappaconitine(**5**)

13-hydroxylappaconitine **(2)** was obtained as a white amorphous powdered, and was assigned the molecular formula $C_{32}H_{44}N_2O_9$, on the basis of HREI-MS (*m/z* 600.7003, calcd. 600.7012). The IR spectrum showed of compound **2** showed absorption bands at 3492 (OH groups), 1700 broadened band, ester carbonyl, amide carbonyl), and bands at 1600, 1280, 1250, and 750 cm^{-1} (1,2-substituted aromatic ring), 1083 (simple ether bonds). The mass fragmentation of **2** is characteristic of alkaloids with aconitine skeleton. The ^{1}H- and ^{13}C-NMR spectra of 13-hydroxylappaconitine (**2**) exhibited a close resemblance to that of the known compound *lappaconitine* (**5**) [13], except the presence of hydroxyl group at C-13, instead of methine group in compound **2**.

The ^{1}H-NMR spectrum of 13-hydroxylappaconitine (**2**) exhibited signals for *N*-ethyl, three methoxy groups and several methine protons with geminal oxygen substituents. In the down field region of the spectrum a singlet of one proton at δ 4.32 was assigned to H-14. Similarly, a broad singlet of one proton in the down field region at δ 10.78 was assigned to the amide proton. Triplet of three protons at δ 1.03 ($\mathcal{J} = 7.0$ Hz), was due to the methyl of *N*-ethyl group. Similarly, a double doublet of two protons at δ 3.21 ($\mathcal{J} = 9.0$, 6.0 Hz), was assigned to the H-1 and H-16, geminal to methoxy group. A broad singlet of one proton at δ 2.45 was assigned to the H-5. The ^{13}C-NMR spectrum (BB, DEPT) (Table I), showed thirty two signals, including five methyls, seven methylene, eleven methine, and nine quaternary carbons. Comparing the ^{13}C-NMR data of compound **2** with those of the reported compound lappaconitine (**5**), (Table I), the appearance of a new quaternary carbon signal at δ 78.1, and the disappearance of CH signal at δ 49.0, indicated the presence of hydroxyl group at C-13. The ^{1}H- ^{13}C correlation was determined by the HMQC spectrum, while the long range ^{1}H-^{13}C connectivities were obtained through HMBC technique (Figure 2). The H-5 (δ 2.45) showed correlation with C-4 (δ 84.6), C-11 (δ 53.1), and C-6 (δ 27.2), whereas H-7 (δ 2.11), exhibited correlation with C-6 (δ 27.2), C-7 (δ 47.8), C-8 (δ 68.4), and C- 17 (δ 58.0), while, H-14 (δ 3.71), showed correlation with C-13 (δ 78.1), C-14 (δ 87.4), C-9 (δ 79.2) and C-8 (δ 68.4), Similarly, H-16 (δ 3.33) exhibited interaction with C-16 (δ 82.2), C-15 (δ 32.4) and C-13 (δ 78.1).

Thus on the basis of above spectral data, the structure of compound **2** was deduced as 13-hydroxylappaconitine.

Antibacterial activity: Compounds **1** and **2** showed significant antibacterial activity against *Staphylococcus aureus*. Compound **3** showed significant activities against *Salmonella typhi* and *Pseudomonas aeruginosa*, while compounds **4** and **5** showed significant activity against *Salmonella typhi* and *Pseudomonas aeruginosa* (Table II).

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