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COMPONENTS FROM *Aconitum karakolicum* ROOTS

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The isolation and structures of 22 alkaloids from the aerial part and roots of *Aconitum karakolicum* Rapaics (Ranunculaceae) growing in Kirgizia (Kungei and Terskei Alatau ridges) have been reported [1–4]. Pharmacological studies found that the plant alkaloids possess spasmolytic, antiarrhythmic, local anesthetic, and other activities [1].

We investigated the chemical composition of this plant growing in Xinjiang Autonomous Region of the PRC taht was identified at the Xinjiang Institute of Ecology and Geography, AS, XAR. Plants of the genus *Aconitum* are frequently used in China to treat fractures, rheumatism, and neuralgia. *A. karakolicum* is used in Chinese folk medicine [5].

Air-dried ground roots (1.08 kg) were extracted with EtOH (80%, 10 × 8 L). The first two extracts were combined. Solvent was distilled off. The resulting crystals were separated with EtOH and recrystallized from acetone:water to afford **1** (0.6 g), mp 189–190°C. Compound **1** was identified as saccharose by comparison of its melting point and ^{13}C NMR spectrum (600 MHz, DMSO) with the literature [6].

Next, condensed mother liquor was diluted with water. The aqueous solution was washed with EtOAc. The EtOAc was distilled off to afford fraction A. Then, the aqueous solution was made basic successively with Na_2CO_3 until the pH was 7 and 10. Alkaloids were extracted from the basic solutions by EtOAc. The remaining eight extracts were worked up analogously to afford fraction A (37.5 g) and total alkaloids (19.46 g) [fraction B, 9.3 g (pH 7) and fraction C 10.16 g (pH 10)]. The yield of total alkaloids was 1.08% of the total dry plant.

Fraction A (37.5 g) was separated over a column of Al_2O_3 with elution by petroleum ether. Compound **2**, mp 140–142°C, was isolated from the first effluents using MeOH and was identified as β-sitosterol by comparison of its PMR and ^{13}C NMR spectra (600 MHz, $CDCl_3$) with the literature [7].

Fraction B (9.3 g, pH 7) was recrystallized. The crystals were separated by acetone and purified over a column of Al_2O_3 with elution by hexane:acetone (10:1, 5:1, 1:1). The 10:1 fractions afforded **3**, mp 195–196°C, which was identified as aconitine [1, 2].

Fraction C (10.16 g, pH 10) was separated over a column of Al_2O_3 (300 g) with elution by petroleum ether and petroleum ether:acetone (1:1). Fraction 13 afforded crystalline **4**, mp 278–280°C; fraction 20, alkaloid **5**. Comparison of PMR and ^{13}C NMR data of **4** identified it as β-sitosterol 3-*O*-β-D-glucopyranoside [7].

Analysis of DEPT, PMR, and ^{13}C NMR spectra of **5** (Table 1) showed that it contained benzoyl, four methoxyls, four hydroxyls, and an *N*-ethyl group. ^{13}C NMR and DEPT spectra of **5** contained resonances for 30 C atoms including 6 singlets, 14 doublets, 5 triplets, and 5 quartets. Because the monosubstituted benzene ring contained two *ortho* and two *meta* C atoms, the base contained 32 C atoms and had the expanded formula $C_{19}H_{19}(N–CH_2–CH_3)(4{\cdot}OH)(4{\cdot}OCH_3)(C_6H_5COO)$, $C_{32}H_{45}NO_{10}$, $[M]^+$ 603.71195. These data for **5** indicated that the base had the lycoctonine skeleton and was an aconitine-type C_{19}-norditerpenoid alkaloid.

The presence in the HMBC spectrum of cross-peaks between C-1 (79.94 ppm) and the 1-OCH_3 protons (3.38 ppm, 3H, s); C-6 (80.82) and the 6-OCH_3 protons (3.39, 3H, s); C-16 (90.67) and the 16-OCH_3 protons (3.73, 3H, s); and C-18 (78.10) and the 18-OCH_3 protons (3.31, 3H, s) placed four methoxyls on C-1, C-6, C-16, and C-18. SSCC of the doublet for H-6 and H-5 was 6.8 Hz and indicated that the C-6 OCH_3 group had the α-orientation [1, 2].

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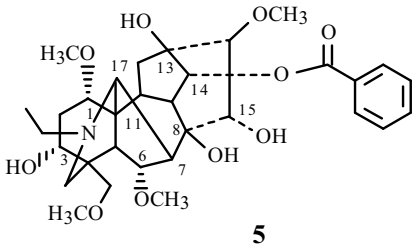
TABLE 1. PMR (399.73 MHz), ^{13}C NMR (100.52 MHz), DEPT, HSQC, and HMBC Spectra of Benzoylaconine ($CDCl_3$, δ, ppm, TMS, J/Hz)

C atom	δ_C	DEPT	HSQC	HMBC
1	79.94	CH	3.54 (1H, d, J = 8.0)	
2	29.70	CH_2	1.24 (1H, m)	
			1.53 (1H, dd, J = 16.0, J = 4.8)	
3	70.36	CH	4.28 (1H, d, J = 4.2)	
4	43.53	C	–	
5	48.05	CH	2.83 (1H, s)	C-6, C-8, C-17, C-11, C-18, C-4, C-19, C-7, C-9
6	80.82	CH	4.24 (1H, d, J = 6.8)	C-18, C-17, 6-OCH_3, C-4, C-9
7	44.63	CH	2.19 (1H, d, J = 6.8)	C-18, C-17, C-19, C-5, C-4, C-10
8	78.39	C	–	
9	43.81	CH	2.61 (1H, t, J = 4.8)	C-14, C-8, C-13, C-10, C-12
10	40.42	CH	2.29 (1H, m)	C-12, C-17, C-14, C-16, C-13, C-11, C-8
11	50.05	C	–	
12	35.56	CH_2	2.32 (1H, m)	C-10, C-11,C-13,C-16
			1.77 (1H, d, J = 9.2)	
13	74.34	C	–	
14	78.48	CH	4.98 (1H, d, J = 4.8)	Ar-C=O, C-16, C-8, C-13, C-9
15	81.34	CH	4.70 (1H, dd, J = 15.6; J = 4.8)	
16	90.67	CH	3.16 (1H, d, J = 6.0)	C-15, C-8, C-13, C-12, C-14, 16-OCH_3
17	64.92	CH	3.43 (1H, s)	C-19, C-6, C-7, C-8, C-3, C-11, N-$\underline{CH_2}$-CH_3, 6-OCH_3
18	78.10	CH_2	3.89 (1H, d, J = 8.8)	C-3, C-4, C-19
			3.26 (1H, d, J = 8.4)	
19	50.45	CH_2	3.49 (1H, d, J = 11.2)	C-3
$N\underline{CH_2}CH_3$	50.20	CH_2	3.53 (1H, m)	$NCH_2\underline{CH_3}$
			3.12 (1H, d, J = 7.6)	
$NCH_2\underline{CH_3}$	11.09	CH_3	1.42 (3H, t, J = 7.2)	$N\underline{CH_2}CH_3$
1-OCH_3	55.27	CH_3	3.38 (3H, s)	C-1
6-OCH_3	58.31	CH_3	3.38 (3H, s)	C-6
16-OCH_3	60.95	CH_3	3.73 (3H, s)	C-16
18-OCH_3	59.29	CH_3	3.31 (3H, s)	C-18
Ar-CO	166.35	C	–	
C-1′	129.60	C	–	
C-2′,6′	129.94	CH	8.03 (2H, 2H, d, J = 8.4)	Ar-CO, Ar-C-4′, Ar-C-1′
C-4′	133.22	CH	7.56 (1H, t, J = 7.4)	Ar-C-1′, Ar-C-2′,6′
C-3′,5′	128.58	CH	7.46 (2H, t, J = 7.6)	Ar-C-1′, Ar-C-2′,6′

The chemical shift, multiplicity, and SSCC of the proton at 4.98 ppm (Table 1) indicated that the C-14 benzoyl had the α-orientation. This was also confirmed by the presence in the HMBC spectrum of cross-peaks between the proton at 4.98 and resonances of Ar–C=O, C-16, C-8, C-9, and C-13. The doublet for H-14 was consistent with a substituent on C-9 or C-13. The presence in the DEPT spectrum of a singlet for a C atom at 74.34 ppm placed a hydroxyl on C-13. The resonance of C-12 was observed at 33.5-38.0 because of the β-effect of the C-13 hydroxyl. The DEPT spectrum of **5** had a triplet for C-12 that was observed at 35.56, confirming that C-13 had a hydroxyl.

The HSQC spectrum shows a doublet of doublets at 4.70 ppm corresponding to a C atom at 81.34 ppm and a doublet at 4.28, to an atom at 70.36 (Table 1). The DEPT spectrum contained a singlet at 78.39. These data enabled three hydroxyls to be placed on C-15, C-3, and C-8, respectively. The resonance of C-4 that appeared at 43.53 as a result of the β-effect of the C-3 OH also indicated that C-3 contained a OH. The C-3 OH had the α-orientation. The C-3 resonance was observed at 70.5–72.0 [8]. Therefore, the C-3 OH had the α-orientation. Atom C-8 resonated at 75.9-81.7; C-15, at 78.6-81.9 due to the OH groups on C-8 and C-15, like for the alkaloids senbusine C [2], benzoyldeoxyaconine [9], benzoylaconine [9], and polyschistine D [9]. The resonance of C-8 with an OH group would have been observed at 72.5-74.5 if C-7, C-9, and C-15 lacked substituents. Therefore, C-8 and C-15 contained OH groups.

According to the results, base **5** was assigned the structure of benzoylaconine.



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Thus, saccharose, β-sitosterol, β-sitosterol 3-*O*-β-D-glucopyranoside, and benzoylaconine were found for the first time in *A. karakolicum*. Benzoylaconine was isolated previously from *A. polyschistum* [9].

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