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TWO NEW ATISINE-TYPE C_{20}-DITERPENOID ALKALOIDS FROM *ACONITUM LEUCOSTOMUM*

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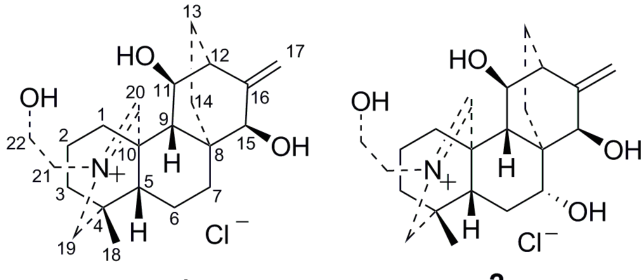
Abstract – Two new atisine-type C_{20}-diterpenoid alkaloids, leucostomine A (**1**) and leucostomine B (**2**) were isolated from the root of *Aconitum leucostomum*. Their structures were determined by spectroscopic methods including 1D-, 2D-NMR, and HR-ESI–MS.

Aconitum leucostomum Worosch. (Ranunculaceae) is a perennial herb distributed in the Gansu Province and Xinjiang Uygur Autonomous Region of China. It has been used as a folk medicine for the treatment of rheumatoid arthritis, cancer, and various types of pains for a long time.[1] The previous phytochemical studies on this herb have led to the isolation of more than a dozen diterpenoid alkaloids.[2] Lappaconitine, one of the main diterpenoid alkaloids, have been developed into a new non-narcotic analgesics drug.[3] Our further chemical investigation of this plant led to the isolation of two new atisine-type C_{20}-diterpenoid alkaloids, named leucostomine A (**1**) and leucostomine B (**2**) (Figure 1). This paper reports the isolation and structural elucidation of these alkaloids.



1 **2**

Figure 1. Structures of compounds **1** and **2**

The 90% EtOH extract of roots of *A. leucostomum* was treated in the usual procedures[4] to give CH_2Cl_2-soluble alkaloid and water-soluble alkaloid fractions. The water-soluble alkaloid fraction was sequentially subjected to column chromatography over D101 macroporous resin, aluminum oxide, and silica gel to give two new compounds.

Compound **1** was obtained as a white solid and gave a positive reaction to Dragendorff's reagent. Its molecular formula was determined as $C_{22}H_{34}NO_3$ by analysis of its HR-ESI-MS spectrum (*m*/*z* 360.2544 $[M]^+$, calcd. for $C_{22}H_{34}NO_3$ 360.2539). The IR spectrum showed absorption bands for hydroxyl group (3411 cm^{-1}) and an imine bond (1706 cm^{-1}). The ^1H NMR spectrum showed signals characteristic for an angular methyl group (δ_H 1.08 s) and an exocyclic methylene group (δ_H 5.25 and 5.33, br s). Its ^{13}C-NMR and DEPT spectra showed twenty-two carbon signals including one methyl (δ_C 26.5), eleven methylenes (δ_C 21.4, 21.6, 25.2, 26.1, 32.1, 37.3, 42.8, 59.9, 61.8, 66.5, 117.4), six methines (δ_C 45.9, 46.7, 52.5, 71.8, 77.0, 185.4) and four typical non-oxygenated quaternary carbons (δ_C 35.6, 40.3, 48.4, 151.2), suggesting compound **1** might be an atisine-type alkaloid.[5] In addition, the resonances at δ_C 35.6 (s, C-4), 48.4 (s, C-10) and 185.4 (d, C-20) strongly indicated the existence of a quaternary-N center in the molecule,[6] which was also supported by its deshielding effects on N-hydroxyethyl (δ_H 4.07 and 4.19), H-20 (δ_H 8.52) and H-19 (δ_H 3.72 and 3.84, ABq, *J* = 18 Hz) compared with the values of free atisine-type alkaloids.[7] From the above-mentioned evidence, compound **1** could be assigned as an atisine-type alkaloid possessing a quaternary nitrogen atom. Comparison of the 1D-NMR data (Table) of **1** with those of the known compound atisinium chloride indicated that there was a hydroxyl group at C-11 in **1** instead of the hydrogen group in atisinium chloride,[8] which was also confirmed by the difference of 16 mass units between the two compounds. Furthermore, the existence of hydroxyl group at C-11 was supported by the cross-peaks between H-11 (δ_H 4.05) and C-9 (δ_C 52.5), C-10 (δ_C 48.4) and C-16 (δ_C 151.2) in the HMBC spectrum. The complete planar structure of **1** was further verified by analysis of the HMBC and ^1H-^1H COSY spectra (Figure 2).

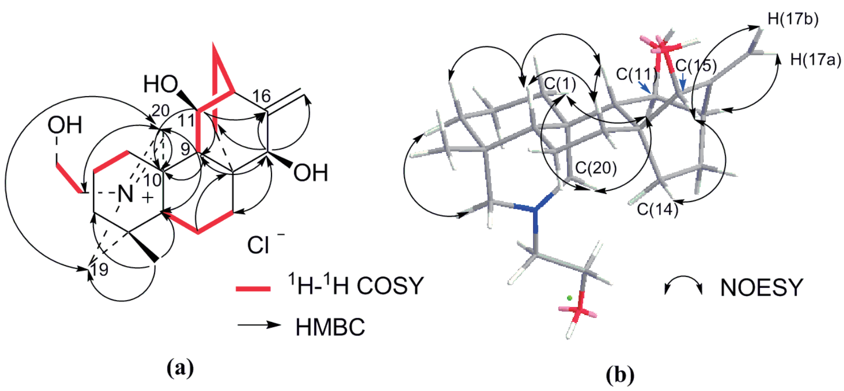


Figure 2. Key ^1H-^1HCOSY, HMBC (**a**) and NOESY (**b**) correlations of **1**

The configuration of compound **1** was deduced from the coupling constants (Table) and NOESY spectrum (Figure 2). The hydroxyl group at C-11 was assigned a β-orientation based on the coupling constants of H-11 at δ_H 4.05 (1H, d, *J* = 4.2 Hz) in the NMR spectra (when the hydroxyl group at C-11 is β-oriented, the coupling of H-11 with H-9 is almost nil and the coupling with H-12 is ~5 Hz; in contrast, H-11 show a large coupling (~9-10 Hz) with H-9 and ~4-5 Hz coupling with H-12[9]). The NOESY correlations of H-11 with H-20 and H-11 with H_{α}-1 also proved a β-orientation of hydroxyl group at C-11. The hydroxyl group at C-15 took β-orientation, since the resonance of C-15 exhibited nearly identical NMR data to those of the known alkaloids, atisinium chloride[10] and brunonine[11] whose structure was unambiguously determined by X-ray crystallography. This conclusion was also supported by the cross-peak between H-15 with H_{β}-14 in the NOESY experiment. Since the absolute configuration of the atisine nucleus was repeatedly confirmed by the X-ray crystallographic analysis of analogues isolated from species of the same genus,[12] it was proposed that the absolute configuration of the atisine nucleus was retained in **1**. Therefore, the structure of compound **1** was characterized as shown in Figure 1, named as leucostomine A.

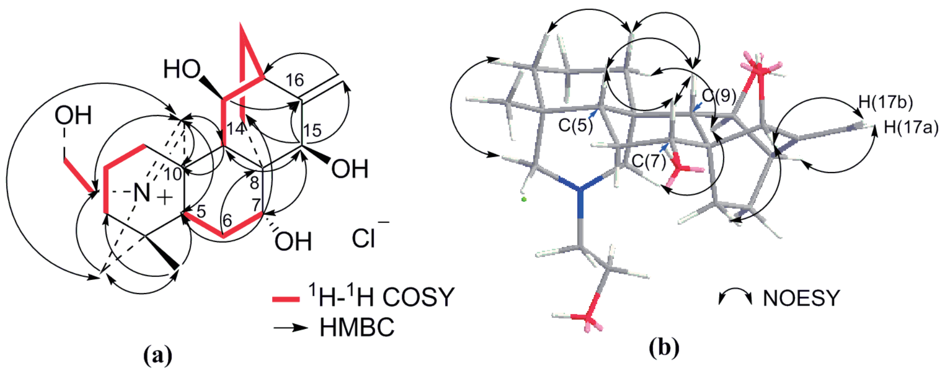


Figure 3. Key ^{1}H-^{1}HCOSY, HMBC (**a**) and NOESY (**b**) correlations of **2**

Compound **2**, a white solid, possessed the molecular formula $C_{22}H_{34}NO_4$, which was determined by the analysis of HR-ESI-MS (*m/z* 376.2494 $[M]^+$, calcd. for $C_{22}H_{34}NO_4$ 376.2488). The IR spectrum showed absorption bands for hydroxyl group (3377 cm^{-1}) and an imine bond (1672 cm^{-1}). It was readily recognized that compound **2** was also an atisine-type quaternary ammonium salt alkaloid[13] according to the NMR spectra (Table), which showed the presence of a methyl group (δ_H 1.10 s, δ_C 24.9 q), an exocyclic double bond (δ_H 5.19 and 5.34, br s; δ_C 115.0 t, 150.7 s), an N-hydroxyethyl group (δ_H 3.99 and 4.15; δ_C 58.4 t, 65.0 t) and four typical non-oxygenated quaternary carbon signals (δ_C 34.2, 44.3, 46.7 and 150.7). The characteristic NMR spectra signals of compound **2** were similar to those of compound **1**, all the differences being due to the hydroxyl group at C-7 in compound **2**. This conclusion was supported by the appearance of a new methine signal at 68.5 ppm, the absence of a methylene signal at 32.1 ppm (compared the ^{13}C-NMR data with compound **1**), and the downfield shift at C-6 ($\Delta\delta_C$ 7.4 ppm) and C-8 ($\Delta\delta_C$ 4.0 ppm). Moreover, the existence of hydroxyl group at C-7 was confirmed by the cross-peaks between H-7 (δ_H 3.97) with C-5 (δ_C 44.0), C-14 (δ_C 18.5) and C-15 (δ_C 70.8) in the HMBC spectrum

(Figure 3). The configuration of **2** was determined to be identical to that of **1**, this being supported not only by their almost similar ^{1}H- and ^{13}C-NMR data (Table), but also by the NOESY data (Figure 3). The correlations among H-7, H-5, and H-9 in the NOESY spectrum indicated that H-7 was β-oriented. Furthermore, the upfield shift[14] of C-14 (Δ δ_C 7.6 ppm) and C-15 (Δ δ_C 6.2 ppm) due to steric compression by the hydroxyl group at C-7 also give the proof of H_{β}-7. Accordingly, the structure of compound **2** was established to be leucostomine B.

Table. ^{1}H- and ^{13}C-NMR data (600 and 150 MHz, resp.) of compounds **1** and **2**

Position	1 in D_2O		**2** in CD_3OD	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	β 1.78 [a] α 2.22 d (15)	37.3 t	β 1.71 m α 2.28 d (13.8)	35.7 t
2	α 1.28 [a] β 1.78 [a]	21.4 t	1.75 m	20.1 t
3	β 1.57 m α 1.78 [a]	42.8 t	β 1.56 [a] α 1.73 m	41.6 t
4	—	35.6 s	—	34.2 s
5	β 1.49 [a]	46.7 d	β 1.56 [a]	44.0 d
6	α 1.05 m β 1.78 [a]	21.6 t	α 1.14 q (13.2) β 1.88 dt (3.6, 13.2)	29.0 t
7	β 1.28 [a] α 1.83 m	32.1 t	β 3.97 br s	68.5 d
8	—	40.3 s	—	44.3 s
9	β 1.88 d (1.8)	52.5 d	β 1.84 d (1.8)	51.3 d
10	—	48.4 s	—	46.7 s
11	α 4.05 d (4.2)	71.8 d	α 3.98 d (3.6)	70.3 d
12	2.50 dd (1.8, 4.2)	45.9 d	2.41 dd (1.8, 3.6)	45.4 d
13	1.72 m	25.2 t	1.65 m	23.4 t
14	α 0.90 m β 1.49 [a]	26.1 t	α 1.27 m β 1.35 m	18.5 t
15	α 3.84 br s	77.0 d	α 4.20 br s	70.8 d
16	—	151.2 s	—	150.7 s
17	a 5.25 br s b 5.33 br s	117.4 t	a 5.19 br s b 5.34 br s	115.0 t
18	1.08 s	26.5 q	1.10 s	24.9 q
19	3.72 ABq (18) 3.84 ABq (18)	61.8 t	3.75 ABq (18) 3.82 ABq (18)	60.3 t
20	8.52 br s	185.4 d	8.62 br s	183.8 d
21	4.19 t (4.8)	66.5 t	4.15 t (4.8)	65.0 t
22	4.07 m	59.9 t	3.99 m	58.4 t

[a] Overlapped signals.

EXPERIMENTAL

General experimental procedure

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. 1D and 2D NMR spectra were recorded on a Bruker AV 600 NMR spectrometer, and IR spectrum on a ThermoFisher Nicolet 6700 spectrometer (KBr discs, cm^{-1}). HR-ESI-MS were carried out on a Q-TOF micro mass spectrometer (Waters, USA). Silica gel (Qingdao Haiyang Chemical Co., Ltd., China, 200–300 mesh), D-101 macroporous resin (Rohm & Haas), and alumina (Qingdao Haiyang Chemical Co., Ltd., China, 100–200 mesh) were used for column chromatography (CC). TLC plates precoated with silica gel GF254 (Qingdao Haiyang Chemical Co., Ltd., China) were visualized under a UV lamp at 254 nm or by spraying the Dragendorff's reagent or by iodine.

Plant Material

A. leucostomum were collected in nileke county, Xinjiang Uygur Autonomous Region of China, in August 2014, and were identified by Prof. Qing-Er Yang of the Institute of Botany, Chinese Academy of Sciences. A voucher specimen (C. Ren & L. Wang 735) was deposited in the School of Life Science and Engineering, Southwest Jiaotong University.

Extraction and isolation

Dried and powder roots of *A. leucostomum* (6.5 kg) were extracted with 95% EtOH three times at room temperature, with each soaking process lasting three days. Evaporation of the solvent under reduced pressure provided a 95% EtOH extract (280.4 g). The extract was treated with 0.5 N hydrochloric acid (2 L) and successively extracted with light petroleum (4×1 L) and EtOAc (4×1 L) to remove non-alkaloid constituents. Then, 28% aqueous ammonia soln. (2 L) was added to the acidic solution to adjust to pH 10. The solutions were extracted with CH_2Cl_2 (4×1 L) to afford the CH_2Cl_2-soluble alkaloid. Then, the aqueous layer was purified using chromatography with D101 macroporous resin, and the column contents were eluted with H_2O, 40% EtOH, 60% EtOH, and 95% EtOH, using a gradient. The 60% EtOH elute was concentrated *in vacuo* to afford a residue (13.7 g), which was subjected to alumina column chromatography, using a $CHCl_3$-MeOH (50:1-0:1) as the eluent to separate it into four fractions, A-D. Fraction B (314.6 mg) was chromatographed on silica gel column and eluted with $CHCl_3$/MeOH/concentrated NH_4OH (3:1:1, v/v/v) to afford **1** (32.3 mg) and **2** (25.0 mg) successively.

Leucostomine A (1)

White solid; $[\alpha]_D^{20}$ +117 (c 0.45, MeOH); IR (KBr) ν_{max} 3411, 2936, 2878, 1706, 1672, 1639, 1452, 1409, 1376, 1110, 1076, 1046, 1018, 864, 580 cm^{-1}. ^1H NMR (600 MHz, D_2O) and ^{13}C NMR (150 MHz, D_2O): see Table; HR-ESI-MS (m/z): 360.2544 $[M]^+$, calcd. for $C_{22}H_{34}NO_3$ 360.2539.

Leucostomine B (2)

White solid; $[\alpha]_D^{20}$ +50 (c 1.5, MeOH); IR (KBr) ν_{max} 3377, 2935, 2875, 1672, 1637, 1431, 1385, 1112, 1076, 1050, 1017, 987, 857, 794, 636 cm^{-1}. ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD): see Table; HR-ESI-MS (*m*/*z*): 376.2494 $[M]^+$, calcd. for $C_{22}H_{34}NO_4$ 376.2488.

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