Tangutidines A–C, Three Amphoteric Diterpene Alkaloids from Aconitum tanguticum

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Abstract
Three new diterpene alkaloids, tangutidines A–C (1–3), and four known alkaloids (4–7) were isolated from the whole plant of Aconitum tanguticum, from which amphoteric diterpene alkaloids (1–3) were obtained for the first time. The structures of 1–3 were elucidated by detailed interpretation of spectroscopic data, including MS and NMR data. All of them were evaluated for their cytotoxic activities.

Keywords Aconitum tanguticum · Amphoteric diterpene alkaloids · Tangutidine · Cytotoxic activity

1 Introduction
Plants in the genus Aconitum of the family Ranunculaceae are abundant in C_{19} and C_{20}-diterpene alkaloids with diverse structural scaffolds and important biological activities, which have long attracted scientists’ attention from chemistry and pharmacology communities [1–3]. Aconitum tanguticum (Maxim.) Stapf is mainly distributed in Tibet, Qinghai, Gansu, Sichuan and Yunnan Provinces in China [4]. Its whole plant has long been used as a traditional Tibetan medicine for treating fever caused by various infectious diseases, influenza and poisoning for thousands of years [5]. In a classic Tibetan Medical book Sman dpyad zla ba’i rgyal po, A. tanguticum is firstly recorded as one of the least toxic plants among other species in the genus Aconitum [5]. Previous
phytochemical investigation of *A. tanguticum* showed that
it contained diterpene alkaloids, flavonoids, phenolic acids,
glycosides, etc. [6–16]. However, most of the studies on diter-
crete alkaloids in *A. tanguticum* focused on its fat-soluble
part and few focused on the water-soluble part. In our phyto-
chemical investigation of the whole plant of *A. tanguticum*,
it resulted in the discovery of three new C20-diterpene alkal-
oids with carboxyl groups, tangutidines A–C (1–3) from the
n-BuOH extract, together with four known alkaloids (4–7)
(Fig. 1). In this paper, we reported their isolation, structure
determination, and cytotoxicity.

2 Results and Discussion

Tangutidine A (1) was isolated as colorless oil with a
molecular formula of C_{23}H_{33}NO_{4}, which was determined
by HRESIMS ([M+H]^+ at m/z 388.2483, calcd 388.2482)
with eight degrees of unsaturation. Its IR spectrum showed
absorptions for hydroxyl (3428 cm⁻¹) and carboxyl (1603
cm⁻¹) groups. Analysis of the \(^1\)H, \(^13\)C NMR, DEPT and
HSQC spectra of 1 revealed the existence of one olefinic
bond (δ_H 5.83, br s; δ_C 128.6, d; 150.7, s), one oxygenated
methylene group (δ_H 4.46, d, J = 2.2 Hz; δ_C 63.4, t), one
tertiary methyl group (δ_H 1.15, s; δ_C 24.9, q), and four sp³
quaternary carbon (one oxygenated) (Table 1). All the men-
tioned evidence suggested that 1 was a hetidine-type dit-
erepene alkaloid containing three extra carbons. Compared
with the structure of naviculine A [8] bearing a double bond
between C-19 and N-atom, 1 had an extra 3-N-propanoic
acid moiety and two hydroxyl groups located at C-5 and
C-17, respectively. The fragment C-1’/C-2’/C-3’ was identi-
ified by the \(^1\)H–\(^1\)H COSY correlation of H-2-1’/H-2-2’, along
with the HMBC correlation from H-2-1’a (δ_H 3.31, m) to C-3’
(δ_C 178.7, s). The connection between the fragment C-1’/C-
2’/C-3’ and N-atom was confirmed by the key HMBC corre-
lation from H_2-1’a (δ_H 3.31, m) to C-20 (δ_C 79.4, d) (Fig. 2)
and the key ROESY correlations from H_2-1’a to H-20 (δ_H
2.70, s), H_2-1’b (δ_H 2.99, m) to H-19b (δ_H 2.62, m) as well
as H_2-2’ (δ_H 2.80, m) to H-19a (δ_H 3.11, br s) (Fig. 3). The
key HMBC correlations of H-18 (δ_H 1.15, s) and H-6 (δ_H
1.86 dd, J = 14.2, 7.0 Hz) with C-5 (δ_C 73.7, s), and H-15
(δ_H 5.83, br s) with C-17 (δ_C 63.4, t) confirmed the attach-
ment of the hydroxyl groups to C-5 and C-17. The linkage of
the hydroxyl groups to C-5 and C-17 was confirmed by the
HMBC correlations from a hydroxyl group (δ_H 4.97, br s) to
C-4, C-5, and C-6, from H-18 to C-5, from H-17 to C-12 and
C-16, and from H-15 to C-17. The relative configuration of 1
was established based on the ROESY spectrum, which was
the same as that of naviculine A (Fig. 3). Thus, the structure
of 1 was elucidated with its assigned NMR spectroscopic
data listed in Table 1.

Compound 2 was obtained as colorless amorphous powder.
The IR spectrum showed absorptions for hydroxyl (3428 cm⁻¹)
and carboxyl (1603 cm⁻¹) groups. Its molecular formula was assigned as C_{23}H_{33}NO_{4} by the analysis of its
HRESIMS (m/z 388.2486 [M+H]^+, calcd 388.2482),
indicating eight degrees of unsaturation. Comparison of the
\(^1\)H and \(^13\)C NMR spectra data of 2 with that of 1 (Table 1)
revealed that 2 was an analogue of 1. The main difference
between them was that an endo double bond at C-15/C-16
and a hydroxyl group at C-17 in 1 were replaced by a double
bond at C-16/C-17 and a hydroxyl group at C-15 in 2.
Compared with the \(^13\)C NMR data of 1, some chemical shifts in that of 2 changed due to the shift of double bond:
a high-field chemical shifts of C-7 (δ_C 31.6, t, Δ = −1.5), C-9
(δ_C 44.3, d, Δ = −6.2), C-11 (δ_C 28.4, t, Δ = −0.7), C-13 (δ_C
37.9, t, Δ = −4.9), C-14 (δ_C 46.8, d, Δ = −2.5) and a low-field
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chemical shifts of C-8 (δ_C 45.7, s, Δ+ 1.3), C-10 (δ_C 49.0, s, Δ + 1.6), and C-12 (δ_C 35.5, d, Δ + 3.1). The main difference between 1 and 2 was further confirmed by the HMBC correlations from H-17 to C-12, C-16, and C-15, and from H-15 to C-7, C-12, and C-14. The presence of C-3' carboxyl group could be further confirmed by the HMBC correlation from both H_2-1' and H_2-2' to C-3'. The fragment C-1'/C-2'/C-3' connection with N-atom was confirmed by the HMBC correlation from both H2-1' and H2-2' to C-3'. The fragment ROESY correlation of H-9β/H-15 indicated that the hydroxyl group at C-15 was α-oriented (Fig. 3). Combined with all the evidence, the structure of compound 2 was established.

Compound 3 was isolated as colorless amorphous powder. Its molecular formula was deduced as C_{32}H_{42}N_{2}O_{4} by the analysis of the positive HRESIMS ion peak at m/z 519.3221 ([M+H]⁺, calc 519.3217). Its IR spectrum showed the presence of hydroxyl (3402 cm⁻¹), phenyl (1583 cm⁻¹, 1513 cm⁻¹, 1456 cm⁻¹), and carboxyl (1637 cm⁻¹) groups. The 1H NMR data of 3 (Table 2) exhibited the signals ascribed to an imine unit (δ_H 7.60, overlap), a p-substituted phenyl (δ_H 7.09, d, J = 8.4 Hz; 7.19, overlap), a trisubstituted vinyl group (δ_H 5.79, s), an oxygen-bearing methylene (δ_H 4.62, s), a nitrogen-bearing methine (δ_H 3.75, s), two methylenes connecting nitrogen (δ_H 2.95, m; 2.67, 2.09, overlap).

### Table 1

| No | Compound 1 | Compound 2 |
|----|------------|------------|
| 1  | 30.0, CH₂ | 2.05 (m)  |
|    | 1.60 (overlap) | 29.6, CH₂ | 1.87 (overlap) |
| 2  | 21.0, CH₂ | 1.67 (overlap) | 21.1, CH₂ | 1.63 (m) |
| 3  | 36.8, CH₂ | 2.14 (m) | 2.17 (overlap) |
|    | 1.04 (d, 12.2) | 36.8, CH₂ | 1.06 (d, 12.2) |
| 4  | 38.7, C  | 38.7, C  |
| 5  | 73.7, C | 4.97 (br s) | 73.6, C |
| 5-OH | 31.6, CH₂ | 2.87 (dt, 14.2, 7.0) | 31.4, CH₂ | 2.87 (dt, 13.5, 7.5) |
|    | 1.86 (dd, 14.2, 7.0) | 1.90 (overlap) | |
| 7  | 33.1, CH₂ | 2.32 (td, 14.2, 7.0) | 31.6, CH₂ | 2.66 (dd, 13.5, 7.5) |
|    | 1.94 (dd, 14.2, 7.0) | 1.94 (dd, 13.5, 7.5) | |
| 8  | 44.4, C | 45.7, C |
| 9  | 50.5, CH | 1.92 (br s) | 44.3, CH | 2.17 (overlap) |
| 10 | 47.4, C | 49.0, C |
| 11 | 29.1, CH₂ | 1.69 (overlap) | 28.4, CH₂ | 1.71 (m) |
|    | 1.46 (m) | |
| 12 | 32.4, CH₂ | 2.55 (s) | 35.5, CH | 2.32 (br s) |
| 13 | 42.8, CH₂ | 1.66 (overlap) | 37.9, CH₂ | 2.44 (d, 9.2) |
|    | 1.76 (m) | |
| 14 | 49.3, CH | 2.10 (m) | 46.8, CH | 2.47 (br s) |
| 15 | 128.6, CH | 5.83 (br s) | 73.9, CH | 4.30 (s) |
| 16 | 150.7, C | 159.1, C |
| 17 | 63.4, CH₂ | 4.46 (d, 2.2) | 106.1, CH₂ | 5.05 (overlap) |
| 18 | 24.9, CH₁ | 1.15 (s) | 24.8, CH₁ | 1.14 (s) |
| 19 | 61.1, CH₂ | 3.11 (br s) | 61.0, CH₂ | 3.10 (d, 11.0) |
|    | 2.62 (m) | 2.62 (d, 11.0) | |
| 20 | 79.4, CH | 2.70 (s) | 79.7, CH | 2.72 (s) |
| 1' | 53.4, CH₂ | 3.31 (m) | 53.4, CH₂ | 3.29 (dt, 12.7, 7.8) |
|    | 2.99 (m) | 2.98 (dt, 12.7, 7.8) | |
| 2' | 33.8, CH₂ | 2.80 (m) | 34.0, CH₂ | 2.79 (t, 6.9) |
| 3' | 178.7, C | 176.2, C |

*a* Recorded at 150 MHz, Recorded in pyridine-d₅

*b* Recorded at 600 MHz, Recorded in pyridine-d₅

*c* Assigned by analysis of the HMBC spectrum
The 13C NMR spectrum displayed the signals for two methyls, twelve methylenes (one oxygenated), ten methines (six olefinic), seven quaternary carbons (three olefinic and one oxygenated). By detailed analyses of its 2D spectra, it revealed the key 1H-1H COSY correlations of H2-1/H2-2/H2-3, H2-6/H2-7, H-9/H2-11/H-12/H2-13/H-14/H-20 (Fig. 2), and the key HMBC correlations of H2-1/C-10, H2-6/C-5, H2-7/C-8, C-9, C-14 and C-15, H-15/C-8, C-9 and C-12, H2-17/C-12, C-15 and C-16, H2-18/C-3, C-4, C-5 and C-19, and H-20/C-1, C-5, C-13, and C-19. All the above evidence showed that 3 was also a hetidine-type diterpene alkaloid. The key 1H-1H COSY correlations of H-2’/H-3’, H-5’/H-6’, H2-7’/H2-8’, and H2-9’/H2-10’, together with the key HMBC correlations from H2-7’ to C-3’, C-4’, C-5’, C-8’, and correlations from H3-12’ to C-8’ and C-9’ further confirmed the existence of this moiety. The linkage of the moiety with C-17 via a C-O bond was confirmed by the key HMBC correlation from H2-17 to C-1’. The carboxyl group was deduced to be linked with C-10’, which accounted for the residual one degree of unsaturation and an IR absorption (1637 cm⁻¹). Thus, the structure of 3 was established, and named as tangutidine C.

Additionally, compounds 1–7 were evaluated for their cytotoxicity against five human cancer cell lines (HL-60, A549, SMMC-7721, MCF-7, SW480), with cis-platin and paclitaxel as positive controls. As a result, no compounds showed activity against the tested cell lines (Table S1).

### 3 Experimental Section

#### 3.1 General

Optical rotations were measured with a JASCO P-1020 polarimeter. UV spectra were obtained using a Shimadzu UV-2401 PC spectrophotometer. A Tensor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. HRESIMS data was acquired on an Agilent 6540 QSTAR TOF time-of-flight mass spectrometer. 1D and 2D NMR spectra were recorded on Bruker DRX-600 spectrometers with TMS as internal standard. Chemical shifts (δ) are expressed in parts per million (ppm) with reference to the solvent signals. Semipreparative HPLC was performed on an Agilent 1260 liquid chromatograph with a COSMOSIL 5C18-MS-II (4.6ID × 250 mm) column. Column chromatography (CC) was performed with silica gel (80–100 and 100–200 mesh; Qingdao Marine Chemical, Inc., Qingdao, People’s Republic of China), Sephadex LH-20 (Pharmacia, Uppsala, Sweden) and SEPAFlash column (Spherical C-18, 20–45 μm, 100 Å).

#### 3.2 Plant Material

The dried whole plants of Aconitum tanguticum (Maxim.) Stapf (Ranunculaceae) were provided by Qinghai Jinke Tibetan Medicine Pharmaceutical Co. Ltd. in 2019 and identified by Prof. Yu-Bi Zhou. A voucher specimen (No. 2019-WZ-01) is deposited in Qinghai Provincial Key Laboratory of Tibetan Medicine Pharmacology and Safety Evaluation, Xining, China.

#### 3.3 Cytotoxicity Assay

Five human cancer cell lines, human myeloid leukemia HL-60, lung cancer A-549 cells, hepatocellular carcinoma SMMC-7721, breast cancer MCF-7, and colon cancer...
3.4 Extraction and Isolation

Dried whole plants of *A. tanguticum* (8.8 kg) were powdered and extracted with 70% EtOH (40 L each) for three times, each time for 3 days. The extract was filtered and concentrated under reduced pressure to give the crude extract. The extract was suspended in water, solution was acidified with 5% aq. HCl to pH 2.0 and the acidic solution was successively extracted with petroleum ether (PE), CHCl₃, and n-BuOH. Then the acidic solution was basified with saturated NaOH solution and extracted with CHCl₃. The n-BuOH part was basified with saturated NaOH solution and extracted with CHCl₃, EtOAc and n-BuOH. The EtOAc part was concentrated to yield the total crude alkaloids (28 g). The part (28 g) was applied to ODS chromatography by eluting with MeOH–H₂O (23:67 to 100:0) to give four fractions E1–E4. Fr. E4 (12 g) was subjected to silica gel CC with CHCl₃-Acetone-DEA (15:1:0.1 to 0:1) to afford eight fractions F1–F8. Then Fr. F3 (800 mg) was chromatographed on flash column by eluting with MeOH-H₂O (3:7 to 10:0) to yield five fractions F3A–F3E. Fr. F3B (100 mg) was subjected on Sephadex LH-20 (CHCl₃-MeOH, 1:1) to give subfractions B1–B6. Fr. B2 (50 mg) was purified by semi-preparative HPLC (3 ml/min, MeCN/H₂O/1% triethylamine 63:37) to yield 4 (19.5 mg, tᵣ = 15.6 min). Fr. F (56.7 mg) was purified by semi-preparative HPLC (3 ml/min, MeCN/H₂O/1% triethylamine 55:45) to yield 5 (15.3 mg, tᵣ = 45 min).

3.5 Physical Constants and Spectroscopic Data of Compounds 1–3

3.5.1 Tangutidine A (1)

Colorless oil; [α]₂⁰D = 21.2 (c 0.10, MeOH); UV (MeOH) λ<sub>max</sub> (log ε) 196 (4.90) nm; For ¹H NMR and ¹³C NMR spectroscopic data, see Table 1; HRESIMS m/z 388.2483 [M+H]<sup>+</sup> (calcd for C₂₃H₃₄NO₄, 388.2482).

3.5.2 Tangutidine B (2)

Colorless amorphous powder; [α]₂⁰D = 50.8 (c 0.09, MeOH); UV (MeOH) λ<sub>max</sub> (log ε) 196 (5.02) nm; For ¹H NMR and ¹³C NMR spectroscopic data, see Table 1; HRESIMS m/z 388.2486 [M+H]<sup>+</sup> (calcd for C₂₃H₃₄NO₄, 388.2482).

3.5.3 Tangutidine C (3)

Colorless amorphous powder; [α]₂⁰D = 19.4 (c 0.10, MeOH); UV (MeOH) λ<sub>max</sub> (log ε) 196 (5.36), 248 (3.85), 275 (4.05) nm; For ¹H NMR and ¹³C NMR spectroscopic data, see Table 2; HRESIMS m/z 519.3221 [M+H]<sup>+</sup> (calcd for C₃₂H₄₃N₂O₄, 519.3217).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13659-021-00310-3.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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