# A new hetisine-type diterpenoid alkaloid from *Aconitum coreanum*

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A new hetisine-type alkaloid, named as guan-fu base $A_1$ (**1**), and five known alkaloids including guan-fu base G (**2**), guan-fu base Y (**3**), guan-fu base Z (**4**), guan-fu base Q (**5**) and coldephnine (**6**) were isolated from the roots of *Aconitum coreanum*. The structure of **1** was established by spectral methods, especially 2D NMR techniques.

**Keywords:** *Aconitum coreanum*, diterpenoid alkaloid, hetisine

The genus of *Aconitum* (Ranunculaceae) is a large genus with about 300 species which are mainly distributed in the temperate regions of the Northern Hemisphere. *Aconitum* species produce highly toxic diterpenoid and norditerpenoid alkaloids which have attracted attention for their unusual structural backbone, and bioactivity. Pharmacologically, they may be developed as analgesic, cardiotonic, anti-inflammatory, anti-rheumatic, and anti-arrhythmic agents.[1] The roots a *Aconitum coreanum*, have been used as a traditional Chinese medicine (Guanbaifu in Chinese) for the treatment of various disorders over many centuries.[2] Pharmacological studies and clinical practice have shown that the extract has anti-arrhythmia,[3] analgesic and anti-inflammatory effects.[4] Previous studies on the chemical constituents of this plant resulted in the isolation a number of diterpenoid alkaloids.[5] In our search for structurally unique and biogenetically interesting alkaloids, a new hetisine-type alkaloid and five known alkaloids were isolated from the roots of *A. corearum*. We report the isolation and structural determination of these compounds from *A. corearum*.

Guan-fu base $A_1$ (**1**) was obtained as an amorphous powder. It had a molecular formula of $C_{24}H_{31}NO_6$ which was established on the basis of NMR and HRESIMS data, implying the existence of 10 degrees of unsaturation. The IR absorption at 3423 and 1735 $cm^{-1}$ were attributed to hydroxyl and ester carbonyl, respectively. Twenty-four carbon signals comprising seven quaternary carbons, eight methines, six methylenes, and three methyls were observed in its $^{13}C$ NMR and DEPT spectra. Two carbonyls ($\delta_C$ 170.9, 170.4), one terminal double bond ($\delta_C$ 144.0, C-16; 108.9, C-17), an oxygenated quaternary carbon ($\delta_C$ 80.0, C-14) were also identified. In the $^1H$ NMR, three methyl groups appeared as three singlets at δ 2.07, 2.06, and 0.87. A low-field methylene at δ 2.95 (1H, d, $J$ = 12.1 Hz, H-19α) and 2.54 (1H, d, $J$ = 12.1 Hz, H-19β), indicated that it was attached to a quaternary carbon. The methine protons which appeared at low-field at δ 5.11 (1H, d, $J$ = 9.1 Hz, H-11) and 5.09 (1H, m, H-2) were assigned to those CH linked to acyloxy groups. The olefinic protons were observed as a typical AB system at δ 4.90 (1H, s, H-17) and 4.75 (1H, s, H-17) and were a typical feature of a terminal double bond. Three degrees of unsaturation were accounted for by the double bond and the two carbonyls. The remaining seven degrees of unsaturation were only attributed to the occurrence of a heptacyclic ring system in **1**. The NMR data mentioned above and the diagnostic carbon signals of C-10 and C-16 indicated **1** was a hetisine-type alkaloid.[6]

Analysis of the $^1H$ NMR, $^{13}C$ NMR and HMQC spectra of **1** enabled us to assign all the protons to their bonding carbons. The three partial structures (C-1 to C-3; C-5 to C-7; C-9 and C-11 to C-13) drawn with bold bond were established by $^1H$–$^1H$ COSY (Fig. 2). The three structural fragments were linked by the HMBC experiment. The linkage of C-6, C-19, and C-20 to each other via the only nitrogen atom was established by the correlations of H-19/C-20 and H-20/C-6. The HMBC correlations of H-18/C-4, H-18/C-3, and H-18/C-5 indicated the connectivity of C-3 and C-5 via the quaternary carbon C-4. The linkage of C-12 and C-15 through C-16 was tentatively established by the HMBC correlations of H-17/C-12 and H-17/C-15. The C-20, C-13 and C-8 were attached to C-14 as judged by the strong HMBC correlation pairs of H-20/C-14, H-13/C-14 and H-13/C-8, respectively. Two quaternary carbons C-8 and C-10 were also connected via C-9 deduced from the HMBC correlations of H-9/C-8, H-9/C-10. The location of the two acetoxy groups at C-2 and C-11 was determined from the crosspeaks of $\delta_H$ 5.09/δc 170.9, and $\delta_H$ 5.11/δc 170.4, respectively. The planar structure of **1** was thus established.

The $^1H$ and $^{13}C$ NMR data of **1** were similar to those of guan-fu base G (**2**),[7] except for the presence of one less acetyl in **1** than **2**. These data indicated that compound **1** was an analogue of **2**, and had the same relative stereochemistry as guan-fu base G, which was confirmed by the NOESY spectrum (Table 1). In the NOESY spectrum, the proton signal of H-18 showed correlations with the signals of H-3β and H-6, indicating that H-3β, H-6, and H-18 were on the same side and had β-orientation. As a consequence, H-19 was placed on the α-face. The correlation pairs of H-3β/H-2, H-3β/H-9,

**Table 1** $^1H$ and $^{13}C$ NMR Data, and NOESY correlations of 1[a]

| No. | **1** | | |
|---|---|---|---|
| | $\delta_H$, $J$ (Hz) | $\delta_C$ | NOESY (H→H) |
| 1α | 2.88 (1H, d, 15.1) | 29.4 | 1β |
| 1β | 1.46 (1H, d, 15.1, 4.5) | | 1α, 2 |
| 2 | 5.09 (1H, m) | 69.6 | 1β, 3β |
| 3α | 1.82 (1H, m) | 36.5 | 3β |
| 3β | 1.57 (1H, m) | | 2, 3α, 6, 9, 18 |
| 4 | – | 37.6 | – |
| 5 | 1.54 (1H, s) | 59.5 | 6 |
| 6 | 3.11 (1H, brs) | 63.2 | 3β, 5, 7β, 18, 19β |
| 7α | 1.40 (1H, d, 13.6) | 31.8 | 7β |
| 7β | 1.84 (1H, m) | | 6, 7α |
| 8 | – | 44.6 | – |
| 9 | 2.18 (1H, d, 8.8) | 51.6 | 3β, 11 |
| 10 | – | 45.9 | – |
| 11 | 5.11 (1H, d, 9.1) | 76.1 | 9, 12 |
| 12 | 2.45 (1H, s) | 49.9 | 11, 13, 17b |
| 13 | 4.07 (1H, s) | 78.6 | 12 |
| 14 | – | 80.0 | – |
| 15 | 2.11 (2H, m) | 30.8 | 11 |
| 16 | – | 144.0 | – |
| 17a | 4.75 (1H, s) | 108.9 | 17b |
| 17b | 4.90 (1H, s) | | 12, 17a |
| 18 | 0.87 (3H, m) | 29.6 | 3β, 6, 19β |
| 19α | 2.95 (1H, d, 12.1) | 63.1 | 19β, 20 |
| 19β | 2.54 (1H, d, 12.1) | | 6, 18, 19α |
| 20 | 3.58 (1H, s) | 68.8 | 19α |
| 2-OAc | – | 170.9 | – |
| 2-OAc | 2.07 (3H, s) | 21.7 | – |
| 11-OAc | – | 170.4 | – |
| 11-OAc | 2.06 (3H, s) | 21.6 | – |

[a]measured in $CDCl_3$ at 400 MHz.

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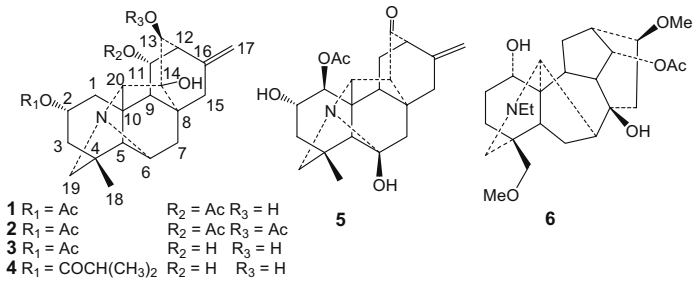


**Fig. 1** Structures of alkaloids from *A. coreanum.*

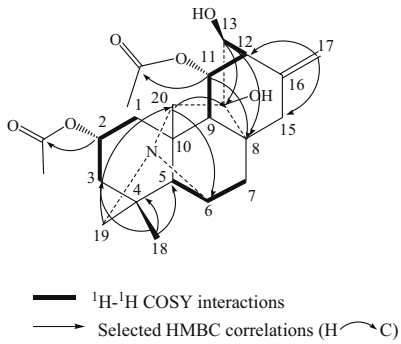


**Fig. 2** Selected 2D NMR correlations of **1**.

H-9/H-11 and H-6/H-5 indicated that the H-2, H-5, H-9, and H-11 also had a β-configuration. The $^{1}H$, $^{13}C$ NMR spectral data and 2D NMR experiments support the assignment of structure **1** to 2,11-diacetoxy-14-hydroxyhetisine. Guan-fu base A, firstly reported by J. H. Liu *et al.* from the same plant as 2,11-diacetoxy-14-hydroxyhetisine,[8] was revised as 2,13-diacetoxy-14-hydroxyhetisine.[9] Hence compound **1** was a new hetisine-type of diterpenoid alkaloid, and named as Guan-fu base $A_1$.

The known alkaloids were identified as guan-fu base G (**2**),[7] guan-fu base Y (**3**),[9] guan-fu base Z (**4**),[10] guan-fu base Q (**5**),[11] and coldephnine (**6**)[12] by comparison with of their NMR data with those reported in the literature, respectively.

## Experimental

Optical rotations were determined on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Thermo Nicolet 6700 spectrometer with KBr disks. NMR spectra were measured on a Bruker Avance-400 spectrometer with TMS as internal standard. ESIMS was recorded on a Finnigan LCQ$^{DECA}$ Mass spectrometer. All solvents used were of analytical grade (Hangzhou Gaojing Fine Chemical Plant, Hangzhou, P. R. China). Silica gel (200–300 mesh) and amino silica gel (NH-DM 1020, 20–45 μm, Fuji Silysia Chemical Ltd.) were used for column chromatography, and a precoated silica gel $GF_{254}$ plate (Qingdao Haiyang Chemical Plant, Qingdao, P. R. China) was used for TLC.

*Plant material*

The roots of *A. coreanum* were collected from Yunnan Province of P. R. China and identified by Prof. Hai-Bo Bai of the College of City, Zhejiang University. A voucher specimen (ZJUT 08316) was deposited at Zhejiang University of Technology, People's Republic of China.

*Extraction and Isolation.* The dry roots (5 kg) of *A. coreanum* were extracted three times with 95% EtOH at room temperature. The extract was evaporated to dryness under reduced pressure to afford a residue (350 g), which was dissolved in water (3 l) to form a suspension, and then adjusted with 0.5 N $H_2SO_4$ to pH≈5. The acidic mixture was extracted with petroleum ether (6 × 500 ml) to remove the non-alkaloidal components. The aqueous phase was brought to pH≈10 by addition of 1 N $Na_2CO_3$ and partitioned with chloroform (6 × 500 ml) to give the crude alkaloids (47 g). The crude alkaloids were then subjected to silica gel column chromatography. Elution with petroleum ether–EtOAc (10 : 1–0 : 1) gave four major fractions 1–4. Fraction 1 (17 g) was separated by column chromatography packed with silica gel and eluted with petroleum ether–EtOAc (4 : 1) to yield alkaloids **1** (105 mg), **2** (2.4 g) and **4** (520 mg). Fraction 2 (8.3 mg) was purified by silica gel column chromatography eluted with petroleum ether–EtOAc (3 : 1) to afford **3** (126 mg). Compound **6** (1.4 g) was purified from fraction 3 (5.5 g) by column chromatography packed with amino-silica gel eluted with $CHCl_3$. Recrystallisation of fraction 4 (8.7 g) from ethanol yielded **5** (1.1 g).

Guan-fu base $A_1$ (**1**), amorphous powder, $[\alpha]^{20}{}_{D}$ –66.1° (*c* 0.16, $CHCl_3$); IR (KBr): 3423, 2929, 1735, 1367, 1221, 1149, 1128, 1066, 942, 890 $cm^{-1}$; ESIMS *m/z*: 430 $[M + H]^+$; HR-ESIMS *m/z*: 430.2225 $[M + H]^+$ (Calcd. for $C_{24}H_{32}NO_6$ 430.2230). $^{1}H$ NMR and $^{13}C$ NMR data: see Table 1.

The financial support of the Natural Science Foundation of P. R. China (20702049) is gratefully acknowledged.

*Received 21 October 2008; accepted 14 November 2008*
*Paper 08/0253 doi: 10.3184/030823409X393673*
*Published online: 16 January 2009*

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