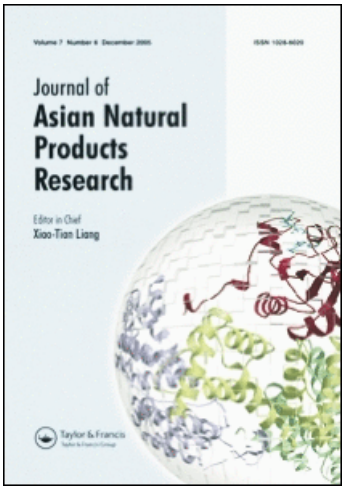


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Two New Diterpenoid Alkaloids, Beiwusines A and B, from *Aconitum kusnezoffii*

Zheng-Bang Li[a]; Feng-Peng Wang[a]
[a] Department of Chemistry of Medicinal Natural Products, School of Pharmacy, West China University of Medical Sciences, Chengdu, China

To cite this Article Li, Zheng-Bang and Wang, Feng-Peng(1998) 'Two New Diterpenoid Alkaloids, Beiwusines A and B, from *Aconitum kusnezoffii*', Journal of Asian Natural Products Research, 1: 2, 87 — 92
To link to this Article: DOI: 10.1080/10286029808039848
URL: http://dx.doi.org/10.1080/10286029808039848

JANPR, Vol. 1, pp. 87–92
Reprints available directly from the publisher
Photocopying permitted by license only

TWO NEW DITERPENOID ALKALOIDS, BEIWUSINES A AND B, FROM *ACONITUM KUSNEZOFFII*

ZHENG-BANG LI and FENG-PENG WANG*

Department of Chemistry of Medicinal Natural Products, School of Pharmacy, West China University of Medical Sciences, Chengdu 610041, China

(Received 27 March 1998; Revised 29 March 1998; In final form 2 April 1998)

Two new atisine-type diterpenoid alkaloids, beiwusine A (**1**) and B (**2**), have been isolated from the roots of *Aconitum kusnezoffii* Reichb. Their structures were established on the basis of spectroscopic data. Beiwusines A and B are the first examples of atisine-type diterpenoid alkaloids having a hydroxyl group at C-1. In addition, one known diterpenoid alkaloid spiramine H (**3**) has been isolated.

Keywords: *Aconitum kusnezoffii*; Diterpenoid alkaloids; Beiwusine A; Beiwusine B

INTRODUCTION

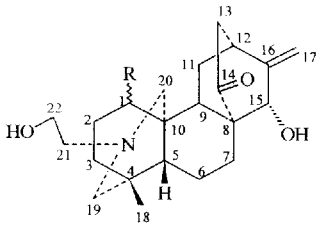
The plant *Aconitum kusnezoffii* Reichb (Ranunculaceae) is native to northern China. The roots are used in native medicine for the treatment of rheumatism and neuralgia [1]. In the previous papers, Wang *et al.* [2] and Uhrin *et al.* [3] reported the isolation of seven alkaloids – aconitine, 3-deoxyaconitine, beiwutine, hypaconitine, mesaconitine, denudatine and lepenine from this plant. Our previous investigation on this plant has led to the isolation of four new norditerpenoid alkaloids: 6-epichasmamine [4], hemsleyanidine and isohemsleyanidine [5] as well as beiwudine [6], together with fifteen known norditerpenoid alkaloids – aconifine, aconitine, anthranoyllycoctonine, beiwutine, 14-benzoylaconine, 14-benzoylmesaconine, chasmanine, 3-deoxyaconitine, foresticine, 15α-hydroxyneoline, hypaconitine, mesaconitine,

*Corresponding author. Tel.: (028)-5501368. Fax: (028)-5582157.
E-mail: wfp@wcums.edu.cn.

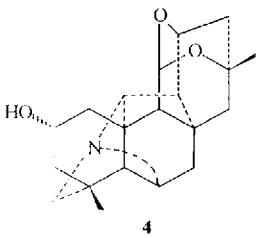
neoline, talatisamine, lycoctonine – and one known diterpenoid alkaloid with no name **4** [7]. Further studies have now led to the isolation of two new atisine-type diterpenoid alkaloids, beiwusine A (**1**) and B (**2**), together with a known diterpenoid alkaloid spiramine H (**3**) [8]. The present paper deals with the isolation and structural determination of the new alkaloids (**1** and **2**).

RESULTS AND DISCUSSION

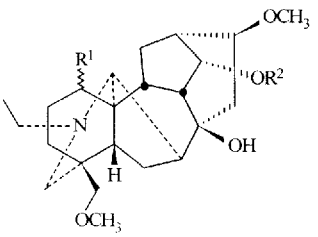
Beiwusine A (**1**) was isolated as a homogeneous amorphous powder, $[\alpha]_D^{17}$ −34.1 (EtOH, *c* 0.41). The HRMS showed $[M]^+$ at *m/z* 375.2415 corresponding to the molecular formula $C_{22}H_{33}NO_4$, which requires *m/z* 375.2381. Spectroscopic analysis showed the presence of an *N*-ethoxyl [δ_H 3.59 (2H, dt, $J = 5.4$, 2.2 Hz); δ_C 50.1t]. an exo-methylene group [δ_H 5.13 (2H, br.s); δ_C 151.8s, 111.4t], a ketone group (δ_C 214.7s; ν 1709 cm^{-1}), and two hydroxyl-bearing methine groups [δ_H 3.49 (1H, dd, $J = 9.6$, 6.4 Hz): δ_C 80.6d; δ_H 4.00 (1H, br.s); δ_C 79.2d]. Thus, the expanded formula is $C_{20}H_{26}$–[1 × *N*-CH_2CH_2OH–2 × OH–O(ketone)]. This suggested that beiwusine A is a diterpenoid alkaloid by considering biogenesis. Besides the unsaturation number of 2 for the exo-methylene and one ketone group, the remainder indicated that its skeleton belongs to a pentacyclic system, leading to an atisine-type or veatchine-type diterpenoid alkaloid. The δ value of C-16 (δ 151.7s) indicated that beiwusine A was of atisine-type, falling in the expected range of δ 150–157 ppm but not δ 158-161 ppm for the veatchines-bearing a 15α-hydroxyl group [9].



1 R = α-OH
2 R = β-OH
3 R = H



4



5 R^1 = α-OH
R^2 = CO–CH=CH–(3,4-dimethoxyphenyl)
6 R^1 = β-OH R^2 = H

The ketone group (δ 214.7s) and one of the secondary hydroxyl group were easily allotted to C-14 and C-15 by comparing with the spectroscopic data of a similar compound spiramine H (**3**) [8].

The MS, ^{1}H- and ^{13}C-NMR of beiwusine A were quite similar to that of spiramine H (**3**) [8], and the difference of 16 mass units showed that beiwusine A (**1**) possesses one more secondary hydroxyl group. This extra hydroxyl group in beiwusine A was assigned at C-1 (δ_C 80.6d) on the basis of the following reasons. First, comparison of the ^{13}C-NMR spectrum of beiwusine A with that of spiramine H (**3**) (Table I) showed that the shift differences were exhibited by the carbons of rings A–B, such as C-1, C-2, C-3, C-5, C-8, C-9, C-10 and C-20, which ruled out other possibilities, such as C-6, C-7, C-11 and C-13, for the location of the extra hydroxyl group. Second, we may note the δ values of C-10 (δ_C 42.3s) with downfield shift and virtually invariant C-18 [δ_H 0.76 (3H, s, 4-CH_3); δ_C 26.2q] by comparison with spiramine H (**3**) (C-10: δ_C 38.0; C-18: δ_C 26.3) [8]. Attention was then focused on the determination of the configuration of the 1-hydroxyl group. The α-configuration of the hydroxyl group at C-1 was suggested by the chemical shift and coupling constant [δ_H 3.49 (1H, dd, $J_1 = 9.6$ Hz, $J_2 = 6.4$ Hz)] of the proton geminal to the hydroxyl group. In addition, this deduction was supported by observations of the small up-field shifts caused by the γ-gauche effects between 1α-OH and C-3 ($\Delta\delta$ −2.1); 1α-OH and C-5 ($\Delta\delta$ −0.2) as well as 1α-OH and C-9 ($\Delta\delta$ −2.4) by comparison with spiramine H (**3**). The structure of beiwusine A (**1**) was thus established.

Beiwusine B (**2**) (amorphous), $[\alpha]_D^{17}$ −42 (EtOH, *c* 0.41) (HRMS *m/z* 375.2440, $C_{22}H_{33}NO_4$ requires 375.2381). The ^{1}H- and ^{13}C-NMR spectra indicated the presence of a methyl group [δ_H 0.78 (3H, s); δ_C 26.0q], an *N*-ethoxyl group [δ_H 3.55 (2H, m); δ_C 57.9t, 58.8t], an exo-methylene group [δ_H 5.14 (2H, d, $J = 1.4$ Hz); δ_C 151.5s, 111.6t], and a carbonyl group (δ_C 215.0s; ν 1705 cm^{-1}). The molecular composition of beiwusine B is the same as that of beiwusine A and exhibited certain spectral similarities. The ^{1}H-NMR spectrum of beiwusine B (**2**) is very similar to that of beiwusine A (**1**) except for some protons such as the one at δ 3.74 ppm (1H, d, $J = 2.6$ Hz). Both beiwusines A and B were especially different in TLC behaviors and comparison of ^{13}C-NMR data shown in Table I are reminiscent of some existent pairs of 1-epimers, e.g., gymnaconitine (**5**) [10] and talatizidine (**6**) [11] shown in Table II. In addition, the doublet ($J = 2.6$ Hz) at δ 3.55 ppm in ^{1}H-NMR spectrum of beiwusine B was assigned to the 1α-H based on the multiplicity, resulting in β-configuration for the 1-hydroxyl group. Structure of beiwusine B therefore was assigned as **2**. It is of interest to note that the plant *Aconitum kusnezoffii* Reichb is probably a typical species of Ser. *Inflata*, which contained aconitine-, lycoctonine-, atisine-, denudatine- and

TABLE I ^{13}C-NMR data of compounds **1**, **2** and **3** [8]

Carbon	**1**	**2**	**3**	*Carbon*	**1**	**2**	**3**
1	80.6d	70.1d	38.8	12	36.8d	36.7d	36.8
2	33.2t	31.8t	22.8	13	44.5t	44.5t	44.5
3	38.9t	35.9t	41.0	14	214.7s	215.0s	214.0
4	33.0s	33.6s	33.5	15	79.2d	79.3d	79.3
5	45.2d	37.0d	45.4	16	151.8s	151.5s	151.7
6	17.5t	17.1t	17.5	17	111.4t	111.6t	111.6
7	27.6t	26.9t	27.1	18	26.2q	26.0q	26.3
8	53.4s	52.0s	53.1	19	60.0t	58.8t	59.3
9	46.8d	40.8d	49.2	20	47.3t	50.3t	52.2
10	42.2s	41.8s	38.0	21	59.6t	60.0t	60.1
11	30.1t	26.2t	27.4	22	58.1t	57.9t	57.7

TABLE II The δ value changes ($\delta > 1$ ppm) caused by configurational changes in epimeric pair **5** [10] — **6** [11]

Carbon	**5**	**6**	$\Delta\delta$ **5** → **6**	*Carbon*	**5**	**6**	$\Delta\delta$ **5** → **6**
1	71.9	68.8	−3.1	7	44.6	41.6	−3.0
2	29.2	27.4	−1.8	9	45.6	46.9	+1.3
3	29.3	30.7	+1.4	10	43.3	44.8	+1.5
4	37.1	38.6	+1.5	12	26.5	29.1	+2.6
5	41.2	39.5	−1.7				

hetisine-type diterpenoid alkaloids. This is helpful for the chemotaxonomy of the genus *Aconitum* plants.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined on the Kofler block (uncorrected). Optical rotations were measured on a Perkin Elmer 241 spectrometer. IR spectra were recorded with a Perkin Elmer 983 spectrometer. LRMS data were recorded a Finnigan TSQ 7000 mass spectrometer, HRMS spectra were measured on a Kratos MS80 mass spectrometer, ^{1}H- and ^{13}C-NMR spectra were determined in $CDCl_3$, with TMS as internal standard, on either a Bruker AC-200 or a Varian Unity INOVA-400 spectrometer. A polyvinyl sulfornic ion resin (H form, cross linking 1×3, Chemical Factory of Nankai University, China) was used in the extraction of total alkaloids. Column chromatography was carried out on silica gel H (10–40 μ), and TLC on silica gel G plates, with solvent system ether–CH_3COCH_3 (85 : 15),

detected with modified Dragendorff reagent. Silica gels H and G were purchased from Marine Chemical Factory, China.

Plant Material

The roots of *A. kusnezoffii* Reichb were collected in September 1991 in Chiferg of Inner Mongolia, China. The plant was identified by Prof. W.T. Wang (Institute of Botany, Chinese Academy of Sciences, Beijing), and voucher specimens have been deposited in the herbarium of the School of Pharmacy, West China University of Medical Sciences.

Extraction and Isolation

Powdered roots (8.5 kg) were percolated with 0.2% HCl (80 l). The percolates were exchanged with a polyvinyl sulfonic ion resin (2.5 kg), which was later washed with deionized water, spread and dried in the air. The resin was then basified by 10% ammonia water (7.35 l) and extracted with ether under reflux. The combined ether solutions were concentrated to a smaller volume (1000 ml). It was allowed to stand at room temperature overnight to give a white powder (total alkaloids I, 16.0 g). The mother liquor was evaporated under reduced pressure to give a light yellow foam (total alkaloids II, 16.5 g).

Using a pH gradient method, total alkaloids II were separated into three parts, part A (pH 7, 9.7 g), part B (pH 9, 4.8 g) and part C (pH 11, 2.0 g). Column chromatography of part B on silica gel H (6 × 60 cm) eluting with $CHCl_3$/MeOH 96 : 4 (2 ml/min) gave fractions 1 (1000–1160 ml, 1.2 g) and 2 (1970–2420 ml, 400 mg). Fraction 1 was chromatographed on silica gel H column (3 × 40 cm) eluting with $CHCl_3$/MeOH 97 : 3. Twenty ml of each fraction were collected and fractions 8 and 9 were evaporated to give a residue, which was crystallized with acetone/cyclohexane solvent to give beiwusine A (colorless needles, 300 mg). Fraction 2 was chromatographed on silica gel H column (3 × 40 cm) eluting with $CHCl_3$/MeOH/Et_2NH 97 : 3 : 0.5. Two fractions between 113–115 and 139–153 ml gave beiwusine B (white foam, 83 mg) and spiramine H (white foam, 29 mg), respectively, after evaporating under reduced pressure. The Rf values of beiwusines A and B on TLC [silica gel G, Et_2O/$(CH_3)_2CO$ 85 : 15] and spiramine H were 0.69, 0.50 and 0.47, respectively.

Beiwusine A (**1**) was obtained as white amorphous powder, 83 mg, $[\alpha]_D^{17}$ −34.1 (*c* 0.41, EtOH); IR (KBr) ν max 3390 (OH), 2849 (CH), 1709 (C=O) cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 0.76 (3H, s, 4-CH_3), J = 2.6 Hz,

12-H), 3.49 (1H, dd, $J=6.4$, 9.6 Hz, 1β-H), 3.59 (2H, dt, $J=11.2$, 1.0 Hz, 22-H_2), 4.00 (1H, br.s, 15β-H), 5.13 (2H, s, 17-H_2); ^{13}C-NMR data, see Table I; EIMS *m/z*: 375 (M^+, 2), 344 (M-31, 100); HRMS *m/z*: 375.2415 ($C_{22}H_{33}NO_4$, calcd 375.2381).

Beiwusine B (**2**) was obtained as white amorphous powder, 29 mg, $[\alpha]_D^{17}$ −42 (*c* 0.41, EtOH); IR (KBr) ν max 3406 (OH), 2944 (CH), 1705 (C=O) cm^{-1}, ^{1}H-NMR (200 MHz, $CDCl_3$): δ 0.78 (3H, s, 4-CH_3), 3.55 (2H, m, 22-H_2), 3.74 (1H, d, $J=2.6$ Hz, 1α-H), 3.99 (1H, s, 15β-H), 5.14 (2H, d, $J=1.4$ Hz, 17-H_2); ^{13}C-NMR data, see Table I; EIMS *m/z*: 375 (M^+, 2), 344 (M-31, 100); HRMS *m/z*: 375.2440 ($C_{22}H_{33}NO_4$, calcd 375.2381).

Spiramine H (**3**) was obtained as colorless needle from acetone/cyclohexane (1 : 1), 30 mg, mp 150–151°C. $[\alpha]_D^{17}$ −39.7 (*c* 0.295, EtOH); IR (KBr) ν max 3332 (OH), 2943 (CH), 1710 (C=O) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.76 (3H, s, 4-CH_3), 3.54 (2H, t, $J=5.4$ Hz, 22-H_2); ^{13}C-NMR data, see Table I; EIMS *m/z*: 328 (M-31, 100); HRMS *m/z*: 359.2447 ($C_{22}H_{33}NO_3$, calcd 359.2431).

Acknowledgements

We thank Professor Xiao-tian Liang, Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, for helpful discussion on this subject. We also acknowledge the skillful assistance of Mr. Thomas Wong, Department of Chemistry, The Hong Kong University of Sciences and Technology, for recording LRMS and HRMS spectra.

References

[1] The Pharmacopeia of People's Republic of China, 1995, pp. 210–212.
[2] Wang, Y.G., Zhu, Y.L. and Zhu, R.H. *Acta Pharm. Sinica*, 1980, **15**, 526–531.
[3] Uhrin, D. Proksa, B. and Zhamiansan, J. *J. Planta Med.* 1991, **57**, 390–391.
[4] Li, Z.B. and Wang, F.P. *Chinese Chem. Lett.* 1996, **7**, 443–444.
[5] Xu, Q.Y., Li, Z.B., Wang, F.P. and Che, C.T. *Heterocycles* 1996, **43**, 1243–1250.
[6] Li, Z.B. and Wang, F.P. *J. Nat. Prod.* 1998 (in press).
[7] Li, Z.B. and Wang, F.P. *Natural Product R & D*, 1997, **9**, 9–14.
[8] Hao, X.J. Node, M. Zhou, J. Chen, S.Y. and Fuji, K. *Acta Botanica Yunnanica*, 1994, **16**, 301–304.
[9] Wang, F.P. *Youji Huaxue* 1982, **3**, 161–169.
[10] Jiang, S.H. Gao, S.H. Zhou, B.N. Wang, S.X. Yi, Y.S. and Ji, L.J. *Acta Pharm. Sinica*, 1986, **21**, 279–284.
[11] Boido, V. Edwards, O.E. Handa, K.L. Kolt, R.J. and Purushothaman, K.K. *Can. J. Chem.* 1984, **62**, 778–784.