Beiwudine, a Norditerpenoid Alkaloid from *Aconitum kusnezoffii*

Feng-Peng Wang,*,† Zheng-Bang Li,† and Chun-Tao Che*,‡

Department of Chemistry of Medicinal Natural Products, School of Pharmacy, West China University of Medical Sciences, Chengdu 610041, China, and Department of Chemistry, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

Received May 22, 1996

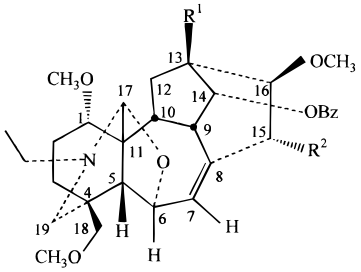
A novel norditerpenoid alkaloid, beiwudine (**1**), was isolated from the roots of *Aconitum kusnezoffii*. Its structure was established on the basis of chemical and NMR spectral studies.

The root part of *Aconitum kusnezoffli* Reichb. (Ranunculaceae), native to northern China, is used in folk medicine for the treatment of rheumatism and neuralgia.[1] In the previous papers,[2−4] a number of norditerpenoid alkaloids and diterpenoid alkaloids were reported from this plant. Continued investigation of this plant has now led to the isolation of a novel norditerpenoid alkaloid, beiwudine (**1**). This paper deals with the isolation and structure elucidation of the compound.

Compound **1** was isolated as an amorphous powder. The HREIMS showed a molecular ion $[M]^+$ at *m/z* 555.2953 consistent with the molecular formula $C_{31}H_{41}NO_8$. The ^1H and ^{13}C NMR spectra indicated the presence of an *N*-ethyl [δ_H 1.00 (3H, t, $J = 7.2$ Hz); δ_C 13.0 q, 49.1 t], three methoxy groups (δ_H, 3.26, 3.30, 3.67; δ_C 57.1, 59.4, 61.3), and a benzoyl ester group [δ_H 7.45 (2H, t, $J = 7.2$ Hz), 7.57 (1H, t, $J = 7.2$ Hz), 8.07 (2H, d, $J = 7$ Hz); δ_C see Table 1]. The spectral data of **1** were quite similar to those of franchetine (**3**),[5] and the compound was considered to possess a norditerpenoid alkaloid structure. Thus, examination of the $^1H-^1H$ and $^1H-^{13}C$ COSY spectra led to the unambiguous assignments of C-1 through C- 3 signals; and the chemical shift of C-1 (δ 86.3) clearly indicated the presence of a methoxyl group. Another methoxyl group (δ 59.4) was assigned to C-18 because the latter was readily identified as an oxymethylene at δ 78.9, consistent with other compounds bearing 18-OCH_3 group.[6,7] The NMR data further suggested the presence of an *N*,*O*-mixed acetal group [δ_H 4.37 (1H, s), 4.55 (1H, d, $J = 6.4$ Hz); δ_C 92.6 d, 74.3 d]. In an NOE experiment, irradiation of the signal at δ 4.55 (H-6) enhanced both the signals for H-5 (δ 2.29, s, 3%) and 18-CH_2 (δ 3.02, 3.12, ABq, 9%), and vice versa. Hence, **1** was considered to be a franchetine derivative bearing an oxygen bridge between C-6 and C-17.

A difference of 32 mass units between **1** and franchetine (**3**) suggests that the former is a dihydroxyl derivative. A hydroxyl group was assigned to C-15 on the basis of the coupling relationship observed between H-15 (δ 5.04, d) and H-16 (δ 2.85, d). This assignment was also supported by the result of an NOE experiment on acetylbeiwudine (**2**) in which NOE was observed between H-7 (δ 6.16, d) and 15-OAc (δ_H 2.15, s). The other OH functional group, locating on a tertiary carbon (δ 76.5), was assigned to C-13 based on the downshift of the C-16 as in other 13,15-dihydroxy derivatives.[8]

Attention was then focused on the determination of the configuration of the 15-OH group. In order to observe the NOE results, **1** was acetylated to **2**. Irradiation of H-7 (δ 5.81) led to the enhancement of the signals of 15-OAc (3.5%) and H-6 (13%); and irradiation of H-15 (δ 6.16) resulted in an enhancement of H-2″/H-6″ of the benzene ring (13%). These results indicated that the 15-OAc was α-oriented.



1 $R^1 = R^2 = OH$

2 $R^1 = OH$ $R^2 = OAc$

3 $R^1 = R^2 = H$

Beiwudine is the third example of a franchetine-type norditerpenoid alkaloid bearing an *N*,*O*-acetal [N−C(17)−*O*−C(6)] with a C-7/C-8 double bond. Assignments of its ^1H and ^{13}C NMR data were made by analysis of COSY, HETCOR, COLOC, NOESY, and NOEDS results.

Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR spectra were determined in $CDCl_3$, with TMS as internal standard, on a Bruker ARX-300 spectrometer. LRMS data were recorded on either a Finnigan TSQ 7000 mass spectrometer or a M-80A GL−MS spectrometer. HRMS spectra were measured on a Kratos MS-80 spectrometer. A polyvinyl sulfonic ion resin (H form, cross linking 1 × 3, Chemical Factory of Nan Kai University, China) was used in the extraction of total alkaloids. Column chromatography was carried out on Si gel H, and TLC on Si gel G plates, with solvent systems S_1($CHCl_3$−MeOH, 9:1) and S_2 (ether−Me_2CO, 9:1), detected with modified Dragendorffs reagent or I_2 vapor. Si gels H and G were purchased from the Qingdao Marine Chemical Factory, China.

Plant Material. *A. kusnezoffii* roots were collected in September 1991, in Chifeng of Inner Mongolia. 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.

* To whom correspondence should be addressed. (F.P.W) Tel.: 86-28-550-1368. Fax: 86-28-558-7960. E-mail: wfp@wcums.edu.cn. (C.T.C.) Tel.: (852) 2358-7351. Fax: (852) 2358-1594. E-mail: chctche@ust.hk.
† West China University of Medical Sciences.
‡ Hong Kong University of Science and Technology.

10.1021/np960481t CCC: $15.00
Published on Web 11/10/1998

Table 1. NMR Data for Beiwudine (**1**) and Franchetine (**3**)[5]

	3	**1**			
position	^{13}C	^{13}C	^{1}H	COLOC	NOESY
1	86.5	86.3 d	ca. 3.30 m		H-5
2	24.3	24.3 t	ca. 1.80 m	C-11	
			ca. 2.40 m		
3	32.7	32.7 t	ca. 1.50 m		
			ca. 1.70 m		H-5
4	37.3	37.3 s			
5	47.9	47.5 d	2.29 s	C-10, C-l , C-17	H-1β, H-3β, 18-CH_2
6	74.8	74.3 d	4.55 d (6.4)		H-7, 18-CH_2
7	128.7	123.9 d	6.08 d (6.4)		H-6
8	136.8	138.6 s			
9	42.9	42.7 d	ca. 3.10 m		
10	49.4	47.5 d	2.65 m	C-11	H-14
11	50.1	50.5 s			
12	29.7	38.9 t	ca. 2.00 m	C-9, C-16	H-17
13	38.3	76.5 s			
14	78.7	74.6 d	5.04 br s ($W_{1/2}$ = 4.6 Hz)	C-13	H-10
15	38.5	83.3 d	5.04 d(6)		
16	85.4	94.2 d	2.85 d (overlapped)		
17	92.2	92.6 d	4.37 s	C-1, C-6	12-CH_2
18	78.8	78.9 t	3.02, 3.25 (ABq, 9.2)		H-5, H-6
19	52.1	52.0 t	2.03, 2.35 (ABq, hidden)		
NCH_2CH$_3$	49.0	49.1 t	2.35 m, 2.55 m		
NCH$_2CH_3$	13.0	13.0 q	1.00 t (7.1)		
1-OCH$_3$	57.6	57.1 q	3.30 s		
16-OCH$_3$	55.9	61.3 q	3.67 s	C-16	
18-OCH$_3$	59.3	59.4 q	3.26 s		
benzoyl ester:					
C=O	166.4	166.6 s			
1″	130.5	129.6 s			
2″, 6″	128.8	129.5 d	8.07 d (7)	C-4″	H-15
3″, 5″	128.2	128.5 d	7.45 d (7.2)		
4″	132.6	133.2 d	7.57 d (7.2)		

Extraction and Isolation. Powdered roots of *A. kusnezoffii* (8 kg) were percolated with 0.15% HCl (33 L). Wet resin (dry wt 2.5 kg) was added to the percolates. After exchange, the resin was washed repeatedly on a suction filter with deionized H_2O and air dried. The dried resin was then mixed with 10% aqueous NH_4OH (total amount 1 L) and extracted in a specially designed extractor[9] with Et_2O under reflux until no alkaloid could be detected with Dragendorff's reagent. The total alkaloid fraction so obtained (32.8 g) appeared as a white powder. The total alkaloid (8 g) was subsequently chromatographed on Si gel H (280 g) eluted with $CHCl_3$−MeOH (96:4) to afford fractions A (1.6 g), B (2.5 g), and C (3.5 g). Fraction A was chromatographed on Si gel H eluted with $CHCl_3$−MeOH (98.5:1.5 to 96:4) (10-mL fractions) to afford fractions A-1 (400 mg), A-2 (250 mg), and A-3 (1.1 g). Fraction A-1 was chromatographed on Si gel H eluting with cyclohexane−Me_2CO−diethylamine (80:20:1) to give a white amorphous powder (**1**, 120 mg) showing one spot on TLC (S_1, S_2). Fraction A-3 was chromatographed on Si gel H eluting with cyclohexane−Me_2CO−diethylamine (70:30:1) to give hypaconitine (117 mg) and aconifine (90 mg).[4] Fraction C was chromatographed repeatly on Si gel H (100 g) eluting with $CHCl_3$−MeOH (99:1 to 9:1) (15-mL fractions) to afford beiwutine (460 mg) and aconifine (300 mg).[4]

Beiwudine (1): white amorphous powder; IR (KBr) ν_{max} 3459 (OH), 1718, 1279 cm^{-1}; ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR, see in Table 1; EIMS *m/z* 555 ([M]$^+$, 8), 540 ([M − Me]$^+$, 11), 524 ([M − OMe]$^+$, 44), 105 ([C_6H_5 − CO]$^+$, 100); HREIMS *m/z* 555.2953 (calcd for $C_{31}H_{41}O_8$, 555.2911).

Acetylation of 1. To **1** (80 mg) was added pyridine (0.5 mL) and acetyl anhydride (0.5 mL). The solution was allowed to stand at room temperature overnight. After removal of the solvent under reduced pressure, the residue was chromatographed on a chromatotron eluting with $CHCl_3$−MeOH (99:1) to give a white amorphous powder of acetylbeiwudine (**2**) (42 mg) showing one spot on TLC (S_1, S_2).

Acetylbeiwudine (2): ^{1}H NMR (300 MHz) δ 1.01 (3H, t, *J* = 7.2 Hz, NCH$_2CH_3$), 2.15 (3H, s, OAc), 3.06, 3.15 (each 1H, ABq, *J* = 9.0 Hz, H_2-18), 3.24, 3.36, 3.51 (each 3H, s, 3 × OCH$_3$), 4.37 (1H, s, H-17), 4.51 (1H, d, *J* = 6.3 Hz, H-6), 5.13 (1H, br s, H-14β), 5.81 (1H, d, *J* = 6.0 Hz, H-7), 6.16 (1H, d, *J* = 6.0 Hz, H-15β), 7.56, 7.45, 8.08 (5H, m, aromatic protons); ^{13}C NMR (75 MHz) δ 86.2 d (C-1), 24.1 t (C-2), 32.5 t (C-3), 37.2 (C-4), 47.2 d (C-5), 74.2 d (C-6), 123.9 d (C-7), 133.0 s (C-8), 42.7 d (C-9), 47.2 d (C-10), 50.4 s (C-11), 38.9 t (C-12), 76.5 s (C-13), 82.7 d (C-14), 76.0 d (C-15), 90.4 d (C-16), 92.5 d (C-17), 78.6 t (C-18), 52.0 t (C-19), 49.0 t (NCH_2CH$_3$), 12.9 q (NCH$_2CH_3$), 56.9 q (C-1′), 60.9 q (C-16′), 59.2 q (C-18′), 169.8 s, 20.9 q (OC-CH$_3$), 166.5 s (C_6H_5-*CO*), 129.7 s (C-1″), 129.7 d (C-2″, C-6″), 128.3 d (C-3″, C-5″), 133.0 d (C-4″). EIMS *m/z* 597 ([M]$^+$, 2), 566 ([M − OCH$_3$]$^+$, 20), 105 ([C_6H_5 − CO]$^+$; HREIMS *m/z* 597.3010 (calcd for $C_{33}H_{43}O_9N$, 597.2937).

Acknowledgment. We thank Professor Xiao-Tian Liang, Institute of Materia Medica, Chinese Academy of Sciences, Beijing, for helpful discussion on the subject. We also acknowledge the skillful assistance of Ms. Eunice Y. L. Chan and Mr. Thomas Wong (Department of Chemistry, The Hong Kong University of Sciences and Technology) for recording 2D NMR and HRMS spectra, respectively.

References and Notes

(1) *Pharmacopeia of People's Republic of China*; 1995; pp 210−212.
(2) Wang, Y. G.; Zhu, Y. L.; Zhu, R. H. *Acta Pharm. Sin.* **1980**, *15*, 526−531.
(3) Uhrin, D.; Proksa, B.; Zhamiansan, J. *Planta Med.* **1991**, *57*, 390−391.
(4) Li, Z. B.; Wang, F. P. *Nat. Prod. R & D* **1997**, *45*, 9−14
(5) Wang, F. P.; Li, Z. B.; Dai, X. P.; Peng, C. S. *Phytochemistry* **1997**, *45*, 1539−1542.
(6) Pelletier, S. W.; Mody, N. V.; Joshi, B. S.; Schramm, L. C. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed. Wiley: New York, 1984; Vol. 2, pp 206−642.
(7) Pelletier, S. W.; Joshi, B. S. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed. Wiley: New York, 1991; Vol. 7, pp 297−564.
(8) Wang, F. P. *Acta Pharm. Sin.* **1981**, *16*, 943−959.
(9) Fang, Q. C.; Hou, Z. M. *Acta Pharm. Sin.* **1966**, *13*, 577−588.

NP960481T