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DITERPENE ALKALOIDS FROM *ACONITUM KIRINENSE*

FENG FENG*, JING-HAN LIU and SHOU-XUN ZHAO

Department of Phytochemistry, China Pharmaceutical University, Nanjing, 210009, China

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Key Word Index—*Aconitum kirinense*; Ranunculaceae; roots diterpene alkaloids; kirinines B and C.

Abstract—Two new C_{20}–diterpenoid alkaloids, kirinines B and C, were isolated from the roots of *Aconitum kirinense*. Their structures were elucidated by spectroscopic methods as 1,19–epoxydenudatine and 1–acetyllepenine azomethine, respectively. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Aconitum kirinense Nakai grows in Jilin Province, north east China. Its root is used in traditional Chinese medicine for the treatment of rheumatic arthritis, rheumatoid disease, etc [1]. Chemical studies on the root of the crude drug resulted in the isolation of nine diterpene alkaloids. Two of them, lepenine(**3**) and kirinine A(**4**), have been reported in former articles [2, 3]. We report here the isolation and structural determination of kirinines B and C, two new C_{20}–diterpene alkaloids.

RESULTS AND DISCUSSION

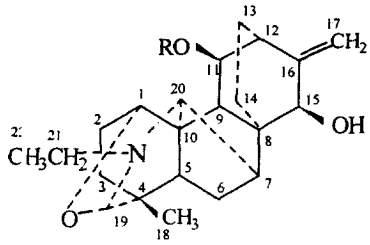
Kirinine B(**1**) was isolated as colourless cubes, mp 157 ~ 158°. EIMS showed the $[M]^+$ at *m/z* 357 and the base peak at 300$[M-C_3H_5O]^+$. The molecular formula $C_{22}H_{31}NO_3$ was assigned on the basis of ^1H NMR, DEPT ^{13}C NMR and the mass spectrum. The ^1H NMR spectrum showed the presence of an exocyclic double bond (δ5.23, 5.04, both 1 H,*t*,$J_1 = J_2 = 2.0$ Hz, H_2–17), an ethyl group bound to N (δ0.99,3 H,*t*,*J* = 7.3 Hz,H_3–22) and an angular methyl group (δ 0.78,3 H,*s*,H_3–18). All of these resonances suggested a C_{20}–diterpene alkaloid. The compound had the same molecular skeleton as lepenine, which was confirmed by the comparison of NMR data with lepenine (Tables 1 and 2). However, instead of a signal for a methylene carbon at *ca* δ 60 (C–19) in this kind of alkaloid, a signal for a methine carbon appeared at δ93.13, which belonged to a tertiary carbon bound to two hetero atoms, a characteristic of an ether bridge between C–1 and C–19 [4]. The base peak *m/z* 300 $[M-C_3H_5O]^+$ and the fragment *m/z* 122 $[C_8H_{12}N]^+$ in the mass spectrum [5], IR absorption at 1110 cm^{-1} and ^1H NMR signals at δ3.68 (1 H,*s*,H–19) and 4.19 (1 H,*d*,*J* = 5.3 Hz,H–1) also indicated the presence of a C_1–C_{19} inner ether in kirinine B [4, 6, 7].

After deuterium–exchange, signals for two protons (δ1.76,1 H,*d*,*J* = 6.8 Hz,OH–15 and 1.40,1 H,*d*,*J* = 6.9 Hz,OH–11) disappeared. This revealed that there were two hydroxyl groups in the molecule. Its ^1H–^1H COSY NMR spectrum indicated coupling between the two hydroxyl groups with H–15 (δ4.28) and H–11 (δ3.74), respectively, and revealed the presence of hydroxyl groups at C–15 and C–11. Thus, the structure of kirinine B was assigned as 1,19–epoxydenudatine (**1**). The NMR data of kirinine B were very similar to those of 11–acetyl–1,19–epoxydenudatine (5) [5]. Compared with the latter, the hydroxyl group at C–11 of kirinine B shifted the C–11 resonance upfield (1.5 ppm) and shifted the C–9 and C–12 resonances downfield (5.3 and 3.9 ppm, respectively) as a result of its β effect.

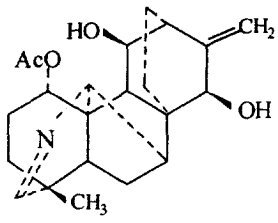
Kirinine C was isolated as colourless needles, mp 218–220°. Its mass spectrum showed the $[M]^+$ at *m/z* 371, with the base peak at 293 $[M-CH_3COOH-H_2O]^+$. The IR spectrum showed the presence of hydroxyl (3200 cm^{-1}), a C=N azomethine group (1640 cm^{-1}) and a carbonyl group (1740 cm^{-1}). Its molecular formula was $C_{22}H_{29}NO_4$ based on DEPT ^{13}C NMR, ^1H NMR and mass spectral data.

The presence of an imine (–CH=N–) group in kirinine C was established by comparison with the ^{13}C NMR chemical shifts of known atisine deriva-

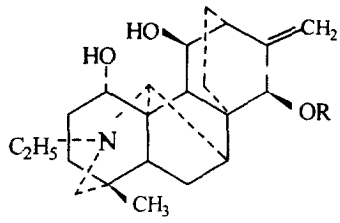
*Author to whom correspondence should be addressed.



(1) R=H kirinine B
(5) R=Ac 11-acetyl-epoxydenudatine



(2) kirinine C



(3) R=H lepenine
(4) R=Ac kirinine A

tives containing an imine group (e.g. atisine azomethine shows a resonance at δ166.4) [8]. The lack of an alkyl substituent linked with N and the absence of a chemical shift in the region of δ60 (C–19) in these alkaloids (compared with 57.1 and 56.8 for lepenine and kirinine A, respectively) were explained by the presence of an imine group (δ169.3, C–19). The downfield shift (10.9 ppm) of the C–4 and the upfield shift (1.1 ppm) of the C–20 in kirinine C relative to lepenine, are due to the presence of this group [9]. This was also confirmed by the downfield shift (0.99 ppm) of H–20 in kirinine C relative to lepenine.

Comparing the ^{13}C NMR data of kirinine C with that of lepenine, the presence of oxygen-groups at C–1,11 and 15 could also be proved. In its ^{1}H NMR spectrum the signal for 1–β H was at far lower field than that of the 11α,15α protons. Therefore, the acetoxyl group should be situated at C–1 and the two hydroxyl groups at C–11 and 15. Thus, the structure of kirinine C was determined as 1–acetyllepenine azomethine (**2**).

Table 1. ^{1}H NMR data of kirinine B (**1**), kirinine C (**2**) and lepenine (**3**) (300 MHz, $CDCl_3$)

H	1	2	3
1β	4.19 *d* (5.3)	5.30 *dd* (10.8,7.2)	4.17 *dt* (10.8,6.9)
2a	1.24 *m*	1.30 *m*	1.82 *m*
2b	1.83 *m*	1.98 *m*	2.35 *m*
3a	1.56 *m*	1.23 *m*	1.32 *m*
3b	1.63 *m*	1.51 *m*	1.64 *m*
5	1.61 *m*	1.43 *m*	1.37 *d* (7.6)
6a	1.67 *m*	1.20 *m*	1.25 *m*
6b	2.35 *ddd* (12.6,8.5,2.0)	2.91 *ddd* (14.0,7.8,1.3)	2.74 *dd* (13.0,7.6)
7	1.84 *m*	2.17 *m*	2.21 *m*
9	1.28 *d* (9.6,6.8)	1.35 *d* (9.2)	1.37 *d* (9.5)
11	3.74 *dd* (9.6,6.8)	3.84 *dd*(9.2,1.4)	4.46 *dd* (9.5,6.7)
12	2.21*dd* (5.3,5.2)	2.15 *m*	2.21 *m*
13a	1.47 *m*	1.48 *m*	1.47 *m*
13b	1.71 *m*	1.69 *m*	1.72 *m*
14a	1.97 *ddd* (14.0,11.7,7.0)	1.96 *m*	1.94 *m*
14b	1.21 *m*	1.25 *m*	1.14 *m*
15α	4.28 *dt* (6.8,2.0,2.0)	4.28 *s*(*br*)	4.28 *dt* (7.7,2.1,2.1)
17a	5.04 *t* (2.0,2.0)	5.22 *t* (2.0,2.0)	5.08 *d* (2.1,2.1)
17b	5.23 *t* (2.0,2.0)	5.22 *t* (2.0,2.0)	5.28 *t* (2.1,2.1)
18(Me)	0.78 *s*	0.98 *s*	0.70 *s*
19	3.68 *s*	7.25 *s*	2.23 *m*,2.50*m*
20	3.04 *dd* (4.1,2.1)	4.67 *s*(*br*)	3.68 *s*(*br*)
21	2.63–2.69 *m*	–	2.30–2.50 *m*
22	0.99 *t* (7.3,7.3)	–	1.05 *t* (7.0,7.0)
OH	1.76 *d* (6.8)	1.86 *s*(*br*)	2.50 *s*(*br*)
OH	1.40 *d* (6.8)	1.81 *s*(*br*)	2.08 *d* (7.7)
*Me*CO	–	2.05 *s*	2.32 *s*(*br*)

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR were recorded at 300 and 75 MHz, respectively. Chemical shifts are given in δ values relative to TMS in $CDCl_3$.

Plant material

Roots of the *A. kirinense* were collected around Yanji city, Jilin province, and were identified by Hui-Zhong Xiao (Department of Pharmacy, Yanbian Medical College). A herbarium voucher

Table 2. ^{13}C NMR data of kirinine B (**1**), kirinine C (**2**) lepenine (**3**) and 11–acetyl–1,19–epoxydenudatine (**5**) (75 MHz, $CDCl_3$)

C	1	2	3	5
1	68.7 *d*	72.6 *d*	70.8 *d*	68.3 *d*
2	24.6 *t*	27.1 *t*	31.2 *t*	24.1 *t*
3	30.0 *t*	33.5 *t*	38.4 *t*	29.7 *tt*
4	37.6 *s*	44.6 *s*	33.7 *s*	37.5 *s*
5	50.2 *d*	49.1 *d*	52.4 *d*	49.6 *d*
6	24.6 *t*	24.5 *t*	23.1 *t*	24.4 *t*
7	47.5 *d*	47.9 *d*	46.9 *d*	47.4 *d*
8	45.9 *s*	47.9 *s*	43.7 *s*	45.5 *s*
9	51.8 *d*	56.3 *d*	54.1 *d*	46.5 *d*
10	49.8 *s*	48.1 *s*	51.0 *s*	49.4 *s*
11	72.7 *d*	73.8 *d*	71.3 *d*	74.2 *d*
12	47.2 *d*	46.7 *d*	42.3 *d*	43.3 *d*
13	24.7 *t*	25.1 *t*	24.6 *t*	24.3 *t*
14	27.2 *t*	26.7 *t*	27.5 *s*	26.9 *t*
15	77.3 *d*	77.3 *d*	78.0 *d*	77.1 *d*
16	154.3 *s*	154.3 *s*	154.5 *s*	153.7 *s*
17	110.4 *t*	109.7 *t*	109.3 *t*	110.7 *t*
18	18.7 *q*	21.0 *q*	26.0 *q*	18.6 *q*
19	93.1 *d*	169.3 *d*	57.1 *t*	92.9 *d*
20	70.1 *d*	68.8 *d*	67.7 *d*	69.8 *d*
21	48.5 *t*	–	50.7 *t*	48.4 *t*
22	14.1 *q*	–	13.6 *q*	14.1 *q*
CO*Me*	–	21.6 *q*	–	–
*CO*Me	–	170.7 *s*	–	–

specimen is deposited in the Department of Phytochemistry, China Pharmaceutical University.

Isolation of alkaloids

Crude alkaloids [2] were chromatographed on neutral Al_2O_3 and eluted with $CHCl_3$–MeOH (10:1) to give fr. 2. Kirinine B (**1**) (40 mg) was obtained from this fr. as colourless cubes. Upon further elution with $CHCl_3$–MeOH (10:3), kirinine C (**2**) (15 mg) was obtained as colourless needles.

*Kirinine B (**1**).* $C_{22}H_{31}NO_3$, mp. 157–158°. IR ν_{max}^{KBr} cm^{-1} 3400, 2940, 2850, 1640, 1460, 1100, 1090, 960, 900. EIMS (probe) 70 eV, *m/z* (rel. int.): 357$[M]^+$(36), 339 $[M-H_2O]^+$(16), 329$[M-C_2H_5O]^+$(100), 122$[C_8H_{12}N]^+$(57). ^1H NMR (300 MHz, $CDCl_3$): Table 1. ^{13}C NMR (75 MHz, $CDCl_3$): Table 2.

*Kirinine C (**2**).* $C_{22}H_{29}NO_4$, mp. 218–220°. IR ν_{max}^{KBr} cm^{-1} 3210, 2980, 1740, 1640, 1220, 1020, 900. EIMS 70 eV, *m/z* (rel. int.): 371$[M]^+$ (44), 353 $[M-H_2O]^+$ (19), 312 $[M-CH_3COOH]^+$ (32), 293 $[M-CH_3COOH-H_2O]^+$ (100). ^1H NMR (300 MHz, $CDCl_3$): Table 1. ^{13}C NMR (75 MHz, $CDCl_3$): Table 2.

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