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Two New Diterpenoid Alkaloids from *Spiraea japonica* L. f. var. *fortunei* (Planchon) Rehd.

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Abstract: Two new hetisine-type C_{20}-diterpenoid alkaloids named spiraqine (**1**) and 6-hydroxylspiraqine (**2**), and four known alkaloids, namely spiredine (**3**), spiradine A (**4**), spiradine B (**5**), and spirasine V/VI (**6**), were isolated from *Spiraea japonica* L. f. var. *fortunei* (Planchon) Rehd. The structures of the alkaloids were elucidated using nuclear magnetic resonance analysis (^{1}H-NMR, ^{13}C-NMR, DEPT, HMQC, and HMBC) and mass spectrometry data.

Key words: C_{20}-diterpenoid alkaloids; hetisine type; 6-hydroxylspiraqine; Rosaceae; *Spiraea japonica* L. f. var. *fortunei* (Planchon) Rehd.; spiraqine.

Spiraea japonica L. (Rosaceae) and its varieties are widespread in the Yunnan Province of China. The young leaves, fruits and roots of these plants have been used as diuretic, detoxicant, and analgesic agents, as well as for the treatment of inflammation, cough, headache and toothache, in traditional Chinese medicine (Jiangsu New Medical College 1986). Previous chemical investigations on *S. japonica* complex have led to the isolation of more than 57 diterpenoid alkaloids, including both atisine-type and hetisine-type alkaloids (Hao *et al.* 2003). The chemotaxonomy of the *S. japonica* complex was proposed on the basis of these chemical data (Hao *et al.* 1997). From further investigation of this plant complex, two new hetisine-type C_{20}-diterpene alkaloids, designated as spiraqine (**1**) and 6-hydroxylspiraqine (**2**), were isolated from *S. japonica* L. f. var. *fortunei* (Planchon) Rehd., along with four known alkaloids, namely spiredine (**3**; Gorbunov *et al.* 1976; Sun *et al.* 1987), spiradine A (**4**; Goto *et al.* 1968; Sun and Yu 1985), spiradine B (**5**; Goto *et al.* 1968) and spirasine V/VI (**6**; Sun *et al.* 1986).

1 Results and Discussion

Compound **1** was determined to have a molecular formula of $C_{20}H_{29}NO$ on the basis of (+)-HRESIMS *m/z* 300.231 8 $[M+H]^+$. Inspection of the nuclear magnetic resonance (NMR) spectra (^{1}H-NMR, ^{13}C-NMR, DEPT, HMQC and HMBC) of compound **1** revealed a hetisine-type C_{20}-diterpenoid alkaloid (Sun and Yu 1985; Sun *et al.* 1986, 1987). The ^{13}C-NMR spectrum of compound **1** showed the presence of 20 carbon signals, including two methyl groups, eight methylene, six methine groups, and four quaternary carbons. The ^{13}C-NMR spectrum of compound **1** suggested an absence of the exocyclic double bond, oxazolidine ring substitutions, and carbonyl, which are present frequently in C_{20}-diterpenoid alkaloids. The N/C-6 bond was validated by ^{1}H-^{13}C long-range correlations between the protons at δ_H 2.68 (H-19), 2.89 (H-20), and the carbon signals at δ_C 65.2 (C-6; Fig. 1), respectively. The ^{1}H-^{13}C long-range correlations between the protons at δ_H 3.74 (H-6) and the carbon signal at δ_C 73.3 (C-20) also validated such an N/C-6 bond. The NMR data of a

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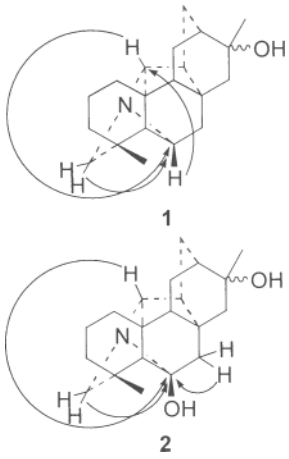

Fig. 1. Key HMBC (from H to C) of compounds **1** and **2**.

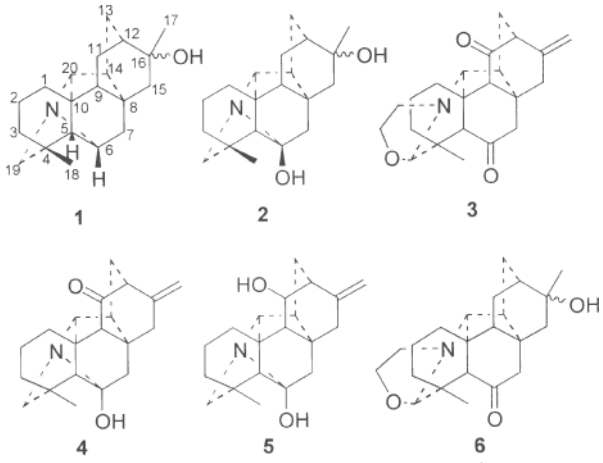

Fig. 2. The structure of compounds **1**–**6**.

tertiary carbon (δ_C 70.2) and a methyl carbon (δ_C 29.2) were assigned to C-16 and C-17, respectively, which revealed that the hydroxyl group is located at C-16 in compound **1**. So, the structure of compound **1** was identified as shown in Fig. 2. This is the simplest C_{20}-diterpenoid alkaloid obtained from the *S. japonica* complex to date.

Compound **2** has a molecular formula of $C_{20}H_{29}NO_2$ on the basis of (+)-HRESIMS *m/z* 316.227 7 $[M+H]^+$. The ^{13}C-NMR spectrum of compound **2**, which has a hetisine-type skeleton, obviously showed 20 peaks, most of which were similar to those of compound **1** (Table 1). Based on comparison with compound **1**, a second hydroxyl group was placed at C-6 (δ_C 101.4). The HMBC correlation among the proton signals of δ_H 2.27, 1.94 (H-7), 2.63 (H-19), 2.87 (H-20), and the carbon signal (δ_C 101.4) supported this assignation (Fig. 1). So, the structure of compound **2** was elucidated as shown in Fig. 2.

Spiradine B (**5**) was first isolated in 1968 (Goto *et al.* 1968), but complete NMR data for spiradine B have not been described. In the present paper, we report the NMR assignments for this compound.

The structures of known compounds **3**, **4** and **6** were established by comparing their spectroscopic data (MS, ^{1}H- and ^{13}C-NMR) with those reported in the literature (Goto *et al.* 1968; Sun and Yu 1985; Sun *et al.* 1986, 1987).

2 Experimental

2.1 General experimental procedures

Optical rotations were measured with a Jasco DIP-370 digital polarimeter (made in Japan). Infrared (IR) spectra were obtained on a Bruker Tensor 27 infrared spectrophotometer (made in Switzerland) with KBr pellets. Mass spectrometry (MS) was performed on an Autospec-3000 spectrometer (made in Manchester of Britain in 1993) and Finnigan LCQ Advantage (made in USA). Nuclear magnetic resonance spectra were recorded on Bruker AM-400 MHz and DRX-500 spectrometers (made in Switzerland) with $(CH_3)_4Si$ as the internal standard.

2.2 Plant materials

The whole plant of *Spiraea japonica* L. f. var. *fortunei* (Planchon) Rehd. was collected in Tengchong County, Yunnan Province, China. A voucher specimen (No. ZZY054) was deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, the Chinese Academy of Sciences.

2.3 Extraction and isolation

The dried powder (6 kg) of the whole plant was extracted with 95% ethanol by refluxing. The resulting extract was concentrated to give a gummy mass (796 g). The gum was suspended in 5% HCl and centrifuged (6 000 r/min, 30 min, 15 °C) to eliminate the

Table 1 ^{1}H- and ^{13}C-NMR data of compounds **1**, **2** and **5** in $CDCl_3$ (*J* in Hz; δ in ppm)

C	1		2		5	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	26.4* (t)	1.91 (1H, d, 13.6)	27.1 (t)	1.83 (1H, d, 13.4)	26.7 (t)	1.70 (m)
		1.29 (m)		1.26 (1H, dd, 4.2, 13.4)		1.35 (m)
2	19.0 (t)	1.76 (m)	18.9 (t)	1.78 (m)	18.7 (t)	1.74 (m)
				1.43 (m)		1.30 (m)
3	33.4 (t)	1.45 (m)	35.4 (t)	1.57 (m)	35.0 (t)	1.53 (m)
		1.26 (m)		1.51 (m)		1.28 (m)
4	36.9 (s)	/	37.7 (s)	/	37.6 (s)	/
5	58.5 (d)	1.62 (1H, s)	59.1 (d)	1.63 (1H, s)	58.5 (d)	1.67 (1H, s)
6	65.2 (d)	3.74 (1H, brs)	101.4 (s)	/	101.4 (s)	/
7	34.7 (t)	2.01 (1H, dd, 3.1, 14.2)	43.0 (t)	2.27 (1H, d, 14.3)	41.8 (t)	2.21 (1H, d, 14.3)
		1.54 (m)		1.94 (1H, d, 14.3)		2.00 (1H, d, 14.3)
8	39.9 (s)	/	40.9 (s)	/	41.4 (s)	/
9	48.9 (d)	1.49 (m)	48.3 (d)	1.43 (m)	58.2 (d)	1.44 (m)
10	50.2 (s)	/	49.4 (s)	/	49.0 (s)	/
11	24.1 (t)	1.72 (m)	24.1 (t)	1.75 (m)	66.9 (d)	3.93 (1H, d, 4.7)
		1.45 (m)		1.51 (m)		
12	35.2 (d)	1.41 (1H, brs)	35.3 (d)	1.33 (m)	41.1 (d)	2.27 (m)
13	26.6* (t)	2.49 (m)	25.7 (t)	2.46 (m)	27.8 (t)	1.92 (m)
		0.72 (m)		0.70 (m)		0.90 (m)
14	41.2 (d)	2.36 (1H, d, 11.3)	40.6 (d)	2.25 (m)	41.1 (d)	2.27 (m)
15	42.8 (t)	1.53 (m)	42.4 (t)	1.60 (m)	32.2 (t)	2.25 (m)
		1.49 (m)		1.57 (m)		
16	70.2 (s)	/	70.1 (s)	/	143.2 (s)	/
17	29.2 (q)	1.27 (3H, s)	29.2 (q)	1.29 (3H, s)	111.5 (t)	4.81 (1H, s)
						4.79 (1H, s)
18	28.4 (q)	1.07 (3H, s)	29.7 (q)	1.47 (3H, s)	29.7 (q)	1.39 (3H, s)
19	59.1 (t)	3.01 (1H, d, 12.4)	56.3 (t)	3.49 (1H, d, 11.6)	56.3 (t)	3.41 (1H, d, 11.8)
		2.68 (1H, d, 12.4		2.63 (1H, d, 11.6)		2.72 (1H, d, 11.8)
20	73.3 (d)	2.89 (1H, s)	71.9 (d)	2.87 (1H, s)	71.7 (d)	2.88 (1H, s)

*, interchangeable.

insoluble part. The supernatants were basified with aqueous ammonia solution to pH 10 and extracted with chloroform to afford the alkaloid part (20 g), which was subjected to column chromatography on silica gel. Elution with petroleum ether-chloroform-diethylamine (80∶20∶2, 70∶30∶3, 60∶40∶4, and 50∶50∶5, v/v/v) yielded 11 fractions. Spiredine (**3**; 1.3 g) and spiradine A (**4**; 0.9 g) were obtained by recrystallization in acetone from fraction 1 and fraction 10, respectively. Fraction 8 was separated by petroleum ether-ethyl acetate-diethylamine (80∶20∶3, v/v/v) to yield spirasine V/VI (**6**; 4 mg). Fraction 11 yielded the compound spiraqine (**1**; 13 mg) by column chromatography on silica gel eluted with chloroform-acetone (8∶2, v/v), chloroform-acetone-diethylamine (80∶20∶2 and 75∶25∶3, v/v/v), chloroform (water-saturated)-methanol (3∶1, 2∶1, and 1∶1, v/v). The residue of fraction 11 was chromatographed using medium pressure liquid chromatography (MPLC) with an RP-18 column eluting with methanol-water (30∶70, 50∶50, and 80∶20, v/v) to give spiradine B (**5**; 5 mg) and 6-hydroxylspiraqine (**2**; 4 mg).

2.4 Identification

Spiraqine (1) Brown gum, $[\alpha]_D^{18}$ 10° (*c* 1.10, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3 396, 1 274, 1 124; ^{1}H-NMR and ^{13}C-NMR see Table 1; EIMS *m/z*: 299 $[M]^+$ (100), 281 (45), 271 (15), 254 (15), 240 (40), 160 (40), 146 (75); (+)-HRESIMS *m/z*: 300.231 8 $[M+H]^+$ (calculated for $C_{20}H_{30}NO$, 300.232 7).

Table 2 ^{13}C-NMR data of compounds **3**, **4** and **6** in $CDCl_3$ (δ in ppm)

C	3	4	6
1	32.5, 33.8 (t)	35.4 (t)	32.5, 31.0 (t)
2	18.1 20.2 (t)	19.1 (t)	20.7, 18.6 (t)
3	36.9, 39.4 (t)	33.6 (t)	39.2, 37.1 (t)
4	36.7 (s)	37.6 (s)	39.7 (s)
5	61.4 (d)	61.6 (d)	62.0, 61.7 (t)
6	207.0 (s)	98.4 (s)	210.0 (s)
7	50.8, 50.1 (t)	44.2 (t)	48.5, 46.8 (t)
8	43.2, 42.0 (s)	45.1 (s)	39.8 (s)
9	64.7 (d)	65.0 (d)	36.0, 35.8, (d)
10	47.5, 46.6 (s)	51.7 (s)	42.0 (s)
11	210.8 (s)	211.5 (s)	24.4, 24.3 (t)
12	53.3, 53.1 (d)	53.2 (d)	48.9, 48.3 (d)
13	29.7, 30.1 (t)	27.3 (t)	28.8 (t)
14	49.6, 44.9 (d)	43.6 (d)	48.0, 43.6 (d)
15	35.2, 34.8 (t)	29.1 (t)	45.6, 44.7 (t)
16	143.1, 142.9 (s)	143.3 (s)	70.6, 70.5 (s)
17	110.6, 110.4 (t)	111.0 (t)	29.8, 29.6 (q)
18	30.2, 23.1 (q)	30.4 (q)	30.4, 23.3 (q)
19	97.6, 93.4 (d)	60.6 (t)	98.0 (d)
20	72.6, 72.3 (d)	74.4 (d)	73.2, 73.1 (d)
21	48.8, 52.1 (t)		52.9, 52.3 (t)
22	62.8, 64.9 (t)		65.6, 62.7 (t)

6-Hydroxylspiraqine (2) Yellow gum, $[\alpha]_D^{25}$–20° (*c* 0.20, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3 416, 1 216, 1 167; ^{1}H-NMR and ^{13}C-NMR see Table 1; EIMS *m/z*: 227 (45), 253 (100), 281 (80), 311 (5); (+)-HRESIMS *m/z*: 316.227 7 $[M+H]^+$ (calculated for $C_{20}H_{30}NO_2$, 316.227 6).

Spiradine B (5) White powder, $[\alpha]_D^{25}$–13° (*c* 0.25, $CHCl_3$); ^{1}H-NMR and ^{13}C-NMR see Table 1; (+)-ESIMS *m/z*: 314 $[M+H]^+$ (100).

Spiredine (3) White powder, ^{13}C-NMR see Table 2; EIMS *m/z*: 353 (100), 338 (22), 325 (22), 310 (12), 91 (21).

Spiradine A (4) White powder, ^{13}C-NMR see Table 2; EIMS *m/z*: 311 (100), 296 (27), 283 (100), 268 (28), 240 (23), 188 (37), 161 (82).

Spirasine V/VI (6) Yellow gum, ^{13}C-NMR see Table 2; EIMS *m/z*: 353 (100), 338 (22), 325 (22), 310 (12), 91 (21).

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