

DITERPENES FROM *SPIRAEA JAPONICA*

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**Key Word Index**—*Spiraea japonica* var. *acuta*; Rosaceae; diterpenes; spiramacetal; spiramadol; spiramilactone C and D.

**Abstract**—Four new diterpenes, spiramacetal, spiramadol, spiramilactone C and D, were isolated from *Spiraea japonica* var. *acuta*. Their structures were elucidated by chemical and spectral means. © 1998 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

The diterpene alkaloids of *Spiraea japonica* complex, which is distributed mainly in south-west of China, have been studied systematically [1–17]. We isolated and identified some new atisane-type diterpenes from this complex distributed in Yunnan province [11, 17, 18]. Further investigation of the diterpenes has yielded four new components from *Spiraea japonica* var. *acuta* Yu. We present here spectroscopic and chemical evidence for the structures of spiraminace(1), spiramadol(2), spiramilactone C(3) and D(4).

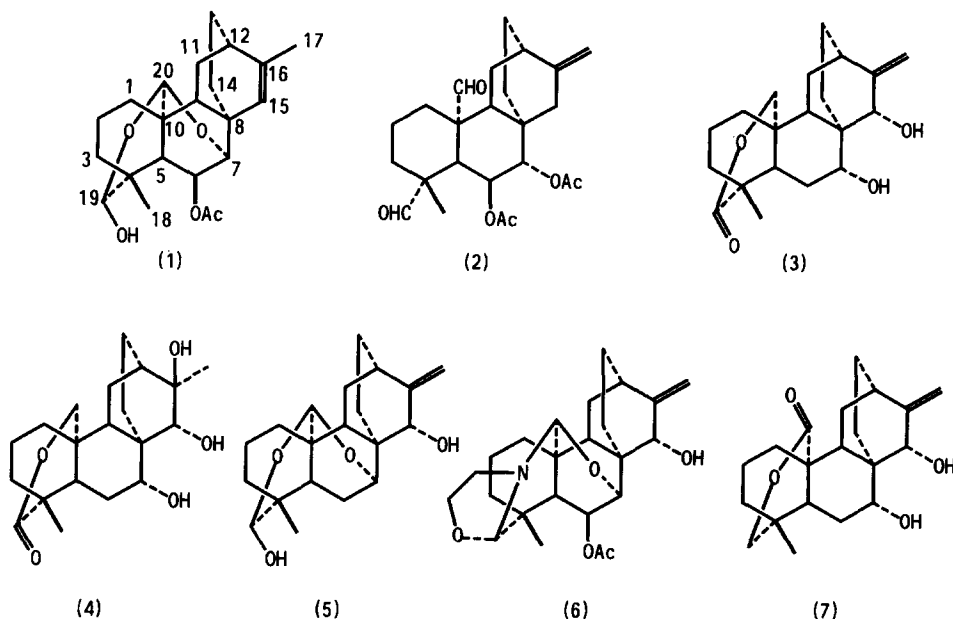
## RESULTS AND DISCUSSION

Spiramacetal(1) was determined as  $C_{22}H_{30}O_5$  on the basis of its HR mass spectrum. The basic skeleton was established by comparing its  $^1H$  NMR and  $^{13}C$  NMR shift values with those of spiraminol (5) [11]. Compound 1 showed the presence of two methyl groups [ $\delta_H$  2.15 and 1.17 (each 3H, s);  $\delta_C$  22.7 and 21.1 (q)], a secondary acetoxyl group [ $1740\text{ cm}^{-1}$ ;  $\delta_H$  5.79 (1H, dd,  $J=5.3, 1.5\text{ Hz}$ , H-6), 1.75 (3H, s);  $\delta_C$  69.8 (d, C-6), 169.9 (s, COCH<sub>3</sub>), 20.7 (q, COCH<sub>3</sub>)], an ether linkage between C-7 and C-20 [ $\delta_H$  5.69 (1H, s, H-20), 4.35 (1H, d,  $J=5.3\text{ Hz}$ , H-7  $\beta$ );  $\delta_C$  97.3 (d, C-20), 71.4 (d, C-7)], acetal group [ $\delta_H$  5.79 (1H, s, H-20);  $\delta_C$  97.3 (d, C-20)], and hemiacetal group [ $\delta_H$  5.47 (1H, d,  $J=1.6\text{ Hz}$ , H-19);  $\delta_C$  95.4 (d, C-19)]. Unlike spiraminol(5), there was no signal of an exo-methylene group for 1, while a methyl carbon ( $\delta_C$  21.1) and tri-substituted double bond were designated as C-15 and C-16, indicated that the migration shift of the exocyclic double bond for 1. In the  $^1H$ - $^1H$  COSY spectrum of 1, the signal of the proton at  $\delta_H$  5.79 (H-6) showed correlation with a proton at  $\delta_H$  4.35 (H-7), which indicated that an acetoxyl group was located at C-6 as in spiradine F(6) [12]. The same coupling constants of  $\delta_H$  5.79 and  $\delta_H$  4.35 with those

of 6 implied the configuration of the acetoxyl group for 1 was  $\beta$ .

Spiramadol(2) was assigned as  $C_{24}H_{32}O_6$  for its molecular formula on the basis of the HR mass spectrum. In addition to an exo-methylene group [ $\delta_H$  4.76, 4.60 (each 1H, d,  $J=2\text{ Hz}$ );  $\delta_C$  149.9 (s), 106.6 (t)], compound 2 showed the presence of two secondary acetoxyl groups [ $1740, 1250\text{ cm}^{-1}$ ;  $\delta_H$  2.00, 2.02 (each 3H, s); 6.00 (1H, dd,  $J=9.8, 11.7\text{ Hz}$ , H-6  $\alpha$ ), 4.78 (1H, d,  $J=9.8\text{ Hz}$ , H-7  $\beta$ );  $\delta_C$  69.1 (d), 80.8 (d), 170.4 (s), 169.9 (s), 21.3 (q), 21.1 (q)], and two aldehyde groups [ $2730\text{ cm}^{-1}$ ;  $\delta_H$  9.84 (1H, s), 9.60 (1H, s);  $\delta_C$  206.0 (d), 200.0 (d)]. In the  $^1H$ - $^1H$  COSY spectrum of 2, the signal at  $\delta_H$  6.00 showed correlation with a signal at  $\delta_H$  4.78, indicating that two acetoxyl groups were located at C-6 and C-7, respectively. The coupling constant of  $\delta_H$  6.00 and 4.78 ( $J=9.8\text{ Hz}$ ) implied H-6 and H-7 were situated in the axial-configuration, while the coupling constant of  $\delta_H$  6.00 ( $J=11.7\text{ Hz}$ ) implied H-6 and H-5 also were situated in the axial-configuration. Since H-5 has the  $\beta$ -configuration, H-6 and H-7 could be designated as  $\alpha$  and  $\beta$ , respectively. Unlike spiraminace(1) or spiraminol(5), compound 2 bears two free-aldehyde groups because there is no free C-7 $\alpha$ hydroxyl group in 2.

Spiramilactone C(3) was assigned the molecular formula  $C_{20}H_{28}O_4$  by the HR mass spectrum. Compound 3 showed the presence of an exo-methylene group [ $\delta_H$  5.34, 5.12 (each 1H, d,  $J=1.5\text{ Hz}$ , H<sub>2</sub>-17);  $\delta_C$  155.4 (s), 109.9 (t)], a methyl group [ $\delta_H$  1.28 (3H, s, H<sub>3</sub>-18);  $\delta_C$  23.6 (q)], two secondary OH groups [ $3400\text{ cm}^{-1}$ ;  $\delta_H$  4.17 (1H, br.s, H-15 $\beta$ ), 3.87 (1H, dd,  $J=4.0, 11.4\text{ Hz}$ , H-7 $\beta$ );  $\delta_C$  77.4 (d), 81.1 (d)], and a lactone moiety [ $1740\text{ cm}^{-1}$ ;  $\delta_H$  4.94 (1H, dd,  $J=2.0, 12.1\text{ Hz}$ , H-20), 4.26 (1H, d,  $J=12.1\text{ Hz}$ , H-20);  $\delta_C$  74.1 (t), 175.7 (s)]. Compound 3 and spiramilactone(7) [18] have the same molecular skeleton and functional groups. The difference between 3 and 7 is the position of carbonyl



groups. The downfield shift of the C-18 methyl group ( $\delta_H$  1.28) of **3** indicated its carbonyl group is located at C-19. The intramolecular Cannizzaro reaction of spiraminol(**5**) gave **3** and **7**, confirming the structure of **3**.

Spiramilactone D(**4**) has the molecular formula  $C_{28}H_{30}O_5$  and showed the same skeleton and functional groups as **3** except for an exo-methylene group by comparing their NMR spectra. The signals of a tertiary carbon [ $\delta_C$  70.3(*s*)] and a methyl carbon [ $\delta_H$  1.47(3H, *s*);  $\delta_C$  31.0(*q*)] indicated **4** was derived from **3** by hydroxylation at the exocyclic double bond, and the configuration of C-16 was designated as *R* comparing with spiramine P(**7**) [17].

## EXPERIMENTAL

### General.

Mps: uncorr. IR spectra were measured as KBr pellets, optical rotation in  $CHCl_3$ .  $^1H$  and  $^{13}C$  NMR and 2D NMR spectra were recorded in pyridine- $d_5$  using TMS as int. standard. EIMS were measured at 70 eV.

### Plant material

Roots of *Spiraea japonica* var. *acuta* Yu were collected in Li-Jiang, Yunnan Province of China in July 1993, and identified by Dr. Hang Sun, a botanist of Kunming Institute of Botany, Chinese Academy of Sciences, where the voucher specimen is deposited (No. 8018).

### Extraction and isolation

Powdered dried roots (5 kg) were extracted with 95% EtOH at room temp. After removal of solvent by evapn, the combined extracts were dissolved in 3% HCl and filtered. The HCl soln was extracted with benzene-petrol (1:1) and made basic with NaOH soln (PH11) and extracted with  $CHCl_3$ . Evapn of  $CHCl_3$  gave 35 g of a basic fr. (0.7%). The fr. (35 g) was chromatographed on a silica gel column eluting with petrol- $Me_2CO$ - $Et_2NH$  (50:1:0.5 to 50:5:4) to give 4 diterpenes and 8 alkaloids.

Spiramacetal (**1**) (20 mg, 0.0004%) as needles (petrol- $CHCl_3$ ), mp 148–150° [ $\alpha_D$ -75 ( $CHCl_3$ , *c* 0.5)]. HRMS: obsd 374.2117 for  $C_{22}H_{30}O_5$ , calc. 374.2093. EIMS *m/z* (rel. int.) 374[M]<sup>+</sup>, 328(55), 314(10), 240(100). IR  $\nu_{KBr}$   $cm^{-1}$ : 3400, 2880, 1740, 1380, 1240, 1020, 890;  $^1H$  NMR (400 Hz):  $\delta$  6.00(1H, *s*, H-15), 5.79(1H, *dd*, *J* = 5.3, 1.5 Hz, H-6  $\alpha$ ), 5.69(1H, *s*, H-20), 5.47(1H, *d*, *J* = 1.6 Hz, H-19), 4.35(1H, *d*, *J* = 5.3 Hz, H-7  $\beta$ ), 2.15(3H, *s*, H<sub>3</sub>-17), 1.75(3H, *s*, H<sub>3</sub>-22), 1.17(3H, *s*, H<sub>3</sub>-18).  $^{13}C$  NMR: Table 1.

Spiramadol(**2**) (20 mg, 0.0004%) as needles (petrol- $CHCl_3$ ), mp 208–210° [ $\alpha_D$ -19 ( $CHCl_3$ , *c* 0.67)]. HRMS( $F_{AB}$ ): obsd 417.2247 for  $C_{24}H_{32}O_6$ , calc. 417.2277. EIMS *m/z* (rel. int.) 416[M]<sup>+</sup>, 399(20), 370(5), 356(40). IR  $\nu_{KBr}$   $cm^{-1}$ : 2990, 2730, 1740, 1710, 1640, 1020, 990, 750;  $^1H$  NMR (400 Hz):  $\delta$  9.84(1H, *s*, H-19), 9.60(1H, *s*, H-20), 6.00(1H, *dd*, *J* = 9.8, 11.7 Hz, H-6 $\alpha$ ), 4.78(1H, *d*, *J* = 9.8 Hz, H-7 $\beta$ ), 4.76, 4.60 (each 1H, *d*, *J* = 2 Hz, H<sub>2</sub>-17), 2.02(3H, *s*, H<sub>3</sub>-24), 2.00(3H, *s*, H<sub>3</sub>-22), 1.06(3H, *s*, H<sub>3</sub>-18).  $^{13}C$  NMR: Table 1.

Spiramilactone C(**3**) (15 mg, 0.0003%) as needles (petrol- $CHCl_3$ ), mp 172–174° [ $\alpha_D$ -50 ( $CHCl_3$ , *c* 0.38)]. HRMS: obsd 332.1986 for  $C_{20}H_{28}O_4$ , calc. 332.1988.

Table 1.  $^{13}\text{C}$  NMR data for spiraminace(1), spiramadol(2), spiramilactone C(3) and spiramilactone D(4)

Carbon	1	2	3	4
1	34.5( <i>t</i> )	42.6( <i>t</i> )	40.1( <i>t</i> )	53.3( <i>t</i> )
2	25.3( <i>t</i> )	32.3( <i>t</i> )	20.8( <i>t</i> )	42.8( <i>t</i> )
3	29.8( <i>t</i> )	35.4( <i>t</i> )	39.9( <i>t</i> )	38.2( <i>t</i> )
4	37.5( <i>s</i> )	53.8( <i>s</i> )	42.6( <i>s</i> )	47.2( <i>s</i> )
5	55.7( <i>d</i> )	52.4( <i>d</i> )	47.7( <i>d</i> )	47.9( <i>d</i> )
6	69.8( <i>d</i> )	69.1( <i>d</i> )	15.3( <i>d</i> )	23.0( <i>d</i> )
7	71.4( <i>d</i> )	80.8( <i>d</i> )	81.1( <i>d</i> )	82.2( <i>d</i> )
8	41.0( <i>s</i> )	39.2( <i>s</i> )	41.8( <i>s</i> )	39.4( <i>s</i> )
9	51.5( <i>d</i> )	48.9( <i>d</i> )	44.8( <i>d</i> )	39.3( <i>d</i> )
10	35.8( <i>s</i> )	47.8( <i>s</i> )	36.6( <i>s</i> )	34.4( <i>s</i> )
11	21.8( <i>t</i> )	25.6( <i>t</i> )	29.0( <i>t</i> )	22.0( <i>t</i> )
12	37.5( <i>d</i> )	35.9( <i>d</i> )	36.2( <i>d</i> )	52.7( <i>d</i> )
13	20.9( <i>t</i> )	22.6( <i>t</i> )	28.3( <i>t</i> )	21.1( <i>t</i> )
14	29.4( <i>t</i> )	19.4( <i>t</i> )	26.8( <i>t</i> )	20.4( <i>t</i> )
15	132.2( <i>d</i> )	27.3( <i>t</i> )	77.4( <i>d</i> )	71.0( <i>d</i> )
16	139.1( <i>s</i> )	149.9( <i>s</i> )	155.4( <i>s</i> )	70.3( <i>s</i> )
17	21.1( <i>q</i> )	106.6( <i>t</i> )	109.9( <i>t</i> )	31.0( <i>q</i> )
18	22.7( <i>q</i> )	27.4( <i>q</i> )	23.6( <i>q</i> )	27.0( <i>q</i> )
19	95.4( <i>d</i> )	206.0( <i>d</i> )	175.7( <i>s</i> )	174.7( <i>s</i> )
20	97.3( <i>d</i> )	200.0( <i>d</i> )	74.1( <i>t</i> )	76.3( <i>t</i> )
COCH <sub>3</sub>	169.9( <i>s</i> )	170.4( <i>s</i> )		
COCH <sub>3</sub>	20.7( <i>q</i> )	21.3( <i>q</i> )		
COCH <sub>3</sub>	169.9( <i>s</i> )			
COCH <sub>3</sub>	20.4( <i>q</i> )			

EIMS  $m/z$  (rel. int.) 332[M]<sup>+</sup>, 314(100), 299(30), 286(60), 273(65). IR  $\nu_{\text{KBr}_{\text{max}}}\text{cm}^{-1}$ : 3400, 2940, 2880, 1740, 1240, 1020, 890.  $^1\text{H}$  NMR(400 Hz):  $\delta$ 5.34(1H, *d*,  $J=1.5$  Hz, H-17), 4.94(1H, *dd*,  $J=2.0$ , 12.1 Hz, H-20), 4.26(1H, *d*,  $J=12.1$  Hz, H-20), 4.17(1H, *br.s.*, H-15  $\beta$ ), 3.87(1H, *dd*,  $J=4.0$ , 11.4 Hz, H-7 $\beta$ ), 5.12(1H, *d*,  $J=1.4$  Hz, H-17), 1.28(3H, *s*, H<sub>3</sub>-18).  $^{13}\text{C}$  NMR: Table 1.

Spiramilactone D(4) (25 mg, 0.0005%) as needles (petrol-CHCl<sub>3</sub>), mp 225–227°.  $[\alpha]_{\text{D}}^{25}$  (CHCl<sub>3</sub>, *c* 0.44). HRMS: obsd 350.2090 for C<sub>20</sub>H<sub>30</sub>O<sub>5</sub>, calc. 350.2093. EIMS  $m/z$  (rel. int.) 350[M]<sup>+</sup>, 332(43), 314(65), 292(100). IR  $\nu_{\text{KBr}_{\text{max}}}\text{cm}^{-1}$ : 3420, 2940, 1710, 920, 890;  $^1\text{H}$  NMR(400 Hz):  $\delta$ 4.54(1H, *dd*,  $J=2.0$ , 11.3 Hz, H-20), 4.04(1H, *d*,  $J=11.3$  Hz, H-20), 4.04(1H, *s*, H-15  $\beta$ ), 3.40(1H, *d*,  $J=9.0$  Hz, H-7 $\beta$ ), 1.47(3H, *s*, H<sub>3</sub>-17), 1.28(3H, *s*, H<sub>3</sub>-18).  $^{13}\text{C}$  NMR: Table 1.

#### Intramolecular Cannizzaro reaction of spiraminol(5).

To the soln of KOH(1 g)-MeOH(10 ml) was added 50 mg(0.15 mmol) of 5. After refluxed 4 hr, solvent

was removed by evapn, the residue was extracted with CHCl<sub>3</sub>. Work-up followed by a short column of silica gel(CHCl<sub>3</sub>-Me<sub>2</sub>CO, 5:1) afforded 3(5 mg, 0.015 mmol) and 7(10 mg, 0.03 mmol), respectively, which were identified by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and TLC.

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