Two New Diterpenoid Lactams from *Spiraea japonica* var. *ovalifolia*

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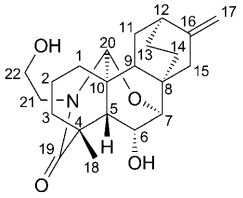
Two new diterpenoid alkaloids, spiramilactams A (**1**) and B (**2**), were isolated from the basic fraction of a MeOH extract of whole plants of *Spiraea japonica* var. *ovalifolia*. Their structures were established on the basis of extensive spectroscopic and mass-spectrometric analyses.

Introduction. – The *Spiraea japonica* complex (Rosaceae) was shown to contain diterpenoid alkaloids and diterpenoids, containing 22 hetisine-type alkaloids, 37 atisine-type alkaloids, and eight atisane-type diterpenoids [1–3]. Among them, some atisine-type alkaloids displayed significant bioactivity concerning anti-inflammation, antiplatelet aggregation, and neuroprotective effects [4–7]. In our ongoing search for additional diterpenoid alkaloids from the above plant complex, a reinvestigation on the chemical constituents of *Spiraea japonica* var. *ovalifolia* collected in Songming County of Yunnan Province, P. R. China, led to the isolation of six compounds including two novel atisine-type diterpenoid lactams, spiramilactams A (**1**) and B (**2**), along with four known diterpenoid alkaloids, namely spiramines A–D. This is the first report of the diterpenoid alkaloids from the *S. japonica* complex with α-configuration for the HO–C(6) group. Details of the isolation and structural elucidation of the two new diterpenoid lactams are presented below.



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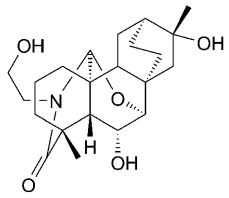

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Results and Discussion. – Spiramilactam A (**1**) was obtained as an optically active, white powder. The molecular formula was established as $C_{22}H_{31}NO_4$ by the HR-ESI-MS (*m*/*z* 374.2326 ($[M+H]^+$; calc. 374.2331)). The IR spectrum indicated the presence of OH groups (3420 cm^{-1}), a CO group (1735 cm^{-1}), and a C=C bond (1632 cm^{-1}). The ^{1}H-NMR spectrum of **1** (*Table*) showed signals for one tertiary Me group at δ(H) 1.53 (*s*, Me(18)), one O-bearing CH_2 group at δ(H) 4.12 (*t*, CH_2(22)), an exo-CH_2 group at δ(H) 4.80 and 4.94 (2 br. *s*, CH_2(17)), and two O-bearing CH groups

at δ(H) 3.75 (*d*, H–C(7)) and 4.46 (*dd*, H–C(6)). The ^{13}C-NMR and DEPT spectrum of **1** (*Table*) indicated 22 C-atom signals, consisting of one Me group, nine CH_2 groups, including one CH_2O group, six CH groups, including two O–CH groups, four quarternary C-atoms, including a CO group at δ(C) 175.4, and two olefinic resonances at δ(C) 108.0 (*t*) and 152.0 (*s*). The above-mentioned ^{1}H- and ^{13}C-NMR data of **1** revealed that compound **1** was an atisine-type lactam derivative [8–11]. Comparison of the ^{1}H- and ^{13}C-NMR spectral data of **1** with those of spiramine Y [8] showed that the two compounds possessed similar C-atom skeletons, except for the absence of an AcO group in the former compound. The two O-bearing CH groups at δ(C) 71.3 (C(6)) and 74.7 (C(7)) correlated with CH groups at δ(H) 4.46 (H–C(6)) and 3.75 (H–C(7)), respectively, in the HMQC experiments, and the two CH groups showed cross peaks in the ^{1}H,^{1}H-COSY spectra, suggesting that their H-atoms are vicinal to each other. The correlations of H–C(7) with C(5), C(9), C(14), C(15), and C(20) in the HMBC spectrum confirmed that C(7) was connected to C(20) through an O-bridge. HMBC correlations of H–C(20) with C(5), C(19), and C(21) suggested that C(19), C(20), and C(21) were connected to each other through a N-atom. Further-

Table. *^{1}H- and ^{13}C-NMR Data of Compounds* **1** *and* **2**. In C_5D_5N; δ in ppm, *J* in Hz.

	1[a])		**2**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	29.2 (*t*)	1.78 (*dd*, *J* = 5.0, 14.5), 1.20 (*ddd*, *J* = 5.0, 6.5, 14.5)	29.1 (*t*)	1.89–1.86 (*m*), 1.37–1.35 (*m*)
CH_2(2)	25.9 (*t*)	1.65–1.63 (*m*), 1.49–1.47 (*m*)	23.9 (*t*)	1.58–1.55 (*m*), 1.53–1.51 (*m*)
CH_2(3)	20.5 (*t*)	1.93–1.89 (*m*), 1.46–1.42 (*m*)	20.6 (*t*)	1.92–1.90 (*m*), 1.43–1.40 (*m*)
C(4)	44.2 (*s*)	–	44.3 (*s*)	–
H–C(5)	58.8 (*d*)	1.60–1.58 (*m*)	59.4 (*d*)	1.76 (br. *s*)
H–C(6)	71.3 (*d*)	4.46 (*dd*, *J* = 2.8, 4.4)	71.7 (*d*)	4.50 (*dd*, *J* = 1.2, 3.0)
H–C(7)	74.7 (*d*)	3.75 (*d*, *J* = 4.4)	75.2 (*d*)	3.78–3.76 (*m*)
C(8)	37.0 (*s*)	–	36.9 (*s*)	–
H–C(9)	46.7 (*d*)	1.70 (*dd*, *J* = 4.0, 8.5)	42.5 (*d*)	1.31–1.29 (*m*)
C(10)	34.6 (s)	–	34.8 (*s*)	–
CH_2(11)	40.0 (*t*)	1.88–1.86 (*m*), 1.40–1.37 (*m*)	40.1 (*t*)	1.84–1.81 (*m*), 1.32–1.30 (*m*)
H–C(12)	37.2 (*d*)	2.30 (*t*, *J* = 4.5)	39.0 (*d*)	1.82–1.80 (*m*)
CH_2(13)	26.9 (*t*)	1.61–1.59 (*m*), 1.33–1.31 (*m*)	23.7 (*t*)	2.18–2.17 (*m*), 1.32–1.30 (*m*)
CH_2(14)	27.4 (*t*)	2.08 (*dd*, *J* = 2.0, 11.0), 1.38–1.35 (*m*)	27.6 (*t*)	2.08–2.06 (*m*), 1.26–1.24 (*m*)
CH_2(15)	40.1 (*t*)	3.37 (*d*, *J* = 15.0), 2.22 (*d*, *J* = 15.0)	47.6 (*t*)	1.44–1.46 (*m*)
C(16)	152.0 (*s*)	–	72.7 (*s*)	–
CH_2(17) / Me(17)	108.0 (*t*)	4.94 (br. *s*), 4.80 (br. *s*)	30.5 (*q*)	1.47 (*s*)
Me(18)	21.6 (*q*)	1.53 (*s*)	21.7 (*q*)	1.53 (*s*)
C(19)	175.4 (*s*)	–	175.8 (*s*)	–
H–C(20)	86.2 (*d*)	5.24 (*d*, *J* = 1.6)	86.7 (*d*)	5.30 (*d*, *J* = 1.7)
CH_2(21)	50.1 (*t*)	4.32 (*ddd*, *J* = 2.0, 6.0, 13.5), 3.71 (*ddd*, *J* = 2.0, 6.0, 13.5)	50.2 (*t*)	4.28 (*ddd*, *J* = 2.0, 5.6, 11.2), 3.75–3.73 (*m*)
CH_2(22)	60.9 (*t*)	4.12 (*t*, *J* = 6.0)	60.9 (*t*)	4.13 (*t*, *J* = 6.2)

[a]) Recorded at 500 MHz. [b]) Recorded at 400 MHz.

more, analysis of the ^{1}H,^{1}H-COSY spectra of **1** established three other fragments: $CH_2(1)-CH_2(2)-CH_2(3)$, $CH(9)-CH_2(11)-CH(12)-CH_2(13)-CH_2(14)$, and $CH_2(21)-CH_2(22)$), as shown with bold bonds in *Fig. 1*. The location of the exo-CH_2 group at C(16) was supported by the correlations between $CH_2(17)$ (δ(H) 4.80, 4.94) and C(12), C(15), and C(16), while the presence of a CO group at C(19) was confirmed by the correlations of $CH_2(3)$, H–C(5), Me(18), H–C(20), and $CH_2(21)$ with the CO group (δ(C) 175.4). The relative configuration of **1** was elucidated by the help of a ROESY spectrum as shown in *Fig. 2*. The ROESY correlations of H–C(5) with H–C(6), H–C(7), and Me(18) implied the α-configuration for the 6-OH group and β-configuration for H–C(7). Thus, the structure of spiramilactam A (**1**) was elucidated to be as shown in **1**.

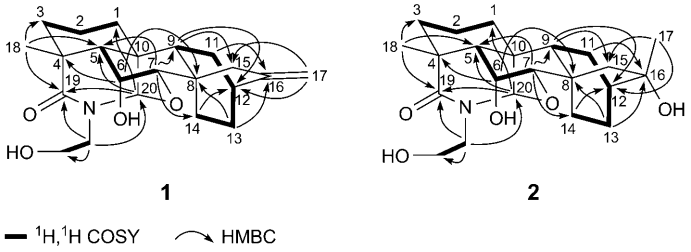

Fig. 1. *Key ^{1}H,^{1}H-COSY and HMBC correlations of compounds* **1** *and* **2**

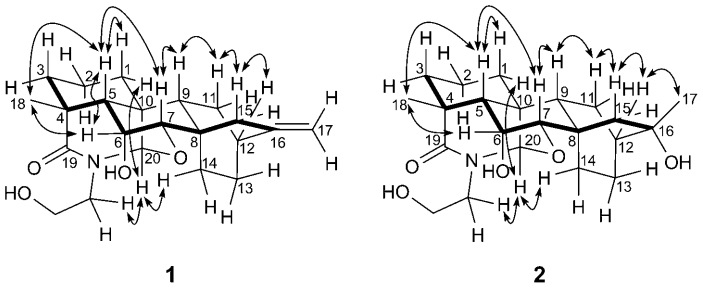

Fig. 2. *Significant ROESY correlations within compounds* **1** *and* **2**

Spiramilactam B (**2**), obtained as a colorless gum, had the molecular formula of $C_{22}H_{33}NO_5$ as determined by a *pseudo*-molecular ion peak in the HR-ESI-MS (392.2440 ($[M+H]^+$; calc. 392.2436)). Step-by-step comparison of the ^{1}H- and ^{13}C-NMR spectral data of **2** with those of **1** (*Table*) revealed that most signals of **2** were similar to those of spiramilactam A (**1**), except for the disappearance of an exocyclic C=C bond and the presence of an O-bearing quarternary C-atom (δ(C) 72.7, C(16)) and a tertiary Me group (δ(H) 1.47) in compound **2**. Therefore, it was supposed that **2** was derived from **1** by hydroxylation at the exocyclic C=C bond, which was confirmed by the mass difference of $\Delta m/z = 18$ and the HMBC spectrum. In the HMBC spectrum of **2**, correlations of Me(17) (δ(H) 1.47) with C(12), C(15), and C(16) were observed (*Fig. 1*). The relative configuration of the OH group attached at C(16) was determined

by a ROESY experiment (*Fig. 2*). The Me(17) signal showed ROESY correlations with H–C(12), suggesting β-orientation for Me(17). Therefore, the structure of spiramilactam B (**2**) was determined as shown in **2**.

Experimental Part

General. MPLC: *Büchi Pump Module C-605*, *Büchi Pump Manager C-615*, and *Büchi Fraction Collector C-660*. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; silica gel *H*, *Qingdao Marine Chemical Ltd.Co.*), or *Sephadex LH-20* (*Pharmacia*). TLC: silica-gel plates; visualization by spraying with *Dragendorff*'s reagent. Optical rotations: *JASCO DIP-370* digital polarimeter. IR Spectra: *Bio-Rad FTS-135* spectrometer, KBr pellets; in cm^{-1}. NMR Spectra: *Bruker AM-400* instrument (^1H: 400, ^{13}C: 100 MHz) or *Bruker DRX-500* instrument (^1H: 500, ^{13}C: 125 MHz); δ in ppm rel. to TMS as internal standard, *J* in Hz. EI-MS: *VG Auto Spec-3000* mass spectrometer; in *m*/*z*. HR-ESI-MS: *API Qstar Pulsar LC*/*TOF* instrument.

Plant Material. The whole plants of *S. japonica* var. *ovalifolia* were collected in July 2007 from Songming, Yunnan Province, P. R. China, and identified by Dr. *Zhao-Yang Zhang* of Kunming Botanical Garden. A voucher specimen was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried whole plants of *S. japonica* var. *ovalifolia* (20 kg) were grounded and extracted with MeOH under reflux (3×50 l, each 3 h). The extracts were condensed *in vacuo* to afford a crude mixture which was dissolved in 3% HCl (5 l) soln. and filtered. The acidic soln. was basified with 5% aq. NaOH to pH 11 and then extracted with $CHCl_3$. Evaporation of $CHCl_3$ gave a crude alkaloid mixture (130 g) which was subjected to CC (SiO_2; petroleum ether (PE)/AcOEt/Et_3N 40:10:1 to 10:10:1) to five fractions (*Fr. I–V*). Repeated separation of *Fr. I* and *II* over SiO_2 by MPLC (PE/AcOEt/Et_3N 80:2:1 to 8:2:1) gave spiramines A (200 mg), B (150 mg), C (140 mg), and D (110 mg), and a mixture of spiramines A and B (4.5 g), as well as a mixture of spiramines C and D (2.3 g). *Fr. V* (3.0 g) was purified by repeated MPLC (silica gel *H*, PE/AcOEt/Et_3N 4:1:0.2 to 1:1:0.2) and *Sephadex LH-20* ($CHCl_3$/MeOH 1:1) to yield **1** (40 mg) and **2** (21 mg).

Spiramilactam A (*=(6α,7α)-6-Hydroxy-21-(2-hydroxyethyl)-4-methyl-7,20-epoxyatid-16-en-19-one*; **1**). White powder. $[\alpha]_D^{18} = -83.3$ ($c = 0.57$, MeOH). IR (KBr): 3420, 2930, 2871, 1735, 1632, 1470, 1316, 1063, 1011, 885. ^1H- and ^{13}C-NMR: *Table*. FAB-MS (pos.): 374 (100, $[M + H]^+$). HR-ESI-MS: 374.2326 ($[M + H]^+$, $C_{22}H_{32}NO_4^+$; calc. 374.2326).

Spiramilactam B (*=(6α,7α)-6,16-Dihydroxy-21-(2-hydroxyethyl)-4-methyl-7,20-epoxyatidan-19-one*; **2**). Colorless gum. $[\alpha]_D^{18} = -53.2$ ($c = 0.85$, MeOH). IR (film): 3406, 2985, 2878, 1593, 1474, 1346, 1066, 1011, 807, 765. ^1H- and ^{13}C-NMR: *Table*. EI-MS: 391 (25, M^+), 373 (29, $[M - H_2O]^+$), 363 (100), 348 (83), 320 (33), 256 (38), 243 (88), 215 (36), 196 (95), 157 (35), 149 (55), 95 (57), 69 (66). HR-ESI-MS: 392.2440 ($[M + H]^+$, $C_{22}H_{34}NO_5^+$; calc. 392.2431).

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