# New Diterpene Alkaloids from the Roots of *Spiraea japonica*

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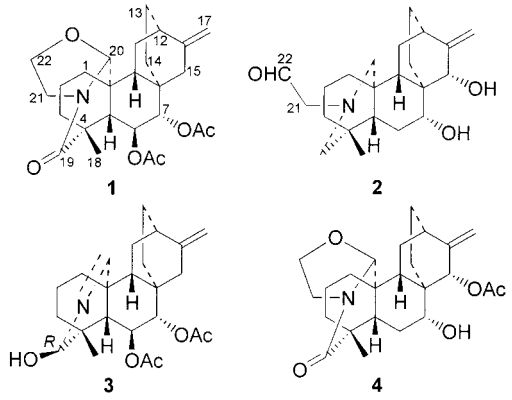
Chemical investigation on an ethanol extract from the roots of *Spiraea japonica* var. *acuta* resulted in the isolation of three new diterpene alkaloids named spiramide (**1**) and spiratine A (**2**) and spiratine B (**3**). Structures of **1**−**3** were elucidated primarily on the basis of 1D and 2D NMR experiments.

*Spiraea japonica* L. (Rosaceae) and its varieties are widespread in Yunnan Province, People's Republic of China. The young leaves, fruits, and roots of these plants have been used as diuretic, detoxicant, and analgesic agents and for the treatment of inflammation, cough, headache, and toothache in traditional Chinese medicine.[1,2] The *Spiraea japonica* complex is shown to contain atisine- and hetisine-type diterpene alkaloids,[3−14] and some of them significantly inhibited rabbit platelet aggregation induced by platelet activating factor and arachidonic acid in vitro and ex vivo.[15] Others, such as spiramine T, exhibited protective effects on cerebral ischemia-reperfusion injury in gerbils.[16] In a search for additional active components from the plant, a large-scale extraction of the roots of *S. japonica* var. *acuta* was conducted, and three new atisine-type diterpene alkaloids, named spiramide (**1**), spiratine A (**2**), and spiratine B (**3**), were isolated from the neutral fraction.




Compound **1** was determined to have the molecular formula $C_{26}H_{35}NO_6$ based on the $[M^+]$ ion at *m/z* 457.2469 (HREIMS) and the presence of carbonyl (1743 $cm^{-1}$) and carbon−carbon double-bond absorptions (1658 $cm^{-1}$) in its IR spectrum. Inspection of the NMR data ($^1H$, $^{13}C$, DEPT, HMQC, and HMBC) indicated an atisine-type amide.[3−8] The $^1H$ NMR spectrum of **1** showed signals for a tertiary alkyl methyl ($\delta$ 1.15) and two acetoxyl methyl ($\delta$ 1.92 and 1.98) groups. The locations of acetoxyl groups were determined by inspecting the HMBC data. The two oxygen-substituted methine carbons at $\delta$ 69.4 and 79.9 correlated with methine protons at $\delta$ 5.33 and 4.76, respectively, in the HMQC experiment, and those methine protons exhibited correlation peaks ($J$ = 9.9 Hz) in the $^1H-^1H$ COSY spectra, revealing that they were on vicinal carbons. The HMBC experiment assigned the resonance at $\delta$ 5.33 to H-6 through $^1H-^{13}C$ long-range correlations between this proton and C-4, C-8, C-10, and the acetoxyl carbonyl carbon of $\delta$ 170.4. The HMBC experiment further correlated H-7 ($\delta$ 4.76) to C-5, C-9, C-14, and C-15, and the acetoxyl carbonyl carbon ($\delta$ 169.8), confirming its vicinal relationship to the $\delta$ 5.33 proton (H-6), and placing the two acetoxyl groups at C-6 and C-7, respectively.

Spiramine S (**4**)[8] and **1** have similar carbon skeletons with a different pattern of substituents as revealed by comparison of their $^1H$ and $^{13}C$ NMR data, particularly the chemical shifts and coupling constants of H-6 and H-7 in **1** and of H-7 and H-15 in **4**. In the HMBC spectrum of **1**, a set of $^1H-^{13}C$ long-range correlations between H-20, H-21, H-22 and related carbons such as C-19, C-20, C-21, and C-22 indicated the presence of an oxazolidine ring. The existence of a C-19 carbonyl group was confirmed by the long-range correlations between the proton signals of H-3, H-5, H-18, H-20, H-21 and the carbonyl carbon signal at $\delta$ 172.7 (C-19) in the HMBC spectrum. The stereochemistry of all chiral centers in **1** was determined on the basis of the absolute structure of spiramine A,[3] which was elucidated on the basis of chemical reactions and single-crystal X-ray analysis. Thus, the structure of **1** was elucidated to be as shown.

Compound **2** had molecular formula $C_{22}H_{33}NO_3$ on the basis of HREIMS (*m/z* 359.2448 $[M]^+$, calcd 359.2460). Inspection of the NMR data ($^1H$, $^{13}C$, DEPT, HMQC, HMBC, and $^1H-^1H$ COSY) indicated an atisine-type alkaloid. The $^{13}C$ NMR and DEPT spectra of **2** showed 22 carbon signals including one methyl, 11 methylene, six methine, and four quaternary carbons. The $^{13}C$ NMR signal at $\delta$ 110.2 (t) revealed an exo-methylene. In the HMBC, the C-17 methylene protons at $\delta$ 5.05 and 4.04 showed correlations with the carbons at $\delta$ 36.5 (C-12), 80.1 (C-15), and 154.8 (C-16), suggesting oxygenation at C-15. This was also supported by the $\gamma$-effect of a hydroxyl at C-15 to C-14 (shifted to lower frequency by 9.3 ppm as compared with **1**). The $^1H-^{13}C$ long-range correlations between the proton at $\delta$ 8.73 (H-22) and the carbons at $\delta$ 60.5 (C-19) and 65.0 (C-21), and between $\delta$ 3.81 (H-19) and $\delta$ 24.8 (C-18), 34.2 (C-4), 41.6 (C-3), 65.0 (C-21), and 182.9 (C-22) indicated an aldehyde at C-22. Thus, the structure of **2** was determined to be as shown.

HREIMS determined the molecular formula of **3** to be $C_{24}H_{33}NO_5$ (*m/z* 415.2359 $[M]^+$, calcd 415.2359). Its $^1H$ and

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$^{13}$C NMR spectra were similar to those of spiramine Z,[13] suggesting that **3** was an atisine-type alkaloid.[3−8] The difference between the $^{13}$C NMR spectra of **3** and spiramine Z was the lack of oxygen-substituted ethyl signals in **3**, indicating that **3** was an analogue of spiramine Z. Assignment of the *R*-configuration for C-19 in **3** was determined on the basis of the $^{13}$C NMR signal at $\delta$ 87.8 (ca. $\delta$ 95 for C-19 in *S*-form in spiramine A[3] and ca. $\delta$ 91 for C-19 in *R*-form in spiramine B[3]). Thus the structure of spiratine B was characterized as **3**, which was also comfirmed by 2D NMR (HMQC, HMBC, and $^1$H−$^1$H COSY).

## Experimental Section

**General Experimental Procedures.** The IR spectra were measured on a Perkin-Elmer-577 spectrophotometer. The optical rotations were measured with a Horiba Sepa-300 polarimeter. MS were performed on an Autospec-3000 spectrometer at 70 eV. The 1D and 2D NMR spectra were recorded on Brucker AM-400 and DRX-500 spectrometers, respectively.

**Plant Material.** The dry roots of *Spiraea japonica* var. *acuta* (200 kg) were collected in Lijiang, Yunnan. A voucher specimen was deposited in Kunming Institute of Botany, Kunming, China.

**Extraction and Isolation.** The 95% EtOH extract of the roots of *S. japonica* var. *acuta* (200 kg) gave a crude extract, which was separated into basic and neutral parts as described previously.[13] The neutral part (1000 g) was chromatographically separated over silica gel to afford 80 fractions. Further chromatography of fraction 17 over silica gel produced one new diterpene amide (**1**) (23 mg). A portion (3.0 g) of fraction 23 (20.2 g) was subjected to column chromatography over silica gel (petroleum ether−acetone−diethylamine, 40:10:1, 30:10:1, 20:10:1) to give the following fractions: A (2100 mg), B (120 mg), and C (183 mg). Fraction C was chromatographed over Sephadex LH-20 eluting with MeOH to afford compound **2** (65 mg). Fraction B was further purified on a silica gel column eluted with $CHCl_3$−MeOH (85:15) to yield compound **3** (52 mg).

**Spiramide** (**1**): amorphous powder; $[\alpha]^{26}_D$ −63.40 (*c* 4.2, $CHCl_3$); IR (KBr) $\nu_{max}$ 3460, 3067, 2952, 2874, 1743, 1658, 1469, 1379, 1252, 1099, 1032, 975, 883 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 5.33 (1H, dd, $J$ = 9.9, 11.6 Hz, H-6), 5.06 (1H, s, H-20), 4.77 (1H, d, $J$ = 1.7 Hz, H-17), 4.76 (1H, d, $J$ = 9.9 Hz, H-7), 4.60 (1H, d, $J$ = 1.7 Hz, H-17), 4.15 (1H, ddd, $J$ = 3.7, 8.2, 8.2 Hz, H-22), 3.97 (1H, ddd, $J$ = 8.2, 8.2, 11.2 Hz, H-21), 3.84 (1H, dt, $J$ = 8.2, 8.2 Hz, H-22), 3.29 (1H, ddd, $J$ = 3.7, 8.2, 11.2 Hz, H-21), 2.41 (1H, br dd, $J$ = 3.2, 13.3 Hz, H-1), 2.25 (1H, br s, H-12), 2.20 (1H, br d, $J$ = 16.2 Hz, H-15), 2.09 (1H, ddd, $J$ = 2.4, 7.2, 13.5 Hz, H-11), 1.98 (1H, br d, $J$ = 16.2 Hz, H-15), 1.98 (3H, s, *O*-6-CO*CH*$_3$), 1.92 (3H, s, *O*-7-CO*CH*$_3$), 1.89 (1H, br d, $J$ = 12.8 Hz, H-14), 1.84 (1H, d, $J$ = 11.6 Hz, H-5), 1.78 (1H, m, H-3), 1.73 (1H, m, H-11), 1.65 (1H, br d, $J$ = 12.1 Hz, H-13), 1.60 (1H, dd, $J$ = 6.5, 12.8 Hz, H-14), 1.52 (1H, dd, $J$ = 7.8, 12.1 Hz, H-13), 1.48 (1H, m, H-9), 1.46 (1H, m, H-2), 1.43 (1H, m, H-3), 1.39 (1H, m, H-2), 1.15 (3H, s, H-18), 0.95 (1H, ddd, $J$ = 4.6, 4.8, 13.3 Hz, H-1); $^{13}$C NMR ($CDCl_3$, 125 MHz) $\delta$ 172.7 (s, C-19), 170.4 (s, *O*-7-*CO*CH$_3$), 169.8 (s, *O*-6-*CO*CH$_3$), 149.3 (s, C-16), 106.6 (t, C-17), 87.9 (d, C-20), 79.9 (d, C-7), 69.4 (d, C-6), 64.7 (t, C-22), 53.1 (d, C-5), 47.4 (d, C-9), 45.5 (t, C-15), 43.0 (s, C-4), 42.5 (t, C-3), 41.5 (s, C-10), 41.5 (t, C-21), 38.3 (s, C-8), 36.0 (d, C-12), 34.3 (t, C-1), 28.8 (t, C-11), 26.2 (t, C-13), 24.6 (q, C-18), 24.1 (t, C-14), 21.3 (q, *O*-6-CO*CH*$_3$), 20.8 (t, C-2), 20.6 (q, *O*-7-CO*CH*$_3$); EIMS *m*/*z* 457 [M]$^+$ (100), 429 (4), 414 (14), 397 (42), 338 (54), 284 (41), 256 (53), 238 (59), 121 (41), 91 (27), 72 (53); HREIMS *m*/*z* 457.2469 (calcd for $C_{26}H_{35}NO_6$, 457.2464).

**Spiratine A** (**2**): amorphous powder; $[\alpha]^{24}_D$ −6.25 (*c* 1.0, $CH_3OH$); IR (KBr) $\nu_{max}$ 3387, 2934, 2875, 1671, 1458, 1102, 1076, 999, 907 cm$^{-1}$; $^1$H NMR ($CD_3OD$, 400 MHz) $\delta$ 8.73 (1H, s, H-22), 5.05 (1H, br s, H-17), 4.19 (1H, m, H-21), 4.16 (1H, m, H-21), 4.04 (1H, m, H-20), 4.04 (1H, br s, H-17), 4.02 (1H, s, H-15), 3.96 (1H, m, H-20), 3.81 (1H, m, H-19), 3.76 (1H, m, H-7), 3.75 (1H, m, H-19), 2.42 (1H, d, $J$ = 2.6 Hz, H-12), 2.02 (1H, m, H-1), 1.99 (1H, m, H-14), 1.82 (1H, m, H-11), 1.79 (1H, m, H-6), 1.79 (1H, m, H-11), 1.74 (1H, m, H-3), 1.73 (2H, m, H-13), 1.67 (1H, m, H-9), 1.65 (1H, m, H-2), 1.59 (1H, m, H-5), 1.58 (1H, m, H-1), 1.48 (1H, m, H-3), 1.36 (1H, m, H-2), 1.17 (1H, m, H-14), 1.08 (3H, s, H-18), 1.05 (1H, m, H-6); $^{13}$C NMR ($CD_3OD$, 100 MHz) $\delta$ 182.9 (d, C-22), 154.8 (s, C-16), 110.2 (t, C-17), 80.1 (d, C-15), 77.2 (d, C-7), 65.0 (t, C-21), 60.5 (t, C-19), 58.6 (t, C-20), 47.0 (s, C-10), 45.8 (d, C-9), 43.9 (d, C-5), 41.9 (s, C-8), 41.6 (t, C-3), 36.5 (d, C-12), 35.2 (t, C-1), 34.2 (s, C-4), 28.7 (t, C-6), 28.2 (t, C-11), 26.1 (t, C-13), 24.8 (q, C-18), 20.0 (t, C-2), 14.8 (t, C-14); EIMS *m*/*z* 359 [M]$^+$ (11), 342 (7), 328 (100), 300 (9), 91 (12); HREIMS *m*/*z* 359.2448 (calcd for $C_{22}H_{33}NO_3$, 359.2460).

**Spiratine B** (**3**)**:** amorphous powder; $[\alpha]^{25}_D$ +129.48 (*c* 5.0, $CHCl_3$); IR (KBr) $\nu_{max}$ 3468, 3070, 2934, 2874, 1747, 1651, 1464, 1373, 1247, 1095, 1030, 975, 883 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 7.76 (1H, br s, H-20), 5.20 (1H, dd, $J$ = 9.5, 11.0 Hz, H-6), 5.11 (1H, s, H-19), 4.79 (1H, br s, H-17), 4.74 (1H, d, $J$ = 9.5 Hz, H-7), 4.62 (1H, br s, H-17), 2.35 (1H, br s, H-12), 2.27 (1H, br d, $J$ = 17.1 Hz, H-15), 2.05 (1H, m, H-3), 2.03 (3H, s, *O*-6-CO*CH*$_3$), 2.00 (1H, br d, $J$ = 17.1 Hz, H-15), 1.96 (3H, s, *O*-7-CO*CH*$_3$), 1.85 (1H, m, H-11), 1.83 (1H, m, H-11), 1.76 (1H, d, $J$ = 11.5 Hz, H-1), 1.67 (1H, m, H-14), 1.66 (1H, m, H-13), 1.65 (1H, d, $J$ = 11.0 Hz, H-5), 1.64 (1H, m, H-14), 1.62 (1H, m, H-9), 1.45 (2H, m, H-2), 1.17 (1H, m, H-1), 1.09 (1H, m, H-13), 1.06 (1H, m, H-3), 0.98 (3H, s, H-18); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 170.5 (s, $OCOCH_3$ of C-7), 170.3 (s, $OCOCH_3$ of C-6), 163.4 (d, C-20), 149.4 (s, C-16), 105.9 (t, C-17), 87.8 (d, C-19), 79.5 (d, C-7), 69.1 (d, C-6), 51.8 (d, C-5), 45.7 (d, C-9), 43.8 (s, C-10), 41.4 (t, C-15), 38.0 (s, C-8), 36.9 (s, C-4), 35.9 (t, C-3), 35.7 (d, C-12), 34.4 (t, C-1), 27.8 (t, C-11), 26.6 (q, C-18), 25.7 (t, C-13), 21.6 (t, C-14), 21.4 (q, $OCOCH_3$ of C-7), 20.7 (q, $OCOCH_3$ of C-6), 19.4 (t, C-2); EIMS *m*/*z* 415 [M]$^+$ (32), 355 (35), 340 (3), 312 (14), 296 (100), 252 (10), 91 (22); HREIMS *m*/*z* 415.2359 (calcd for $C_{24}H_{33}NO_5$, 415.2359).

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