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DITERPENE ALKALOIDS FROM ROOTS OF *SPIRAEA JAPONICA*

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Key Word Index—*Spiraea japonica* var. *incisa*; Rosaceae; roots; atisine-type diterpene alkaloids; spiramines P, Q and R.

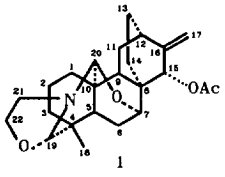
Abstract—Three new diterpene alkaloids, namely spiramines P, Q and R, together with the known compounds, spiramines A and B, were isolated from roots of *Spiraea japonica* var. *insica Yu*. Their structures were elucidated by chemical and spectroscopic means.

INTRODUCTION

In previous papers [1–6] we reported 15 new atisine-type diterpene alkaloids and one new atisane-type diterpenoid isolated from roots of *Spiraea japonica* var. *acuminata* (Franch); some of them showed anti-inflammatory activities [7]. Further investigation of the constituents of roots of *Spiraea japonica* var. *incisa Yu* has yielded three new atisine-type diterpene alkaloids, together with two known compounds, spiramines A (**1**) and B (**2**). We present here spectroscopic and chemical evidence for the structures of the three new alkaloids designated as spiramines P (**3**), Q (**4**) and R (**5**).

RESULTS AND DISCUSSION

Spiramine P (**3**) was determined as $C_{22}H_{33}O_4N$ on the basis of its mass spectrum and elemental analysis. The basic skeleton was established by comparing its ^1H NMR and ^{13}C NMR shift values with those of spiramine C (**6**) [2]. Alkaloid **3** showed the presence of two methyl groups [δ_H 1.16 and 1.35 (each 3H, *s*): δ_C 22.5 and 31.3 (2 × Me)], a secondary hydroxyl group [3420 cm^{-1}; δ_H 4.63 (1H, *br s*, *m*, H-15); δ_C 74.0 (C-15)], an oxazolidine ring and an ether linkage between C-7 and C-20 [δ_H 3.30 (1H *d*, *J* = 5Hz, H-7), 3.84 (1H, *s*, H-19), 4.52 (1H, *s*, H-20), 3.27, 3.59 (each 1H, *m*, 2H-22), 3.40, 3.55 (each 1H, *m*, 2H-21);



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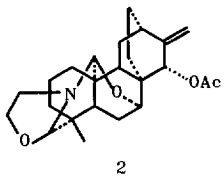

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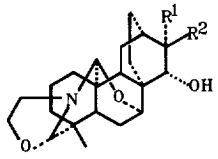
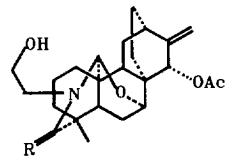
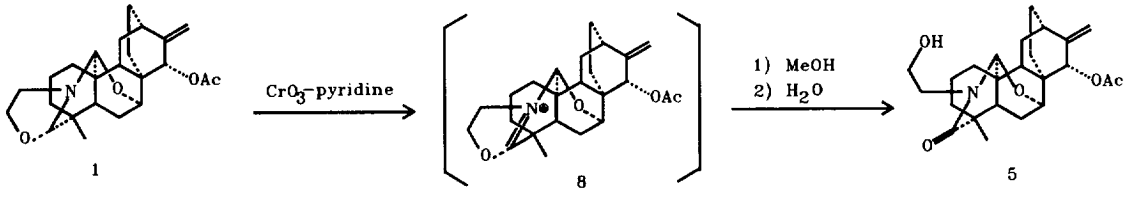


3, R^1=Me, R^2=OH
4. R^1=OH, R^2=Me
6. R^1, R^2= $=CH_2$



5. R= O
7. R=H,H



1 CrO_3–pyridine 8 1) MeOH 2) H_2O 5

δ_C 69.5 (C-7), 95.0 (C-19), 85.2 (C-20), 51.0 (C-21), 63.2 (C-22)]. The closely similar ^{13}C NMR shift values for **3** and **6** indicated the S-configuration of C-19 in the oxazolidine ring of **3**, while the α-hydroxyl group at C-15 was presumed from the ^{13}C NMR shift values of C-7 and C-15, which were very close to those of **6** [2]. Comparing the ^{13}C NMR data of **3** with those of spiramines [1–6], there was no signal for an exo-methylene group for **3**, while a tertiary carbon (δ_C 72.6) and a methyl carbon (δ_C 31.3) which were designated as C-16 and C-17 respectively, indicated that a hydroxyl group is located at C-16 in **3**. Namely, **3** was derived from **6** by hydroxylation at the exocyclic double bond.

Spiramine Q (**4**) had the same skeleton and functional groups as **3**. Mass spectrometry, ^1H NMR and ^{13}C NMR of **3** and **4** indicated they are a pair of epimers at C-16. The IR spectrum of **4** showed absorptions for intramolecular hydrogen bonding (3360, 3330 and 3250 cm^{-1}), which was distinguishable from that of **3**. In the ^1H NOESY spectrum of **3**, the signal for the proton at C-12 [δ_H 2.30 (1H, *dd*, J = 3, 12 Hz)] showed cross-peaks with protons of the methyl group at C-16 [δ_H 1.35 (3H, *s*)], which was different from the ^1H NOESY spectrum of **4**. Thus, these results indicated the configuration of C-16 in **3** to be *R*, that of **4**, *S*.

Spiramine *R* (**5**) was assigned as $C_{24}H_{33}O_5N$ for its molecular formula on the basis of mass spectral and elemental analysis. The basic skeleton of **5** was established by comparing its ^{13}C NMR shift values with those of spiramine F (**7**). These showed the presence of the following groups: a methyl group [δ_H 1.09 (3H, *s*); δ_C 21.1], an exo-methylene group [IR 1650 cm^{-1}; δ_H 5.01 (2H, *d*, J = 3 Hz); δ_C 114.7, 149.3], a secondary acetoxy group, IR 1725, 1230 cm^{-1}; δ_H 5.17 (1H, *br s*, H-15), 1.99 (3H, *s*); δ_C 74.0 (C-15), 171.0, 21.1 (OCOMe)], an ether linkage between C-7 an C-20 [δ_H 4.77 (1H, *d*, J = 2 Hz, H-20), 3.46 (1H, *d*, J = 4 Hz, H-7); δ_C 86.7 (C-20), 69.4 (C-7)], and a primary hydroxyl group [IR 3405 cm^{-1}; δ_H 3.90, 3.75 (each 1H, *m*, 2H-22); δ_C 61.8 (C-22)]. The structure of **5** is very close to that of **7** except that the former has a lactam moiety (IR 1615 cm^{-1}; δ_C 175.5). Oxidation with CrO_3 in pyridine [8], converted **1** to the intermediate immonium ion (**8**), which was followed by quenching with MeOH and water to give **5**. This result confirmed the structure of 5.

EXPERIMENTAL

General. Mps: uncorr. IR spectra were measured as KBr pellets, optical rotations in $CHCl_3$. ^1H and ^{13}C NMR and 2D NMR spectra were recorded in $CDCl_3$ using TMS as int. standard. EIMS were measured at 70 eV.

Plant material. Roots of *Spiraea japonica* var. *incisa Yu* were collected in Da-Li, Yunnan Province of China in July 1993.

Extraction and isolation of spiramines. Powdered dried roots (2 kg) were extracted with 75% EtOH under reflux (× 3). After removal of solvent by evapn, the combined extracts were dissolved in 0.4 N HCl (400 ml) and filtered. The HCl soln was extracted with benzene, then made basic with $NaHCO_3$ (pH 9–10) and extracted with $CHCl_3$. Evapn of $CHCl_3$ gave 10 g of a basic fr. (0.5%).

The basic fr. (10 g) was chromatographed on a silica gel column eluting with petrol–Et_2NH (8:1 to 6:1) to give spiramine A (**1**) (50 mg, 0.028%) and B (**2**) (25 mg, 0.0013%), which were identified by comparison with authentic samples by mp, IR and ^1H NMR. Continued elution with petrol–Et_2NH (6:1 to 2:1) yielded four frs in increasing order of polarity.

Fr. 1 was chromatographed on a silica gel flash column eluting with petrol–Et_2NH (8:1) to give spiramine Q (**4**) (50 mg, 0.0025%) as needles, mp 197–199°. $[\alpha]_D$ −70° ($CHCl_3$; *c* 0.84). EIMS *m/z* (rel. int.) 375 $[M]^+$ 319 (20), 288 (10), 180 (30). Anal. calcd. for $C_{22}H_{33}O_4N$: C, 69.78; H, 8.90. Found: C, 70.40; H, 8.80. IR ν_{max} cm^{-1}: 3360, 3330, 3250, 1460, 1090, 1040. ^1H NMR (400 MHz): δ 1.15 (3H, *s*, 3H-18), 1.28 (3H, *s*, 3H-17), 2.70 (1H, *br s*, OH), 3.18, 3.60 (each 1H, *m*, 2H-22), 3.28 3.46 (each 1H, *m*, 2H-21), 3.30 (1H, *d*, J = 5 Hz, H-7), 3.50 (1H, *br s*, OH), 3.84 (1H, *s*, H-19), 4.52 (1H, *s*, H-20), 4.56 (1H, *br s*, H-15). ^{13}C NMR (100.6 MHz): δ 40.9 (C-1), 22.7 (C-2), 47.3 (C-3), 35.2 (C-4), 42.2 (C-5), 27.3 (C-6), 69.4 (C-7), 36.3 (C-8), 40.8 (C-9), 35.6 (C-10), 29.1 (C-11), 55.9 (C-12), 23.5 (C-13), 20.3 (C-14), 73.9 (C-15), 73.9 (C-16), 30.1 (C-17), 22.7 (C-18), 95.1 (C-19), 85.5 (C-20), 51.0 (C-21), 63.1 (C-22).

Fr. 2 was recrystallized from MeOH to give spiramine P (**3**) (30 mg, 0.0015%) as needles, mp 237–239°. $[\alpha]_D$ −49° ($CHCl_3$; *c* 0.81). EIMS *m/z* (rel. int.) 375 $[M]^+$ 345 (18), 319 (18), 278 (10), 180 (30). Anal. calcd. for $C_{22}H_{33}O_4N$: C, 70.25; H, 8.86. Found: C, 70.40; H, 8.80. IR ν_{max} cm^{-1}: 3420, 1520, 1200, 1120. ^1H NMR (400 MHz): δ 1.16 (3H, *s*, 3H-18), 1.35 (3H, *s*, 3H-17), 2.30 (1H, *dd*, J = 3, 12 Hz, H-12), 3.27, 3.59 (each 1H, *m*, 2H-22), 3.40 3.55 (each 1H, *m*, 2H-21), 3.30 (1H, *d*, J = 5 Hz, H-7), 3.84 (1H, *br s*, H-19), 4.52 (1H, *s*, H-20), 4.63 (1H, *br s*, H-15). ^{13}C NMR (100.6 MHz): δ 40.7 (C-1), 22.8 (C-2), 47.9 (C-3), 35.3 (C-4), 43.1 (C-5), 26.6 (C-6), 69.5 (C-7), 36.7 (C-8), 39.2 (C-9), 35.6 (C-10), 29.2 (C-11), 56.1 (C-12), 21.3 (C-13), 20.3 (C-14), 74.0 (C-15), 72.6 (C-16), 31.3 (C-17), 22.5 (C-18), 95.1 (C-19), 85.2 (C-20), 51.0 (C-21), 63.2 (C-22).

Fr. 3 was chromatographed on a silica gel flash column eluting with petrol–Et_2O (1:4) to give spiramine R (**5**), which was recrystallized from EtOAc to give needles (100 mg, 0.005%), mp 190–192°. $[\alpha]_D$ −180° ($CHCl_3$; *c* 0.39). EIMS *m/z* (rel. int.) 415 $[M]^+$ 384 (25), 372 (70), 312 (10). Anal. calcd. for $C_{24}H_{33}O_5N$: C, 69.34; H, 8.08. Found: C, 69.40; H, 7.95. IR ν_{max} cm^{-1}: 3405, 1720, 1650, 1615, 1260. ^1H NMR (400 MHz): δ 1.09 (3H, *s*, 3H-18), 1.99 (3H, *s*, OCOMe), 2.42 (1H, *m*, H-12), 3.22, 3.60 (each 1H, *m*, 2H-21), 3.46 (1H, *d*, J = 4 Hz, H-7), 3.75, 3.90 (each 1H, *m*, 2H-22), 4.77 (1H, *d*, J = 2 Hz, H-20), 5.01 (2H, *d*, J = 3 Hz, 2H-17), 5.17 (1H, *br s*, *H*-15). ^{13}C NMR (100.6 MHz): δ 39.4 (C-1), 20.6 (C-2), 29.4 (C-3), 44.4 (C-4), 45.2 (C-5), 25.3 (C-6), 69.4 (C-7), 40.7 (C-8), 45.2 (C-9), 33.2 (C-10), 25.6 (C-11), 36.5 (C-12), 25.6 (C-13), 19.8 (C-14), 74.0 (C-15), 149.3 (C-16), 114.7 (C-17), 21.1 (C-18), 175.5 (C-19), 86.7 (C-20), 51.6 (C-21), 61.8 (C-22), 171.0 (OCOMe), 21.1 (OCOMe).

Oxidation of spiramine A (**1**). To 50 ml of pyridine was added 300 mg of CrO_3 at 0°. To this soln was added 500 mg of spiramine A (**1**) and the reaction mixt. stirred for 4 hr at room temp. The reaction mixt. was quenched with MeOH and poured into ice-H_2O and extracted with $CHCl_3$. The $CHCl_3$ soln was washed with brine and dried with Na_2SO_4. Evapn of $CHCl_3$ and pyridine gave a crude product which was subjected to silica gel flash CC eluting with petrol–Et_2O (1:1 to 1:4) to afford **5** (200 mg, 40%), which was recrystallized from EtOAc to give needles. Its mp, IR, ^1H and ^{13}C NMR were identical to those of an authentic specimen.

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