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THE STRUCTURES OF FOUR NEW DITERPENE ALKALOIDS: SPIRASINES XII-XV

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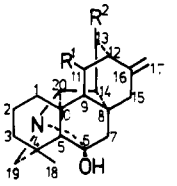
ABSTRACT.—The structures and absolute configurations of four minor diterpene alkaloids in *Spiraea japonica*, spirasines XII [**1**], XIII [**2**], XIV [**3**], and XV [**4**], were established by spectroscopic and chemical methods.

In a previous paper (1) we reported the structure of spirasine X, a diterpene alkaloid isolated from *Spiraea japonica* L. var. *fortunei* (Pl.) Rehd. (Rosaceae). We now present the structural elucidation of another four new diterpene alkaloids of this series designated as spirasines XII [**1**], XIII [**2**], XIV [**3**], and XV [**4**] isolated from the same plant.

All four alkaloids have a masked keto group at C-6. *N*-Methylation restores the C-6 keto group, which gives rise to an acid-quenchable negative Cotton effect at about 290 nm, thus allowing the assignment of absolute configuration as shown. Then the sign of the Cotton effect at about 305 nm can be used to discriminate C-11 and C-13 carbonyl groups (2-4).



1 R^1==O, R^2=αOH
2 R^1==O, R^2=βOH
3 R^1=H, R^2=αOH
4 R^1=H, R^2=βOH
5 R^1=H, R_2==O

Spirasine XII [**1**], $C_{20}H_{25}NO_3$ (M^+ 327.1809, calcd 327.1834), had mp 226-228° (crystallized from CH_2Cl_2/MeOH), $[\alpha]^{17}D$ +17.9 (*c* 1.2, $CHCl_3$). Its nmr spectra showed the presence of an exocyclic methylene group (δ_C 142.8 s and δ_C 111.3 t) and a methyl group (δ_H 1.32 3H, s), typical for a C_{20}-diterpene alkaloid. The ir spectrum of spirasine XII exhibited a keto group absorption at 1720 cm^{-1}, which was assigned to the C-11 position from a positive Cotton effect at 304 nm in the cd spectrum. The ^{13}C-nmr spectrum of spirasine XII (see Table 1) showed 20 peaks, most of which were close to those of spiradine A [**5**] and spirasine X [**1**]. The masked C-6 keto group was evidenced by δ_C 98.5 (s) and 3380 cm^{-1} absorptions. The other hydroxyl group was placed at the C-13 α position because of the δ_C 67.3 (d) and δ_H 4.12 (1H q, J=3.5, J= 10.0 Hz) values as compared with spirasine X (1). Double irradiation experiments on spirasine XII also supported this postulation. The peak at δ_H 4.12 ppm was found to couple with the doublet at 2.83 ppm (C-12) and the quartet at 2.40 ppm (C-14). The α configuration for the C-13 hydroxyl is in accord with the magnitude of the coupling constants. The structure **1** is, thus, assigned to spirasine XII.

Spirasine XIII [**2**] had mp 188-189° (from CH_2Cl_2), $[\alpha]^{17}D$ +25.7 (*c* 0.93, $CHCl_3$). Its molecular formula $C_{20}H_{25}NO_3$ was identical with spirasine XII (M^+ 327.1858, calcd 327.1834). The ir spectrum exhibited the following signals: 3420 (OH), 1710 (C=O), 1650 (C=C) cm^{-1}. The keto group was similarly assigned to C-

TABLE 1. ^{13}C-nmr Spectra of Spirasines XII [**1**], XIII [**2**], XIV [**3**], XV [**4**], and [**5**].[a]

Carbon atom	Compounds				
	1[b]	**2**[c]	**3**[c]	**4**[d]	**5**[c]
1	36.4	35.3	35.4	35.1	35.6
2	19.8	19.0	18.6	18.3	19.1
3	27.3	28.6	24.2	26.1	26.7
4	38.4	37.6	37.2	37.3	37.6
5	62.3	60.7	59.1	58.2	60.1
6	98.5	98.9	99.5	101.5	99.5
7	44.3	43.3	42.5	41.2	44.1
8	46.6	45.1	43.1	41.2	44.1
9	65.7	73.9	48.8	47.5	48.2
10	51.2	52.3	49.4	49.4	50.4
11	210.8	209.2	21.6	23.3	22.7
12	64.3	61.9	48.2	53.7	52.9
13	67.3	67.4	65.9	69.3	210.4
14	51.7	56.0	41.6	41.1	61.6
15	33.9	32.8	33.0	32.3	33.6
16	142.8	137.6	147.1	143.1	142.6
17	111.3	114.9	106.6	109.4	110.9
18	31.2	30.5	29.6	29.4	29.0
19	61.9	61.1	58.0	56.7	62.4
20	68.9	72.0	69.0	71.0	68.6

[a]Chemical shifts in ppm downfield from TMS.
[b]In C_5D_5N.
[c]In $CDCl_3$.
[d]In $CDCl_3$-CD_3OD.

11 based on a positive Cotton effect at 304 nm. By comparison of ^{13}C-nmr data with spirasine XII [**1**], one of the hydroxyl groups can be assigned at C-6, supported by the signal at δ_C 98.9 (s). The other hydroxyl group was assigned as C-13β by a corresponding signal δ_C 67.4 (d) and δ_H 3.65 (d). The β-configuration as well as the location was confirmed by a 2D-nmr COSY experiment. Thus, the signal at δ_H 3.65 (1H, d, J=4.0 Hz) was coupled to the signal at δ_H 3.05 (1H, d, J=4.0 Hz) at C-12. The vicinal coupling between C-13H and C-14H was undetectably small with a dihedral angle of about 90°. Long-range couplings were revealed between 17-CH_2 (5.02, 4.97, br s) and 15-CH_2 (2.48, 2.38, br d) and also between the latter and 9-H (1.95, s). Other signals that can be assigned were 19-CH_2 (3.11, 2.39) and 7-CH_2 (1.92, 1.87). Based upon the above data, spirasine XIII was shown to have structure **2**.

Spirasine XIV [**3**] was obtained as colorless needles, mp 244-246° (from CH_2Cl_2/EtOAc), $[\alpha]^{17}D$ −18.8 (c=1.6, EtOH) with molecular formula $C_{20}H_{27}NO_2$ by hrms. The ^{1}H-nmr spectrum showed the following signals: δ_H 1.36 (3H, s), 2.08, 2.30 (each 1H, d, J=12.6 Hz), 3.96 (1H, br d, J=10.0 Hz), 4.62, 4.77 (each 1H, br s). Because the splitting pattern of the signal at δ_H 3.96 was very similar to those of spirasine X (1), spirasine XI (5), and spirasine XII, an α-OH at C-13 was suggested.

The ^{13}C-nmr spectrum of spirasine XIV [**3**] (Table 1) showed 20 peaks in agreement with the basic skeleton of spiradine A (5). The C-6 OH was assigned with δ_C 99.5. Hence, structure **3** is assigned to spirasine XIV.

Spirasine XV [**4**] had a molecular formula $C_{20}H_{27}NO_2$ identical with that of spirasine XIV, mp 156-158° (from CH_2Cl_2/EtOAc). Its spectral data were as follows: ir 3450-3200 (OH), 1665 (C=C) cm^{-1}; ^{1}H nmr δ_H 1.49 (3H, s), 4.85 (2H, br s). The ^{13}C-nmr spectrum of spirasine XV was very close to that of spirasine XIV [**3**]. Oxidation of both spirasine XIV and spirasine XV with CrO_3/pyridine (6) afforded the same

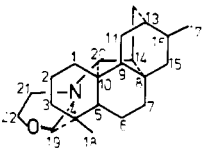
compound **5** with molecular ion at *m/z* 311. As expected, the uv spectrum of **5** exhibited the absorption of a β,γ-unsaturated ketone and gave rise to a negative Cotton effect at 304 nm in the cd spectrum. Hence, spirasine XV is the C-13 epimer of spirasine XIV.

Due to the presence of the 11-keto group, attempted oxidation of spirasines XII and XIII under similar conditions gave rise to a complex array of products that were not further studied.

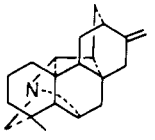
The stereochemistry of the C-13 OH groups also finds expression in the ^{13}C-nmr spectra. Here the preferential shielding of the γ-carbon that lies in closer proximity in space to the C-13 OH group can be readily seen. Comparison between spirasine XII and XIII showed that the signal of C-16 suffered an upfield shift of 4-5 ppm due to the presence of the β-hydroxyl group, and C-20 was affected to a similar extent by the α-hydroxyl group.

From the plant *S. japonica*, we have thus far isolated spiradine A and spiredine with known structures and fifteen new alkaloids designated as spirasines I to XV. This paper concludes the description of the new isolates. For ready reference, the structures of spirasines I to XI are shown in Table 2.

TABLE 2. Structures of Spirasines I to XI.



I, 9β-OH, 6-keto, $\Delta^{15(16)}$ [Sun *et al.* (7)] VI, 16β-OH, 6-keto [Sun *et al.* (9)]
II, 9β-OH, 6-keto, $\Delta^{16(17)}$ [Sun *et al.* (7)] VII, 9β, 16α-diOH, 6-keto [Sun *et al.* (7)]
III, 9β-OH, 6, 11-diketo, $\Delta^{16(17)}$ [Sun *et al.* (8)] VIII, 9β, 16β-diOH, 6-keto [Sun *et al.* (7)]
V, 16α-OH, 6-keto [Sun *et al.* (9)]



IV, 13-keto [Sun and Yu (5)], X, 13α-OH, 11-keto [Sun *et al.* (1)]
IX, 11-keto [Sun and Yu (5)] XI, 13α-OH [Sun and Yu (5)]

EXPERIMENTAL

GENERAL METHODS AND PLANT MATERIAL.—See Sun *et al.* (1).

EXTRACTION OF TOTAL ALKALOIDS.—Powdered roots of *S. japonica* (40 kg) were percolated with 800 liters 0.05 N HCl, and the percolate was run through a column of 34 kg wet resin (sulfonic type). After exchange, the resin was washed repeatedly on a suction filter with deionized H_2O, spread out, and air dried overnight. The resin was wetted with 10% NH_3 water until it contained 83% H_2O and continuously extracted in a specially designed extractor (10) with Et_2O under reflux for 8 h. White deposits of crude alkaloids (270 g) from the Et_2O extracts were collected by evaporation. Crude alkaloids (70 g) were developed on a dry silica column (2700 g) with a mixture of $CHCl_3$-MeOH (10:1). The last three sections (fractions 15-17) of the extruded column were combined and extracted to give 4.4 g of solid. The tlc showed five spots.

ISOLATION AND IDENTIFICATION OF THE ALKALOIDS.—The solid thus obtained (4.4 g) was chromatographed on a column of alumina (250 g) using a mixture of $CHCl_3$-MeOH (100:1) as the eluent in 50-ml fractions. Fractions 55-64 were combined (1 g) and rechromatographed on preparative silica plates with $CHCl_3$-MeOH (8:2.5). The four main bands were separately extracted with 50% MeOH in $CHCl_3$.

Spirasine XII [**1**] (band 2), 140 mg colorless needles; eims *m/z* 327 (M^+ 100%), 299 (90%), 282 (89%), 161 (33%); uv λ max (EtOH) (log ε) 305 (2.1); cd Δε 0 (270), +2.40 (304), sh, +2.03 (316), 0 (330); ^{1}H nmr (100 MHz) 1.32 (3H, s), 1.89 (2H, d, J=1.5 Hz), 2.02 (1H, d, J=2.0 Hz), 2.28 (1H, d, J=2.0 Hz), 2.40 (1H, q, J=2.0 Hz, J=10.0 Hz), 2.42 (1H, d, J=11.5 Hz), 2.83 (1H, d, J=3.5 Hz), 3.08 (1H, d, J=11.5 Hz), 4.12 (1H, q, J=3.5 Hz, J=10.0 Hz), 4.86 (1H, br s), 4.96 (1H, br s).

Spirasine XIII [**2**] (band 1), 80 mg; eims *m/z* 327 (M^+ 100%), 299 (95%), 282 (35%), 161 (26%); uv λ max (EtOH) (log ε) 305 (2.27); cd Δε 0 (261), +1.85 (304), sh. +1.77 (315), 0 (338); ^{1}H nmr (100 MHz) 1.38 (3H, s), 2.39, 3.11 (each 1H, d, J=12.0 Hz), 2.94 (1H, s), 3.05 (1H, d, J=4.0 Hz), 3.65 (1H, d, J=4.0 Hz), 5.02, 4.97 (each 1H, br s), 4.57 (OH, s).

Spirasine XIV [**3**] (band 4), 121 mg; eims *m/z* (ei) 313 (M^+ 100%); ir 3350, 1660, 890 cm^{-1}.

Spirasine XV [**4**] (band 3), 42 mg; $[\alpha]^{17}D$ 0 (EtOH); eims *m/z* 313 (M^+ 100%).

OXIDATION OF SPIRASINES XIV [**3**] AND XV [**4**].—Spirasine XIV [**3**] (10 mg) in anhydrous pyridine (0.5 ml) was treated with CrO_3/pyridine complex (40 mg), and the reaction mixture was kept at room temperature with stirring for 4 h. The mixture was filtered through an alumina column (1 g) and evaporated in vacuo to give 8 mg of **5**; ir 3150, 1715, 1650 cm^{-1}; uv λ max (EtOH) (log ε) 300 (2.4); cd Δε 0 (257), −5.18 (304), sh −4.91 (311), 0 (330). Spirasine XV also furnished **5** by oxidation.

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