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A 2D-NMR STRUCTURE DETERMINATION OF SPIRASINE X, A NEW DITERPENE ALKALOID FROM *SPIRAEA JAPONICA*

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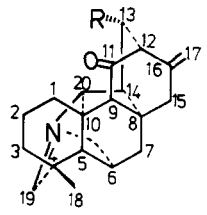
In previous papers (1-4) we have reported ten new structures of the spirasine series, all C_{20}-diterpene alkaloids, isolated from *Spiraea japonica* L. f. var. *fortunei* (Pl.) Rehd. (Rosaceae). We now report the structural elucidation of another new C_{20}-diterpene alkaloid designated as spirasine X [**1**].

Spirasine X [**1**], $C_{20}H_{25}NO_2$ (M^+ 311.1853, calc. 311.1885), mp 224-227°, $[\alpha]^{17}D+51.0°$ (*c* 1.5, $CHCl_3$), showed the presence of an exocyclic methylene group (δ_c 140.5 s, 112.4 t) and a methyl group (δ_H 1.01 3H, s) suggestive of a C_{20}-diterpene alkaloid. The ir spectrum of spirasine X exhibited ketone absorption at 1714 cm^{-1}, which was assigned to the C_{11} position based on the uv absorption of a β,γ-unsaturated ketone (ca. 300 nm) and on the positive Cotton effect at 304 nm ($\Delta\epsilon+2.95$). Here the use of the cd data involves the tacit assumption of "normal" absolute configuration for the alkaloid (5), whereas a C-13 keto group would have been implicated for an unprecedented antipodal skeleton. However, the absolute configuration is jus-



1 R=OH
2 R=H

TABLE 1. 2D-nmr Correlations of Spirasine X [**1**]

COSY				HECTOR (^1J)		
Correlations		Bond Separation	comments	Correlations		
H	to H			C	to H	Carbon atom
19	19	2	AB	33.5	1.22, 1.41	1
17a	17b	2		19.2	1.30, 1.60	2
15	17b	4	allylic	25.7	1.53	3
15	17a	4	allylic	37.9		4
15	15	2	AB	60.1	1.61	5
14	13	3		65.1	3.30	6
14	20	3		34.8	1.68, 1.87	7
13	12	3		44.9		8
9	12	4	planar "w"	67.2	3.25	9
7	7	2	AB	50.3		10
7	6	3		211.0		11
7	6	3		62.5	2.94	12
6	18	5	weak	67.7	4.24	13
				51.6	2.41	14
				33.7	2.26, 2.32	15
				140.5		16
				112.4	4.85, 5.02	17
				28.8	1.01	18
				62.5	2.47, 2.58	19
				65.1	2.04	20

tified by, inter alia, unequivocal δ_c values for C-9 and C-14 from 2D-nmr (see Table 1), which would be subject to unreasonable reversal for the antipodal case.

The signals δ_c 65.1 (d) and δ_H 3.30 (1H, br. s) excluded the possibility of accomodating a hydroxyl group at C-6 as compared with spiradine A (2). An α-OH at C-13 was evidenced by δ_c 67.7 (d) and the quartet at δ_H 4.24 (1H).

The ^{1}H-nmr spectrum of spirasine X

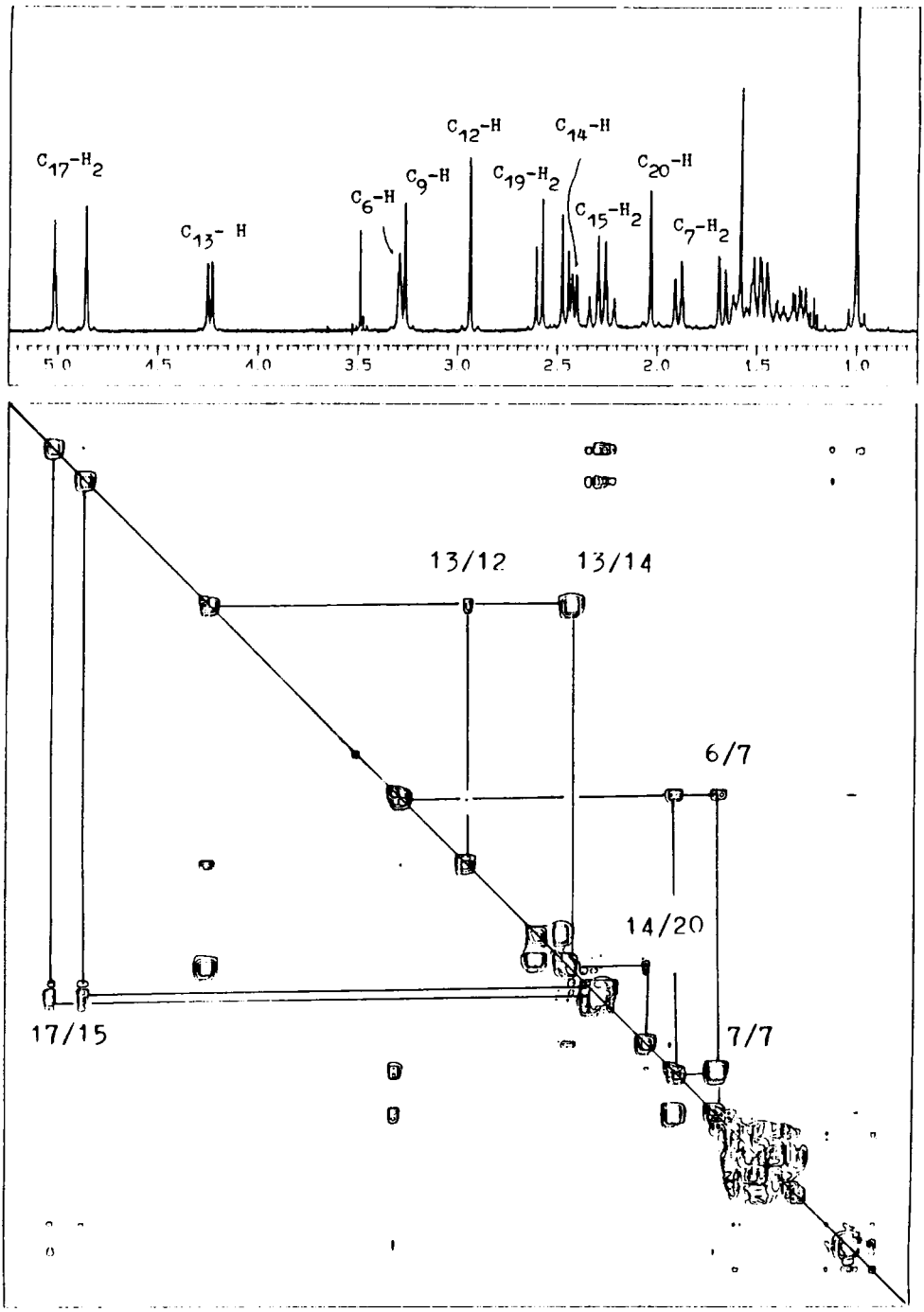


FIGURE 1. HOMCOR (COSY) spectrum of spirasine X [1]

(Table 1 and Figure 1) contains well-resolved peaks downfield of 1.50 ppm, attributable to olefinic, allylic, and neighboring protons (α and β) of oxygen and nitrogen functions.

As shown in the COSY spectrum in Figure 1, assignment of C-12 H and C-14 H, and, thus, also C-20 H, follows from coupling with C-13 H. The C-15 protons are characterized by allylic coupling. The relationship between C-6 H and C-7 2H is also readily discernible.

From the 2D-heteronuclear proton-carbon chemical shift correlation spectrum shown in Figure 2, C-3 and C-15 resonate at 25.7 and 33.7 ppm, respectively, in marked contrast with published shift data of C_{20}-diterpene alkaloids such as nominine (C-3 at 34.2) (6). The upfield shift of ca. 5 ppm for C-20 can be adequately accounted for by the presence of C-13 αOH giving rise to the γ-effect (7), as compared with spirasine IX [**2**].

The C-13 OH is designated as "α" here. To avoid any possible confusion,

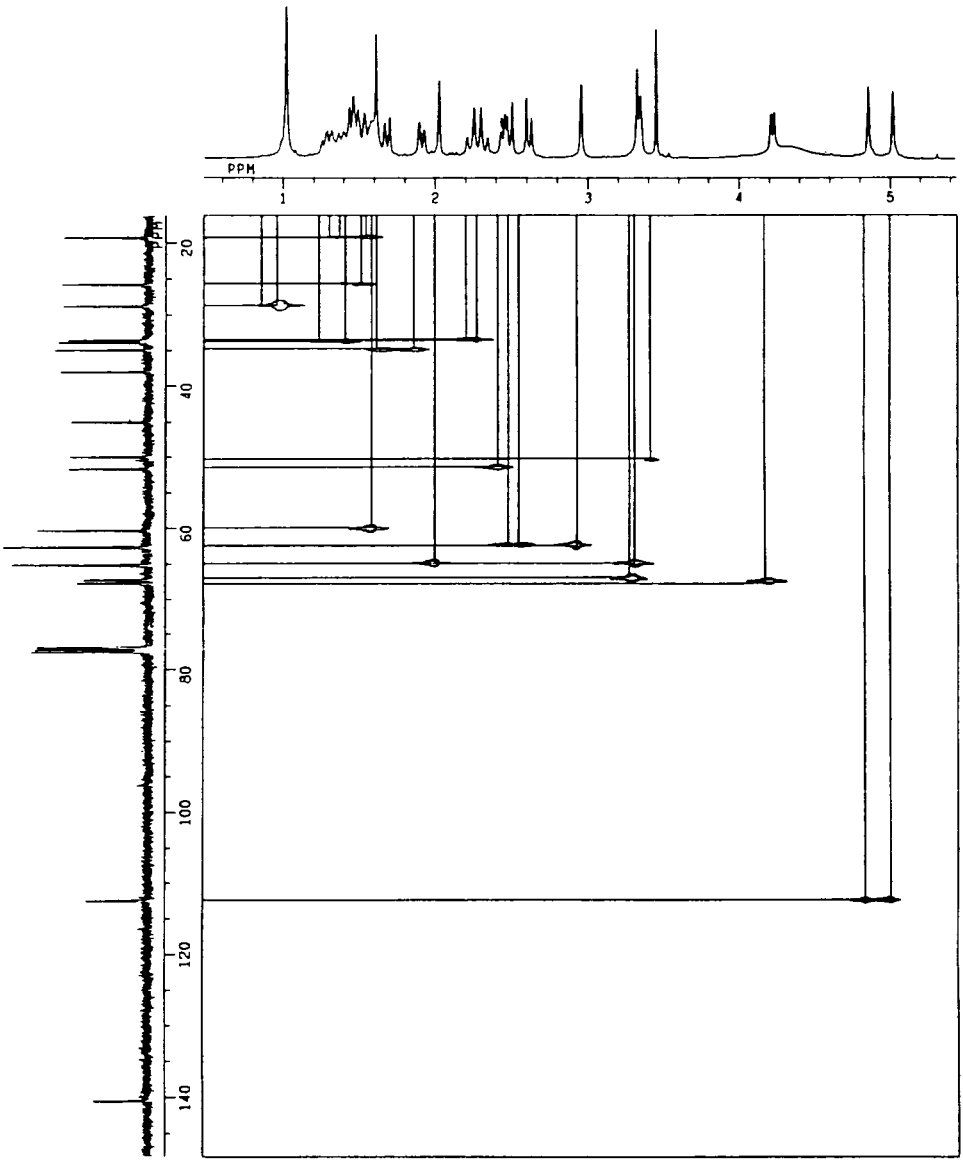


FIGURE 2. 2D- proton-carbon heteronuclear chemical shift correlation nmr spectrum of spirasine X [**1**]

one has to hold the molecular model with the N-atom away from the viewer.

EXPERIMENTAL

GENERAL METHODS.—The melting point was determined on a Kofler hot-stage microscope and is reported uncorrected. Optical rotation was measured on a Perkin-Elmer model 241 polarimeter; the ir spectrum on a Perkin-Elmer model 399B as a KBr pellet; mass spectra on ZAB-2F; cd spectra on Duospec CD-V; ^{1}H-nmr and ^{13}C-nmr spectra were recorded on a JEOL FT model GX-400Q spectrometer in $CDCl_3$ solution. The HOMCOR spectrum (Figure 1) was obtained with an N type selection, using a symmetrized data matrix with an initial matrix of 256×1024 points (zero filled). A polyvinyl-sulfonic ion exchange resin H^+-form (linking 1×1.1 from Chemical Factory of Nankai University) was used in isolation of the total alkaloids. Si gel 140-160 mesh for chromatography and Si gel G for tlc were from Qindao Haiyang Chemical Factory. Alumina for chromatography (180-200 mesh) were purchased from Shanghai Chemical Reagents Factory.

PLANT MATERIAL.—The plant material was collected from Guiyang, Guizhou Province, China, and the sample was identified by Prof. Z.Y. Zhu of our institute. A voucher specimen is located in the Department of Pharmacognosy of our institute.

EXTRACTION OF TOTAL ALKALOIDS.—Powdered roots of *S. japonica* (40 kg) were percolated with 0.05 N HCl with about 800 liters, and the percolate was run through a column of 34 kg wet resin. 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 continously extracted in a specially designed extractor with Et_2O under reflux for 8 h. White deposits of crude alkaloids (270 g) from the Et_2O extracts were collected by evaporation.

ISOLATION AND IDENTIFICATION OF THE ALKALOID.—The crude alkaloids (70 g) were developed on a dry silica column (2700 g) with a mixture of $CHCl_3$-MeOH (10:1). Sections 13-14 (total 17) were combined and extracted to give 2.4 g of solid. Tlc showed three spots. Rechromatography on an alumina column (150 g) with CH_2Cl_2-MeOH (50:1) in 50-ml fractions gave 405 mg (in fractions 4-5) which when crystallized from Me_2CO gave 50 mg of colorless needles of spirasine X [**1**]; mp 224-227°, $[\alpha]^{17}D$+51.0 (*c* 1.5, $CHCl_3$); eims *m/z* 311 (M 100%), 266 (79%); ir (KBr) 3584, 1714, 1640, 891 cm^{-1}; cd $\Delta\epsilon$ 0 (257), +2.95 (304), +2.03 (sh, 314); ^{1}H nmr 1.01 (3H, s, C_4-CH_3), 1.68, 1.87 (each 1H, dd, C_7-2H), 2.04 (1H, d, C_{20}-H), 2.26, 2.32 (each 1H, br, d, C_{15}-2H), 2.41 (1H, q, C_{14}-H), 2.47, 2.58 (each 1H, d, C_{19}-2H), 2.94 (1H, d, C_{12}-H), 3.25 (1H, s, C_9-H), 3.30 (1H, br, s, C_6-H), 4.24 (1H, q, C_{13}-H) and 4.85, 5.02 (each 1H, br, s, C_{17}-2H); ^{13}C nmr (INEPT) see Table 1.

ACKNOWLEDGMENTS

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