(17) 209-213

云 南 植 物 研 究 2000, 22 (2): 209 ~ 213
Acta Botanica Yunnanica

急尖绣线菊中一微量新二萜生物碱*

Q949.751.8
Q946.88

王斌贵, 刘 斌**, 左国营, 郝小江***
(中国科学院昆明植物研究所植物化学开放研究实验室, 云南 昆明 650204)

摘要: 从蔷薇科绣线菊属植物急尖绣线菊 (*Spiraea japonica* var. *acuta* Yu) 的根部分离得到 6 个二萜生物碱, 经光谱分析, 其中 5 个分别鉴定为 spiramines A (1), B (2), P (3) 和 U (4) 及 spiradine F (5), 另一微量成分被鉴定为一新的二萜生物碱, 命名为 spiramine W (6)。

关键词: 蔷薇科; 绣线菊; 急尖绣线菊; 二萜生物碱; 绣线菊碱 W
中图分类号: Q 946 **文献标识码**: A **文章编号**: 0253 - 2700(2000)02 - 0209 - 05

A New Minor Diterpenoid Alkaloid from *Spiraea japonica* var. *acuta**

WANG Bin - Gui, LIU Bin**, ZUO Guo - Ying, HAO Xiao - Jiang***
(*Laboratory of Phytochemistry, Kunming Institute of Botany, The Chinese Academy of Sciences*, Kunming 650204)

Abstract: A new minor diterpenoid alkaloid, named spiramine W (6), together with five known diterpenoid alkaloids, spiramines A (1), B (2), P (3), U (4), and spiradine F (5), was isolated from the roots of *Spiraea japonica* var. *acuta* Yu. Their structures were determined by detailed interpretation of spectral data.

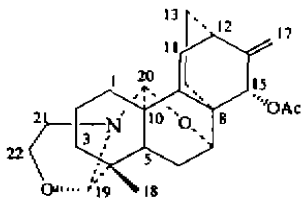
Key words: Rosaceae; *Spiraea*; *Spiraea japonica* var. *acuta*; Diterpenoid alkaloid; Spiramine W

The species of *Spiraea japonica* and its varieties are widely distributed in Yunnan Province, China. Some of them have been used in traditional medicine in China for a long time (Jiangsu College of New Medicine, 1977). In previous papers (Hao *et al*, 1992a; 1992b; 1993; 1994; 1995a; 1995b; Nie *et al*, 1997a; 1997b; Node *et al*, 1990), we reported the isolation and structural elucidation of twenty - two new atisine - type diterpenoid alkaloids, spiramines A - V, from the roots of *Spiraea japonica* and its varieties. Recently, we investigated the constituents of the roots of *Spiraea japonica* var. *acuta* Yu collected in Dali, Yunnan Province, and a new minor diterpenoid alkaloid, named spiramine W (6), together with five known compounds, spiramines A (1), B (2), P (3), U (4), and spiradine F (5), was obtained. This paper describes the isolation and structural elucidation of the compounds.

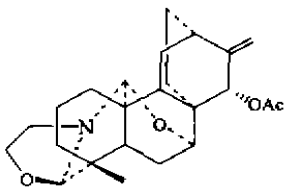
* 基金项目: 国家杰出青年科学基金资助项目 (39525025)
** 贵阳医学院 99 届毕业实习生
*** 通讯联系人
Received date: 1999 - 08 - 04, Accepted date: 1999 - 10 - 15

RESULTS AND DISCUSSION

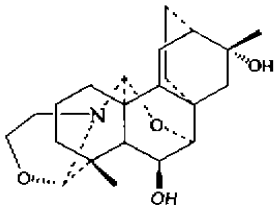
An ethanolic extract from dried roots of *Spiraea japonica* var. *acuta* was treated in the usual manner to give alkaloid and non－alkaloid fractions [see **Experimental**]. Spiramines A (**1**), B (**2**), P (**3**), U (**4**), W (**6**), and spiradine F (**5**), were isolated from the alkaloid fraction by means of repeated silica gel column chromatography.



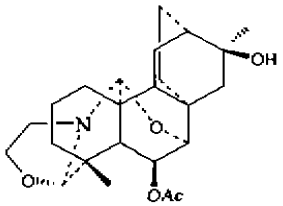
1 spiramine A C-19 S



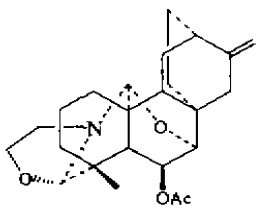
2 spiramine B C-19 R



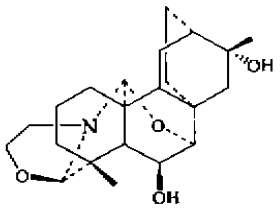
3 spiramine P C-19 S



4 spiramine U C-19 S



5 spiradine F C-19 S



6 spiramine W C-19 R

Fig. 1 Diterpenoid alkaloids obtained from *Spiraea japonica* var. *acuta*

The structures of known compounds **1** ~ **5** were determined by spectroscopic techniques or by comparison with the authentic samples. Here it deserves to mention that one of the hydroxyl groups of spiramine P (**3**) and the acetoxyl group of spiramine U (**4**) have been re－assigned from C－15 to C－6 position according to the detailed NMR spectral analysis and 2D NMR experiments (Wang *et al*, 1999).

Apart from the pure compound **3**, we also obtained a mixture of compound (**3**) and (**6**) in the procedures of isolation. The ^{1}H and ^{13}C spectra showed that this mixture contains two structural－related compounds in a ratio of *ca* 3:1, which is a common phenomenon in the diterpenoid alkaloids of spiramine series (Nie, 1996). The IR spectrum exhibited the presence of hydroxyl group (3433 cm^{-1}), and ether linkage (1037, 1062 cm^{-1}) in the molecule. Its EIMS revealed the molecular ion peak at m/z 375, suggested the molecular formula $C_{22}H_{33}NO_4$ for both of the compounds, and this

suggestion was further confirmed by ^{13}C NMR and DEPT data. The ^{1}H NMR spectrum exhibited the presence of an oxazolidine ring system by a five – proton multiplet pattern at δ4. 22 (1H, s, H – 19), 3.87 (1H, m, H – 22a), 3.40 (1H, m, H – 22b), 3.20 (1H, m, H – 21a), and 3.09 (1H, m, H – 21b). The carbon signals at δ92.3 (d, C – 19), 65.0 (t, C – 22), and 45.9 (t, C – 21) in the ^{13}C NMR spectrum also suggested the presence of oxazolidine ring in (6). Detailed analysis of the ^{1}H – and ^{13}C NMR data revealed that compound (3) and (6) are two epimeric isomers at C – 19. It is reported that in the case of C – 19s configuration, the proton signal of H – 19S appeared at *ca* δ3.8, in contrast the H – 19R signal at *ca* δ4.2 in the ^{1}H NMR spectrum. In addition, the chemical shifts of C – 19 and C – 20 signals are also useful to identify the configuration of C – 19 by the signals at δ91 and 83 (C – 19R and C – 20, respectively), and δ95 and 86 (C – 19S and C – 20, respectively) (Nie *et al*, 1997a). In ^{1}H NMR spectrum of compound 6, the signal for H – 19 appeared at δ4.22 (1H, S, H – 19) and, in the ^{13}C NMR spectrum, C – 19 and C – 20 signals appeared at δ92.3 (d, C – 19) and 82.9 (d, C – 20), respectively, suggested the 19R configuration of compound (6), which means compound (6) was the C – 19 epimer of spiramine P (3). Compound (6) was named as spiramine W since it has not been reported previously.

EXPERIMENTAL

IR spectra were recorded on KBr discs with a Bio – Rad FTS – 135 spectrometer. EIMS were measured on a VG AutoSpec – 3000 spectrometer with direct inlet on 70 ev. NMR were taken on a Bruker AM – 400 spectrometer using TMS as internal standard in $CDCl_3$ or C_5D_5N.

Plant materials The roots of *Spiraea japonica* var. *acuta* Yu were collected in Dali, western region of Yunnan Province, in July 1998. The specimen was identified by Prof. Zheng – Wei Lu of Kunming Botanical Garden and deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN).

Extraction and isolation of compounds Air dried roots of *Spiraea japonica* var. *acuta* (18 kg) were extracted with 95 % ethanol at room temperature for three times (6 days for each time) and the EtOH solution was concentrated under reduced pressure to give a crude residue (1090 g). The residue was treated with 3 % HCl. The acidic solution was basified with 5% NaOH to pH 11 and then extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O and then dried with Na_2SO_4. A total of 90g mixture of crude base were obtained after removal $CHCl_3$ in vacuum. The crude base was subjected to cc on silica gel. Elution was carried out with mixtures of solvents of increasing polarity starting with petroleum ether – acetone – diethylamine. The fractions eluted with petroleum ether – acetone – diethylamine (50 : 10 : 1) were further separated by repeated flash cc to afford compound 1 (1.0g), 2 (1.0g), and 5 (2.0g). The fractions eluted with petroleum ether – acetone – diethylamine (20 : 10 : 1) were further purified by repeated flash cc to afford compound 3 (200mg) and 4 (800mg). The elution of petroleum ether – acetone – diethylamine (15 : 10 : 1) were also further purified by repeated flash cc to afford a mixture of compounds 3 and 6 (100mg). It deserves mention that this mixture cannot be separated into pure compounds although we attempted several times using various solvents.

Spiramine P (3), needles, IRν_{max}^{KBr} cm^{-1}: 3443, 2936, 2909, 2882, 1463, 1406, 1371, 1205, 1119, 1098, 1037, 1023, 981, 909, 873; EI－MS m/z (%): 375 (90), 346 (50), 319 (75), 278 (35), 180 (100), 92 (50), 72 (78). HREIMS: m/z 375.2386 (calcd for $C_{22}H_{33}NO_4$: 375.2409). ^{1}H and ^{13}C NMR spectral data, see Table 1.

Table 1 NMR assignments of Spiramine P and W*

Atom No	^{1}H NMR of Spiramine P*,** δ (Jin Hz)	^{13}CNMR of spiramine P and W Spiramine P**, δ	DEPT	HMBC (H→C)	Spiramine W δ	DEPT
1	1.32 (1H, m)	29.6	CH_2	2, 3, 9, 10, 20	29.6	CH_2
	1.21 (1H, m)					
2	2.26 (1H, m)	20.9	CH_2	1, 3	21.3	CH_2
	1.39 (1H, m)					
3	1.40 (1H, m)	41.3	CH_2	1, 2, 4, 5	34.6	CH_2
	1 52 (1H, m)					
4		35.8	C		35.2	C
5	1.38 (1H, br.s)	56 8	CH	3, 4, 6, 7, 19, 20	60.6	CH
6	5.09 (1H, dd, J=2.1, 4.9)	69 1	CH	4, 5, 7, 8, 10	69.1	CH
7	3.70 (1H, d, J=4.9)	75.2	CH	5, 6, 8, 9, 14, 15, 20	75.0	CH
8		37.5	C		37.4	C
9	2.03 (1H, dd, J=2.9, 10.5)	43.5	CH	5, 8, 11, 14, 15, 20	42.3	CH
10		36.0	C		36 9	C
11	1.60 (1H, m)	23.3	CH_2	9, 12, 16	23.3	CH_2
	1.23 (1H, m)					
12	1.83 (1H, m)	40.0	CH	9, 11, 13, 14, 15, 16, 17	40.0	CH
13	2.65 (1H, m)	22.3	CH_2	11, 12, 14, 16	22.3	CH_2
	1.48 (1H, m)					
14	2.12 (1H, m)	27.8	CH_2	8, 15	27.8	CH_2
	1.50 (1H, m)					
15[a]	3.06 (1H, dd, J=3.2, 12.4)	48.9	CH_2	8, 9, 14, 16, 17	48.9	CH_2
	1.89 (1H, d, J=12.4)					
16		71.7	C		71.7	C
17	1.71 (3H, s)	32 0	CH_3	12, 15, 16	32.0	CH_3
18	1.40 (3H, s)	23.3	CH_3	3, 4, 5, 19	23.1	CH_3
19	3.91 (1H, s)	95.4	CH	3, 5, 20, 22	92 3	CH
20	4.64 (1H, s)	85.5	CH	5, 7, 9, 10, 19, 21	82.9	CH
21	3.38 (1H, m)	51.5	CH_2	20	45.9	CH_2
	3.18 (1H, m)					
22	3.75 (1H, m)	63.4	CH_2	19, 21	65.0	CH_2
	3.40 (1H, m)					

* using C_5D_5N as solvent, δin ppm

** Assignments by 2D NMR experiments (^{1}H－^{1}H cosy, HMQC, and HMBC)

[a] one of the H－15 protons showed W－type coupling (J=3.2Hz) with one of the H－14 protons

Spiramine W (6), $C_{22}H_{33}NO_4$, Mw 375, needles, IRν_{max}^{KBr} cm^{-1}: 3433, 2957, 2923, 2854, 1462, 1118, 1098, 1062, 1037; EI－MS m/z (%): 375 (M^+, 80), 346 (20), 319 (25), 278 (15), 180 (35), 91 (40), 72 (100); ^{1}H NMR (400 MHz, C_5D_5N): 5.09 (1H, dd, J=

2.1, 4.9 Hz, H-6α), 4.94 (1H, s, H-20), 4.22 (1H, s, H-19), 3.87 (1H, m, H-22a), 3.70 (1H, d, J = 4.9 Hz, H-7β), 3.40 (1H, m, H-22b), 3.20 (1H, m, H-21a), 3.09 (1H, m, H-21b), 1.74 (3H, s, H-17), 1.20 (3H, s, H-18). ^{13}C NMR spectral data, see Table 1.

Acknowledgements: This work was financially supported by National Natural Science Foundation of China (NSFC) for Outstanding Young Scientists to Prof. Dr. Hao Xiao-Jiang (39525025). The first author, Dr. Wang Bin-Gui, also wishes to thank the K. C. Wong Education Foundation, Hong Kong, for partial financial support. The spectral data were recorded by the analytical group of Kunming Institute of Botany, which acknowledgements should also be addressed.

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