Chinese Chemical Letters Vol. 11, No. 9, pp. 789–792, 2000

Spiramide, a New Diterpene Amide from the Roots of *Spiraea japonica* var. *acuta*

Yue Mao SHEN[1,2], Hong Ping HE[1], Yong Sheng ZHANG[3], Bin Gui WANG[1], Xiao Jiang HAO[1,*]

[1]Laboratory of Phytochemistry and [2]Department of Plant Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204
[3]Chinese Medical College of Yunnan Province, Kunming 650031

Abstract: Chemical investigation on the neutral fraction of the ethanol extract from the roots of *Spiraea japonica* var. *acuta* has resulted in the isolation of one new diterpene amide, spiramide (**1**). Structure elucidation for **1** was carried out on the basis of 1D and 2D NMR spectra.

Keywords: *Spiraea japonica* var. *acuta*, spiramide.

Previous chemical investigations on *Spiraea japonica* and its varieties have led to the report of 7 new atisane-type diterpenoids[1] and 30 new diterpene alkaloids of atisine- and hetisine-type[2]. The chemotaxonomy of *S. japonica* complex was proposed based on those chemical data[3]. A hypothetical pathway of the biosynthesis of atisine-type alkaloids was suggested to be formed from diterpene directly to the corresponding alkaloids through double Mannich reactions (unpublished data). In order to search for biosynthetic intermediates from the plant, a large amount of the roots of *S. japonica* var. *acuta* (200 Kg) were collected in Lijiang of Yunnan Province. The 95% EtOH extract of the roots of *S. japonica* var. *acuta* gave a crude extract which was separated into basic and neutral parts. The neutral part (1000g) was chromatographically separated over silica gel to afford eighty fractions. TLC on silica gel revealed that fraction 18 mainly contained spiraminol[1a], a relatively low oxygenated diterpene. According to the hypothesis above, the diterpenoids having light polarity might be the intermediates. Therefore, isolation was concentrated on fraction 17 and resulted in the isolation of one new diterpene amide **1**.

Compound **1** was determined to have the molecular formula $C_{26}H_{35}NO_6$ based on the high resolution positive fast atom bombardment mass spectrometry (HRFABMS$^+$ *m/z* 458.2609, calcd: 457.2543, -6.6mmu error) and showed the presence of carbonyl group (1743 cm^{-1}) and carbon-carbon double bond (1658 cm^{-1}) in its IR spectrum. Inspection of the NMR data (proton, carbon, DEPT, HMQC and HMBC) revealed an atisine-type amide[2g]. The ^{1}H NMR spectrum of **1** showed signals for a tertiary alkyl methyl group (δ

1.15) and two acetoxyl methyls (δ 1.92 and 1.98). The ^{13}C NMR and DEPT spectra of **1** showed twenty-six carbon signals, including three methyls, ten methylenes, six methines, and seven quaternary carbon atoms. The ^{1}H and ^{13}C NMR resonances of **1** were assigned by a HMQC experiment. The locations of acetoxyl groups were determined by inspecting the HMBC data of **1**. The ^{13}C NMR and DEPT of **1** displayed two methine carbons at δ 69.4 and 79.3 linked to ether/ester oxygens. Those resonances correlated with methine protons in the ^{1}H NMR spectrum at δ 5.33 and 4.76 in the HMQC experiment. These two methine protons exhibited correlation peaks (J=9.9Hz) in the ^{1}H-^{1}H COSY spectrum of **1**, revealing that those protons were on vicinal oxygen substituted carbons. The HMBC experiment (**Table 1**) established the δ 5.33 resonances to be H6 through long-range correlations between the proton and C4, C8, C10, and the acetoxyl carbonyl carbon of δ 170.4. The HMBC experiment further correlated the H7 proton (δ 4.76) to C5, C9, C14 and C15, and the acetoxyl carbonyl carbon of δ 169.8, confirming its vicinal relationship to the δ 5.33 proton. H5 had ^{1}H-^{13}C long-range correlation with C7, placing the two acetoxyl groups at C6 and C7, respectively. In an HMQC experiment, ^{1}H-^{13}C direct correlations were observed between the proton signals at δ 3.84 and 4.15 and the oxygenated methylene carbon at δ 64.7 (C-22). These proton signals at δ 3.84 and 4.15 in the ^{1}H NMR was correlated with signals of a methylene at δ 3.29 and 4.01 in the ^{1}H-^{1}H COSY spectrum. These methylenes showed no other ^{1}H-^{1}H correlations thereby defining an isolated ethyl group in **1**.

Spiramine S[2g] and **1** possessed similar carbon skeleton with a different pattern of substituents revealed by the above-mentioned information and the comparison of their ^{1}H and ^{13}C NMR data, particularly the chemical shifts and the coupling constants of H6 and H7 in **1**, and of H7 and H15 in spiramine S[2g]. In the HMBC spectrum of **1**, one set of ^{1}H-^{13}C long-range correlations between H20, H21, H22 and related carbons such as C19, C20, C21 and C22 (**Table 1**) indicated the presence of an oxazolidine ring. The existence of a C19 carbonyl group was confirmed by the long-range correlations between the proton signals of H3, H5, H18, H20, H21 and the carbonyl carbon signal at δ 172.7 (C19) in the HMBC spectrum. Thus, **1** was elucidated to be spiramide as shown in **Figure 1**.

Figure 1. Spiramide (**1**)

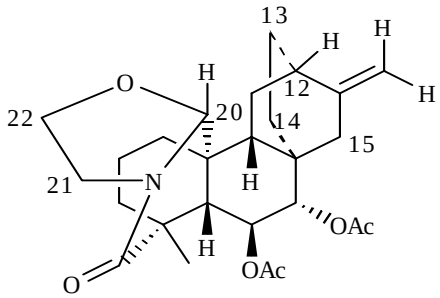

Table 1. The ^{1}H, ^{13}C NMR Assignments and ^{1}H-^{13}C Long-Range Correlation (HMBC) Data for **1**[a]

Carbon	^{13}C	^{1}H[b]	HMBC
1	34.3 t	0.95 (ddd, 4.6, 4.8, 13.3)	
		2.41 (br dd, 3.2, 13.3)	C-2, C-3, C-5, C-10, C-20
2	20.8 t	1.39 (m)	
		1.46 (m)	C-1, C-3, C-4, C-10
3	42.5 t	1.43 (m)	
		1.78 (m)	C-1, C-2, C-4, C-5, C-19
4	43.0 s	/	/
5	53.1 d	1.84 (d, 11.6)	C-1, C-3, C-7, C-9, C-18, C-19, C-20
6	69.4 d	5.33 (dd, 9.9, 11.6)	C-4, C-8, C-10, Ac-CO
7	79.9 d	4.76 (d, 9.9)	C-5, C-9, C-14, C-15, Ac-CO
8	38.3 s	/	/
9	47.4 d	1.48 (m)	C-1, C-5, C-7, C-12, C-14, C-15, C-20
10	41.5 s	/	
11	28.8 t	1.73 (m)	
		2.09 (ddd, 2.4, 7.2, 13.5)	C-8, C-9, C-10, C-12, C-13, C-16
12	36.0 d	2.25 (br s)	C-9, C-14, C-15, C-17
13	26.2 t	1.52 (dd, 7.8, 12.1)	
		1.65 (br d, 12.1)	C-8, C-11, C-12, C-14, C-16
14	24.1 t	1.60 (dd, 6.5, 12.8)	
		1.89 (br d, 12.8)	C-7, C-8, C-9, C-12, C-13, C-15
15	45.5 t	1.98 (br d, 16.0)	
		2.20 (br d, 16.4)	C-7, C-8, C-9, C-12, C-16, C-17
16	149.3 s	/	/
17	106.6 t	4.60 (d, 1.7)	
		4.77 (d, 1.7)	C-12, C-15, C-16
18	24.6 q	1.15 (s, 3H)	C-3, C-4, C-5, C-19
19	172.7 s	/	/
20	87.9 d	5.06 (s)	C-1, C-5, C-9, C-19, C-21, C-22
21	41.5 t	3.29 (ddd, 3.7, 8.2, 11.2)	
		3.97 (ddd, 8.2, 8.2, 11.2)	C-19, C-20, C-22
22	64.7 t	3.84 (dt, 8.2, 8.2)	
		4.15 (ddd, 3.7, 8.2, 8.2)	C-20, C-21
Ac	20.6 q	1.98 (s, 3H)	Ac-CO
	170.4 s		
Ac	21.3 q	1.92 (s, 3H)	Ac-CO
	169.8 s		

[a] ^{1}H NMR, ^{13}C NMR and HMBC spectra were obtained at 400MHz, 100MHz and 500MHz, and recorded in CD_3OD at room temperature, respectively.
[b] Coupling constants are presented in hertz. Unless otherwise indicated, all proton signals integrate to 1H.

Acknowledgments

This research was financially supported by the National Natural Science Foundation of China. The authors are grateful to Prof. D. Z. Wang, Mr. Y. N. He and Ms. H. L. Liang in Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences, for measuring 2D NMR, 1D NMR and HRFABMS data, respectively.

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Received 28 February 2000