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NEW SPIRAMINES FROM *SPIRAEA JAPONICA* VAR. *OVALIFOLIA*

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Abstract- Eleven atisine-type diterpene alkaloids, including five new ones 19-*O*-deethylspiramine N (**1**), 15-deacetylspiramine S (**2**), spiramine Z-2 (**3**), spiramine Z-3(**4**), deacetylspiramine F(**5**), and six known ones, spiramines A, B, C, D, F(**6**), H were isolated from the air dried *Spiraea japonica* var. *ovalifolia.*

INTRODUCTION

Spiraea japonica is a shrub of varieties of Rosaceae which are widespead in Yunnan, P. R. China. Previous investigation of several of these varieties has led to the isolation of 27 new atisine-type diterpene alkaloids spiramines A~Z-1.[1, 2] So far, the chemical constitution of *S. japonica* var. *ovalifolia* has not been investigated. As a part of serial studies of the varieties of the plants, we undertook isolation of the components of *S. japonica* var. *ovalifolia* and obtained eleven atisine-type diterpene alkaloids, including five new ones 19-*O*-deethylspiramine N (**1**), 15-deacetylspiramine S (**2**), spiramine Z-2 (**3**), spiramine Z-3 (**4**), deacetyl spiramine F (**5**), and six known ones spiramines A(**9**), B(**10**), C, D,[3] F (**6**) ,[4] H.[5] This note deals with the isolation, structural determination of these compounds. The structures of the known compounds were established by comparison with reported data. Here we described the structural elucidation of the new components (**1~5**)(Figure 1).

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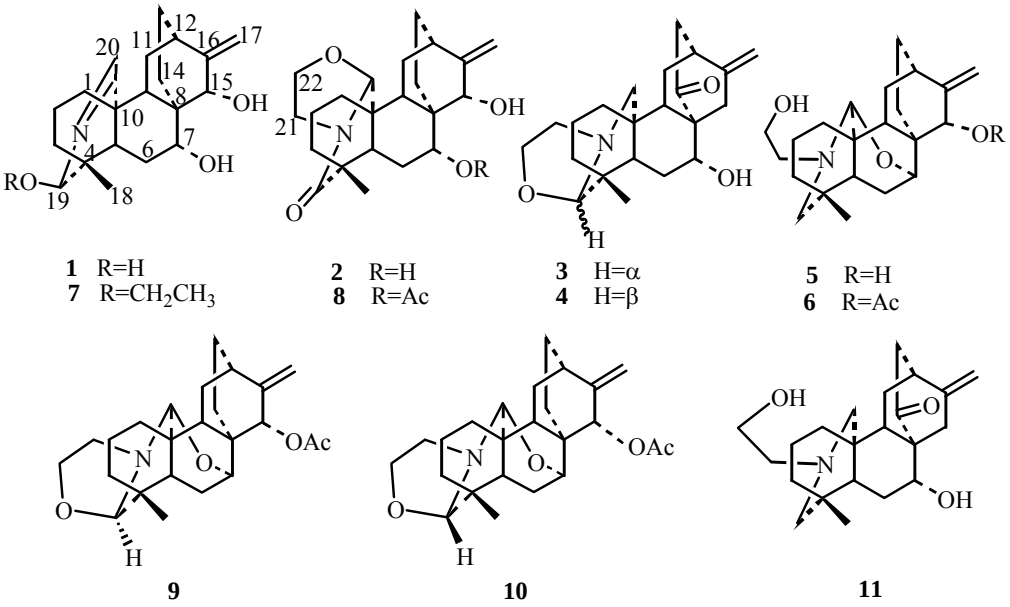

Figure 1. The Structures of diterpene alkaloids (**1-11**)

RESULTS AND DISCUSSION

The 90% EtOH extract of whole plants of *S. japonica* was separated into an alkaloid and a non-alkaloid fractions. The alkaloid fraction was chromatographed over silica gel to afford the eleven spiramines.

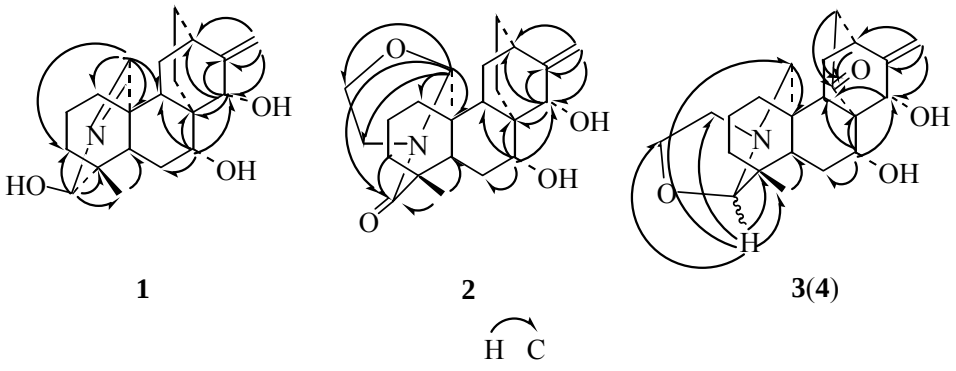

Figure 2 . Selected HMBC correlations of **1** ~ **3**(**4**)

Compound (**1**) was determined to have the molecular formula of $C_{20}H_{29}NO_3$ by HREIMS (331.2161, calcd 331.2147). In the ^{1}H and ^{13}C NMR spectra, three oxygen-substituted methines were demonstrated by δ_C 88.9 (d, C19) and δ_H 5.30 (1H, s, H19), δ_C 80.1 (d, C15) and δ_H 4.26 (1H, s, H15β), as well as δ_C 77.6 (d, C7) and δ_H 3.90 (1H, dd, J 4.1, 7.4 Hz, H7β). The signal at δ_C 163.2 (s) was attributed to the

imine (C=N, 1647cm^{-1} in IR) of C20. The ^{1}H and ^{13}C NMR spectra of **1** were similar to spiramine N (**7**) .[6] The difference between the ^{13}C NMR spectra of **1** and **7** was the missing of the 19-*O*-ethyl signals in **1**. Furthermore, the absence of ethyl group was also supported by its EIMS (M^+ *m/z* 331). Hence, **1** was determined to be 19-*O*-deethylspiramine N. The NMR assignments (**Tables 1** and **2**) were thoroughly carried out on the basis of 2D NMR experiments.

HREIMS showed the molecular ion of compound (**2**) at m/z 373.2237 (calcd for $C_{22}H_{31}NO_4$, 373.2253). The ^{13}C NMR and DEPT spectra showed 22 carbon signals including one carbonyl group at δ_C173.2 (1626cm^{-1} in IR). The two oxygen-substituted methines at δ_C 77.3 (C7) and 80.7 (C15) were similar to those in **1**, and the HMQC spectra demonstrated their corresponding ^{1}H NMR signals at δ_H 3.73 (1H, d, J 5.3, 8.8 Hz, H7β) and 3.93 (1H, s, H-15β), respectively. In the HMBC spectrum of **2**, one set of ^{1}H-^{13}C long-range correlations between H20, H21, H22 and related carbons such as C19, C20, C21 and C22 indicated the presence of an oxazolidine ring (-*N*-C20-*O*-C22-C21-) which was the same as that of spiramine S (**8**).[7, 8] The position of the C19 carbonyl group was confirmed by the long-range correlations with the proton signals of H3, H5, H18, H20, H21 in the HMBC spectrum and the carbonyl carbon signal at δ_C 173.2 (C19) (**Figure 2**). In the ^{13}C NMR and MS spectra, the difference between **2** and spiramine S (**8**) was the missing of the acetyl signals in **2**, indicating **2** to be the 15-deacetylspiramine S.

Compounds (**3**) and (**4**) were obtained as a mixture. HREIMS indicated both possessed the same molecular formula $C_{22}H_{31}NO_3$, M^+ *m/z* 357.2289 (calcd 357.2304). The NMR signals were in pairs, particularly those at δ_C 97.8/95.5 (C19), 147.1/146.3 (C16), and 107.5/107.0 (C17), indicating that **3** and **4** were C19 epimers. The signals at δ_C 97.8/95.5 and δ_H 3.89/3.70 (1H, br s, H19), together with δ_C 54.7 (d, C20) and δ_H 2.65, 3.04 (m, H20) (**Tables 1** and **2**) revealed the presence of an oxazolidine-ring (-*N*-C19-*O*-C22-C21-), similar to that in spiramines A and B.[3] The *R*- and *S*-configurations for C19 of **3** and **4** were proposed on the basis of the ^{13}C NMR data in comparison with those in spiramines A and B, respectively.[9] The δ_{C19} and $\Delta\delta_{C19}$ (2.3 ppm) of **3** and **4** were all in the ranges of the corresponding reported Iso-Type Oxazolidine-Ring-Containing Derivatives 99.5~96.3 and 1.6~3.0 ppm,[10] although the $\Delta\delta_{C19}$ was smaller than that in spiramines A and B (3.9 ppm). And the inspection of the HMBC spectrum revealed that C20~22 were all correlated with H19, which added evidences to the deduction. The signals at δ_C 219.4 (s) and 76.5 (d), δ_H (3.03,1H, d, J 2.5 Hz) were assigned to C14 carbonyl group (1708 cm^{-1} in IR) and C7α OH, the same ones as in spiramine G (**11**),[4] which was confirmed by the

HMBC correlation of C-14 with H7, 9, 12, 13, 15 and H7β to C5, 6, 8, 9, 14, 15, respectively. All the data revealed that **3** and **4** were the 19-*O*-22-epoxy of spiramine G (**11**).

Compound (**5**) was shown to be $C_{22}H_{33}NO_3$ with M^+ at m/z 359.2444 (calcd 359.2460) by HREIMS, and all its spectral data were in consistent with those of the deacetyl product of spiramine F (**6**)[4] which was primarily obtained in the process of elucidating spiramine F. However, **5** was isolated as a component naturally occurring in the plant for the first time.

EXPERIMENTAL

General Experimental Procedures. Melting points were determined using a Kofler micro-melting point apparatus and are uncorrected. Optical rotations were determined on a Horiba SEPA-300 polarimeter. IR spectra were obtained on KBr pellets using a Bio-Rad FTS-135 spectrophotometer. 1D and 2D NMR spectra were recorded on a Bruker AM-400 and Bruker DRX-500 spectrometers, respectively, using TMS as internal standard. EIMS and HREIMS measurements were carried out on a VG Auto Spec-3000 spectrometer.

Plant Material. The *S. japonica* var. *ovalifilia* was collected in Songming, Yunnan Province, in July, 1999. A voucher specimen has been deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air dried whole plants of *S. japonica* (14 kg) were grounded and refluxed with 90% EtOH (25 L×3 in the sequences of 3, 2 and 1 h each time). The ethanolic extracts were combined and the distillation of most of the solvent formed a dilutedly ethanolic water suspension (3000 mL). The suspension was acidified to pH 2 with 36% HCl and filtered, the filtrate was defatted with peteroleum ether (bp 60-90^{o}C)-benzene (1:1, 1000 mL × 3 times). Then it was made alkaline (pH 11) with 28% NH_3 (H_2O), followed by exhaustive extraction with $CHCl_3$ to afford total alkaloids (31 g). The total alkaloids were chromatographed over silica gel H through dry column chromatography with the solvent system petroleum ether-acetone-diethylamine (15:2:1) to afford five sections (S1~5). Repeated separation of each section over silica gel by vacuum liquid chromatography (VLC) eluted with different ratios of the solvent systems petroleum ether-acetone-diethylamine (100:2:1~8:2:1) to afford the main components spiramines A and B (5.2 g), C and D (7.0 g), F (**6)** (70 mg), deacetylspiramine F (**5**) (302 mg) and the minor components deethylspiramine N (**1**) (125 mg), deacetylspiramine S (**2**) (224 mg), spiramines Z-2 (**3**) and Z-3 (**4**) (60 mg) and H (22 mg).

Table 1 ^{1}H NMR data for **1-4** and **8** (δ in ppm, 400 MHz)

No.	**1** [a]	**2** [b]	**3** (**4**) [b, c]	**8** [b]
1	1.04 (m), 2.46 (m)	0.89 (m), 2.37 (m)	1.43 (m), 1.22 (m)	/
2	1.41 (m), 1.76 (m)	1.30 (m), 1.45 (m)	2.28 (m), 1.45 (m)	/
3	0.99 (m), 1.61 (m)	1.42 (m), 1.84 (m)	1.97 (m), 1.91 (m)	/
5	1.21 (d, 14.0)	1.52 (d, 9.6)	0.89 (0.71), m	/
6	1.65 (m), 2.34 (m)	1.75 (m), 1.95 (m)	1.43 (m), 1.58 (m)	/
7	3.90 (dd, 4.1, 7.4)	3.73 (dd, 5.3, 8.8)	3.03 (d, 2.5)	3.68 (m)
9	1.27 (d, 5.4)	1.12 (dd, 5.9, 8.6)	1.50 (m)	/
11	2.03 (m), 1.56 (m)	1.42 (m), 1.69 (m)	1.98 (m), 1.58 (m)	/
12	2.36 (m)	2.36 (m)	2.86 (m)	/
13	1.15 (m), 1.62 (m)	1.30 (m), 1.41 (m)	2.16 (m) 2.23 (m)	/
14	1.18 (m), 1.76 (m)	1.42 (m), 1.68 (m)	/	/
15	4.26 (s)	3.93 (s)	2.99 (s), 2.24 (dd, 2.5, 8)	5.31 (d, 2)
17	5.11 (s), 5.33 (s)	5.09 (s), 5.06 (s)	4.87, 4.69 (4.83, 4.62) (br s)	5.05 (s), 5.03 (d, 1.5)
18	1.10 (s, 3H)	1.21 (s, 3H)	1.04 (1.01) (s)	1.19 (s)
19	5.30 (s)	/	3.89 (3.70), (br s)	/
20	7.99 (s)	5.11 (s)	2.65 (m), 3.04 (m)	4.91 (br s)
21	/	3.28 (d, 2.6), 3.90 (m)	3.46 (m), 3.41 (m)	/
22	/	3.87 (m), 4.18 (d, 3.2)	3.75 (m)	4.17 (dt, 4.0, 7.0), 3.90 (m)

[a] Data were obtained in C_5D_5N. [b] Data were obtained in $CDCl_3$.

[c] δ in ( ) was different value for compound (**4**) , others of **3** and **4** were the same.

Table 2 ^{13}C NMR data of **1-5** and **8-11** (ppm, 100 MHz in $CDCl_3$)

C	**1** [a]	**2**	**3** (**4**) [b]	**5** [a]	**8**	**9**	**10**	**11**
1	35.4t	33.6t	40.0t	41.7t	33.9t	41.0t	33.9t	39.5t
2	20.1t	20.4t	21.6 (21.5)t	21.5t	20.8t	22.9t	22.9t	22.8t
3	34.3t	40.0t	29.8t	30.4t	41.4t	29.8t	29.8t	41.1t
4	36.8s	41.2s	35.4s	34.9s	42.5s	35.4s	35.4s	33.5s
5	49.8d	49.7d	48.5 (46.9)d	44.8d	46.2d	45.2d	47.4d	48.4d
6	14.6t	15.3t	27.7 (27.2)t	25.7t	16.7t	25.2t	25.3t	28.1t
7	77.6d	77.3d	76.5d	74.5d	80.9d	74.2d	74.3d	76.2d
8	41.6s	41.1s	51.9s	41.7s	41.9s	40.8s	41.0s	51.8s
9	44.8d	45.7d	49.6 (49.5)d	44.8d	40.3d	43.0d	43.9d	49.4d
10	43.2s	39.5s	39.3s	34.9s	39.8s	34.2s	34.9s	38.2s
11	28.6t	26.0t	27.7 (27.3)t	24.1t	26.3t	23.5t	23.1t	27.3t
12	36.3d	35.5d	38.5d (38.4)	37.9d	35.7d	36.7d	36.4d	38.6d
13	27.5t	27.2t	45.5 (45.4)t	25.4t	28.0t	21.1t	21.2t	45.6t
14	26.3t	27.6t	219.4s	21.2t	28.6t	20.9t	20.8t	219.8s
15	80.1d	80.7d	38.2 (38.1)d	70.2d	75.4d	69.2d	69.7d	38.2t
16	156.0s	147.1s	147.1 (146.3)s	156.5s	150.3s	150.1s	150.1s	146.3s
17	108.7t	109.0t	107.5 (107.0)t	111.6t	111.0t	114.2t	114.3t	107.7t
18	25.7q	21.8q	24.0q	26.8q	22.2q	26.0q	25.9q	26.3q
19	88.9d	173.2s	97.8 (95.5)d	53.9t	173.2s	95.2t	91.3t	59.6t
20	163.2s	88.7d	54.7 (54.6)t	88.0d	88.9d	85.8d	83.5d	52.4t
21	-	42.2t	58.7 (58.6)t	58.4t	49.1t	51.0t	45.7t	58.0t
22	-	64.4t	64.5 (63.0)t	59.9t	64.8t	63.1t	64.9t	60.3t

[a] Data were obtained in C_5D_5N.

[b] δ in ( ) was different value for compound (**4**) , others of **3** and **4** were the same.

Compound (**1**), white amorphous, $[\alpha]_D^{23}$ +17.99^o (c, 0.26, MeOH), $C_{20}H_{29}NO_3$ by HREIMS 331.2161,

(calcd 331.2147), EIMS: 331 (M^+, 16), 313 (M-H_2O, 85), 286 (100), 268 (20), 239 (26), 229 (30), 185 (17), 141 (25), 129 (33), 105 (32); IR ν_{max}^{KBr} 3394 (OH), 2940, 2867, 1647, 1423, 1367, 1325, 1304,1062, 1024, 995, 905. ^{1}H NMR (Table **1**), ^{13}C NMR (Table **2**).

Compound (**2**), colorless prisms, mp 113-115°C ($CHCl_3$-MeOH), $[\alpha]_D^{21}$ -74.35° (c, 0.27, MeOH), $C_{22}H_{31}NO_4$ by HREIMS 373.2237 (calcd 373.2253), EIMS: 373 (M^+, 14), 356 (M-OH, 29), 345 (M-CO, 93), 328 (15), 284 (13), 256 (16), 159 (23), 105 (36), 91 (71), 72 (100); IR ν_{max}^{KBr} 3503, 3365 (OH), 2940, 2864, 1626 (C=O), 1473, 1425, 1319, 1270, 1102, 1059, 916, 905. ^{1}H NMR (Table **1**), ^{13}C NMR (Table **2**).

Compound (**3**) and (**4**), colorless crystals (MeOH), $C_{22}H_{31}NO_3$ by HREIMS 357.2289 (calcd 357.2304), EIMS: 357 (M^+, 100), 340 (M-OH, 52), 326 (M-CO, 93), 314 (15), 241 (7), 159 (4), 105 (21), 91 (37); IR ν_{max}^{KBr} 3501 (OH), 3073, 2937, 2844, 1708 (C=O), 1657, 1458, 1407, 1373, 1230, 1105, 1020, 887; ^{1}H NMR (Table **1**), ^{13}C NMR (Table **2**).

Compound (**5**), colorless plates (Me_2CO), mp 149-151°C, $C_{22}H_{33}NO_3$ by HREIMS 359.2444 (calcd 359.2460), EIMS 359 (M^+, 52), 342 (M-OH, 35), 328 (100), 316 (27), 300 (44), 288 (20), 250 (15), 195 (15), 164 (25), 91 (36), 84 (50). IR ν_{max}^{KBr} 3400 (OH), 3280, 2945, 2920, 2866, 1651 (C=O), 1458, 1462, 1375, 1230, 1106, 1062, 946, 878; ^{13}C NMR (Table **2**).

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