Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Systematics and Ecology 32 (2004) 75–78
biochemical systematics and ecology
www.elsevier.com/locate/biochemsyseco

Five diterpene alkaloids from *Spiraea japonica* (Rosaceae)

Liming Fan, Zhaoyang Zhang, Yuemao Shen, Xiaojiang Hao *

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Heilongtan, Kunming, Yunnan 650204, China

Received 22 October 2002; accepted 7 July 2003

Keywords: *Spiraea japonica*; Diterpene alkaloids; Hetisine-type

1. Subject and source

The complex *Spiraea japonica* L. (Rosaceae) includes seven varieties of small foliose shrubs widespread in eastern Asia, and used as folk medicines to treat malaria, inflammation and analgesics (Zhang et al., 1986). Morphologically, the complex has high diversity. To investigate the relationship between its chemical diversity, and the physical environmental changes in eastern Asia, a comparative phytochemical study on this complex has been performed. In the course of this work, *S. japonica* var. *fortunei*, and var. *glabra* were collected in Zhejiang Province, east of China. The voucher specimens (KUN NO. ZZY051 and ZZY008) are deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

2. Previous work

Previous studies on the secondary metabolites from *S. japonica* and its varieties showed that the complex *S. japonica* produce a rich array of C_{20}-skeleton diterpene alkaloids (Goto et al., 1968; Goto and Hirata, 1968; Toda and Hirata, 1968; Sun et al., 1986a,b, 1987a, 1988; Hao et al., 1987, 1992a,b, 1993, 1995; Yang and Hao, 1993). These diterpene alkaloids, isolated from plants collected from different geo-

* Corresponding author. Tel.: +86-871-5219684; fax: +86-871-5150227.
E-mail address: yshen@public.km.yn.cn (Y. Shen).

0305-1978/$ - see front matter
doi:10.1016/j.bse.2003.07.001

graphic localities, can be classified into two categories: atisine- and hetisine-types. However, the diterpene alkaloids of *S. japonica* of Zhejiang Province, in eastern China, have not been previously investigated.

3. Present study

Eight kilograms of dried powder of the whole plant of *S. japonica* var. *fortunei* and 12 kg of dried powder of the whole plant of *S. japonica* var. *glabra* were individually extracted with ethanol by reflux. The ethanol extracts were suspended in 5% hydrochloric acid, and centrifuged. The supernatants were neutralized with aqueous ammonia solution to pH 10, and extracted with chloroform to afford the alkaloids, 28.3 and 27.6 g, respectively. Through repeated column chromatography over silica gel (200–300 mesh) eluted with petroleum ether-acetone-diethylamine (100:5:1, 100:10:2, 85:15:5, v/v/v), and petroleum-ether-chloroform-diethylamine (100:3:1, 90:10:3, 80:20:5, 75:25:5, 60:40:5, v/v/v); and over Sephadex LH-20, reversed-phase C-18 silica gel, and finally, recrystallization from petroleum ether-acetone, two hetisine-type diterpene alkaloids, spiredine (**1**, 900 mg) (Sun et al., 1987b) and spirasine 3 (**2**, 700 mg) (Sun et al., 1987b) were obtained from *S. japonica* var. *fortunei*, while the five hetisine-type diterpenoid alkaloids spiredine (**1**, 130 mg), spirasine 3 (**2**, 190 mg), spirasine 2 (**3**, 60 mg) (Sun et al., 1986b), spiradine C (**4**, 5 mg) (Goto et al., 1968) and spirasine 5/6 (**5**, 190 mg) (Sun et al., 1986a) were obtained from *S. japonica* var. *glabra*. Their structures (Scheme 1) were established by comparing spectroscopic data (MS, ^{1}H and ^{13}C NMR) with those reported in literature. The ^{13}C NMR spectral data were given in Table 1.

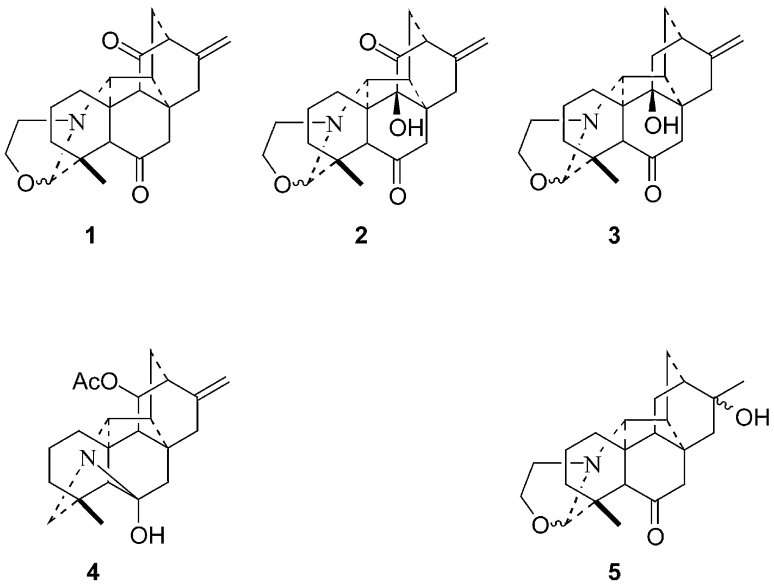
4. Chemotaxonomic significance

Previous chemical investigations on *S. japonica* and its varieties collected mainly from Yunnan Province and Guizhong Province in western China have led to the reports of seven new atisane-type diterpenoids and more than 40 new diterpene alkaloids of atisine- and hetisine-type (Sun et al., 1986a,b, 1987a, 1988; Hao et al., 1987, 1992a,b, 1993, 1995; Yang and Hao, 1993). From an inspection of the structural characteristics of atisane-type, atisine-type and hetisine-type diterpene alkaloids, a hypothetical biosynthetic pathway of C_{20} diterpene alkaloids was proposed in which atisine-type diterpene alkaloids were formed from atisane-type diterpenoids and then transformed to hetisine-type diterpene alkaloids (Ichinohe, 1978).

Based on these chemical data, a systematic hypothesis was suggested that the complex *S. japonica* originated in the southwest of China, and showed an evolutionary tendency from west to east (Hao et al., 1997). This previous work involved only populations of *S. japonica* from southwest of China. The results reported here, which show that all the diterpene alkaloids of *S. japonica* varieties collected in Zhejiang Province, eastern China, are hetisine-type, provides further support for the above hypothesis.

Table 1
The ^{13}C NMR data for compounds **1–5** in $COCL_3$

Carbon	Spiredine	Spirasine 3	Spirasine 2	Spiradine C	Spirasine 5/6
1	33.8, 32.5	48.6	48.9	28.5	51.9
2	20.2, 18.1	20.1, 18.0	20.7, 18.6	18.8	20.7, 18.6
3	39.4, 36.6	39.4, 36.5	32.8, 32.5	35.2	32.6, 32.4
4	36.7	41.1, 35.9	41.1	37.9	42.0
5	61.4, 60.8	55.3	56.2, 55.8	59.4	62.1, 61.6
6	207.0	207.3	209.3	100.6	209.9
7	50.8, 50.1	47.8, 47.2	48.3	42.9	48.9
8	43.2, 42.0	45.1, 44.8	43.0, 42.6	49.7	42.0
9	64.7, 64.5	85.4, 84.9	79.9, 79.5	70.8	36.0
10	46.6	48.7, 48.4	47.7	41.8	46.7/45.0
11	210.8, 210.5	214.5, 214.3	37.0	77.0	21.2/20.7
12	53.3, 53.1	53.0, 52.9	36.4	41.6	47.9/47.7
13	30.1, 29.7	31.9, 31.7	35.2, 35.0	26.9	37.1
14	44.9, 49.6	54.6	43.7	55.9	44.3
15	35.2, 34.8	29.5, 29.2	30.0, 28.7	32.7	32.1, 31.3
16	143.1, 142.9	143.4, 143.2	150.6, 150.4	144.2	70.3
17	110.6, 110.4 t	111.1, 111.0	103.9, 103.7	108.9	29.7/29.6
18	30.2, 23.1	30.5, 23.2	30.4, 23.3	29.9	30.4, 23.3
19	97.6, 93.4	97.9, 93.6	97.8, 93.8	57.5	97.9, 93.8
20	72.6, 72.3	70.1, 70.0	71.3, 71.2	72.3	73.1, 72.7
21	48.8, 52.1 t	51.6	52.0	170.5	52.8, 52.3
22	64.9, 62.8	64.7, 62.6	64.7, 62.6	21.3	65.0, 62.7



Scheme 1. Chemical formulae

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (NSFC) for Outstanding Young Scientists to Hao, X. J. (No. 39525025) and for Gu, Z. J. (No. 30270120). All spectra were recorded by the analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

References

Goto, G., Sasaki, K., Sakabe, N., Hirata, Y., 1968. Tetrahedron Lett 11, 1369.
Goto, G., Hirata, Y., 1968. Tetrahedron Lett 25, 2989.
Hao, X.J., Hong, X., Yang, X.S., Zhao, B.T., 1995. Phytochemistry 38, 545.
Hao, X.J., Nie, J.L., Sun, H., Gu, Z.J., 1997. Acta Botanica Yunnanica 19, 297.
Hao, X.J., Node, M., Taga, T., Miwa, Y., Zhou, J., Chen, S.Y., Fuji, K., 1987. Chem Pharm. Bull 35, 1670.
Hao, X.J., Node, M., Zhou, J., Chen, S.Y., Taga, T., Miwa, Y., Fuji, K., 1993. Heterocycles 36, 825.
Hao, X.J., Zhou, J., Fuji, K., Node, M., 1992a. Chin Chem Lett 3, 427.
Hao, X.J., Zhou, J., Fuji, K., Node, M., 1992b. Acta Botanica Yunnanica 14, 314.
Ichinohe, Y., 1978. Kagaku no Lyoiki 32, 27.
Sun, F., Liang, X.T., Yu, D.Q., 1986a. Tetrahedron Lett 27, 275.
Sun, F., Liang, X.T., Yu, D.Q., 1986b. Heterocycles 24, 2105.
Sun, F., Liang, X.T., Yu, D.Q., 1987a. J Nat Prod 50, 923.
Sun, F., Liang, X.T., Yu, D.Q., 1987b. Heterocycles 26, 19.
Sun, F., Liang, X.T., Yu, D.Q., 1988. J Nat Prod 51, 50.
Toda, M., Hirata, Y., 1968. Tetrahedron Lett 53, 5565.
Yang, X.S., Hao, X.J., 1993. Acta Botanica Yunnanica 15, 421.
Zhang, X.S., Wang, B.D., 1986. Chinese Medicine Dictionary. Shanghai Science and Technology Publishing House, Shanghai, (pp. 1117–1118 and 1978).