Pergamon

Tetrahedron Letters 40 (1999) 4239–4242

TETRAHEDRON
LETTERS

Staminol A, a Novel Diterpene from *Orthosiphon stamineus*

Pavlos Stampoulis,[a] Yasuhiro Tezuka,[a] Arjun H. Banskota,[a] Kim Qui Tran,[b]
Ikuo Saiki[a] and Shigetoshi Kadota*[a]

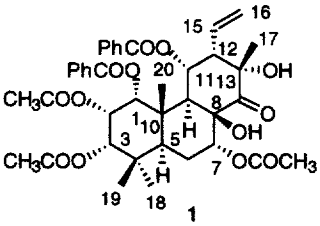
[a]*Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630-Sugitani, Toyama 930-0194, Japan.*
[b]*National University Hochiminh City, Hochiminh, Vietnam.*

Received 1 March 1999; revised 23 March 1999; accepted 26 March 1999

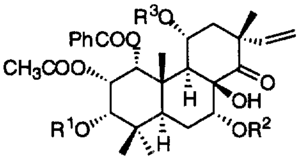
Abstract: From the aerial part of a Vietnamese medicinal plant, *Orthosiphon stamineus* BENTH. (Lamiaceae), staminol A (**1**), a diterpene with a novel carbon framework, was isolated together with four new isopimarane-type diterpenes, orthosiphols F—I (**2**—**5**). Their structures were elucidated by the spectroscopic analyses.

Keywords: natural products; terpenes and terpenoids; NMR

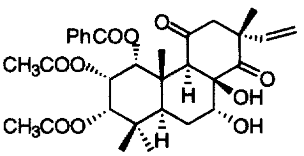
Orthosiphon stamineus BENTH. (Lamiaceae) is a medicinal plant grown in Southeast Asia and is currently cultivated in Indonesia. The plant has been used in treating urinary lithiasis, edema, eruptive fever, influenza, rheumatism, hepatitis, jaundice and biliary lithiasis.[1,2] In our continued studies on Vietnamese medicinal plants,[3] the MeOH extract of the aerial part of this plant was found to exhibit cytotoxic activity against a highly liver-metastatic colon 26-L5 carcinoma cells.[4] Thus we fractionated the MeOH extract into hexane–soluble, $CHCl_3$–soluble, AcOEt–soluble, BuOH–soluble and H_2O–soluble fractions. Among these fractions, the $CHCl_3$–soluble fraction showed the strongest activity against colon 26-L5 cells. Separation by silica gel column chromatography followed by preparative TLC procedures afforded five new diterpenes, named staminol A (**1**) and orthosiphols F—I (**2**—**5**). Here we would like to communicate the structure elucidation of them by the spectroscopic analyses.



1



2 $R^1 = COCH_3$, $R^2 = H$, $R^3 = COPh$
3 $R^1 = COCH_3$, $R^2 = R^3 = H$
4 $R^1 = R^2 = COCH_3$, $R^3 = COPh$
6 $R^1 = H$, $R^2 = COCH_3$, $R^3 = COPh$



5

Staminol A (**1**)[5] was obtained as colorless amorphous solid and showed $[\alpha]_D^{25}$ −24.3° ($c = 0.51$, $CHCl_3$). Its molecular formula was determined by high-resolution FAB-MS measurement to be $C_{40}H_{46}O_{13}$ (*m/z* 734). The IR spectrum of **1** showed absorptions of hydroxy (3550, 3430, 3300 cm^{-1}), ester carbonyl (1725 cm^{-1}) and phenyl (1600, 1450 cm^{-1}) groups. The ^{1}H-NMR spectrum of **1** (Table 1) revealed signals due to four tertiary methyl, a vinyl, five oxygen-substituted methine and three aliphatic methine groups together with signals of two benzoyl and three acetyl groups. Moreover, its ^{13}C-NMR spectrum (Table 1) indicated the presence of a ketone and five ester carbonyl groups and four (two oxygen-substituted and two oxygen-non-substituted) quaternary carbons. Analyses of these signals by the ^{1}H–^{1}H and ^{1}H–^{13}C COSY experiments led to the partial structures depicted in Fig. 1.

---

* Author to whom corresponding should be addressed. Fax: +81-764-34-5059. E-mail: kadota@ms.toyama-mpu.ac.jp.

0040-4039/99/$ - see front matter
PII: S0040-4039(99)00685-1

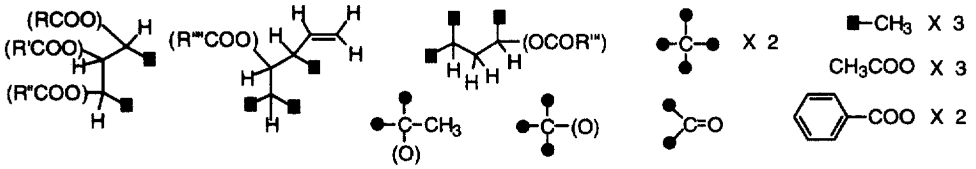


Fig. 1. Partial Structures for Staminol A (**1**).

These partial structures were connected based on the long-range correlations observed in the FG-pulsed HMBC spectrum (Fig. 2). The methyl protons at δ 1.40 (H_3-20) showed the long-range correlations with three methine carbons at δ 35.8 (d, C-5), 40.7 (d, C-9) and 74.4 (d, C-1) and with a quaternary carbon at δ 43.5 (s, C-10), indicating that the carbons, C-1, C-5, C-9, and the methyl group (H_3-20) should be connected with the quaternary carbon (C-10). Furthermore, the methyl protons at δ 0.91 (H_3-18) revealed the long-range correlations with the carbons at δ 22.3 (q, C-19), 35.8 (d, C-5), 37.3 (s, C-4) and 76.1 (d, C-3), and the protons at δ 1.09 (H_3-19) with the carbons at δ 28.3 (q, C-18), C-3, C-4 and C-5. Thus the carbons C-3, C-5, C-18 and C-19 were connected with the quaternary carbon C-4. Moreover, the connectivity of C-12 (δ 54.3), C-14 (δ 209.0) and C-17 (δ 29.1) with the oxygen-substituted quaternary carbon C-13 (δ 76.8) has established on the basis of long-range correlations observed between the methyl protons at δ 1.68 (H_3-17) and the carbons C-12, C-13 and C-14. Nineteen out of twenty carbons in the diterpene skeleton thus have been accounted, and the remaining one carbon at δ 77.4 (s) was assigned to C-8.

The locations of five ester (three acetyl and two benzoyl) groups were also determined by the analyses of the FG-pulsed HMBC spectrum. Significant correlations were observed between the ester carbonyl carbon at δ 170.5 (3-OCO-) with the protons at δ 1.60 (3-$OCOCH_3$) and 5.01 (H-3); the ester carbonyl carbon at δ 169.9 (2-OCO-) with the protons at δ 1.93 (2-$OCOCH_3$) and 5.38 (H-2) and the ester cabonyl carbon at δ 169.3 (7-OCO-) with the protons at δ 2.19 (7-$OCOCH_3$) and 5.37 (H-7) suggested that three acetyl groups should be located at C-2, C-3 and C-7. On the other hand, two benzoyl groups were located at C-1 and C-11, based on the long-range correlations between the ester carbonyl carbon at δ 164.0 (1-OCO-) with the protons at δ 8.13 (H-2',6') and 5.79 (H-1) and between the ester carbonyl carbon at δ 166.8 (11-OCO-) with the protons at δ 8.26 (H-2',6') and 6.29 (H-11).

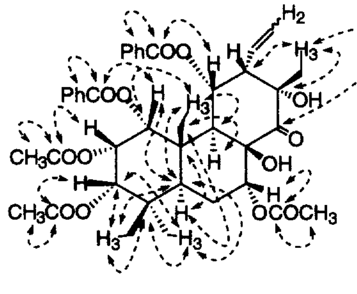


Fig. 2. Significant Long-range Correlations Observed in the FG-pulsed HMBC Spectrum of **1**

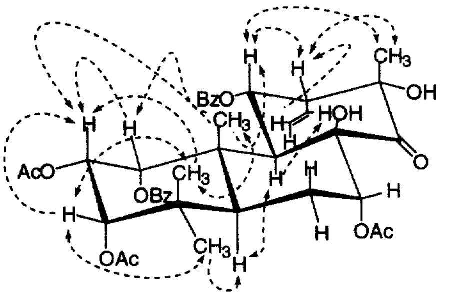


Fig. 3. NOE's Observed in the Difference NOE Experiment of **1**

The stereostructure of **1** was elucidated from the *J* values of each proton and the results of difference NOE experiments. The large *J* values for H-5 ($J_{5,6ax}$ = 13.5 Hz), H-9 and H-11 ($J_{9,11}$ = 11 Hz) indicated them to be ***axial***, while the small *J* values of H-7 ($J_{6eq,7}$ = 3 Hz, $J_{6ax,7}$ = 0 Hz) and H-12 ($J_{11,12}$ = 3.5 Hz) suggested them to be ***equatorial***. In the difference NOE experiments, the methyl protons H_3-18, H_3-19, H_3-20 and H_3-17 showed NOE's with the protons H-3 and H-5, with H-2, H-3 and H_3-20, with H-1, H-2, H-11 and H_3-19 and with H-11 and H-12, respectively. These and other NOE's depicted in Fig. 3 indicated the stereostructure of **1**.

Orthosiphols F (**2**),[6] G (**3**),[7] H (**4**)[8] and I (**5**)[9] were also obtained as amorphous solids, and they showed the ^1H- and ^{13}C-NMR data (Table 1) similar to each other and to those of orthosiphol A (**6**), a diterpene isolated

Table 1. ^{1}H- and ^{13}C-NMR Data for Compounds **1**—**5** in $CDCl_3$ (*J* values in parentheses)

	1		**2**		**3**		**4**		**5**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	5.79 d (2)	74.4	5.29 d (3)	71.9	5.60 d (2.5)	74.4	5.16 d (2)	72.5	6.39 d (3)	73.4
2	5.38 dd (3, 2)	66.4	5.54 t (3)	66.3	5.52 dd (3.5, 2.5)	66.8	5.51 dd (4, 2)	66.4	5.61 t (3)	65.9
3	5.01 d (3)	76.1	4.99 d (3)	76.1	5.05 d (3.5)	76.2	4.99 d (4)	76.1	5.08 d (3)	76.1
4		37.3		37.5		37.4		37.2		37.2
5	2.63 dd (13.5, 2)	35.8	2.69 dd (13, 2.5)	35.6	2.58 m	35.2	2.48 dd (12.5, 3)	36.7	2.47 dd (13.5, 2)	34.8
6	1.88 br d (13)	21.0	2.03 ddd (14, 13, 2.5)	23.4	1.82 m	23.4	2.04 m	21.3	2.04 ddd (15, 13.5, 2.5)	20.8
	2.08 br t (13)		1.89 dt (14, 2.5)		1.95 m		2.02 m		1.84 dt (15, 2.5)	
7	5.37 t (3)	70.3	4.22 t (2.5)	69.3	4.14 t (3)	69.3	5.45 t (3)	70.6	4.29 t (2.5)	69.0
8		77.4		78.1		78.1		75.8		78.0
9	3.11 d (11)	40.7	3.04 d (4.5)	42.5	2.60 d (5)	44.6	3.18 d (6.5)	41.8	3.49 s	51.0
10		43.5		44.0		43.6		43.7		42.8
11	6.29 dd (11, 3.5)	70.2	5.64 ddd (4.5, 4, 2.5)	68.9	4.43 m	64.7	5.80 ddd (6.5, 5, 2)	68.6		205.7
12	2.96 dd (9.5, 3.5)	54.3	2.21 dd (15, 2.5)	39.1	2.35 dd (14.5, 5.5)	43.9	2.58 dd (15.5, 5)	39.7	2.66 d (18)	47.1
			2.73 dd (15, 4)		1.73 dd (14.5, 4)		1.95 dd (15.5, 2)		2.76 d (18)	
13		76.8		47.9		48.9		47.9		49.5
14		209.0		214.0		213.5		208.8		211.1
15	5.15 dt (17, 9.5)	131.2	5.77 dd (17.5, 11)	141.9	5.87 dd (17.5, 11)	141.7	5.67 dd (17, 11)	141.9	5.35 dd (17.5, 11)	138.9
16	4.51 dd (10, 1.5)	121.1	4.71 d (11)	113.3	4.69 d (11)	114.3	4.75 d (11)	113.2	4.16 d (11)	116.2
	4.83 dd (17, 1.5)		4.88 d (17.5)		4.87 d (17.5)		4.81 d (17)		4.67 d (17.5)	
17	1.68 s	29.1	1.26 s	27.7	1.23 s	26.1	1.13 s	26.4	1.14 s	25.1
18	0.91 s	28.3	0.99 s	27.9	1.00 s	28.0	0.90 s	27.9	0.98 s	27.8
19	1.09 s	22.3	1.12 s	22.1	1.13 s	22.5	1.14 s	22.3	1.12 s	22.1
20	1.40 s	15.5	1.47 s	17.2	1.45 s	16.1	1.52 s	16.5	1.42 s	16.3
2-$OCOCH_3$	1.93 s	169.9	1.82 s	170.1	1.97 s	170.8$^{a)}$	1.84 s	170.1	1.95 s	170.1
		20.7		20.7		21.0$^{b)}$		20.7		23.6
3-$OCOCH_3$	1.60 s	170.5	1.50 s	170.7	1.66 s	170.6$^{a)}$	1.44 s	170.5	1.77 s	170.6
		20.5		20.5		20.6$^{b)}$		20.3		23.6
7-$OCOCH_3$	2.19 s	169.3					2.19 s	168.7		
		21.2						20.9		
1-OCOPh										
1'		128.5		130.3$^{a)}$		130.8		130.8		130.4
2',6'	8.13 dd (8, 1)	129.5	7.59 dd (8, 1)	129.7	8.09 d (7.5)	129.8	7.70 dd (8, 1)	129.6	8.11 dd (8, 1)	130.0
3',5'	7.42 t (8)	128.3	7.21 t (8)	128.0	7.43 t (7.5)	128.3	7.32 t (8)	127.8	7.46 t (8)	128.2
4'	7.58 tt (8, 1)	133.0	7.44 dt (8, 1)	132.4	7.55 t (7.5)	132.8	7.54 tt (8, 1)	132.7	7.58 tt (8, 1)	132.9
7'		164.0		164.5		166.1		163.7		164.3
11-OCOPh										
1"		128.5		130.2$^{a)}$				130.9		
2",6"	8.26 dd (8, 1)	130.6	7.49 dd (8, 1)	129.5			7.60 dd (8, 1)	129.7		
3",5"	7.48 t (8)	128.3	6.99 t (8)	127.6			7.12 t (8)	127.9		
4"	7.61 tt (8, 1)	133.3	7.31 dt (8, 1)	132.1			7.42 tt (8, 1)	132.3		
7"		166.8		165.8				166.1		

a),b) Assignments may be interchanged within each column.

from the plant cultivated in Okinawa.[10] The analyses of the ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectra of **2**—**5** led to the partial structures similar to those for **1**, except for the CH–CH(O)–CH$_2$ and vinyl (for **2**—**4**) groups, CH—CO—CH$_2$ and vinyl (for **5**) groups and a non-oxygenated quaternary carbon instead of the CH–CH(O)–CH–CH=CH$_2$ group and an oxygenated quaternary carbon in **1**. These facts suggested **2**—**5** to be pimarane- or isopimarane-type diterpenes, which was supported by the HMBC correlations. The HMBC correlations of the ester carbonyl carbons elucidated the location of each ester group. The configuration of the rings A and B was indicated by NOEs observed in difference NOE experiments to be the same as **1** and **6**. About the configuration of the ring C, NOEs from H-9 to H-16 indicated the α-orientation of the vinyl group, *i.e.*, **2**—**5** are isopimarane-type diterpenes, while NOEs from H$_3$-20 to H-11 in **2**—**4** suggested the α-orientation of H-11. From these data, orthosiphols F—I were concluded to be 3-*O*-acetyl-7-*O*-deacetylorthosiphol A (**2**), 3-*O*-acetyl-7-*O*-deacetyl-11-*O*-debenzoyl-orthosiphol A (**3**), 3-*O*-acetylorthosiphol A (**4**) and 3-*O*-acetyl-7-*O*-deacetyl-11-debenzoyloxy-11-oxo-orthosiphol A (**5**).

To the best of our knowledge, staminol A (**1**) represents the first example of the novel carbon framework, to which we propose the name "staminane". The co-existence of the novel type diterpene **1** with isopimarane-type diterpenes **2**—**5** suggest that the former should be biosynthesized from an isopimarane-type precursor through a migration of the vinyl group from C-13α to C-12α.

The new diterpenes **1**—**5** showed moderate cytotoxic activity against colon 26-L5 carcinoma with a ED$_{50}$ of 61.7, 51.6, 89.7, 56.7 and >100 μg/ml, respectively.[11] These and related diterpenes may have contributed to the cytotoxic activity of the MeOH extract of *O. stamineus*.

Acknowledgements This work was supported in part by a Grant-in-Aid for International Scientific Research (No.09041177) from the Ministry of Education, Sports, and Culture, Japan.

References and Notes

1. Sumaryono, W.; Proksch, P.; Wray, V.; Witte, L.; Hartmann, T. *Planta Med.* **1991**, 57, 176—180.
2. WHO Regional Office for the Western Pacific Malina, and Institute of Material Medica Hanoi, In *Medicinal Plants in Vietnam*, Science and Technology Publishing House: Hanoi, 1990, pp.271.
3. Banskota, A. H.; Tezuka, Y.; Phung, L. K.; Tran, K. Q.; Saiki, I.; Miwa, Y.; Taga, T.; Kadota, S. *Bioorg. Med. Chem. Lett.* **1998**, 8, 3519—3524.
4. Colon 26-L5 carcinoma is a highly liver-metastatic variant cell line derived from murine colon 26 carcinoma and offers a good model for human colon cancers where the prognosis depends heavily on the occurrence of liver-metastasis; Ohnishi, Y.; Sakamoto, T.; Fujii, H.; Kimura, F.; Murata, J.; Tazawa, K.; Fujimaki, M.; Sato, Y.; Kondo, M.; Une, Y.; Uchino, J.; Saiki, I. *Tumor Biol.* **1997**, 18, 113—122.
5. **Staminol A (1)**: $[\alpha]_D^{25}$ −24.3° (c = 0.51, CHCl$_3$). IR ν_{max} cm^{-1}: 3550, 3430, 3300, 1725, 1600, 1450, 1370, 1200—1270, 1090, 1070, 1040. FAB-MS *m/z*: 757 (M+Na)$^+$, 735 (M+H)$^+$. High-resolution FAB-MS: 735.2970 (calcd. for $C_{40}H_{47}O_{13}$ (MH$^+$), 735.3017).
6. **Orthosiphol F (2)**: $[\alpha]_D^{25}$ −82.8° (c = 2.10, CHCl$_3$). IR ν_{max} cm^{-1}: 3550, 3450, 1725, 1455, 1370, 1280, 1110, 1050. FAB-MS *m/z*: 699 (M+Na)$^+$, 677 (M+H)$^+$. High-resolution FAB-MS: 677.2958 (calcd. for $C_{38}H_{45}O_{11}$ (MH$^+$), 677.2962).
7. **Orthosiphol G (3)**: $[\alpha]_D^{25}$ −63.3° (c = 0.47, CHCl$_3$). IR ν_{max} cm^{-1}: 3550, 3400, 1720, 1455, 1370, 1280, 1115, 1045. FAB-MS *m/z*: 595 (M+Na)$^+$, 573 (M+H)$^+$. High-resolution FAB-MS: 573.2668 (calcd. for $C_{31}H_{41}O_{10}$ (MH$^+$), 573.2700).
8. **Orthosiphol H (4)**: $[\alpha]_D^{25}$ −58.0° (c = 0.63, CHCl$_3$). IR ν_{max} cm^{-1}: 3550, 3400, 1725, 1455, 1370, 1280, 1240, 1110, 1045. FAB-MS *m/z*: 741 (M+Na)$^+$, 719 (M+H)$^+$. High-resolution FAB-MS: 719.3051 (calcd. for $C_{40}H_{47}O_{12}$ (MH$^+$), 719.3067).
9. **Orthosiphol I (5)**: $[\alpha]_D^{25}$ −108.9° (c = 0.55, CHCl$_3$). IR ν_{max} cm^{-1}: 3550, 3400, 1725, 1455, 1375, 1270, 1110, 1045. FAB-MS *m/z*: 593 (M+Na)$^+$, 571 (M+H)$^+$. High-resolution FAB-MS: 571.2568 (calcd. for $C_{31}H_{39}O_{10}$ (MH$^+$), 571.2543).
10. Masuda, T.; Masuda, K.; Shiragami, S.; Jitoe, A.; Nakatani, N. *Tetrahedron* **1992**, 48, 6787—6792.
11. Cytotoxicity was determined by a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide (MTT) assay method as described previously; Banskota, A. H.; Tezuka, Y.; Prasain, J. K.; Matsushige, K.; Saiki, I.; Kadota, S. *J. Nat. Prod.* **1998**, 61, 896—900.