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Staminolactones A and B and Norstaminol A: Three Highly Oxygenated Staminane-Type Diterpenes from *Orthosiphon stamineus*

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

Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630-Sugitani, Toyama 930-0194, Japan, and National University Hochiminh City, Hochiminh, Vietnam

kadota@ms.toyama-mpu.ac.jp

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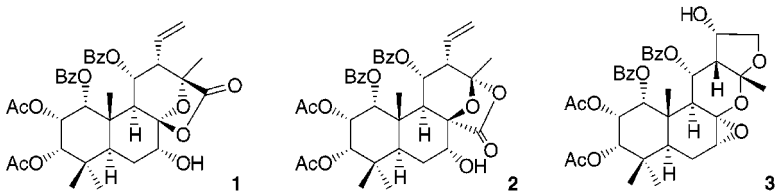
ABSTRACT



Staminolactones A (1) and B (2) and norstaminol A (3), three highly oxygenated staminane-type diterpenes having mild cytotoxic activities against highly liver-metastatic colon 26-L5 carcinoma cells, were isolated from the aerial part of the Vietnamese medicinal plant *Orthosiphon stamineus* (Lamiaceae). Their structures were elucidated on the basis of the extensive spectral analyses.

Orthosiphon stamineus BENTH. (Lamiaceae) is a medicinal plant grown in Southeast Asia and is used in treating urinary lithiasis, edema, eruptive fever, influenza, rheumatism, hepatitis, jaundice, and biliary lithiasis.[1] This plant has been known to contain highly oxygenated isopimarane-type diterpenes, orthosiphols A−E.[2] As part of our continued studies on Vietnamese medicinal plants,[3] we have isolated two new diterpenes, staminols A (**4**) and B (**5**), having the novel carbon framework "staminane" [4] along with four new isopimarane-type diterpenes, orthosiphols F−I, from a $CHCl_3$-soluble fraction of an MeOH extract of the aerial part.[5] Interestingly, **4** and **5**, containing the novel carbon framework, show a mild cytotoxicity against highly liver-metastatic colon 26-L5 carcinoma cells.[6] We thus further separated the cytotoxic constituents of the $CHCl_3$-soluble fraction by a combination of silica gel column chromatography and

[†] Institute of Natural Medicine, Toyama Medical and Pharmaceutical University.

[‡] National University Hochiminh City, Hochiminh, Vietnam.

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(4) After our communication had been reported, Shibuya et al. also reported two "staminane"-type diterpenes, neoorthosiphols A and B. Shibuya, H.; Bohgami, T.; Ohashi, K. *Chem. Pharm. Bull.* **1999**, *47*, 911.

(5) (a) Stampoulis, P.; Tezuka, Y.; Banskota, A. H.; Tran, K. Q.; Saiki, I.; Kadota, S.*Tetrahedron Lett.* **1999**, *40*, 4239. (b) Stampoulis, P.; Tezuka, Y.; Banskota, A. H.; Tran, K. Q.; Saiki, I.; Kadota, S. *Abstract of Papers*, The 100th Meeting of the Hokuriku Branch of the Pharmaceutical Society of Japan; Hokuriku Branch of Pharmaceutical Society of Japan: Kanazawa, 1999; p 57.

(6) 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.

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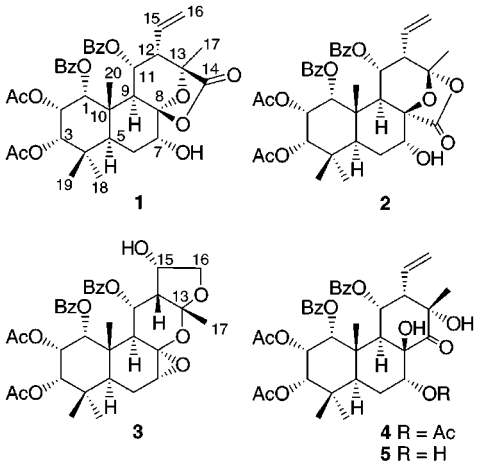
preparative TLC techniques and isolated three highly oxygenated staminane-type diterpenes exhibiting a mild cytotoxicity. They were identified as two secostaminane-type diterpenes, staminolactones A (**1**) and B (**2**), and a norstaminane-type diterpene, norstaminol A (**3**), by spectroscopic analyses. Here we wish to communicate the structure elucidation of these compounds.

Staminolactones A[7] (**1**) and B[8] (**2**) were obtained as colorless amorphous solids, and their molecular formulas were determined to be identical ($C_{38}H_{42}O_{12}$, *m*/*z* 691) by HR-FAB-MS. The IR spectra of **1** and **2** are similar and also closely match those of **4** and **5**, showing the absorptions characteristic of a hydroxy group (**1**, 3570 cm^{-1}; **2**, 3500 cm^{-1}), an ester carbonyl (1730 cm^{-1}), and a phenyl ring (1600, 1455 cm^{-1}). They are, however, characterized by the presence of an absorption of γ-lactone carbonyl (**1**, 1800 cm^{-1}; **2**, 1770 cm^{-1}).



The ^1H NMR spectra of **1** and **2** are also similar and closely match that of **5** (Table 1), showing the signals of one vinyl, five oxymethines, three methines, one methylene, and four methyls in addition to those of two benzoyl and two acetyl groups. The ^{13}C NMR spectra are also similar and reveal signals corresponding to the above groups (Table 1), but those of **1** and **2** are characterized by the presence of the carbon signals of one lactone carbonyl (**1**, δ 172.5; **2**, 176.4) and one acetal (**1**, δ 107.9; **2**, δ 110.9) and by the disappearance of the signals of one ketone (δ 214.1) and one of two oxygen-substituted quaternary carbon (δ 77.2, 78.7). These spectral data and the $^1H-^1H$ COSY and FG-pulsed HMQC spectra [Figure 1a] suggest that **1** and **2** should be isomers on ring C, having the same rings A and B as **5**.

The long-range correlations of the *tert*-methyls H_3-18, H_3-19, and H_3-20 in the FG-pulsed HMBC spectra of **1** and **2** confirm the structures of the rings A and B. On rings C and D, **1** and **2** show different correlations which correspond to the structural differences. In the case of **1**, the acetal carbon (δ 107.9) is correlated with the methine protons at δ 3.10 (H-9) and 4.00 (H-7), and the lactone carbonyl at δ 172.5 (C-14) and the oxygen-substituted quaternary carbon at δ 79.4 (C-13) both are correlated with the *tert*-methyl at δ 1.32 (H_3-17). In the case of **2** a correlation is observed between the acetal carbon (δ 110.9) and the *tert*-methyl at δ 1.59 (H_3-17), and the oxygen-substituted quaternary carbon at δ 78.9 is correlated with the oxymethine at δ 2.62 (H-9). Thus the acetal and oxygen-substituted quaternary carbons of **1** are concluded to be C-8 and C-13, respectively, and those of **2** are C-13 and C-8. Similarly, the location of the two benzoyl groups was determined to be at C-1 and C-11 and that of the two acetyl groups at C-2 and C-3, based on the long-range correlations of the ester carbonyl carbons. Thus the planar structures of staminolactones A and B were determined to be **1** and **2**.

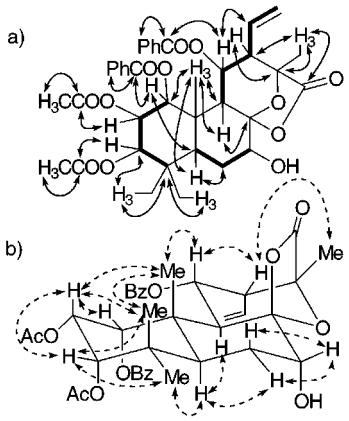


Figure 1. Connectivities (bold line) established by the $^1H-^1H$ COSY and FG-pulsed HMQC spectra and key long-range correlations (arrows) from the FG-pulsed HMBC spectrum of **1** (a) and ROESY correlations (dotted arrows) observed in the phase-sensitive ROESY spectrum (mixing time, 0.5 s) of **1** (b).

The relative stereochemistries of **1** and **2** were elucidated on the basis of the ROESY correlations and the analyses of the coupling constants. The coupling pattern of H-1-H-2-H-3 and H-5-H-6_{ax},H-6_{eq}-H-7 of both **1** and **2** suggests the former three protons to be *cis*, H-5 to be *axial*, and H-7 to be equatorial. In the ROESY spectra of **1** and **2**, on the other hand, correlations are observed between the methyl protons H_3-19 and the protons H-2, H-3 and H_3-20, between the methyl protons H_3-20 and the protons H-1, H-2 and H-6_{ax}, and between H-5 and H-9, indicating the protons H-1, H-2, H-3, H-6_{ax}, H-7, H_3-19, and H_3-20 to be *cis* (β); the protons H-5 and H-9 to be *cis* (α); and the two groups to be *trans*. The coupling constant between H-9 and H-11 of **1** is large (J = 11 Hz), while that of **2** is small (nearly zero). Thus H-11 of **1** should be axial and that of **2** equatorial. In the ROESY spectrum of **1**, on the other hand, correlations are observed between H_3-20 and H-11, between H-11 and H-12. and between H-12 and H_3-17 [Figure 1b], while the ROESY spectrum of **2** shows the correlations between H_3-20 and

(7) **Characterization Data.** Colorless amorphous solid, $[\alpha]_D^{25}$ −97.24° (c = 0.067, $CHCl_3$). IR ν_{max} cm^{-1}: 3570, 1800, 1730, 1600, 1455, 1370, 1270, 1200−1240. FAB-MS *m*/*z*: 713 $(M + Na)^+$, 691 $(M+H)^+$. HR-FAB-MS: 691.2731 [calcd for $C_{38}H_{43}O_{12}$ $(M + H)^+$, 691.2754].

(8) **Characterization Data.** Colorless amorphous solid, $[\alpha]_D^{25}$ −98.88° (c = 0.12, $CHCl_3$). IR ν_{max} cm^{-1}: 3500, 1770, 1730, 1600, 1455, 1390, 1370, 1270, 1200−1240. FAB-MS *m*/*z*: 713 $(M + Na)^+$, 691 $(M + H)^+$. HR-FAB-MS: 691.2746 [calcd for $C_{38}H_{43}O_{12}$ $(M + H)^+$, 691.2754].

Table 1. ^{1}H and ^{13}C NMR Data for Diterpenes **1**−**3** in $CDCl_3$ (*J* Values Are Given in Parentheses)

	1		**2**		**3**		**5**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	5.13 br s	73.8	5.57 br s	71.8	5.29 br s	71.2	5.69 br s	74.0
2	5.34 br s	66.6	5.57 br s	66.1	5.50 br s	66.0	5.38 br s	66.5
3	4.99 d (3.1)	75.8	5.08 br s	76.0	5.05 d (2.9)	75.8	4.99 d (2.9)	76.1
4		37.2		37.4		37.3		37.3
5	2.80 br d (13.8)	34.7	2.74 d (11.7)	34.6	2.62 br d (13.3)	35.1	2.85 d (12.7)	34.3
6	1.89 br t (13.8)	24.5	1.86 br t (13.9)	24.3	1.82 br t (13.3)	24.4	1.83 br t (12.0)	22.6
	2.02 br d (13.8)		1.93 br d (13.9)		1.91 br d (13.3)		1.99 br t (12.0)	
7	4.00 br s	69.7	4.17 br s	67.9	3.80 br s	69.7	4.19 br s	69.4
8		107.9		78.9		94.9		78.7
9	3.10 d (11.0)	39.8	2.62 s	42.5	2.74 d (3.4)	45.3	3.10 d (10.2)	40.6
10		42.8		43.0		41.7		43.7
11	5.71 dd (11.0, 6.1)	68.4	5.53 d (5.2)	66.9	5.58 t (3.4)	64.6	6.21 dd (10.2, 3.1)	70.4
12	2.79 dd (10.5, 6.1)	48.6	2.78 dd (10.2, 5.2)	50.2	2.41 t (3.4)	43.8	3.05 dd (9.5, 3.1)	54.7
13		79.4		110.9		104.8		77.2
14		172.5		176.4				214.1
15	5.56 dt (17.3, 10.5)	129.7	5.47 dt (16.9, 10.2)	131.0	4.59 br s	72.84	5.14 dt (16.6, 10.2)	144.7
16	4.88 d (17.3)	122.9	5.19 d (10.2)	122.4	3.69 bd d (10.4)	72.77	4.58 d (10.2)	121.2
	5.03 d (10.5)		5.31 d (16.9)		3.81 bd d (10.4)		4.86 d (16.6)	
17	1.32 s	18.2	1.59 s	22.1	1.66 s	21.4	1.67 s	28.2
18	1.03 s	28.2	1.04 s	27.8	1.04 s	27.9	1.09 s	22.3
19	1.11 s	22.7	1.15 s	22.2	1.12 s	22.4	1.00 s	28.1
20	1.40 s	14.1	1.49 s	17.3	1.33 s	15.8	1.38 s	15.7
1-OBz								
1′		129.7		129.37		129.7		130.7[a]
2′,6′	8.06 d (7.3)	129.7	7.86 d (7.6)	129.3	7.68 d (7.3)	129.9	8.04 d (7.6)	129.7
3′,5′	7.52 t (7.3)	128.4	7.10 t (7.6)	127.9	7.29 t (7.3)	128.1	7.42 t (7.6)	128.4
4′	7.63 t (7.3)	132.9	7.21 t (7.6)	132.55	7.53 t (7.3)	132.7	7.48 t (7.6)	132.7
7′		163.3		164.5		164.6		164.2
2-OAc								
1″		170.1		170.6		169.9		170.2
2″	1.75 s	20.7	1.99[a] s	20.7	1.82 s	20.7	1.98 s	20.8
3-OAc								
1‴		170.6		170.5		170.6		170.8
2‴	1.57 s	20.5	1.74[a] s	20.9	1.70 s	20.8	1.59 s	20.5
11-OBz								
1⁗		130.6		128.5		129.9		130.3[a]
2⁗,6⁗	7.72 d (7.6)	130.7	7.52 d (7.3)	129.44	7.58 d (7.6)	129.3	8.16 d (7.5)	130.5
3⁗,5⁗	7.33 t (7.6)	127.6	7.18 t (7.3)	128.0	7.07 t (7.6)	128.0	7.42 t (7.5)	128.2
4⁗	7.53 t (7.6)	132.9	7.37 t (7.3)	132.64	7.41 t (7.6)	132.6	7.55 t (7.5)	133.2
7⁗		166.2		163.1		165.5		166.7

[a] May be interchanged in each column.

H-11, between H_3-20 and H-12, between H-11 and H-12, and between H-12 and H_3-17. Thus the protons H-11, H-12, and H_3-17 should be β in both compounds. From these data, together with a consideration of the Dreiding stereomodel, ring C of **1** is determined to be chair and that of **2** boat; i.e., the lactone bridge of **1** has a β-orientation and that of **2** an α-orientation.

Norstaminol A[9] (**3**), $[\alpha]_D^{25}$ −38.00° (c = 0.41, $CHCl_3$), was isolated as a colorless amorphous solid. It gave a quasimolecular ion at m/z 701 $(M + Na)^+$ and 679 $(M + H)^+$ in FAB-MS, and its molecular formula was determined by HR-FAB-MS to be $C_{37}H_{42}O_{12}$, one carbon less than that of **1** and **2**. The IR spectrum of **3** indicates the absorption of a hydroxy (3550 cm^{-1}) and an ester carbonyl (1725 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **3** are partially similar to those of **1** and **2** (Table 1), but they are characterized by the lack of the signals of a vinyl group (C-15, C-16), a lactone carbonyl (C-14), and an oxygen-substituted quaternary carbon (C-13) and the presence of the signals of an oxymethylene (δ 3.69, 3.81), an oxymethine (δ 4.59), and an additional ketal carbon. The ^{1}H−^{1}H COSY and FG-pulsed HMQC spectra led to the structural units depicted by bold lines, which units are connected on the basis of the long-

(9) **Characterization Data.** Colorless amorphous solid, $[\alpha]_D^{25}$ −38.00° (c =0.41, $CHCl_3$). IR ν_{max} cm^{-1}: 3550, 1725, 1600, 1455, 1370, 1280, 1200−1240, 1110. FAB-MS m/z: 701 $(M + Na)^+$, 679 $(M + H)^+$. HR-FAB-MS: 679.2788 [calcd for $C_{37}H_{43}O_{12}$ $(M + H)^+$, 679.2754].

range correlations in the FG-pulsed HMBC spectrum [Figure 2a]. The acetal carbon at δ 104.8 was located at C-13 on the basis of the long-range correlations with the methyl protons at δ 1.66 (H_3-17) and the oxymethine proton at δ 4.59 (H-15), while the other acetal carbon (δ 94.9) is assigned as C-8 on the basis of the long-range correlations with the protons H-7 and H-9. Moreover, the oxymethylene proton H-16 shows a correlation with the former acetal carbon (C-13), indicating the presence of a furane ring. Although there is no long-range correlation to deduce the pyrane ring, it would be reasonable to connect the two acetal carbons (C-8 and C-13) through an oxygen and to suspect the presence of an epoxide ring at C-7 and C-8, because the molecular formula of **3** ($C_{37}H_{42}O_{12}$) indicates that there is no other atom. The locations of the ester groups were also determined by the analyses of the FG-pulsed HMBC spectrum, i.e., two benzoyl groups at C-1 and C-11 and two acetyl groups at C-2 and C-3.

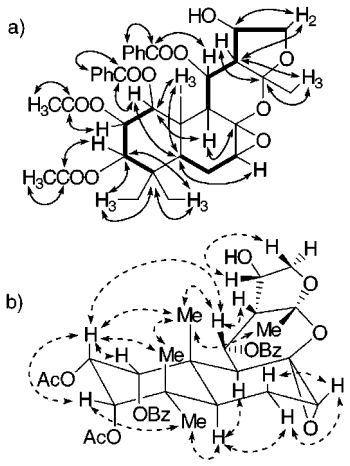


Figure 2. Connectivities (bold line) established by the $^1H-^1H$ COSY and FG-pulsed HMQC spectra and key long-range correlations (arrows) from the FG-pulsed HMBC spectrum of **3** (a) and ROESY correlations (dotted arrows) observed in the phase-sensitive ROESY spectrum (mixing time, 0.5 s) of **3** (b).

The configurations at the chiral centers C-1, C-2, C-3, C-5, C-9, and C-10 were determined to be the same those in **1** and **2**, on the basis of the ROESY correlations [Figure 2b] and the coupling constants. For the configuration of ring C, on the other hand, the ROESY correlations of H-11 with H_3-20, H-12, and H_3-17 indicated that H-11, H-12, and H_3-17 should be β. The proton H-12 also show ROESY correlations with H-15 and H-16β, and the small coupling constants of H-15 with H-12 and H_2-16 suggest that the hydroxy group at C-15 should have α-configuration and that ring C should have a boat conformation. At last, the configuration of the epoxy ring, i.e., C-7 and C-8, was concluded to be α on the basis of the ROESY correlation between H_3-17 and H_3-20. Thus, the structure of norstaminol A was concluded to be represented by the structure formula **3**.

Staminolactone A (**1**) is 8,14-secostaminane-type and staminolactone B (**2**) is 13,14-secostaminane-type, while norstaminol A (**3**) is 14-norstaminen-type with an α-alkyloxy-epoxide. Usually, an α-alkyloxy-epoxide is easily hydrolyzed to an α-hydroxy-ketone. The α-alkyloxy-epoxide in **3**, however, is very stable, because the alkyloxy group constitutes the rigid pyrane ring. The coexistence of staminolactones A (**1**) and B (**2**) with staminol B (**5**) suggested that the former two should be biosynthesized from the latter through a Baeyer−Villiger oxidation,[10] and further oxidation, including oxidative decarboxylation, would lead to norstaminol A (**3**).

The new diterpenes **1**−**3** showed moderate cytotoxicity against colon 26-L5 carcinoma with ED_{50} values of 68.5, 79.8, and 56.1 μg/mL, respectively,[11] which were comparable with those of orthosiphols F−I and staminols A (**4**) and B (**5**). These and related diterpenes may contribute to the cytotoxicity of the MeOH extract of *O. stamineus*.

Acknowledgment. This work was supported in part by a Grant-in-Aid for International Scientific Research (Grant 09041177) from the Ministry of Education, Sports, and Culture, Japan.

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(10) Baeyer−Villiger oxidation was reported to correspond to the biosynthesis of antibiotics and brassinolides. (a) Prado, L.; Fernandez, E.; Weissbach, U.; Blanco, G.; Quiros, L. M.; Brana, A. F.; Mendez, C.; Rohr, J.; Salas, J. A. *Chem. Biol.* **1999**, *6*, 19. (b) Lacave, C.; Laneelle, M. A.; Daffe, M.; Montrozier, H.; Laneelle, G. *Eur. J. Biochem.* **1989**, *181*, 459. (c) Lee, J. J.; Lee, J. P.; Keller, P. J.; Cottrell, C. E.; Chang, C.; Zahner, H.; Floss, H. G. *J. Antibiot.* **1986**, *39*, 1123. (d) Grove, M. D.; Spencer, G. F.; Rohwedder, W. K.; Mandava, N.; Worley, J. F.; Warthen Jr., J. D.; Steffens, G. L.; Flippen-Anderson, J. L.; Cook, J. C., Jr. *Nature* **1979**, *281*, 216.

(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.