Constituents of the Vietnamese Medicinal Plant *Orthosiphon stamineus*

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From the MeOH extract of the aerial part of Vietnamese *Orthosiphon stamineus*, five new isopimarane-type diterpenes [orthosiphols F—J (1—5)] and two new diterpenes [staminols A (6) and B (7)] with a novel carbon-framework, to which we proposed the name "staminane", and three new highly-oxygenated staminane-type diterpenes [staminolactones A (8) and B (9) and norstaminol A (10)] were isolated. Moreover, staminolactone A (8) is 8,14-secostaminane-type and staminolactone B (9) is 13,14-secostaminane-type, while norstaminol A (10) is 14-norstaminen-type. Together with these new diterpenes, sixteen known compounds were also isolated and identified to be: 7,3′,4′-tri-*O*-methylluteolin (11), eupatorin (12), sinensetin (13), 5-hydroxy-6,7,3′,4′-tetramethoxyflavone (14), salvigenin (15), ladanein (16), tetramethylscutellarein (17), 6-hydroxy-5,7,4′-trimethoxyflavone (18), vomifoliol (19), aurantiamide acetate (20), rosmarinic acid (21), caffeic acid (22), oleanolic acid (23), ursolic acid (24), betulinic acid (25), and β-sitosterol (26). All the isolated compounds were tested for their cytotoxicity towards highly liver metastatic murine colon 26-L5 carcinoma cells, and the new diterpenes, except for 4, and flavonoids (11, 12, 16, 18) showed cytotoxicity with an ED_{50} value between 10 and 90 μg/ml.

Key words *Orthosiphon stamineus*; staminane-type diterpene; Vietnamese medicinal plant; isopimarane-type diterpene; cytotoxicity; colon 26-L5 carcinoma

Orthosiphon (*O.*) *stamineus* BENTH. [syn.: *O. grandiflorus* BOLD., *O. spicatus* (THUNB.) BAK., *O. aristatus* (BL.) MIQ.; Lamiaceae] is a medicinal plant grown in Southeast Asia and is currently cultivated in Indonesia.[1)] In Indonesia, the leaves of this plant are used by the name of "Kumis kucing" as a diuretic, and to treat rheumatism, diabetes, hypertension, *etc*.,[1a)] while in Vietnam its aerial part is used by the name of "Râu mèo" in treating urinary lithiasis, edema, eruptive fever, influenza, rheumatism, hepatitis, jaundice, and biliary lithiasis.[1b)] From its popularity and demonstrated effectiveness, phytochemical and pharmacological studies have been conducted since the 1930's,[2,3)] and highly-oxygenated isopimarane-type diterpenes, orthosiphols A—E,[2a—c)] were reported, together with monoterpenes, triterpenes, saponins, flavonoids, hexoses, organic acids, rosmarinic acid, chromene, and myo-inositol. More recently, Shibuya *et al*. reported two isopimarane-type diterpenes,[4)] two migrated pimarane-type diterpenes,[5)] and a benzochromene[4)] from the leaves of *O. aristatus* (=*O. stamineus*) in Indonesia. Recently, in the course of our study on Vietnamese medicinal plants,[6)] we found that a MeOH extract from the aerial part of *O. stamineus* showed a cytotoxic activity against highly liver metastatic murine colon 26-L5 carcinoma cells.[7)] Thus, we examined its chemical constituents and identified ten new diterpenes **1**—**10**, including five (**6**—**10**) with a novel carbon-framework, together with sixteen known compounds. In this paper, we report the isolation and structural elucidation of the new diterpenes by spectroscopic techniques, together with their cytotoxic activities.[8)]

Isolation The dried aerial part of Vietnamese *O. stamineus* was extracted with refluxing MeOH, and the MeOH extract (ED_{50} for cytotoxicity, 73.6 μg/ml) was separated into hexane- (ED_{50}, >100 μg/ml), $CHCl_3$- (ED_{50}, 76.2 μg/ml), EtOAc- (ED_{50}, 82.4 μg/ml), BuOH- (ED_{50}, >100 μg/ml), and water-soluble (ED_{50}, >100 μg/ml) fractions. The $CHCl_3$- and EtOAc-soluble fractions with cytotoxicity were separated, respectively, by a combination of silica-gel column chromatography and normal- and reversed-phase preparative TLC techniques to give five new isopimarane-type diterpenes [orthosiphols F—J (**1**—**5**)], two new diterpenes [staminols A (**6**) and B (**7**)] with a novel carbon-framework, to which we proposed the name "staminane", and three new highly-oxygenated staminane-type diterpenes [staminolactones A (**8**) and B (**9**), norstaminol A (**10**)] together with sixteen known compounds. The known compounds were identified by analyses of their spectroscopic data and comparison of the spectral data with those in the literature (**11**—**19**)[2c,9)] or in our laboratory (**20**—**26**)[6a,10)] to be: 7,3′,4′-tri-*O*-methylluteolin (**11**), eupatorin (**12**), sinensetin (**13**), 5-hydroxy-6,7,3′,4′-tetramethoxyflavone (**14**), salvigenin (**15**), ladanein (**16**), tetramethylscutellarein (**17**), 6-hydroxy-5,7,4′-trimethoxyflavone (**18**), vomifoliol (**19**), aurantiamide acetate (**20**), rosmarinic acid (**21**), caffeic acid (**22**), oleanolic acid (**23**), ursolic acid (**24**), betulinic acid (**25**), and β-sitosterol (**26**). The presence of **19** and **20** in *O. stamineus* is reported here for the first time.

Structures of Isopimarane-Type Diterpenes Orthosiphol F (**1**) was obtained as a colorless amorphous solid with an $[\alpha]_D^{25}$ value of −82.8° ($CHCl_3$). High-resolution FAB-MS (HR-FAB-MS) of **1** indicated the molecular formula $C_{38}H_{44}O_{11}$ (*m*/*z* 676), the same as that of orthosiphol A (**27**), and its IR spectrum showed absorption due to hydroxyl (3550, 3450 cm^{-1}) and ester carbonyl (1725 cm^{-1}) groups, similar to those of **27**.[6)] The ^1H- and ^{13}C-NMR data (Tables 1, 2) also closely resembled those of **27**,[6)] but they were characterized by a downfield shift of H-3 (**1**, δ 4.99; **27**, δ 3.49) and an upfield shift of H-7 (**1**, δ 4.22; **27**, δ 5.43). Thus, orthosiphol F appeared to be 3-*O*-acetyl-7-*O*-deacetylorthosiphol A, which was confirmed by the ^1H–^1H sift correlation spectroscopy (COSY), heteronuclear shift correlation (HETCOR), and heteronuclear multiple bond correlation (HMBC) spectra (Fig. 1a, Table 2). Based on the nuclear Overhauser effects (NOEs) observed in the difference NOE experiment (Fig. 1c), the rings A and B were determined to have a chair conformation with the same configuration as **27**, while the ring C was indicated, by the NOEs from H-9 to H-

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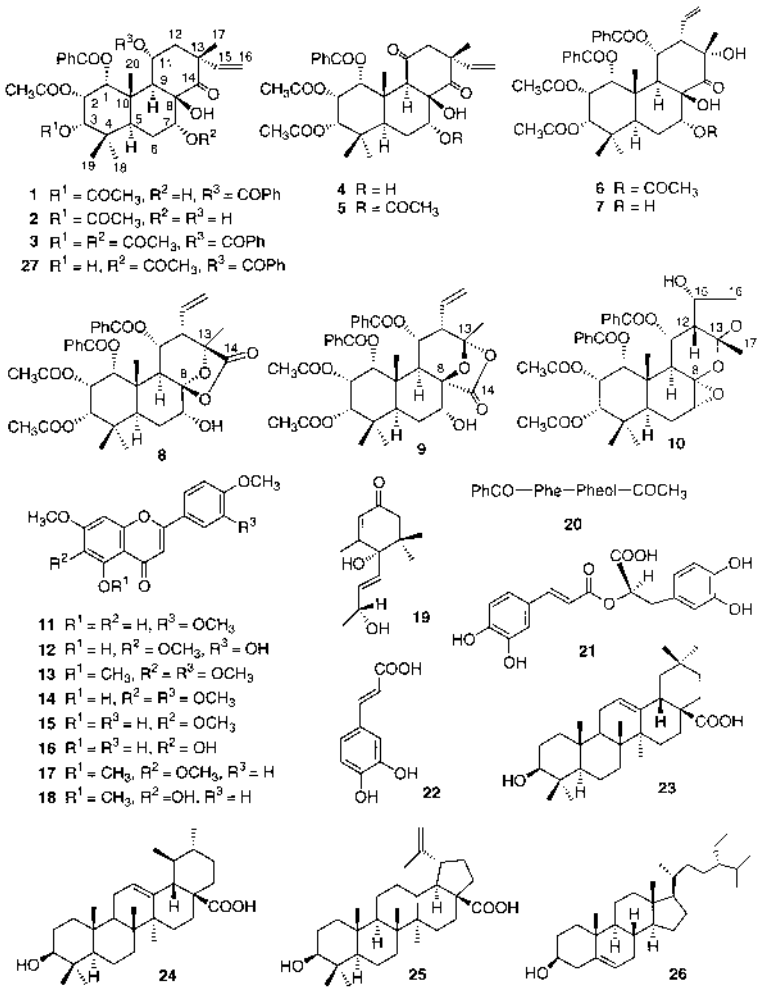

Chart 1. Structures of Constituents of Aerial Part of *Orthosiphon stamineus*

16 and from H_3-20 to H-11, to have a boat conformation with the vinyl and 11-*O*-benzoyl groups at the α-orientation. From these spectral data, orthosiphol F was concluded to be 3-*O*-acetyl-7-*O*-deacetylorthosiphol A (**1**).

Orthosiphol G (**2**), $[\alpha]_D^{25}$ −63.3° ($CHCl_3$), and orthosiphol H (**3**), $[\alpha]_D^{25}$ −58.0° ($CHCl_3$), were obtained as amorphous solids and displayed almost identical IR spectrum with that of **1**. Their molecular formulas were determined by HR-FAB-MS to be $C_{31}H_{40}O_{10}$ (M.W. 572) and $C_{40}H_{46}O_{12}$ (M.W. 718), respectively, which suggested that **2** has one less benzoyl group and **3** has one more acetyl group than **1**. The ^{1}H- and ^{13}C-NMR data for **2** and **3** (Tables 1, 2) were also similar to each other and to those of **1**, but those of **2** were characterized by the lack of signals of one of two benzoyl groups and those of **3** by presence of the signals of one more acetyl group. The locations of the debenzoylation in **2** and of the additional acetyl group in **3** were supposed to be at C-11 and at C-7, respectively, based on the upfield shift of H-11 in **2** (δ 4.43) and the downfield shift of H-7 in **3** (δ 5.45), compared to those of **1** (H-11, δ 5.64; H-7, δ 4.22). From these data, together with the analyses of the COSY, HETCOR, HMBC, and difference NOE spectra, orthosiphols G and H were determined to be 11-*O*-debenzoylorthosiphol F (**2**) and 3-*O*-acetylorthosiphol A (**3**), respectively.

Orthosiphol I (**4**), $[\alpha]_D^{25}$ −108.9° ($CHCl_3$), and orthosiphol J (**5**), $[\alpha]_D^{25}$ −58.5° ($CHCl_3$), were also obtained as amorphous solids. Their molecular formulas were determined by HR-FAB-MS to be $C_{31}H_{38}O_{10}$ (MW 570) and $C_{33}H_{40}O_{11}$ (MW 612), respectively. They showed ^{1}H- and ^{13}C-NMR data similar to each other and to those of **1**—**3**, and those of **5** showed the presence of one more acetyl group than **4** (Tables 1, 2). The ^{1}H- and ^{13}C-NMR data for **4** were similar to those of orthosiphol G (**2**), but they were characterized by a lack of the signals of one (C-11) of two hydroxymethines in **2** and by the presence of a signal due to a ketone-carbonyl carbon. Thus, **4** was believed to have a carbonyl group instead of a hydroxyl group at C-11 in **2**, and **5** was a 7-*O*-acetate of **4**, which was confirmed by analyses of the COSY, heteronuclear multiple-quantum coherence (HMQC), and HMBC spectra. Based on these data and the results of difference NOE experiments, orthosiphols I and J were determined to be 11-dehydroxy-11-oxoorthosiphol G (**4**) and its 7-*O*-acetate (**5**), respectively.

Structures of Staminane-Type Diterpenes Staminol A (**6**) was obtained as a colorless amorphous solid with an $[\alpha]_D^{25}$ value of −24.3° ($CHCl_3$), and its molecular formula was determined by HR-FAB-MS to be $C_{40}H_{46}O_{13}$ (MW 734). The IR spectrum of **6** showed the absorption of hydroxyl (3550, 3430, 3300 cm^{-1}), ester carbonyl (1725 cm^{-1}), and phenyl (1600, 1450 cm^{-1}) groups. The ^{1}H-NMR spectrum of **6** revealed signals due to four tertiary methyls, a vinyl, five oxygenated methines, and three aliphatic methines, together

Table 1. ^{1}H-NMR Data for Orthosiphols F—J (**1**—**5**) in $CDCl_3$ (J in Hz)

	1	**2**	**3**	**4**	**5**
1	5.29 d (3)	5.60 d (2.5)	5.16 d (2)	6.39 d (3)	6.35 d (3)
2	5.54 t (3)	5.52 dd (3.5, 2.5)	5.51 dd (4, 2)	5.61 t (3)	5.60 t (3)
3	4.99 d (3)	5.05 d (3.5)	4.99 d (4)	5.08 d (3)	5.06 d (3)
5	2.69 dd (13, 2.5)	2.58 m	2.48 dd (12.5, 3)	2.47 dd (13.5, 2.5)	2.26 m
6	2.03 ddd (14, 13, 2.5)	1.82 m	2.04 m	2.04 ddd (15, 13.5, 2.5)	2.24 m
	1.89 dt (14, 2.5)	1.95 m	2.02 m	1.84 dt (15, 2.5)	1.98 m
7	4.22 t (2.5)	4.14 br t (3)	5.45 t (3)	4.29 br t (2.5)	5.33 br t (2.2)
9	3.04 d (4.5)	2.60 d (5)	3.18 d (6.5)	3.49 s	3.52 s
11	5.64 ddd (4.5, 4, 2.5)	4.43 m	5.80 ddd (6.5, 5, 2)		
12	2.21 dd (15, 2.5)	2.35 dd (14.5, 5.5)	2.58 dd (15.5, 5)	2.66 d (18)	2.63 d (18.3)
	2.73 dd (15, 4)	1.73 dd (14.5, 4)	1.95 dd (15.5, 2)	2.76 d (18)	2.69 d (18.3)
15	5.77 dd (17.5, 11)	5.87 dd (17.5, 11)	5.67 dd (17, 11)	5.35 dd (17.5, 11)	5.28 dd (17.3, 10.7)
16	4.71 d (11)	4.69 d (11)	4.75 d (11)	4.16 d (11)	4.12 d (10.7)
	4.88 d (17.5)	4.87 d (17.5)	4.81 d (17)	4.67 d (17.5)	4.66 d (17.3)
17	1.26 s	1.23 s	1.13 s	1.14 s	1.11 s
18	0.99 s	1.00 s	0.90 s	0.98 s	0.90 s
19	1.12 s	1.13 s	1.14 s	1.12 s	1.12 s
20	1.47 s	1.45 s	1.52 s	1.42 s	1.44 s
1-OCOPh					
2′,6′	7.59 dd (8, 1)	8.09 d (7.5)	7.70 dd (8, 1)	8.11 dd (8, 1)	8.11 d (7.3)
3′,5′	7.21 t (8)	7.43 t (7.5)	7.32 t (8)	7.46 t (8)	7.43 t (7.3)
4′	7.44 dt (8, 1)	7.55 t (7.5)	7.54 tt (8, 1)	7.58 tt (8, 1)	7.58 t (7.3)
2-OCOCH_3					
1″	1.82 s	1.97 s	1.84 s	1.95 s	1.95 s
3-OCOCH_3					
1‴	1.50 s	1.66 s	1.44 s	1.77 s	1.76 s
7-OCOCH_3					
1⁗			2.19 s		2.06 s
11-OCOPh					
2⁗,6⁗	7.49 dd (8, 1)		7.60 dd (8, 1)		
3⁗,5⁗	6.99 t (8)		7.12 t (8)		
4⁗	7.31 dt (8, 1)		7.42 tt (8, 1)		

with those of two benzoyl and three acetyl groups (Table 3). Moreover, its ^{13}C-NMR spectrum (Table 4) indicated the presence of six (a ketone and five ester) carbonyl groups and four quaternary carbons, including two oxygenated ones. Analyses of these signals by the COSY and HETCOR spectra led to the partial structures depicted in Fig. 1b, which were connected based on the long-range correlations observed in the HMBC spectrum (Fig. 1b, Table 4). The stereostructure of **6** was elucidated from the J values of each proton and from the results of difference NOE experiments. The large J values for H-5, H-9, and H-11 ($J_{5,6ax}$=13.5 Hz; $J_{9,11}$=11 Hz) indicated their axial nature, while the small J values for H-7 and H-12 ($J_{6eq,7}$=3 Hz, $J_{6ax,7}$=0 Hz; $J_{11,12}$=3.5 Hz) suggested them to be equatorial. In the difference NOE experiments, the same NOEs were detected on rings A and B, while on ring C NOEs were observed from H_3-20 to H-11, from H-9 to H-15, and from H_3-17 to H-11 and H-12 (Fig. 1d). Thus, ring C was determined to have a chair conformation with the configuration indicated in Fig. 1d. From these data, the structure of staminol A was concluded to be **6**.

Staminol B (**7**), $[\alpha]_D^{25}$ −27.8° ($CHCl_3$), showed the molecular formula $C_{38}H_{44}O_{12}$, C_2H_2O less than that of **6**, in HR-FAB-MS. The ^{1}H- and ^{13}C-NMR data for **7** were similar to those of **6** (Tables 3, 4), but they showed the presence of only two acetyl groups instead of the three in **6**. Analysis of the COSY and HMQC spectra revealed an upfield shift of H-7 (δ 4.19), compared to that of **6** (δ 5.37), indicating that the free hydroxyl group should be located at C-7. Thus, the structure of staminol B was determined to be 7-*O*-deacetylstaminol A (**7**).

Structures of Highly-Oxygenated Staminane-Type Diterpenes Staminolactones A (**8**) and B (**9**) were obtained as colorless amorphous solids, and their molecular formulas were determined to be the same ($C_{38}H_{42}O_{12}$, MW 690) by HR-FAB-MS. Their IR spectra were similar to each other and also to that of **7**, and showed the absorption of a hydroxyl group, an ester carbonyl, and a phenyl ring. They were, however, characterized by the presence of an absorption of γ-lactone carbonyl (**8**, 1800 cm^{-1}; **9**, 1770 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **8** and **9** resembled and were similar to those of **7** (Tables 3, 4), and showed signals of a vinyl, eight methines including five oxygenated ones, a methylene, and four methyls, together with those of two benzoyl and two acetyl groups. On the other hand, the ^{13}C-NMR spectra of **8** and **9** were characterized by the presence of signals of a lactone carbonyl (**8**, δ 172.5; **9**, 176.4) and an ketal (**8**, δ 107.9; **9**, δ 110.9), and by the disappearance of the signals of a ketone (δ 214.1) and one of two oxygenated quaternary carbons (δ 77.2, 78.7) in **7**. These spectral data and the COSY and HMQC spectra suggested that **8** and **9** should be isomers on ring C, having the same rings A and B as **7**.

The long-range correlations of the tertiary methyls H_3-18, H_3-19 and H_3-20 in the HMBC spectra of **8** and **9** confirmed the structures of rings A and B (Table 4). On rings C and D, **8** and **9** showed different correlations which correspond to structural differences. In the case of **8**, the ketal carbon (δ 107.9) was correlated with H-7 and H-9, and the lactone carbonyl (δ 172.5) and the oxygenated quaternary carbon (δ

Table 2. ^{13}C-NMR Data for Orthosiphols F—J (**1**—**5**) in $CDCl_3$

	1		**2**		**3**		**4**		**5**
	δ	HMBC[a)]	δ	HMBC[a)]	δ	HMBC[a)]	δ	HMBC[a)]	δ
1	71.9	2, 3, 9, 20	74.4	3, 20	72.5	3, 9, 20	73.4	2, 3, 20	73.5
2	66.3	1, 3	66.8	1, 3	66.4	1, 3	65.9	1, 3	65.7
3	76.1	1, 2, 18, 19	76.2	1, 18, 19	76.1	18, 19	76.1	1, 2, 18, 19	75.9
4	37.5	3, 5, 18, 19	37.4	3, 5, 18, 19	37.2	3, 18, 19	37.2	3, 5, 18, 19	37.0
5	35.6	1, 3, 7, 18, 19, 20	35.2	1, 3, 18, 19, 20	36.7	1, 3, 18, 19, 20	34.8	1, 3, 9, 18, 19, 20	36.0
6	23.4	5	23.4		21.3		20.8		22.7
7	69.3	9	69.3		70.6		69.0		71.4
8	78.1	7, 9	78.1	9	75.8	9	78.0	9	76.3
9	42.5	7, 12	44.6	20	41.8	12, 20	51.0	20	51.7
10	44.0	1, 5, 9, 20	43.6	9, 20	43.7	1, 9, 11, 20	42.8	1, 5, 9, 20	42.8
11	68.9	9, 12	64.7	9	68.6	9, 12	205.7	9, 12	204.5
12	39.1	15, 17	43.9	9, 17	39.7	15, 17	47.1	17	47.0
13	47.9	12, 15, 16, 17	48.9	12, 15, 16	47.9	12, 15, 16	49.5	12, 15, 16	49.5
14	214.0	9, 12, 15, 17	213.5	12, 15, 17	208.8	12, 15, 17	211.1	12, 17	208.2
15	141.9	12, 16, 17	141.7	12, 16, 17	141.9	12, 16, 17	138.9	12, 16, 17	138.3
16	113.3		114.3		113.2		116.2		116.4
17	27.7	12, 15	26.1	12	26.4	12	25.1	12	25.0
18	27.9	3, 5, 19	28.0	19	27.9	3, 19	27.8	19	28.0
19	22.1	5, 18	22.5	18	22.3	18	22.1	18	22.1
20	17.2	5, 9	16.1	5, 9	16.5	5, 9	16.3	5, 9	16.1
1-OCOPh									
1′	130.3[b)]	3′, 5′	130.8	3′, 5′	130.8	2′, 6′	130.4	3′, 5′	129.9
2′,6′	129.7	3′, 4′, 5′	129.8	4′	129.6	4′	130.0	3′, 5′	129.9
3′,5′	128.0		128.3		127.8		128.2	2′, 4′, 6′	128.2
4′	132.4	2′, 6′	132.8	2′, 6′	132.7	2′, 6′	132.9	2′, 6′	133.1
CO	164.5	1, 2′, 6′	166.1	1, 2′, 6′	163.7	1, 2′, 6′	164.3	1, 2′, 6′	163.9
2-OCOCH$_3$									
1″	170.1		170.6		170.1		170.1		169.9
CO	20.7	2, 1″	21.0	2, 1″	20.7	2, 1″	23.6	2, 1″	20.7
3-OCOCH$_3$									
1‴	170.7		170.8		170.5		170.6		170.3
CO	20.5	3, 1‴	20.6	3, 1‴	20.3		23.6	3, 1‴	21.1
7-OCOCH$_3$									
1⁗					168.7				168.2
CO					20.9	7, 1⁗			20.7
11-OCOPh									
1⁗′	130.2[b)]	3⁗′, 5⁗′			130.9	2⁗′, 6⁗′			
2⁗′,6⁗′	129.5	3⁗′, 4⁗′, 5⁗′			129.7	4⁗′			
3⁗′,5⁗′	127.6				127.9				
4⁗′	132.1	2⁗′, 6⁗′			132.3	2⁗′, 6⁗′			
CO	165.8	11, 2⁗′, 6⁗′			166.1	11, 2⁗′, 6⁗′			

a) Long-range correlated protons observed in the HMBC spectra.

79.4) both were correlated with H_3-17 (δ 1.32) (Fig. 2a), while in the case of **9** a correlation was observed between the ketal carbon (δ 110.9) and H_3-17 (δ 1.59), and the oxygenated quaternary carbon (δ 78.9) was correlated with H-9 (δ 2.62) (Fig. 2b). Thus, the ketal and oxygenated quaternary carbons of **8** were concluded to be C-8 and C-13, respectively, and those of **9** were C-13 and C-8. Similarly, the location of two benzoyl groups was determined to be at C-1 and C-11 and that of two acetyl groups at C-2 and C-3, based on the long-range correlations of the ester carbonyl carbons (Table 4). Thus, the planar structures of staminolactones A and B were determined to be **8** and **9**, respectively.

The relative stereochemistries of **8** and **9** were elucidated based on analyses of the coupling constants (Table 3) and the rotating-frame Overhauser enhancement spectroscopy (ROESY) correlations (Figs. 2d, e). On the rings A and B, the coupling pattern and ROESY correlations, similar to those of **1**—**7**, were observed and indicated H-1, H-2, H-3, H-6_{ax}, H-7, H_3-19, and H_3-20 to be *cis* (β); 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 **8** was large (J=11 Hz), while that of **9** was small (nearly zero), indicating that H-11 of **8** and **9** are axial and equatorial, respectively. On ring C, on the other hand, both **8** and **9** showed the same ROESY correlations, except for that between H_3-20 and H-12 of **9** (Figs. 2d, e). These data, together with consideration of the Dreiding stereomodel, indicated H-11 and H-12 to be β in both compounds; ring C of **8** to have a chair conformation (*i.e.*, lactone bridge has β-orientation); and that of **9** to have a boat conformation (*i.e.*, lactone bridge has α-orientation). Thus, the structures of staminolactones A and B were determined to be **8** and **9**, respectively.

Norstaminol A (**10**), $[\alpha]_D^{25}$ −38.0° ($CHCl_3$), 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 **8** and **9**. The ^{1}H- and ^{13}C-NMR spectra of **10** were partially similar to those of **8** and **9**

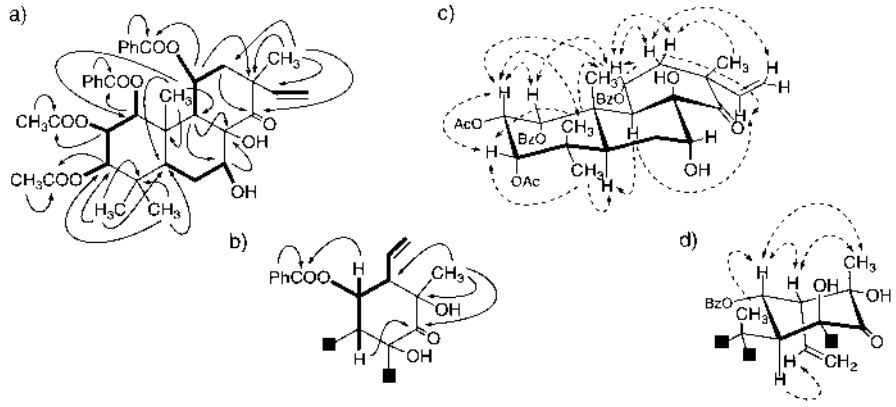

Fig. 1. Connectivities (Bold Line) Deduced by the COSY and HETCOR Spectra and Key Long-Range Correlations (Arrows, H→C) from the HMBC Spectrum of **1** (a) and **6** (b) and NOEs Observed in the Difference NOE Spectra of **1** (c) and **6** (d)

On rings A and B of **6**, the same long-range correlations and NOEs as those of **1** were observed.

Table 3. ^{1}H-NMR Data for Staminane-Type Diterpenes **6**—**10** in $CDCl_3$ (*J* in Hz)

	6	**7**	**8**	**9**	**10**
1	5.79 d (2)	5.69 br s	5.13 br s	5.57 br s	5.29 br s
2	5.38 dd (2, 3)	5.38 br s	5.34 br s	5.57 br s	5.50 br s
3	5.01 d (3)	4.99 d (2.9)	4.99 d (3.1)	5.08 br s	5.05 d (2.9)
5	2.63 dd (13.5, 2)	2.85 br d (12.2)	2.80 br d (13.8)	2.74 br d (11.7)	2.62 br d (13.3)
6	1.88 br t (13)	1.83 br t (12.2)	1.89 br t (13.8)	1.86 br t (13.9)	1.82 br t (13.3)
	2.08 br d (13)	1.99 br d (12.2)	2.02 br d (13.8)	1.93 br d (13.9)	1.91 br d (13.3)
7	5.37 d (3)	4.19 br s	4.00 br s	4.17 br s	3.80 br s
9	3.11 d (11)	3.10 d (10.2)	3.10 d (11)	2.62 s	2.74 d (3.4)
11	6.29 dd (11, 3.5)	6.21 dd (10.2, 3.1)	5.71 dd (11, 6.1)	5.53 d (5.2)	5.58 t (3.4)
12	2.96 dd (9.7, 3.5)	3.05 dd (9.7, 3.1)	2.79 dd (10.5, 6.1)	2.78 dd (10.2, 5.2)	2.41 t (3.4)
15	5.15 dt (17, 9.7)	5.14 dt (16.6, 9.7)	5.56 dt (17.3,10.5)	5.47 dt (16.9, 10.2)	4.59 br s
16	4.51 dd (9.7, 1.5)	4.58 d (9.7)	5.03 d (10.5)	5.19 d (10.2)	3.81 br d (10.4)
	4.83 dd (17, 1.5)	4.86 d (16.6)	4.88 d (17.3)	5.30 d (16.9)	3.69 br d (10.4)
17	1.68 s	1.67 s	1.32 s	1.59 s	1.66 s
18	0.91 s	1.09 s	1.03 s	1.04 s	1.04 s
19	1.09 s	1.00 s	1.11 s	1.15 s	1.12 s
20	1.40 s	1.38 s	1.40 s	1.49 s	1.33 s
7-OH				4.40 s	
1-OCOPh					
2′,6′	8.13 dd (8, 1)	8.04 d (7.6)	8.06 d (7.3)	7.86 d (7.6)	7.68 d (7.3)
3′,5′	7.42 t (8)	7.42 d (7.6)	7.52 t (7.3)	7.10 t (7.6)	7.29 t (7.3)
4′	7.58 tt (8, 1)	7.48 t (7.6)	7.63 t (7.3)	7.21 t (7.6)	7.53 t (7.3)
2-$OCOCH_3$					
1″	1.93 s	1.98 s	1.75 s	1.99 s	1.82 s
3-$OCOCH_3$					
1‴	1.60 s	1.59 s	1.57 s	1.74 s	1.70 s
7-$OCOCH_3$					
1⁗	2.19 s				
11-OCOPh					
2⁗,6⁗	8.26 dd (8, 1)	8.16 d (7.5)	7.72 d (7.6)	7.52 d (7.3)	7.58 d (7.6)
3⁗,5⁗	7.48 t (8)	7.42 d (7.5)	7.33 t (7.6)	7.18 t (7.3)	7.07 t (7.6)
4⁗	7.61 tt (8, 1)	7.55 t (7.5)	7.53 t (7.6)	7.37 t (7.3)	7.41 t (7.6)

(Tables 3, 4), but they were characterized by a lack of the signals of a vinyl group, a lactone carbonyl, and an oxygenated quaternary carbon, and by the presence of signals of an oxymethylene, an oxymethine, and an additional ketal carbon. These data were analyzed by the COSY, HMQC, and HMBC spectra and suggested the planar structure. The ketal carbon at δ 104.8 was located at C-13 based on the long-range correlations with H_3-17 and H-15, while the other ketal carbon (δ 94.9) was assigned as C-8 on the basis of the correlations with H-6, H-7, and H-9 (Fig. 2c, Table 4). Moreover, the former ketal carbon (C-13) showed a correlation with H-16, indicating the presence of a furan ring. Finally, the ketal carbons (C-8, C-13) would be connected through an oxygen to construct an epoxide ring at C-7 and C-8, because the molecular formula of **10** ($C_{37}H_{42}O_{12}$) indicated that there is no other atom. The locations of two benzoyl and two acetyl groups were also determined by analyses of the HMBC spectrum to be at C-1 and C-11 and at C-2 and C-3, respectively (Table 4). The configuration at the chiral centers in rings A and B was determined to be the same as that in **8** and **9** on the basis of the coupling constants and the ROESY correlations (Fig. 2f) of each proton. On the configuration of ring C,

Table 4. ^{13}C-NMR Data for Staminane-Type Diterpenes **6**—**10** in $CDCl_3$

	6		**7**	**8**		**9**		**10**	
	δ	HMBC[a)]	δ	δ	HMBC[a)]	δ	HMBC[a)]	δ	HMBC[a)]
1	74.4	3, 20	74.0	73.8	3, 20	71.8	3, 9, 20	71.2	3, 9, 20
2	66.4	1, 3	66.5	66.6	1, 3	66.1	1, 3	66.0	1, 3
3	76.1	1, 18, 19	76.1	75.8	1, 18, 19	76.0	1, 2, 18, 19	75.8	1, 18, 19
4	37.3	5, 18, 19	37.3	37.2	5, 18, 19	37.4	5, 18, 19	37.3	3, 5, 18, 19
5	35.8	1, 3, 18, 19, 20	34.3	34.7	1, 3, 7, 18, 19, 20	34.6	1, 3, 18, 19, 20	35.1	1, 3, 6, 7, 18, 19, 20
6	21.0		22.6	24.5		24.3	5, 7-OH	24.4	5
7	70.3	9	69.4	69.7		67.9		69.7	
8	77.4		78.7	107.9	7, 9	78.9	9, 11	94.9	6, 7, 9
9	40.7	20	40.6	39.8	11, 12, 20	42.5	11, 12, 20	45.3	20
10	43.5	5, 9, 20	43.7	42.8	1, 9, 5, 20	43.0	5, 9, 20	41.7	1, 5, 6, 9, 11, 20
11	70.2	9	70.4	68.4	9, 12	66.9	9	64.6	9
12	54.3	9, 16, 17	54.7	48.6	16, 17	50.2	9, 11, 15, 16, 17	43.8	16, 17
13	76.8	17	77.2	79.4	17	110.9	12, 17	104.8	15, 16, 17
14	209.0	9, 17	214.1	172.5	17	176.4			
15	131.2	11	144.7	129.7		131.0		72.8	11, 16
16	121.1		121.2	122.9	12	122.4	12, 15	72.8	15
17	29.1		28.2	18.2		22.1	12	21.4	
18	28.3	5, 19	28.1	28.2	5, 19	27.8	19	27.9	19
19	22.3	18	22.3	22.7	18	22.2	3, 5, 18	22.4	5, 18
20	15.5	5, 9	15.7	14.1	5, 9	17.3	1, 5, 9	15.8	5, 9
1-OCOPh									
1′	128.5	2′, 6′	130.7[b)]	129.7	2′, 6′	129.4	2′, 6′	129.7	2′, 6′
2′,6′	129.5	4′	129.7	129.7	4′	129.3	4′	129.9	4′
3′,5′	128.3		128.4	128.4		127.9	2′, 6′	128.1	2′, 6′
4′	133.0	2′, 6′	132.7	132.9	2′, 6′	132.6	2′, 6′	132.7	2′, 6′
CO	164.0	1, 2′, 6′	164.2	163.3	1, 2′, 6′	164.5	1, 2′, 6′	164.6	1, 2′, 6′
2-OCOCH$_3$									
1″	169.9		170.2	170.1		170.6[b)]		169.9	
CO	20.7	2, 1″	20.8	20.7	2, 1″	20.7[c)]	2, 1″	20.7	2, 1″
3-OCOCH$_3$									
1‴	170.5		170.8	170.6		170.5[b)]		170.6	
CO	20.5	3, 1‴	20.5	20.5	3, 1‴	20.9[c)]	3	20.8	3, 1‴
7-OCOCH$_3$									
1⁗	169.3								
CO	21.2	7, 1⁗							
11-OCOPh									
1⁗	128.5	2⁗, 6⁗	130.3[b)]	130.6	2⁗, 6⁗	128.5	2⁗, 6⁗	129.9	2⁗, 6⁗
2⁗,6⁗	130.6	4⁗	130.5	130.6	4⁗	129.4	4⁗	129.3	4⁗
3⁗,5⁗	128.3		128.2	127.6		128.0		128.0	2⁗, 6⁗
4⁗	133.3	2⁗, 6⁗	133.2	132.9	2⁗, 6⁗	132.6	2⁗, 6⁗	132.6	2⁗, 6⁗
CO	166.8	11, 2⁗, 6⁗	166.7	166.2	11, 2⁗, 6⁗	163.1	11, 2⁗, 6⁗	165.5	11, 2⁗, 6⁗

a) Long-range correlated protons observed in the HMBC spectra. *b*, *c*) Assignments may be interchanged in each column.

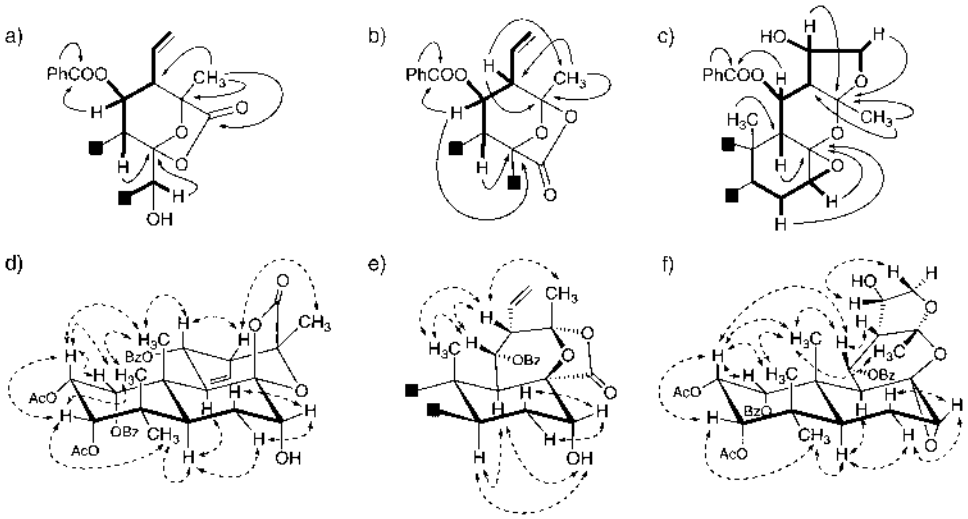

Fig. 2. Connectivities (Bold Line) Deduced by the COSY and HMQC Spectra and Key Long-Range Correlations (Arrows, H→C) on Ring C from the HMBC Spectra of **8**—**10** (a—c) and ROESY Correlations Observed in the ROESY Spectra (Mixing time, 0.5 s) of **8**—**10** (d—f)

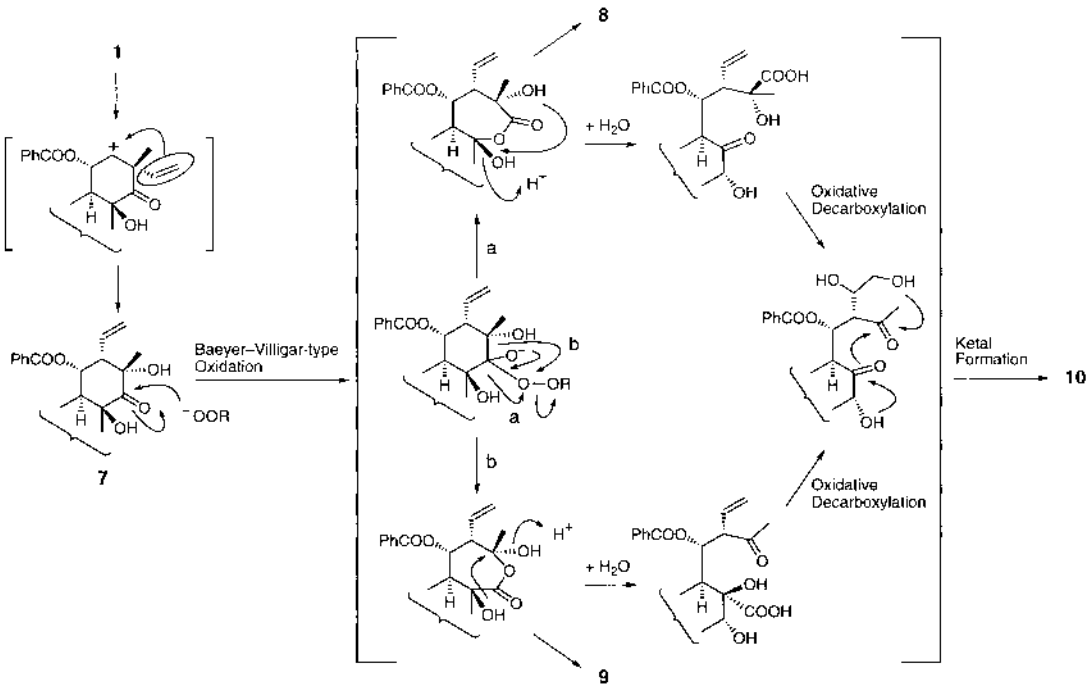

Chart 2. Possible Biogenetic Pathway of Novel Diterpenes **7**—**10**

on the other hand, the ROESY correlations of H-11 indicated H-11, H-12, H_3-17, and H_3-20 should have a β-orientation, and the correlations of H-12 with H-15 and H-16β and the broad singlet nature of H-15 suggested that H-15 also has a β-orientation and that ring C has a boat conformation. Finally, the configuration of the epoxy ring was concluded to be α based on 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 **10**.

Biogenetic Consideration In this paper, we have reported the isolation and structures of ten new diterpenes; orthosiphols F—J (**1**—**5**), staminols A (**6**) and B (**7**), staminolactones A (**8**) and B (**9**), and norstaminol A (**10**). Among them, **1**—**5** are isopimarane-type diterpenes, while others (**6**-**10**) have a novel carbon-framework, to which we proposed the name "staminane". Moreover, staminolactone A (**8**) is 8,14-secostaminane-type and staminolactone B (**9**) is 13,14-secostaminane-type, while norstaminol A (**10**) is 14-norstaminan-type. The co-existence of a staminane-type (**6**—**10**) with isopimarane-type (**1**—**5**) suggests that the former is biosynthesized from the latter through the migration of the vinyl group from C-13α to C-12α (Chart 2). Then, staminolactones A (**8**) and B (**9**) would be biosynthesized from a staminane-type diterpene such as staminol B (**7**) through a Baeyer–Villiger-type oxidation[11]; migration of C-8 leads to **8** (route a) and migration of C-13 leads to **9** (route b). At last, further oxidation of **8** or **9** with oxidative decarboxylation would lead to norstaminol A (**10**).

Cytotoxic Activity All the isolated compounds were tested for their cytotoxicity towards highly liver metastatic murine colon 26-L5 carcinoma cells. The new diterpenes, except for **4**, showed weak cytotoxicity with an ED_{50} value between 50 and 90 μg/ml, while on flavonoids 7,3′,4′-tri-*O*-methylluteolin (**11**), eupatorin (**12**), ladanein (**16**), and 6-hydroxy-5,7,4′-trimethoxyflavone (**18**) showed cytotoxic activities (ED_{50}: **11**, 87.7 μg/ml; **12**, 11.3 μg/ml; **16**, 50.4 μg/ml; **18**, 56.5 μg/ml). Three flavonoids, except for **11**, contained a free hydroxyl group in a molecule and their cytotoxicity was stronger than that of **11**. This would indicate that the presence of free hydroxyl group in a molecule would enhance their antioxidative activity and lead to enhanced cytotoxicity. This tendency was also observed in previous cases.[12] These compounds would contribute to the cytotoxic activity of the MeOH extract of *O. stamineus*.

Experimental

Optical rotations were recorded on a JASCO DIP-140 digital polarimeter. IR spectra were measured with a Shimadzu IR-408 spectrophotometer in $CHCl_3$ solutions. FAB-MS measurements were performed on a JEOL JMS-700T spectrometer with *m*-nitrobenzylalcohol (NBA) or glycerol as a matrix. NMR spectra were taken on a JEOL JNM-GX400 spectrometer with tetramethylsilane (TMS) as the internal standard, and chemical shifts are expressed in δ values. Column chromatography was performed with silica-gel (Fuji Silysia BW-820MH), while TLC and preparative TLC were carried out on pre-coated Merck Kieselgel $60F_{254}$ (0.25 or 0.50 mm) or RP-$18F_{254}$ (0.25 mm) plates.

Extraction and Isolation The aerial part of *Orthosiphon stamineus* Benth. was purchased at the local market at Ho Chi Minh City, Vietnam in 1997. The voucher sample (TMPW No. 18623) is preserved in the Museum for Materia Medica, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University. The air dried aerial part of *O. stamineus* (4 kg) was extracted with MeOH (60 l, reflux, 3 h, ×2), and the MeOH extract (150 g) was suspended on water and extracted successively with hexane, $CHCl_3$, EtOAc, and BuOH to yield hexane- (15.8 g), $CHCl_3$- (47.3 g), EtOAc- (11.5 g), and BuOH-soluble (14 g) fractions, respectively.

Treatment of $CHCl_3$-Soluble Fraction $CHCl_3$-soluble fraction (47.3 g) was chromatographed over silica gel with an EtOAc–hexane solvent system to give five fractions (fr. 1, 4.2 g; fr. 2, 3.6 g; fr. 3, 23.3 g; fr. 4, 9.4 g; fr. 5, 2.7 g).

Fraction 1 afforded oleanolic acid (**23**, 4 mg) and β-sitosterol (**26**, 9 mg), while fr. 2 gave 7,3′,4′-tri-*O*-methylluteolin (**11**, 4.5 mg) and betulinic acid (**25**, 6 mg).

Fraction 3 [hexane–EtOAc (7 : 3) eluate, 8.2 g] was rechromatographed over silica gel with a benzene–acetone solvent system to afforded four subfractions [fr. 3-1: benzene–acetone (95 : 5) eluate, 1.94 g; fr. 3-2: benzene–acetone (92.5 : 7.5) eluate, 1.87 g; fr. 3-3: benzene–acetone (90 : 10) eluate,

2.07 g; fr. 3-4, benzene–acetone (80 : 20) eluate, 0.93 g]. Subfraction 3-1 was separated by preparative TLC with hexane–acetone (3 : 1) to give orthosiphol H (**3**, 9 mg), norstaminol A (**10**, 30 mg), 5-hydroxy-6,7,3′,4′-tetramethoxyflavone (**14**, 3 mg), salvigenin (**15**, 7 mg), and tetramethylscutellarein (**17**, 3 mg). Subfraction 3-2 was separated by preparative TLC with hexane–acetone (3 : 2) to yield orthosiphol F (**1**, 20 mg), **3** (228 mg), and aurantiamide acetate (**20**, 7.5 mg), together with crude orthosiphol J (**5**), which was purified by reversed-phase preparative TLC with H_2O–MeOH (1 : 9) to give pure **5** (3 mg). Subfractions 3-3 and 3-4 were separately subjected to preparative TLC with hexane–acetone (1 : 1), and **1** (100 mg) and orthosiphol G (**2**, 5 mg) were obtained from subfraction 3-3; **1** (22 mg), staminol A (**6**, 26 mg), and 6-hydroxy-5,7,4′-trimethoxyflavone (**18**, 3 mg) were from subfraction 3-4.

Fraction 4 [hexane–EtOAc (7 : 4) eluate, 7.0 g] was also rechromatographed over silica-gel with a benzene–acetone solvent system to give four subfractions [fr. 4-1: benzene–acetone (95 : 5) eluate, 0.2 g; fr. 4-2: benzene–acetone (92.5 : 7.5) eluate, 0.5 g; fr. 4-3: benzene–acetone (90 : 10) eluate, 1.9 g; fr. 4-4, benzene–acetone (80 : 20) eluate, 3.4 g]. Each subfraction was separated by preparative TLC with hexane–acetone (1 : 1) to give the following compounds: fr. 4-1: staminolactone A (**8**, 7.8 mg), staminolactone B (**9**, 18 mg), **14** (16 mg); fr. 4-2: **1** (54 mg), eupatorin (**12**, 24 mg), ladanein (**16**, 24 mg); fr. 4-3: orthosiphol I (**4**, 7 mg), **12** (13 mg), **16** (13 mg); fr. 4-4: staminol B (**7**, 14 mg), **12** (13 mg), sinensetin (**13**, 75 mg).

Orthosiphol F (**1**): Colorless amorphous solid, $[\alpha]_D^{25}$ −82.8° (c=2.10, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3550, 3450, 1725, 1455, 1370, 1280, 1110, 1050. FAB-MS *m/z*: 699 $(M+Na)^+$, 677 $(M+H)^+$. HR-FAB-MS *m/z*: 677.2958 [Calcd for $C_{38}H_{45}O_{11}$: 677.2962 $(M+H)^+$]. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Orthosiphol G (**2**): Colorless amorphous solid, $[\alpha]_D^{25}$ −63.3° (c=0.47, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3550, 3400, 1720, 1455, 1370, 1280, 1115, 1045. FAB-MS *m/z*: 595 $(M+Na)^+$, 573 $(M+H)^+$. HR-FAB-MS *m/z*: 573.2668 [Calcd for $C_{31}H_{41}O_{10}$: 573.2700 $(M+H)^+$]. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Orthosiphol H (**3**): Colorless amorphous solid, $[\alpha]_D^{25}$ −58.0° (c=0.63, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3550, 3400, 1725, 1455, 1370, 1280, 1240, 1110, 1045. FAB-MS *m/z*: 741 $(M+Na)^+$, 719 $(M+H)^+$. HR-FAB-MS *m/z*: 719.3051 [Calcd for $C_{40}H_{47}O_{12}$: 719.3067 $(M+H)^+$]. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Orthosiphol I (**4**): Colorless amorphous solid, $[\alpha]_D^{25}$ −108.9° (c=0.55, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3550, 3400, 1725, 1455, 1375, 1270, 1110, 1045. FAB-MS *m/z*: 593 $(M+Na)^+$, 571 $(M+H)^+$. HR-FAB-MS *m/z*: 571.2568 [Calcd for $C_{31}H_{39}O_{10}$: 571.2543 $(M+H)^+$]. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Orthosiphol J (**5**): Colorless amorphous solid, $[\alpha]_D^{25}$ −58.5° (c=0.24, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3550, 1725, 1460, 1370, 1270, 1230, 1110, 1050. FAB-MS *m/z*: 612 $(M+H)^+$. HR-FAB-MS *m/z*: 613.2646 [Calcd for $C_{33}H_{41}O_{11}$: 613.2648 $(M+H)^+$]. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Staminol A (**6**): Colorless amorphous solid, $[\alpha]_D^{25}$ −24.3° (c=0.51, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3550, 3430, 3300, 1725, 1600, 1450, 1370, 1200—1270, 1090, 1070, 1040. FAB-MS *m/z*: 757 $(M+Na)^+$, 735 $(M+H)^+$. HR-FAB-MS *m/z*: 735.2970 [Calcd for $C_{40}H_{47}O_{13}$: 735.3017 $(M+H)^+$]. ^{1}H- and ^{13}C-NMR: Tables 3 and 4.

Staminol B (**7**): Colorless amorphous solid, $[\alpha]_D^{25}$ −27.8° (c=0.69, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3550, 3430, 3300, 1725, 1600, 1450, 1200—1270, 1090, 1070, 1040. FAB-MS *m/z*: 715 $(M+Na)^+$, 693 $(M+H)^+$. HR-FAB-MS *m/z*: 715.2740 [Calcd for $C_{38}H_{44}O_{12}Na$: 715.2730 $(M+Na)^+$]. ^{1}H- and ^{13}C-NMR: Tables 3 and 4.

Staminolactone A (**8**): Colorless amorphous solid, $[\alpha]_D^{25}$ −97.2° (c= 0.067, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3570, 1800, 1730, 1600, 1455, 1370, 1270, 1200—1240. FAB-MS *m/z*: 713 $(M+Na)^+$, 691 $(M+H)^+$. HR-FAB-MS *m/z*: 691.2731 [Calcd for $C_{38}H_{43}O_{12}$: 691.2754 $(M+H)^+$]. ^{1}H- and ^{13}C-NMR: Tables 3 and 4.

Staminolactone B (**9**): Colorless amorphous solid, $[\alpha]_D^{25}$ −98.9° (c=0.12, $CHCl_3$). IR ν_{max} ($CHCl_3$) 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 *m/z*: 691.2746 [Calcd for $C_{38}H_{43}O_{12}$: 691.2754 $(M+H)^+$]. ^{1}H- and ^{13}C-NMR: Tables 3 and 4.

Norstaminol A (**10**): Colorless amorphous solid, $[\alpha]_D^{25}$ −38.0° (c=0.41, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3550, 1725, 1600, 1455, 1370, 1280, 1200—1240, 1110. FAB-MS *m/z*: 701 $(M+Na)^+$, 679 $(M+H)^+$. HR-FAB-MS *m/z/m/z*: 679.2788 [Calcd for $C_{37}H_{43}O_{12}$: 679.2754 $(M+H)^+$]. ^{1}H- and ^{13}C-NMR: Tables 3 and 4.

Treatment of EtOAc-soluble Fraction EtOAc-soluble fraction (11.5 g) was chromatographed over silica-gel with a hexane–EtOAc solvent system to give three subfractions (fr. 4-1, 2 g; fr. 4-2, 1.5 g; fr. 4-3, 2.4 g). Fraction 4-1 was separated by preparative TLC with hexane–EtOAc (3 : 1) to afford **11** (3 mg) and **15** (11 mg). Fraction 4-2 gave **1** (223 mg), **3** (12.7 mg), **6** (33 mg), **12** (10 mg), **14** (3 mg), and **16** (3 mg) by preparative TLC with acetone–benzene (5 : 95). On the other hand, fr. 4-3 yielded vomifoliol (**19**, 7.5 mg), rosmarinic acid (**21**, 10 mg), caffeic acid (**22**, 4 mg), and ursolic acid (**24**, 5 mg) by preparative TLC with benzene–acetone–AcOH (70 : 29 : 1).

Cytotoxic Assay A cytotoxic assay was done using the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide (MTT) assays as described previously,[13] with slight modification. In brief, exponentially growing cells were harvested and 100 μl of cell suspension containing 2000 cells was plated in 96-well plates (Falcon, Becton Dickinson, NJ, U.S.A.). After 24 h incubation at 37 °C under 5% CO_2, the cells were treated with varying concentrations of test specimens in 100 μl medium and incubated for 3 d under the same conditions. At 3 h after adding an MTT solution, UV absorption of the formazan formed was measured at 590 nm using a Perkin–Elmer HTS-7000 plate reader. Test specimens were dissolved in dimethyl sulfoxide (DMSO) and then diluted by the medium. DMSO less than 0.25% in the test solution had no effect on the all. 5-Flurouracil (5-FU) was used as a positive control, and ED_{50} values were calculated from the mean values of data from three wells. The following compounds showed cytotoxicity with an ED_{50} value less than 100 μg/ml: **1**, 51.6 μg/ml; **2**, 89.7 μg/ml; **3**, 56.7 μg/ml; **5**, 48.2 μg/ml; **6**, 61.7 μg/ml; **7**, 78.9 μg/ml; **8**, 68.5 μg/ml; **9**, 79.8 μg/ml; **10**, 56.1 μg/ml; 11, 87.7 μg/ml; **12**, 11.3 μg/ml; **16**, 50.4 μg/ml; **18**, 56.5 μg/ml; **20**, 70.1 μg/ml; **21**, 53.3 μg/ml; **22**, 13.5 μg/ml; **25**, 75.4 μg/ml; **5-FU**, 0.24 μg/ml.

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References and Notes

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