Five Novel Highly Oxygenated Diterpenes of *Orthosiphon stamineus* from Myanmar

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Received December 19, 2000

Five novel highly oxygenated diterpenes, orthosiphols K (**1**), L (**2**), M (**3**), and N (**4**) and norstaminone A (**5**), were isolated from the aerial part of *Orthosiphon stamineus*, together with three known diterpenes, orthosiphols A (**6**) and B (**7**) and neoorthosiphol A (**8**). Orthosiphol L (**2**) is an isopimarane-type diterpene with a hydroxyl group at C-12, which supports the biogenesis of staminane-type diterpenes, i.e., migration of a vinylic group from C-13 of isopimarane to C-12. Norstaminone A (**5**) has a staminane carbon framework and supports the biosynthetic pathway from staminols to norstaminols via staminolactones. All the isolated compounds showed mild to weak antiproliferative activities toward highly liver metastatic colon 26-L5 carcinoma and human HT-1080 fibrosarcoma cell lines.

Orthosiphon stamineus [syn.: *O. grandiflorus*, *O. spicatus*, *O. aristatus*; Lamiaceae] is a medicinal herb grown in Southeast Asia.[1] It has gained popularity by the name of "Kumis Kucing" in Indonesia, and the leaves of this plant are used as a diuretic and to treat rheumatism, diabetes, hypertension, tonsillitis, epilepsy, menstrual disorders, gonorrhea, syphilus, renal calculus, gallstones, etc.[1] In Vietnam, the aerial part is used by the name of "Rau meo" for the treatment of urinary lithiasis, edema, eruptive fever, influenza, hepatitis, jaundice, and biliary lithiasis.[2] In Myanmar, this plant is known by the name "Se-cho" or "Myit-Shwe", and the leaves are locally reputed as an antidiabetic drug,[3] while decoctions of the air-dried leaves are used to cure urinary tract and renal diseases.[4] From its popularity and demonstrated effectiveness, phytochemical and pharmacological studies have been conducted since 1930s, and highly oxygenated isopimarane-type diterpenes, orthosiphols A−E, were reported together with monoterpenes, triterpenes, saponins, flavonoids, hexoses, organic acids, rosmarinic acid, chromene, and myo-inositol from this plant in Indonesia and Okinawa.[5] In addition, two migrated pimarane-type diterpenes,[6] along with two isopimarane-type diterpenes and a benzochromene, were recently reported from the leaves of Indonesian *O. aristatus* (= *O. stamineus*).[7] On the other hand, as a part of our study on the medicinal plants from Southeast Asia, we also reported two diterpenes with a novel carbon framework named "staminane" (staminols A and B), two *seco*-staminanes (staminolactones A and B), and a norstaminane (norstaminolactone), along with five isopimarane-type diterpenes (orthosiphols F−J) from Vietnamese *O. stamineus*.[8] On the constituents of *O. stamineus* from Myanmar, however, there has been no previous report. Thus, we examined the constituents of *O. stamineus* from Myanmar and isolated five novel highly oxygenated diterpenes along with three known diterpenes and five known phenolics. In this paper we report the isolation and structure elucidation of the new diterpenes and their antiproliferative activities.

Results and Discussion

Air-dried aerial parts of *O. stamineus* from Myanmar were extracted with refluxing MeOH, and the MeOH extract was successively partitioned into hexane, $CHCl_3$, EtOAc, BuOH, and H_2O fractions. The $CHCl_3$ fraction was subjected to a series of chromatographic separations and preparative TLC to afford five new highly oxygenated diterpenes, orthospihols K (**1**), L (**2**), M (**3**), and N (**4**) and norstaminone A (**5**) and eight known compounds. The known compounds were identified by analysis of their spectroscopic data and comparison with literature data to be orthosiphols A (**6**) and B (**7**),[5b] neoorthosiphol A (**8**),[7a] tetramethylscutellarein,[9] eupatorin,[10] 5,6-dihydroxy-4′,7-dimethoxyflavone,[10] 3′,4′,6,7,8-pentamethoxyflavone,[11] and (4-hydroxyphenyl)ethyl *trans*-ferulate.[12]

Orthosiphol K (**1**) showed the quasimolecular ion at *m*/*z* 633.2700 $(M - H)^-$ in HRFABMS, which corresponded to the molecular formula $C_{36}H_{42}O_{10}$. The IR spectrum of **1** showed absorptions of hydroxyl (3550 cm^{-1}), ester carbonyl (1705 cm^{-1}), and phenyl (1600, 1450 cm^{-1}) groups. The ^1H NMR spectrum of **1** displayed signals due to four tertiary methyls, a vinyl, and five oxygen-substituted and two aliphatic methines, together with those of an acetyl and two benzoyl groups (Table 1), while its ^{13}C NMR spectrum revealed the signals of a ketone, three ester carbonyl groups, and an oxygen-substituted and three oxygen-nonsubstituted quaternary carbons (Table 2). These ^1H and ^{13}C NMR spectra were similar to those of orthosiphols A (**6**) and B (**7**), but they were characterized by the lack of one of two acetyl groups. Analysis of the COSY and HMQC spectra indicated the high-field shift of H-2 (δ_H 4.35) or H-3 (δ_H 3.50), compared to H-2 of **6** (δ_H 5.54) or H-3 of **7** (δ_H 5.04), respectively. Thus, **1** was assumed to be 2-*O*-deacetylorthosiphol A (= 3-*O*-deacetylorthosiphol B), which was confirmed by the HMBC spectrum. The stereochemistry of **1** was determined to be the same as that of **6** and **7**, based on the ROESY correlations H-2/H_3-19, H-5/H-9, and H-9/H-12 and coupling constants of each proton. Thus, orthosiphol K (**1**) was concluded as 2-*O*-deacetylorthosiphol A (= 3-*O*-deacetylorthosiphol B).

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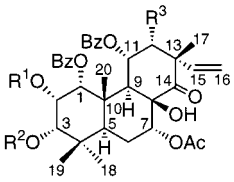
10.1021/np000607t CCC: $20.00
Published on Web 04/21/2001



1 $R^1 = R^2 = R^3 = H$
2 $R^1 = Ac$, $R^2 = H$, $R^3 = OH$
6 $R^1 = Ac$, $R^2 = R^3 = H$
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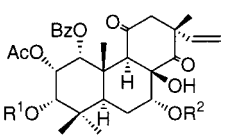
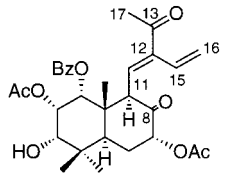


3 $R^1 = H$, $R^2 = Ac$
4 $R^1 = Bz$, $R^2 = H$



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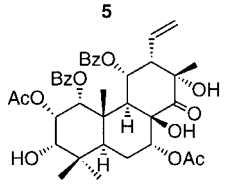

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The HRFABMS of orthosiphol L (**2**) showed the quasi-molecular ion at *m*/z 691.2749 $(M - H)^-$, consistent with the molecular formula $C_{38}H_{44}O_{12}$. The IR spectrum of **2** closely resembled that of **6** and showed absorptions of hydroxyl, ester carbonyl, and phenyl groups. The ^1H and ^{13}C NMR spectra of **2** were also similar to those of **6** (Tables 1, 2), but they showed the presence of one more oxymethine (δ_H 3.80, δ_C 76.0) and the lack of a methylene than in **6** (δ_H 1.96, 2.27; δ_C 39.7), indicating the presence of one more hydroxyl group in **2**. The location of the oxymethine, and also of other substituents, was determined to be the same as that of **6** by the analysis of the COSY, HMQC, and HMBC spectra. The rings A−C were determined to have a chair conformation based on ROESY correlations of H-2 with H-1, H-3, H_3-19, and H_3-20, of H-11 with H_3-20, and of H-5 with H-9. The broad singlet nature of the signal suggested the α-orientation of 12-OH. From these data, orthosiphol L (**2**) was concluded to be 12-hydroxyorthosiphol A, the most oxygenated isopimarane-type diterpene.

Orthosiphol M (**3**) displayed an IR spectrum almost identical with **6**, and its molecular formula, $C_{31}H_{38}O_{10}$, was determined by HRFABMS. The ^1H and ^{13}C NMR spectra of **3** were similar to those of **6**, but the ^1H NMR spectrum of **3** showed a sharp singlet at δ_H 2.65 (2H), due to a methylene group, and signals for one fewer of two benzoyl groups in **6** (Table 1), while the ^{13}C NMR spectrum showed the presence of two ketone carbonyl carbons (Table 2). These data suggested that **3** would have the same 1,4-diketone functionarity on ring C as orthosiphols I and J, isolated from Vietnamese *O. stamineus*.[8a] Indeed, the ^1H and ^{13}C NMR spectra of **3** resembled those of orthosiphol J, but they were characterized by the lack of signals due to one of three acetyl groups. The high-field shift of H-3 (**3**, δ_H 3.58; orthosiphol J, δ_H 5.06) indicated the deacetylation to be at C-3, which was confirmed by the HMBC correlations of the ester carbonyl carbon at δ_C 169.9 with H-2 and the acetyl methyl protons (Table 2). The coupling constants of each proton and the ROESY correlations H-2/H-3, H-2/H_3-19, H-2/H_3-20, H_3-19/H_3-20, H_3-20/H-6, and H-5/H-9 indicated the same stereochemistry as that of orthosiphols I and J. From these data, orthosiphol M (**3**) was determined to be 3-*O*-deacetylorthosiphol J.

Orthosiphol N (**4**) showed an IR spectrum almost identical with that of **3**, and its molecular formula was determined to be $C_{36}H_{40}O_{10}$ by HRFABMS. The ^1H and ^{13}C NMR spectra of **4** were similar to those of **3** (Tables 1, 2), but they were characterized by the lack of signals due to an acetyl group and the presence of the signals of one more benzoyl group than **3**. The location of the deacetylation and the benzoylation was determined to be at C-7 and at C-3, respectively, based on the high-field shift of H-7 (**4**, δ_H 4.35; **3**, δ_H 5.33) and the downfield shift of H-3 (**4**, δ_H 5.33; **3**, δ_H 3.58). These, and also the location of other ester groups, were confirmed by the HMBC correlations of the ester carbonyl carbons (Table 2). Thus, orthosiphol N (**4**) was determined to be 3-*O*-benzoyl-7-*O*-deacetylorthosiphol M.

Norstaminone A (**5**) was determined to have the molecular formula $C_{30}H_{36}O_9$ by HRFABMS, and its IR spectrum showed absorptions of hydroxyl (3550 cm^{-1}), ester carbonyl (1720 cm^{-1}), and phenyl (1600, 150 cm^{-1}) groups. The ^1H NMR spectrum of **5** revealed signals due to three tertiary methyls, a vinyl, and four oxygenated and two aliphatic methines, together with those of a benzoyl and two acetyl groups (Table 1). On the other hand, its ^{13}C NMR spectrum showed the signals of 19 carbons, including those of two ketone carbonyl, four oxygenated sp^3 and four sp^2 carbons, together with those of a benzoyl and two acetyl groups (Table 2). Thus, compound **5** was a norditerpene having a benzoyl and two acetyl groups. The planar structure of **5** was deduced by the COSY, HMQC, and HMBC experiments (Figure 2a), while the stereochemistry of **5** was elucidated from ROESY and NOE correlations (Figure 2b). The ROESY correlations H-2/H_3-19, H-5/H-9, and H-6/H_3-20 indicated that rings A and B have a chair conformation and that the ring junction is *trans*. The difference NOE experiments, on the other hand, indicated the geometry of the olefin at C-11(12) to be *E*: observed NOEs were from H-9 to H-5 (7.8%), H-11 (1.8%), H-15 (3.2%), and H-16 at δ_H 4.68 (3.5%); H-11 to H-1 (5.0%), H-9 (1.5%), and H_3-17 (4.8%); H-15 to H-9 (1.7%), H-11 (0.4%), and H-16 at δ_H 4.75 (2.9%); H-16 at δ_H 4.75 to H-15 (4.2%) and H-16 at δ_H 4.68 (13.5%); H-16 at δ_H 4.88 to H-9 (4.8%), H-11 (2.5%), and H-16 at δ_H 4.75 (16%); and H_3-17 to H-11 (2.2%) and H-15 (1.4%). Thus, norstaminone A was determined to have the structure formula **5**.

In this paper we have identified five novel diterpenes, orthosiphols K−N (**1**−**4**) and norstaminone A (**5**), and three known diterpenes, orthosiphols A (**6**) and B (**7**) and neoorthosiphol A (**8**). Among them, six (**1**−**4**, **6**, **7**) were isopimarane-type diterpenes, while the other two (**5**, **8**) were diterpenes with a staminane carbon framework. As both staminane-type and isopimarane-type diterpenes coexist, we and Shibuya et al. have independently proposed that the former type may be biosynthesized from the latter type, through a migration of the vinylic group from C-13 to C-12.[7a,8a] Isolation of orthosiphol L (**2**), along with isopimarane-type (**1**−**4**, **6**, **7**) and staminane-type diterpenes, would support the proposed biosynthetic route, which may be through an oxidation by cytochrome P-450[13] (Scheme 1 in Supporting Information). On the other hand,

Table 1. ^{1}H NMR Data ($CDCl_3$, 400 MHz) for Diterpenes **1**−**5**, *J* Values (in Hz) in Parentheses

position	**1**	**2**	**3**	**4**	**5**
1	5.05 d (2.8)	5.19 d (2.9)	6.42 d (3.2)	6.42 d (3.0)	5.24 d (3.0)
2	4.35 t (2.8)	5.47 t (2.9)	5.55 t (3.2)	5.72 t (3.0)	5.38 t (3.0)
3	3.50 d (2.8)	3.50 br d (2.9)	3.58 br d (3.2)	5.33 d (3.0)	5.63 d (3.0)
5	2.46 dd (16.2, 6.2)	2.46 dd (12.0, 2.5)	2.29 dd (11.7, 4.1)	2.68 dd (14.0, 2.0)	2.84 dd (13.4, 3.0)
6	2.03 m; 2.00 m	2.11 m (2H)	2.27 m; 1.99 m	2.05 td (14.0, 2.0); 1.88 dt (14.0, 2.0)	2.32 m; 2.05 m
7	5.46 br s	5.50 t (2.4)	5.33 t (2.9)	4.35 br s	5.07 t (3.0)
9	3.26 d (7.2)	3.17 d (6.6)	3.45 s	3.50 s	4.32 d (9.5)
11	5.95 t (7.2)	5.74 d (6.6)			6.43 d (9.5)
12	2.59 dd (15.5, 7.2); 1.85 d (15.5)	3.80 br s	2.65 s (2H)	2.86 d (17.5); 2.60 d (17.5)	
15	5.76 dd (17.5, 10.6)	4.87 dd (17.6, 10.7)	5.24 dd (17.6, 10.5)	5.33 dd (17.5, 10.5)	5.45 dd (17.8, 11.5)
16	4.88 d (17.5); 4.81 d (10.6)	5.59 d (10.7); 5.55 d (17.6)	4.63 d (17.6); 4.10 d (10.5)	4.70 d (17.5); 4.21 d (10.5)	4.75 d (11.5); 4.68 d (17.8)
17	1.11 s	1.29 s	1.09 s	1.69 s	2.31 s
18	1.01 s	1.06 s	1.05 s	1.93 s	1.21 s
19	1.06 s	1.06 s	1.02 s	1.03 s	1.05 s
20	1.48 s	1.60 s	1.42 s	1.94 s	1.25 s
1-OCOPh					
2′, 6′	7.68 br d (7.3)	7.50 d (7.6)	8.03 d (7.8)	8.01 dd (7.5, 1.3)	8.01 d (7.7)
3′, 5′	7.33 t (7.3)	7.24 t (7.6)	7.45 t (7.8)	7.33 t (7.5)	7.47 t (7.7)
4′	7.56 t (7.3)	7.50 t (7.6)	7.59 t (7.8)	7.02 t (7.5)	7.61 t (7.7)
2-$OCOCH_3$					
1″		1.92 s	2.06 s	1.94 s	2.00 s
3-OCOPh					
2‴, 6‴				7.86 br d (7.7)	
3‴, 5‴				7.05 t (7.7)	
4‴				7.36 t (7.7)	
7-$OCOCH_3$					
1⁗	2.17 s	2.16 s	2.03 s		2.13 s
11-OCOPh					
2⁗′, 6⁗′	7.56 br d (7.3)	7.44 d (7.6)			
3⁗′, 5⁗′	7.33 t (7.3)	7.06 t (7.6)			
4⁗′	7.48 t (7.3)	7.40 t (7.6)			

Table 2. ^{13}C NMR Data ($CDCl_3$, 100 MHz) for Diterpenes **1**−**5**

position	1	2	3	4	5
1	78.0	73.8	75.0	73.9	74.0
2	67.2	68.0	67.4	65.9	67.6
3	77.6	77.2	76.3	76.6	77.2
4	37.3	38.3	37.9	37.1	38.4
5	35.2	35.6	34.7	34.9	36.7
6	21.4	21.2	21.2	23.5	21.9
7	70.9	70.9	71.2	68.4	76.0
8	75.6	74.6	77.1	77.0	204.4
9	41.2	42.7	51.9	50.9	54.7
10	43.7	43.6	42.7	43.4	47.8
11	68.6	72.7	204.8	210.3	131.9
12	40.3	76.0	47.0	47.7	144.7
13	47.9	53.8	49.3	49.2	198.0
14	208.5	205.4	208.1	213.9	
15	141.3	114.4	138.4	138.9	130.4
16	114.1	138.7	116.3	116.5	120.4
17	25.9	22.2	24.9	25.4	26.8
18	28.9	22.0	28.7	22.4	20.7
19	22.8	28.9	21.9	27.9	20.8
20	16.5	17.0	16.0	16.7	14.6
1-OCOPh					
1′	129.7	130.0	130.1	130.2	129.6
2′, 6′	130.6	129.9	129.7	130.6	129.6
3′, 5′	128.1	128.1	128.5	128.1	128.7
4′	133.3	132.9	133.2	132.6	130.6
CO	167.7	164.2	163. 9	164.8	164.6
2-OCOCH$_3$					
1′		20.9	20.8	21.0	20.7
CO		170.4	169.9	170.5	169.8
3-OCOPh					
1‴				129.9	
2‴, 6‴				129.7	
3‴, 5‴				127.9	
4‴				132.6	
CO				166.0	
7-OCOCH3					
1⁗	20.9	21.0	21.0		20.7
CO	168.7	168.6	168.5		169.1
11-OCOPh					
1⁗′	130.6	129.8			
2⁗′, 6⁗′	129.6	129.5			
3⁗′, 5⁗′	128.1	127.9			
4⁗′	132.6	132.7			
CO	166.5	166.8			

the isolation of norstaminone A (**5**) would support a participation of the Baeyer−Villiger-type oxidation, which we proposed previously.[8a]

All of the isolated compounds were tested for their antiproliferative activity toward highly liver metastatic murine colon 26-L5 carcinoma[14] and human HT-1080 fibrosarcoma[15] cell lines (Table 3). The diterpenes displayed mild activities against both cell lines, while the flavones, except for 3′,4′,6,7,8-pentamethoxyflavone, showed more potent antiproliferative activities than diterpenes. The highest activities were observed for 5,6-dihydroxy-4′,7-dimethoxyflavone, with ED_{50} values of 2.3 and 3.0 μg/mL against murine colon 26-L5 carcinoma and HT-1080 fibrosarcoma cell lines, respectively. Comparing the present data with our earlier observations indicated that an increase in the number of free hydroxyl groups in flavones leads to enhanced antiproliferative activity toward the tested cell lines. Moreover, hydroxyl groups at positions 3′, 5, and 7 have a greater contribution toward antiproliferative activity, which is in agreement with our previous findings.[12]

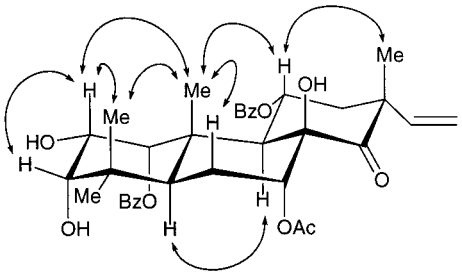

Figure 1. Significant correlations observed in the ROESY spectrum of **1**.

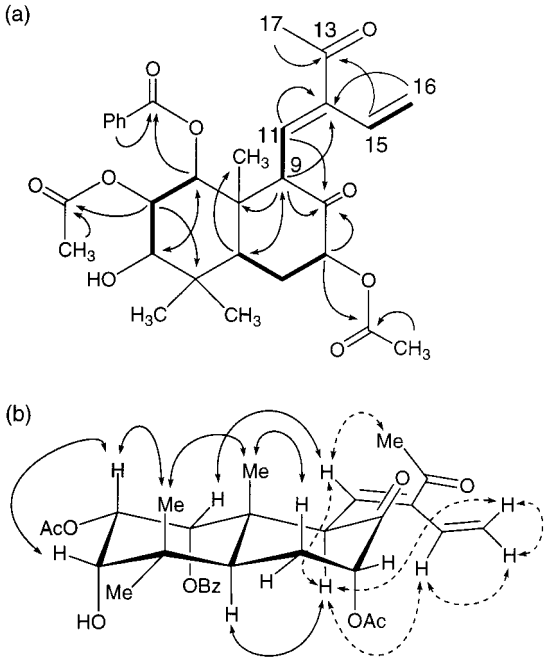

Figure 2. Connectivities (bold line) deduced by the COSY and HMQC spectra and significant HMBC correlations (arrow) (a) and ROESY correlations (solid arrow) and NOEs (dashed arrow) (b) observed in the ROESY and difference NOE experiments of **5**.

Table 3. Antiproliferative Activity for the Isolated Compounds (ED_{50} Values in μg/mL)[a]

compound	colon 26-L5	HT-1080
1	13.8	21.8
2	24.7	20.6
3	31.3	81.7
4	35.1	18.6
5	12.8	23.2
6	63.1	>100
7	28.1	57.9
8	38.8	96.3
b	8.1	4.5
c	3.7	8.8
d	2.3	3.0
e	31.4	74.8
f	33.7	47.1
5-fluorouracil	0.015	0.48

[a] ED_{50} values were calculated from the mean of data of four determinations. [b] Tetramethylscutellarein. [c] Eupatorin. [d] 5,6-Dihydroxy-4′,7-dimethoxyflavone. [e] 3′,4′,6,7,8-Pentamethoxyflavone. [f] (4-Hydroxyphenyl)ethyl *trans*-ferulate.

Experimental Section

General Experimental Procedures. 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. NMR spectra were taken on a JEOL JNM-LA400 spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed in δ values. HRFABMS measurements were carried out on a JEOL JMS-700T spectrometer, and glycerol was used as a matrix. Column chromatography was performed with BW-820MH Si gel (Fuji Silysia, Aichi, Japan). Analytical and

preparative TLC were carried out on precoated Merck Si gel 60 F_{254} plates (0.25 or 0.50 mm).

Plant Material. The aerial part of cultivated *Orthosiphon stamineus* Benth. was collected at Ye Ta Guing Traditional Herbal Garden, Mandalay, Myanmar, in December 1999. A voucher sample (TMPW 20303) is preserved in the Museum for Materia Medica, Analytical Research Center for Ethnomedicines, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan.

Extraction and Isolation. Air-dried aerial parts of *O. stamineus* (490 g) were extracted with MeOH (3 L, reflux, 3 h × 3). The MeOH extract (61 g) was suspended in H_2O and partitioned successively with hexane, $CHCl_3$, EtOAc, and BuOH to yield hexane (17 g), $CHCl_3$ (22 g), EtOAc (3 g), and BuOH (4 g) fractions, respectively. The $CHCl_3$ fraction (22 g) was chromatographed with EtOAc−hexane to give six fractions.

Fraction 4 (EtOAc−hexane = 1:1 eluate, 705 mg) was rechromatographed (1 × 35 cm) with hexanes−EtOAc (2:1) to afford three subfractions (fraction 4-1, 50 mg; fraction 4-2, 193 mg; fraction 4-3, 452 mg). Subfraction 4-1 was separated by preparative TLC with 2.5% MeOH−$CHCl_3$ to give tetramethylscutellarein (9.5 mg), eupatorin (1.4 mg), and 5,6-dihydroxy-4′,7-dimethoxyflavone (24.6 mg). Subfraction 4-2 was subjected to preparative TLC with 2.5% MeOH−$CHCl_3$ and then with 15% acetone−benzene to give orthosiphols A (**6**, 8.9 mg) and M (**3**, 18.0 mg), while subfraction 4-3 was separated by preparative TLC with 15% acetone−benzene and then with 2.5% MeOH−$CHCl_3$ to give orthosiphols B (**7**, 10.0 mg), K (**1**, 7.9 mg), L (**2**, 16.0 mg), and N (**4**, 36.0 mg), norstaminone A (**5**, 9.7 mg), and neoorthosiphol A (**8**, 53.1 mg).

Fraction 5 (hexanes−EtOAc = 4:6 eluate, 895 mg) was chromatographed (1 × 35 cm) with 2.5% MeOH−$CHCl_3$ to yield three subfractions (fraction 5-1, 98 mg; fraction 5-2, 200 mg; fraction 5-3, 320 mg). Each subfraction was subjected to preparative TLC with 2.5% MeOH−$CHCl_3$ to give tetramethylscutellarein (4.0 mg), **5** (4.4 mg), and **8** (12.0 mg) and 5,6-dihydroxy-4′,7-dimethoxyflavone (4.0 mg), respectively.

Fraction 6 (hexanes−EtOAc = 1:3 eluate, 200 mg) was subjected to repeated preparative TLC with 2.5% MeOH−$CHCl_3$ to yield 3′,4′,6,7,8-pentamethoxyflavone (40.4 mg) and (4-hydroxyphenyl)ethyl *trans*-ferulate (5.0 mg).

Orthosiphol K (1): colorless amorphous solid; $[\alpha]_D^{25}$ −18.8° (*c* 0.08, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3550, 3400, 1725, 1455, 1368, 1280, 1110, 1065 cm^{-1}; ^1H NMR, Table 1; ^{13}C NMR, Table 2; HRFABMS *m/z* 633.2700 [calcd for $C_{36}H_{41}O_{10}$, 633.2700 $(M - H)^-$].

Orthosiphol L (2): colorless amorphous solid; $[\alpha]_D^{25}$ −68.1° (*c* 0.11, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3550, 3450, 1720, 1450, 1365, 1275, 1090, 1040 cm^{-1}; ^1H NMR, Table 1; ^{13}C NMR, Table 2; HRFABMS *m/z* 691.2749 [calcd for $C_{36}H_{41}O_{10}$, 691.2744 $(M - H)^-$].

Orthosiphol M (3): colorless amorphous solid; $[\alpha]_D^{25}$ −50.0° (*c* 0.06, $CHCl_3$), IR ν_{max} ($CHCl_3$) 3550, 3450, 1725, 1450, 1360, 1265, 1110, 1065 cm^{-1}; ^1H NMR, Table 1; ^{13}C NMR, Table 2; HRFABMS *m/z* 569.2405 [calcd for $C_{36}H_{41}O_{10}$, 569.2423 $(M - H)^-$].

Orthosiphol N (4): colorless amorphous solid; $[\alpha]_D^{25}$ −67.3° (*c* 0.38, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3550, 3450, 1720, 1450, 1370, 1285, 1110, 1040 cm^{-1}; ^1H NMR, Table 1; ^{13}C NMR, Table 2; HRFABMS *m/z* 631.2533 [calcd for $C_{36}H_{41}O_{10}$, 631.2543 $(M - H)^-$].

Norstaminone A (5): colorless amorphous solid; $[\alpha]_D^{25}$ +28.9° (*c* 0.10, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1}: 3500, 3450, 1720, 1450, 1370, 1260, 1100, 1065; ^1H NMR, Table 1; ^{13}C NMR, Table 2; HRFABMS *m/z* 539.2318 [calcd for $C_{36}H_{41}O_{10}$, 539.2354 $(M - H)^-$].

Antiproliferative Assay. The antiproliferative assay was carried out, using standard 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium (MTT) method,[16] by the procedure reported previously.[17] 5-Fluorouracil, a clinically used drug,[18] was used as a positive control. The cultured cells were treated with the isolated compounds at five different concentrations ranging from 1 to 100 μg/mL, while for the positive control, concentrations ranging from 1 to 0.001 μg/mL were used. The assay was performed in quadruplicate, and results are expressed as ED_{50} values (μg/mL).

Supporting Information Available: Scheme 1, showing the possible biogenetic pathway to diterpenes **2**, **5**, and **8** from **1**, and Table 4, showing HMBC correlations of the novel diterpenes **1**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP000607T