Staminane- and Isopimarane-Type Diterpenes from *Orthosiphon stamineus* of Taiwan and Their Nitric Oxide Inhibitory Activity

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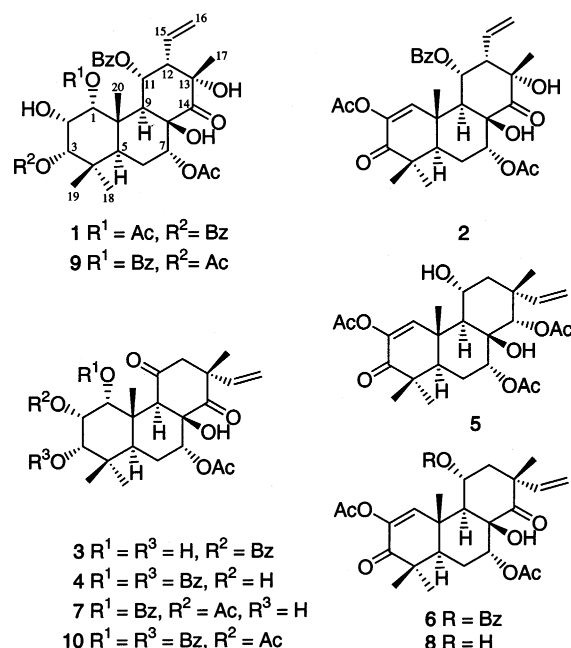
Received October 23, 2003

From the MeOH extract of Taiwanese *Orthosiphon stamineus*, two new staminane-type diterpenes, staminols C (**1**) and D (**2**), and three new isopimarane-type diterpenes, orthosiphonone C (**3**) and D (**4**) and 14-deoxo-14-*O*-acetylorthosiphol Y (**5**), have been isolated together with 16 known diterpenes, orthosiphols A, B, D, K, M, N, O, X, and Y, nororthosiphonolide A, neoorthosiphol B, orthosiphonone A, secoorthosiphols B and C, 3-*O*-deacetylorthosiphol I, and 2-*O*-deacetylorthosiphol J. Their structures were determined on the basis of the spectroscopic data. All the newly isolated diterpenes exhibited dose-dependent inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-activated macrophage-like J774.1 cells, and 2-*O*-deacetylorthosiphonone A showed the most potent activity, with an IC_{50} value of 35.0 μM, comparable to that of the positive control N^G-monomethyl-L-arginine (L-NMMA; IC_{50}, 35.7 μM).

Orthosiphon stamineus Benth. [Syn.: *O. aristatus* (Bl.) Miq., *O. gradiflorus* Bold., *O. spicatus* (Thumb) Bak.; Lamiaceae] is one of the popular traditional folk medicines extensively used in Southeast Asia for the treatment of diabetes, hypertension, rheumatism, tonsillitis, menstrual disorder, etc.[1−3] In the course of our study on bioactive constituents from this plant species, we previously reported a series of highly oxygenated isopimarane- and staminane-type diterpenes from Vietnam,[4] Myanmar,[5,6] Indonesia,[7,8] and Okinawa,[9] and their nitric oxide (NO) inhibitory activity.[10] Our study revealed the characteristic variation of diterpenoid constituents of this plant species with respect to its geographical location. This plant species is also present in Taiwan, but there is no report on the constituents of *O. stamineus* from Taiwan. Thus, we studied the constituents of *O. stamineus* from Taiwan and isolated two new staminane-type diterpenes (**1**, **2**) and three new isopimarane-type diterpenes (**3**−**5**), together with 16 previously reported diterpenes. In this paper, we report the isolation and structure elucidation of the new diterpenes by spectroscopic techniques, together with their NO production inhibitory activity.



Results and Discussion

Air-dried aerial parts of *O. stamineus* from Taiwan were extracted with refluxing MeOH, and the MeOH extract was successively partitioned into hexane, $CHCl_3$, EtOAc, and H_2O fractions. The $CHCl_3$ fraction was subjected to a series of chromatographic separation and preparative TLC to afford two new staminane-type diterpenes, staminols C and D (**1** and **2**), and three new isopimarane-type diterpenes, orthosiphonones C and D (**3** and **4**) and 14-deoxo-14-*O*-acetylorthosiphol Y (**5**), together with 16 known compounds. The known compounds were identified by analysis of their spectroscopic data and comparison with literature data to be orthosiphols A,[5,11] B,[5,11] D (**6**),[6,12] K,[5] M (**7**),[5] N,[5] O,[6] X,[7] and Y (**8**),[7] nororthosiphonolide A,[6] neoorthosiphol B (**9**),[13] orthosiphonone A (**10**),[6] secoorthosiphols B[9] and C,[9] 3-*O*-deacetylorthosiphol I,[8] and 2-*O*-deacetylorthosiphol J.[8]

Staminol C (**1**) showed a quasimolecular ion at *m*/*z* 693.2951 $(M + H)^+$, corresponding to the molecular formula $C_{38}H_{44}O_{12}$ in HRFABMS. The IR spectrum of **1** showed absorptions of hydroxyl (3500 cm^{-1}), ester carbonyl (1720 cm^{-1}), and phenyl (1600, 1460 cm^{-1}) groups. The ^{1}H NMR spectrum of **1** displayed signals due to four tertiary methyls, a vinyl, a methylene, five oxygen-substituted methines, and three aliphatic methines, together with those of two acetyl and two benzoyl groups (Table 1), while its ^{13}C NMR spectrum revealed the signals of a ketone and four ester carbonyl carbons, seven oxygen-substituted carbons, and two aliphatic quaternary carbons (Table 2). These data were similar to those of neoorthosiphol B (**9**),[13] isolated from the same extract and having the same molecular formula. The partial connectivities $C_1-C_2-C_3$, $C_5-C_6-C_7$, and $C_9-C_{11}-C_{12}-C_{15}-C_{16}$ deduced by the COSY spectrum (Figure 1a) indicated that **1** has the same staminane carbon framework as **9**. However, analysis of

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10.1021/np030471+ CCC: $27.50 © 2004 American Chemical Society and American Society of Pharmacognosy
Published on Web 03/31/2004

Table 1. ^{1}H NMR Data (δ) for Compounds **1**−**5** in $CDCl_3$ (*J* values in parentheses)

position	**1**	**2**	**3**	**4**	**5**
1	5.04 d (2.7)	7.34 s	4.94 br s	6.45 d (2.9)	7.97 s
2	4.40 t (2.7)		5.56 t (3.2)	4.66 br s	
3	5.19 d (2.7)		3.74 br s	5.35 d (2.9)	
5	2.48 dd (13.2, 2.2)	2.34 dd (13.4, 2.4)	1.97 d (2.2)	2.42 dd (11.2, 2.9)	2.17 dd (10.8, 2.4)
6	2.06 m; 1.99 t (2.2)	2.18 m; 1.84 dt (13.4, 2.4)	2.02 m; 1.90 m	2.09 m; 2.04 t (2.9)	2.22 m; 1.61 m
7	5.41 t (2.2)	5.37 t (2.4)	5.31 t (2.2)	5.45 t (2.9)	4.87 t (2.4)
9	3.29 d (9.5)	2.74 d (10.8)	3.81 d (2.2)	3.58 s	2.07 d (4.4)
11	6.30 dd (9.5, 4.6)	6.22 dd (10.8, 4.4)			4.63 dd (10.8, 4.4)
12	3.11 dd (9.5, 4.6)	3.19 dd (10.0, 4.4)	2.81 d (18.1)	2.83 d (17.8)	2.08 d (10.8)
			2.73 d (18.1)	2.64 d (17.8)	1.73 dd (10.8, 4.4)
14					4.68 s
15	5.33 dt (16.6, 9.5)	5.61 dt (16.8, 10.0)	5.77 dd (17.6, 10.6)	5.34 dd (17.3, 10.2)	5.70 dd (17.3, 10.3)
16	5.07 d (16.6)	5.14 d (16.8)	5.19 d (17.6)	4.79 d (17.3)	4.94 d (17.3)
	4.82 d (9.5)	5.34 d (10.0)	5.29 d (10.6)	4.41 d (10.2)	4.95 d (10.3)
17	1.33 s	1.70 s	1.25 s	1.17 s	1.33 s
18	1.09 s	1.16 s	0.97 s	0.96 s	1.10 s
19	0.89 s	1.15 s	1.01 s	1.15 s	1.17 s
20	1.55 s	1.42 s	1.33 s	1.44 s	1.49 s
1-OCOPh					
2′,6′				8.03 d (7.3)	
3′,5′				7.33 t (7.3)	
4′				5.76 t (7.3)	
2-OCOPh					
2″,6″			8.12 dd (7.7, 1.4)		
3″,5″			7.46 t (7.7)		
4″			7.59 tt (7.7, 1.4)		
3-OCOPh					
2‴,6‴	7.90 d (7.8)			7.82 d (8.0)	
3‴,5‴	7.42 t (7.8)			7.01 t (8.0)	
4‴	7.63 t (7.8)			7.35 t (8.0)	
11-OCOPh					
2⁗,6⁗	7.76 d (7.8)	8.05 d (7.3)			
3⁗,5⁗	7.34 t (7.8)	7.45 t (7.3)			
4⁗	7.57 t (7.8)	7.63 t (7.3)			
1-OCOC*H*$_3$	2.22 s				
2-OCOC*H*$_3$		2.08 s			2.21 s
7-OCOC*H*$_3$	1.48 s	2.10 s	2.00 s	2.12 s	1.98 s
14-OCOC*H*$_3$					2.01 s

the COSY and HMQC spectra indicated an upfield shift of H-1 (δ_H 5.04) and downfield shift of H-3 (δ_H 5.19), compared to those of **9** (H-1, δ_H 5.49; H-3, δ_H 5.03), suggesting the presence of the acetyl and benzoyl groups at C-1 and C-3, respectively. This was confirmed by the HMBC correlations between the ester carbonyl carbon at δ_C 169.2 (1-OCO) and the protons at δ_H 2.22 (1-$OCOCH_3$) and δ_H 5.04 (H-1) and between the ester carbonyl carbon at δ_C 168.4 (C-7‴) and the protons at δ_H 7.90 (H-2‴,6‴) and 5.19 (H-3), respectively (Figure 1a). The relative stereochemistry of **1** was assigned on the basis of the ROESY correlations and the coupling constant data. The ROESY correlations H_3-19/H_3-20, H-2/H_3-20, H-2/H_3-19, and H-5/H-9 indicated that rings A and B are *trans*-fused with β-axial orientation of H-2, H_3-19, and H_3-20 and α-axial orientation of H-5 and H-9, while the small coupling constant for H-7 (J = 2.2 Hz) indicated it to be in β-equatorial orientation. These ROESY correlations also indicated the rings A and B of **1** to be in chair conformation. The signals due to 20-H_3 (δ_H 1.55) and 17-H_3 (δ_H 1.33) were observed at lower fields than expected, which was assumed to be due to an anisotropic effect of the 8β-axial-OH. The large J value between H-9 and H-11 (J = 9.5 Hz) and the ROESY correlation between H_3-20 and H-11 indicated H-11 to have β-axial orientation, while the ROESY correlation H-11/H-12 indicated the vinyl group to be α-oriented. From these data, the structure of staminol C was concluded to be **1**.

The HRFABMS of staminol D (**2**) showed a quasimolecular ion at m/z 569.2394 $(M + H)^+$, consistent with the molecular formula $C_{31}H_{36}O_{10}$. The IR spectrum of **2** showed absorptions of hydroxyl (3500 cm^{-1}), ester carbonyl (1730 cm^{-1}), α,β-unsaturated carbonyl (1680 cm^{-1}), and phenyl (1600, 1450 cm^{-1}) groups. The ^{1}H NMR spectrum of **2** displayed signals due to four tertiary methyls, two acetyl methyls, a benzoyl, a vinyl, two methylenes, and an isolated olefinic proton (Table 1). These data resembled those of orthosiphol D (**6**),[6,12] isolated from the same extract. However, the partial connectivities $C_9-C_{11}-C_{12}-C_{15}-C_{16}$ deduced from the COSY spectrum indicated the vinyl group to be located at C-12, indicating **2** to have a staminane carbon framework, which was confirmed by the HMBC spectrum. The coupling constants of each proton and the ROESY correlations H_3-19/H_3-20, H-5/H-9, and H-11/H-12 indicated that rings B and C have the same stereochemistry as staminol C (**1**). From these data, the structure of staminol D was concluded as **2**.

The ^{1}H and ^{13}C NMR spectra of orthosiphonone C (**3**) closely resembled those of orthosiphol M (**7**),[5] isolated from the same extract, but they differ from each other due to the disappearance of signals of one of the two acetyl groups in **7**. The partial connectivity determined from the COSY and HMQC spectra indicated the upfield shift of H-1 (**3**, δ_H 4.94; **7**, δ_H 6.42), suggesting the presence of a hydroxyl group instead of the benzoyl substituent at C-1. On the other hand, the presence of a benzoyl group instead of the acetyl substituent at C-2 was determined from the significant HMBC correlations between the ester carbonyl carbon at δ_C 165.9 (2-OCO) and the protons at δ_H 5.56 (H-2) and 8.12 (H-2″,6″). The ROESY correlations H-1/H-2, H-2/H_3-19, H-2/H_3-20, H_3-19/H_3-20, and H-5/H-9 and their coupling constants indicated the stereochemistry of **3** to be the same as that of **7**. From these data, the structure of ortho-

Table 2. ^{13}C NMR Data (δ) for Compounds **1**−**5** in $CDCl_3$

position	1	2	3	4	5
1	79.1	144.3	75.0	77.8	147.9
2	66.8	142.7	68.7	65.6	141.9
3	80.3	196.5	78.8	78.5	196.9
4	37.9	45.2	38.5	37.6	44.9
5	37.3	44.2	33.9	36.3	43.7
6	21.7	22.8	21.7	21.2	23.5
7	71.7	69.7	71.6	71.0	76.2
8	78.0	76.6	77.2	76.3	75.7
9	42.6	44.5	52.6	51.9	46.9
10	45.2	40.3	44.1	43.5	39.9
11	70.9	70.2	206.6	206.0	67.3
12	52.8	54.1	47.7	48.0	41.8
13	76.9	76.2	49.8	49.1	42.5
14	202.4	207.9	208.6	207.2	78.6
15	134.4	131.3	138.9	138.5	145.3
16	123.2	121.9	116.8	117.1	111.6
17	30.5	28.2	25.1	25.3	25.2
18	23.1	28.0	28.5	28.3	30.0
19	28.8	21.3	21.4	22.3	21.4
20	16.8	20.8	16.3	16.9	19.8
1-OCOPh					
1′				129.7	
2′,6′				130.2	
3′,5′				128.1	
4′				133.2	
7′				166.9	
2-OCOPh					
1″			129.8		
2″,6″			129.8		
3″,5″			128.5		
4″			133.2		
7″			165.9		
3-OCOPh					
1‴	130.6			129.9	
2‴,6‴	130.7			129.5	
3‴,5‴	129.1			127.8	
4‴	134.0			132.6	
7‴	168.4			166.8	
11-OCOPh					
1⁗	130.3	129.6			
2⁗,6⁗	130.4	129.8			
3⁗,5⁗	129.1	128.7			
4⁗	134.0	133.6			
7⁗	167.0	165.3			
1-OCOCH$_3$					
CO*C*H$_3$	21.8				
*C*OCH$_3$	169.2				
2-OCOCH$_3$					
CO*C*H$_3$		20.2			20.4
*C*OCH$_3$		169.8			169.2
7-OCOCH$_3$					
CO*C*H$_3$	21.2	21.2	21.0	20.8	21.2
*C*OCH$_3$	171.4	168.9	168.9	168.2	170.2
14-OCOCH$_3$					
CO*C*H$_3$					21.7
*C*OCH$_3$					169.7

siphonone C (**3**) was assigned to be 2-*O*-benzoyl-2-*O*-deacetyl-1-*O*-debenzoylorthosiphol M. This is the first example of a compound with a benzoyl substituent at C-2 in highly oxygenated diterpenes isolated from *O. stamineus*.

Orthosiphonone D (**4**) showed the quasimolecular ion at *m*/*z* 633.2673 (M + H)$^+$ in HRFABMS, which corresponded to the molecular formula $C_{36}H_{40}O_{10}$. The ^{1}H NMR spectrum of **4** closely resembled those of orthosiphonone A (**10**),[6] isolated from the same extract, and displayed the signals of two benzoyl groups, an acetyl group, a vinyl, four oxygen-substituted methines, and four tertiary methyls. However it lacks the signals due to one of the two acetyl groups. Analysis of the COSY and HMQC spectra of **4** indicated the upfield shift of H-2 (δ_H 4.66), compared to that of **10** (δ_H 5.74). On the basis of the chemical shift and coupling constant data, C-2 was assumed to have a hydroxyl group

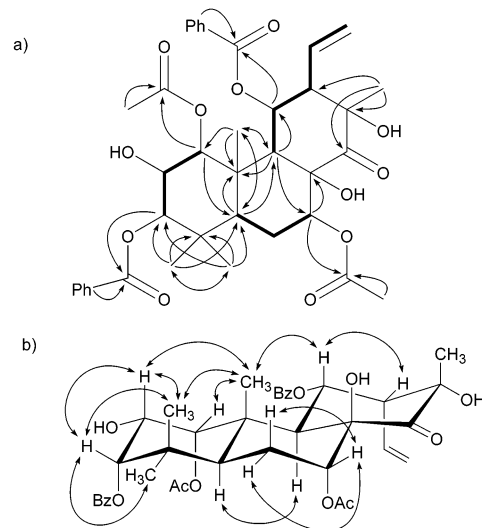

Figure 1. (a) Connectivities (bold line) deduced by the COSY spectrum and significant HMBC correlations (arrow) and (b) ROESY correlations observed for **1**.

instead of the *O*-acetyl substituent in **10**, which was confirmed by the HMBC spectroscopic analysis. The relative stereochemistry of **4** was deduced to be the same as that of **10** by the ROESY spectrum. Thus, the structure of orthosiphonone D (**4**) was concluded to be 2-*O*-deacetylorthosiphonone A.

14-Deoxo-14-*O*-acetylorthosiphol Y (**5**) showed a quasimolecular ion at *m*/*z* 493.2473 (M + H)$^+$ in HRFABMS, which corresponded to the molecular formula $C_{26}H_{36}O_9$. The IR spectrum of **5** closely resembled that of orthosiphol Y (**8**),[7] also isolated from the same extract, and showed absorptions of hydroxyl, ester carbonyl, and α,β-unsaturated carbonyl groups. The ^{1}H NMR spectrum of **5** (Table 1) was also similar to that of **8**, but it displayed signals due to one more oxymethine (δ_H 4.68) and one more acetyl methyl (δ_H 2.01) than **8**. On the other hand, the ^{13}C NMR spectrum of **5** (Table 2) showed the disappearance of a signal due to one of the two ketone carbons and the presence of one more oxygen-substituted carbon. The additional oxygen-substituted carbon showed HMBC correlations with H-9, H-12, H-15, and H-17, indicating its location to be at C-14. This was confirmed by the HMBC correlations of the ester carbonyl carbon at δ_C 169.7 with the oxymethine proton at δ_H 4.68 (H-14) and the acetyl methyl protons at δ_H 2.01 ($OCOCH_3$). The ROESY correlations H-6/H-7, H-11/H$_3$-20, H-11/H$_3$-17, H$_3$-19/H$_3$-20, and H-14/H$_3$-17 indicated the same stereochemistry on ring A and B as **8**, while on ring C, the correlations H-11/H-17 and H-14/H$_3$-17 suggested the α-axial orientation of the 14-OAc group. Thus, **5** was concluded to be 14-deoxo-14-*O*-acetylorthosiphol Y. This is the first example of a compound with an acetyl substituent at C-14 in the diterpenes isolated from *O. stamineus*.

All the newly isolated diterpenes were tested for their inhibitory activities against NO production in lipopolysaccharide (LPS)-activated macrophage-like J744.1 cells. All of them displayed significant dose-dependent inhibition, and **4** showed the most potent activity, with an IC_{50} value of 35.0 μM, comparable to that of the positive control L-NMMA (Table 3). As evident from a previously studied structure−activity relationship,[10] the potent activity of **4** may be due to the presence of the benzoyl group at C-3. Nitric oxide (NO) is an important signaling molecule that acts in many tissues to regulate a diverse range of physiological processes. However, excessive production has

Table 3. Inhibitory Effects of Diterpenes on NO Production in LPS-Activated Macrophage-like J774.1 Cells

compound	IC_{50} (μM)[a]
1	61.1
2	92.0
3	81.8
4	35.0
5	118.7
L-NMMA	35.7

[a] IC_{50} values were calculated from the mean of data of four determinations.

detrimental effects on many organ systems of the body, leading to tissue damage, even causing a fetal event (septic shock). Therefore, effective inhibition of NO accumulation by inflammatory stimuli presents a beneficial therapeutic strategy. In this regard, the diterpenoid constituents from *O. stamineus* have been shown to be effective inhibitors of NO production, suggesting its anti-inflammatory utility.

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 Silisia, Aichi, Japan). Analytical and preparative TLC were carried out on precoated Merck Kieselgel 60 F_{254} and RP-18 F_{254} plates (0.25 or 0.5 mm thickness).

Plant Material. The aerial parts of cultivated *Orthosiphon stamineus* Benth. were collected at Alian Hsiang, Kaohsiung Hsien, Taiwan, in June 2002 and were identified by one of the authors (C.C.-H.). A voucher sample (CNPR 523-033-01-0-05) is preserved in the Herbarium Center of Pharmacy, Chianan University of Pharmacy and Science, Jen-te, Taiwan.

Extraction and Isolation. Air-dried aerial parts of *O. stamineus* (2.2 kg) were extracted with MeOH (6 L, reflux, 3 h, × 2). The MeOH extract (200 g) was suspended in H_2O and partitioned successively with hexane, $CHCl_3$, EtOAc, and H_2O to yield hexane (37 g), $CHCl_3$ (46 g), EtOAc (13 g), and H_2O (100 g) fractions, respectively. The $CHCl_3$ fraction (46 g) was chromatographed on Si gel with an EtOAc−hexane solvent system to give seven fractions.

Fraction 3 [EtOAc−hexane (4:6) eluate, 1.2 g] was rechromatographed (2.5 × 20 cm) with 2% MeOH−$CHCl_3$ to afford two subfractions (3-1, 18 mg; 3-2, 198 mg). Subfraction 3-1 was separated by preparative TLC with hexane−acetone (4:1) to give orthosiphols B[5,11] (1.0 mg) and D (**6**, 4.2 mg),[6,12] while subfraction 3-2 was subjected to preparative TLC with 2% MeOH−$CHCl_3$ to give nororthosiphonolide A (71.7 mg).[6]

Fraction 4 [EtOAc−hexane (6:4) eluate, 5.9 g] was rechromatographed (4 × 30 cm) with 2% MeOH−$CHCl_3$ to give two subfractions (4-1, 4.1 g; 4-2, 1.1 g). Subfraction 4-1 was rechromatographed with 2% MeOH−$CHCl_3$, followed by reversed-phase preparative TLC with CH_3CN−MeOH−H_2O (1:1:1), to give orthosiphols A[5,11] (33.3 mg) and M (**7**, 5.4 mg).[5] Subfraction 4-2 was rechromatographed on reversed-phase silica gel with CH_3CN−MeOH−H_2O (1:1:2), followed by reversed-phase preparative TLC with CH_3CN−MeOH−H_2O (1:1:1), to give orthosiphonone C (**3**, 2.4 mg), 14-deoxo-14-*O*-acetylorthosiphol Y (**5**, 8.3 mg), **7** (5.6 mg), orthosiphol Y (**8**, 1.5 mg),[7] orthosiphonone A (**10**, 8.4 mg),[6] orthosiphols A (9.6 mg), B (1.0 mg), O (7.9 mg),[6] and X (32.5 mg),[7] nororthosiphonolide A (2.4 mg), and secoorthosiphol C (1.5 mg).[9]

Fraction 5 [EtOAc−hexane (7:3) eluate, 1.8 g] was rechromatographed (2 × 25 cm) with 2% MeOH−$CHCl_3$ to afford two subfractions (5-1, 274 mg; 5-2, 1.1 g). Subfraction 5-1 was separated by preparative TLC with 2% MeOH−$CHCl_3$, to give **7** (37.3 mg), while subfraction 5-2 was rechromatographed with 2% MeOH−$CHCl_3$, followed by preparative TLC with 3% MeOH−$CHCl_3$, to give staminols C (**1**, 1.1 mg) and D (**2**, 4.0 mg), orthosiphonone D (**4**, 4.0 mg), orthosiphols B (70.2 mg), K (7.1 mg),[5] and N (44.2 mg),[5] and secoorthosiphol B (20.6 mg).[9]

Fraction 6 [EtOAc−hexane (9:1) eluate, 4.4 g] was rechromatographed (4 × 40 cm) with 2% MeOH−$CHCl_3$, followed by reversed-phase preparative TLC with MeOH−H_2O (3:1), to give neoorthosiphol B (**9**, 50.4 mg),[13] 3-*O*-deacetylorthosiphol I (1.0 mg),[8] and 2-*O*-deacetylorthosiphol J (4.0 mg).[8]

Staminol C (1): colorless amorphous solid, $[\alpha]_D^{25}$ −81.7° (*c* 0.067, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3500, 1720, 1600, 1460, 1370, 1280, 1090 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS 693.2951 [calcd for $C_{38}H_{45}O_{12}$ $(M + H)^+$, 693.2911].

Staminol D (2): colorless amorphous solid, $[\alpha]_D^{25}$ −18.8° (*c* 0.293, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3500, 1730, 1680, 1600, 1450, 1370, 1260, 1090, 1040 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS 569.2394 [calcd for $C_{31}H_{37}O_{10}$ $(M + H)^+$, 569.2387].

Orthosiphonone C (3): colorless amorphous solid, $[\alpha]_D^{25}$ −117.7° (*c* 0.093, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3500, 1710, 1600, 1450, 1370, 1270, 1110, 1060 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS 529.2463 [calcd for $C_{29}H_{37}O_9$ $(M + H)^+$, 529.2438].

Orthosiphonone D (4): colorless amorphous solid, $[\alpha]_D^{25}$ −105.3° (*c* 0.393, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3500, 1720, 1600, 1450, 1370, 1280, 1110, 1030 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS 633.2673 [calcd for $C_{36}H_{41}O_{10}$ $(M + H)^+$, 633.2700].

14-Deoxo-14-*O*-acetylorthosiphol Y (5): colorless amorphous solid, $[\alpha]_D^{25}$ −29.1° (*c* 0.340, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3500, 1730, 1670, 1370, 1250, 1040 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS 493.2473 [calcd for $C_{26}H_{37}O_9$ $(M + H)^+$, 493.2438].

Nitric Oxide Inhibitory Assay. The macrophage-like J774.1 cell line was purchased from Riken Cell Bank (Tsukuba, Japan) and propagated in 75 cm^2 plastic culture flasks (Falcone, Becton Dickinson, NJ), containing RPMI-1640 medium supplemented with penicillin G (100 units/mL), streptomycin (100 μg/mL), and 10% fetal calf serum. The cells were harvested with trypsin and diluted to a suspension in fresh medium. The cells were seeded in 96-well plastic plates with 1×10^5 cells/well and allowed to adhere for 2 h at 37 °C in a humidified atmosphere containing 5% CO_2. Then the medium was replaced with fresh medium, containing LPS (10 μg/mL) and test compounds at indicated concentrations, and the cells were incubated for 24 h. NO production was determined by measuring the accumulation of nitrite in the culture supernatant with Griess reagent.[14] Briefly, 50 μL of the supernatant from each well of the 96-well plate was incubated with an equal volume of Griess reagent (0.5% sulfanilamide and 0.05% naphthylenediamide dihydrochloride in 2.5% H_3PO_4) and then allowed to stand for 10 min at room temperature. Absorbance at 550 nm was measured using a HTS 7000 microplate reader. The blank correction was carried out by subtracting the absorbance due to medium only from the absorbance reading of each well.

Acknowledgment. This work was supported in part by a Grant-in-Aid for International Scientific Research (No. 13576-027) from The Ministry of Education, Culture, Sports, Science and Technology, Japan.

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NP030471+