Nitric Oxide Inhibitory Isopimarane-type Diterpenes from *Orthosiphon stamineus* of Indonesia

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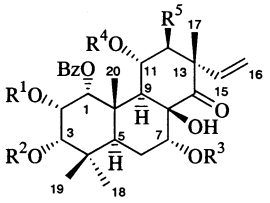
A methanolic extract of *Orthosiphon stamineus* yielded six new highly oxygenated isopimarane-type diterpenes, orthosiphols U−Z (**1**−**6**), and 15 previously reported diterpenes. The isolated diterpenes all showed significant dose-dependent inhibitory effects on the nitric oxide (NO) production in lipopolysaccharide (LPS)-activated macrophage-like J774.1 cells. Orthosiphols A (**7**), B (**8**), D (**9**), and X (**4**) showed more potent inhibitory activities than a positive control, N^G-monomethyl-L-arginine (L-NMMA), and **1** displayed the strongest activity with an IC_{50} value of 6.4 μM.

Orthosiphon stamineus Benth. [syn.: *O. aristatus* (Bl.) Miq., *O. grandiflorus* Bold., *O. spicatus* (Thumb.) Bak.; Lamiaceae] is one of the popular traditional folk medicines extensively used in Southeast Asia for the treatment of a wide range of diseases: in Indonesia to treat rheumatism, diabetes, hypertension, tonsillitis, epilepsy, menstrual disorder, gonorrhea, syphilis, renal calculus, gallstone, etc.;[1] in Vietnam for urinary lithiasis, edema, eruptive fever, influenza, hepatitis, jaundice, and biliary lithiasis;[2] and in Myanmar to alleviate diabetes and urinary tract and renal diseases.[3] In Okinawa, this herb is consumed as a healthy Java tea to facilitate body detoxification. In our search for biologically active compounds from *O. stamineus*,[4−7] we found that the methanolic extract of an aerial part of *O. stamineus* collected from Indonesia showed significant inhibitory activity on NO production in lipopolysaccharide (LPS)-activated J774.1 macrophage-like cells (IC_{50}, 42 μg/mL). Further fractionation of the methanol extract led to the isolation of six novel highly oxygenated isopimarane-type diterpenes named orthosiphols U−Z (**1**−**6**). In this paper, we report the isolation and structure elucidation of the new diterpenes together with their NO inhibitory activity.



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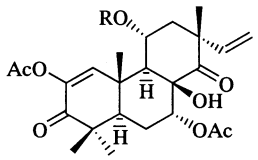
3 $R^1 = R^3 =$ Ac, $R^2 = R^4 = R^5 =$ H
4 $R^1 =$ Ac, $R^2 =$ Bz, $R^3 = R^4 = R^5 =$H
7 $R^1 = R^3 =$ Ac, $R^2 = R^5 =$ H, $R^4 =$ Bz
8 $R^1 = R^5 =$ H, $R^2 = R^3 =$ Ac, $R^4 =$ Bz
10 $R^1 = R^2 =$ Ac, $R^3 = R^5 =$ H, $R^4 =$ Bz
11 $R^1 = R^2 =$ Ac, $R^3 = R^4 = R^5 =$H
14 $R^1 = R^3 =$ Ac, $R^2 =$ Bz, $R^4 = R^5 =$H
15 $R^1 = R^5 =$ H, $R^2 = R^3 =$ Ac, $R^4 =$ Bz, $R^5 =$ OH
16 $R^1 =$ Ac, $R^2 = R^3 = R^5 =$ H, $R^4 =$ Bz



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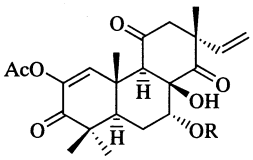
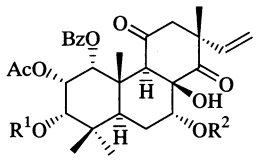
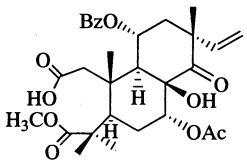


6 R = H
19 R = Ac



12 $R^1 =$ Ac, $R^2 =$ H
13 $R^1 = R^2 =$ Ac
17 $R^1 =$ Bz, $R^2 =$ Ac



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Results and Discussion

Air-dried aerial parts of *O. stamineus* from Indonesia were extracted by refluxing with MeOH, and the H_2O suspension of the MeOH extract was then successively partitioned into hexane, $CHCl_3$, EtOAc, BuOH, and H_2O. The $CHCl_3$ fraction was subjected to a series of chromatographic separations and preparative TLC to afford six new highly oxygenated isopimarane-type diterpenes, orthosiphols U−Z (**1**−**6**), together with 16 previously reported compounds. The known compounds were identified by spectroscopic analysis as orthosiphols A[5,8] (**7**), B[5,8] (**8**), D[6,9] (**9**), F[4] (**10**), G[4] (**11**), I[4] (**12**), J[4] (**13**), O[6] (**14**), R[7] (**15**), and T[7] (**16**), orthosiphonone A[6,10] (**17**), and secoorthosiphol B[7] (**18**).

Orthosiphonol U (**1**) was obtained as a colorless amorphous solid and showed the quasimolecular ion at *m/z* 615.2836 $(M + H)^+$ in HRFABMS, which corresponds to the molecular formula $C_{33}H_{42}O_{11}$. The IR spectrum of **1** showed absorptions of hydroxyl (3450 cm^{-1}), carbonyl (1720 cm^{-1}), and phenyl (1605, 1455 cm^{-1}) groups. The ^1H NMR spectrum of **1** displayed signals due to four methyls, a vinyl, five oxygen-substituted methines, and two methylenes, together with those of three acetyl groups and a benzoyl group (Table 1), while its ^{13}C NMR spectrum revealed the signals of a ketone, four ester carbonyls, six oxygen-substituted carbons, and three aliphatic quaternary carbons (Table 2). Analysis of a COSY spectrum led to the partial structures depicted by bold lines in Figure 1a, which

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Table 1. ^{1}H NMR Data (δ) for Compounds **1**−**6** in $CDCl_3$ (J values in parentheses)

position	1	2	3	4	5	6
1	5.53 d (2.7)	5.52 d (3.1)	5.60 d (3.0)	5.57 d (3.4)	7.53 s	7.6 s
2	5.51 t (2.7)	4.48 t (3.1)	5.46 t (3.0)	5.66 t (3.4)		
3	5.04 d (2.7)	5.07 d (3.1)	3.55 d (3.0)	5.35 d (3.4)		
5	2.40 dd (13.1, 2.4)	2.34 dd (10.2, 4.6)	2.36 dd (10.4, 5.1)	2.76 dd (14.4, 1.9)	2.24 dd (13.5, 2.0)	2.41 dd (13.9, 2.2)
6	2.06 dd (13.1, 2.4)	1.96 m (2H)	1.98 m (2H)	2.06 m	2.19 m	1.76 td (13.9, 2.2)
	1.93 td (13.1, 2.4)			1.95 m	1.90 dt (14.6, 2.0)	2.03 d (13.9)
7	5.34 d (2.4)	5.30 br s	5.29 t (2.8)	4.22 br s	5.32 t (2.8)	4.31 br s
9	2.76 d (8.3)	2.86 d (7.1)	2.72 d (8.1)	2.82 d (4.9)	2.31 d (7.5)	3.11 s
10						
11	4.46 br t (8.3)	4.44 t (7.1)	4.41 br t (8.1)	4.50 br t (4.9)	4.47 br t (7.5)	
12	2.31 dd (14.6, 8.3)	2.33 dd (15.1, 7.1)	2.28 dd (14.7, 8.1)	2.39 dd (14.3, 4.9)	2.50 dd (15.2, 7.5)	2.95 d (17.8)
	1.80 dd (14.6, 1.9)	1.81 d (15.1)	1.77 dd (14.7, 2.2)	1.76 m	2.05 dd (15.2)	2.84 d (17.8)
15	5.85 dd (17.6, 10.5)	5.84 dd (17.6, 10.5)	5.79 dd (17.7, 10.7)	5.91 dd (17.7, 10.7)	6.14 dd (17.7, 10.7)	5.76 dd (17.6, 10.5)
16	4.78 dd (17.6)	4.85 d (17.6)	4.78 d (17.7)	4.94 d (17.7)	5.19 d (10.7)	5.25 d (10.5)
	4.54 d (10.5)	4.63 d (10.5)	4.56 d (10.7)	4.78 d (10.7)	5.01 d (17.7)	5.15 d (17.6)
17	1.12 s	1.14 s	1.11 s	1.23 s	1.24 s	1.30 s
18	0.91 s	0.89 s	1.07 s	1.03 s	1.14 s	1.17 s
19	1.14 s	1.09 s	1.04 s	1.21 s	1.16 s	1.21 s
20	1.48 s	1.41 s	1.43 s	1.51 s	1.44 s	1.44 s
1-OBz						
2′,6′	8.09 d (7.3)	8.11 d (7.6)	8.02 dd (7.4, 1.0)	8.05 d (7.3)		
3′,5′	7.40 t (7.3)	7.43 t (7.6)	7.42 t (7.4)	7.40 t (7.3)		
4′	7.57 t (7.3)	7.60 t (7.6)	7.57 tt (7.4, 1.0)	7.56 t (7.3)		
3-OBz						
2″,6″				7.77 d (7.2)		
3″,5″				6.89 t (7.2)		
4″				7.29 t (7.2)		
2-OAc						
CO*CH*$_3$	1.99 s		2.05 s	1.95 s	1.98 s	2.21 s
3-OAc						
CO*CH*$_3$	1.63 s	1.62 s				
7-OAc						
CO*CH*$_3$	2.08 s	2.09 s	2.10 s		2.21	

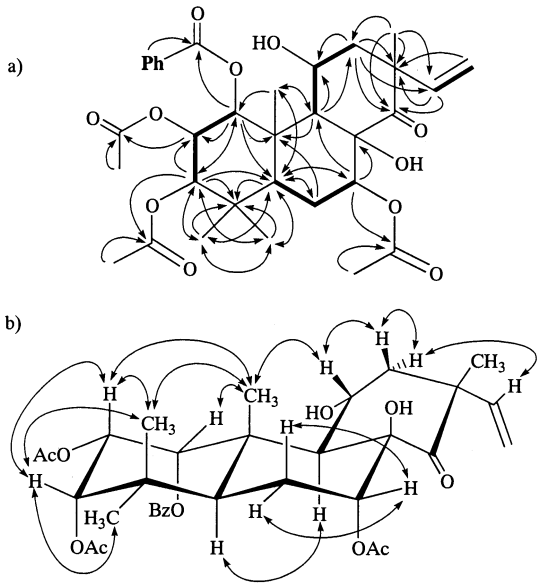

Figure 1. (a) Connectivities (bold lines) deduced by the COSY spectrum and significant HMBC correlations (arrow) and (b) ROESY correlations observed for **1**.

were connected on the basis of the long-range correlations observed in the HMBC spectrum (Figure 1a). Significant correlations were observed between the ester carbonyl carbon at δ 170.53 (2-OCO) and the protons at δ 1.99 (2-OCO*CH*$_3$) and 5.51 (H-2), between the ester carbonyl carbon at δ 170.51 (3-OCO) and the protons at δ 1.63 (3-OCO*CH*$_3$) and 5.04 (H-3), between the ester carbonyl carbon at δ 168.8 (7-OCO) and the protons at δ 2.08 (7-OCO*CH*$_3$) and 5.34 (H-7), and between the ester carbonyl carbon at δ 165.4 (1-OCO) and the protons at δ 8.09 (H-2′,6′) and 5.53 (H-1), allowing the locations of three acetoxyl groups to be at C-2, C-3, and C-7 and the benzoyloxy group at C-1, respectively.

The relative stereochemistry of **1** was assigned on the basis of ROESY correlations and coupling constant data. The ROESY correlations H-1/H-2, H-2/H-3, H-2/H$_3$-19, H-2/H$_3$-20, H-3/H$_3$-19, H$_3$-19/H$_3$-20,H-5/H-9, and H-5/H$_3$-18 indicated that rings A and B were *trans*-fused and H-2 was β-axial oriented (Figure 1b). On the other hand, the axial−equitorial coupling constant (J = 2.7 Hz) for H-1, H-2, and H-3 indicated the benzoyloxy substituent at C-1 and acetoxyl substituents at C-2 and C-3 to be α-equatorial oriented. Similarly, a small coupling constant observed for H-7 (br s) indicated it to be in β-equatorial orientation. As for ring C, the ROESY correlations H-9/H-11, H$_3$-20/H-11, H-11/H-12β, H-12α/H-12β, and H-12α/H$_3$-15 indicated the boat conformation of ring C, and a small coupling constant between H-9 and H-11 (J = 5.1 Hz) indicated the β-equatorial orientation of H-11. This is also supported by the absence of *trans*-diaxial coupling between H-11 and H-12α.

The HRFABMS of orthosiphol V (**2**) showed the quasi-molecular ion at m/z 573.2732 $(M + H)^+$, consistent with the molecular formula $C_{31}H_{40}O_{10}$. The IR spectrum of **2** was similar to that of **1** and showed absorptions of hydroxyl, ester carbonyl, and phenyl groups. The ^{1}H and ^{13}C NMR spectra of **2** also closely resembled those of **1**, but they were characterized by the disappearance of signals due to one of three acetyl groups in **1**. The deacetylation was determined to be at C-2 on the basis of the upfield shift of H-2 (**2**, δ 4.48; **1**, δ 5.51), which was confirmed by the HMBC spectrum. The relative stereochemistry of **2** was determined to be the same as **1**, on the basis of the 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 and the coupling constant value of H-9/H-11 (J = 7.1 Hz). Thus, **2** is 2-*O*-deacetylorthosiphol U.

Table 2. ^{13}C NMR Data (δ) for Compounds **1**−**6** in $CDCl_3$

position	1	2	3	4	5	6
1	74.5	79.2	76.3	74.7	144.3	143.2
2	66.8	66.1	68.1	66.8	142.3	141.9
3	76.1	78.2	77.5	76.5	196.6	196.6
4	37.2	37.1	38.4	37.9	44.9	44.9
5	36.6	36.7	35.4	35.4	44.7	42.3
6	21.2	21.4	21.3	23.5	22.9	24.7
7	71.2	71.4	71.3	69.0	70.8	68.8
8	75.6	75.8	75.8	77.8	75.4	77.6
9	44.1	44.1	44.3	44.2	49.0	52.6
10	43.4	43.4	43.4	44.0	40.3	39.8
11	64.3	64.9	64.4	64.7	65.6	206.5
12	43.3	43.5	43.5	43.7	43.3	47.9
13	48.3	48.4	48.4	48.6	48.6	49.5
14	210.1	209.9	210.0	213.4	209.4	209.1
15	140.9	140.7	140.7	141.5	141.2	138.3
16	114.7	115.3	115.0	114.7	115.9	116.1
17	26.9	26.0	26.0	26.1	26.1	25.4
18	28.0	28.0	29.0	27.9	27.6	27.6
19	22.4	22.6	22.4	22.8	21.5	21.5
20	15.8	16.0	16.0	16.4	20.2	20.0
1-OBz						
1′	131.3	130.5	130.8	131.4		
2′,6′	129.6	129.8	129.6	130.1		
3′,5′	128.0	128.3	128.4	128.3		
4′	132.7	133.3	133.0	132.5		
7′	165.4	168.4	16.5	166.6		
3-OBz						
1"				131.4		
2″,6″				129.7		
3″,5″				127.8		
4″				132.5		
7″				166.6		
2-OAc						
$COCH_3$	20.9		20.9	21.1	21.0	20.3
$COCH_3$	170.53		168.8	171.0	170.0	168.6
3-OAc						
$COCH_3$	20.5	20.6				
$COCH_3$	170.51	168.6				
7-OAc						
$COCH_3$	20.8	20.9	21.2		20.5	
$COCH_3$	168.8	170.9	170.5		169.1	

The ^1H and ^{13}C NMR spectra of orthosiphol W (**3**) also closely resembled those of **1** and were also characterized by the disappearance of signals of one of three acetyl groups in **1**. The deacetylation was determined to be at C-3 on the basis of the upfield shift of H-3 (**3**, δ 3.55; **1**, δ 5.04), as indicated by the COSY and HMQC spectra. Thus, **3** was concluded to be 3-*O*-deacetylorthosiphol U, which was confirmed by the COSY, HMQC, HMBC, and ROESY spectra.

Orthosiphol X (**4**) showed the quasimolecular ion at *m*/*z* 635.2836 $(M + H)^+$ in HRFABMS, which corresponded to the molecular formula $C_{36}H_{42}O_{10}$. The IR spectrum of **4** showed absorptions of hydroxyl (3450 cm^{-1}), carbonyl (1720 cm^{-1}), and phenyl (1605, 1455 cm^{-1}) groups. The ^1H NMR spectrum of **4** displayed signals due to four methyls, a vinyl, and five oxygen-substituted and two aliphatic methines, together with those of an acetyl and two benzoyl groups (Table 1). On the other hand, its ^{13}C NMR spectrum revealed the signals of a ketone and three ester carbonyl carbons, six oxygen-substituted carbons, and three aliphatic quaternary carbons (Table 2). These ^1H and ^{13}C NMR data were similar to those of orthosiphol O[6] (**14**). However, they were characterized by the disappearance of signals due to one of two acetyl groups in **14**. Analysis of the COSY and HMQC spectra indicated a low-field shift of H-7 (δ 4.22) in **4** compared to that (δ 5.38) in **14**, indicating the deacetylation to be at C-7. Thus, orthosiphol X was 7-*O*-deacetylorthosiphol O (**4**), which was confirmed by the HMBC spectrum. Significant ROESY correlations H-2/H-3, H-2/H_3-19, H-2/H_3-20, H-5/H-9, H-11/H_3-20, and H_3-19/H_3-20 and a small coupling constant between H-9 and H-11 (J = 4.9 Hz) indicated the same relative stereochemistry as that of **1**.

Orthosiphol Y (**5**) was obtained as a colorless amorphous solid, and its HRFABMS displayed the quasimolecular ion at *m*/*z* 449.2189 $(M + H)^+$, indicating the molecular formula $C_{24}H_{32}O_8$. The IR spectrum of **5** showed the absorptions of hydroxyl (3450 cm^{-1}), ester carbonyl (1735 cm^{-1}), and α,β-unsaturated carbonyl (1680 cm^{-1}) groups. The ^1H NMR spectrum of **5** displayed signals due to four methyls, two acetyl methyls, a vinyl, two methylenes, and an isolated olefinic proton (Table 1). These data and the ^{13}C NMR data (Table 2) also resembled those of orthosiphol D[11] (**9**), except for the absence of signals due to a benzoyl group. Thus, orthosiphol Y was hypothesized to be 11-debenzoyloxy-orthosiphol D (**5**), which was confirmed by the HMBC correlations. The relative stereochemistry of **5** was assigned on the basis of the ROESY correlations H-1/H-11, H-9/H-11 (J = 7.5 Hz), and H-11/H-12$_\beta$, NOE correlations H-1/H_3-20, H_3-19/H_3-20, and H-5/H-9, and coupling constant of each proton.

Orthosiphol Z (**6**) displayed the quasimolecular ion at *m*/*z* 405.1914 $(M + H)^+$, indicating the molecular formula $C_{22}H_{28}O_7$. The IR spectrum of **6** showed the absorptions of hydroxyl and carbonyl groups. The ^1H NMR spectrum of **6** displayed signals due to four methyls, an acetyl methyl, a vinyl, and an isolated olefinic proton (Table 1), and its ^{13}C NMR spectrum displayed signals due to four olefinic, two methylene, four quaternary, and three ketone carbons (Table 2). These data resembled those of orthosiphol Q[6] (**19**), isolated from *O. stamineus* from Myanmar, except for the absence of signals due to one of two acetyl groups in **19**. The deacetylation was determined to be at C-7 on the basis of the upfield shift of H-7 in **6** (**6**, δ 4.31; **19**, δ 5.39) as determined by the COSY and HMQC spectra. Thus, orthosiphol Y was concluded to be 7-*O*-deacetylorthosiphol Q (**6**).

In this paper, we have reported six new diterpenes, orthosiphols U−Z (**1**−**6**), together with 16 previously reported diterpenes, orthosiphols (**7**−**16**), orthosiphonone A (**17**), and secoorthosiphol B (**18**). All of the isolated compounds, except for **6**,[11] were tested for their inhibitory activities against NO production by LPS-activated macrophage-like J774.1 cells. All of them displayed significant dose-dependent inhibition, and **4** and **7**−**9** were more potent inhibitors than the positive controls N^G-monomethyl-L-arginine (L-NMMA), polymixin B, and dexamethasone. Among the isolated compounds, **4** displayed the most potent activity with an IC_{50} value of 6.4 μM (Table 3).

The diterpenes isolated from this plant species have been shown to exhibit suppressive effect on contractile responses in rat thoracic aorta[10] and inhibitory activity against the inflammation induced by a tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) on mouse ears.[8] The NO inhibitory activity in endotoxin-activated macrophages by these diterpenes further verifies the antiinflammatory utility of *O. stamineus*.

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 $CHCl_3$. 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 matrix. Column chromatography was performed with BW-820MH silica gel (Fuji Silysia, Aichi, Japan). Analytical and prepara-

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

compound	IC_{50} (μM)[a]
1	59.7
2	54.5
3	57.6
4	6.4
5	37.9
7	11.5
8	20.5
9	14.4
10	34.5
11	145
12	102
13	66.3
14	27.7
15	35.7
16	35.9
17	32.1
18	127
L-NMMA	26.0
polymixin B (μg/mL)	27.8
dexamethasone	170

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

tive TLC were carried out on precoated silica gel plates (Merck, 0.25 or 0.50 mm thickness).

Plant Material. The aerial parts of cultivated *O. stamineus* Benth. were collected at Bangdung, Indonesia, in August 2000. A voucher sample (TMPW 20628) 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* (2.8 kg) were extracted with MeOH (6 L, reflux, 3 h × 3). The MeOH extract (200 g) was suspended in H_2O (1 L) and partitioned successively with hexane, $CHCl_3$, EtOAc, and BuOH (each 1 L × 3) to yield hexane (25 g), $CHCl_3$ (90 g), EtOAc (15 g), and H_2O (68 g) fractions, respectively. The $CHCl_3$ fraction (87 g) was chromatographed (8 × 46 cm) with an EtOAc−hexane solvent system to give six fractions.

Fraction 3 (10 g) was rechromatographed (5 × 45 cm) with hexane−EtOAc (2:1) to afford four subfractions (fractions 3-1, 1.6 g; 3-2, 1.8 g; 3-3, 4.2 g; 3-4, 1.5 g). Subfraction 3-1 was rechromatographed with 2.5% MeOH−$CHCl_3$, followed by preparative TLC with 15% acetone−benzene, to give orthosiphols A[5,8] (**7**, 8.0 mg), D[6,9] (**9**, 9.0 mg), F[4] (**10**, 150 mg), I[4] (**12**, 5.0 mg), J[4] (**13**, 2.0 mg), and Y (**5**, 1.5 mg) and orthosiphonone A[6,10] (**17**, 50 mg). Subfraction 3-2 was rechromatographed with 2.5% MeOH−$CHCl_3$, followed by preparative TLC with hexane−EtOAc (2:1), to give **7** (10 mg), **10** (120 mg), and orthosiphols G[4] (**11**, 8.0 mg) and Z (**6**, 0.6 mg). Subfraction 3-3 was rechromatographed with 2.5% MeOH−$CHCl_3$, followed by preparative TLC with hexane−EtOAc (2:1), to give **7** (36 mg), **8** (30 mg), **10** (27 mg), **11** (21 mg), orthosiphols O[6] (**14**, 49 mg), R[7] (**15**, 2.2 mg), T[7] (**16**, 5.0 mg), U (**1**, 22 mg), and V (**2**, 1.2 mg), and secoorthosiphol B[7] (**18**, 5.0 mg), respectively. Subfraction 3-4 was rechromatographed with 2.5% MeOH−$CHCl_3$ followed by preparative TLC with 15% acetone−benzene, to give **8** (10 mg), **14** (29 mg), **16** (6.0 mg), and orthosiphols I[4] (**12**, 5.0 mg), J[4] (**13**, 2.0 mg), W (**3**, 3.0 mg), and X (**4**, 15 mg).

Orthosiphol U (1): colorless amorphous solid, $[\alpha]_D^{25}$ −170.0° (*c* 0.161, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3450, 1720, 1605, 1590, 1495, 1455, 1370, 1210, 1180, 1120, 1045 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS 615.2836 [calcd for $C_{33}H_{43}O_{11}$ $(M + H)^+$, 615.2805].

Orthosiphol V (2): colorless amorphous solid, $[\alpha]_D^{25}$ −63.4° (*c* 0.028, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3450, 1720, 1605, 1590, 1495, 1455, 1370, 1210, 1180, 1110, 1040 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS 573.2732 [calcd for $C_{31}H_{41}O_{10}$ $(M + H)^+$, 573.2700].

Orthosiphol W (3): colorless amorphous solid, $[\alpha]_D^{25}$ −99.2° (*c* 0.025, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3400, 1725, 1605, 1590, 1495, 1455, 1370, 1210, 1180, 1110 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS 573.2675 [calcd for $C_{31}H_{41}O_{10}$ $(M + H)^+$, 573.2700].

Orthosiphol X (4): colorless amorphous solid, $[\alpha]_D^{25}$ −376.8° (*c* 0.029, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3450, 1720, 1605, 1585, 1510, 1455, 1370, 1315, 1210, 1175, 1120 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS 635.2836 [calcd for $C_{36}H_{43}O_{10}$ $(M + H)^+$, 635.2856].

Orthosiphol Y (5): colorless amorphous solid, $[\alpha]_D^{25}$ −55.54° (*c* 0.033, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3450, 1735, 1720, 1680, 1510, 1420, 1370, 1210, 1040, 930 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS 449.2189 [calcd for $C_{24}H_{33}O_8$ $(M + H)^+$, 449.2175].

Orthosiphol Z (6): colorless amorphous solid, $[\alpha]_D^{25}$ −120.7° (*c* 0.025, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3450, 1725, 1455, 1370, 1360, 1265, 1115, 1065 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; HRFABMS 405.1914 [calcd for $C_{22}H_{29},O_7$ $(M + H)^+$, 405.1913].

Nitric Oxide Inhibitory Assay. 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.[12] 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% naphthylene-diamide 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 nitrite concentration in the medium was determined from the calibration curve ($r = 0.9998$) obtained by using different concentrations of sodium nitrite ($NaNO_2$) in the culture medium as standard. The blank correction was carried out by subtracting the absorbance due to medium only from the absorbance reading of each well.

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