Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 31–35

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Siphonols A–E: Novel Nitric Oxide Inhibitors from *Orthosiphon stamineus* of Indonesia

Suresh Awale, Yasuhiro Tezuka, Arjun H. Banskota and Shigetoshi Kadota*

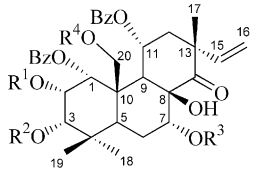
Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630-Sugitani, Toyama 930-0194, Japan

Received 30 August 2002; accepted 4 October 2002

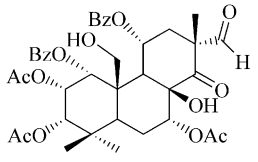
Abstract—From the methanolic extract of *Orthosiphon stamineus*, four novel highly oxygenated isopimarane-type diterpenes named siphonols A–D (**1**–**4**) and a novel biogenetically interesting norisopimarane-type diterpene named siphonol E (**5**) were isolated. The new compounds **1**–**3** and **5** showed more potent inhibitory effects on the nitric oxide (NO) production in lipopolysaccharide (LPS)-activated macrophage-like J774.1 cells than a positive control N^G-monomethyl-L-arginine (L-NMMA). Siphonols A–E (**1**–**5**) represent the first examples of isopimaranes oxygenated at C-20.

Nitric oxide (NO) is an important signaling molecule that acts in many tissues to regulate a diverse range of physiological processes. When certain cells are activated by specific proinflammatory agents such as endotoxin, tumor necrosis factor (TNF), interferon-gamma (IFN-γ), and interleukin-1 (IL-1), NO is produced by inducible nitric oxide synthase (iNOS) and acts as a host defense by damaging pathogenic DNA and as a regulatory molecule with homeostatic activities.[1] However, excessive production has detrimental effects on many organ systems of the body leading to tissue damage, even leading to a fetal development (septic shock).[2] Therefore, effective inhibition of NO accumulation by inflammatory stimuli presents a beneficial therapeutic strategy.

Orthosiphon stamineus Benth. [syn.: *O. aristatus* (Bl.) Miq., *O. grandiflorus* Bold., *O. spicatus* (Thumb) Bak.; Lamiaceae] is one of the popular traditional folk medicine extensively used in Southeast Asia for the treatment of wide range of diseases: in Indonesia for rheumatism, diabetes, hypertension, tonsillitis, epilepsy, menstrual disorder, gonorrhea, syphilis, renal calculus, gallstone, etc.;[3] in Vietnam for urinary lithiasis, edema, eruptive fever, influenza, hepatitis, jaundice and biliary lithiasis;[4] and in Myanmar to alleviate diabetes, urinary tract and renal diseases.[5] While in Okinawa prefecture of Japan, it is consumed as a healthy Java tea to facilitate body detoxification. In our search of biologically active compounds from *O. stamineus*,[6–9] we found that the methanolic extract of an aerial part of *O. stamineus* collected from Indonesia showed significant inhibitory activity on the NO production in lipopolysaccharide (LPS)-activated macrophage-like J774.1 cells (IC_{50}, 40.1 μg/mL). Further separation of the methanol extract led to the isolation of four novel highly oxygenated isopimarane-type diterpenes named siphonols A–D (**1**–**4**) and a novel norisopimarane-type diterpene, siphonol E (**5**). In this paper, we wish to report the identification of the novel diterpenes as potent NO inhibitors.



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



5

Siphonol A (**1**)[10] was obtained as a colorless amorphous solid and showed the quasimolecular ion at *m/z* 735.3030 $(M+H)^+$ in HRFABMS, which corresponds to the molecular formula $C_{40}H_{46}O_{13}$. The IR spectrum of **1** showed absorptions of hydroxyl (3450 cm^{-1}), ester carbonyl (1720 cm^{-1}) and phenyl (1605, 1455 cm^{-1}) groups. The ^1H NMR spectrum of **1** displayed signals due to three tertiary methyls (δ_H 1.22, 1.20, 0.94), a

*Corresponding author. Tel.: +81-76-434-7625; fax: +81-76-434-5059; e-mail: kadota@ms.toyama-mpu.ac.jp

0960-894X/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00854-5

Table 1. ^{1}H NMR data for compounds **1**–**5** in $CDCl_3$, 400 MHz (*J* values in parentheses)

Position	1	2	3	4	5
1	5.47 d (2.9)	5.76 d (2.9)	5.49 d (3.2)	5.66 br s	5.70 br s
2	5.49 t (2.9)	5.5 t (2.9)	4.49 t (3.2)	5.41 t (3.0)	5.51 t (3.4)
3	5.03 d (2.9)	5.04 d (2.9)	5.03 d (3.2)	4.94 d (3.0)	5.03 d (3.4)
5	2.60 dd (13.5, 2.2)	2.82 dd (15.2, 2.8)	2.53 d (12.4)	2.76 dd	2.63 d (12.4)
6	2.01 m	1.90 m	2.01 d (12.4)	1.80 m	2.00 m
	2.34 d (13.5)	2.22 m	2.22 m	1.91 m	2.23 dd (12.4, 4.5)
7	5.51 br s	4.31 br s	5.51 br s	3.56 br s	5.51 br s
9	3.35 d (5.6)	3.26 d (2.7)	3.44 d (5.6)	3.10 d (3.9)	3.14 d (2.6)
11	6.04 t (5.6)	5.82 t (2.7)	6.13 t (5.6)	5.86 t (3.9)	5.83 t (2.6)
12	2.05 d (15.6)	2.40 dd (16.2, 2.7)	2.06 d (15.6)	2.14 d (11.5)	2.65 dd (12.6, 2.6)
	2.72 dd (15.6, 5.6)	2.86 dd (16.2, 2.7)	2.73 dd (15.6, 5.6)	2.73 dd (11.5, 3.9)	2.74 dd (12.6, 2.6)
15	5.66 dd (17.5, 10.9)	5.86 dd (16.6, 10.8)	5.70 dd (17.5, 10.6)	5.71 dd (17.6, 10.7)	9.34 s
16	4.60 (10.9)	4.58 d (10.8)	4.66 d (10.7)	4.55 d (10.7)	
	4.83 (17.5)	4.86 d (16.6)	4.90 d (17.5)	4.80 d (17.6)	
17	1.22 s	1.39 s	1.24 s	1.88 s	1.33 s
18	0.94 s	1.03 s	0.92s	0.95 s	0.94 s
19	1.20 s	1.09 s	1.13 s	1.08 s	1.09 s
20	4.22 d (12.2)	4.19 d (12.1)	4.25 d (12.0)	4.58 d (12.7)	4.21 d (12.0)
	4.45 d (12.2)	4.31 d (12.1)	4.48 d (12.0)	5.01 d (12.7)	4.32 d (12.0)
1-OBz					
2′,6′	7.71 d (7.1)	7.63 d (7.3)	7.25 d (7.4)	7.59 d (7.8)	7.59 d (7.5)
3′,5′	7.28 t (7.1)	7.11 t (7.3)	7.26 t (7.4)	7.13 t (7.8)	7.11 t (7.5)
4′	7.51 t (7.1)	7.36 t (7.3)	7.52 t (7.4)	7.35 dt (7.8, 1.2)	7.4 t (7.5)
11-OBz					
2″,6″	7.63 d (7.5)	7.41 d (7.3)	7.53 d (7.6)	7.40 dd (7.6, 1.0)	7.33 d (7.5)
3″,5″	7.12 t (7.5)	6.96 t (7.3)	7.13 t (7.6)	6.95 t (7.6)	6.97 t (7.5)
4″	7.40 t (7.5)	7.25 t (7.3)	7.43 t (7.6)	7.25 dt (7.6, 1.0)	7.27 t (7.5)
2-OAc					
$COCH_3$	1.84 s	1.85 s		1.75 s	1.87 s
3-OAc					
$COCH_3$	1.51 s	1.57 s	1.52 s	1.45 s	1.54 s
7-OAc					
$COCH_3$	2.22 s		2.22 s		2.27 s
20-OAc					
$COCH_3$				2.20 s	

vinyl (δ_H 5.66, 4.83, 4.60) and five oxygen-substituted methines (δ_H 6.04, 5.51, 5.49, 5.47, 5.03), one oxygen-substituted methylene (δ_H 4.45, 4.22) and two oxygen-nonsubstituted methylenes (δ_H 2.35, 2.01; δ_H 2.72, 2.05), together with those of three acetyl and two benzoyl groups (Table 1), while its ^{13}C NMR spectrum revealed the signals of a ketone and five ester carbonyl and seven oxygen-substituted carbons and three oxygen-non-substituted quaternary carbons (Table 2). Analysis of these signals by the COSY and HMQC spectra led to the partial structures (bold line), which were connected based on the long-range correlations observed in the HMBC spectrum (Fig. 1a).

Significant correlations were observed between the ester carbonyl carbon at δ_C170.6 (2-OCO) and the protons at δ_H 1.84 (2-OCOCH_3) and δ_H 5.49 (H-2), between the ester carbonyl carbon at δ_C170.4 (3-OCO) and the protons at δ_H 1.51 (3-OCOCH_3) and δ_H 5.03 (H-3) between the ester carbonyl carbon at δ_C169.0 (7-OCO) and the protons at δ_H 2.22 (7-OCOCH_3) and δ_H 5.51 (H-7), between the ester carbonyl carbon at δ_C 164.1 (1-OCO) and the protons at δ_H 7.71 (H-2′, 6′) and δ_H 5.47 (H-1), and between the ester carbonyl carbon at δ_C 166.4 (11-OCO) and the protons at δ_H 7.63 (H-2″,6″) and δ_H 6.04 (H-11), allowing the locations of three acetoxyl groups to be at C-2, C-3 and C-7 and of two benzoyloxy groups at C-1 and C-11, respectively. The relative stereochemistry of **1** was assigned on the basis of the ROESY correlations and the coupling constant data. The ROESY correlations H-2/H-3, H-2/H_3-19, H-2/H_2-20, H-3/H_3-19, H_3-19/H_2-20, H_2-20/H-6β and H-5/H-9 indicated rings A and B to have a chair conformation (Fig. 1b) with *trans*-fused ring junctions and β-axial orientation of H-2. On the other hand, a small axial-equitorial coupling constants (2.9 Hz each) between H-1, H-2 and H-3 indicated a benzoyloxy and two acetoxy substituents at C-1, C-2 and C-3 to be in α-orientated. Similarly, a small coupling constant observed for H-7 (br s) indicated it to be in β-equitorial orientation. As for ring C, the ROESY correlations H-1/H-11, H_2-20/H-11, H-11/H-12 and H-12/H_3-17 indicated a boat conformation of ring C and a small axialequitorial coupling constant between H-9 and H-11 ($J = 5.6$ Hz) indicated the β-equitorial orientation of H-11. This is also supported by absence of *trans*-diaxial coupling between H-11 and H-12α. The significant ROESY correlations between H-16 [δ_H 4.83 (1H, d, $J = 17.5$)] and an acetyl methyl signal [δ_H 2.22 (3H, s)] indicated that the vinyl group at C-13 was α-oriented.

The absolute configuration of **1** was established by application of exciton chirality method.[11,12] In the circular dichroism (CD) spectrum of **1**, a positive maximum

Table 2. ^{13}C NMR data for compounds **1**–**5** in $CDCl_3$, 100 MHz

Position	1	2	3	4	5
1	69.8	69.3	74.4	68.5	69.1
2	67.6	67.3	66.6	67.0	67.0
3	75.8	75.7	78.1	75.7	75.6
4	37.1	37.5	37.0	35.8	37.4
5	36.6	36.3	36.7	37.0	37.5
6	21.7	23.5	21.6	23.3	22.0
7	70.7	68.6	70.7	68.4	69.4
8	74.0	76.6	74.1	77.7	74.8
9	43.2	44.6	43.0	42.5	45.1
10	48.4	49.3	48.7	47.1	49.0
11	69.5	70.3	70.1	69.6	69.0
12	38.6	37.3	38.5	38.2	32.8
13	47.3	47.3	47.3	47.8	56.3
14	207.2	215.3	207.3	213.99	204.9
15	142.3	142.5	142.2	141.9	199.0
16	112.9	112.7	113.2	113.2	
17	27.4	29.2	27.4	28.1	23.9
18	28.0	27.5	27.9	27.9	27.6
19	21.7	21.9	22.0	21.9	21.8
20	61.9	62.6	61.9	63.6	62.7
1-OBz					
1′	130.1	129.5	129.8	130.0	129.5
2′,6′	129.6	129.6	129.7	129.6	129.3
3′,5′	127.9	128.0	128.0	127.9	127.9
4′	132.8	132.7	133.2	132.5	132.9
7′	164.1	165.2	166.9	164.3	165.3
11-OBz					
1″	130.0	129.5	129.6	129.9	129.2
2″,6″	129.5	129.4	129.5	129.4	129.1
3″,5″	127.7	127.5	127.9	127.5	127.8
4″	132.4	132.1	132.6	132.0	132.4
7″	166.4	165.9	166.0	165.7	164.6
2-OAc					
$COCH_3$	20.7	20.7		20.6	20.7
$COCH_3$	170.6	170.8		169.9	170.5
3-OAc					
$COCH_3$	20.3	20.6	21.1	20.4	20.5
$COCH_3$	170.4	170.6	171.0	170.7	170.3
7-OAc					
$COCH_3$	21.1		20.4		21.3
$COCH_3$	169.0		169.0		169.3
20-OAc					
$COCH_3$				21.1	
$COCH_3$				171.4	

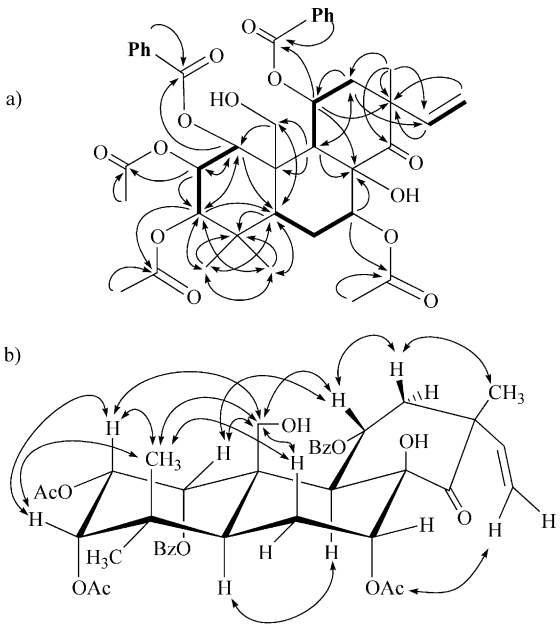


Figure 1. (a) Partial structures (bold line) deduced by the COSY and HMQC spectra and significant HMBC correlations (arrows) and (b) ROESY correlations for **1**.

([θ]$_{241}$ +29815) due to two chromophoric benzoates at C-1 and C-11 was observed and indicated that C-11 has an *R* configuration.[13,14]

The HRFABMS of siphonol B (**2**)[15] showed the quasi-molecular ion at *m*/z 693.2943 $(M+H)^+$, consistent with the molecular formula $C_{38}H_{44}O_{12}$. The IR spectrum of **2** was similar to that of siphonol A (**1**) and showed absorptions of hydroxyl, ester carbonyl and phenyl groups. The ^1H 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 location of the deacetylation was determined to be at C-7 based on the upfield shift of H-7 (δ_H 4.31), which was confirmed by the HMBC spectrum. The stereochemistry of **2** was determined to be the same as **1**, that is chair conformation in rings A and B and boat conformation in ring C, based on the ROESY correlations of H-2 with H-1, H-3, H_3-19 and H_2-20, of H-11 with H_3-20 and of H-5 with H-9 and small coupling constant value of H-9/H-11 (J = 2.8 Hz). Thus, the structure of siphonol B was assigned to be 7-*O*-deacetylsiphonol A (**2**). A positive cotton effect ([θ]$_{241}$ + 34704) observed in the CD spectrum revealed the absolute stereostructure of **2** to be same as **1**.

The ^1H and ^{13}C NMR spectra of siphonol C (**3**)[16] also closely resembled those of siphonol A (**1**), but they were characterized by the disappearance of signals of one of three acetyl groups in **1**. The location of deacetylation was determined to be at C-2 based on the upfield shift of H-2 (δ_H 4.49), as indicated by COSY and HMQC spectra. Thus, orthosiphonol C (**3**) was concluded as 2-*O*-deacetylsiphonol A, which was confirmed by the COSY, HMQC, HMBC, ROESY and CD spectra.

The molecular formula of siphonol D (**4**)[17] was determined by HRFABMS to be $C_{40}H_{46}O_{13}$, the same as that of orthosiphonol A (**1**). The ^1H NMR spectrum of **4** displayed signals due to three tertiary methyls, five oxygen-substituted methines, one oxygen-substituted methylene and two oxygen-nonsubstituted methylenes, together with those of three acetyl and two benzoyl groups (Table 1). These data were similar to those of siphonol A (**1**). However, the ^1H NMR spectrum of **4** showed upfield shift of H-7 (δ_H 3.56) and downfield shift of H_2-20 (δ_H 5.01, 4.58). These data suggested that the acetoxyl group at C-7 in **1** should be replaced by the hydroxyl group in **4**, and the hydroxyl group at C-20 in **1** should be replaced by acetoxyl group in **4**. This was confirmed by the HMBC correlations between the ester carbonyl carbon at δ_C171.4 (20-OCO) and the protons at δ_H 2.20 (20-OCOCH_3) and δ_H 5.01 and 4.58 (H_2-20). The relative stereochemistry was assigned by the ROESY experiment and a positive maximum ([θ]$_{243}$ +18414) in CD spectrum confirmed its absolute configuration to be the same as **1**.

Siphonol E (**5**)[18] was obtained as a colorless amorphous solid. The positive ion HRFABMS showed a quasimolecular ion at *m*/z 737.2818 $(M+H)^+$, consistent with the molecular formula $C_{39}H_{44}O_{14}$. The IR spectrum of **5** showed absorptions due to hydroxyl (3400 cm^{-1}), aldehyde (2850, 2750 cm^{-1}), ester carbonyl (1725 cm^{-1}) and phenyl (1605, 1455 cm^{-1}) groups. The ^1H NMR spectrum of **5** displayed signals due to an aldehyde proton (δ_H 9.34), three tertiary methyls, five oxygen-substituted and two aliphatic methines and one oxygen-substituted methylene, together with those of three acetyl and two benzoyl groups (Table 1), while its ^{13}C NMR spectrum revealed the signals of a ketone carbonyl, an aldehyde carbonyl, five ester carbonyls, seven oxygen- substituted carbons and two oxygen-nonsubstituted quaternary carbons (Table 2). Excluding the ^{13}C NMR signals for two benzoyl and three acetyl groups, **5** possessed only 19 carbon signals in its main carbon framework, suggesting it to be a norditerpene.

The partial connectivities C_1–C_2–C_3, C_5–C_6–C_7 and C_9–C_{11}–C_{12} were obtained by the analysis of the COSY and HMQC spectra, and these were connected from the long-range correlations observed in the HMBC spectrum (Fig. 2a). Significant long-range correlations between the aldehyde carbon (δ_C 199.0) with H_3-17 and H_2-12 confirmed the aldehyde group to be C-15. On the other hand, the locations of the two benzoyl and three acetyl groups were determined to be at C-1 and C-11 and at C-2, C-3 and C-20, respectively, based on the HMBC correlations between the ester carbonyl carbon at δ_C 165.3 (1-OCO) and the protons at δ_H 5.70 (H-1) and 7.59 (H-2′,6′), between the ester carbonyl carbon at δ_C 170.5 (2-OCO) and the protons at δ_H 5.51 (H-2) and 1.87 (2-$OCOCH_3$), between the ester carbonyl carbon at δ_C 170.3 (3-OCO) and the protons at δ_H 5.03 (H-3) and 1.54 (3-$OCOCH_3$),between the ester carbonyl carbon at δ_C 169.3 (7-OCO) and the protons at δ_H 5.51 (H-7) and 2.27 (7-$OCOCH_3$) and between the ester carbonyl carbon at δ_C 164.6 (11-OCO) and the protons at δ_H 5.83 (H-11) and 7.33 (H-2″,6″).

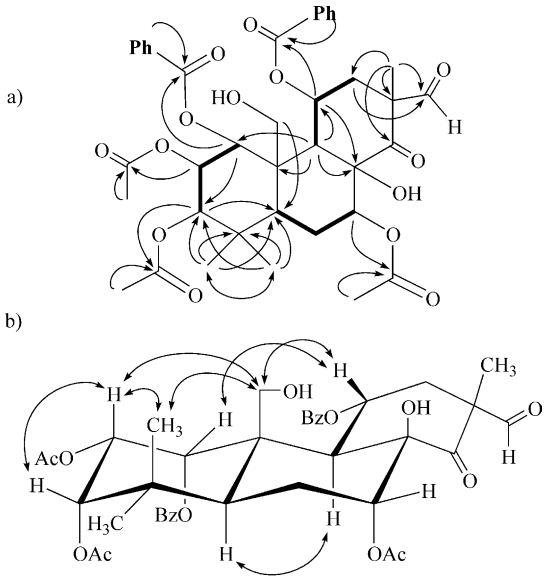


Figure 2. (a) Partial structures (bold line) deduced by the COSY and HMQC spectra and significant HMBC correlations (arrows) and (b) ROESY correlations for **5**.

The relative stereochemistry of **5** was determined to be the same as **1**, on the basis of the ROESY correlations (Fig. 2b) and the coupling constant data. The absolute stereochemistry was established by the application of the exciton chirality method in the CD spectrum ($[\theta]_{244}+14249$).[13,14]

To the best of our knowledge, siphonols A–E (**1**–**5**) represent the first examples of isopimarane-type diterpenes oxygenated at C-20. Among them, **1**–**4** are isopimarane-type and **5** is biogenetically interesting norisopimarane-type, which might have been produced by the oxidative cleavage of the vinylic group in **1**. All these compounds were tested for their inhibitory activities against NO production by LPS-activated macrophage-like J774.1 cells.[19] Siphonols A–E (**1**–**5**) displayed significant dose-dependent inhibition (Fig. 3), and the activities of **1**–**3** and **5** (IC_{50}: **1**, 10.8 μM; **2**, 17.3 μM; **3**,

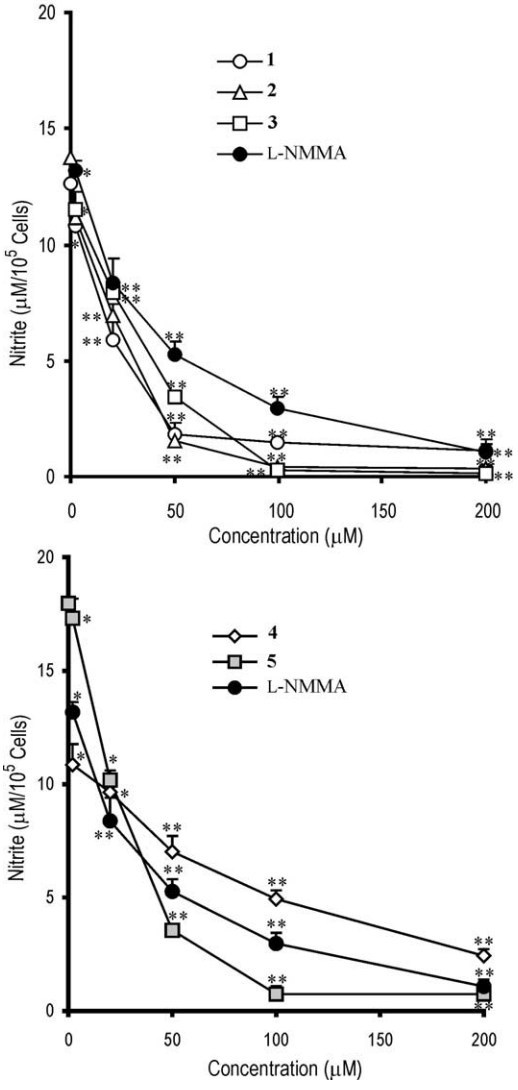


Figure 3. Dose-dependent inhibition of NO production in macrophage-like J774.1 cells by orthosiphonols A–E (**1**–**5**) and L-NMMA. Each data is the mean ± SD of quadruplet experiment. $^*P<0.05$, $^{**}P<0.01$.

22.9 μM; **5**, 23.0 μM) were more than the positive controls N^G-monomethyl-L-arginine (L-NMMA; IC_{50}, 26.0 μM), polymixin B (IC_{50}, 27.8 μg/mL) and dexamethasone (IC_{50}, 169.5 μM), and **1** displayed the most potent activity with an IC_{50} value of 10.8 μM. The decrease in the inhibitory activity of **4** (IC_{50}, 46.5 μM) compared to the other diterpenes may indicate the importance of the C-20 hydroxyl group for the activity.

The diterpenes isolated from this plant species have been shown to exhibit antiproliferative activities,[6–9] suppressive effect on contractile responses in rat thoracic aorta[14] and inhibitory activity against the inflammation induced by a tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) on mouse ears.[13] The NO inhibitory activity in endotoxin-activated macrophages by the diterpenes further verifies the anti-inflammatory utility of *O. stamineus*.

Acknowledgements

This work was supported in part by a Grant-in-Aid for International Scientific Research (No. 13576027) from The Ministry of Education, Culture, Sports, Science and Technology, Japan.

References and Notes

1. Kuo, P. C.; Schroeder, R. A. *Ann. Surg.* **1995**, *221*, 220.
2. Vincent, J. L.; Zhang, H.; Szabo, C.; Preiser, J. C. *Am. J. Respir. Crit. Care. Med.* **2000**, *161*, 1781.
3. Bwin, D. M.; Gwan, U. S. Eds. *Burmese Indigenous Medicinal Plants: 1. Plants With Reputed Hypoglycemic Action*; Yangon. Ministry of Health, Health and Myanmar Traditional Medicine, Burma Medical Research Institute: 1967; p 126.
4. PT Eisai Indonesia. *Medicinal Herb Index in Indonesia*, 2nd ed.; 1995.
5. WHO Regional Office for the Western Pacific Manila and Institute of Material Medica Hanoi. In *Medicinal Plants in Viet Nam;* Tran, K., Ed.; Science and Technology Publishing House: Hanoi, 1970.
6. Tezuka, Y.; Stampoulis, P.; Banskota, A. H.; Awale, S.; Tran, K. Q.; Saiki, I.; Kadota, S. *Chem. Pharm. Bull.* **2000**, *48*, 1711.
7. Awale, S.; Tezuka, Y.; Banskota, A. H.; Kouda, K.; Tun, K. M.; Kadota, S. *J. Nat. Prod.* **2001**, *64*, 592.
8. Awale, S.; Tezuka, Y.; Banskota, A. H.; Kouda, K.; Tun, K. M.; Kadota, S. *Planta Med.* **2002**, *68*, 286.
9. Awale, S.; Tezuka, Y.; Banskota, A. H.; Shimoji, S.; Taira, K.; Kadota, S. *Tetrahedron* **2002**, *58*, 5503.
10. **Siphonol A (1)**. Colorless amorphous solid, $[\alpha]_D^{25}$ −146.5°, (*c* 0.07, $CHCl_3$). IR $\nu_{,max}$ ($CHCl_3$) 3450, 1720, 1605, 1590, 1495, 1455, 1370, 1320–1200, 1180, 1110, 1040 cm^{-1}. CD (EtOH) $[\theta]_{241}$ +29815. HRFABMS 735.3030 [calcd for $C_{40}H_{47}O_{13}$ $(M+H)^+$, 735.3017]. ^1H and ^{13}C NMR, see Tables 1 and 2.
11. Harada, N.; Chen S.-M., L.; Nakanishi, K. *J. Am. Chem. Soc.* **1975**, *97*, 5345.
12. Harada, N.; Iwabuchi, J.; Yokota, Y.; Uda, H.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 5590.
13. Masuda, T.; Masuda, K.; Shiragami, S.; Jitoe, A.; Nakatani, N. *Tetrahedron* **1992**, *48*, 6787.
14. Shibuya, H.; Bohgami, T.; Ohashi, K. *Chem. Pharm. Bull.* **1999**, *47*, 911.
15. **Siphonol B (2)**. Colorless amorphous solid, $[\alpha]_D^{25}$ −103.4° (*c* 0.08, $CHCl_3$). IR ν_{max} ($CHCl_3$) 3450, 1720, 1605, 1590, 1495, 1455, 1370, 1320–1200, 1180, 1110, 1040 cm^{-1}. CD $[\theta]_{241}$ +34704. HRFABMS 693.2943 [calcd for $C_{38}H_{45}O_{12}$ $(M+H)^+$, 693.2911]. ^1H and ^{13}C NMR, see Tables 1 and 2.
16. **Siphonol C (3)**. Colorless amorphous solid, $[\alpha]_D^{25}$ −49.9° (*c* 0.06, $CHCl_3$). IR ν_{max} ($CHCl_3$) 3400, 1725, 1605, 1590, 1495, 1455, 1370, 1320–1200, 1180, 1110 cm^{-1}. CD $[\theta]_{240}$ +31485 .HRFABMS 693.2875 [calcd for $C_{38}H_{45}O_{12}$ $(M+H)^+$, 693.2911]. ^1H and ^{13}C NMR, see Tables 1 and 2.
17. **Siphonol D (4)**. Colorless amorphous solid, $[\alpha]_D^{25}$ −92.8° (*c* 0.09, $CHCl_3$). IR ν_{max} ($CHCl_3$) 3450, 1720, 1605, 1585, 1510, 1455, 1370, 1315, 1285–1200, 1175, 1120 cm^{-1}. CD $[\theta]_{243}$ +18414. HRFABMS 735.3030 [calcd for $C_{40}H_{47}O_{13}$ $(M+H)^+$, 735.3017]. ^1H and ^{13}C NMR, see Tables 1 and 2.
18. **Siphonol E (5)**. Colorless amorphous solid, $[\alpha]_D^{25}$ −135.7° (*c* 0.06, $CHCl_3$). IR ν_{max} ($CHCl_3$) 3400, 2850, 2750, 1725, 1605, 1590, 1495, 1455, 1370, 1320, 1280, 1240–1210, 1115, 1040, 980 cm^{-1}. CD $[\theta]_{244}$ +14249. HRFABMS 737.2818 [calcd for $C_{39}H_{45}O_{14}$ $(M+H)^+$, 737.2809]. ^1H and ^{13}C NMR, see Tables 1 and 2.
19. The J774.1 cell line was propagated in 75-cm^2 plastic culture flasks (Falcone, Becton Dickinson, NJ, USA), 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. Briefly, 50 μL of the supernatant from 96-well plate were incubated with equal volume of Griess reagent (0.5% sulfanilamide and 0.05% naphthylene-diamide dihydrochloride in 2.5% H_3PO_4) and were allowed to stand for 10 min at room temperature. Absorbance at 550 nm was measured using HTS 7000 microplate reader. The nitrite concentration in the medium was determined from the calibration curve ($Y=0.0038-X0.0043$, $r=0.9998$) 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 wells.