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Absolute Stereostructures of Novel Norcadinane- and Trinoreudesmane-Type Sesquiterpenes with Nitric Oxide Production Inhibitory Activity from *Alpinia oxyphylla*

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Abstract—Novel 14-norcadinane-type sesquiterpenes, oxyphyllenodiols A and B, and 11,12,13-trinoreudesmane-type sesquiterpenes, oxyphyllenones A and B, were isolated from the methanolic extract of kernels of *Alpinia oxyphylla*. The absolute stereostructures of these norsesquiterpenes were determined on the basis of physicochemical and chemical evidence. In addition, oxyphyllenodiol A and oxyphyllenone A were found to inhibit the NO production in lipopolysaccharide-activated macrophages. © 2001 Elsevier Science Ltd. All rights reserved.

Previously, we have reported the bioactive constituents from Zingiberaceae plants, Zingiberis Rhizoma[1] and Zedoariae Rhizoma.[2] As a continuation of the study, we have isolated two new 14-norcadinane-type sesquiterpenes, oxyphyllenodiols A (**1**, 0.0060% from the natural medicine) and B (**2**, 0.0010%), and two new 11,12,13-trinoreudesmane-type sesquiterpenes, oxyphyllenones A (**3**, 0.0008%) and B (**4**, 0.0004%), from the methanolic extract of kernels of *Alpinia oxyphylla* MIQUEL cultivated in Hainan island (China) together with seven sesquiterpenes, one diarylheptanoid, one flavone, and two glycosides.[3] The whole fruit of *A. oxyphylla* (yakuchi in Japanese) have been used as Chinese and Japanese herbal medicine, and is listed in the Japanese Pharmacopoeia XIII as an aromatic stomachic. In the ancient Chinese herbal literatures,[4] kernels were reported to be utilized for medication of dieresis, ulceration as well as dementia, and examination of the kernels' extracts on these activities has also been reported by the author.[5]

This communication deals with the elucidation of absolute stereostructure of **1**–**4**, and their inhibitory effect on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages.

Oxyphyllenodiol A (**1**),[6] a colorless oil, $[\alpha]_D^{26}$ +66.3 (*c* 0.5, MeOH), $C_{14}H_{22}O_3$, showed absorption bands due to hydroxyl and enone functions [UV (MeOH, nm, log ε) 246 (2.30), IR (KBr) 3432, 1655 cm^{-1}]. ^{1}H NMR (500 MHz, CDCl$_3$) and ^{13}C NMR (Table 1) spectra of **1** indicated the presence of a *tert*-methyl [δ 1.22 (s, 15-H$_3$)], an isopropyl [δ 0.92, 1.06 (both d, *J* = 7.0 Hz, 12 and 13-H$_3$), 2.21 (m, 11-H)], and a methine bearing a hydroxyl group [δ 4.17 (br s, 4-H)] together with four methylenes and a methine. The connectivities of the ^{1}H–^{1}H and quaternary carbons (C-3, 5, 9, 10) in **1** were clarified by ^{1}H–^{1}H correlation spectroscopy (H–H COSY) and heteronuclear multiple bond correlation (HMBC) and nuclear Overhauser effect spectroscopy (NOESY) experiments as shown in Figure 1. Oxyphyllenodiol B (**2**),[7] the 6-position isomer of **1**, has been characterized in the same way. The absolute stereostructures of **1** and **2** were determined by the application of the circular dichroic (CD) exciton chirality method, a method commonly used for the determination of chirality of allylic alcohol benzoates.[8] The CD spectra of the 4-*p*-bromobenzoyl esters (**1a**, **2a**)[9] showed the positive Cotton effect [CD Δε (*n*-hexane, nm): **1a**; −0.95 (232), +4.33 (250), **2a**; −2.59 (233), +10.42

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(249)]. This evidence indicated the 4-position of **1a** and **2a** to be *S*, so that the absolute stereostructures of **1** and **2** were determined.

Oxyphyllenone A (**3**),[10] colorless needles (from AcOEt), mp 184–186 °C, $[\alpha]_D^{23}$ +75.4 (*c* 1.2, MeOH), $C_{12}H_{18}O_3$, showed absorption bands due to hydroxyl and enone functions [UV (MeOH, nm, log ε) 235 (1.90), IR (KBr) 3359, 1646 cm^{-1}]. ^{1}H NMR (CD$_3$OD) and ^{13}C NMR (Table 1) spectra of **3** showed the presence of two *tert*-methyls [δ 1.41, 1.46 (both s, 15 and 14-H$_3$)], a methine bearing a hydroxyl group [δ 3.69 (dd, *J* = 2.8, 3.0 Hz, 3-H)], and an olefin [δ 6.01 (br s, 6-H)] together with four methylenes and four quaternary carbons (C-4, 5, 7, 10), whose connectivity was clarified by H–H COSY and HMBC experiments as shown in Figure 2. Small coupling constants of the signal at δ 3.69 due to the carbinol proton indicated that the hydroxyl was axially oriented, and the observed NOE correlation between 15-H$_3$ and 6-H suggested the equatorial configuration of the methyl moiety at C-4. Finally, the relative stereo-

structure of **3** was elucidated by X-ray crystallographic analysis (Fig. 3).[11]

Oxyphyllenone B (**4**),[12] a colorless oil of the same molecular formula as **3**, showed similar spectroscopic properties to those of **3**, the same skeletal structure being suggested. The large coupling constant (*J* = 12.0) of the carbinol proton and the observed NOE correlation between the proton and 2β-H indicated that the proton was at the β-side of the cyclohexane ring which is in the twist boat conformation. Furthermore, NOE correlations between the signals of the following proton pairs (3-H and 15-H$_3$; 6-H and 15-H$_3$) supported the equatorial orientation of the methyl moiety at C-4. Thus, the relative stereostructure of **4** was elucidated as the 4-position isomer of **3**.

Table 1. ^{13}C NMR data for **1–4**

	1[a]	2[a]	3[b]	4[a]
C-1	21.4	20.8	35.2	37.8
C-2	32.0	31.3	25.3	26.5
C-3	72.2	70.3	76.1	76.8
C-4	75.0	72.5	73.9	76.3
C-5	158.2	156.5	172.6	173.8
C-6	39.9	41.3	126.0	123.3
C-7	22.1	22.0	203.4	200.5
C-8	34.9	35.6	34.8	33.5
C-9	200.0	199.9	40.9	40.6
C-10	132.4	133.5	36.5	36.0
C-11	29.6	29.2		
C-12	19.1^c	18.6^c		
C-13	21.5^c	21.5^c		
C-14			25.4	24.7
C-15	21.7	25.2	25.5	22.8

^{a}The spectra were taken in CDCl$_3$ at 125 MHz.
^{b}The spectra were taken in CD$_3$OD at 125 MHz.
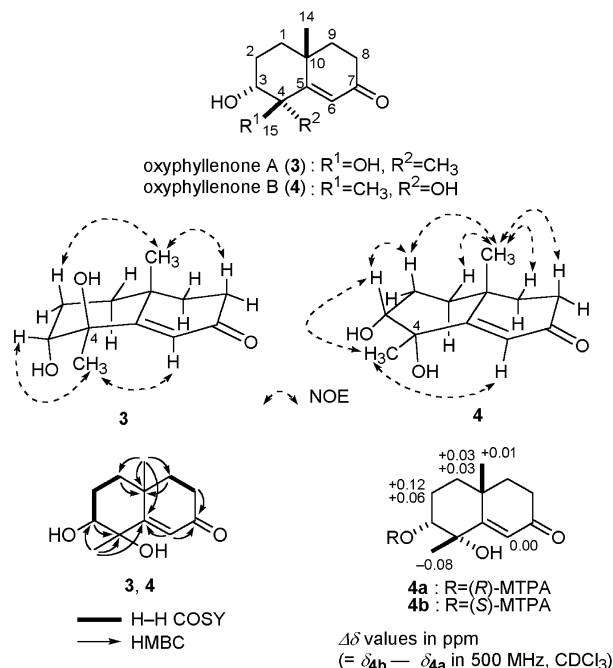^{c}May be interchangeable within the same column.

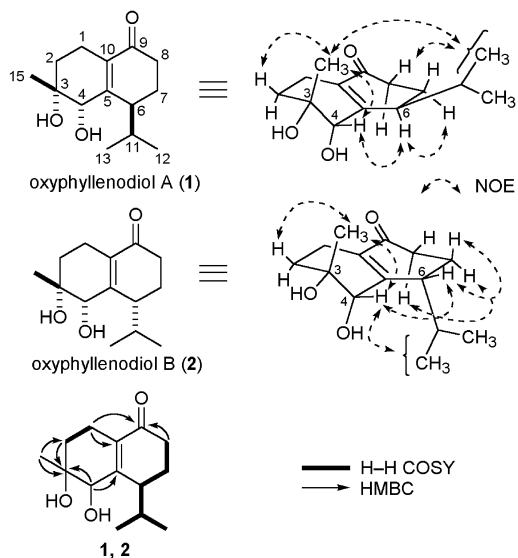**Figure 2.** Structural elucidation of oxyphyllenones (**3** and **4**).

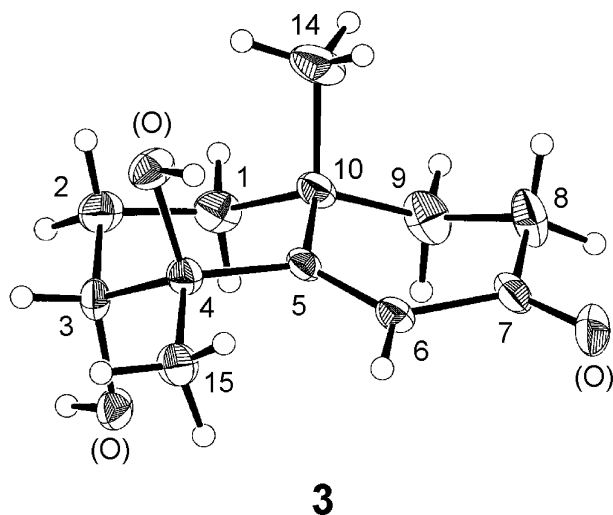**Figure 1.** Structural elucidation of oxyphyllenodiols (**1** and **2**).

Figure 3. Perspective view of oxyphyllenone A (**3**).

Table 2. Inhibitory effects of oxyphyllenodiols A (**1**) and B (**2**), and oxyphyllenones A (**3**) and B (**4**) on NO production in LPS-activated macrophages

Compounds	Inhibition (%)				
	$3\,\mu M$	$10\,\mu M$	$30\,\mu M$	$100\,\mu M$	$IC_{50}\,(\mu M)$
Oxyphyllenodiol A (**1**)	11.8 ± 3.3	$16.9\pm3.8^*$	$52.5\pm5.9^{**}$	$101.7\pm0.4^{**}$	28
Oxyphyllenodiol B (**2**)	-4.2 ± 1.9	-0.4 ± 6.2	$19.6\pm3.2^{**}$	$40.1\pm3.6^{**}$	>100
Oxyphyllenone A (**3**)	1.6 ± 3.9	-2.9 ± 3.5	$43.0\pm2.2^{**}$	$85.7\pm3.5^{**}$	35
Oxyphyllenone B (**4**)	-8.1 ± 3.9	0.4 ± 3.4	$19.2\pm2.3^*$	$41.2\pm4.9^{**}$	>100
L-NMMA	2.0 ± 1.6	$17.7\pm2.8^{**}$	$52.3\pm1.5^{**}$	$79.2\pm0.9^{**}$	28

Each value represents the mean$\pm$SEM ($n=4$).
Significantly different from the control: $^*p<0.05$, $^{**}p<0.01$.

Next, the absolute stereostructures of **4** were determined by application of the modified Mosher's method.[13] Namely, signals due to protons at C-1, 2, and 14 of the (*R*)-MTPA ester (**4a**) appeared at higher fields than those of the (*S*)-MTPA ester (**4b**) ($\Delta\delta$: positive), while signals due to protons attached to C-15 of **4a** were observed at lower field as compared to those of **4b** [$\Delta\delta$: negative].[14] Consequently, the absolute configuration at the 3-position in **4** has been elucidated to be *R* and the absolute stereostructure of **4** has been determined as shown in Figure 2. The absolute configurations at C-3 and C-10 of **3** were speculated to be the same as those of **4** on the basis of the specific rotation properties.

The NO production inhibitory effects of **1**–**4** were examined using a bioassay to test the inhibitory activity against NO production in LPS-activated mouse peritoneal macrophages.[15] As shown in Table 2, **1** and **3** were found to inhibit the NO production, and their activities were similar to that of L-NMMA (N^G-monomethyl-L-arginine), which is commonly used as the reference compound, in efficacy.[16]

References and Notes

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2. (a) Matsuda, H.; Ninomiya, K.; Morikawa, T.; Yoshikawa, M. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 339. (b) Yoshikawa, M.; Murakami, T.; Morikawa, T.; Matsuda, H. *Chem. Pharm. Bull*. **1998**, *46*, 1186. (c) Matsuda, H.; Morikawa, T.; Ninomiya, K.; Yoshikawa, M. *Bioorg. Med. Chem*. **2001**, *9*, 909. (d) Matsuda, H.; Morikawa, T.; Toguchida, I.; Ninomiya, K.; Yoshikawa, M. *Heterocycles* **2001**, *55*, 841.
3. The following components were isolated: sesquiterpenes: nootkatone, nootkatol, α-humulene diepoxide, 6,15α-epoxy 1β,4β-dihydroxyeudesmane, 3-murolene 2α,9β-diol, and oxyphyllenonic acids A and B; diarylheptanoid: yakuchinone-A; flavone: techtochrysin; and glycosides: 2S-butanol 2-*O*-β-D-glucopyranoside, and 1S-hydroxyethylbenzene 1-*O*-β-D-glucopyranoside.
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6. **1**: ^{1}H NMR (CDCl$_3$) δ 0.92, 1.06 (both d, *J*=7.0 Hz, 12,13-H$_3$), 1.22 (s, 15-H$_3$), 1.66 (ddd, *J*=6.4, 8.5, 14.9 Hz, 2β-H), 1.79 (ddd, *J*=5.2, 6.4, 14.9 Hz, 2α-H), 1.96 (m, 7-H$_2$), 2.21 (m, 11-H), 2.24 (ddd, *J*=6.4, 8.5, 15.9 Hz, 1α-H), 2.31 (ddd, *J*=6.1, 6.1, 17.4 Hz, 8α-H), 2.49 (ddd, *J*=5.2, 6.4, 15.9 Hz, 1β-H), 2.52 (ddd, *J*=6.7, 8.9, 17.4 Hz, 8β-H), 2.65 (br dd, *J*=ca. 5, 8 Hz, 6-H), 4.17 (br s, 4-H). Negative-ion FAB-MS *m/z* 237 (M−H)$^-$. EI-MS *m/z* (%) 238 (M$^+$, 27), 177 (100).
7. **2**: Colorless oil, $[\alpha]_D^{25}$ +27.0 (*c* 0.3, CHCl$_3$), C$_{14}$H$_{22}$O$_3$. UV (MeOH, nm, log ε) 245 (3.76). IR (film) 3436, 1661 cm^{-1}. ^{1}H NMR (CDCl$_3$) δ 0.73, 0.86 (both d, *J*=7.0 Hz, 12,13-H$_3$), 1.28 (s, 15-H$_3$), 1.58 (ddd, *J*=6.4, 11.0, 17.7 Hz, 2β-H), 1.86 (ddd, *J*=6.1, 7.1, 17.7 Hz, 2α-H), 1.94 (dddd, *J*=4.0, 6.4, 13.1, 14.9 Hz, 7α-H), 2.00 (dddd, *J*=5.2, 8.9, 10.7, 14.9 Hz, 7β-H), 2.26 (dq, *J*=5.5, 7.0 Hz, 11-H), 2.34 (m, 8α-H), 2.36 (m, 1-H$_2$), 2.53 (ddd, *J*=4.0, 8.9, 17.1 Hz, 8β-H), 2.62 (br s, 6-H), 3.97 (br s, 4-H). ^{13}C NMR (CDCl$_3$) δ$_c$ given in Table 1. EI-MS *m/z* (%) 238 (M$^+$, 8), 177 (42), 83 (100).
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9. **1a**: C$_{21}$H$_{25}$O$_4$Br. ^{1}H NMR (CDCl$_3$) δ 0.92, 1.02 (both d, *J*=7.0 Hz, 12,13-H$_3$), 1.24 (s, 15-H$_3$), 1.80 (ddd, *J*=5.8, 8.0, 14.1 Hz, 2β-H), 1.88 (ddd, *J*=2.1, 8.0, 14.1 Hz, 2α-H), 1.95 (m, 7-H$_2$), 2.09 (dq, *J*=5.4, 7.0 Hz, 11-H), 2.23 (br dd, 6-H), 2.36 (ddd, *J*=5.5, 11.6, 18.0 Hz, 8α-H), 2.40 (ddd, *J*=5.8, 8.0, 15.9 Hz, 1α-H), 2.52 (ddd, *J*=2.1, 8.0, 15.9 Hz, 1β-H), 2.57 (ddd, *J*=5.5, 11.0, 18.0 Hz, 8β-H), 5.74 (br s, 4-H), 7.62, 7.91 (2H each, both d, *J*=8.7 Hz, ph-H). EI-MS *m/z* (%) 420, 422 (M$^+$, 3), 237 (M$^+$−*p*-BrBz, 13), 183 (100), 177 (76); **2a**: C$_{21}$H$_{25}$O$_4$Br. ^{1}H NMR (CDCl$_3$) δ 0.91, 1.02 (both d, *J*=7.1 Hz, 12,13-H$_3$), 1.29 (s, 15-H$_3$), 1.70 (ddd, *J*=5.2, 10.7, 17.2 Hz, 2β-H), 1.93 (ddd, *J*=6.1, 6.4, 17.2 Hz, 2α-H), 1.98 (m, 7-H$_2$), 2.08 (dq, *J*=6.4, 7.1 Hz, 11-H), 2.20 (br dd, 6-H), 2.35, 2.54 (both m, 1-H$_2$), 2.40, 2.59 (both m, 8-H$_2$), 5.71 (br s, 4-H), 7.62, 7.93 (2H each, both d, *J*=8.5 Hz, ph-H). EI-MS *m/z* (%) 420, 422 (M$^+$, 7), 237 (M$^+$−*p*-BrBz, 23), 183 (52), 177 (100).
10. **3**: C$_{12}$H$_{18}$O$_3$. ^{1}H NMR (CD$_3$OD) δ 1.41, 1.46 (both s, 15, 14-H$_3$), 1.43 (ddd, *J*=3.3, 3.7, 14.0 Hz, 1β-H), 1.56 (dddd, *J*=2.8, 3.3, 4.3, 14.0 Hz, 2α-H), 1.72 (ddd, *J*=2.4, 4.9, 13.1 Hz, 9β-H), 1.82 (ddd, *J*=4.3, 13.7, 14.0 Hz, 1α-H), 1.91 (ddd, *J*=4.5, 13.1, 15.0 Hz, 9α-H), 2.31 (br d, *J*=ca. 17 Hz, 8α-H), 2.40 (dddd, *J*=3.0, 3.7, 13.7, 14.0 Hz, 2β-H), 2.62 (ddd, *J*=4.9, 15.0, 17.4 Hz, 8β-H), 3.69 (dd, *J*=2.8, 3.0 Hz, 3-H), 6.01 (br s, 6-H). EI-MS *m/z* (%) 210 (M$^+$, 6), 192 (M$^+$−H$_2$O, 16), 167 (100).
11. Crystal data for **3**: monoclinic, space group P2$_1$, *a*=7.664(2), *b*=19.716(3), *c*=8.192(2) Å, β=114.53(2)°, *V*=1126.1(4) Å^3, *Z*=2, μ(Mo-K_α)=0.88 cm^{-1}, *F*(000)=456, D_c=1.240 g/cm^3, crystal dimensions: 0.30×0.20×0.15 mm. A total of 2855 reflections (2668 unique) were collected using the

ω–2θ scan technique to a maximum 2θ value of 55°, and 1587 reflections with $I > 3\sigma(I)$ were used in the structure determination. Final R and R_w values were 0.052 and 0.033, respectively. The maximum and minimum peaks in the difference map were 0.26 e Å^{-3} and -0.29 e Å^{-3}, respectively. The data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 161716.

12. **4**: $[\alpha]_D^{26}$ +58.8 (*c* 1.9, CHCl$_3$), C$_{12}$H$_{18}$O$_3$. UV (MeOH, nm, log ε) 237 (2.30). IR (KBr) 3401, 1667 cm^{-1}. ^{1}H NMR (CDCl$_3$) δ 1.32, 1.37 (both s, 14,15-H$_3$), 1.45 (ddd, J = 4.0, 14.0, 14.0 Hz, 1α-H), 1.68 (ddd, J = 3.5, 4.0, 14.0 Hz, 1β-H), 1.75 (dddd, J = 3.5, 4.0, 4.0, 15.0 Hz, 2α-H), 1.80 (ddd, J = 2.0, 5.0, 14.0 Hz, 9β-H), 1.88 (ddd, J = 3.5, 14.0, 14.0 Hz, 9α-H), 1.89 (dddd, J = 4.0, 12.0, 14.0, 15.0 Hz, 2β-H), 2.37 (ddd, J = 2.0, 4.0, 18.0 Hz, 8α-H), 2.55 (ddd, J = 5.0, 15.0, 18.0 Hz, 8β-H), 3.63 (dd, J = 4.0, 12.0 Hz, 3-H), 6.35 (s, 6-H). EI-MS *m/z* (%) 210 (M$^+$, 8), 192 (M$^+$ $-$H$_2$O, 34), 167 (100).

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14. **4a**: ^{1}H NMR (CDCl$_3$) δ 1.33, 1.38 (both s, 14, 15-H$_3$), 1.58, 1.74 (m, 1-H$_2$), 1.83 (m, 9β-H), 1.86 (m, 2α-H), 1.93 (br dd, 9α-H), 1.98 (dddd, J = 3.4, 12.2, 12.2, 15.6 Hz, 2β-H), 2.42 (ddd, J = 2.4, 3.3, 18.0 Hz, 8α-H), 2.56 (ddd, J = 5.2, 14.6, 18.0 Hz, 8β-H), 3.56 (s, –OCH$_3$), 5.04 (dd, J = 4.5, 12.2 Hz, 3-H), 6.40 (s, 6-H), 7.42–7.59 (5H, m, ph-H); **4b**: ^{1}H NMR (CDCl$_3$) δ 1.30, 1.34 (both s, 15, 14-H$_3$), 1.61, 1.77 (m, 1-H$_2$), 1.83 (ddd, J = 2.5, 5.0, 13.3 Hz, 9β-H), 1.93 (br dd, 9α-H), 1.98 (dddd, J = 3.1, 4.6, 4.6, 16.2 Hz, 2α-H), 2.04 (dddd, J = 3.3, 12.2, 12.2, 16.2 Hz, 2β-H), 2.41 (ddd, J = 2.5, 3.0, 8.3 Hz, 8α-H), 2.55 (ddd, J = 5.0, 15.0, 18.3 Hz, 8β-H), 3.59 (s, –OCH$_3$), 5.06 (dd, J = 4.6, 12.2 Hz, 3-H), 6.40 (s, 6-H), 7.42–7.58 (5H, m, ph-H).

15. Naturally occurring products with NO production inhibitory effects including those with higher activity (cf., ref 15c, IC$_{50}$ 1.2–3.8 μM for sesquiterpenoids) were recently reported: (a) Matsuda, H.; Kageura, T.; Morikawa, T.; Toguchida, I.; Harima, S.; Yoshikawa, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 323. (b) Yoshikawa, M.; Morikawa, T.; Toguchida, I.; Harima, S.; Matsuda, H. *Chem. Pharm. Bull.* **2000**, *45*, 651. (c) Matsuda, H.; Kageura, T.; Toguchida, I.; Ueda, H.; Morikawa, T.; Yoshikawa, M. *Life Sci.* **2000**, *66*, 2151. (d) Lee, M.-W.; Kim, N.-Y.; Park, M.-S.; Ahn, K.-H.; Toh, S.-H.; Hahn, D.-R.; Kim, Y.-C.; Chung, H.-T. *Planta Med.* **2000**, *66*, 551.

16. Nitrite concentration in the medium without inhibitors (control group) was 22.1±4.9 μM and that without LPS (unstimulated group) was 2.2±1.8 μM (mean±SD of five experiments).