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EREMOPHILANE-TYPE SESQUITERPENES FROM *Alpinia oxyphylla* WITH INHIBITORY ACTIVITY AGAINST NITRIC OXIDE PRODUCTION

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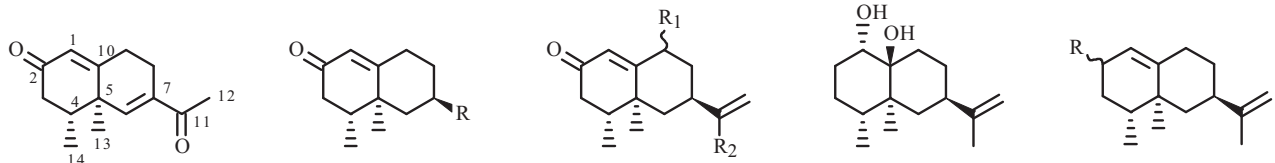
Phytochemical investigation of the fruits of Alpinia oxyphylla *led to the isolation of 12 eremophilane sesquiterpenes, including a novel noreremophilane sesquiterpene, (4*R**,5*R**)-7-acetyl-4,5-dimethyl-4,5,8,9-tetrahydronaphthalen-2(3*H*)-one (oxyphyllanone A, **1**), along with eleven known compounds (**2–12**). Among them, compounds **2–6**, **9**, and **11** were isolated from this genus for the first time. Their structures were determined on the basis of spectroscopic analysis, including 2D NMR spectroscopic techniques. The tested compounds **2–12** showed different inhibitory activity against NO production in interferon-γ- and lipopolysaccharide-induced RAW264.7 macrophage cells, and compound **11** was the most active one with IC_{50} value of 4.61 μg/mL.*

Keywords: Zingiberaceae, *Alpinia oxyphylla*, eremophilane sesquiterpene, oxyphyllanone A, inhibition of NO production.

Alpinia oxyphylla Miq. (Zingiberaceae) is a plant indigenous to southern China. Its fruits are eaten as food and also widely used in folk medicine to treat intestinal disorders, urosis, diuresis, ulcer and dementia [1–3]. Previous investigations on the constituents of *A. oxyphylla* have revealed the presence of sesquiterpenes, diterpenes, flavonoids, and diarylheptanoids, some of which showed inhibitory activity on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages [1, 2, 4, 5]. Furthermore, we recently reported a series of new sesquiterpenes, including seven new eudesmane sesquiterpenes, against NO production from *A. oxyphylla* [6–8]. In continuation of our search for bioactive metabolites from the fruits of *A. oxyphylla*, we have further isolated 12 eremophilane-type sesquiterpenes, including a new compound, (4*R**,5*R**)-7-acetyl-4,5-dimethyl-4,5,8,9-tetrahydronaphthalen-2(3*H*)-one (oxyphyllanone A, **1**), along with eleven known sesquiterpenes, (4*R**,5*S**,7*R**)-7-acetyl-4,5-dimethyl-4,5,6,7,8,9-hexahydronaphthalen-2(3*H*)-one (**2**) [9], (11*R*)-nootkatone-11,12-diol (**3**) [9], (11*S*)-nootkatone-11,12-diol (**4**) [9], 11-hydroxyvalenc-1(10)-en-2-one (**5**) [10], 9β-hydroxynootkatone (**6**) [9], oxyphyllol B (**7**) [2], oxyphyllol C (**8**) [2], epinootkatol (**9**) [11, 12], nootkatol (**10**) [12, 13], 12-hydroxynootkatone (**11**) [9, 14], and nootkatone (**12**) [11]. Among them, compounds **2–6**, **9**, and **11** were isolated from this genus for the first time. Compounds **2–12** were evaluated for their inhibitory activity against NO production, the IC_{50} values of **2**, **3**, and **5–12** ranged from 4.61 to 17.90 μg/mL. This paper deals mainly with the isolation and structural determination of the new compound **1** and the inhibitory activity of compounds **2–12** against NO production in interferon-γ(IFN-γ)- and lipopolysaccharide(LPS)-induced RAW264.7 macrophage cells.



1 **2 - 5** **6, 7, 11, 12** **8** **9, 10**

2: R = C(O)CH_3; **3:** R = (βCH_3)-C-(αOH)CH_2OH; **4:** R = (αCH_3)-C-(βOH)-CH_2OH; **5:** R = $C(CH_3)_2$-OH; **6:** R_1 = βOH, R_2 = CH_3 **7:** R_1 = αOH, R_2 = CH_3; **11:** R_1 = H, R_2 = CH_2OH; **12:** R_1 = H, R_2 = CH_3; **9:** R = αOH; **10:** R = βOH

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TABLE 1. NMR Data of **1** (400 MHz for ^1H, 100 MHz for ^{13}C, $CDCl_3$, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
1	5.88 (1H, s)	124.8 (d)	8	2.15 (1H, m, H-*α*),	24.6 (t)
2		198.5 (s)		2.80 (1H, ddd, J = 2.7, 5.4, 17.4, H-*β*)	
3	2.40 (2H, m)	42.1 (t)	9	2.48 (2H, m)	29.8 (t)
4	2.15 (1H, m)	37.1 (d)	10		168.0 (s)
5		40.6 (s)	11		198.3 (s)
6	6.76 (1H, s)	143.3 (d)	12	2.35 (3H, s)	25.3 (q)
7		137.8 (s)	13	1.23 (3H, s)	18.7(q)
			14	1.12 (3H, d, J = 6.8)	15.0 (q)

TABLE 2. Inhibitory Activities of Compounds **2–12** on NO Production in LPS and IFN-γ-induced RAW 264.7 Macrophages

Compound	IC_{50}, μg/mL	Compound	IC_{50}, μg/mL
(4*R**,5*S**,7*R**)-7-Acetyl-4,5-dimethyl-4,5,6,7,8,9-hexahydronaphthalen-2(3*H*)-one (**2**)	10.08	Oxyphyllol C (**8**)	15.33
		Epinootkatol (**9**)	12.03
(11*R*)-Nootkatone-11,12-diol (**3**)	17.90	Nootkatol (**10**)	14.89
(11*S*)-Nootkatone-11,12-diol (**4**)	>25.0	12-Hydroxynootkatone (**11**)	4.61
11-Hydroxyvalenc-1(10)-en-2-one (**5**)	10.42	Nootkatone (**12**)	11.67
9*β*-Hydroxynootkatone (**6**)	12.27	MG-132	11.67
Oxyphyllol B (**7**)	10.50		

MG-132: positive control for LPS and IFN-γ stimulated NO production.

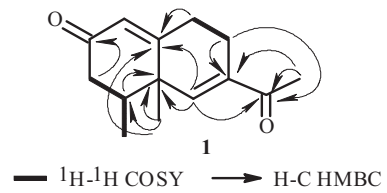

Fig. 1. ^1H-^1H COSY and key HMBC correlations for compound **1**.

(4*R**,5*R**)-7-Acetyl-4,5-dimethyl-4,5,8,9-tetrahydronaphthalen-2(3*H*)-one (oxyphyllanone A, **1**) ($[\alpha]_D^{22.0}$ +78.89°) was obtained as a colorless oil. The molecular formula $C_{14}H_{18}O_2$ was deduced by HR-ESI-MS *m/z* 219.1380 $[M + H]^+$; calcd 219.1385. The ^1H NMR spectrum (Table 1) of **1** exhibited signals for three methyls at δ 2.35 (3H, s, H-12), 1.23 (3H, s, H-13), and 1.12 (3H, d, J = 6.8 Hz, H-14), two olefinic protons at δ 5.88 (1H, s, H-1) and 6.76 (1H, s, H-6), one methine, and three methylenes. The ^{13}C NMR (Table 1) indicated the presence of five quaternary carbons, including two conjugated ketone carbons at δ 198.5 (s, C-2) and 198.3 (s, C-11). In addition, the moieties of $-CH_2-CH-CH_3$ and $-CH_2-CH_2-$ were identified by the ^1H–^1H COSY spectrum (Fig. 1). Moreover, the HMBC experiment showed the correlations between H-3 with C-2, H-4 with C-5, H-13 with C-4, C-5, and C-10, H-6 with C-10 and C-11, H-8 with C-7 and C-11, and H-9 with C-10 (Fig. 1). The above information suggested that compound **1** was a noreremophilane sesquiterpene, and the cross peak of Me (13) with Me (14) in the NOESY spectrum revealed the presented configuration of **1** as shown in Fig. 1.

EXPERIMENTAL

General Procedures. Column chromatography (CC) was performed on silica gel (100–200 or 200–300 mesh, Qingdao Marine Chemical Ltd. Co., China), silica gel H (60 μm, Qingdao Marine Chemical Ltd. Co., China), Lichroprep RP-18 gel (40-63 μM, Merck, Germany), and MCI gel CHP20P (75–150 μm, Mitsubishi Chemical Co., Japan). TLC was performed on silica gel GF254 (Qingdao Marine Chemical Ltd. Co., China). Semipreparative reverse-phase (RP) HPLC was performed on

an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18} column. Solvents were of industrial purity and distilled prior to use. NMR spectra were determined using a Bruker AM-400 instrument with TMS as internal standard. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were determined on a Shimadzu 210A double-beam spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. MS were recorded on an API Qstar Pulsar instrument.

Plant Material. The fruits of *Alpinia oxyphylla* were bought from Kunming medicinal market, Kunming, Yunnan Province, People′s Republic of China in 2006 and identified by Prof. Ning-Hua Tan.

Extraction and Isolation. The dried and powdered fruits of *A. oxyphylla* (15 kg) were extracted with acetone–water (7:3) under reflux for 8 h (five volumes, each 30 L). The resulting residue was partitioned between petroleum ether and water, ethyl acetate and water, and then *n*-butanol and water. The ethyl acetate extract (480 g) was subjected to column chromatography on silica gel, eluting with petroleum–acetone (9:1–1:1), to yield 10 fractions (Fr. 1–Fr. 10). Fraction 1 (16 g) was subjected to column chromatography (silica gel, petroleum ether–chloroform–acetone 3:1:0.1) and then further purified by HPLC (methanol–water 6:4 and ethane nitrile–water 55:45) to yield **12** (38 mg). Fraction 2 (24 g) was subjected to column chromatography (silica gel, chloroform–ethyl acetate 100:1–9:1) to afford four subfractions (Fr. 2.1–Fr. 2.4). Fraction 2.2 (4.8 g) was subjected to column chromatography (RP-18, methanol–water 6:4–8:2, and silica gel, petroleum ether–acetone 50:1 and petroleum ether–ethyl acetate 40:1) to yield **9** (134 mg), **10** (288 mg). Fraction 4 (49 g) was subjected to column chromatography (silica gel, chloroform–ethyl acetate 100:1–7:3) to afford four subfractions (Fr. 4.1–Fr. 4.4). Fraction 4.3 (11g) was further purified by column chromatography (MCI) and HPLC (ethane nitrile–water 2:8 and methanol–water 55:45) to yield **1** (2 mg), **2** (60 mg), and **8** (65 mg). Fraction 5 (20 g) was subjected to column chromatography (silica gel, chloroform–acetone 50:1–8:2) to afford four subfractions (Fr. 5.1–Fr. 5.4). Fraction 5.2 (5 g) was subjected to column chromatography (RP-18, methanol–water 2:8–1:1, and silica gel, chloroform–ethyl acetate 40:1) and further purified by HPLC (ethane nitrile–water 2:8) to yield **5** (36 mg), **6** (64 mg), **7** (43 mg). Fraction 8 (5 g) was subjected to column chromatography (RP-18, methanol–water 85:15–1:1) to afford three subfractions (Fr. 8.1–Fr. 8.3). Fraction 8.3 (1.2 g) was further purified by column chromatography (silica gel, chloroform–acetone 3:1 and petroleum ether–ethyl acetate 1:2) to yield **11** (33 mg). Fraction 10 (5 g) was subjected to column chromatography (RP-18, methanol–water 2:8–7:3) to afford five subfractions (Fr. 10.1– Fr. 10.5). Fraction 10.2 (1.5 g) was further purified by column chromatography (silica gel, chloroform–acetone 2:1 and RP-18, methanol–water 25:75) to yield **3** (40 mg) and **4** (14 mg).

(4*R,5*R**)-7-Acetyl-4,5-dimethyl-4,5,8,9-tetrahydronaphthalen-2(3*H*)-one (oxyphyllanone A, 1)**. $C_{14}H_{18}O_2$, colorless oil, $[\alpha]_D^{22.0}$ +78.89° (*c* 0.30, $CHCl_3$). UV spectrum (MeOH, λ_{max}, nm) (log ε): 231 (4.25). IR spectrum (KBr, ν, cm^{-1}): 3625, 2927, 1710, 1659, 1378, 1290, 1209. HR-ESI-MS $[M + H]^+$ *m/z* 219.1380 (calcd for $C_{14}H_{19}O_2$, 219.1385). ^1H NMR and ^{13}C NMR, see Table 1.

(4*R,5*S**,7*R**)-7-Acetyl-4,5-dimethyl-4,5,6,7,8,9-hexahydronaphthalen-2(3H)-one (2)**. $C_{14}H_{20}O_2$, colorless oil, $[\alpha]_D^{25.5}$ +133.75° (*c* 0.40, $CHCl_3$). EI-MS (*m/z*, I_{rel}, %): 220 (M^+, 94), 205 (45), 177 (50), 161 (45), 135 (100), 121 (25), 107 (44), 91 (39), 79 (25), 71 (26). PMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.73 (1H, s, H-1), 2.71 (1H, tt, J = 3.0, 12.6, H-7), 2.50 (1H, m, H-9α), 2.39 (1H, m, H-9β), 2.22 (2H, m, H-3), 2.16 (3H, s, H-12), 2.07 (1H, m, H-8α), 2.05 (1H, m, H-6α), 2.00 (1H, m, H-4), 1.40 (1H, m, H-8β), 1.21 (1H, m, H-6β), 1.07 (3H, s, H-13), 0.94 (3H, d, J = 6.8, H-14). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 210.5 (s, C-11), 199.2 (s, C-2), 168.6 (s, C-10), 125.1 (d, C-1), 46.6 (d, C-7), 41.9 (t, C-3), 40.1 (d, C-4), 39.8 (t, C-6), 38.8 (s, C-5), 31.9 (t, C-9), 28.4 (t, C-8), 28.1 (q, C-12), 16.6 (q, C-14), 14.8 (q, C-15).

(11*R*)-Nootkatone-11,12-diol (3). $C_{15}H_{24}O_3$, colorless oil, $[\alpha]_D^{25.6}$ +83.33° (*c* 0.10, $CHCl_3$). Positive FAB (*m/z*, I_{rel}, %): 253 ($[M + H]^+$, 100), 235 (10). PMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.75 (1H, s, H-1), 3.46, 3.61 (each 1H, d, J = 11.0, H_2-12), 2.49 (1H, m, H-9α), 2.37 (1H, m, H-9β), 2.29 (1H, m, H-3α), 2.24 (1H, m, H-3β), 2.12 (1H, dt, J = 2.8, 13.0, H-6α), 2.01 (1H, m, H-4), 1.97 (1H, m, H-7), 1.86 (1H, m, H-8α), 1.17 (1H, m, H-8β), 1.10 (3H, s, H-13), 1.09 (3H, s, H-14), 1.05 (1H, m, H-6β), 0.98 (3H, d, J = 6.9, H-15). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 199.8 (s, C-2), 170.7 (s, C-10), 124.5 (d, C-1), 74.3 (s, C-11), 68.4 (t, C-12), 42.1 (t, C-3), 40.6 (d, C-4), 39.2 (s, C-5), 39.2 (d, C-7), 38.6 (t, C-6), 32.9 (t, C-9), 27.9 (t, C-8), 19.9 (q, C-13), 16.8 (q, C-14), 15.0 (q, C-15).

(11*S*)-Nootkatone-11,12-diol (4). $C_{15}H_{24}O_3$, colorless oil, $[\alpha]_D^{25.6}$ +74.44° (*c* 0.15, $CHCl_3$). Positive FAB (*m/z*, I_{rel}, %): 253 ($[M + H]^+$, 100), 235 (17). PMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.76 (1H, s, H-1), 3.46, 3.60 (each 1H, d, J = 11.0, H-12), 1.11 (3H, s, H-13), 1.09 (3H, s, H-14), 0.96 (3H, d, J = 6.9, H-15). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 199.7 (s, C-2), 170.6 (s, C-10), 124.5 (d, C-1), 74.4 (s, C-11), 68.2 (t, C-12), 42.0 (t, C-3), 40.5 (d, C-4), 39.7 (t, C-6), 39.5 (d, C-7), 39.2 (s, C-5), 32.9 (t, C-9), 26.7 (t, C-8), 20.3 (q, C-13), 16.9 (q, C-14), 14.9 (q, C-15).

11-Hydroxyvalenc-1(10)-en-2-one (5). $C_{15}H_{24}O_2$, colorless oil, $[\alpha]_D^{26.8}$ +112.50° (*c* 0.10, $CHCl_3$). Positive FAB-MS *m/z* (%): 237 ($[M + H]^+$, 1). PMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.71 (1H, s, H-1), 2.43 (1H, m, H-9α), 2.33 (1H, m, H-9β),

2.24 (1H, dd, J = 13.8, 17.0, H-3α), 2.17 (1H, m, H-3β), 2.00 (1H, m, H-8α), 2.00 (1H, m, H-6α), 1.95 (1H, m, H-4), 1.69 (1H, m, H-7), 1.17 (3H, s, H-12), 1.15 (1H, m, H-8β), 1.15 (3H, s, H-13), 1.04 (3H, s, H-14), 0.93 (1H, m, H-6β), 0.93 (3H, d, J = 6.8, H-15). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 199.8 (s, C-2), 171.1 (s, C-10), 124.3 (d, C-1), 72.2 (s, C-11), 43.7 (d, C-7), 41.9 (t, C-3), 40.4 (d, C-4), 39.5 (t, C-6), 39.1 (s, C-5), 32.9 (t, C-9), 27.6 (t, C-8), 27.2 (q, C-12), 26.8 (q, C-13), 16.8 (q, C-14), 14.9 (q, C-15).

9β-Hydroxynootkatone (6). $C_{15}H_{22}O_2$, colorless oil, $[\alpha]_D^{26.0}$ +86.84° (*c* 0.15, $CHCl_3$). Positive FAB-MS (*m/z*, I_{rel}, %): 235 ($[M + H]^+$), 217 (22). PMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.19 (1H, br.s, H-1), 4.70, 4.69 (each 1H, br.s, H_2-12), 4.41 (1H, m, H-9), 2.37 (1H, m, H-7), 2.25 (1H, dd, J = 13.9, 17.1, H-3α), 2.20 (1H, m, H-8α), 2.16 (1H, m, H-3β), 1.97 (1H, m, H-4), 1.90 (1H, m, H-6α), 1.69 (3H, s, H-13), 1.39 (1H, m, H-8β), 1.08 (1H, m, H-6β), 1.07 (3H, s, H-14), 0.92 (3H, d, J = 6.8, H-15). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 200.4 (s, C-2), 172.5 (s, C-10), 147.8 (s, C-11), 120.6 (d, C-1), 109.7 (t, C-12), 68.8 (d, C-9), 43.7 (t, C-6), 41.7 (t, C-3), 40.7 (d, C-4), 40.5 (t, C-8), 39.6 (s, C-5), 38.2 (d, C-7), 20.7 (q, C-13), 17.5 (q, C-14), 15.0 (q, C-15).

Oxyphyllol B (7). $C_{15}H_{22}O_2$, colorless oil, $[\alpha]_D^{26.3}$ +51.92° (*c* 0.15, $CHCl_3$). EI-MS (*m/z*, I_{rel}, %): 234 (M^+, 100), 216 (39), 191 (46), 177 (55), 151 (30), 137 (46). PMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.83 (1H, br.s, H-1), 4.75, 4.73 (each 1H, br.s, H-12), 4.43 (1H, br.s, H-9), 2.79 (1H, m, H-7), 2.30 (1H, dd, J = 14.0, 17.4, H-3α), 2.25 (1H, dd, J = 4.0, 17.4, H-3β), 2.04 (1H, ddd, J = 2.7, 2.8, 15.8, H-8α), 1.97 (1H, ddq, J = 4.0, 6.8, 14.0, H-4), 1.93 (1H, m, H-6α), 1.75 (3H, s, H-13), 1.50 (1H, ddd, J = 3.0, 12.5, 15.8, H-8β), 1.29 (3H, s, H-14), 1.07 (1H, m, H-6β), 0.93 (3H, d, J = 6.8, H-15). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 200.9 (s, C-2), 168.9 (s, C-10), 148.9 (s, C-11), 126.9 (d, C-1), 109.2 (t, C-12), 72.9 (d, C-9), 43.5 (t, C-6), 42.1 (t, C-3), 41.0 (d, C-4), 38.7 (s, C-5), 37.8 (t, C-8), 33.9 (d, C-7), 20.9 (q, C-13), 18.0 (q, C-14), 14.4 (q, C-15).

Oxyphyllol C (8). $C_{15}H_{26}O_2$, colorless oil, $[\alpha]_D^{26.5}$ +34.06° (*c* 1.6, $CHCl_3$). Positive FAB-MS (*m/z*, I_{rel}, %): 239 ($[M + H]^+$, 4), 221 (100), 83 (55). PMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.73, 4.69 (each 1H, br.s, H_2-12), 3.52 (1H, br.s, H-1), 2.27 (1H, m, H-7), 2.25 (1H, m, H-9α), 2.14 (1H, m, H-2β), 1.80 (1H, m, H-4), 1.73 (3H, s, H-13), 1.65 (2H, m, H-8), 1.59 (1H, m, H-3α), 1.57 (1H, m, H-2α), 1.40 (2H, m, H-6), 1.30 (1H, m, H-3β), 1.25 (1H, m, H-9β), 1.08 (3H, s, H-14), 0.76 (3H, d, J = 6.8, H-15). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 150.6 (s, C-11), 108.5 (t, C-12), 75.9 (d, C-1), 73.8 (s, C-10), 39.3 (d, C-7), 39.2 (s, C-5), 38.6 (t, C-6), 34.9 (d, C-4), 31.4 (t, C-9), 29.2 (t, C-2), 25.7 (t, C-8), 25.3 (t, C-3), 20.9 (q, C-13), 15.8 (q, C-14), 15.1 (q, C-15).

Epinootkatol (9). $C_{15}H_{24}O$, colorless oil, $[\alpha]_D^{26.4}$ +122.45° (*c* 0.26, $CHCl_3$). Positive FAB-MS (*m/z*, I_{rel}, %): 220 (M^+, 15), 203 (100). PMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.30 (1H, br.s, H-1), 4.66 (2H, br.s, H_2-12), 4.22 (1H, m, H-2), 1.68 (3H, br.s, H-13), 0.97 (3H, s, H-14), 0.87 (3H, d, J = 6.0, H-15). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 150.1 (s, C-10), 145.7 (s, C-11), 124.3 (d, C-1), 108.5 (t, C-12), 67.8 (d, C-2), 44.5 (t, C-9), 40.7 (d, C-7), 39.2 (d, C-4), 38.1 (s, C-5), 37.0 (t, C-3), 32.8 (t, C-8), 32.3 (t, C-6), 20.8 (q, C-13), 18.2 (q, C-14), 15.4 (q, C-15).

Nootkatol (10). $C_{15}H_{24}O$, colorless oil, $[\alpha]_D^{26.4}$ +147.78° (*c* 1.35, $CHCl_3$). EI-MS (*m/z*, I_{rel}, %): 213 ($M + 1 - H_2O$, 12), 185 (19), 129 (44), 97 (66), 69 (90), 55 (100). PMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.46 (1H, d, J = 4.9, H-1), 4.65 (2H, s, H_2-12), 4.02 (1H, m, H-2), 1.68 (1H, m, H-4), 1.68 (3H, s, H-13), 0.86 (3H, s, H-14), 0.86 (3H, d, J = 6.0, H-15). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 150.0 (s, C-10), 148.4 (s, C-11), 121.5 (d, C-1), 108.5 (t, C-12), 64.2 (d, C-2), 44.4 (t, C-6), 40.5 (d, C-7), 38.1 (s, C-5), 36.0 (t, C-3), 34.8 (d, C-4), 32.4 (t, C-8), 32.4 (t, C-9), 20.7 (q, C-13), 16.7 (q, C-14), 15.1 (q, C-15).

12-Hydroxynootkatone (11). $C_{15}H_{22}O_2$, colorless oil, $[\alpha]_D^{26.4}$ +8.52° (*c* 0.19, MeOH). Positive FAB-MS *m/z* (%): 235 ($[M + H]^+$, 18). PMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.76 (1H, s, H-1), 5.07, 4.90 (each 1H, br.s, H_2-12), 4.14 (2H, t, H_2-13), 2.49 (1H, m, H-9α), 2.41 (1H, m, H-7), 2.38 (1H, m, H-9β), 2.18 (2H, m, H-3), 1.97 (1H, m, H-6β), 1.96 (1H, m, H-4), 1.94 (1H, m, H-8β), 1.34 (1H, m, H-8α), 1.12 (1H, t, J = 13.0, H-6α), 1.07 (3H, s, H-14), 0.93 (3H, d, J = 6.8, H-15). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 199.9 (s, C-2), 170.8 (s, C-10), 152.5 (s, C-11), 124.6 (t, C-12), 124.6 (d, C-1), 65.0 (t, C-13), 44.3 (t, C-6), 41.9 (t, C-3), 40.3 (d, C-4), 39.4 (s, C-5), 35.8 (d, C-7), 33.1 (t, C-9), 32.0 (t, C-8), 16.8 (q, C-14), 14.9 (q, C-15).

Nootkatone (12). $C_{15}H_{22}O$, colorless oil, $[\alpha]_D^{26.4}$ +103.65° (*c* 0.22, MeOH). Positive ESI-MS *m/z*: 219 ($[M + H]^+$). PMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.84 (1H, d, J = 4.9, H-1), 4.81, 4.79 (each 1H, br.s, H-12), 1.80 (3H, br.s, H_3-13), 1.20 (3H, s, H-14), 1.03 (3H, d, J = 6.8, H-15). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 199.6 (s, C-2), 170.5 (s, C-10), 149.1 (s, C-11), 124.6 (d, C-1), 109.2 (t, C-12), 43.9 (t, C-9), 42.1 (t, C-3), 40.4 (d, C-7), 40.3 (d, C-4), 39.3 (s, C-5), 32.9 (t, C-6), 31.6 (t, C-8), 20.9 (q, C-13), 16.8 (q, C-14), 14.9 (q, C-15).

Assessment of Inhibitory Activity against NO Production. It has been reported that several sesquiterpenes from *A. oxyphylla* showed inhibitory effects on NO production [1, 2, 8]. Therefore, the inhibitory activity on NO production of isolated compounds **2–12** was tested according to the published method [15]. The IC_{50} values of **2**, **3**, and **5–12** ranged from

4.61 to 17.90 μg/mL (Table 2). Among them, compound **11** was the most active, and **4** showed no inhibitory activity. This is the first report on the inhibitory activity of the above compounds, except for **12**.

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