Inhibitory Effects of Terpenoids from the Fermented Broth of the Ascomycete *Stilbohypoxylon elaeicola* YMJ173 on Nitric Oxide Production in RAW264.7 Macrophages

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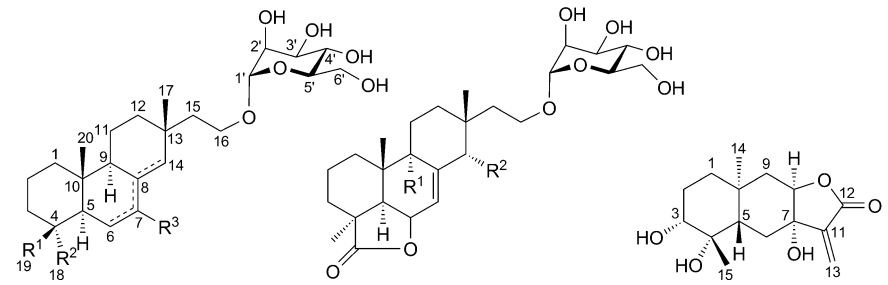
A series of six isopimarane-type diterpene glycosides, along with an eremophilane-type sesquiterpene, *i.e.*, elaeicolasides A–C (**1**–**3**, resp.), 16-(α-D-mannopyranosyloxy)isopimar-7-en-19-oic acid (**4**), hymatoxin K (**5**), hymatoxin L (**6**), and elaeicolalactone (**7**), were isolated from the AcOEt extract of the fermented broth of *Stilbohypoxylon elaeicola* YMJ173. Among these, **1**–**3** and **7** are new compounds based on their spectroscopic data and sugar composition analysis. The effects of **1**–**7** on the inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-activated murine macrophage RAW264.7 cells were evaluated. All these compounds inhibited NO production, detected as nitrite in the culture medium, in activated macrophages without any cytotoxicity at a concentration of 100 μM. Among these compounds, **2** showed a significant activity with the average maximum inhibition (E_{max}) and median inhibitory concentration (IC_{50}) values of 93.3 ± 0.5% and 79.3 ± 0.4 μM, respectively.

Introduction. – *Stilbohypoxylon elaeicola* (HENN.) L. E. PETRINI is geographically distributed in tropical and subtropical areas, and belongs to the ascomycete family Xylariaceae [1][2]. *Petrini* listed *Astrocystis cocoës* (HENN.) LÆSSØE & SPOONER, *Rosellinia elaeicola* HENN., *R. samoensis* HENN., *R. cocoës* HENN., and *S. moelleri* as synonyms of *S. elaeicola* [2]. So far, no report regarding the chemical constituents of this fungus has been found in the literature. During a preliminary human disease-based biological screening, it was found that the AcOEt extract from the fermented broth of *S. elaeicola* strain No. YMJ173 exhibited a potent inducible nitric oxide synthase (iNOS) inhibitory activity (E_{max} = 98%) without any cytotoxicity against RAW264.7 cells at a concentration of 100 μg/ml [3]. We thus set out to isolate the active principles from the fermented broth of this strain and to assess the bioactivities of the pure isolates.

Results and Discussion. – The AcOEt extract of the fermented broth of *S. elaeicola* YMJ173 was fractionated by a *Sephadex LH-20* column into five portions. Portions II and III were then subjected to HPLC to give four new compounds **1**–**3** and **7** (*Fig. 1*), together with three known ones. Compound **4** was identified as 16-(α-D-mannopyranosyloxy)isopimar-7-en-19-oic acid [4], obtained previously from *Xylaria polymor-*



	R^1	R^2	R^3	
1	Me	OOH	α-OH	C(8)=C(14)
2	Me	OH	H	C(7)=C(8)
3	COOH	Me	H	C(6)=C(7), C(8)=C(14)
4	COOH	Me	H	C(7)=C(8)

	R^1	R^2
5	OH	H
6	H	OH

7

Fig. 1. *Chemical structures of compounds* **1**–**7**

pha, a fungus of the family Xylariaceae to which *S. elaeicola* belongs. Compounds **5** and **6** were identified as hymatoxins K and L, respectively, two rare isopimarane mannopyranosides with a γ-lactone moiety between C(4) and C(6), which were previously isolated from the fungus *Hypoxylon mammatum* [5].

Compound **1**, obtained as amorphous white powder, was determined to have the molecular formula $C_{25}H_{42}O_9$ deduced from the HR-ESI-MS and ^{13}C-NMR data. The IR spectrum of **1** revealed the presence of a C=C bond (1643 cm^{-1}) and a OH group (3348 cm^{-1}). The ^{13}C-NMR spectrum of **1** exhibited 25 C-atom signals attributable to a C_6 sugar unit and a C_{19} aglycone (*Table 1*). The ^{13}C-NMR data of **1** were quite similar to those of **4** except that conspicuous differences, including the disappearance of a COOH signal at δ(C) 180.2 (C(19)), and the signal of an olefinic C-atom at δ(C) 137.2 (C(14)), a lower field-shifted signal at δ(C) 84.9 (C(4)), and the signal of a carbinol C-atom at δ(C) 74.0 (C(7)) were observed (*Table 1*). Thus, the COOH group at C(4) in **4** was replaced by a OOH group in **1** as evidenced by the lower-field shift of the signal of C(4) at δ(C) 84.9, quite similar to that of the previously reported norabietane analogs [6]. The differences observed in the ^{13}C-NMR spectra of **4** and **1** were also reflected in the ^{1}H-NMR data of **1** (*Table 2*), in which a higher-field shift of the Me(18) signal from δ(H) 1.48 to 1.12, an olefinic H–C(7) signal from δ(H) 5.44 (*d*, J = 4.4) to 4.11 (*t*, J = 2.7), and CH_2(14) signal at δ(H) 1.95–1.99 (*m*) to 5.55 (*s*) were observed. These data unambiguously revealed that the aglycone of **1** had a norisopimarane skeleton with a OOH group at C(4), a OH group at C(7), and a C(8)=C(14) bond as evidenced from key HMBCs Me(18)/C(4), H–C(7)/C(5), C(8), C(9), and C(14), and Me(17)/C(14) (*Fig. 2*). In the NOESY spectrum of **1** (*Fig. 2*), cross-peaks Me(20)/Me(17) and Me(18), H_{ax}–C(1)/H–C(5) and H–C(9), and H–C(7)/H–C(14) indicated that Me(17), Me(18), Me(20), and H–C(7) were β-oriented, and HOO–C(4), H–C(5), and H–C(9) were α-oriented. The sugar moiety was identified as α-D-mannopyranose by comparing the ^{1}H- and ^{13}C-NMR data with those in the literature [5][7], and this was supported by the optical-rotation measurement and sugar composition analysis of the acid hydrolysate of **1**. The sugar was at C(16) as corroborated by correlations H–C(1′)/C(16) and

Table 1. *^{13}C-NMR Data* (125 MHz) *of Compounds* **1**–**3** *and* **7**

	1[a])	**2**[a])	**3**[b])	**7**[b])
1	39.3	40.4	38.6	34.8
2	20.7	21.3	20.7	25.5
3	36.7	43.8	38.7	75.6
4	84.9	73.1	44.3	73.9
5	43.0	53.2	56.5	43.2
6	29.6	23.5	129.0	29.4
7	74.0	122.2	128.8	76.1
8	139.2	137.2	136.3	84.9
9	47.5	53.7	50.6	49.5
10	40.1	37.4	38.7	34.8
11	19.7	21.7	20.1	141.5
12	35.2	38.3	35.3	172.1
13	34.5	34.1	35.4	122.9
14	137.2	49.0	134.7	18.9
15	43.6	45.2	43.8	26.6
16	65.4	65.1	65.7	
17	26.5	22.2	28.4	
18	19.4	23.5	28.8	
19			181.0	
20	14.6	15.1	12.3	
1′	101.6	101.7	101.7	
2′	72.3	72.3	72.3	
3′	72.7	72.7	72.7	
4′	68.8	68.7	68.6	
5′	74.7	74.7	74.6	
6′	63.0	63.0	62.9	

[a]) Recorded in C_5D_5N. [b]) Recorded in CD_3OD.

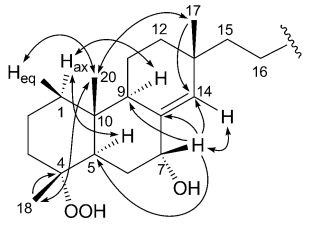


Fig. 2. *Key NOESY correlations* (↔) *and HMBCs* (H→C) *of* **1**

CH_2(16)/C(1′) in the HMBC experiment. Accordingly, **1** was conclusively characterized as 7α-hydroxy-16-(α-D-mannopyranosyloxy)norisopimar-8(14)-ene 4-hydroperoxide, and named elaeicolaside A.

The molecular formula of **2**, $C_{25}H_{42}O_7$, was established by analysis of the ^{13}C-NMR and HR-ESI-MS data. The IR spectrum revealed the presence of a OH group (3402 cm^{-1}) and a C=C bond (1680 cm^{-1}). The ^{1}H- and ^{13}C-NMR data of **2** closely resemble those of **1** except for a carbinol C-atom resonance at δ(C) 73.1 (C(4)), an

Table 2. *^{1}H-NMR Data* (500 MHz) *for Compounds* **1**–**3** *and* **7**. δ in ppm, J in Hz.

	1[a])	**2**[a])	**3**[b])	**7**[b])
1	1.08–1.12 (*m*), 1.63–1.69 (*m*)	1.08 (*dt*, *J* = 13.1, 3.3), 1.80 (*d*, *J* = 13.1)	1.10–1.14 (*m*), 1.73 (*d*, *J* = 12.7)	1.13–1.17 (*m*), 1.46–1.50 (*m*)
2	1.46–1.49 (*m*), 1.60–1.64 (*m*)	1.41–1.45 (*m*), 1.53–1.59 (*m*)	1.50–1.54 (*m*), 1.87–1.91 (*m*)	1.40–1.44 (*m*), 2.08–2.12 (*m*)
3	1.60–1.64 (*m*), 1.84–1.90 (*m*)	1.37–1.41 (*m*), 1.71–1.77 (*m*)	1.06–1.10 (*m*), 2.14–2.18 (*m*)	3.44 (br. *s*)
5	2.18–2.21 (*m*)	1.40–1.44 (*m*)	2.13–2.16 (*m*)	1.30 (*dd*, *J* = 3.3, 14.0)
6	1.50–1.53 (*m*), 1.80–1.85 (*m*)	1.83–1.87 (*m*), 2.11–1.15 (*m*)	6.07 (*dd*, *J* = 1.6, 10.0)	2.01 (*t*, *J* = 14.0), 2.28 (*dd*, *J* = 3.3, 14.0)
7	4.11 (*t*, *J* = 2.7)	5.37 (br. *d*, *J* = 4.4)	5.89 (*dd*, *J* = 2.9, 10.0)	
8				4.59 (*dd*, *J* = 6.6, 12.3)
9	2.20–2.23 (*m*)	1.69–1.73 (*m*)	1.95–1.98 (*m*)	0.78 (*t*, *J* = 12.3), 1.87 (*dd*, *J* = 12.3, 6.6)
11	1.38–1.42 (*m*), 1.53–1.57 (*m*)	1.37–1.41 (*m*), 1.72–1.76 (*m*)	1.39–1.43 (*m*), 1.60–1.64 (*m*)	
12	1.39–1.42 (*m*)	1.27–1.31 (*m*), 1.51–1.55 (*m*)	1.50–1.54 (*m*)	
13				5.79 (*s*), 6.25 (*s*)
14	5.55 (*s*)	1.89–1.93 (*m*)	5.28 (*s*)	1.13 (*s*)
15	1.58–1.62 (*m*)	1.49–1.53 (*m*)	1.58–1.62 (*m*)	1.23 (*s*)
16	3.47–3.51 (*m*), 3.73–3.77 (*m*)	3.47–3.51 (*m*), 3.81–3.85 (*m*)	3.42 (*dt*, *J* = 9.9, 6.7), 3.79–3.83 (*m*)	
17	1.01 (*s*)	0.80 (*s*)	0.99 (*s*)	
18	1.12 (*s*)	1.21 (*s*)	1.27 (*s*)	
20	0.85 (*s*)	0.83 (*s*)	0.64 (*s*)	
1′	4.71 (*d*, *J* = 1.6)	4.72 (*d*, *J* = 1.5)	4.68 (*s*)	
2′	3.76 (*dd*, *J* = 3.4, 1.6)	3.75 (*dd*, *J* = 3.3, 1.5)	3.73–3.77 (*m*)	
3′	3.63–3.69 (*m*)	3.64–3.68 (*m*)	3.65–3.69 (*m*)	
4′	3.57 (*t*, *J* = 9.5)	3.58 (*t*, *J* = 9.5)	3.61 (*t*, *J* = 9.6)	
5′	3.50–3.54 (*m*)	3.49–3.53 (*m*)	3.48–3.52 (*m*)	
6′	3.67–3.71 (*m*), 3.81–3.85 (*m*)	3.67–3.71 (*m*), 3.81–3.85 (*m*)	3.69–3.73 (*m*), 3.77–3.81 (*m*)	

[a]) Recorded in C_5D_5N. [b]) Recorded in CD_3OD.

olefinic resonance at δ(H) 5.37 (*d*, *J* = 4.4) and δ(C) 122.2 (H–C(7)), and a CH_2 signal at δ(H) 1.91 (*s*) and δ(C) 49.0 (CH_2(14)). These data indicated that **2** was also a norisopimarane α-D-mannopyranoside with a OH group at C(4) and a C(7)=C(8) bond, as confirmed by key HMBCs Me(18)/C(4), and H–C(7)/C(5), C(6), C(9), and C(14). After assignment of the full 2D-NMR data, the structure of compound **2**, named elaeicolaside B, was established as 16-(α-D-mannopyranosyloxy)norisopimar-7-en-4-ol.

Compound **3** was assigned to have a molecular formula of $C_{26}H_{40}O_8$ on the basis of the ^{13}C-NMR and HR-ESI-MS data. By comparison of the ^{13}C-NMR data of **3** (*Table 1*) and **4**, major differences of the resonances at δ(C) 28.4 (C(17)), 56.5 (C(5)), 128.8 (C(7)), 129.0 (C(6)), and 134.7 (C(14)) indicated conjugated C(6)=C(7) and C(8)=C(14) bonds in **3**. The location of the olefinic bonds was further supported by key

HMBCs H–C(7)/C(6), C(8), and C(14), and Me(17)/C(14). These assignments were in accordance with the UV spectrum, in which an absorption maximum at 241 nm confirmed the presence of conjugated double bonds in **3**. After further analysis of the HMBC and NOESY of **3**, the locations of all functionalities and relative configuration were determined. Thus, **3** was deduced as 16-(α-D-mannopyranosyloxy)isopimar-6,8(14)-dien-19-oic acid, and was named elaeicolaside C.

Compound **7**, a minor component, was obtained as an amorphous white powder, and had a molecular formula of $C_{15}H_{22}O_5$, as determined by HR-ESI-MS. Its IR spectrum exhibited absorption bands at 3402, 1753, and 1666 cm^{-1}, indicating the presence of a OH and a γ-lactone CO group, and a C=C bond, respectively. The ^{1}H- (*Table 2*), ^{13}C-NMR (*Table 1*), and HSQC spectra revealed that **7** contained a CO group (δ(C) 172.1 (C(12))), an exocyclic C=CH_2 moiety (δ(C) 122.9 (C(13)) and 141.5 (C(11))), two O-bearing quaternary C-atoms (δ(C) 73.9 (C(4)) and 76.1 (C(7))), two O-bearing CH groups (δ(C) 75.6 (C(3)) and 84.9 (C(8))), an aliphatic quaternary C-atom (δ(C) 34.8 (C(10))), a CH group (δ(C) 43.2 (C(5))), four CH_2 groups (δ(C) 25.5 (C(2)), 29.4 (C(6)), 34.8 (C(1)), and 49.5 (C(9))), and two Me groups (δ(C) 18.9 (C(14)) and 26.6 (C(15))). In the HMBC spectrum of **7** (*Fig. 3*), key cross-peaks H–C(8)/C(12), CH_2(13)/C(7), C(11), and C(12), Me(14)/C(1), C(5), C(9), and C(10), and Me(15)/C(3), C(4), and C(5) established the locations of all the functionalities. The presence of a decalin moiety fused with a γ-lactone ring to form a linear 6/6/5 ring system was deduced from the HMBC and COSY correlations H–C(1)/H–C(2), H–C(2)/H–C(3), H–C(5)/H–C(6), and H–C(8)/H–C(9) (*Fig. 3*). The relative configuration of **7** was determined by means of the NOESY experiment (*Fig. 4*), in which key cross-peaks H–C(3)/Me(15), Me(15)/H_{eq}–C(6), H_{eq}–C(6)/H_a–C(13), H_{ax}–C(6)/Me(14), and Me(14)/H–C(8) indicated that HO–C(3), HO–C(4), HO–C(7), Me(14), and H–C(8) were α-oriented, and Me(15) and H–C(5) were β-oriented. Thus, **7** was determined as 3α,4α,7α-trihydroxyeremophil-11(13)-en-12,8β-olide and named elaeicolalactone.

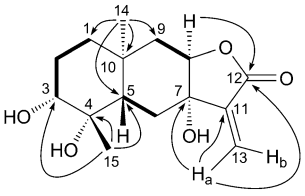


Fig. 3. *Key COSY* (—) *correlations and HMBCs* (H→C) *of* **7**

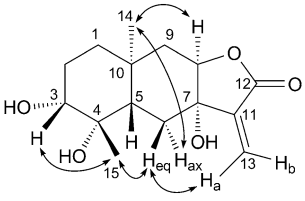


Fig. 4. *Key NOESY* (↔) *correlations of* **7**

The effects of **1**–**7** on the inhibition of NO production in lipopolysaccharide (LPS)-activated murine macrophage RAW264.7 cells were evaluated. All these compounds inhibited NO production, detected as nitrite in the culture medium, in activated

macrophages to various degrees without affecting the cellular viability. As shown in *Fig. 5*,*a*, the E_{max}, the mean maximum inhibitory effect, was evaluated at a concentration of 100 μM and expressed as a percentage inhibition of nitrite production induced by LPS (200 ng/ml) in the presence of vehicle for 24 h. Of all compounds tested, **2** possessed a significant activity with the E_{max} and median inhibitory concentration (IC_{50}) values of 93.3 ± 0.5% and 79.3 ± 0.4 μM, respectively. The inhibitory effects were compared with those of the reference compounds, aminoguanidine, a selective iNOS inhibitor, and N^{ω}-nitro-L-arginine (L-NNA), a non-selective NOS inhibitor. Under the same conditions, aminoguanidine exhibited an E_{max} of 85.7 ± 0.2% and an IC_{50} value of 27.5 ± 0.4 μM. L-NNA revealed an E_{max} value of 47.3 ± 0.7% and an IC_{50} value of 145.2 ± 16.7 μM, similar to our previous report [8]. The other compounds are only marginally active. It is noteworthy that compounds **1** and **2** exhibit strongly different activities, although they possessed the same C-atom skeleton.

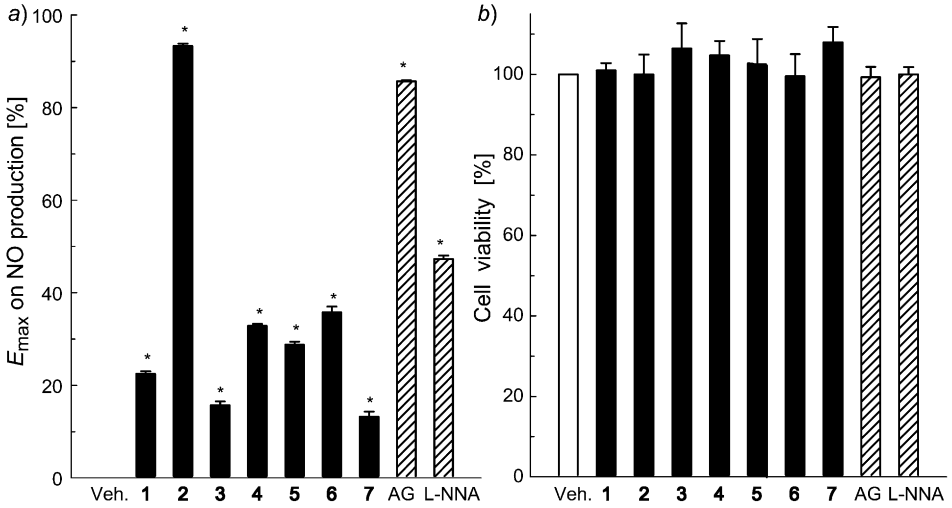


Fig. 5. *The* E_{max} *on NO production* (*a*) *and cell viability* (*b*) *of compounds* **1**–**7**, *and two positive controls, aminoguanidine* (AG) *and* N^{ω}*-nitro-*L*-arginine* (L-NNA) *in LPS-activated RAW 264.7 cells.* Cells were co-incubated with LPS (200 ng/ml) and various compounds (100 μM) or vehicle (0.1%, DMSO) for 24 h. $n = 5–6$ in each group. *: $P < 0.05$ when compared with vehicle-treated cells.

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Experimental Part

General. Optical rotation: *JASCO P-1020* polarimeter (Tokyo, Japan). UV Spectra: *Thermo Helios α* spectrophotometer (Waltham, USA). IR Spectra: *JASCO FT/IR 4100* spectrometer (Tokyo, Japan). ^{1}H- and ^{13}C-NMR: *Bruker DRX-500 SB* spectrometer (D-Ettlingen). LR- and HR-MS: *VG Platform Electrospray* ESI/MS (*VG*, England) and *Waters Synapt* high-definition mass spectrometer (Miford, MA, USA), resp.

Fermentation of Stilbohypoxylon elaeicola. *Stilbohypoxylon elaeicola* (HENN.) L. E. PETRINI strain No. YMJ173 was isolated by one of us (*Y.-M. J.*) from a specimen collected by Dr. *Sabine Huhndorf*, Field Museum, Chicago, USA, in French Guiana. This strain was deposited with the Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan. The mycelium of *S. elaeicola* YMJ173 was inoculated into 1-l *Erlenmeyer* flasks, each containing 10 g of *Bacto*™ malt extract (*Becton, Dickinson and Company*, Sparks, USA) and 500 ml of deionized H_2O. The fermentation was conducted at 25–30° for 30 d.

Extraction and Isolation. The filtered fermented broth (90 l) of *S. elaeicola* YMJ173 was partitioned three times with 50 l of recycled AcOEt, then concentrated in vacuum to dryness (13.0 g). Subsequently, this residue was re-dissolved in 25 ml of MeOH and applied onto a *Sephadex LH-20* column (3 cm i.d. × 65 cm) eluted by MeOH with a flow rate of 2.5 ml/min. Each subfraction (23 ml) collected was checked for its compositions by TLC with AcOEt/AcOH/H_2O 85 : 10 : 10 (*v*/*v*/*v*) for development. Compounds were detected by dipping the plate in vanillin–H_2SO_4 and heating [8]. Subsequently, subfractions were combined into five portions *I–V. Portion II* (*Subfrs. 11* and *12*; 560 mg) was purified by HPLC on a semi-prep. reversed-phase (RP) column (*Biosil PRO-ODS-U*, 10 × 250 mm, 5 μM, *Biotic Chemical Co.*, Taipei, Taiwan) with MeOH/H_2O 7 : 3 (*v*/*v*); 2 ml/min, to afford **5** (52.8 mg; t_R 11.7 min), **6** (7.2 mg; t_R 16.0 min), and **7** (4.9 mg; t_R 8.5 min). *Portion III* (*Subfrs. 13–17*, 875 mg) was purified by HPLC on the same column with MeOH/H_2O 4 : 1 (*v*/*v*); 2 ml/min to give **1** (9.6 mg; t_R 18.5 min), **2** (5.0 mg; t_R 20.9 min), **3** (4.9 mg; t_R 24.1 min), and **4** (24.0 mg; t_R 22.5 min).

Monosaccharide Composition Analysis of **1**–**6**. Compounds **1**–**6** (each 2 mg) were treated with 8M CF_3COOH (TFA) at 110° for 4 h to give an acid hydrolysate. The acid hydrolysate was then vacuum-evaporated to remove the residual TFA. Subsequently, the hydrolysate was added to 2 ml of 16 mM NaOH, and this soln. was analyzed by high pH anion-exchange chromatography–pulsed amperometric detection (HPAEC-PAD, *DIONEX ICS 3000*, Sunyvale, CA, USA) [9]. HPAEC-PAD Analysis was performed on a *Carbo Pac PA10* column (2 i.d. × 250 mm) with 16 mM NaOH as the eluent at a flow rate of 0.25 ml/min. Three authentic monosaccharides including glucose, mannose, and galactose were used as standards. When compared with the standards, the monosaccharide composition of **1**–**6** was confirmed to be mannopyranose. Under above conditions, the t_R value of the mannopyranose was 11.3 min.

Optical Rotation of the Mannopyranose in **1**. Compound **1** (2 mg) was hydrolyzed with 1M H_2SO_4 (2 ml) at r.t. overnight. The mixture was then partitioned with AcOEt (2 × 2 ml). The lower layer was neutralized with 2 ml of 1M $Ba(OH)_2$ and filtered through glass wool, and the filtrate was evaporated to give D-mannose: $[\alpha]_D^{23} = +11.1$ ($c = 0.1$, H_2O).

Nitrite Determination and Cell Viability Assay. The methods were essentially the same as reported in [10]. To assess the effects on LPS-induced NO production, compounds **1**–**7** (purity > 98% as checked by their ^{1}H-NMR), both reference inhibitors aminoguanidine (a specific inhibitor of iNOS) and N^{ω}-nitro-L-arginine (L-NNA, a non-selective NOS inhibitor), or vehicle (0.1%, DMSO) were added in the presence of LPS (200 ng/ml) to the RAW 264.7 cells for 24 h. The nitrite concentration in the culture medium was determined spectrophotometrically as an index of NO production. Maximum inhibition (E_{max}) is expressed as the percentage inhibition at 100 μM calculated *vs.* vehicle plus LPS-treated cells. Both reference inhibitors were purchased from *Sigma–Aldrich Chemical Co.*, and the purity of each compound was more than 98%. A redox indicator, alamarBlue, was used to determine cytotoxicity as reported in [11]. After the removal of culture supernatant for nitrite measurement as described above, a soln. of 10% alamarBlue in culture medium was added to each well containing RAW264.7 cells. The plates were incubated at 37° in a humidified 5% CO_2 atmosphere for 3 h. Following incubation, the absorbance of the alamarBlue was read spectrophotometrically at dual wavelengths of 570 and 600 nm. The absorbance in cultures treated with LPS plus vehicle was regarded as 100% cell viability.

Statistical Analyses. Comparisons of the concentration and treatment effects were conducted with ANOVA, followed by *post hoc* comparisons by *Newman–Keuls* test as appropriate. The average IC_{50} value was determined by data fitting with GraFit (Erithacus Software, UK).

7α-Hydroxy-16-(α-D-mannopyranosyloxy)norisopimar-8(14)-ene 4-Hydroperoxide (*=2-[(2*R,*4a*R, *4b*R,*8*R,*8a*R,*10*R*)-2,3,4,4a,4b,5,6,7,8,8a,9,10-Dodecahydro-8-hydroperoxy-10-hydroxy-2,4b,8-trimethyl-phenanthren-2-yl]ethyl α-D-Mannopyranoside*; **1**). Amorphous white powder. $[\alpha]_D^{24} = -10.8$ ($c = 0.5$, MeOH). IR (KBr): 3348, 2933, 2868, 1643, 1445, 1377, 1126, 1084, 1038, 974, 868, 807. ^{1}H-NMR

(500 MHz): see *Table 2*. ^{13}C-NMR (125 MHz): see *Table 1*. ESI-MS: 509 ($[M+Na]^+$). HR-ESI-MS: 509.2721 ($[M+Na]^+$, $C_{25}H_{42}NaO_9^+$; calc. 509.2727).

*16-(α-*D*-Mannopyranosyloxy)norisopimar-7-en-4-ol* (*=2-[(2*S*,4a*S*,4b*R*,8*R*,8a*R*)-1,2,3,4,4a,4b,5,6,7,8,8a,9-Dodecahydro-8-hydroxy-2,4b,8-trimethylphenanthren-2-yl]ethyl α-*D*-Mannopyranoside*; **2**). Amorphous white powder. $[\alpha]_D^{24} = +47.2$ ($c = 0.5$, MeOH). IR (KBr): 3402, 2925, 1681, 1523, 1456, 1198, 1131, 1079, 821, 685. ^{1}H-NMR (500 MHz): see *Table 2*. ^{13}C-NMR: see *Table 1*. ESI-MS: 453 ($[M-H]^-$). HR-ESI-MS: 453.2853 ($[M-H]^-$, $C_{25}H_{41}O_7^-$; calc. 453.2852).

*16-(α-*D*-Mannopyranosyloxy)isopimar-6,8(14)-dien-19-oic Acid* (*=(13α)-16-(α-*D*-Mannopyranosyloxy)pimara-6,8(14)-dien-19-oic Acid*; **3**). Amorphous white powder. $[\alpha]_D^{21} = +12.2$ ($c = 0.5$, MeOH). UV (MeOH): 241 (4.3). IR (KBr): 3375, 2934, 1689, 1461, 1381, 1203, 1187, 1134, 1086, 1058, 1027, 974, 799. ^{1}H-NMR (500 MHz): see *Table 2*. ^{13}C-NMR (125 MHz): see *Table 1*. ESI-MS: 479 ($[M-H]^-$). HR-ESI-MS: 479.2655 ($[M-H]^-$, $C_{26}H_{39}O_8^-$; calc. 479.2645).

3α,4α,7α-Trihydroxyeremophil-11(13)-en-12,8β-olide (*=(3a*S*,4a*S*,7*R*,8*S*,8a*S*,9a*R*)-Dodecahydro-7,8,9a-trihydroxy-4a,8-dimethyl-1-methylidene-2*H*-cyclopenta[*b*]naphthalen-2-one*; **7**). Amorphous white powder. $[\alpha]_D^{24} = -36.0$ ($c = 0.55$, MeOH). UV (MeCN): 198 (3.8). IR (KBr): 3402, 2925, 1753, 1666, 1453, 1394, 1300, 1277, 1228, 1124, 1070, 1032, 1001, 908, 623. ^{1}H-NMR (500 MHz): see *Table 2*. ^{13}C-NMR (125 MHz): see *Table 1*. ESI-MS: 281 ($[M-H]^-$). HR-ESI-MS: 281.1394 ($[M-H]^-$, $C_{15}H_{21}O_5^-$; calc. 281.1389).

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