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Sesquiterpene Glycosides from *Cosmospora joca*

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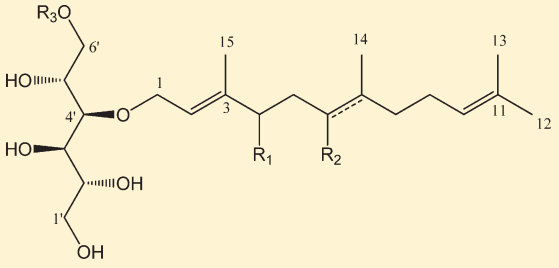
Supporting Information

ABSTRACT: Five novel sesquiterpene glycosides, namely, cosmosporasides A−E (**1**−**5**), were isolated from the ethyl acetate extract of the fermented broth of *Cosmospora joca*. The structures of **1**−**5** were elucidated on the basis of spectroscopic data analyses, monosaccharide composition analyses, and monosaccharide chirality analyses. The relative configuration of the sesquiterpene moiety of **3**−**5** was determined by *J*-based configuration analyses and supported by NOESY assignments. The inhibitory effects of **1**−**5** on the nitric oxide (NO) production in lipopolysaccharide (LPS)-activated murine macrophage RAW264.7 cells were evaluated; all except **3** inhibited NO production at 100 μM. **4** was the most potent, with an average maximum inhibition and a median inhibitory concentration value of 71.70 ± 1.23% and 65.98 ± 0.53 μM, respectively.



Cosmospora joca (Samuels) Rossman & Samuels (Nectriaceae), a fungicolous species, is rarely encountered and has been reported only in Brazil[1] and Taiwan.[2] In Taiwan, it was found on the stromata of *Biscogniauxia* spp.[2] Previous chemical investigations on *Cosmospora* spp. afforded several antibiotics including cephalochromin[3] and parnafungins[4−6] together with a protein tyrosine phosphatase 1B inhibitory compound, namely, aquastatin A.[7] However, the secondary metabolites of *C. joca* have not been studied thus far. Our preliminary pharmacological experiments demonstrated that the ethyl acetate extract of the fermented broth of *C. joca* 89041201 at a concentration of 100 μg/mL significantly inhibited murine macrophage production of the inflammatory mediator nitric oxide (NO) without any cytotoxicity against RAW264.7 cells. In an attempt to disclose the bioactive principles of the fermented broth of this fungus, a series of fermentation, extraction, isolation, and structural elucidation experiments were undertaken, which resulted in the characterization of five novel sesquiterpene glycosides, **1**−**5**. This paper addresses the isolation and spectroscopic analysis of these compounds. Their NO production-inhibitory activities in cultured RAW 264.7 macrophages stimulated with bacterial lipopolysaccharides (LPS) were evaluated.

RESULTS AND DISCUSSION

An ethyl acetate extract of the fermented broth of *C. joca* 89041201 was dried to give a brown residue. Sephadex LH-20 column separation of this sample followed by HPLC purification afforded five sesquiterpene glycosides, **1**−**5**.

Compound **1** was obtained as an amorphous white solid, and its IR absorptions at 3401 and 1636 cm^{-1} indicated the presence of hydroxy groups and double bonds, respectively. Twenty-one carbon resonances observed in the ^{13}C NMR spectrum coupled with the DEPT spectrum of **1** were attributable to four methyls, seven methylenes, seven methines, and three quaternary carbons (Table 1). On account of the molecular formula, $C_{21}H_{38}O_6$, as assigned by HRESIMS, the double-bond equivalent (DBE) of **1** was determined to be three. This corresponded to distinctive features of three sets of trisubstituted olefinic functionalities at δ_C 122.6 (C-2)/140.6 (C-3), δ_C 125.2 (C-6)/136.2 (C-7), and δ_C 125.4 (C-10)/132.1 (C-11) in the ^{13}C NMR spectrum of **1** (Table 1). The ^{1}H NMR spectrum exhibited signals for four primary methyl groups [δ_H 1.69 (H_3-15), 1.66 (H_3-12), 1.60 (H_3-14), and 1.59 (H_3-13)], four methylene groups [δ_H 2.13 (H_2-5), 2.07 (H_2-9), 2.04 (H_2-4), and 1.97 (H_2-8)], three oxymethylene groups [δ_H 4.22 (H_2-1), 3.83, 3.67 (each 1 H, H_2-1′), and 3.77, 3.66 (each 1H, H_2-6′)], three olefinic methines [δ_H 5.40 (H-2), 5.12 (H-6), and 5.08 (H-10)], and four carbinoyl protons [δ_H 3.79 (H-3′), 3.73 (H-2′), 3.69 (H-4′), and 3.68 (H-5′)] (Table 2). The above assignments were characteristic for a farnesol moiety[8] bearing a C_6 monosaccharide. Due to the complex ^{1}H NMR signals for the carbinols of **1**, its monosaccharide composition was confirmed to be mannitol by a high-pH

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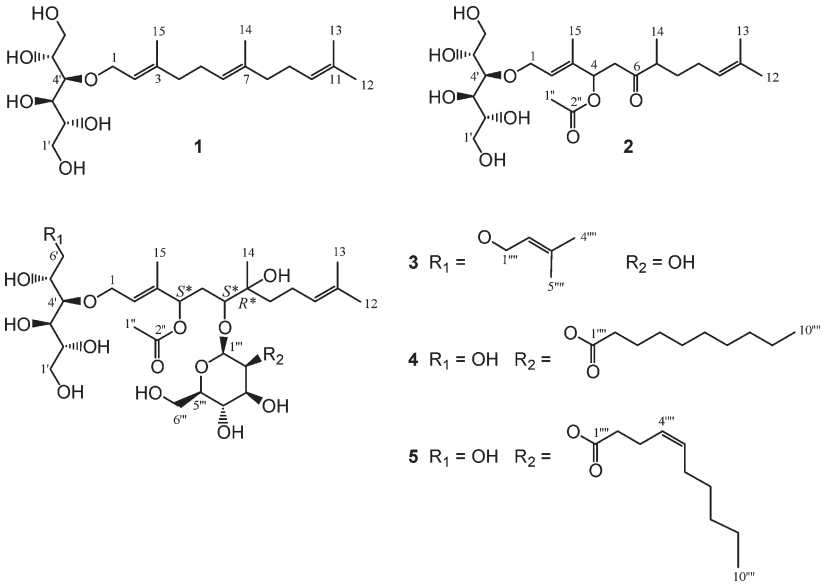
Chart 1



anion exchange chromatography−pulsed amperometric detection (HPAEC-PAD) method.[9] The structure of **1** was further deduced by key COSY (H-2′/H-3′; H-4′/H-5′; H-1/H-2; H-5/H-6; H-9/H-10) and HMBC (H_2-1′/C-2′, -3′; H_2-6′/C-4′, -5′; H-1/C-4′; H-5/C-3, -4; H-9/C-7, -8; H_3-15/C-2, -3, and -4; H_3-14/C-6, -7, and -8; H_3-13/C-10, -11, and -12; H_3-12/C-10, -11, and -13) correlations (Figure 1). The configurations of both Δ^2 and Δ^6 were shown to be *E* from the δ_C of C-14 and C-15 at 16.1 and 16.6 ppm, respectively, in contrast to those of the *Z* configurations at around δ_C 25.0.[10] Accordingly, the structure of **1** was assigned as a (2*E*,6*E*)-farnesol moiety with its C-1 linked with C-4′ of a mannitol via an ether functionality as shown, namely, cosmosporaside A.

The molecular formula for **2**, $C_{23}H_{40}O_9$, was determined by ^{13}C NMR and HRESIMS data. Its IR absorption bands at 3418, 1732, 1708, and 1651 cm^{-1} indicated the presence of hydroxyls, an ester carbonyl, a ketone carbonyl, and double bonds, respectively. When the ^1H and ^{13}C NMR spectra of **2** were compared with those of **1**, major differences involved two methines [δ_H 6.01 (H-4), 2.61 (H-7); δ_C 74.6 (C-4), 46.7 (C-7)], an acetoxy [δ_H 1.99 (H_3-1″); δ_C 21.4 (C-1″), 170.2 (C-2″)], and a ketone (δ_C 211.2, C-6) in **2**, instead of a methylene [δ_H 2.04 (H_2-4); δ_C 40.7 (C-4)] and a Δ^6 [δ_H 5.12 (H-6); δ_C 125.2 (C-6), 136.2 (C-7)] in **1** (Tables 1 and 2). These data revealed that the methylene of C-4 in **1** was assigned as an oxygenated methine and bearing an acetoxy in **2**, as corroborated by cross-peaks of H_3-15/C-4 and H-4/C-2″ in the HMBC spectrum of **2**, and a double bond at Δ^6 in **1** was a ketone (C-6) and a methine (C-7) in **2**, as confirmed by cross-peaks of H_3-14/C-6 and H_3-14/C-7 in the same experiment (Figure 2). The monosaccharide composition and configuration of the Δ^2 in **2** were deduced to be the same as those of **1** on the basis of the monosaccharide composition analysis together with the ^{13}C NMR assignments (Table 1). After considering all the spectroscopic data of **2**, its structure was thus determined as shown and was named cosmosporaside B.

Compound **3**, with the molecular formula $C_{34}H_{60}O_{15}$, was assigned as a derivative of **2** from its spectroscopic data. Its ^1H NMR data were compatible with those of **2** except an extra set of resonances for a monosaccharide [δ_H 5.03 (H-1‴), 4.88 (H-2‴), 4.56 (H-4‴), 4.59, 4.32 (each 1H, H_2-6‴), 4.25 (H-3‴), and 3.94 (H-5‴)] as well as an additional hemiterpene moiety [δ_H 5.49 (H-2⁗), 4.15 (H-1⁗), 1.66 (H-4⁗), and 1.58 (H-5⁗)] (Table 2), which was also reflected in its ^{13}C NMR data (Table 1), as supported by the HSQC experiment of **3**. The extra monosaccharide of **3** was determined to be D-mannopyranose by means of sugar composition analysis of the acid hydrolysate of **3** and subsequent sugar configuration analysis of the derivatized manno-naphthimidazole (manno-NAIM)[11] and by comparison of the ^1H and ^{13}C NMR data with the literature.[12,13] The orientation of the anomeric hydroxyl group was deduced to be β as shown from the mutual NOE correlations between H-1‴, H-3‴, and H-5‴, indicating the axial orientations of H-1‴, -3‴, and -5‴ (Figure 3). The connectivities of the β-D-mannopyranose and hemiterpene moieties with the main structure were elucidated by the interpretations of the HMBC spectrum of **3**, where the anomeric H-1‴ signal at δ_H 5.03 correlated with the oxygenated C-6 resonance at δ_C 88.7, and the oxymethylene signal H-1⁗ at δ_H 4.15 interacted with the C-6′ resonance of the mannitol at δ_C 73.5 (Figure 3). Analysis of the relative stereochemistry of the C-3−C-8 segment of **3** was approached using the $^3J_{H,H}$, long-range $J_{C,H}$, and NOESY correlations.[14,15] The $^3J_{H,H}$ coupling constants were extracted from the E.COSY experiment,[16] and the measurement of heteronuclear coupling constants $^{2,3}J_{C,H}$ relied on analysis of the G-BIRD$_{R,X}$-HSQMBC spectrum.[17] As shown in Figure 4, $^3J_{H\text{-}4,H\text{-}5b}$ (9.1 Hz) and $^3J_{H\text{-}5a,H\text{-}6}$ (9.5 Hz) revealed values that are typical of an anti-orientation of a 1,3-methine system,[15] while the small coupling constants observed for $^3J_{H\text{-}4,H\text{-}5a}$ (2.4 Hz) and $^3J_{H\text{-}5b,H\text{-}6}$ (1.7 Hz) suggested both H-4/H-5a and H-5b/H-6 adopted a gauche conformation. With regard to the C-4−C-5 bond, the values for $^3J_{C\text{-}3,H\text{-}5a}$ (3.2 Hz), $^3J_{C\text{-}3,H\text{-}5b}$ (0−2 Hz), $^3J_{C\text{-}6,H\text{-}4}$ (3.3 Hz), $^2J_{C\text{-}4,H\text{-}5a}$ (−2.9 Hz), and $^2J_{C\text{-}4,H\text{-}5b}$ (−5.9 Hz) indicated that both H-5a and H-5b were gauche to C-3, and H-5a was anti to the acetoxy functionality. With respect to the C-5−C-6 bond, the values of $^3J_{C\text{-}7,H\text{-}5a}$, $^3J_{C\text{-}7,H\text{-}5b}$, $^3J_{C\text{-}4,H\text{-}6}$, $^2J_{C\text{-}6,H\text{-}5b}$, and $^2J_{C\text{-}4,H\text{-}5a}$ were measured to be 2.3, 0−2, 0−2, −2.6, and −6.3 Hz, respectively, indicating that both H-5a and H-5b were gauche to

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Table 1. ^{13}C NMR Spectroscopic Data for Compounds 1−5

no.	1[a,d]	2[b,d]	3[c,d]	4[c,d]	5[c,d]
1	69.8 t	69.1 t	69.2 t	69.4 t	69.4 t
2	122.6 d	125.9 d	126.0 d	125.2 d	125.1 d
3	140.6 s	136.2 s	136.9 s	137.4 s	137.4 s
4	40.7 t	74.6 d	76.0 d	75.7 d	75.7 d
5	27.4 t	45.2 t	36.6 t	36.7 t	36.7 t
6	125.2 d	211.2 s	88.7 d	88.5 d	88.4 d
7	136.2 s	46.7 d	73.8 s	73.8 s	73.8 s
8	40.8 t	33.5 t	37.2 t	37.6 t	37.6 t
9	27.8 t	26.4 t	22.8 t	22.9 t	22.9 t
10	125.4 d	124.9 d	126.7 d	126.7 d	126.6 d
11	132.1 s	132.5 s	130.1 s	131.2 s	131.2 s
12	25.9 q	26.2 q	26.2 q	26.3 q	26.3 q
13	17.7 q	18.1 q	18.2 q	18.2 q	18.2 q
14	16.1 q	16.5 q	24.3 q	24.1 q	24.0 q
15	16.6 q	13.6 q	13.3 q	13.9 q	13.9 q
1′	65.4 t	66.1 t	66.2 t	66.2 t	66.2 t
2′	71.2 d	73.1 d	73.0 d	73.1 d	73.1 d
3′	72.9 d	73.4 d	73.1 d	73.4 d	73.4 d
4′	78.6 d	79.7 d	79.6 d	79.8 d	79.8 d
5′	72.6 d	73.2 d	71.5 d	73.3 d	73.3 d
6′	64.7 t	65.1 t	73.5 t	65.1 t	65.1 t
1″		21.4 q	21.4 q	21.5 q	21.5 q
2″		170.2 s	170.9 s	171.0 s	171.0 s
1‴			104.8 d	102.5 d	102.4 d
2‴			72.9 d	73.6 d	73.9 d
3‴			76.4 d	74.0 d	74.0 d
4‴			69.5 d	69.5 d	69.5 d
5‴			79.7 d	79.7 d	79.7 d
6‴			63.3 t	63.1 t	63.1 t
1⁗			68.5 t	174.0 s	173.5 s
2⁗			132.0 d	26.0 t	35.3 t
3⁗			136.4 s	35.3 t	23.8 t
4⁗			26.1 q	29.9−30.2 t	128.8 d
5⁗			18.4 q	29.9−30.2 t	131.7 d
6⁗				29.9−30.2 t	27.8 t
7⁗				29.9−30.2 t	30.0 t
8⁗				32.5 t	32.0 t
9⁗				23.4 t	23.2 t
10⁗				14.7 q	14.7 q

[a] Measured in methanol-d_4 (125 MHz). [b] Measured in pyridine-d_5 (200 MHz). [c] Measured in pyridine-d_5 (125 MHz). [d] Multiplicities were obtained from DEPT experiments.

C-7, and H-5b was anti to the mannopyranose moiety. Concerning the C-6−C-7 bond, the coupling constants of $^3J_{C\text{-}8,H\text{-}6}$ (4.8 Hz), $^3J_{C\text{-}14,H\text{-}6}$ (2.7 Hz), and $^2J_{C\text{-}7,H\text{-}6}$ (−4.5 Hz) accompanied by the correlations of H_3-14/H-5b and H_3-14/H-6 in the NOESY spectrum of **3** corroborated that C-8 was anti to H-6, and both H-6 and C-5 were gauche to C-14. Assembling the above evidence unambiguously established the relative configurations of C-4, C-6, and C-7 to be *S**, *S**, and *R**, respectively. Thus, **3** was assigned conclusively as the shown structure and was named cosmosporaside C.

Compound **4** was assigned a molecular formula of $C_{39}H_{70}O_{16}$ by HRESIMS and ^{13}C NMR. Its IR absorptions at 3397 (hydroxy), 1734, 1717 (carbonyl), and 1647 (double bond) cm^{-1} indicated that **4** adopted a skeleton analogous to that of **3**. The ^{13}C NMR and DEPT spectra of **4** were consistent with those of **3**, with the exception of the disappearance of the C_5 hemiterpene signals (C-1⁗−C-5⁗), and 10 additional signals ascribable to an aliphatic chain (C-1⁗−C-10⁗) linked by an ester functionality (δ_C 174.0, C-1⁗) were observed (Table 1). In the ^{1}H NMR spectrum of **4**, the signals corresponding to the sesquiterpene (H-1−H_3-15), mannitol (H_2-1′−H_2-6′), acetyl (H_3-1″), and mannopyranose (H-1‴−H_2-6‴) were closely related to those of **3**, except that the H-2‴ signal shifted from higher field (δ_H 4.88) to lower field (δ_H 6.42), and nine signals in the region δ_H 2.48−0.86 were also attributed to the resonances of the saturated aliphatic chain (H-2⁗−H-10⁗) (Table 2). The location of the aliphatic chain was deduced to be at C-2‴ by the distinctive cross-peak of H-2‴/C-1⁗ in the HMBC spectrum of **4**, corresponding to the downfield shift of H-2‴. The chiralities of C-4, -6, and -7 were determined to be the same as those of **3** by the same *J*-based configuration analysis as described above. Accordingly, the structure of **4** was characterized as shown and was named cosmosporaside D.

The molecular formula for **5**, $C_{39}H_{68}O_{16}$, was determined by ^{13}C NMR and HRESIMS, and its molecular weight was only 2 amu less than that of **4**, suggesting the existence of an additional double bond in **5**. When comparing the ^{1}H and ^{13}C NMR spectra of **5** with those of **4** (Tables 1 and 2), differences involved only two olefinic methines [δ_H 5.52 (H-4⁗) and 5.44 (H-5⁗); δ_C 128.8 (C-4⁗) and 131.7 (C-5⁗)] in **5** instead of two methylenes [δ_H 1.19 or 1.30 (H-4⁗, -5⁗); δ_C 29.9−30.2 (C-4⁗, -5⁗)] in **4**. In the HMBC spectrum of **5** (Figure 4), the cross-peaks of H-5⁗/C-6⁗, H-5⁗/C-3⁗, H-5⁗/C-7⁗, H-4⁗/C-3⁗, and H-2⁗/C-4⁗ indicated the double bond was located at Δ^4. The configuration of Δ^4 was determined to be cis as judged from the $^3J_{H4,H5}$ (10.3 Hz) as well as the cross-peaks of H-4⁗/H-5⁗ and H-3⁗/H-6⁗ in the NOESY spectrum of **5** (Figure 5). Conclusively, **5** was identified as shown and was named cosmosporaside E.

To assess the anti-inflammatory effects of these sesquiterpene glycosides, **1**−**5** were tested for their effect on NO production in LPS-activated macrophages. Without LPS, RAW264.7 cells released undetectable levels of NO, measured as nitrite concentration, after 24 h incubation (data not shown). When LPS (200 ng/mL) was added to RAW 264.7 cells, NO production was dramatically increased to 35−40 μM for the 24 h incubation period. Vehicle did not affect the NO production induced by LPS. The significant inhibitory effects on NO production have been shown for the test compounds at a concentration of 100 μM except **3**. Compound **1** appeared to inhibit NO production with an average maximum inhibition (E_{max}) value of 54.52 ± 0.93%, but this effect was comparable with its cytotoxicity. Of all the compounds tested, **4** was the most potent. When **4** (0.1 to 100 μM) was combined with LPS for 24 h, this compound significantly attenuated NO production in a concentration-related manner, with an E_{max} and an IC_{50} value of 71.70 ± 1.23% and 65.98 ± 0.53 μM, respectively.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-2000 polarimeter (Tokyo, Japan). ^{1}H and ^{13}C NMR spectra were acquired on a Bruker DRX-500 SB and an AVIII-800 spectrometer (Ettlingen, Germany). Low- and high-resolution mass spectra were obtained using a VG Platform Electrospray ESI/MS

Table 2. ^{1}H NMR Spectroscopic Data for Compounds 1−5 [δ in ppm, mult. (J in Hz)]

no.	1[a,d]	2[b,d]	3[c,d]	4[c,d]	5[c,d]
1	4.22 d (6.3)	4.73 d (6.2)	4.67 d (6.2)	4.78 d (6.3)	4.78 d (6.3)
2	5.40 t (6.3)	6.07 t (6.2)	5.95 t (6.2)	6.26 t (6.3)	6.25 t (6.3)
3					
4	2.04 t (7.9)	6.01 dd (9.3, 3.4)	5.77 br d (10.4)	5.99 br d (9.9)	5.98 br d (9.8)
5	2.13 m	2.80 dd (17.1, 3.4)	1.90 m	2.00 m	1.99 m
		3.05 dd (17.1, 9.3)	2.16 m	2.14 m	2.14 m
6	5.12 t (6.8)		4.06 d (10.0)	4.03 d (10.2)	4.04 d (10.6)
7		2.61 m			
8	1.97 t (7.1)	1.40 m	1.56 m	1.66 m	1.65 m
		1.82 m	2.12 m	2.09 m	2.06 m
9	2.07 m	2.03 m	2.29 m	2.33 m	2.32 m
			2.47 m	2.52 m	2.50 m
10	5.08 t (7.1)	5.16	5.14 t (6.6)	5.27 t (6.9)	5.26 t (7.0)
11					
12	1.66 s	1.68 s	1.74 s	1.71 s	1.70 s
13	1.59 s	1.59 s	1.59 s	1.63 s	1.63 s
14	1.60 s	1.09 d (7.0)	1.34 s	1.34 s	1.34 s
15	1.69 s	1.70 s	1.67 s	1.82 s	1.82 s
1′	3.67	4.38 dd (10.9, 5.9)	4.38 dd (10.8, 6.0)	4.38 dd (10.9, 5.9)	4.38 dd (10.5, 5.2)
	3.83	4.59 dd (10.9, 2.9)	4.61	4.59 dd (10.9, 3.1)	4.59 dd (10.5, 3.0)
2′	3.73	4.50 m	4.49 m	4.54	4.53
3′	3.79	4.68	4.68	4.69	4.68
4′	3.69	4.66	4.55	4.66	4.66
5′	3.68	4.74 d (9.3)	4.71 d (8.4)	4.74 d (9.2)	4.74 d (9.6)
6′	3.66	4.29 dd (11.0, 5.4)	4.00	4.33	4.31
	3.77	4.41 dd (11.0, 3.8)	4.02	4.42	4.41
1″		1.99 s	2.02 s	2.01 s	2.01 s
2″					
1‴			5.03 s	5.21 s	5.21 s
2‴			4.88 d (2.8)	6.42 d (3.1)	6.42 d (2.9)
3‴			4.25 dd (9.2, 2.8)	4.43	4.43 dd (9.4, 2.9)
4‴			4.56	4.51	4.50 t (9.4)
5‴			3.94 m	3.98 m	3.98 m
6‴			4.32 dd (11.4, 6.2)	4.36	4.35
			4.59	4.61	4.62
1⁗			4.15 d (6.5)		
2⁗			5.49 t (6.5)	1.72	2.56 m
3⁗				2.49	2.54 m
4⁗			1.66 s	1.19 or 1.30	5.52 m
5⁗			1.58 s	1.19 or 1.30	5.44 dt (10.3, 7.5)
6⁗				1.19 or 1.30	1.98
7⁗				1.19 or 1.30	1.27
8⁗				1.19	1.19
9⁗				1.24	1.21
10⁗				0.86 t (7.2)	0.84 t (6.8)

[a] Measured in methanol-d_4 (500 MHz). [b] Measured in pyridine-d_5 (800 MHz). [c] Measured in pyridine-d_5 (500 MHz). [d] Signals without multiplicity were overlapped and were picked up from COSY or HMBC spectra.

(VG, England) and a high-definition mass spectrometry system with an ESI interface and a TOF analyzer (Waters Corp., Manchestwe, UK), respectively. IR spectra were recorded on a JASCO FT/IR 4100 spectrometer (Tokyo, Japan). Sephadex LH-20 (Amersham Biosciences, Filial Sverige, Sweden) was used for open column chromatography. TLC was performed using silica gel 60 F254 plates (200 μm, Merck).

Fermentation of *Cosmospora joca* 89041201. *Cosmospora joca* (Samuels) Rossman & Samuels (strain no. 89041201 from Pingtung, Taiwan) was collected, isolated, and identified by one of us (Y.M.J.)[2] and was deposited at the Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan. The mycelium of *C. joca* 89041201 was inoculated into 5 L serum bottles, each containing 60 g of Bacto malt extract (Becton, Dickinson and Company, Sparks, USA) and 3 L of

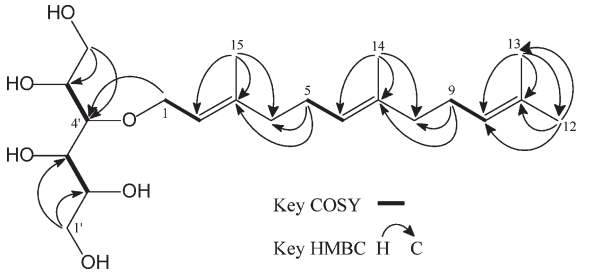


Figure 1. Key COSY and HMBC correlations of **1**.

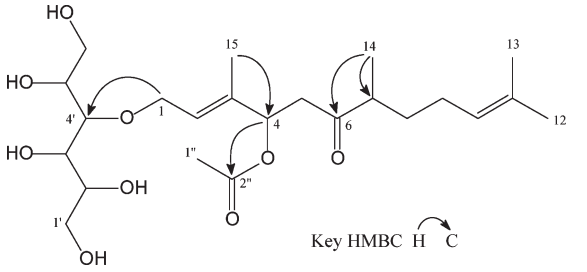


Figure 2. Key HMBC correlations of **2**.

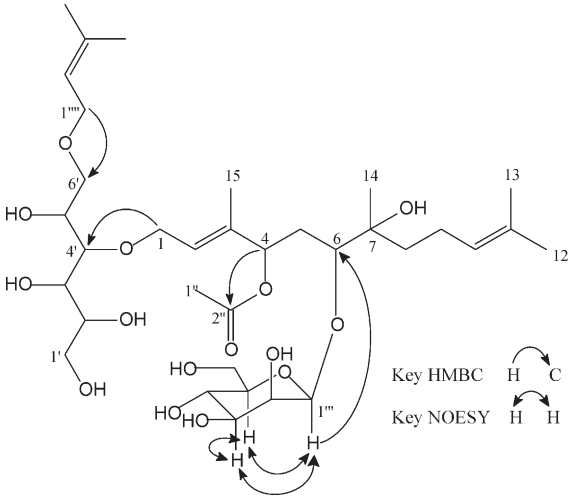
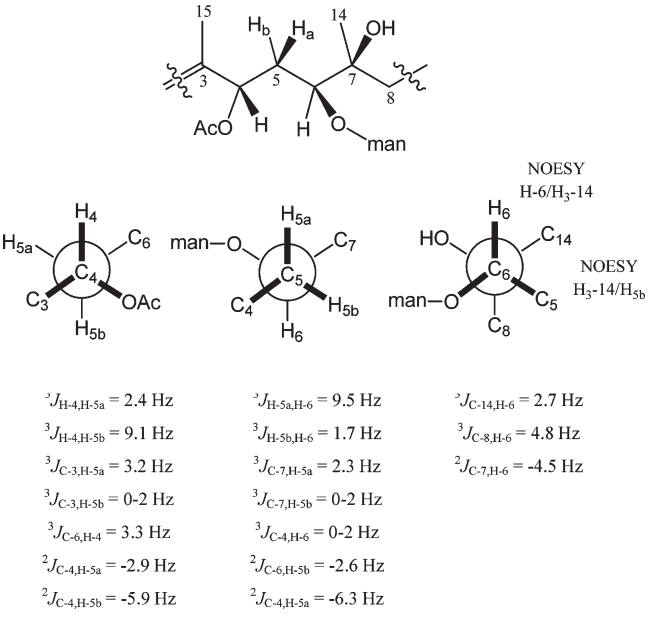


Figure 3. Key HMBC and NOESY correlations of **3**.

deionized water. The fermentation was conducted with aeration at 25−30 °C for 30 days.

Extraction and Isolation. The filtered fermented broth (50 L) of *C. joca* 89041201 was partitioned three times with 50 L of recycled ethyl acetate, then concentrated under vacuum to dryness (3.0 g). Subsequently, the crude extract was redissolved in 20 mL of MeOH, then applied onto a Sephadex LH-20 column (3 cm i.d. × 35 cm) eluted by MeOH−H_2O (4:1, v/v) with a flow rate of 2.5 mL/min. Each subfraction (7.5 mL) collected was checked for its composition by TLC using EtOAc−AcOH−H_2O (85:10:10, v/v/v) for development, and dipping in vanillin−sulfuric acid was used in the detection of compounds with similar skeletons. All fractions were combined into three portions, I−III. Portion II (fr. 11−14) was purified by HPLC on a semipreparative reversed-phase column (Hyperprep HSC18 Thermo Quest, 10 × 250 mm, Bellefonte, CA, USA) with MeOH−H_2O (7:3, v/v) as eluent, 2 mL/min, giving **3** (11.8 mg, t_R = 11.18 min), **5** (7.3 mg, t_R = 23.80 min), and **4** (5.8 mg, t_R = 29.60 min). Portion III (fr. 15−18) was



$^2J_{H\text{-}4,H\text{-}5a}$ = 2.4 Hz
$^3J_{H\text{-}4,H\text{-}5b}$ = 9.1 Hz
$^3J_{C\text{-}3,H\text{-}5a}$ = 3.2 Hz
$^3J_{C\text{-}3,H\text{-}5b}$ = 0-2 Hz
$^3J_{C\text{-}6,H\text{-}4}$ = 3.3 Hz
$^2J_{C\text{-}4,H\text{-}5a}$ = -2.9 Hz
$^2J_{C\text{-}4,H\text{-}5b}$ = -5.9 Hz
$^2J_{H\text{-}5a,H\text{-}6}$ = 9.5 Hz
$^3J_{H\text{-}5b,H\text{-}6}$ = 1.7 Hz
$^3J_{C\text{-}7,H\text{-}5a}$ = 2.3 Hz
$^3J_{C\text{-}7,H\text{-}5b}$ = 0-2 Hz
$^3J_{C\text{-}4,H\text{-}6}$ = 0-2 Hz
$^2J_{C\text{-}6,H\text{-}5b}$ = -2.6 Hz
$^2J_{C\text{-}4,H\text{-}5a}$ = -6.3 Hz
$^2J_{C\text{-}14,H\text{-}6}$ = 2.7 Hz
$^3J_{C\text{-}8,H\text{-}6}$ = 4.8 Hz
$^2J_{C\text{-}7,H\text{-}6}$ = -4.5 Hz

Figure 4. The C-3−C-8 segment and the Newman projections for the C-4−C-5, C-5−C-6, and C-6−C-7 conformations (upper) of **3** deduced by the characteristics of $^3J_{H,H}$, $^3J_{C,H}$, $^2J_{C,H}$ (lower), and NOESY data.

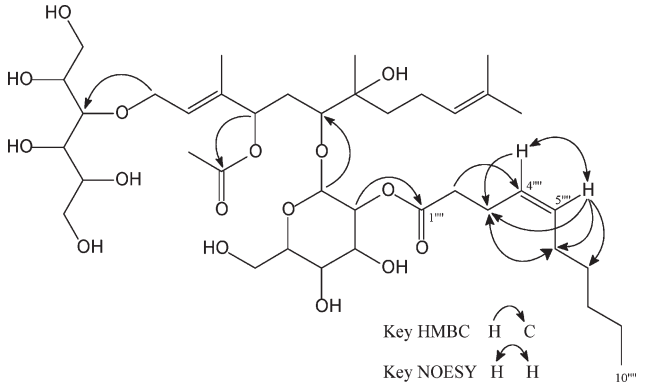


Figure 5. Key HMBC and NOESY correlations of **5**.

purified by HPLC on a semipreparative reversed-phase column (Biosil PRO-ODS-U, 10 × 250 mm, Biotic Chemical Co., Taipei, Taiwan) with MeCN−H_2O (58:42, v/v) as eluent, 2 mL/min, affording **1** (6.3 mg, t_R = 14.98 min) and **2** (3.5 mg, t_R = 8.55 min).

Monosaccharide Composition Analyses of 1−5. Compounds **1**−**5** (each 1 mg) were treated with 4 M trifluoroacetic acid (TFA) at 110 °C for 4 h to give an acid hydrolysate. The acid hydrolysate was then vacuum evaporated to remove the residual TFA. Subsequently, the hydrolysate was mixed with 2 mL of 16 mM NaOH, and this solution was used for DIONEX ICS 3000 high-pH anion exchange chromatography−pulsed amperometric detection (HPAEC-PAD, Sunyvale, CA, USA).[9] HPAEC-PAD analysis was performed on a Carbo Pac PA10 column (2 i.d. × 250 mm) using 16 mM NaOH as the eluent at a flow rate of 0.25 mL/min. Two authentic monosaccharides including mannitol and mannopyranose were used as standards. When compared with the standards, the monosaccharide in **1** and **2** was confirmed to be mannitol, while the monosaccharides in **3**−**5** were determined to be mannitol and mannopyranose. Under the above conditions, the retention times of the mannitol and mannopyranose were 3.25 and 12.80 min, respectively.

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Chirality Analysis of Mannnopyranose in 3−5. Compounds **3**−**5** (each 1 mg) were treated with 4 M TFA at 110 °C for 4 h to give their acid hydrolysates. The acid hydrolysate of each compound was then derivatized with 2,3-naphthalenediamine using molecular iodine as catalyst to obtain the fluorescent naphthimidazole derivative of the mannopyranose (manno-NAIM) as previously reported.[11] The fluorescent product was further applied for chiral resolution using the ligand exchange capillary electrophoresis (LECE) method.[18,19] Electrophoretic experiments were carried out using a Beckman Coulter capillary electrophoresis system (model P/ACE MDQ, USA) equipped with a photodiode array UV detector. The background electrolyte in the electrophoretic experiments was composed of 10% acetonitrile, 200 mM sulfated-α-CD, and 200 mM phosphate buffer (pH 3.4). An uncoated fused-silica capillary (30/40.2 cm × 50 μM) was used. The capillary temperature was kept at 30 °C, and the analyte was detected by UV absorption at 254 nm. The sample loading is set at 40 ppm at 0.5 psi, 3 s for each injection. The D- and L-forms of manno-NAIM were used as standards and were enantioseparated in the above LECE system individually. The retention times of L- and D-form standards were 21.05 and 23.15 min, respectively. Under the same conditions, the retention time of the manno-NAIM originating from **3**−**5** was 23.15 min. Thus, the mannopyranose in **3**−**5** was determined to be the D-form.

*Cosmosporaside A (**1**):* amorphous white solid; $[\alpha]^{24}_{D}$ +5.0 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3401, 2959, 2924, 2855, 1636, 1576, 1457, 1416, 1084, 1039 cm^{-1}; ^{1}H NMR data, see Table 2, and ^{13}C NMR data, see Table 1; ESIMS $[M - H]^-$ *m*/*z* 385; HREIMS $[M - H]^-$ *m*/*z* 385.2592 (calcd for $C_{21}H_{37}O_6$, 385.2590).

*Cosmosporaside B (**2**):* amorphous white solid; $[\alpha]^{27}_{D}$ −1.82 (*c* 0.55, MeOH); IR (KBr) ν_{max} 3418, 2959, 2925, 2852, 1732, 1708, 1651, 1541, 1457, 1257, 1205, 1185, 1136 cm^{-1}; ^{1}H NMR data, see Table 2, and ^{13}C NMR data, see Table 1; ESIMS $[M + Na]^+$ *m*/*z* 483; HRESIMS $[M + Na]^+$ *m*/*z* 483.2564 (calcd for $C_{23}H_{40}O_9Na$, 483.2570).

*Cosmosporaside C (**3**):* amorphous white solid; $[\alpha]^{28}_{D}$ +13.9 (*c* 0.75, MeOH); IR (KBr) ν_{max} 3384, 2965, 2923, 2858, 1732, 1650, 1375, 1242, 1069, 1029 cm^{-1}; ^{1}H NMR data, see Table 2, and ^{13}C NMR data, see Table 1; ESIMS $[M + H]^+$ *m*/*z* 709; HREIMS $[M + H]^+$ *m*/*z* 709.4011 (calcd for $C_{34}H_{61}O_{15}$, 709.4010).

*Cosmosporaside D (**4**):* amorphous white solid; $[\alpha]^{27}_{D}$ +10.5 (*c* 0.80, MeOH); IR (KBr) ν_{max} 3397, 2953, 2925, 2855, 1734, 1717, 1647, 1541, 1375, 1249, 1064, 1035 cm^{-1}; ^{1}H NMR data, see Table 2, and ^{13}C NMR data, see Table 1; ESIMS $[M + H]^+$ *m*/*z* 795; HRESIMS $[M + H]^+$ *m*/*z* 795.4747 (calcd for $C_{39}H_{71}O_{16}$, 795.4742).

*Cosmosporaside E (**5**):* amorphous white solid; $[\alpha]^{26}_{D}$ −1.75 (*c* 0.80, MeOH); IR (KBr) ν_{max} 3378, 2956, 2925, 2855, 1734, 1717, 1653, 1373, 1249, 1062, 1040 cm^{-1}; ^{1}H NMR data, see Table 2, and ^{13}C NMR data, see Table 1; ESIMS $[M + H]^+$ *m*/*z* 793; HRESIMS $[M + H]^+$ *m*/*z* 793.4586 (calcd for $C_{39}H_{69}O_{16}$, 793.4586).

Nitrite Measurement and Cell Viability Assay. The methods were essentially the same as reported previously.[20] To assess the effects on LPS-induced NO production, compounds **1**−**5** (purity >98% as checked by their ^{1}H NMR), the two positive controls aminoguanidine (a specific inhibitor of iNOS) and N^{ω}-nitro-L-arginine (L-NNA, a nonselective NOS inhibitor), or vehicle (0.1%, DMSO) was added in the presence of LPS (200 ng/mL) to the RAW 264.7 cells. Both inhibitors were purchased from Sigma-Aldrich Chemical Co., and the purity of each compound was more than 98%. The nitrite concentration in the culture medium was determined spectrophotometrically as an index of NO production. The Alamar Blue assay was used to examine whether the amount of the test specimens used in this study caused cell damage. Results are expressed as percentage of inhibition calculated versus vehicle plus LPS-treated cells.

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

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra of the new compounds **1**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Samuels, G. J.; Rossman, A. Y.; Lowen, R.; Rogerson, C. T. *Mycol. Pap.* **1991**, *164*, 1–47.
(2) Guu, J. R.; Ju, Y. M.; Shieh, H. J. *Bot. Stud.* **2007**, *48*, 187–203.
(3) Kim, W. G.; Jung, C. G.; Son, M. J. *Repub. Korean Kongkae Taeho Kongbo* Pat. No. KR 20100614, 2010 (CAN 153:106799 AN 2010:762574).
(4) Parish, C. A.; Smith, S. K.; Calati, K.; Zink, D.; Wilson, K.; Roemer, T.; Jiang, B.; Xu, D.; Bills, G.; Platas, G.; Pelaez, F.; Diez, M. T.; Tsou, N.; McKeown, A. E.; Ball, R. G.; Powles, M. A.; Yeung, L.; Liberator, P.; Harris, G. *J. Am. Chem. Soc.* **2008**, *130*, 7060–7066.
(5) Overy, D.; Calati, K.; Kahn, J. N.; Hsu, M.-J.; Martin, J.; Collado, J.; Roemer, T.; Harris, G.; Parish, C. A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1224–1227.
(6) Bills, G. F.; Platas, G.; Overy, D. P.; Collado, J.; Fillola, A.; Jimenez, M. R.; Martin, J.; Gonzales del Val, A.; Vicente, F.; Tormo, J. R.; Pelaez, F.; Calati, K.; Harris, G.; Parish, C.; Xu, D.; Roemer, T. *Mycologia* **2009**, *101*, 449–472.
(7) Seo, C.; Sohn, J. H.; Oh, H.; Kim, B. Y.; Ahn, J. S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6095–6097.
(8) Kempin, U.; Henning, L.; Weizel, P.; Marzian, S.; Müller, D.; Fehlhaber, H.-W.; Markus, A.; van Heijenoort, Y.; van Heijenoort, A. *Tetrahedron* **1995**, *51*, 8471–8482.
(9) Lee, Y. C. *Anal. Biochem.* **1990**, *189*, 151–162.
(10) Yang, S. S.; Wang, G. J.; Wang, S. Y.; Lin, Y. Y.; Kuo, Y. H.; Lee, T. H. *Planta Med.* **2009**, *75*, 512–516.
(11) Lin, C.; Lai, P. T.; Liao, S. K. S.; Hung, W. T.; Yang, W. B.; Fang, J. M. *J. Org. Chem.* **2008**, *73*, 3848–3853.
(12) Kohno, J.; Nishio, M.; Sakurai, M.; Kawano, K.; Hiramatsu, H.; Kameda, N.; Kishi, N.; Yamashita, T.; Okuda, T.; Komatsubara, S. *Tetrahedron* **1999**, *55*, 7771–7786.
(13) Ju, Y. M.; Juang, S. H.; Chen, K. J.; Lee, T. H. *Z. Naturforsch.* **2007**, *62b*, 561–564.
(14) Lu, C. K.; Chen, Y. M.; Wang, S. H.; Wu, Y. Y.; Cheng, Y. M. *Tetrahedron Lett.* **2009**, *50*, 1825–1827.
(15) Hanif, N.; Ohno, O.; Kitamura, M.; Yamada, K.; Uemura, D. *J. Nat. Prod.* **2010**, *73*, 1318–1322.
(16) Griesinger, C.; Sørensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1985**, *107*, 6394–6396.
(17) Williamson, R. T.; Marquez, B. L.; Gerwick, W. H.; Kover, K. E. *Magn. Reson. Chem.* **2000**, *38*, 265–273.

(18) Sundin, N. G.; Dowling, T. M.; Grinberg, N.; Bicker, G. *J. Microcol. Sep.* **1996**, *8*, 323–329.
(19) Aizawa, S.; Yamamoto, A.; Kodama, S. *Electrophoresis* **2006**, *27*, 880–886.
(20) Wang, G. J.; Chen, Y. M.; Wang, T. M.; Lee, C. K.; Chen, K. J.; Lee, T. H. *J. Ethnopharmacol.* **2008**, *118*, 71–78.