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Isochromans and Related Constituents from the Endophytic Fungus *Annulohypoxylon truncatum* of *Zizania caduciflora* and Their Anti-Inflammatory Effects

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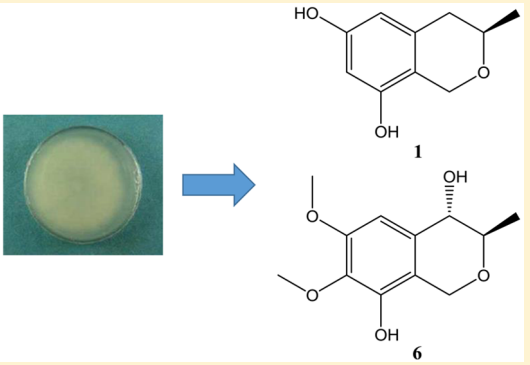
Supporting Information

ABSTRACT: Six new isochroman derivatives (annulohypoxylomans A–C, **1**–**3**; annulohypoxylomanols A and B, **6** and **7**; and annulohypoxyloside, **8**), an isocoumarin (annulohypoxylomarin A, **4**), and an azaphilone derivative (xylariphilone, **5**) were isolated from an ethyl acetate extract derived from cultures of the endophytic fungus JS540 found in the leaves of *Zizania caduciflora*. The JS540 strain was identified as *Annulohypoxylon truncatum*. The structures of the isolated compounds were elucidated by one- and two-dimensional nuclear magnetic resonance and mass spectrometry and by comparison with related compounds from the literature. The anti-inflammatory activities of the isolated compounds were evaluated in lipopolysaccharide (LPS)-stimulated bone marrow-derived dendritic cells. Xylariphilone (**5**) exhibited significant inhibitory effects on LPS-induced interleukin (IL)-6, IL-12 p40, and tumor necrosis factor (TNF)-α production with IC_{50} values of 5.3, 19.4, and 37.6 μM, respectively.



Endophytes are microbes that colonize living, internal tissues of plants without causing any immediate negative effects.[1] The ecological roles and chemical interactions of endophytes are currently under investigation, particularly in relation to their host plants. This is changing rapidly, however, as it is now evident that plants have intricate relationships with an array of fungi, which can lead to an increase in plant vigor, growth, and development as well as changes in plant metabolism.[2,3] Therefore, such beneficial endophytic fungi may be very valuable for the development of more productive and sustainable agricultural practices or for the production of chemicals for pharmacological purposes.[4,5] Moreover, endophytes are a rich and reliable source of genetic diversity and may include previously undescribed species.[6,7] Novel microbes (as defined at the morphological and/or molecular level) are often associated with novel natural products. In an effort to investigate natural compounds with intriguing structures in endophytic fungi, we selected plants from distinct environmental settings. Several endophytes were isolated from the leaves and stems of aquatic reed plants. Among them, a strain of endophyte, *Annulohypoxylon truncatum* (JS540), was isolated from the leaves of *Zizania caduciflora*. A previous report showed that the metabolites of *A. truncatum*, such as truncaquinones, display antibacterial activity.[8] In the course of investigating novel bioactive constituents, eight secondary metabolites were isolated from the ethyl acetate extract of *A. truncatum* (JS540). The anti-inflammatory effects of the isolated compounds on lipopolysaccharide (LPS)-induced expression of the pro-inflammatory cytokines interleukin (IL)-6, IL-12 p40, and tumor necrosis factor (TNF)-α in bone marrow derived dendritic cells (BMDCs) were investigated. To our knowledge, this is the first report on the isochroman components of *A. truncatum* and their anti-inflammatory activity.

Eight secondary metabolites were isolated from an ethyl acetate extract derived the endophytic fungus *A. truncatum*. Their structures were identified as annulohypoxyloman A (**1**), annulohypoxyloman B (**2**), annulohypoxyloman C (**3**), annulohypoxylomarin A (**4**), xylariphilone (**5**),[10] annulohypoxylomanol A (**6**), annulohypoxylomanol B (**7**), and annulohypoxyloside (**8**). Their structures were elucidated by one- and two-dimensional NMR as well as mass spectrometry. Among them, compounds **1**–**3** and **6**–**8** are new compounds, and compound **4** was isolated from nature for the first time.

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Table 1. ^{1}H NMR Data of Compounds 1−4 and 6−8 (600 MHz)[a]

	1[b]	2[c]	3[c]	4[d]	6[b]	7[b]	8[c]
1	5.02, d (14.8)	4.52, d (14.8)	4.55, d (15.0)		5.08, d (15.1)	5.16, d (15.0)	4.52, d (14.9)
	5.44, d (14.8)	4.79, d (14.8)	4.80, d (15.0)		5.39, d (15.1)	5.49, d (15.0)	4.79, d (14.9)
3	3.80, m	3.31, m	3.71, m	4.60, m	3.93, m	3.98, m	3.70, m
4	2.62, dd (16.1, 10.5)	2.45, dd (16.1, 10.5)	2.52, dd (16.1, 10.6)	2.63, dd (16.6, 11.6)	4.81, t (8.0)	4.83, t (8.0)	2.40, dd (16.1, 10.5)
	2.70, dd (16.1, 2.7)	2.50, dd (16.1, 2.8)	2.61, dd (16.1, 3.0)	2.86, dd (16.6, 3.3)			2.63, dd (16.1, 2.8)
5	6.64, br s	6.10, s	6.24, s	6.72, d (8.5)	7.26, s	7.29, s	6.11, s
6				7.20, d (8.5)			
7	6.83, br s						
11	1.32, d (6.1)	1.25, d (6.1)	1.28, d (6.2)	1.47, d (6.3)	1.69, d (6.1)	1.68, d (6.1)	1.26, d (6.2)
6-OMe			3.78, s		3.75, s	3.72, s	
7-OMe		3.72, s	3.73, s		3.83, s		
7-Me				2.11, s			
8-OMe							3.58, s
1′							5.59, d (4.2)
2′							4.21, d (5.5)
3′							4.10, dd (5.8, 1.6)
4′							4.21, d (4.2)
5′							3.56, d (4.2)

[a]Assignments were done by HMQC and HMBC experiments; *J* values (Hz) are in parentheses. [b]Measured in pyridine-d_5. [c]measured in methanol-d_4. [d]measured in chloroform-*d*.

Compound **1** was isolated as a yellow, amorphous powder. The molecular formula was determined to be $C_{10}H_{12}O_3$ by HRESIMS. The ^{1}H NMR data of **1** (Table 1) revealed two aromatic proton signals at δ_H 6.64 (br s, H-5) and 6.83 (br s, H-7), an oxygenated methylene signal at δ_H 5.02 (d, J = 14.8 Hz, H-1a) and 5.44 (d, J = 14.8 Hz, H-1b), an oxygenated methine signal at δ_H 3.80 (m, H-3), a methylene signal at δ_H 2.62 (dd, J = 16.1, 10.5 Hz, H-4a) and 2.70 (dd, J = 16.1, 2.7 Hz, H-4b), and a methyl signal at δ_H 1.32 (d, J = 6.1 Hz, H-11). Correspondingly, the ^{13}C NMR spectrum (Table 2) showed the signals of six aromatic carbons at δ_C 101.7 (C-7), 107.4 (C-5), 114.1 (C-9), 136.7 (C-10), 155.9 (C-8), and 158.7 (C-6),

Table 2. ^{13}C NMR Data of Compounds 1−4 and 6−8 (150 MHz)[a]

	1[b]	2[c]	3[c]	4[d]	6[b]	7[b]	8[c]
1	65.7	65.4	65.9	170.2	65.4	65.7	64.1
3	71.1	72.1	72.1	75.3	76.9	77.2	70.9
4	36.9	36.3	37.1	31.8	71.5	71.6	35.1
5	107.4	107.8	104.7	115.5	102.3	102.1	106.3
6	158.7	149.8	153.4	137.8	153.0	148.8	148.6
7	101.7	134.8	135.9	124.8	136.2	134.4	128.6
8	155.9	147.0	147.5	160.3	147.1	143.0	146.5
9	114.1	114.2	116.2	107.9	117.3	117.4	112.5
10	136.7	130.3	130.7	136.9	135.4	130.3	129.4
11	22.3	21.6	22.1	20.8	19.6	19.6	20.4
6-OMe			56.7		56.1	56.1	
7-OMe		60.9	61.6		60.8		
7-Me				17.9			
8-OMe							62.9
1′							102.5
2′							72.8
3′							70.6
4′							87.2
5′							61.9

[a]Assignments were done by HMQC and HMBC experiments; *J* values (Hz) are in parentheses. [b]Measured in pyridine-d_5. [c]Measured in methanol-d_4. [d]Measured in chloroform-*d*.

an oxygenated methine carbon at δ_C 71.1 (C-3), an oxygenated methylene carbon at δ_C 65.7 (C-1), a methylene carbon at δ_C 36.9 (C-4), and a methyl carbon at δ_C 22.3 (C-11). Thus, both ^{1}H and ^{13}C NMR data demonstrated that **1** is an isochroman derivative. Key HMBC correlations between H-5 (δ_H 6.64)/C-9 (δ_C 114.1) and C-10 (δ_C 136.7) and between H-7 (δ_H 6.64) and C-9 (δ_C 114.1) indicated two hydroxy groups located at C-6 and C-8 (Figure 2), which was similar to (3*S*)-6-hydroxy-8-methoxy-3-methylisochroman.[11] The obvious difference between these compounds was the configuration of C-3. This was further confirmed by comparing the $[\alpha]_D$ values for both compounds, which were found to be +45 for (3*S*)-6-hydroxy-8-methoxy-3-methylisochroman and −72.6 for **1**. Thus, *R* was deduced as the absolute configuration of C-3. Therefore, compound **1** was named (3*R*)-6,8-dihydroxy-3-methylisochroman (annulohypoxyloman A).

Compound **2** was obtained as a yellow, amorphous powder. Its molecular formula was established as $C_{11}H_{14}O_4$ using HRESIMS. The ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of compound **2** were similar to those **1**, except for a substituent group (methoxy group) at C-7. Its HMBC spectrum displayed key correlations between 7-OMe (δ_H 3.72) and C-7 (δ_C 149.8), suggesting that the methoxy group is located at C-7 (Figure 2). Compound **2** was identified as (3*R*)-6,8-dihydroxy-7-methoxy-3-methylisochroman (annulohypoxyloman B).

Compound **3** was isolated as a yellow, amorphous powder, and its molecular formula was determined to be $C_{12}H_{16}O_4$ based on a pseudomolecular ion peak in the HRESIMS spectrum. The ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) closely resemble those of **1** and **2**, the only difference being that **3** possesses a methoxy group at C-6 instead of an OH group. There were key HMBC correlations between 6-OMe (δ_H 3.78) and C-6 (δ_C 153.4) and between 7-OMe (δ_H 3.73) and C-7 (δ_C 135.9), suggesting that two methoxy groups are located at C-6 and C-7 (Figure 2). Thus, compound **3** was identified as (3*R*)-6,7-dimethoxy-8-hydroxy-3-methylisochroman (annulohypoxyloman C).

The synthesis of compound **4** has been reported, but its NMR spectroscopic data have not been assigned. For the first

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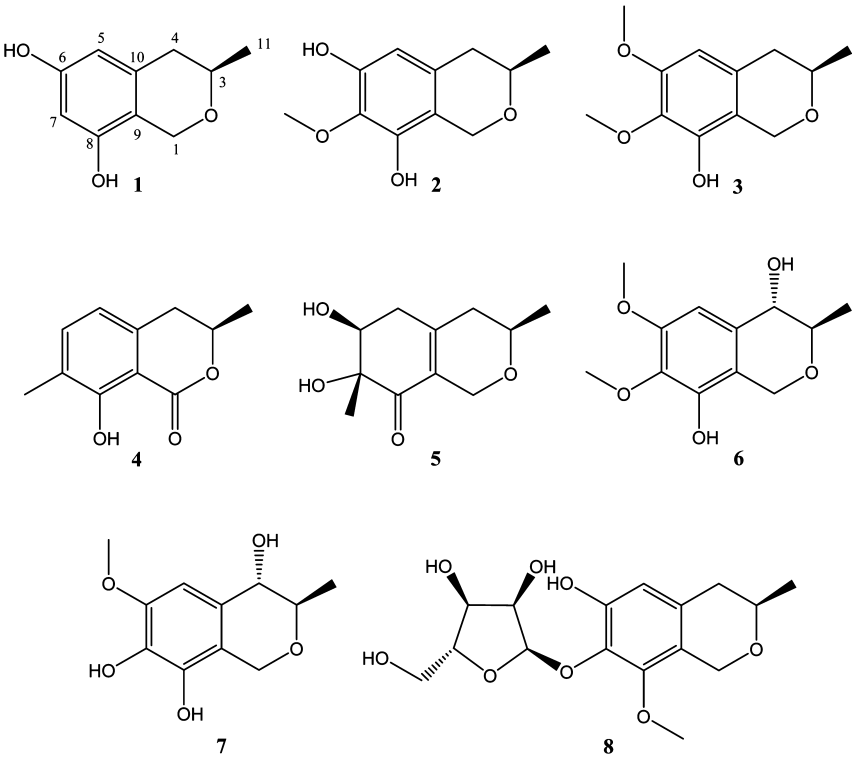

Figure 1. Structures of compounds **1**–**8** from *A. truncatum*.

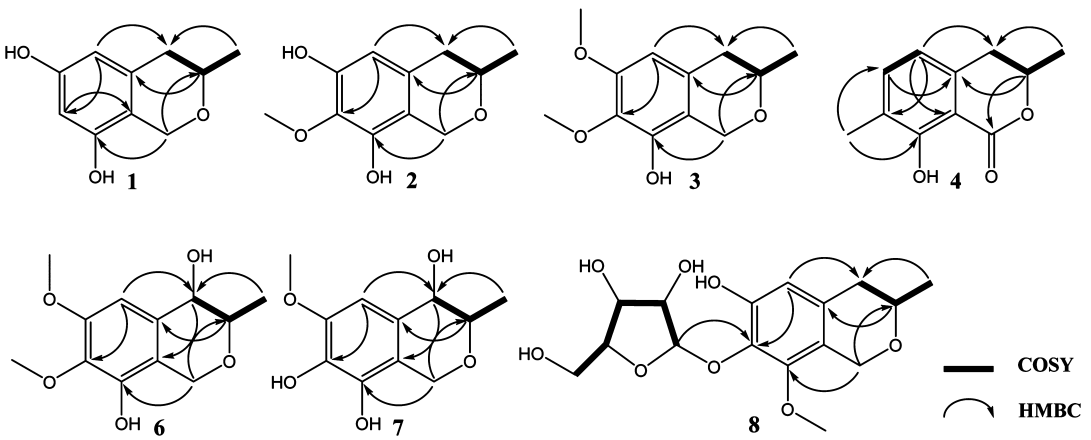

Figure 2. $^{1}H-^{1}H$ COSY and key HMBC correlations of compounds **1**–**4** and **6**–**8**.

time, we report ^{1}H and ^{13}C NMR data for annulohypoxylomarin A (**4**) (Tables 1 and 2).

The molecular formula of compound **6** was determined to be $C_{12}H_{16}O_5$ based on a pseudomolecular ion peak from HRESIMS. The ^{1}H NMR data of **6** (Table 1) showed an aromatic proton signal at δ_H 7.26 (s, H-5), an oxygenated methylene signal at δ_H 5.08 (d, J = 15.1 Hz, H-1a) and 5.39 (d, J = 15.1 Hz, H-1b), two oxygenated methine signals at δ_H 3.93 (m, H-3) and 4.81 (t, J = 8.0 Hz, H-4), two methoxy signals at δ_H 3.75 (s, 6-OMe) and 3.83 (s, 7-OMe), and a methyl signal at δ_H 1.69 (d, J = 6.1 Hz, H-11). The ^{13}C NMR data (Table 1) showed signals for six aromatic carbons at δ_C 102.3 (C-5), 117.3 (C-9), 135.4 (C-10), 136.2 (C-7), 147.1 (C-8), and 153.0 (C-6), two oxygenated methine carbons at δ_C 71.5 (C-4) and 76.9 (C-3), an oxygenated methylene carbon at δ_C 65.4 (C-1), two methoxy carbons at δ_C 56.1 (6-OMe) and 60.8 (7-OMe), and a methyl carbon at δ_C 19.6 (C-11). The ^{1}H and ^{13}C NMR spectroscopic data of **6** were very similar to those of **3**, which was coisolated with **6**. The only difference is that **6** possesses an oxymethine group (δ_H 4.83; δ_C 71.5) instead of a geminal coupling at C-4, which is supported by key HMBC correlations between H-5 (δ_H 7.26) and C-4 (δ_C 71.5) and between H-4 (δ_H 4.81) and C-9 (δ_C 117.3) (Figure 2). NOE correlations were observed between H-11 (δ_H 1.69) and H-4 (δ_H 4.81), indicating their cofacial position. The large coupling constant (8.0 Hz) between H-3 and H-4 is indicative of an axial–axial coupling. Chiral center C-3 is assumed to have an *R* configuration, as found in its coisolated compound (annulohypoxyloman C, **3**), and the absolute configuration at C-4 was deduced to be *S*.[12] Thus, **6** was identified as (3*R*,4*S*)-6,7-dimethoxy-8-hydroxy-3-methylisochromanol (annulohypoxylomanol A). In a similar manner, compound **7** was indentified as (3*R*,4*S*)-6-methoxy-7,8-dihydroxy-3-methylisochromanol (an-

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nulohypoxylomanol B) via comparison of its HRESIMS and ^{1}H and ^{13}C NMR data with those of compound **6**.

Compound **8** was obtained as a yellow, amorphous solid. Its molecular formula, $C_{16}H_{22}O_8$, was determined based on positive HRESIMS. Comparison of the ^{1}H and ^{13}C NMR data (Tables 1 and 2) with those of compounds **1**−**3** suggested that they have the same isochroman skeleton. Further comparison of their 1D NMR data with several ribofuranosides indicated that **8** is a ribofuranoside.[13,14] The connection between the sugar moiety and the skeleton via the O bond was established by the key HMBC correlation between H-1′ (δ_H 5.59) and C-7 (δ_C 128.6). D-Ribose was identified as the sugar moiety by measurement of its optical rotation following acid hydrolysis ($[\alpha]_D^{25}$ −21.2 (*c* 0.095, H_2O)).[15,16] The sugar moiety was further determined as α-D-ribofuranose through comparison of the $J_{1',2'}$ value (4.2 Hz) with those of methyl-α-D-ribofuranoside ($J_{1,2}$ = 4.3 Hz) and methyl-β-D-ribofuranoside ($J_{1,2}$ = 1.2 Hz).[17] Thus, compound **8** was identified as (3*R*)-6-hydroxy-8-methoxy-3-methylisochroman 7-*O*-α-D-ribofuranose (annulohypoxyloside).

To investigate the active anti-inflammatory constituents in the ethyl acetate extract from *A. truncatum*, the production of IL-12 p40, IL-6, and TNF-α in LPS-stimulated BMDCs was evaluated. The cytotoxicity of compounds **1**−**8** (at a concentration of 50 μM) against BMDCs was evaluated using the MTT assay (Sigma, St. Louis, MO, USA). The results showed that these compounds were inactive at the concentrations evaluated. To examine the effects of compounds **1**−**8** on the secretion of cytokines, their ability to inhibit the production of IL-6, IL-12 p40, and TNF-α at a concentration of 50 μM was assessed. Xylariphilone (**5**) significantly decreased the production of IL-6, IL-12 p40, and TNF-α; several of the isolated compounds (**1**−**4** and **6**−**8**) had no effect at the concentrations evaluated (IC_{50} > 100 μM). The effect of compound **5** on the production of IL-6, IL-12 p40, and TNF-α at various concentrations (2, 5, 10, 25, and 50 μM) was then determined. SB203580, 4-[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1*H*-imidazol-5-yl]pyridine, an inhibitor of p38 kinase, which inhibits IL-6, IL-12 p40, and TNF-α production with IC_{50} values of 3.5, 5.0, and 7.2 μM, respectively, was used as a positive control.[18] The results showed that compound **5** significantly inhibited the production of IL-6, IL-12 p40, and TNF-α, with IC_{50} values of 5.3 ± 0.8, 19.4 ± 0.5, and 37.6 ± 0.9 μM, respectively. Moreover, the observed anti-inflammatory activities and structural features of compounds **1**−**8** provide information regarding structure−activity relationships. Xylariphilone (**5**) contains a ketone group (C-8), showed strong activity. This suggests that the ketone group (C-8) of isochroman derivatives plays an important role in the anti-inflammatory activity.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined using a Jasco DIP-370 automatic polarimeter. UV spectra were recorded using a Beckman Du-650 UV−vis recording spectrometer. The NMR spectra were recorded using a JEOL ECA 600 spectrometer (^{1}H, 600 MHz; ^{13}C, 150 MHz). The LCQ Advantage trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA) was equipped with an electrospray ionization (ESI) source, and high-resolution electrospray ionization mass spectra (HRESIMS) were obtained using an Agilent 6530 Accurate-Mass Q-TOF LC/MS system. Column chromatography was performed using silica gel (Kieselgel 60, 70−230 and 230−400 mesh, Merck, Darmstadt, Germany) and YMC RP-18 resins, and thin-layer chromatography (TLC) was performed using precoated silica gel 60 F_{254} and RP-18 F_{254S} plates (both 0.25 mm, Merck, Darmstadt, Germany).

Fungal Material. JS540 was isolated from the leaves of *Z. caduciflora*, collected from a swamp at Suncheon, South Korea. The fungal strain (GenBank accession No. FJ478107.1) was identified by one of the authors (S.K.). Parts of the material were deposited in the Wildlife Genetic Resources Bank at NIBR. Leaf tissues were cut into small pieces (0.5 × 0.5 cm), and surfaces were sterilized with 2% sodium hypochlorite for 1 min and 70% ethanol for 1 min and then washed with sterilized distilled water. Fungal strains were grown out from plant tissues after about 7 days' incubation on malt extract agar (Difco) added to 50 ppm kanamycin, 50 ppm chloramphenicol, and 50 ppm Rose Bengal at 22 °C. Fungal strains were cultured by transferring actively growing edges to a new potato dextrose agar (Difco) and were stored as 20% glycerol stocks in a liquid nitrogen tank.

Fermentation, Extraction, and Isolation. The fermentation was performed in Erlenmeyer flasks (20 × 500 mL) on solid rice medium containing 80 g of rice, 2.0 g of sea salt, and 80 mL of demineralized water. After autoclaving at 121 °C for 20 min and then cooling to room temperature, each flask was inoculated and then incubated at 28 °C under static conditions. After 30 days, the fermentation was stopped by adding 500 mL of EtOAc to each flask. The extraction was completed after the flasks had been shaken on a laboratory shaker at 150 rpm for 2 h.

The EtOAc solution was then evaporated under reduced pressure at 45 °C to give an EtOAc extract (28.5 g). The EtOAc extract (26.0 g) was subjected to silica gel (5 × 30 cm) column chromatography with a gradient of hexane−EtOAc−MeOH (30:1:0, 10:1:0, 4:1:0, 2:1:0; 1.5:1:0.12, 1:1:0.2, 0:5:1, 0:0:1; 1.5 L for each step) to give eight fractions (Fr. 1A−1H). Fraction 1B (3.3 g) was separated using YMC (2.0 × 80 cm) column chromatography with a MeOH−acetone−H_2O (0.2:0.2:1, 0.5:0.5:1, 1:1:1, 2:2:1, 4:4:1, 8:8:1; 1.0 L for each step) elution solvent to give 16 fractions (Fr. 1B1−1B16). Fraction 1B2 (50.0 mg) was separated using silica gel (1 × 80 cm) column chromatography with a hexane−EtOAc (2.5:1; 650 mL) elution solvent to give compound **4** (39.0 mg). Fraction 1B4 (103.0 mg) was separated using silica gel (1 × 80 cm) column chromatography with a hexane−acetone−MeOH (6:1:0.1; 1.0 L) elution solvent to give compound **5** (12.0 mg). Fraction 1B5 (110.0 mg) was separated using silica gel (1 × 80 cm) column chromatography with a hexane−acetone−MeOH (6:1:0.1; 1.0 L) elution solvent to give compound **7** (21.0 mg). Fraction 1B7 (22.0 mg) was separated using silica gel (1 × 80 cm) column chromatography with a hexane−acetone (2:1; 800 mL) elution solvent to give compound **3** (16.0 mg). Fraction 1D (0.6 g) was separated using silica gel (1 × 80 cm) column chromatography with a gradient of hexane−EtOAc−MeOH (6:1:0, 5:1:0, 3.5:1:0, 2:1:0.1; 550 mL for each step) elution solvent to give compounds **2** (71.0 mg) and **6** (14.0 mg) and three fractions (Fr. 1D1−1D3). Fraction 1D1 (18.0 mg) was separated using silica gel (1 × 80 cm) column chromatography with a hexane−acetone (5:1; 800 mL) elution solvent to give compound **1** (2.5 mg). Fraction 1D3 (70.0 mg) was separated using silica gel (1 × 80 cm) column chromatography with a hexane−acetone−MeOH (6:1:0.1; 1.0 L) elution solvent to give compound **8** (3.6 mg).

*Annulohypoxyloman A (**1**):* yellow, amorphous powder; $[\alpha]_D^{25}$ −72.6 (*c* 0.1, MeOH); UV (MeOH) 204, 280 nm; ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR data (pyridine-d_5, 150 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 203.0678 $[M + Na]^+$ (calcd for 203.0679, $C_{10}H_{12}NaO_3$).

*Annulohypoxyloman B (**2**):* yellow, amorphous powder; $[\alpha]_D^{25}$ −55.8 (*c* 0.1, MeOH); UV (MeOH): 205, 280 nm; ^{1}H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR data (methanol-d_4, 150 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 233.0786 $[M + Na]^+$ (calcd for 233.0784, $C_{11}H_{14}NaO_4$).

*Annulohypoxyloman C (**3**):* yellow, amorphous powder; $[\alpha]_D^{25}$ −63.1 (*c* 0.1, MeOH); UV (MeOH) 204, 280 nm; ^{1}H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR data (methanol-d_4, 150 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 247.0946 $[M + Na]^+$ (calcd for 247.0941, $C_{12}H_{16}NaO_4$).

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*Annulohypoxylomarin A (**4**):* orange solid; $[\alpha]_D^{25}$ −39.2 (*c* 0.1, MeOH); UV (MeOH) 215, 268 nm; ^{1}H NMR (chloroform-*d*, 600 MHz) and ^{13}C NMR data (chloroform-*d*, 150 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 215.0684 $[M + Na]^+$ (calcd for 215.0679, $C_{11}H_{12}NaO_3$).

*Annulohypoxylomanol A (**6**):* brown, amorphous powder; $[\alpha]_D^{25}$ −22.8 (*c* 0.1, MeOH); UV (MeOH) 203, 290 nm; ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR data (pyridine-d_5, 150 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 263.0896 $[M + Na]^+$ (calcd for 263.0890, $C_{12}H_{16}NaO_5$).

*Annulohypoxylomanol B (**7**):* brown, amorphous powder; $[\alpha]_D^{25}$ −27.6 (*c* 0.1, MeOH); UV (MeOH) 205, 290 nm; ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR data (pyridine-d_5, 150 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 249.0738 $[M + Na]^+$ (calcd for 249.0733, $C_{11}H_{14}NaO_5$).

*Annulohypoxyloside A (**8**):* yellow, amorphous solid; $[\alpha]_D^{25}$ −17.9 (*c* 0.1, MeOH); UV (MeOH) 245, 280 nm; ^{1}H NMR (methanol-d_4, 600 MHz) and ^{13}C NMR data (methanol-d_4, 150 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 365.1209 $[M + Na]^+$ (calcd for 365.1207, $C_{16}H_{22}NaO_8$).

Acid Hydrolysis. A solution of compound **8** (1.5 mg) in 1 M hydrochloric acid (HCl) (1 mL) was reacted for 3 h at 90 °C. The reaction mixture was extracted with EtOAc repeatedly to remove the aglycone fraction. The water layer was then concentrated to furnish the sugar residue (0.7 mg). The rotation recorded for the ribose was $[\alpha]_D^{25}$ −21.2 (*c* 0.095, H_2O), which closely matched that for D-ribose (lit. −20).[9]

Cell Culture. BMDCs were grown from wild-type C57BL/6 mice (Orient Bio Inc., South Korea) as previously described. All animal procedures were approved and performed according to the guidelines of the Institutional Animal Care and Use Committee of Jeju National University (#2010-0028). Briefly, the mouse tibia and femur were obtained by flushing with Dulbecco's modified Eagle medium to yield bone marrow cells. The cells were cultured in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS; Gibco, NY, USA), 50 μM β-ME, and 2 mM glutamine supplemented with 3% J558L hybridoma cell culture supernatant containing granulocyte-macrophage colony stimulating factor. The culture medium was replaced with fresh medium every second day. At day 6 of culture, nonadherent cells and loosely adherent DC aggregates were harvested, washed, and resuspended in RPMI 1640 supplemented with 5% FBS.

Cell Toxicity Assay. Cell-Counting Kit (CCK)-8 (Dojindo, Kumamoto, Japan) was used to analyze the effect of compounds on cell toxicity according to the manufacturer's instructions. Cells were cultured overnight in a 96-well plate (~1 × 10^4 cells/well). Cell toxicity was assessed after the addition of compounds in a dose-dependent manner. After 24 h of treatment, 10 μL of the CCK-8 solution was added to triplicate wells and incubated for 1 h. Absorbance was measured at 450 nm to determine viable cell numbers in wells.

Cytokine Production Measurements. BMDCs were incubated in 48-well plates in 0.5 mL containing 1 × 10^5 cells per well and then treated with the isolated compounds **1**−**8** at the indicated concentration for 1 h before stimulation with 10 ng/mL LPS from *Salmonella minnesota* (Alexis, NY, USA). Supernatants were harvested 18 h after stimulation. Concentrations of murine TNF-α, IL-6, and IL-12 p40 in the culture supernatants were determined by ELISA (BD PharMingen, CA, USA) according to the manufacturer's instructions. The data are presented as means plus SD of at least three independent experiments performed in triplicate.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00698.

^{1}H and ^{13}C NMR, HMQC, HMBC, COSY, NOESY, and HRESIMS spectra of compounds **1**−**4** and **6**−**8** (PDF)

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Notes

The authors declare no competing financial interest.

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