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Diterpene Glycosides and Polyketides from *Xylotumulus gibbisporus*

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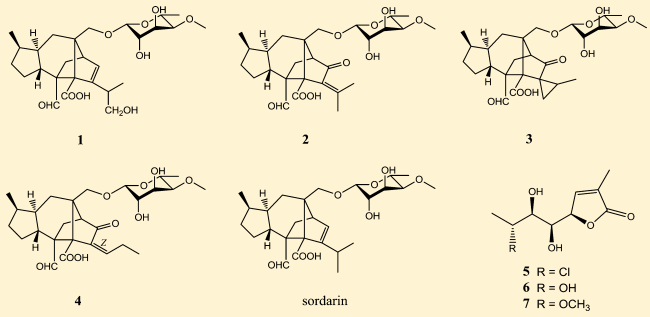
Supporting Information

ABSTRACT: Four new tetracyclic diterpene glycosides, namely, sordarins C−F (**1**−**4**), and three new γ-lactone polyketides, namely, xylogiblactones A−C (**5**−**7**), along with sordarin were isolated from the ethyl acetate extracts of the fermented broths of *Xylotumulus gibbisporus* YMJ863. The structures of **1**−**7** were elucidated on the basis of spectroscopic data analyses. The configurations of **1**−**4** were deduced by NOESY, molecular modeling, and comparison with the literature. The relative configurations of **5**−**7** were deduced by X-ray crystallographic analysis of **5**. Compounds **1**−**5** and sordarin were evaluated in an antifungal assay using *Candida albicans* ATCC 18804, *C. albicans* ATCC MYA-2876, and *Saccharomyces cerevisiae* ATCC 2345, and only sordarin exhibited significant antifungal activities against these fungal strains, with MIC values of 64.0, 32.0, and 32.0 μg/mL, respectively. The effect of compounds **1**−**7** and sordarin on the inhibition of NO production in lipopolysaccharide-activated murine macrophages was also evaluated. Compounds **2** and sordarin inhibited NO production with IC_{50} values of 327.2 ± 46.6 and 157.1 ± 24.1 μM, respectively.



The family Xylariaceae is one of the most commonly encountered groups of ascomycetes and is found throughout the temperate and tropical regions of the world. Some species categorized in this family have long been used in traditional Chinese medicine preparations, suggesting that fungi of the Xylariaceae could potentially be developed for medicinal uses.[1,2] *Xylotumulus gibbisporus* J. D. Rogers, Y.-M. Ju & Hemmes, classified in Xylariaceae, was described for the first time from dead angiosperm wood collected from the Bird Park area in Hawaii Volcanoes National Park in 2006.[3] It has affinities with certain *Xylaria* species and is closely related morphologically to *Amphirosellinia* Y.-M. Ju, J.D. Rogers, H.-M. Hsieh & Vasilyeva in the same family.[4] Although bioactive compounds have been reported from *Xylaria* spp.,[5−8] no reports of secondary metabolites from this fungal species were found in our survey of the scientific literature. Preliminary pharmacological screenings demonstrated that the ethyl acetate extracts of the fermented broths of *X. gibbisporus* YMJ863 displayed significant antifungal activities. In an attempt to identify the bioactive constituents of the fermented broths of this fungus, a series of fermentation, extraction, isolation, and structural elucidation experiments were performed, which resulted in the characterization of seven new compounds, **1**−**7** (Figure 1). In this report we describe the isolation and identification of these compounds as well as their bioactivities.

RESULTS AND DISCUSSION

From the EtOAc extracts of the fermented broths of *X. gibbisporus* YMJ863, eight major compounds including four new tetracyclic diterpene glycosides, **1**−**4**, three new γ-lactone polyketides, **5**−**7**, and a known diterpene glycoside were isolated by sequential separation on Sephadex LH-20, Diaion HP-20, and reversed-phase HPLC. The known compound was obtained as an amorphous white solid whose ^{1}H and ^{13}C NMR, IR, optical rotation, and MS were consistent with those of sordarin isolated originally from *Sordaria araneosa*.[9] So far, a

Received: July 9, 2013
Published: March 5, 2014

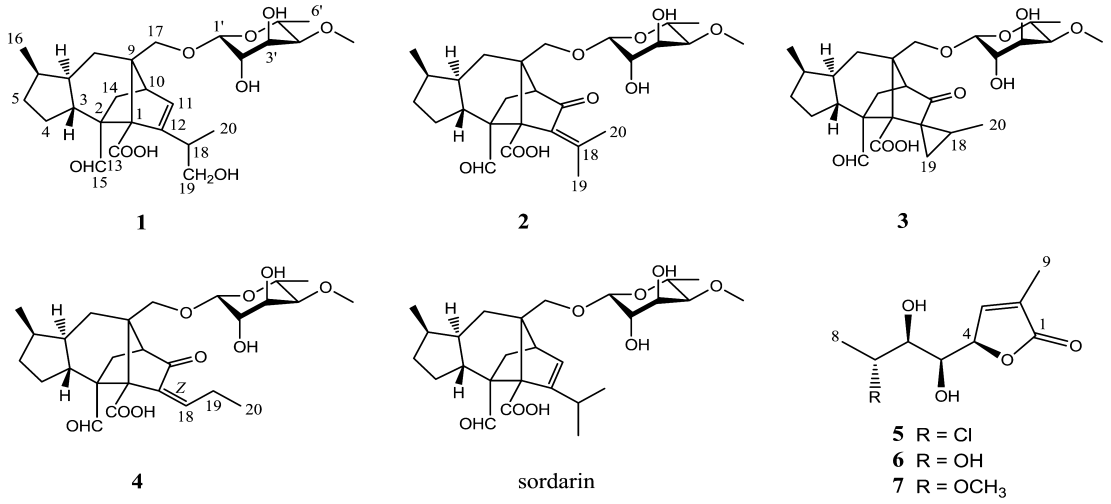


Figure 1. Chemical structures of **1**−**7** and sordarin identified in this report.

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for Compounds 1−4 [δ in ppm, mult (J in Hz)]

	1		2		3		4	
no.	^{13}C[a]	^{1}H[b]	^{13}C[a]	^{1}H[b]	^{13}C[a]	^{1}H[b]	^{13}C[a]	^{1}H[c]
1	74.0		64.8		65.3		67.4	
2	58.9		62.5		61.9		60.5	
3	41.3	2.03 m	38.9	2.86 m	41.8	2.02 m	41.5	2.08 m
4	26.4	0.97 m	24.6	0.91 m	24.4	0.87 m	24.7	0.95 m
		1.88 m		1.60 m		1.54 m		1.73 m
5	32.0	1.22 m	31.6	1.23 m	31.5	1.20 m	31.6	1.21 m
		2.02 m		2.01 m		2.01 m		2.02 m
6	31.3	2.06 m	31.3	2.09 m	31.3	2.13 m	31.2	2.11 m
7	41.3	1.74 m	41.1	1.54 m	41.3	1.67 m	41.4	1.66 m
8	28.8	1.72 m	30.4	1.49 m	30.0	1.23 m	29.5	1.87 m
		1.89 m		2.18 m		2.06 m		2.05 m
9	65.3		55.0		56.1		54.2	
10	47.6	2.57 t (3.4)	53.8	2.68 d (5.7)	54.2	2.64 d (5.0)	54.5	2.68 d (5.9)
11	133.3	6.15 d (3.4)	201.8		211.5		201.1	
12	145.1		130.8		37.1		133.5	
13	175.1		174.1		171.6		171.8	
14	28.9	1.25 m	23.8	1.69 d (15.1)	22.0	1.88 dd (5.0, 14.6)	23.6	1.70 d (14.9)
		2.01 m		1.83 dd (5.7, 15.1)		1.94 d (14.6)		1.95 dd (5.9, 14.9)
15	204.9	9.72 s	202.5	9.98 s	203.0	10.03 s	203.3	9.82 s
16	17.4	0.79 d (6.8)	17.4	0.79 d (7.1)	17.3	0.80 d (7.1)	17.3	0.79 d (7.0)
17	74.2	3.46 m	73.5	3.60 d (10.8)	73.0	3.66 d (10.4)	73.0	3.58 d (10.7)
		4.20 d (9.4)		3.93 d (10.8)		4.19 d (10.4)		4.08 d (10.7)
18	35.0	2.53 m	150.9		21.9	1.37 tq (6.3, 6.1)	144.5	5.99 t (7.6)
19	68.1	3.43 m	23.4	1.72 s	22.4	1.06 d (6.3)	21.6	2.60 qd (7.5, 7.6)
								2.78 qd (7.5, 7.6)
20	15.7	1.02 d (7.0)	22.4	2.23 s	10.7	1.08 d (6.1)	13.5	0.98 t (7.5)
1′	98.4	4.67 brs	99.0	4.67 d (1.0)	98.7	4.68 d (1.0)	98.7	4.65 brs
2′	69.8	3.89 d (3.3)	69.6	3.88 dd (1.0, 3.5)	69.7	3.90 dd (1.0, 3.4)	69.8	3.86 d (3.3)
3′	67.0	4.17 brt (3.3)	67.0	4.19 brt (3.5)	67.0	4.18 brt (3.4)	67.0	4.17 brt (3.3)
4′	79.4	3.18 dd (3.3, 9.3)	79.8	3.20 dd (3.5, 9.1)	79.8	3.18 dd (3.4, 9.1)	79.8	3.18 dd (3.3, 9.1)
5′	68.8	3.69 qd (6.2, 9.3)	68.8	3.72 qd (6.3, 9.1)	68.7	3.68 qd (6.3, 9.1)	68.7	3.68 qd (6.2, 9.1)
6′	17.9	1.27 d (6.2)	18.1	1.27 d (6.3)	18.1	1.27 d (6.3)	18.1	1.26 d (6.2)
4′-OCH$_3$	57.5	3.38 s	57.5	3.39 s	57.5	3.40 s	57.5	3.39 s

[a]Measured in chloroform-*d* (125 MHz). [b]Measured in chloroform-*d* (500 MHz). [c]Measured in chloroform-*d* (800 MHz).

large number of semisynthetic sordarin derivatives have been reported,[10] and some of the synthetic analogues were developed as antifungal agents such as zofimarin[11] and FR231956.[12]

Compound **1** was obtained as an amorphous white solid, and its IR absorptions at 3400−2500 coupled with 1705 and 1636 cm^{-1} indicated the presence of a carboxylic acid group and a double bond, respectively. Its molecular formula, $C_{27}H_{40}O_9$,

dx.doi.org/10.1021/np400523k | J. Nat. Prod. 2014, 77, 751−757

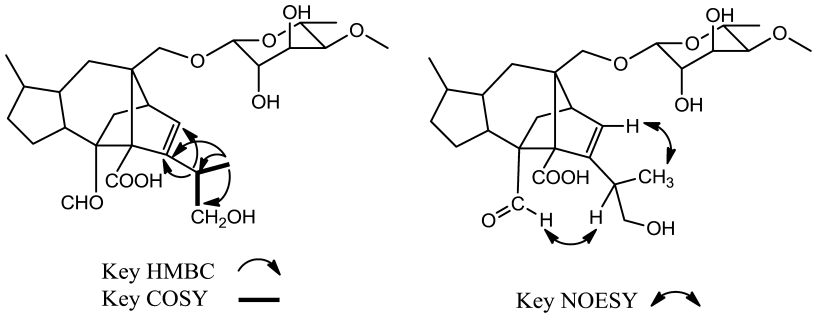


Figure 2. Key HMBC, COSY, and NOESY of **1**.

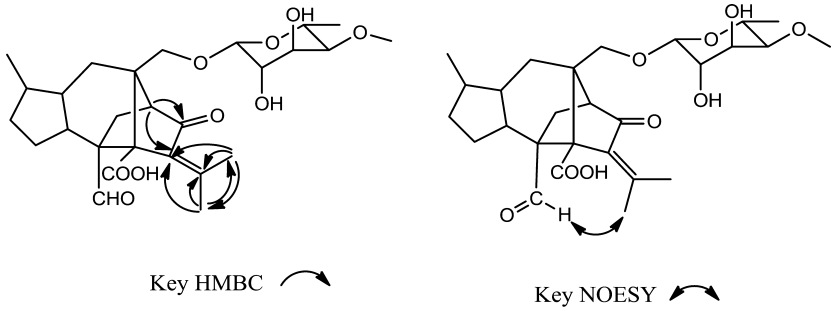


Figure 3. Key HMBC and NOESY of **2**.

was deduced by ^{13}C NMR and HRESIMS data. The ^{13}C NMR coupled with DEPT revealed 27 resonances including a set of deoxy-sugar signals at δ_C 98.4 (d), 79.4 (d), 69.8 (d), 68.8 (d), 67.0 (d), and 17.9 (q), a methoxyl signal at δ_C 57.5 (q), a residual C_{20} diterpenoid containing two carbonyl groups at δ_C 175.1 (s) and 204.9 (s), an olefinic functionality at δ_C 145.1 (s) and 133.3 (d), and two oxymethylenes at δ_C 74.2 (t) and 68.1 (t) (Table 1). The ^1H NMR of **1** showed a set of deoxy-monosaccharide signals at δ_H 4.67 (1H, brs), 4.17 (1H, brt, J = 3.3 Hz), 3.89 (1H, d, J = 3.3 Hz), 3.69 (1H, qd, J = 6.2, 9.3 Hz), 3.18 (1H, dd, J = 3.3, 9.3 Hz), and 1.27 (3H, d, J = 6.2 Hz), a methoxy at δ_H 3.38 (s), an aldehyde at δ_H 9.72 (1H, s), a trisubstituted olefinic resonance at δ_H 6.15 (1H, d, J = 3.4 Hz), two oxymethylenes at δ_H 4.20 (1H, d, J = 9.4 Hz), 3.46 (1H, m), and 3.43 (2H, m), and two terminal methyls at δ_H 1.02 (3H, d, J = 7.0 Hz) and 0.79 (3H, d, J = 6.8 Hz). All the above data indicated a sordaricin-type diterpene aglycone bearing a 6-deoxy-4-*O*-methylaltrose moiety.[9] Further comparison of the ^1H and ^{13}C NMR data of **1** with those of sordarin showed the difference was solely the H_3-19 located on C-18 of sordarin substituted by a $-CH_2OH$ [δ_C 68.1, C-19; δ_H 3.43, m, H_2-19] in **1** (Table 1). This was corroborated by key cross-peaks of H_3-20/C-18, -19, and -12 and H-18/C-12 and -11 in the HMBC spectrum and key cross-peaks of H_3-20/H-18 and H-18/H_2-19 in the COSY spectrum of **1** (Figure 2). The configuration of the chiral C-18 of **1** was determined to prefer the shown conformer by distinctive cross-peaks of H-18/H-15 and H_3-20/H-11 in the NOESY spectrum of **1** (Figure 2) and supported by the three-dimensional molecular modeling. Accordingly, the structure of **1** was characterized as shown, and was named sordarin C.

The physical data of **2** were similar to those of sordarin. Its molecular formula, $C_{27}H_{38}O_9$, deduced from HRESIMS and UV λ_{max} at 247 nm, were the major differences. When comparing the ^1H and ^{13}C NMR data of **2** with those of sordarin, **2** was almost identical with sordarin except that the H-11 in sordarin was oxidized to give a carbonyl (δ_C 201.8) in **2** and a double bond at Δ^{11} in sordarin was changed to $\Delta^{12(18)}$ (δ_C 130.8 and 150.9) of **2** (Table 1), as shown by key HMBC correlations of H-10/C-11 and -12, H_3-19/C-18, -20, and -12, and H_3-20/C-18, -19, and -12 (Figure 3). The H_3-19 at δ_H 1.72 in the ^1H NMR of **2** was assigned as *trans* to the carbonyl attached at C-11 due to the cross-peak of H_3-19/H-15 in the NOESY spectrum (Figure 3) and from molecular modeling, and the H_3-20 downfield shifted to δ_H 2.23 was caused by the anisotropic effect of the same carbonyl group at C-11. Thus, **2** was determined as shown and was named sordarin D.

The physical data of **3** were similar to those of **2**, and the major difference was its UV λ_{max} adopted a hypsochromic shift to lower than 210 nm, indicating a structural change of the α,β-unsaturated carbonyl at C-11 and $\Delta^{12(18)}$ of **3**. The differences of ^1H and ^{13}C NMR data between **3** and **2** were due to the disappearance of an olefinic functionality at $\Delta^{12(18)}$ and a methyl group and the presence of a methylene [δ_H 1.06 (2H, d, J = 6.3 Hz); δ_C 22.4 (t)] as well as a methine [δ_H 1.37 (1H, tq, J = 6.3, 6.1 Hz); δ_C 21.9 (d)] (Table 1). Key HMBC cross-peaks of H-10/C-11 and -12, H_3-20/C-12, -18, and -19, and H_2-19/C-12 (Figure 4) corroborated that C-12−C-19−C-18−C-20 formed a *spiro*-methylcyclopropane moiety connected with the core structure of **3** by C-12. The chemical shift of the methylene in cyclopropane at δ_H 0.22[13] downfield shifted to δ_H

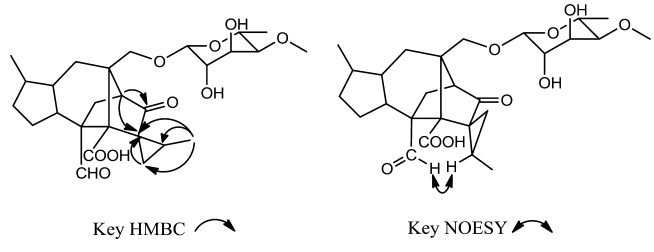


Figure 4. Key HMBC and NOESY of **3**.

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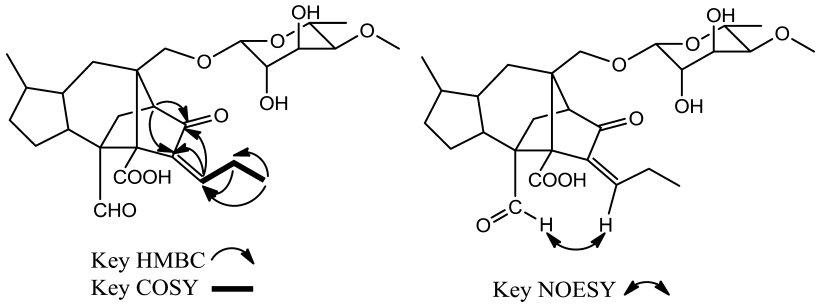

Figure 5. Key HMBC, COSY, and NOESY of **4**.

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data for Compounds 5−7 [δ in ppm, mult (J in Hz)]

	5		6		7	
no.	$^{13}C^a$	$^1H^b$	$^{13}C^a$	$^1H^b$	$^{13}C^a$	$^1H^b$
1	176.2		176.4		176.4	
2	131.3		131.1		131.1	
3	150.1	7.42 dq (1.7, 1.7)	150.4	7.43 dq (1.7, 1.7)	150.4	7.42 dq (1.7, 1.7)
4	83.2	5.03 ddq (6.3, 1.7, 1.7)	83.3	5.05 ddq (6.3, 1.7, 1.7)	83.3	5.03 ddq (6.2, 1.7, 1.7)
5	72.7	3.73 dd (2.4, 6.3)	73.8	3.66 dd (2.0, 6.3)	73.1	3.64 dd (2.3, 6.2)
6	76.5	3.68 dd (2.4, 7.0)	76.0	3.48 dd (2.0, 6.3)	75.3	3.56 dd (2.3, 6.6)
7	61.5	4.18 dq (6.3, 7.0)	69.8	3.85 dq (6.3, 6.3)	79.7	3.48 dq (6.3, 6.6)
8	21.5	1.51 d (6.3)	19.4	1.18 d (6.3)	15.1	1.16 d (6.3)
9	10.6	1.89 t (1.7)	10.6	1.89 t (1.7)	10.6	1.88 t (1.7)
7-OCH_3					57.1	3.38 s

^{a}Measured in methanol-d_4 (125 MHz). ^{b}Measured in methanol-d_4 (500 MHz).

1.06 for H_2-19 in **3** was speculated to be caused by the anisotropic effect of the carbonyl at C-11. The shown configurations of C-12 and C-18 were deduced based on the key cross-peak of H-18/H-15 in the NOESY spectrum (Figure 4) and from molecular modeling. The structure of **3**, with a unique 5/6/5/5/3-fused ring system, was assigned as shown and was named sordarin E.

The physical data for compound **4** was similar to those of **2**. It also showed a UV maximum absorption at 242 nm indicating the presence of the same α,β-unsaturated carbonyl group as that of **2**. The ^{1}H NMR data of **4** coincided well with those of **2** except that a methyl group at δ_H 1.72 (3H, s, H_3-19) in **2** was replaced by an olefinic proton at δ_H 5.99 (1H, t, J = 7.6 Hz, H-18) and a methylene group at δ_H 2.60 and 2.78 (each 1H, qd, J = 7.5, 7.6 Hz, H_2-19), and the other methyl group at δ_H 2.23 (3H, s, H_3-20) in **2** shifted upfield to δ_H 0.98 (3H, t, J = 7.5 Hz, H_3-20) in **4**. These changes are also reflected in the ^{13}C NMR (Table 1). Key HMBC cross-peaks of H-10/C-11 and -12, H-18/C-11 and -12, H_2-19/C-18, and H_3-20/C-18 and -19 along with key COSY correlations of H_3-20/H_2-19 and H_2-19/H-18 (Figure 5) corroborated that the 2-propylene attached at C-12 of **2** was changed to a 1-propylene in **4**. The configuration of $\Delta^{12(18)}$ was deduced to be Z due to a distinctive cross-peak of H-18/H-15 in the NOESY spectrum (Figure 5) and from molecular modeling. The anisotropic effect and steric hindrance of the carbonyl located at C-11 would account for the downfield shifts and nonequivalence of the H_2-19 *cis* to the carbonyl. The structure of compound **4** was thus deduced to be as shown and was named sordarin F.

Compound **5** was obtained as one colorless crystal, and its IR absorptions at 3338 and 1734 cm^{-1} indicated the presence of hydroxyl group and conjugated γ-lactone carbonyl functionalities. HRESIMS of **5** showed two prominent pseudomolecular ions, $[M + Na]^+$ at m/z 243.0397 (calcd for $C_9H_{13}{}^{35}ClO_4Na$, 243.0400) and $[M + 2 + Na]^+$ at m/z 245.0409 (calcd for $C_9H_{13}{}^{37}ClO_4Na$, 245.0400), with a ratio of around 3:1, suggesting the presence of a chloride in **5**. Nine carbon resonances observed in the ^{13}C NMR coupled with DEPT spectra of **5** were attributable to two primary carbons at δ_C 10.6 (C-9) and 21.5 (C-8), five tertiary carbons at δ_C 61.5 (C-7), 72.7 (C-5), 76.5 (C-6), 83.2 (C-4), and 150.1 (C-3), and two quaternary carbons at δ_C 131.3 (C-2) and 176.2 (C-1) (Table 2). The ^{1}H NMR spectrum of **5** exhibited signals for two methyls at δ_H 1.51 (H_3-8) and 1.89 (H_3-9), four carbinoyl protons at δ_H 3.68 (H-6), 3.73 (H-5), 4.18 (H-7), and 5.03 (H-4), and one olefinic proton at δ_H 7.42 (H-3) (Table 2). The lower field shifts of a methine at $\delta_{H\text{-}7}$ 4.18 and $\delta_{C\text{-}7}$ 61.5 and a nearby methyl at $\delta_{H\text{-}8}$ 1.51 and $\delta_{C\text{-}8}$ 21.5 accompanied by key COSY cross-peaks of H_3-8/H-7 and H-7/H-6 and key HMBC correlations of H_3-8/C-7 and -6 (Figure 6) suggested the chloride was located at C-7. Further complete interpretations of other COSY signals of H-6/H-5 and H-5/H-4 as well as residual HMBC cross-peaks of H_3-9/C-1, -2 and -3, H-3/C-2, H-4/C-1 and -6, and H-5/C-3 (Figure 6) established the gross structure of **5** as shown. The relative configurations of C-4−C-7

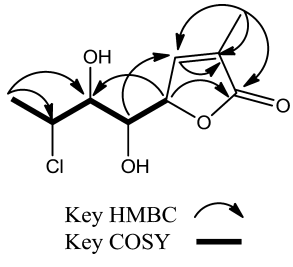

Figure 6. Key HMBC and COSY of **5**.

dx.doi.org/10.1021/np400523k | J. Nat. Prod. 2014, 77, 751−757

of **5** were determined by the single-crystal X-ray diffraction analysis to be *R**, *S**, *S**, and *R** (Figure 7), respectively.

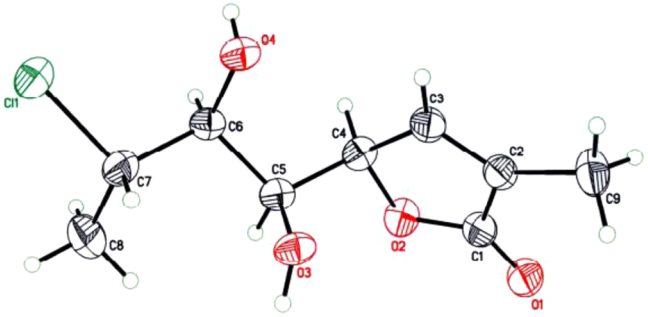

Figure 7. ORTEP drawing of **5**.

The ^{1}H and ^{13}C NMR data of **6** were similar to those of **5** except signals for C-7 and −8 were shifted to δ_C 69.8 and 19.4, respectively, with their corresponding H-7 and H_3-8 shifted upfield to δ_H 3.85 and 1.18, respectively, implying the −Cl at C-7 of **5** was substituted by −OH in **6** and supported by a prominent sodium adduct at *m*/*z* 225.0733 (calcd for $C_9H_{14}O_5Na$, 225.0739) in the HRESIMS of **6**. The ^{1}H and ^{13}C NMR data of **7** were consistent with those of **6** except for an additional methyl group at δ_H 3.38 (−OC<u>H</u>$_3$) and δ_C 57.1 (−O<u>C</u>H$_3$) (Table 2). The methoxyl group was assigned to be located at C-7 due to the key cross-peak of −OC<u>H</u>$_3$/C-7 in the HMBC spectrum of **7**. Thus, **7** was assigned to be the methyl ether of **6**, as evidenced from the pseudomolecular ion [M + Na]$^+$ at *m*/*z* 239.0893 (calcd for $C_{10}H_{16}O_5Na$, 239.0895), 14 Da more than that of **6**, in the HRESIMS of **7**. Due to the NMR data consistency and biogenetic relationship of **5**−**7**, the relative configurations of C-4−C-7 of **6** and **7** were determined to be consistent with those of **5**. Accordingly, **5**−**7** were determined to possess a C_9 polyketide skeleton containing a γ-lactone moiety, as shown in Figure 1, and were named xylogiblactones A−C, respectively. To our knowledge, only one analogue of **5**−**7**, namely, hypoxylactone, was isolated from the marine fungus *Hypoxylon croceum*.[14]

Compounds **1**−**5** and sordarin were subjected to an antifungal assay against *Candida albicans* ATCC 18804, *C. albicans* ATCC MYA-2876, and *Saccharomyces cerevisiae* ATCC 2345, and only sordarin exhibited significant antifungal activities against the three fungal strains, with MIC values of 64.0, 32.0, and 32.0 μg/mL, respectively. Under the same conditions, the MIC values of amphotericin B were 0.5, 1.0, and 1.0/mL, respectively. The effect of compounds **1**−**7** and sordarin on the inhibition of NO production in lipopolysaccharide-activated RAW264.7 cells was further evaluated. The most potent compounds, **2** and sordarin, inhibited NO production with IC_{50} values of 327.2 ± 46.6 and 157.1 ± 24.1 μM, respectively, without any cytotoxicity. The IC_{50} values of the positive inhibitors aminoguanidine and L-NNA were 22.3 ± 0.3 and 146.4 ± 0.7 μM, respectively.

The natural sordarin analogues, soradrins C−F (**1**−**4**), described in this report varied at the C-11 position and at the branch attached to C-12 of the sordaricin-type diterpene skeleton. Soradrins C−F exhibited a mild antifungal effect compared to that of sordarin. It was reported that sordarin inhibits fungal protein synthesis by blocking the function of translation elongation factor 2 (EF2).[15] Co-crystallization of sordarin and EF2 showed that no van der Waals interactions between the C-12−isopropyl moiety and EF2 were involved, indicating the branch on C-12 does not play a role in antifungal activity.[16] The structure−activity relationship studies of sordarins have been confined mostly to the glycosyl moiety, which makes the antifungal activity more potent, while modification of the isopropyl group at C-12 does not effect the antifungal activity.[17] Our results were consistent with the findings reported in the literature.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-2000 polarimeter (Tokyo, Japan). ^{1}H and ^{13}C NMR were acquired on a Bruker Avance DRX-500 and AVIII-800 spectrometer (Ettlingen, Germany). Low-resolution and high-resolution mass spectra were obtained using an API4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) and Synapt High Definition Mass Spectrometry system with an ESI interface and a TOF analyzer (Waters Corp., Manchester, UK), respectively. IR spectra were recorded on a JASCO FT/IR 4100 spectrometer (Tokyo, Japan). Single-crystal data were collected on an Oxford Diffraction Gemini A Ultra diffractometer using Cu Kα radiation (Oxford Diffraction, Oxford, UK). Sephadex LH-20 (Amersham Biosciences, Filial Sverige, Sweden) and Diaion HP-20 (Mitsubishi Chemical, Tokyo, Japan) were used for open column chromatography. TLC was performed using silica gel 60 F_{254} plates (200 μm, Merck). A reflective index detector (Bischoff, Leonberg, Germany) was used in HPLC purification.

Fermentation of *Xylotumulus gibbisporus* YMJ863. *Xylotumulus gibbisporus* J. D. Rogers, Y.-M. Ju & Hemmes YMJ863 was isolated from dead angiosperm wood collected from the Bird Park area in Hawaii Volcanoes National Park.[3] This fungal strain was deposited at the Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan. The mycelium of *X. gibbisporus* 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 deionized water. The fermentation was conducted with aeration at 25−30 °C for 30 days.

Extraction and Isolation. The filtered fermented broth (60 L) of *X. gibbisporus* was partitioned three times with 60 L of recycled ethyl acetate, then concentrated in vacuum to dryness (3.7 g). Subsequently, the crude extract was redissolved in 25 mL of MeOH, then applied onto a Sephadex LH-20 column (2.8 cm i.d. × 70 cm) eluted by MeOH with a flow rate of 2.2 mL/min. Each subfraction (22 mL) collected was checked for its compositions 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 the fractions were combined into five portions, I−IV. Portion II (frs. 5−12) was purified by HPLC on a semipreparative reversed-phase column (Phenomenex Luna 5 μ PFP, 10 × 250 mm, Torrance, CA, USA) with MeCN−H_2O (37:100, v/v) containing 0.05% trifluoroacetic acid as eluent, 2 mL/min, giving **1** (5.3 mg, t_R = 20.07 min), **2** (5.8 mg, t_R = 27.59 min), **3** (15.8 mg, t_R = 29.83 min), **4** (7.8 mg, t_R = 35.41 min), and sordarin (25.8 mg, t_R = 56.96 min). Portion III (frs. 13−19) was rechromatographed on a Diaion HP-20 resin (2.0 cm i.d. × 20 cm) eluted with aqueous MeOH in a stepwise gradient mode with a flow rate of 5.0 mL/min. Each subfraction (125 mL) collected was checked for its composition by the above-mentioned TLC system and combined into four subportions, III-I−III-V. Subportion III-I (subfrs. 1 and 2) was purified by HPLC on the same column with MeCN−H_2O (10:100, v/v) as eluent, 2 mL/min, affording **6** (9.7 mg, t_R = 20.42 min). Subportion III-III (subfrs. 5−7) was purified by HPLC on the same column with MeCN−H_2O (15:100, v/v) as eluent, 2 mL/min, affording **5** (11.3 mg, t_R = 33.28 min) and **7** (6.8 mg, t_R = 18.33 min).

X-ray Crystallographic Analysis of 5 (CCDC 949280). Xylogiblactone A (**5**) was crystallized from $CHCl_3$ to give colorless crystals. The crystal data were recorded at 295 K on an Oxford Diffraction Gemini A Ultra diffractometer with Cu Kα radiation (λ =

dx.doi.org/10.1021/np400523k | J. Nat. Prod. 2014, 77, 751−757

1.54178 Å). The structure was solved by direct methods (SHELXS-97) and refined using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Crystal data: $C_9H_{13}ClO_4$, M_r = 220.64, monoclinic, space group $P2_1$, a = 9.6443(6) Å, b = 4.7764(3) Å, c = 11.5683(7) Å, α = 90°, β = 94.929(5)°, γ = 90°, V = 530.92(6) Å^3, Z = 2, D_{calc} = 1.380 Mg/m^3. Crystal dimensions: 0.20 × 0.15 × 0.10 mm^3. Independent reflections: 1299 (R_{int} = 0.0162). The final R_1 values were 0.0523, wR_2 = 0.1409 [$I > 2\sigma(I)$]. Flack parameter = −0.03(3).

Sordarin C (1): amorphous, white solid; $[\alpha]^{26}_D$ −51.3 (*c* 0.75, MeOH); IR (ZnSe) ν_{max} 3400−2500, 2937, 2878, 1705, 1635, 1588, 1543, 1453, 1374, 1258, 1175, 1077, 1018 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS $[M - H]^-$ *m/z* 507; HREIMS $[M - H]^-$ *m/z* 507.2590 (calcd for $C_{27}H_{39}O_9$, 507.2594).

Sordarin D (2): amorphous, white solid; $[\alpha]^{26}_D$ −68.0 (*c* 0.55, MeOH); IR (ZnSe) ν_{max} 3400−2500, 2934, 2871, 1709, 1630, 1578, 1547, 1454, 1377, 1240, 1177, 1087, 1028 cm^{-1}; UV λ_{max} (log ε, MeOH) 247 (3.7); ^{1}H and ^{13}C NMR data, see Table 1; ESIMS $[M - H]^-$ *m/z* 505; HRESIMS $[M - H]^-$ *m/z* 505.2439 (calcd for $C_{27}H_{37}O_9$, 505.2438).

Sordarin E (3): amorphous, white solid; $[\alpha]^{26}_D$ −8.73 (*c* 0.90, MeOH); IR (ZnSe) ν_{max} 3400−2500, 2944, 2899, 2875, 1714, 1631, 1447, 1371, 1307, 1269, 1175, 1143, 1090, 1025 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS $[M - H]^-$ *m/z* 505; HRESIMS $[M - H]^-$ *m/z* 505.2440 (calcd for $C_{27}H_{37}O_9$, 505.2438).

Sordarin F (4): amorphous, white solid; $[\alpha]^{26}_D$ −50.5 (*c* 1.10, MeOH); IR (ZnSe) ν_{max} 3400−2500, 2935, 2875, 2855, 1715, 1642, 1602, 1560, 1454, 1370, 1265, 1188, 1146, 1086, 1026 cm^{-1}; UV λ_{max} (log ε, MeOH) 242 (3.7); ^{1}H and ^{13}C NMR data, see Table 1; ESIMS $[M - H]^-$ *m/z* 505; HRESIMS $[M - H]^-$ *m/z* 505.2440 (calcd for $C_{27}H_{37}O_9$, 505.2438).

Xylogibloactone A (5): colorless crystal; $[\alpha]^{26}_D$ +62.1 (*c* 1.50, MeOH); IR (ZnSe) ν_{max} 3338, 3120, 2981, 2931, 2856, 1734, 1659, 1586, 1545,1436, 1355, 1265, 1206, 1119, 1049 cm^{-1}; UV λ_{max} (log ε, MeOH) 209 (3.9); ^{1}H and ^{13}C NMR data, see Table 2; ESIMS $[M + Na]^+$ *m/z* 243, $[M + 2 + Na]^+$ *m/z* 245; HRESIMS $[M + Na]^+$ *m/z* 243.0397 (calcd for $C_9H_{13}{}^{35}ClO_4Na$, 243.0400), $[M + 2 + Na]^+$ *m/z* 245.0409 (calcd for $C_9H_{13}{}^{37}ClO_4Na$, 245.0400).

Xylogiblactone B (6): amorphous, white solid; $[\alpha]^{26}_D$ +78.9 (*c* 0.75, MeOH); IR (ZnSe) ν_{max} 3389, 2982, 2925, 2857, 1739, 1673, 1580, 1540, 1410, 1331, 1260, 1200, 1104, 1058 cm^{-1}; UV λ_{max} (log ε, MeOH) 212 (3.9); ^{1}H and ^{13}C NMR data, see Table 2; ESIMS $[M + Na]^+$ *m/z* 225; HRESIMS $[M + Na]^+$ *m/z* 225.0733 (calcd for $C_9H_{14}O_5Na$, 225.0739).

Xylogiblactone C (7): amorphous, white solid; $[\alpha]^{26}_D$ +94.5 (*c* 0.85, MeOH); IR (ZnSe) ν_{max} 3404, 2925, 2857, 1744, 1654, 1571, 1445, 1386, 1326, 1204, 1062, 1007, 955 cm^{-1}; UV λ_{max} (log ε, MeOH) 212 (4.0); ^{1}H and ^{13}C NMR data, see Table 2; ESIMS $[M + Na]^+$ *m/z* 239; HRESIMS $[M + Na]^+$ *m/z* 239.0893 (calcd for $C_{10}H_{16}O_5Na$, 239.0895).

Antimicrobial Bioassay. Antifungal assays were conducted with the National Committee for Clinical Laboratory Standards (NCCLS) reference method.[18−20] All the compounds except **6** and **7** were tested against the microorganisms *Candida albicans* (ATCC 18804), *C. albicans* (ATCC MYA-2876), and *Saccharomyces cerevisiae* (ATCC 2345). Test samples were dissolved in DMSO and were serially diluted using RPMI-1640 medium (Sigma-Aldrich R8755). These were transferred to 96-well microplates in triplicate, and the suspension of the test organisms was added to each well, achieving a final volume of 200 μL. Amphotericin B (Sigma-Aldrich A2411) was used as positive control for testing antifungal agents, and blank (medium only) controls were added to each test plate. The plates were read turbidimetrically at 612 nm using a TECAN Sunrise ELISA reader (TECAN Group Ltd., Männedorf, Switzerland) prior to and after incubation. The percent growth was calculated and plotted versus concentration to afford the MIC. The MIC values were determined as the lowest concentration where test compounds inhibited fungal growth.[20]

Nitric Oxide Production Inhibition Assay. RAW 264.7 cells obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan) were maintained by twice-weekly passage in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS) and penicillin−streptomycin. The methods of nitrite measurement were the same as reported previously.[21] Cell aliquots (5 × 10^5 cells/mL) were grown to confluence on 24-well plates for 24 h. The medium was switched to the serum-free DMEM for another 4 h to render attached cells quiescent. To assess the effects on lipopolysaccharide-induced NO production, compounds **1**−**7** and sordarin (purity >98% as checked by their ^{1}H NMR) and two positive controls, N^{ω}-nitro-L-arginine (L-NNA, a NOS inhibitor) and aminoguanidine (a specific inhibitor of iNOS), or vehicle (0.1%, DMSO) were added in the presence of LPS (200 ng/mL) to the RAW 264.7 cells. The nitrite concentration in the culture medium was determined spectrophotometrically as an index of NO production.[22] The Alamar Blue assay was used to examine whether the amount of the test specimens used in this study caused cytotoxicity.[23] Both positive controls were purchased from Sigma-Aldrich Chemical Co., and the purity of each compound was more than 98%.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra of the new compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by a grant from the National Science Council of the Republic of China. We thank Ms. S.-H. Wang of the Instrumentation Center of Taipei Medical University and Ms. S.-L. Huang of the Instrumentation Center of the College of Science, National Taiwan University, for the NMR data acquisition and Ms. Y.-C. Wu of the Small Molecule Metabolomics Core Facility, the Institute of Plant and Microbial Biology and Academia Sinica Scientific Instrument Center, Academia Sinica, for the MS data acquisition.

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