New α-Tetralone Galloylglucosides from the Fresh Pericarps of *Juglans sigillata*

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Three new α-tetralone galloylglucosides, **1**–**3**, were isolated from the fresh pericarps of *Juglans sigillata* (Juglandaceae), together with six known compounds. The structures of the new compounds were determined as 1,2,3,4-tetrahydro-7-hydroxy-4-oxonaphthalen-1-yl 6-*O*-[(3,4,5-trihydroxyphenyl)carbonyl]-β-D-glucopyranoside (**1**), (1*S*)-1,2,3,4-tetrahydro-8-hydroxy-4-oxonaphthalen-1-yl 6-*O*-[(3,4,5-trihydroxyphenyl)carbonyl]-β-D-glucopyranoside (**2**), and 1,2,3,4-tetrahydro-7,8-dihydroxy-4-oxonaphthalen-1-yl 6-*O*-[(3,4,5-trihydroxyphenyl)carbonyl]-β-D-glucopyranoside (**3**), respectively, on the basis of detailed spectroscopic analyses, and acidic and enzymatic hydrolysis. The antimicrobial activities of the isolated compounds **2**, **4**, and **7**–**9** were evaluated.

Introduction. – The genus *Juglans* (Juglandaceae) comprising about 20 species is widely distributed in the temperate and subtropical areas of the world [1]. The seeds of the *Juglans* species, particularly *J. regia*, known as walnuts, are excellent sources of unsaturated fatty acids and polyphenols, and used as folk remedies for cancer, kidney, and stomach diseases in Asia and Europe [2]. The fresh pericarp of some species, *e.g.*, *J. mandshurica* and *J. regia*, commonly named as 'Qing-Long-Yi', have been medicinally used for thousands of years in China, Japan, and Korea, owing to its anti-tumor, anti-inflammatory, antinociceptive, and antioxidant effects. The roots and leaves of these plants are also used as folk medicine for the treatment of cancer, rheumatic pains, and eczema [3][4]. Some naphthalene glucosides [5], α-tetralones and their derivatives [6–8], and diarylheptanoids [9] have been reported from the fresh pericarps or fruits of *Juglans* species.

Juglans sigillata DODE, known as the iron walnut, has been widely cultivated for its edible nuts in the southwest of China. To date, no chemical work has been carried out on this species. Our detailed chemical investigation on the fresh pericarps of *J. sigillata* led to the isolation of three new galloylglucosides, **1**–**3**, together with six known compounds, **4**–**9** (*Fig. 1*). The isolated compounds **2**, **4**, and **7**–**9** were evaluated for their antimicrobial activities.

Results and Discussion. – The fresh pericarps of *J. sigillata* were extracted three times with 80% aqueous acetone at room temperature. After removal of the organic

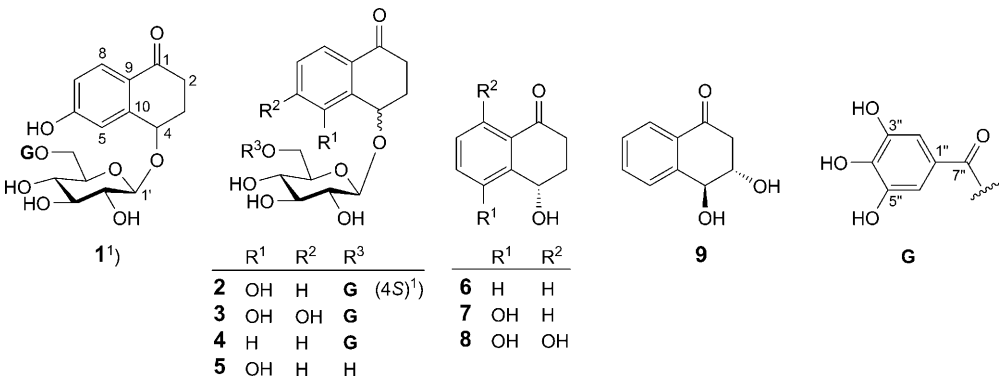

Fig. 1. *α-Tetralone derivatives from the fresh pericarps of* Juglans sigillata

solvent, the aqueous fraction was extracted with $CHCl_3$ and AcOEt, successively. The AcOEt fraction was subjected to column chromatography (CC) over *Sephadex LH-20*, silica gel, and *MCI-gel CHP20P* to afford compounds **1**–**9**. Compounds **4**–**9** were known, and identified as (1*S*)-1,2,3,4-tetrahydro-4-oxonaphthalen-1-yl 6-*O*-[(3,4,5-trihydroxyphenyl)carbonyl]-*β*-D-glucopyranoside (**4**) [6], (1*S*)-1,2,3,4-tetrahydro-8-hydroxy-4-oxonaphthalen-1-yl 6-*O*-[(3,4,5-trihydroxyphenyl)carbonyl]-*β*-D-glucopyranoside (**5**) [7], (4*S*)-4-hydroxy-*α*-tetralone (**6**) [7], (4*S*)-4,5-dihydroxy-*α*-tetralone (**7**) [7], (4*S*)-4,5,8-trihydroxy-*α*-tetralone (**8**) [7], and (3*S*,4*S*)-3,4-dihydro-3,4-dihydroxynaphthalen-1(2*H*)-one (**9**) [8], respectively, by comparison of their spectroscopic and physical data with those reported previously in the literature.

Compound **1** was obtained as a white amorphous powder. The molecular formula of $C_{23}H_{24}O_{12}$ was determined on the basis of the HR-ESI-MS (*m*/*z* 491.1210, $[M-H]^-$). The IR spectrum showed absorptions at 3439, 1627, and 1606 cm^{-1}, suggesting the presence of OH and CO groups. Acidic hydrolysis of **1** gave D-glucose as the sole sugar residue. The ^1H- and ^{13}C-NMR data (*Table*) of **1** exhibited the signals arising from a CO group (δ(C) 197.8), a benzene ring (δ(H) 7.74, 7.08, and 6.81), a *β*-glucopyranosyl moiety (anomeric H-atom at δ(H) 4.60 (*d*, *J* = 7.9, H–C(1′)[1]) and C-atom signals at δ(C) 103.7 C(1′), 74.3 C(2′), 77.1 C(3′), 71.1 C(4′), 76.5 C(5′), and 63.4 C(6′)), a galloyl (δ(H) 7.11 (*s*, 2 H)) group, two CH_2 groups (δ(C) 35.0 (C(2)) and 31.2 (C(3))), and one O-bearing CH group (δ(C) 74.4 (C(4))). These NMR characteristics resembled those observed for **4**. However, instead of a 1,2-disubstituted benzene ring in **4**, compound **1** had a 1,2,4-trisubstituted benzene ring in view of the coupling patterns of the aromatic H-atoms (δ(H) 7.74 (*d*, *J* = 8.6, H–C(8)), 7.08 (*d*, *J* = 2.4, H–C(5)), and 6.81 (*dd*, *J* = 8.6, 2.4, H–C(7))). In the HMBC spectrum of **1**, correlations of δ(H) 7.74 (H–C(8)) with δ(C) 197.8 (C(1)) and δ(H) 7.08 (H–C(5)) with δ(C) 74.4 (C(4)) were observed. These HMBCs in conjunction with the multiplicities in the ^1H-NMR spectrum of **1** demonstrated that C(6) of the benzene ring was substituted with a OH group. In addition, the HMBC of H–C(4) (δ(H) 4.82) with C(1′) (δ(C) 103.7, glucosyl unit) indicated the glucose moiety to be attached to C(4) of the aglycone. The location of the

[1]) Arbitrary atom numbering. For systematic names, see *Exper. Part*.

galloyl group at HO–C(6) of the glucosyl unit was also determined by the HMBC experiment, in which correlations of $CH_2(6')$ (δ(H) 4.57, 4.36, glucosyl unit) with the CO C-atom C(7″) (δ(C) 167.3) of the galloyl group were observed. Other HMBCs (*Fig. 2*) also confirmed the structure of **1**. The configuration at C(4) could not be established due to insufficient amounts of compound **1**. Accordingly, compound **1** was determined to be 1,2,3,4-tetrahydro-7-hydroxy-4-oxonaphthalen-1-yl 6-*O*-[(3,4,5-trihydroxyphenyl)carbonyl]-β-D-glucopyranoside.

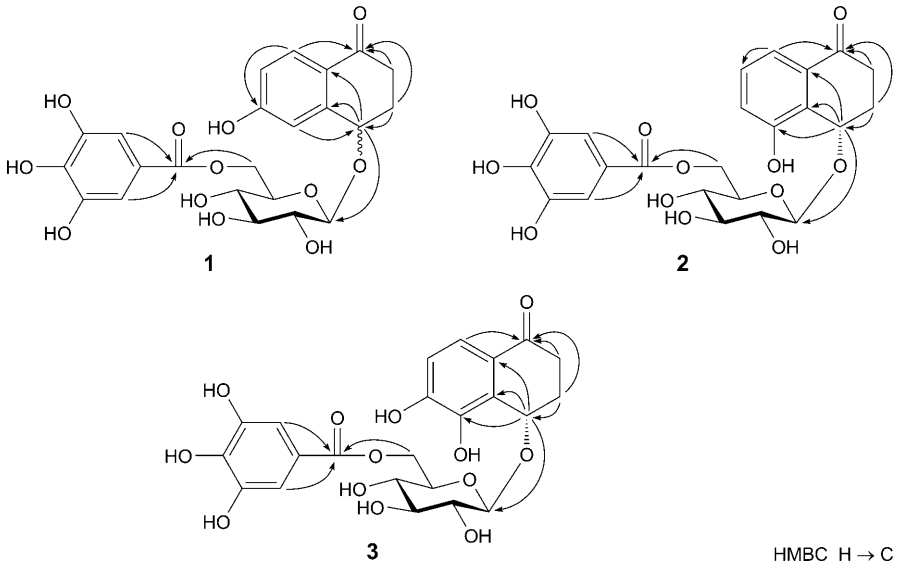

Fig. 2. *Key HMBCs of compounds* **1**–**3**

Compound **2** was isolated as a white amorphous powder. The molecular formula $C_{23}H_{24}O_{12}$ was deduced from the HR-ESI-MS (*m*/*z* 491.1191, $[M-H]^-$) data. The ^{1}H- and ^{13}C-NMR spectra of **2** (*Table*) were very similar to those of **1**, except for the substitution of the benzene ring, which showed H-atom signals at δ(H) 7.37 (*d*, $J = 7.8$, H–C(8)[1])), 7.26 (*t*, $J = 7.8$, H–C(7)), and 7.14 (*d*, $J = 7.8$, H–C(6)). These observations indicated that the benzene ring was a 1,2,3-trisubstituted system. In the HMBC spectrum of **2** (*Fig. 2*), correlations of H–C(4) (δ(H) 5.24) with C(5) (δ(C) 156.0), C(9) (δ(C) 133.5), and C(10) (δ(C) 128.7), and H–C(8) (δ(H) 7.37) with C(1) (δ(C) 199.7) revealed that a OH group was attached to the C(5) position. Connectivities of the sugar moiety with the 4-hydroxy-α-tetralone skeleton and the galloyl unit were confirmed by the HMBC experiment, in which correlations of H–C(4) (δ(H) 5.24) with C(1′) (δ(C) 103.5), and H–C(6′) (δ(H) 4.57, 4.35) with C(7″) (δ(C) 167.3) were observed. Furthermore, enzymatic hydrolysis of **2** gave **7** as the aglycone, whose absolute configuration was determined as (4*S*) by the positive $[\alpha]_D$ value [7]. Acidic hydrolysis, followed by GC analysis, revealed D-glucose as the sugar residue. Thus, the structure of **2** was established as (1*S*)-1,2,3,4-tetrahydro-8-hydroxy-4-oxonaphthalen-1-yl 6-*O*-[(3,4,5-trihydroxyphenyl)carbonyl]-β-D-glucopyranoside.

Table. *^{1}H- and ^{13}C-NMR Data of* **1**–**3**[1]) (in (D_6)acetone, 500 MHz and 125 MHz for ^{1}H- and ^{13}C-NMR, resp.; δ in ppm, J values in Hz)

	1		**2**		**3**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
1	197.8		199.7		199.0	
2	35.0	2.63 (*ddd*, *J* = 4.5, 7.0, 17.0), 2.28–2.37 (*m*)	33.4	2.88 (*dt*, *J* = 19.0, 6.0), 2.36–2.44 (*m*)	33.1	2.78 (*ddd*, *J* = 4.5, 9.5, 18.5), 2.34 (*ddd*, *J* = 4.5, 8.5, 18.5)
3	31.2	2.28 (*dt*, *J* = 17.0, 5.0), 2.08–2.13 (*m*)	29.0	2.33 (*dt*, *J* = 21.0, 4.7), 2.11 (*dt*, *J* = 16.0, 4.7)	29.2	2.28 (*dt*, *J* = 17.5, 4.5), 2.05–2.09 (*m*)
4	74.4	4.82 (*dd*, *J* = 10.0, 4.3)	71.0	5.24 (*t*, *J* = 4.0)	71.0	5.25 (*t*, *J* = 4.0)
5	114.5	7.08 (*d*, *J* = 2.4)	156.0		143.0	
6	163.1		121.8	7.14 (*d*, *J* = 7.8)	151.6	
7	116.3	6.81 (*dd*, *J* = 8.6, 2.4)	130.2	7.26 (*t*, *J* = 7.8)	115.8	6.89 (*d*, *J* = 8.4)
8	130.0	7.74 (*d*, *J* = 8.6)	118.2	7.37 (*d*, *J* = 7.8)	120.6	7.35 (*d*, *J* = 8.4)
9	124.3		133.5		124.9	
10	147.0		128.7		129.3	
1′	103.7	4.60 (*d*, *J* = 7.9)	103.5	4.64 (*d*, *J* = 8.0)	103.2	4.64 (*d*, *J* = 7.9)
2′	74.3	3.31 (*dd*, *J* = 8.9, 7.9)	74.4	3.22 (*dd*, *J* = 8.0, 9.0)	74.2	3.22 (*t*, *J* = 8.5, 7.9)
3′	77.1	3.48 (*t*, *J* = 8.9)	76.9	3.48 (*t*, *J* = 8.9)	76.8	3.47 (*t*, *J* = 8.9)
4′	71.1	3.42 (*t*, *J* = 9.4)	70.7	3.41 (*t*, *J* = 9.4)	71.3	3.43 (*t*, *J* = 9.4)
5′	76.5	3.67–3.71 (*m*)	74.7	3.68–3.70 (*m*)	74.6	3.65–3.69 (*m*)
6′	63.4	4.57 (*dd*, *J* = 11.8, 2.5), 4.36 (*dd*, *J* = 11.8, 7.2)	64.4	4.57 (*dd*, *J* = 12.0, 2.5), 4.35 (*dd*, *J* = 12.0, 8.5)	64.4	4.55 (*dd*, *J* = 11.8, 2.0), 4.34 (*dd*, *J* = 11.8, 7.1)
1″	121.0		120.8		120.8	
2″	109.7	7.11 (*s*)	109.7	7.12 (*s*)	109.7	7.10 (*s*)
3″	145.8		145.8		145.6	
4″	139.0		139.1		138.9	
5″	145.8		145.8		145.6	
6″	109.7	7.11 (*s*)	109.7	7.12 (*s*)	109.7	7.10 (*s*)
7″	167.3		167.3		167.4	

Compound **3** was obtained as a white amorphous powder. The molecular formula was determined to be $C_{23}H_{24}O_{13}$ by HR-ESI-MS (*m*/*z* 507.1118, $[M-H]^-$). The ^{1}H- and ^{13}C-NMR spectra (*Table*) displayed signals due to a 4-hydroxy-α-tetralone skeleton, a glucosyl moiety, and a galloyl unit, suggesting that **3** was also a 4-hydroxy-α-tetralone derivative. The *ortho* coupled aromatic H-atom signals at δ(H) 7.35 and 6.89 (each 1 H, *d*, *J* = 8.4) indicated a 1,2,3,4-tetrasubstituted benzene ring in **3**. Acidic hydrolysis of **3** gave D-glucose as the sole sugar residue. In the HMBC spectrum of **3** (*Fig. 2*), correlations of H–C(8)[1]) (δ(H) 7.35) with C(1) (δ(C) 199.0) and C(4) (δ(C) 71.0) and H–C(4) (δ(H) 5.25) with C(5) (δ(C) 143.0) assigned the *ortho* aromatic H-atoms as H–C(7) and H–C(8). Furthermore, HMBCs of H–C(4) (δ(H) 5.25) with C(1′) (δ(C) 103.2) and H–C(6′) (δ(H) 4.55, 4.34) with C(7″) (δ(C) 167.4) confirmed the connectivities of the sugar moiety with the 4-hydroxy-α-tetralone aglycone and the galloyl moiety. Accordingly, the structure of **3** was established as 1,2,3,4-tetrahydro-7,8-dihydroxy-4-oxonaphthalen-1-yl 6-*O*-[(3,4,5-trihydroxyphenyl)carbonyl]-β-D-glucopyranoside.

Compounds **1**–**4**, with a 4-hydroxy-α-tetralone aglycone and a 6-*O*-galloylglucosyl moiety in the molecule, were isolated for the first time from fresh pericarps of the

Juglans species. The known compound **4** was reported to have strong inhibitory activity against the protein tyrosine phosphatase 1B [6], which plays a major role in the dephosphorylation of the insulin receptor. Thus, it may have a potential for the development of new pharmacological agents for the treatment of type-2 diabetes and obesity.

The isolated compounds **2**, **4**, and **7**–**9** were evaluated for *in vitro* antifungal (*Aspergillus fumigatus*, *Candida albicans*, *C. glabrata*, *C. krusei*, and *Cryptococcus neoformans*) and antibacterial (*Escherichia coli*, *Mycobacterium intracellulare*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and methicillin-resistant *S. aureus*) activities using a modified version of the CLSI (formerly NCCLS) methods [10–12]. Only compound **8** exhibited moderate antibacterial activity against *S. aureus* and methicillin-resistant *S. aureus* with 50% inhibitory concentrations (IC_{50})/minimum inhibitory concentrations (*MIC*) of 4.96/10.0 μg/ml, and 3.46/5.0 μg/ml. The IC_{50}/*MIC* values of the positive antibacterial control ciprofloxacin were 0.13/0.50 μg/ml and 0.14/0.50 μg/ml.

Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* (*Pharmacia Fine Chemical Co., Ltd.*), silica gel (SiO_2; 200–300 mesh, *Qingdao Makall Group Co., Ltd.*), or *MCI gel CHP 20P* (*Mitsubishi Chemical Co.*). Thin layer chromatography (TLC): on silica gel *H*-precoated plates (*Qingdao Makall Group Co., Ltd.*) with $CHCl_3$/MeOH/H_2O (8:2:0.2); spots were detected by spraying with 10% H_2SO_4 reagent followed by heating. GC Analysis: *Shimadzu GC-14C* gas chromatograph. Optical rotations: *SEPA-3000* automatic digital polarimeter. UV Spectra: *Jasco V-560 UV/VIS* spectrophotometer. IR Spectra: *Bio-Rad FTS-135* spectrometer; in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker AM-400* or *DRX-500* instruments operating at 400 or 500 MHz for ^1H, 100 or 125 MHz for ^{13}C, resp.; coupling constants in Hertz (Hz), and chemical shifts are given on a ppm scale with TMS as internal standard. FAB-MS (negative-ion mode) and HR-ESI-MS (negative-ion mode) spectra: *VG AutoSpec 3000* and *API Qstar Pulsar LC/TOF* spectrometers, resp.; the matrix for FAB-MS was glycerol.

Plant Material. The fresh pericarps of *J. sigillata* were collected in August 2007 in the botanical garden of Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan Province, P. R. China, and identified by Prof. *Xiao Cheng* (Botanical Garden, Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen (KIB-ZL2007002) has been deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences. The fresh material was soaked into 80% aq. acetone at r.t. as soon as collected.

Extraction and Isolation. The fresh pericarps of *J. sigillata* (37 kg) were extracted three times with 80% aq. acetone (30 l) at r.t. (each 7 d). After removal of the org. solvent under reduced pressure, the aq. fraction was extracted with equivoluminal $CHCl_3$ and AcOEt, successively, to give a $CHCl_3$ fraction (24 g) and an AcOEt fraction (240 g).

The AcOEt fraction (240 g) was subjected to CC over *Sephadex LH-20*, eluted with MeOH/H_2O (0:1 to 1:0), to afford four fractions (*Frs. 1–4*). Further CC on *MCI-gel CHP20P* and *Sephadex LH-20*, both eluted with MeOH/H_2O (0:1 to 4:6), gave **1** (2.0 mg) and **3** (3.0 mg) from *Fr. 1* (4.9 g), **2** (44 mg) from *Fr. 2* (3.8 g), and **4** (114 mg) from *Fr. 3* (21.8 g); resp. *Fr. 4* (3.3 g) was subjected to CC over SiO_2, eluted with petroleum ether (PE)/AcOEt (7:3, 1:1, and 2:8, successively), and then over *Sephadex LH-20* and *MCI-gel CHP20P*, eluted with MeOH/H_2O (0:1 to 3:7), to afford compounds **5** (35 mg), **6** (6 mg), **7** (48 mg), **8** (25 mg), and **9** (20 mg).

*1,2,3,4-Tetrahydro-7-hydroxy-4-oxonaphthalen-1-yl 6-O-[(3,4,5-Trihydroxyphenyl)carbonyl]-β-*D*-glucopyranoside* (**1**). White amorphous powder. $[\alpha]_D^{16} = 0$ ($c = 0.08$, MeOH). UV (MeOH): 290 (3.69), 224 (3.86), 207 (3.85). IR (KBr): 3439, 1627, 1606, 1042, 577. ^1H- and ^{13}C-NMR: *Table*. FAB-MS (neg.): 491 ($[M-H]^-$). HR-ESI-MS (neg.): 491.1210 ($[M-H]^-$, $C_{23}H_{23}O_{12}^-$; calc. 491.1190).

(*1S*)*-1,2,3,4-Tetrahydro-8-hydroxy-4-oxonaphthalen-1-yl 6-*O*-[(3,4,5-Trihydroxyphenyl)carbonyl]-β-*D*-glucopyranoside* (**2**). White amorphous powder. $[\alpha]_D^{26} = +10.3$ ($c = 0.20$, MeOH). UV (MeOH): 321 (3.87), 235 (4.24), 198 (4.14). IR (KBr): 3422, 1679, 1609, 1348, 1319, 1232, 1074, 1038, 589. ^{1}H- and ^{13}C-NMR: *Table*. FAB-MS (neg.): 491 ($[M-H]^-$). HR-ESI-MS (neg.): 491.1191 ($[M-H]^-$, $C_{23}H_{23}O_{12}^-$; calc. 491.1190).

*1,2,3,4-Tetrahydro-7,8-dihydroxy-4-oxonaphthalen-1-yl 6-*O*-[(3,4,5-Trihydroxyphenyl)carbonyl]-β-*D*-glucopyranoside* (**3**). White amorphous powder. $[\alpha]_D^{16} = 14.2$ ($c = 0.11$, MeOH). UV (MeOH): 321 (3.87), 220 (4.03), 203 (4.08). IR (KBr): 3425, 1611, 1318, 1225, 1038, 592. ^{1}H- and ^{13}C-NMR: *Table*. FAB-MS: 507 ($[M-H]^-$). HR-ESI-MS: 507.1118 ($[M-H]^-$, $C_{23}H_{23}O_{13}^-$; calc. 507.1139).

Acidic Hydrolysis of Compounds **1**–**3**. Compounds **1** (1.5 mg), **2** (4.0 mg), and **3** (1.5 mg) were separately hydrolyzed with 2M HCl/dioxane (1:1, 4 ml) under reflux for 6 h. The mixture was extracted with AcOEt 4 times. The aq. layer was neutralized with 2M NaOH and dried under reduced pressure. The residue was dissolved in pyridine (2 ml). L-Cysteine methyl ester hydrochloride (about 1.5 mg) was added, and the mixture was kept at 60° for 1 h. Next, trimethylsilylimidazole (about 1.5 ml) was added to the mixture in ice water and kept at 60° for 30 min. Then, the mixture was subjected to GC analysis, run on a *Shimadzu GC-14C* gas chromatograph equipped with a 30 m × 0.32 mm i.d. *30QC2/AC-5* quartz capillary column and an H_2 flame ionization detector with the following conditions: column temp. 180–280°, programmed increase 3°/min, carrier gas N_2 (1 ml/min), injector and detector temp. 250°; injection volume 4 µl, and split ratio 1:50. The configuration of the sugar moiety was determined by comparing the retention time with the derivatives of authentic samples. The retention times of D- and L-glucose were 19.21 and 14.94 min.

Enzymatic Hydrolysis of Compound **2**. A soln. of **2** (10 mg) in H_2O was incubated with β-glucosidase (*Sigma Chemical Co.*, 8.92 U/mg, 8 mg) at 37° for 7 d. The mixture was extracted with $CHCl_3$ for 5 times. The $CHCl_3$ fraction was evaporated to dryness and subjected to CC over *MCI-gel CHP20P*, eluted successively with H_2O and MeOH, to afford **7** (2 mg), $[\alpha]_D^{18} = +16.7$ ($c = 0.03$, MeOH).

Antifungal and Antibacterial Bioassays. All organisms were obtained from the *American Type Culture Collection* (Manassas, VA) and included the fungi *Candida albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 204305, and the bacteria *Staphylococcus aureus* ATCC 29213, methicillin-resistant *S. aureus* ATCC 33591 (MRS), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068. Susceptibility testing was performed using a modified version of the CLSI methods [10][11]. *M. intracellulare* was tested using a modified method of *Franzblau et al.* [13]. Samples **2**, **4**, and **7**–**9** were serially diluted in 20% DMSO/saline and transferred in duplicate to 96-well flat bottom microplates. Microbial inocula were prepared by correcting the OD_{630} of the microbe suspensions in incubation broth to afford final target inocula after addition to the samples. All organisms were read spectrometrically prior to and after incubation. The detailed protocol has been described in a previous article [14].

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