

Acetylated Flavanone Glycosides from the Rhizomes of *Cyclosorus acuminatus*Wei Fang,[†] Jinlan Ruan,^{*,†} Zhong Wang,[†] Zhongxiang Zhao,[†] Jian Zou,[‡] Daonian Zhou,[†] and Yaling Cai[†]

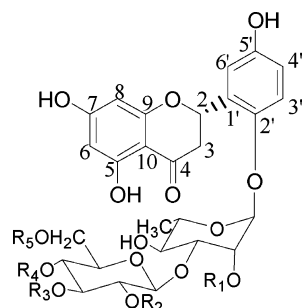
College of Pharmacy, Tongji Medical Center, Huazhong University of Science and Technology, Wuhan 430030, People's Republic of China, and Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, People's Republic of China

Received July 11, 2006

Six new flavanone glycosides (**1–6**) were isolated from the methanol extract of the rhizomes of *Cyclosorus acuminatus*, together with the parent flavanone glycoside **2a**. Their structures were established on the basis of spectroscopic and chemical methods. All compounds showed moderate activity against *Streptococcus pneumoniae* and *Haemophilus influenzae*.

Cyclosorus acuminatus (Houtt.) Nakai (Thelypteridaceae) is widely distributed in the south of China.^{1,2} The rhizomes of this plant have been used in Chinese folk medicine for the treatment of diarrhea, rheumatism, and wounds.² A previous phytochemical investigation on the *Cyclosorus* genus has been reported;³ however, no reports on *C. acuminatus* have been published. As part of our search for bioactive constituents from the *Cyclosorus* species, the rhizomes of *C. acuminatus* have been studied and six new flavanone glycosides (**1–6**) were isolated. We herein report the isolation and structure elucidation of **1–6**, as well as the antibacterial activities of **1–6** and the parent flavanone glycoside **2a**.

The rhizomes of *C. acuminatus* were extracted with MeOH. The extract was concentrated in vacuo, and the residue was suspended in H₂O and extracted with petroleum ether, CHCl₃, EtOAc, and *n*-BuOH, sequentially. Column chromatography of the CHCl₃ and EtOAc extract led to the isolation of **1–6**. Their structures were elucidated by spectroscopic methods, including 2D NMR experiments (COSY, HSQC, and HMBC), and chemical methods.



	R ₁	R ₂	R ₃	R ₄	R ₅
1	Ac	H	H	H	H
2	Ac	H	H	H	Ac
2a	H	H	H	H	H
3	Ac	Ac	H	H	Ac
4	Ac	H	Ac	H	Ac
5	Ac	H	H	Ac	Ac
6	Ac	Ac	Ac	H	Ac

Compound **1** was obtained as a white, amorphous powder with the molecular formula C₂₉H₃₄O₁₆, determined on the basis of NMR and HRESIMS data (*m/z* 661.1734 [M + Na]⁺). The IR spectrum of **1** showed strong absorptions at 1639 and 3423 cm⁻¹ (OH). UV absorption maxima at λ_{max} 290 and 330 (sh) nm were indicative of a flavanone structure.^{4,5} Examination of the ¹H and ¹³C NMR

spectroscopic data of **1** (Tables 1 and 2) indicated that the molecule consisted of a flavanone, two sugars, and one acetyl moiety.

The spin systems located at δ 5.61 (1H, dd, *J* = 13.0, 3.0 Hz, H-2), 3.08 (1H, dd, *J* = 17.0, 13.0 Hz, H-3α), and 2.81 (1H, dd, *J* = 17.0, 3.0 Hz, H-3β) in the ¹H NMR spectrum together with a carbonyl resonance at δ 198.6 in the ¹³C NMR confirmed the presence of a flavanone skeleton. The ABX spin system was found at δ 7.04 (1H, d, *J* = 8.8 Hz, H-3'), 6.99 (1H, d, *J* = 3.0 Hz, H-6'), and 6.74 (1H, dd, *J* = 8.8, 3.0 Hz, H-4'), and the correlations of H-2 to C-1', C-2', and C-6' in the HMBC spectrum (Figure 1) supported **1** having 2',5'-dihydroxy substitution in the B ring. Furthermore, the ¹H NMR spectrum showed two one-proton doublets (*J* = 2.2 Hz) at δ 5.99 (H-8) and 5.92 (H-6). On the basis of the above analyses of spectroscopic data, the flavanone aglycone was suggested as 5,7,2',5'-tetrahydroxyflavanone. Acid hydrolysis of **1** yielded D-glucose and L-rhamnose, as determined by TLC and GC analyses. The ¹H NMR spectrum also revealed one three-proton singlet at δ 2.09 (2''-OCOCH₃), and this methyl group was assigned to the acetyl moiety, which was connected to the C-2'' site of the rhamnose unit by the HMBC correlation of H-2''/2''-OCOCH₃.

Finally, the connection of these substructures was completed by the HMBC spectrum of **1**. The β-D-O-glucopyranosyl unit was linked to C-3' of the α-L-acetylramnopranosyl moiety by the ³*J* interactions of H-1'''/C-3'' and H-3'''/C-1'''. The sugar chain was connected to the C-2' position of the flavanone aglycone by the HMBC correlation of H-1'''/C-2' (Figure 1). The absolute configuration at C-2 was confirmed by a positive Cotton effect at 326 nm and a negative Cotton effect at 287 nm in the CD spectrum, which is characteristic for (2*S*)-flavanones.⁶ Thus, the structure of **1** was determined as (2*S*)-5,7,5'-trihydroxyflavanone 2'-O-β-D-glucopyranosyl-(1→3)-α-L-2-O-acetylramnopranoside.

Compound **2** was obtained as a white, amorphous powder with the molecular formula C₃₁H₃₆O₁₇ deduced from HRESIMS (*m/z* 703.1827 [M + Na]⁺). The ¹H and ¹³C NMR spectra of **2** were similar to those of **1**, except for the presence of two acetyl groups at δ_C 173.8, 21.2 (6'''-OCOCH₃) and 172.6, 21.1 (2''-OCOCH₃) in the ¹³C NMR spectrum of **2**. The acetyl groups were linked to the C-2'' and C-6''' positions by HMBC correlations of H-2''/2''-OCOCH₃ and H-6'''/6'''-OCOCH₃. All the protons and carbons of **2** could be assigned on the basis of the analyses of the 2D NMR spectroscopic data of **2** including ¹H–¹H COSY, HMQC, and HMBC spectra. The configuration at C-2 was defined as *S* from the CD spectrum, which showed a positive Cotton effect at 326 nm and a negative Cotton effect at 287 nm.⁶ Thus, compound **2** was determined to be (2*S*)-5,7,5'-trihydroxyflavanone 2'-O-β-D-6-O-acetylglucopyranosyl-(1→3)-α-L-2-O-acetylramnopranoside.

Compounds **3**, **4**, and **5** were isolated as white, amorphous powders having the same molecular formula C₃₃H₃₈O₁₈ on the basis of HRESIMS. Comparing the ¹H and ¹³C NMR spectroscopic data

* To whom correspondence should be addressed. Tel/Fax: 86-27-83692892. E-mail: jinlan8152@163.com.

[†] Huazhong University of Science and Technology.

[‡] Shanghai Institute of Materia Medica.

Table 1. ^1H NMR (400 MHz) Data of **1–6** and **2a** (δ values, J in Hz)

position	1 ^a	2 ^a	3 ^a	4 ^a	5 ^a	6 ^b	2a ^a
2	5.61 dd (13.0, 3.0)	5.69 dd (13.0, 3.0)	5.67 dd (13.0, 3.0)	5.71 dd (13.0, 3.0)	5.61 dd (13.0, 3.0)	5.81 dd (13.0, 3.0)	5.57 dd (13.2, 3.0)
3	3.08 dd (17.0, 13.0)	3.10 dd (17.0, 13.0)	3.08 dd (17.0, 13.0)	3.11 dd (17.0, 13.0)	2.91 dd (17.0, 13.0)	3.12 dd (17.0, 13.0)	3.07 dd (17.8, 13.2)
	2.81 dd (17.0, 3.0)	2.81 dd (17.0, 3.0)	2.81 dd (17.0, 3.0)	2.82 dd (17.0, 3.0)	2.74 dd (17.0, 3.0)	2.84 dd (17.0, 3.0)	2.77 dd (17.8, 3.0)
6	5.92 d (2.2)	5.92 d (2.2)	5.93 d (2.2)	5.94 d (2.2)	5.88 d (2.2)	6.00 d (2.2)	5.96 d (2.4)
8	5.99 d (2.2)	6.00 d (2.2)	5.99 d (2.2)	5.99 d (2.2)	5.94 d (2.2)	6.05 d (2.2)	5.90 d (2.4)
3'	7.04 d (8.8)	7.04 d (8.8)	7.03 d (8.8)	7.05 d (8.8)	6.99 d (8.8)	7.05 d (8.8)	7.07 d (9.0)
4'	6.74 dd (8.8, 3.0)	6.74 dd (8.8, 3.0)	6.74 dd (8.8, 3.0)	6.74 dd (8.8, 3.0)	6.68 dd (8.8, 3.0)	6.80 dd (8.8, 3.0)	6.72 dd (9.0, 3.0)
6'	6.99 d (3.0)	7.00 d (3.0)	6.98 d (3.0)	7.01 d (3.0)	6.94 d (3.0)	7.07 d (3.0)	6.96 d (3.0)
1''	5.31 d (1.7)	5.34 ^c	5.28 ^d	5.32 ^e	5.28 ^f	5.34 d (1.7)	5.27 br s
2''	5.40 m	5.34 m ^c	5.28 m ^d	5.32 m ^e	5.28 m ^f	5.30 m	4.24 m
3''	4.00 m	3.97 m	3.96 m	3.97 m	3.94 m	4.06 m	3.80 m
4''	3.60 m	3.60 m	3.50 m	3.60 m	3.57 m	3.56 m	3.62 m
5''	3.80 m	3.69 m	3.73 m	3.78 m	3.75 m	3.76 m	3.72 m
6''	1.30d (7.4)	1.30 d (7.4)	1.28 d (7.4)	1.30 d (7.4)	1.24 d (7.4)	1.28 d (6.0)	1.27 d (6.0)
1'''	4.35 d (7.7)	4.39 d (7.7)	4.41 d (8.0)	4.42 d (8.0)	4.22 d (7.8)	4.76 ^g	4.36 d (7.8)
2'''	3.19 m	3.21 m	4.66 m	3.31 m	3.23 m	4.76 m ^g	3.25 m
3'''	3.31 m	3.32 m	3.46 m	4.90 m	3.44 m	5.01 m	3.39 m
4'''	3.30 m	3.21 m	3.31 m	3.40 m	4.63 m	3.62 m	3.33 m
5'''	3.09 m	3.21 m	3.26 m	3.35 m	3.25 m	3.58 m	3.06 m
6'''	3.62 m	4.20 br d	4.23 dd	4.23 dd	3.84 dd	4.26 dd	3.65 dd
		(11.6)	(12.0, 2.0)	(12.0, 2.0)	(12.0, 5.0)	(11.8, 2.2)	(12.0, 3.6)
	3.58 m	3.95 m	3.95 dd	3.94 dd	3.75 dd	4.08 m	3.53 dd
			(12.0, 5.7)	(12.0, 5.0)	(12.0, 2.0)		(12.0, 1.8)
2''-OCOCH ₃	2.10 s	2.05 s	2.04 s	2.05 s	2.05 s	2.02 s ^h	
2'''-OCOCH ₃			2.10 s			1.97 s ^h	
3'''-OCOCH ₃				2.10 s		1.96 s ^h	
4'''-OCOCH ₃					2.10 s		
6'''-OCOCH ₃		1.91 s	1.94 s	1.91 s	1.90 s	1.94 s	

^a In CD₃OD. ^bIn DMCO-*d*₆. ^{c–g}Overlapping signals. ^hValues may be interchanged.

of **3**, **4**, and **5** with those of **2** (Tables 1 and 2), the obvious differences were the additional acetyl moieties in compounds **3**, **4**, and **5**. The acetyl groups of **3** were linked to C-2'', C-2''', and C-6''' by the HMBC correlations of H-2''/2'''-OCOCH₃, H-2'''/2'''-OCOCH₃, and H-6'''/6'''-OCOCH₃. The HMBC correlations between H-2''/2'''-OCOCH₃, H-3'''/3'''-OCOCH₃, and H-6'''/6'''-OCOCH₃ indicated that the acetyl moieties were connected to C-2'', C-3''', and C-6''' of **4**, and the acetyl groups of **5** were linked to C-2'', C-4''', and C-6''' by the HMBC correlations of H-2''/2'''-OCOCH₃, H-4'''/4'''-OCOCH₃, and H-6'''/6'''-OCOCH₃. The CD spectra of **3**, **4**, and **5** showed a positive Cotton effect at 326 nm and a negative Cotton effect at 287 nm, suggesting the C-(2*S*) configuration.⁶ Thus, **3**, **4**, and **5** were identified as (2*S*)-5,7,5'-trihydroxyflavanone 2'-*O*-β-D-2,6-di-*O*-acetylglucopyranosyl-(1→3)-α-L-2-*O*-acetylramnopyranoside, (2*S*)-5,7,5'-trihydroxyflavanone 2'-*O*-β-D-3,6-di-*O*-acetylglucopyranosyl-(1→3)-α-L-2-*O*-acetylramnopyranoside, and (2*S*)-5,7,5'-trihydroxyflavanone 2'-*O*-β-D-4,6-di-*O*-acetylglucopyranosyl-(1→3)-α-L-2-*O*-acetylramnopyranoside.

Compound **6** was obtained as a white, amorphous powder and assigned the molecular formula C₃₅H₄₀O₁₉, which was determined by HRESIMS (m/z 787.2094 [$M + Na$]⁺). The ^1H and ^{13}C NMR spectra of **6** were similar to these of **2**, except for the additional acetyl groups of **6**. Alkaline hydrolysis of **2** and **6** with 1% KOH gave the same deacetylated glycoside **2a**, which was identified by TLC. In the HMBC spectrum, the correlations between H-2''/2'''-OCOCH₃, H-3'''/3'''-OCOCH₃, H-4'''/4'''-OCOCH₃, and H-6'''/6'''-OCOCH₃ indicated that the acetyl moieties were connected to C-2'', C-3''', C-4''', and C-6'''. The configuration at C-2 was defined as *S* from the CD spectrum, which showed a positive Cotton effect at 326 nm and a negative Cotton effect at 285 nm.⁶ These results indicated that **6** is (2*S*)-5,7,5'-trihydroxyflavanone 2'-*O*-β-D-3,4,6-tri-*O*-acetylglucopyranosyl-(1→3)-α-L-2-*O*-acetylramnopyranoside.

The antibacterial test results of **1–6** and **2a** are shown in Table 3. These compounds showed weak activity against *Staphylococcus*

Table 2. ^{13}C NMR (100 MHz) Data of **1–6** and **2a** (δ values)

position	1 ^a	2 ^a	3 ^a	4 ^a	5 ^a	6 ^b	2a ^a
2	77.3	77.4	77.6	77.4	77.3	75.3	77.3
3	43.5	43.7	43.8	43.6	43.8	42.4	43.6
4	198.3	198.6	198.3	198.6	198.6	196.6	198.2
5	166.1	166.2	165.9	166.1	166.2	164.5	166.0
6	98.0	98.0	97.9	98.0	98.0	96.1	97.9
7	169.6	169.3	169.0	169.3	169.3	167.4	196.0
8	97.1	97.1	97.0	97.1	97.1	95.7	96.9
9	165.4	165.6	165.4	165.6	165.6	163.8	165.4
10	103.8	104.0	103.9	103.9	104.0	102.5	103.8
1'	131.0	131.0	130.9	131.0	131.1	129.7	130.8
2'	148.2	148.3	148.2	148.3	148.4	146.5	148.4
3'	118.5	118.3	118.2	118.3	117.4	117.0	118.2
4'	117.4	117.3	117.3	117.4	117.4	116.0	117.3
5'	154.8	154.7	154.6	154.7	154.8	153.3	154.4
6'	115.5	115.4	115.3	115.5	115.4	114.1	115.3
1''	98.6	98.7	98.6	98.8	98.8	97.3	101.4
2''	74.3	74.4	74.1	74.4	74.3	72.3	72.5
3''	81.0	81.3	80.4	80.9	81.9	78.3	83.8
4''	73.1	72.9	72.8	73.0	72.8	71.3	73.0
5''	71.1	71.2	71.7	71.2	71.4	70.2	71.2
6''	18.5	18.6	18.4	18.6	18.7	17.7	18.7
1'''	106.9	106.9	104.6	106.4	107.1	101.8	106.9
2'''	75.8	75.7	75.5	74.1	75.5	71.9	75.9
3'''	78.1	78.0	76.2	78.9	75.8	75.6	78.3
4'''	71.2	71.7	71.8	71.0	72.5	68.4	71.0
5'''	78.1	75.5	75.5	75.4	73.4	74.1	78.1
6'''	62.5	64.6	64.5	64.2	64.1	62.8	62.2
2''-OCOCH ₃	173.5	172.6	172.7	172.6	172.4	170.2 ^c	
2'''-OCOCH ₃	21.3	21.1	21.2	21.2	21.3	20.3 ^d	
2'''-OCOCH ₃			173.2			170.4 ^c	
2'''-OCOCH ₃			21.5			20.4 ^d	
3'''-OCOCH ₃				173.5		170.3 ^c	
3'''-OCOCH ₃				21.5		20.4 ^d	
4'''-OCOCH ₃					172.5		
4'''-OCOCH ₃					21.4		
6'''-OCOCH ₃		173.8	173.8	173.7	173.0	170.8	
6'''-OCOCH ₃		21.2	21.1	21.1	21.3	20.3 ^d	

^a In CD₃OD. ^bIn DMCO-*d*₆. ^{c,d}Values may be interchanged.

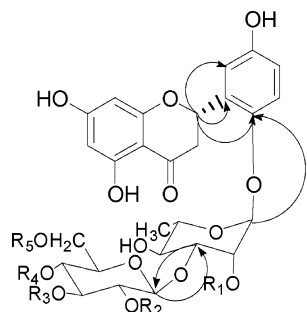


Figure 1. Selected HMBC correlations for compounds **1–6**.

Table 3. Antibacterial Effects of **1–6** and **2a** against Bacteria

compound	MIC (μg/mL)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. pneumoniae</i>	<i>H. influenzae</i>
1	128	64	32	32
2	128	64	32	32
3	128	64	32	32
4	128	64	32	32
5	128	64	32	32
6	128	64	32	32
2a	128	64	32	32
azithromycin	0.5	0.5	4	8
erythromycin	0.25	0.25	64	64

aureus and *Escherichia coli* and moderate activity against *Streptococcus pneumoniae* and *Haemophilus influenzae*.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer model 341 polarimeter, and CD data were recorded on a JASCO J-810 spectropolarimeter. UV and IR spectra were obtained on a Shimadzu UV-260 and a Perkin-Elmer Spectrum 577 spectrophotometer. NMR spectra were obtained on a Bruker AM-400 spectrometer using TMS as the internal standard. HRESIMS were obtained on a Marine instrument. GC was carried out on a GC-14C gas chromatograph (Shimadzu, Japan) with a AC-1 fused silica capillary column (30 m × 0.25 mm × 0.25 μm) (SGE); detection, FID; carrier gas, N₂; temperature for injector and detector, 230 °C; temperature gradient system for the oven, 150 °C for 1 min and then raised to 230 °C at the rate of 5 °C/min. Silica gel plates for TLC and silica gel for column chromatography were produced by Qingdao Marine Chemical Company, Qingdao, People's Republic of China.

Plant Material. The rhizomes of *C. acuminatus* were collected in July 2005 from Rusan County of Jianxi Province, People's Republic of China, and identified by Prof. Changgong Zhang, College of Pharmacy, Huangzhong University of Science and Technology. A specimen (CAC0121) was deposited in the College of Pharmacy, Tongji Medical Center, Huangzhong University of Science and Technology.

Extraction and Isolation. The air-dried rhizomes (5.0 kg) were ground and extracted with MeOH (5 × 10 L) at room temperature, and 500 g of extract was obtained. The extract was suspended in distilled H₂O and extracted with petroleum ether, CHCl₃, EtOAc, and *n*-BuOH, sequentially. The CHCl₃ extract (5.0 g) was subjected to silica gel column chromatography (200–300 mesh, 120 g) eluted with a CHCl₃–MeOH system (20:1) to yield fractions I–VI. Fraction III (0.5 g) was passed through a Sephadex LH-20 column with CHCl₃–MeOH (1:1) and then further purified on a silica gel (300–400 mesh) column, eluting with CHCl₃–MeOH (15:1) to give **6** (30 mg). The EtOAc extract (12.0 g) was separated by column chromatography on silica gel (200–300 mesh, 200 g) eluting with a CHCl₃–MeOH gradient (20:1, 10:1, 5:1, 2:1) to yield fractions I–X. Fraction III (0.8 g) was then repeatedly chromatographed on a silica gel (200–300 mesh) column using CHCl₃–MeOH (10:1) as eluent to give **3** (20 mg), **4** (19 mg), and **5** (27 mg). Compounds **1** (10 mg) and **2** (40 mg) were obtained from fraction IV (0.6 g) by chromatography on a silica column (CHCl₃–MeOH, 6:1).

Compound 1: white, amorphous powder; [α]_D²⁵ –36.0 (*c* 0.053, MeOH); CD (*c* 0.0048, MeOH), λ (Δε) 216 (+31.2), 230 (–9.2), 287 (–27.6), 326 (+3.2) nm; UV (MeOH) λ_{max} (log ε) 216 (4.12), 290

(4.04), 330 (3.48) nm; IR (KBr) ν_{max} 3423, 2929, 1733, 1639, 1498, 1457, 810 cm^{–1}; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS *m/z* 661.5 [M + Na]⁺; HRESIMS (positive-ion mode) *m/z* 661.1734 [M + Na]⁺ (calcd for C₂₉H₃₄O₁₆Na, 661.1745).

Compound 2: white, amorphous powder; [α]_D²⁵ –20.0 (*c* 0.30, MeOH); CD (*c* 0.0032, MeOH), λ (Δε) 216 (+26.4), 229 (–8.8), 287 (–23.9), 326 (+3.3) nm; UV (MeOH) λ_{max} (log ε) 217 (4.40), 290 (4.38), 330 (3.78) nm; IR (KBr) ν_{max} 3415, 2929, 1727, 1639, 1498, 1457, 810 cm^{–1}; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS *m/z* 703.5 [M + Na]⁺; HRESIMS (positive-ion mode) *m/z* 703.1827 [M + Na]⁺ (calcd for C₃₁H₃₆O₁₇Na, 703.1850).

Compound 3: white, amorphous powder; [α]_D²⁵ –33.5 (*c* 0.27, MeOH); CD (*c* 0.0048, MeOH), λ (Δε) 216 (+25.9), 230 (–7.4), 287 (–21.1), 326 (+3.5) nm; UV (MeOH) λ_{max} (log ε) 227 (4.40), 289 (4.45), 330 (3.85) nm; IR (KBr) ν_{max} 3430, 2937, 1731, 1639, 1498, 1457, 810 cm^{–1}; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS *m/z* 745.4 [M + Na]⁺; HRESIMS (positive-ion mode) *m/z* 745.1955 [M + Na]⁺ (calcd for C₃₃H₃₈O₁₈Na, 745.1956).

Compound 4: white, amorphous powder; [α]_D²⁵ –34.0 (*c* 0.27, MeOH); CD (*c* 0.0048, MeOH), λ (Δε) 216 (+40.6), 229 (–11.4), 287 (–30.8), 326 (+4.8) nm; UV (MeOH) λ_{max} (log ε) 225 (4.31), 290 (4.31), 330 (3.76) nm; IR (KBr) ν_{max} 3423, 2939, 1727, 1639, 1498, 1457, 810 cm^{–1}; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS *m/z* 745.5 [M + Na]⁺; HRESIMS (positive-ion mode) *m/z* 745.1965 [M + Na]⁺ (calcd for C₃₃H₃₈O₁₈Na, 745.1956).

Compound 5: white, amorphous powder; [α]_D²⁵ –35.0 (*c* 0.27, MeOH); CD (*c* 0.0048, MeOH), λ (Δε) 216 (+28.5), 229 (–7.9), 287 (–23.8), 326 (+3.7) nm; UV (MeOH) λ_{max} (log ε) 227 (4.16), 290 (4.20), 330 (3.59) nm; IR (KBr) ν_{max} 3401, 2936, 1741, 1640, 1498, 1460, 810 cm^{–1}; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS *m/z* 745.3 [M + Na]⁺; HRESIMS (positive-ion mode) *m/z* 745.1926 [M + Na]⁺ (calcd for C₃₃H₃₈O₁₈Na, 745.1956).

Compound 6: white, amorphous powder; [α]_D²⁵ –33.5 (*c* 0.27, MeOH); CD (*c* 0.0080, MeOH), λ (Δε) 217 (+28.5), 230 (–21.7), 285 (–71.9), 326 (+8.7) nm; UV (MeOH) λ_{max} (log ε) 230 (4.18), 290 (4.28), 330 (3.78) nm; IR (KBr) ν_{max} 3438, 2939, 1743, 1639, 1498, 1457, 810 cm^{–1}; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS *m/z* 787.4 [M + Na]⁺; HRESIMS (positive-ion mode) *m/z* 787.2094 [M + Na]⁺ (calcd for C₃₅H₄₀O₁₉Na, 787.2061).

Compound 2a: white, amorphous powder; [α]_D²⁵ –55.0 (*c* 0.32, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.20), 290 (4.35), 330 (3.61) nm; IR (KBr) ν_{max} 3382, 2931, 1639, 1498, 1457, 811 cm^{–1}; ¹H and ¹³C NMR data, see Tables 1 and 2; ESIMS *m/z* 619.5 [M + Na]⁺.

Alkaline Hydrolysis of 1–6. Compound **2** (15 mg) was hydrolyzed with 1% KOH (1.0 mL) for 1 h at room temperature. After acidification with 1% HCl until pH 5, the reaction mixture was extracted with *n*-BuOH. The *n*-BuOH extract was purified on silica gel (CHCl₃–MeOH–H₂O, 4:1:0.1) to give **2a** (7 mg). Compounds **1** and **3–6** (0.5 mg) were treated in the same manner as **2** to afford **2a**, which was determined by co-TLC (CHCl₃–MeOH–H₂O, 4:1:0.1, *R*_f = 0.25).

Acid Hydrolysis of 1–6. A solution of each compound (3 mg) in 10% HCl was stirred at 90 °C for 5 h. After cooling, the reaction mixture was filtered. The filtrate was examined by TLC together with authentic sugar samples (EtOAc–MeOH–H₂O–HOAc, 6:1:1:1, glucose, *R*_f = 0.31; rhamnose, *R*_f = 0.55). The remaining filtrate was concentrated to dryness to give a residue, which was dissolved in dry pyridine,⁷ to which was added L-cysteine methyl ester hydrochloride. The mixture was stirred at 60 °C for 1 h, then hexamethyldisilazane–trimethylchlorosilane (2:1) was added, and the mixture was stirred at 60 °C for 30 min. After centrifugation, the supernatant was directly subjected to GC analysis. The sugar derivatives obtained from compounds **1–6** were detected in each case by co-injection of the D-glucose and L-rhamnose derivatives, giving single peaks at 23.14 and 19.17 min, respectively.

Antibacterial Activity. The antibacterial activities of **1–6** and **2a** were determined by the 2-fold dilution method.⁸ Laboratory standard ATCC strains (*S. aureus* ATCC # 25923, *E. coli* ATCC # 25922, *S. pneumoniae* ATCC # 49619, and *H. influenzae* ATCC # 49247) were used as the test bacteria. Erythromycin and azithromycin were used as the positive controls.

Acknowledgment. The authors are grateful to the members of the analytical group in Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, for measurements of the mass and NMR spectra.

Supporting Information Available: This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- (1) The Institute of Botany Chinese Academy of Sciences. *Chinese Flora*; Science Publisher: Beijing, 1999; Vol. 4, Chapter 1, pp 234–235.
- (2) Administration Bureau of National Chinese Traditional Medicine. *China Herbal*; Shanghai Scientific and Technical Publisher: Shanghai, 1999; Vol. 4, pp 159–160.
- (3) Quadri-spinelli, T.; Heilmann, J.; Rali, T.; Sticher, O. *Planta Med.* **2000**, *66*, 728–733.
- (4) Somdej, K.; KwanJai, K.; Komkrich, N.; Palangpon, K. *J. Nat. Prod.* **2004**, *67*, 968–972.
- (5) Harborne, J. B. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*; Chapman & Hall: London, 1972; p 78.
- (6) Gaffield, W. *Tetrahedron* **1970**, *26*, 4093–4108.
- (7) Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1986**, *34*, 1843–1845.
- (8) Ma, X. R.; Su, D. M. *Medicine Analysis Handbook of Microbiology*; Science Publisher: Beijing, 2000; pp 211–212.

NP060334N