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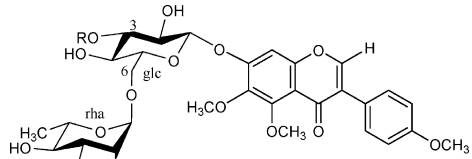

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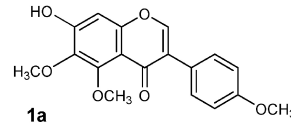
2 rha-(1→6)-glc-

3 H



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Chemical investigation of the methanol extract of the roots of *Baphia bancoensis* led to the isolation and characterization of three new isoflavonoid glycosides (**1**−**3**). Their structures were determined on the basis of spectroscopic studies and chemical evidence. Antibacterial activity of isolated compounds was evaluated against *Staphylococcus aureus*, *Enterococcus faecalis, Escherichia coli*, and *Pseudomonas aeruginosa*.

The genus *Baphia* (Fabaceae) is represented by more than 65 species distributed in tropical regions of Africa, Madagascar, and Indonesia. The presence of aminosugars and isoflavonoids in *B. nitida* and iminoacids in *B. racemosa* was reported.[1−4] *B. bancoensis* Aubréville is a small tree endemic to the Ivory Coast and is frequently found in the humid dense coastal forest.[5] The juice of the plant is used in traditional medicine as a remedy for suppurating eyes.[6] The chemical composition of the roots of *B. bancoensis* has not yet been explored. In this report, we describe the isolation and structure elucidation of three new isoflavonoid glycosides (**1**−**3**) from the roots of this plant, as well as their antibacterial activities toward *Staphylococcus aureus*, *Enterococcus faecalis, Escherichia coli*, and *Pseudomonas aeruginosa.*

The MeOH extract prepared from the defatted dried powdered roots of *B. bancoensis* was fractionated by silica gel VLC to give seven fractions (I−VII). TLC analysis of these fractions in $CHCl_3$−MeOH−H_2O (14:6:1) showed that fraction VI contained three spots. Purification of this fraction by semipreparative HPLC afforded three isoflavonoid glycosides (**1**−**3**). Acid hydrolysis of fraction IV afforded the aglycone **1a** (see Experimental Section) and two sugar units. These were identified as D-glucose and L-rhamnose by TLC analysis with authentic samples and by measurement of their optical rotation after purification. Structural confirmation of the sugar moieties in the intact compounds was achieved by analysis of 1D and 2D NMR experiments.

The positive ion mode HRESIMS of compound **1** exhibited a pseudomolecular ion peak at *m*/*z* 1129.3580 $[M + Na]^+$, consistent with a molecular formula $C_{48}H_{66}O_{29}Na$. The UV spectrum (λ_{max} 260 and 317 nm) of **1** was typical of compounds having an isoflavone skeleton.[7] In the ^1H NMR spectrum of **1**, a characteristic resonance for H-2 of an isoflavone was observed at δ 8.25.[8,9] This assignment was confirmed by long-range connectivities to the quaternary carbons at δ 177.5 (C-4), 155.7 (C-9), 126.1 (C-3), and 125.6 (C-1′) in the HMBC spectrum. A second singlet at δ 7.22, corresponding to H-8, showed HMBC correlations with quaternary carbons at δ 157.1 (C-7), 155.7 (C-9), 142.1 (C-6), and 115.3 (C-10). The remaining aromatic proton resonances comprised two doublets at δ 7.50 and 7.01, representing a *p*-substituted ring B. The proton at δ 7.50 (H-2′, H-6′) showed correlations with C-3, C-1′, and C-4′ (δ 161.1). Additionally, the ^1H NMR spectrum showed the resonances of three *O*-methyl singlets at δ 3.85, 3.94, and 3.96. The HMBC correlations between these three singlets and C-4′, C-6, and C-5, respectively, indicated the location of the *O*-methyl groups. As expected, the C-5 and C-6 *O*-methyl groups resonated at δ 62.7 and 62.2, respectively, which is characteristic of sterically hindered *O*-methyl groups with substituents in both *ortho* positions; this contrasts with the C-4′ *O*-methyl signal at δ 55.7 (unsubstituted *ortho* positions).[9] The ROESY spectrum of **1** showed interactions from H-2 to H-2′, 6′, from 4′-OCH_3 to H-3′, 5′, and from H-2′, 6′ to H-3′, 5′. In the HMBC spectrum of **1a**, H-2 (δ 7.80) showed long-range HMBC connectivities to the carbonyl carbon (δ 175.4, C-4), two quaternary carbons (δ 125.1, C-3 and 124.2, C-1′), and the quaternary oxygenated carbon C-9 (δ 154.6). The later resonance was further correlated with the singlet at δ 6.78 (H-8). The 7-OH proton (δ 6.50) showed $^3J_{C-H}$ correlations with the carbons at δ 99.0 (C-8) and 138.2 (C-6), and the later showed HMBC connectivity to the protons of the *O*-methyl group at δ 4.03, indicating the location of this *O*-methyl group at C-6. A NOESY experiment of the aglycone (**1a**) showed a correlation between the C-6-*O*-methyl and an *O*-methyl group at δ 3.95 (5-OCH_3). The 5-OCH_3 showed a HMBC correlation with the carbon at 151.7 (C-5). Additional NOE correlations were observed in the NOESY spectrum of **1a** between 7-OH/H-8, H-2/H-2′,H-6′, 4′-OCH_3/H-3′,H-5′, and H-2′,H-6′/H-3′,H-5′. From the above data, the aglycone of **1** could be identified as 7-hydroxy-4′,5,6,-trimethoxyisoflavone (**1a**). The ^{13}C NMR data of **1a** were in good agreement with those reported for a synthetic compound.[9]

Further analysis of the ^1H and ^{13}C NMR spectra of **1** revealed the presence of five anomeric protons at δ 5.21, 4.78, 4.74, 4.56, and 4.52, which were correlated in the HSQC spectrum with five anomeric carbons at δ 101.6, 103.3, 101.9, 106.4, and 105.5, respectively. Complete assignment of each glycosidic proton system was realized from the combined analysis of COSY, TOCSY, and HSQC spectra. Three β-D-glucopyranose units were assigned starting from the anomeric protons at δ 5.21 (d, $J = 8.0$ Hz) (glc), 4.52 (d, $J = 7.8$ Hz) (glc′), and 4.56 (d, $J = 7.7$ Hz) (glc″) (Table 1). The chemical shifts of the anomeric carbons and the ROE interactions between H-1/H-3 and H-1/H-5 of each glucose unit confirmed the β-configuration of the glycosidic linkages.[10] The first glucose unit (glc) with its C-3 and C-6 deshielded at δ 89.4 and 67.5, respectively, suggested a 3,6-disubstituted glucose. The signal for C-6 of the second glucose (glc′) was downfield shifted to δ 70.4, indicating the presence of a glucosidic linkage at position 6 of glc′, whereas the last glucose unit (glc″) was in a terminal position (Table 1).[10] Two α-L-rhamnose units were identified starting from the anomeric protons at δ 4.74 (d, $J = 1.6$ Hz) (rha) and 4.78 (d, $J = 1.7$ Hz) (rha′), characterized by their methyl groups at δ 18.1 (δ 1.27 and 1.42, each d, $J = 6.2$ Hz) (Table 1). Their C-5 resonated at δ 69.9 and 68.6, indicating an α configuration for the anomeric center of each rhamnose.[10] The first rhamnose (rha) was in a terminal position, whereas the second (rha′) was substituted

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Table 1. NMR Data of Compounds **1**, **2**, and **3** (in CD_3OD)

	1		**2**		**3**	
position	δ_C, mult	δ_H (*J* in Hz)	δ_C, mult	δ_H (*J* in Hz)	δ_C, mult	δ_H (*J* in Hz)
aglycone						
2	154.0, CH	8.25, s	154.1, CH	8.19, s	154.0, CH	8.20, s
3	126.1, qC		126.2, qC		126.1, qC	
4	177.5, qC		177.4, qC		177.5, qC	
5	154.1, qC		154.1, qC		154.1, qC	
6	142.1, qC		142.2, qC		142.1, qC	
7	157.1, qC		157.1, qC		157.1, qC	
8	102.1, CH	7.22, s	102.2, CH	7.17, s	102.1, CH	7.19, s
9	155.7, qC		155.7, qC		155.7, qC	
10	115.3, qC		115.3, qC		115.3, qC	
1′	125.6, qC		125.6, qC		125.6, qC	
2′, 6′	131.6, CH	7.50, d (8.7)	131.6, CH	7.46, d (8.8)	131.6, CH	7.47, d (8.8)
3′, 5′	114.7, CH	7.01, d (8.7)	114.7, CH	6.97, d (8.8)	114.7, CH	6.98, d (8.8)
4′	161.1, qC		161.1, qC		161.1, qC	
5-*O*-Me	62.7, CH_3	3.96, s	62.7, CH_3	3.92, s	62.7, CH_3	3.92, s
6-*O*-Me	62.2, CH_3	3.94, s	62.2, CH_3	3.91, s	62.2, CH_3	3.91, s
4′-*O*-Me	55.7, CH_3	3.85, s	55.7, CH_3	3.83, s	55.7, CH_3	3.83, s
7-*O*-Glc						
1	101.6, CH	5.21, d (8.0)	101.6, CH	5.14, d (8.0)	102, CH	5.06, d (7.7), CH
2	73.6, CH	3.84, t (8.0)	73.6, CH	3.79, t (8.0)	74.5, CH	3.59, t (7.8)
3	89.4, CH	3.69, t (8.7)	89.3, CH	3.66, t (8.7)	78.0, CH	3.55, t (8.7)
4	70.3, CH	3.50, dd (9.6, 8.7)	70.2, CH	3.49, dd (9.7, 8.7)	71.5, CH	3.40, dd (9.7, 8.7)
5	76.6, CH	3.89, m	76.7, CH	3.76, m	77.0, CH	3.70, m
6	67.5, CH_2	3.59, dd (10.4, 7.3)	67.8, CH_2	3.62, m	67.5, CH_2	3.62, m
		4.16, dd (10.6, 2.7)		4.10, dd (11.1, 2.5)		4.10, dd (11.2, 2)
Rha						
1	101.9, CH	4.74, d (1.6)	102.2, CH	4.71, d (1.1)	103.0, CH	4.70, d (1.1)
2	71.9, CH	3.95, dd (3.4, 1.6)	72.0, CH	3.89, dd (3.4, 1.4)	71.8, CH	3.90, dd (3.4, 1.4)
3	72.3, CH	3.90, dd (9.4, 3.4)	72.4, CH	3.78, dd (9.1, 3.1)	72.5, CH	3.80, dd (9.1, 3.3)
4	74.4, CH	3.40, t (9.6)	74.0, CH	3.38, t (9.6)	74.0, CH	3.38, t (9.6)
5	69.9, CH	3.74, dq (9.5, 6.2)	69.9, CH	3.67, m	70.0, CH	3.65, m
6	18.1, CH_3	1.27, d (6.2)	18.1, CH_3	1.21, d (6.2)	18.0, CH_3	1.28, d (6.2)
Glc′						
1	105.5, CH	4.52, d (7.8)	105.4, CH	4.54, d (7.8)		
2	75.2, CH	3.37, dd (8.9, 7.7)	71.8, CH	3.32, m		
3	77.7, CH	3.45, t (9.1)	77.6, CH	3.43, t (9.1)		
4	72.3, CH	3.27, t (9.1)	75.2, CH	3.33, t (8.9)		
5	76.8, CH	3.64, m	76.7, CH	3.55, m		
6	70.4, CH_2	3.71, dd (11.0, 7.8)	68.7, CH_2	3.57, m		
		4.06, dd (11.1, 2.2)		4.05, dd (11.3, 1.5)		
Rha′						
1	103.3, CH	4.78, d (1.7)	102.5, CH	4.74, d (1.1)		
2	71.9, CH	3.95, dd (3.4, 1.6)	72.1, CH	3.92, m		
3	72.0, CH	4.06, dd (9.3, 3.4)	72.2, CH	3.71, dd (9.5, 3.4)		
4	85.0, CH	3.63, t (9.2)	74.1, CH	3.39, t (9.6)		
5	68.6, CH	3.84, m	69.9, CH	3.63, m		
6	18.1, CH_3	1.42, d (6.2)	18.1, CH_3	1.28, d (6.2)		
Glc″						
1	106.4, CH	4.56, d (7.7)				
2	76.3, CH	3.35, t (8.5)				
3	78.2, CH	3.41, t (9.1)				
4	71.6, CH	3.18, t (9.5)				
5	78.4, CH	3.28, m				
6	62.8, CH_2	3.55, dd (12.0, 6.0)				
		3.80, dd (12.1, 2.4)				

at C-4 due to the downfield shift at δ 85.0. Sites of attachment and sequence of the pentasaccharide chain were obtained from an HMBC experiment, which provided cross-peaks between glc″-H-1/rha′-C-4, between rha′-H-1/glc′-C-6, between glc′-H-1/glc-C-3, between rha-H-1/glc-C-6, and between glc-H-1/C-7 of the aglycone. On the basis of the foregoing evidence, compound **1** was elucidated as 4′,5,6-trimethoxyisoflavone-7-*O*-β-D-glucopyranosyl(1→4)-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranosyl(1→3)-[α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside.

The positive HRESIMS of compound **2** displayed a molecular ion peak $[M + Na]^+$ at *m/z* 967.3051 ($C_{42}H_{56}O_{24}Na$), suggesting the lack of one hexose unit compared to **1**. Comparison of the UV, IR, and ^1H and ^{13}C NMR values and the analysis of the HMBC and ROESY correlations showed that **1** and **2** contained the same aglycone **1a** (Table 1). The positive HRESIMS suggested the presence of four sugar units, two hexoses and two deoxyhexoses, and this was confirmed by the presence of four anomeric proton and carbon signals in the ^1H and ^{13}C NMR spectra (Table 1). The detailed analysis of the 2D NMR spectra led to the identification of two terminal α-L-rhamnose units (rha and rha′) and two β-D-glucose units, one of which (glc) was bisubstituted at C-3 and C-6 and the other one (glc′) substituted at C-6 (Table 1). The sequence of the tetrasaccharide chain was deduced from the HMBC experiment, which showed long-range correlations between rha′-H-1/glc′-C-6, between glc′-H-1/glc-C-3, between rha-H-1/glc-C-6, and between glc-H-1/C-7 of the aglycone. These findings led to the assignment of compound **2** as 4′,5,6-trimethoxyisoflavone-7-*O*-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranosyl(1→3)-[α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside.

Table 2. Antibacterial Activities of Compounds **1**−**3** and **1a**[a]

	minimal inhibitory concentration							
	S. aureus		*E. faecalis*		*E. coli*		*P. aeruginosa*	
	μg/mL	μM	μg/mL	μM	μg/mL	μM	μg/mL	μM
1	>128	>115.6	>128	>115.6	>128	>115.6	>128	>115.6
2	>128	>135	>128	>135	>128	>135	>128	>135
3	32	48.4	32	48.4	>128	>196.3	128	196.3
1a	32	97.5	32	97.5	128	390	64	195

[a] Each concentration was done in triplicate, and each time the same result was obtained.

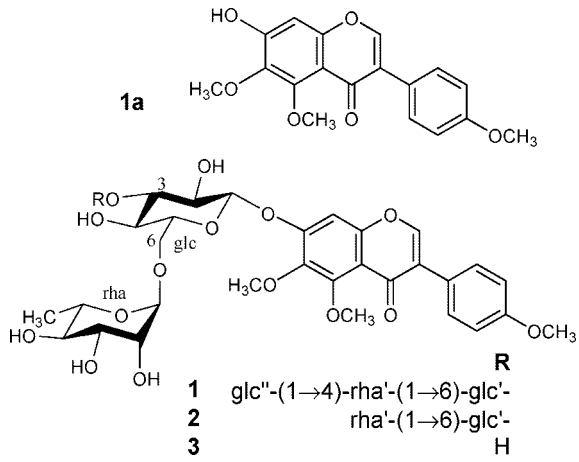

Figure 1. Structures of compounds **1a**, **1**, **2**, and **3**.

Compound **3** was assigned the molecular formula $C_{30}H_{36}O_{15}Na$ from its positive HRESIMS, which exhibited a molecular ion peak $[M + Na]^+$ at *m/z* 659.1945, suggesting the lack of one hexose and one deoxyhexose units compared to **2**. Inspection of the NMR data (Table 1) indicated that **3** contained the same aglycone unit **1a**. Compound **3** was a diglycoside, as shown by the presence in the ^{13}C NMR spectrum of two resonances at δ 102.0 and 103.0. From analysis of COSY, TOCSY, and HSQC experiments, a β-D-glucose and a terminal α-L-rhamnose were identified (Table 1). The downfield shifts of glc-C-6 (δ 67.5) and the HMBC correlations between rha-H-1/glc-C-6 and between glc-H-1/C-7 of the aglycone showed that compound **3** was 4′,5,6-trimethoxyisoflavone-7-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside.

Compounds **1**−**3** and **1a** were assayed for their antibacterial activities against four bacteria including two Gram-positive bacteria (*S. aureus* and *E. faecalis*) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*). Two antibacterial agents, vancomycin and imipenem, were used as positive controls in these tests. The minimal inhibitory concentration (MIC) values are reported in Table 2. Compounds **1** and **2** showed moderate antibacterial activities with MICs of >115.6 and >135 μM, respectively. Compounds **3** and **1a** showed a mild activity against the Gram-positive bacteria, with MIC values of 48.4 and 97.5 μM, respectively (Table 2).

Experimental Section

General Experimental Procedures. Optical rotations were measured in MeOH or H_2O on a Perkin-Elmer241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. UV spectra were obtained using a Philips PU 8720 spectrophotometer. IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer. ^1H and ^{13}C NMR spectra were recorded in CD_3OD or $CDCl_3$ on a Bruker Avance DRX-500 spectrometer (^1H at 500 MHz and ^{13}C at 125 MHz), and 2D-NMR experiments were performed using Bruker's standard microprograms (XWIN-NMR version 2.6 software). ESIMS and HRESIMS experiments were performed using a Micromass Q-TOF micro instrument (Manchester, UK). TLC was carried out on precoated silica gel 60 F254 (Merck), and spots were visualized by heating after spraying with 50% H_2SO_4. Column chromatography was carried out on silica gel 60 (63−200 μm, Merck). HPLC was performed on a Dionex apparatus equipped with an ASI-100 autosampler, a STH 585 column oven, a P580 pump, a UVD 340S diode array detector, and the Chromeleon software. A prepacked RP-18 column (201SP510, 250 × 10 mm, 5 μm, 90 Å, Dionex, Vydac, France) was used for semipreparative HPLC with a binary gradient elution (solvent A: H_2O with 0.0025% TFA, solvent B: MeCN) at 25 °C and a flow rate of 3 mL min^{-1}; the chromatogram was monitored at 260 and 315 nm.

Plant Material. *B. bancoensis* roots were collected in September 2003 in Adiopodoumé near Abidjan (Ivory Coast) and authenticated by Prof A. Assi of the University of Cocody-Abidjan (Ivory Coast). A voucher specimen (AKE ASSI 15117) has been deposited at the Centre National Floristique de l'Université de Cocody-Abidjan.

Extraction and Isolation. The dried, powdered roots (362 g) were extracted successively with petroleum ether, EtOAc, and MeOH (each 5 L) for 48 h to yield after solvent evaporation the corresponding extracts: petroleum ether (1.1 g), EtOAc (2 g), and MeOH (20.4 g). A part of the MeOH extract (9.6 g) was fractionated by silica gel VLC and eluted successively with $CHCl_3$−MeOH−H_2O (9:1:0, 8:2:0, 7:3:0, 70:30:1, 70:30:2, 70:30:3, and 70:30:5, each 1 L) to yield fractions I−VII, respectively. A part of fraction III (30 mg) was purified with semipreparative HPLC using a linear gradient (10 to 30% B during 30 min) at 3 mL/min to give **3** (10 mg), whereas the purification of 60 mg of fraction VI using the same conditions afforded 13 mg of **1**, 18 mg of **2**, and 10 mg of **3**.

Compound 1: brown lacquer; $[\alpha]^{25}_D$ −8 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 260 (4.35), 317 (3.82) nm; IR (KBr) ν_{max} 3450, 2959, 1658, 1623, 1575, 1460, 1371, 1295, 1231, 1153, 1067, 1036, 812 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD) see Table 1; HRESIMS (positive ion mode) *m/z* 1129. 3580 $[M + Na]^+$ (calcd for $C_{48}H_{66}O_{29}Na$, 1129.3587).

Compound 2: brown lacquer; $[\alpha]^{25}_D$ −19 (*c* 0.42, MeOH); UV (MeOH) λ_{max} (log ε) 259 (3.8), 317 (3.2) nm; IR (KBr) ν_{max} 3451, 2958, 1648, 1622, 1571, 1463, 1373, 1297, 1229, 1147, 1071, 1042, 817 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD) see Table 1; HRESIMS (positive ion mode) *m/z* 967.3051 $[M + Na]^+$ (calcd for $C_{42}H_{56}O_{24}Na$, 967.3059).

Compound 3: brown lacquer; $[\alpha]^{25}_D$ −13 (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ε) 260 (4.9), 316 (4.3) nm; IR (KBr) ν_{max} 3450, 2959, 1658, 1623, 1575, 1460, 1371, 1295, 1231, 1153, 1067, 1036, 812 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD) see Table 1; HRESIMS (positive ion mode) *m/z* 659.1945 $[M + Na]^+$ (calcd for $C_{30}H_{36}O_{15}Na$, 659.1952).

Compound 1a: amorphous, white powder; $[\alpha]^{25}_D$ −12 (*c* 0.5, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 265 (3.9), 316 (3.1) nm; + NaOAc 272 (4.4), 337 (4.1) nm; IR (KBr) ν_{max} 3450, 2959, 1658, 1623, 1575, 1460, 1371, 1295, 1231, 1153, 1067, 1036, 812 cm^{-1}; ^1H NMR ($CDCl_3$ 500 MHz) δ 3.95 (3H, s, CH_3-5), 4.03 (3H, s, CH_3-6), 3.84 (3H, s, CH_3-4′), 6.50 (1H, s, OH-7), 6.78 (1H, s H-8), 6.96 (2H, d, J = 8.5 Hz, H-3′,5′), 7.40 (2H, d, J = 8.5 Hz, H-2′,6′), 7.80 (1H, s, H-2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 55.3 (CH_3, OCH_3-4′), 61.8 (CH_3, OCH_3-6), 62.0 (CH_3, OCH_3-5), 99.0 (CH, C-8), 113.4 (C, C-10), 113.9 (C, C-3′, C-5′), 124.2 (C, C-1′), 125.1 (C, C-3), 130.3 (C, C-2′, C-6′), 138.2 (C, C-6), 150.7 (CH, C-2), 151.7 (C, C-5), 153.8 (C, C-7), 154.6 (C, C-9), 159.5 (C, C-4′), 175.4 (C, C-4); ESIMS (positive ion mode) *m/z* 329 $[M + H]^+$.

Acid Hydrolysis. A part of fraction VI (1 g), from which **1**, **2**, and **3** were isolated, was refluxed with 2 N HCl (30 mL) for 4 h 30 min. The mixture was extracted with EtOAc (3 × 25 mL). The EtOAc layer was washed with H_2O and evaporated to dryness (230 mg) and then crystallized in MeOH to afford **1a** (90 mg). The acidic aqueous layer was neutralized with 2 N KOH and freeze-dried. Two sugars were identified by TLC with authentic sugar samples in MeCOEt−*i*-PrOH−Me_2CO−H_2O (20:10:7:6) as glucose and rhamnose. After preparative TLC of the sugar mixture (100 mg) in this solvent,

measurement of the optical rotation indicated the presence of a D-glucose ($[\alpha]^{25}_D$ +10 (*c* 0.94, H_2O)) and L-rhamnose ($[\alpha]^{25}_D$ +4 (*c* 1, H_2O)).

Antibacterial Assays. The assay for antibacterial activity against standard strains *Staphylococcus aureus* (ATCC 2523), *Enterococcus faecalis* (29212), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) was performed using the liquid microdilution growth inhibition method.[11] The MICs of test compounds were determined as follows: the test compound was dissolved in MeOH to a final concentration of 512 μg/mL. This was serially diluted 2-fold with Muller-Hinton medium to obtain concentration ranges of 1 to 128 μg/mL. Fifty microliters of each concentration was added in a well (96-well microplate) containing 150 μL of Mueller-Hinton medium and 5 μL of the standard inoculum. The final concentration of MeOH in the well was less than 6% (preliminary analysis with 6% (v/v) MeOH/Mueller-Hinton medium affected neither the growth of the test organisms nor the change of color due to this growth). The negative control well consisted of 12.5 μL of MeOH, 187.5 μL of Mueller-Hinton medium, and 5 μL of the standard inoculum. The plates were covered with a sterile plate sealer, then agitated and incubated at 37 °C for 18 h. Microbial growth was determined by observing the change of color in the wells. The lowest concentration showing no color change was considered as the MIC. The experiments were run in triplicate, and each time the MIC values were identical. Two antibacterial agents were used as positive controls: vancomycin for *S. aureus* and *E. faecalis* and imipenem for *E. coli* and *P. aeruginosa*.

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