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Potential Anti-inflammatory Constituents of the Stems of *Gordonia chrysandra*

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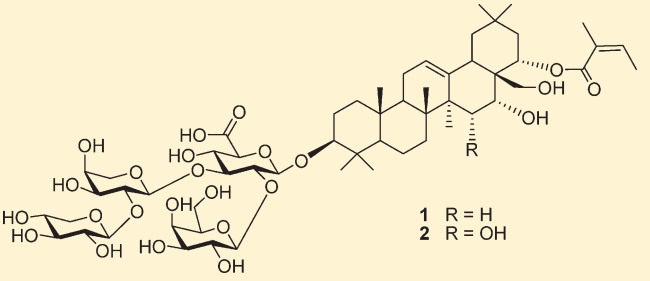
Supporting Information

ABSTRACT: Eight new oleanane-type triterpenoid glycosides, gordonosides I−P (**1**−**8**), and two new phenolic glycosides (**9** and **10**) were isolated from the stems of *Gordonia chrysandra*. Their structures were elucidated by spectroscopic and chemical methods. In an in vitro bioassay, compound **1** showed a strong inhibitory effect on nitric oxide production in LPS-activated macrophages with an IC_{50} value of 0.14 μM.



Gordonia chrysandra Cowan, belonging to the family Theaceae, is distributed widely in Sichuan, Guizhou, and Yunnan Provinces of mainland China. *G. chrysandra* is used in folk medicine for treating diarrhea, gastralgia, and arthritis.[1] Previously, two active flavanonol glycosides and eight cytotoxic acylated triterpenoid saponins from *G. chrysandra* roots have been reported.[2,3] Until now, no work has been done on the constituents of the stems of *G. chrysandra.* As part of a continuing search for bioactive constituents from *G. chrysandra*, a 95% EtOH extract of the stems of *G. chrysandra* has been investigated with 10 new compounds obtained (**1**−**10**). Reported herein are the isolation, structure elucidation, and biological activity of these new compounds.

RESULTS AND DISCUSSION

Compound **1** was obtained as a white, amorphous powder. The IR spectrum of **1** showed hydroxy (3402 cm^{-1}), carbonyl (1678 cm^{-1}), and α,β-unsaturated carbonyl (1080 cm^{-1}) absorption signals. The molecular formula, $C_{57}H_{90}O_{24}$, was determined by HRESIMS (*m*/*z* 1181.5703 $[M + Na]^+$, calcd for *m*/*z* 1181.5714). The NMR data (Tables 1 and 2) analysis of **1** indicated that it has the same aglycone, glucuronic acid (GluA), and arabinose (Ara) moieties as gordonoside C, which has been reported previously.[3] The difference between **1** and gordonoside C is the number of sugars and substituent groups. The ^1H NMR spectrum of **1** displayed four anomeric proton signals at δ_H 5.27 (1H, d, J = 7.5 Hz), 5.37 (1H, d, J = 8.0 Hz), 6.09 (1H, d, J = 7.5 Hz), and 6.11 (1H, d, J = 7.0 Hz) and signals for an angeloyl group [δ_H 2.25 (3H, s), 2.41 (3H, d, J = 7.0 Hz), and 6.24 (1H, q, J = 7.0 Hz); δ_C 167.8, 136.3, 129.3, 20.7, and 15.6]. Acid hydrolysis of **1** with 2 M HCl afforded monosaccharides, which were identified by GC analysis of their trimethylsilyl L-cysteine derivatives[4] and the coupling constants of the anomeric protons as β-D-glucuronic acid, α-L-arabinose, β-D-xylose, and β-D-galactose.

The sequence of the glycosidic chains in **1** was determined by the analysis of the 2D NMR spectroscopic data. HMBC correlations from H-22 to C-1′ indicated unambiguously that the angeloyloxy ester group is attached to C-22 of the aglycone. In addition, a HMBC correlation from GlcA-H-1 (δ_H 5.27) to C-3 (δ_C 89.4) confirmed that the β-D-glucuronopyranosyl unit is located at C-3. The long-range correlations observed between the ^1H NMR resonances at δ_H 6.09 (Gal-H-1) and the ^{13}C NMR resonance at δ_C 78.8 (GlcA-C-2), between δ_H 6.11 (Ara-H-1) and δ_C 83.7 (GlcA-C-3), and between δ_H 5.37 (Xyl-H-1) and δ_C 81.8 (Ara-C-2) indicated that the tetrasaccharide residue at C-3 of the aglycone is β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-glucuronopyranoside. Thus, compound **1** was determined as 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-glucuronopyranosyl-22α-angeloyloxyolean-12-ene-16α,28-diol and has been named gordonoside I.

Compound **2** was isolated as a white, amorphous powder. The HRESIMS peak at *m*/*z* 1197.5668 $[M + Na]^+$ indicated the molecular formula of **2** to be $C_{57}H_{90}O_{25}$, with one oxygen atom more than that of **1** (1181 $[M + Na]^+$). The IR and NMR data of **2** were almost identical to those of **1** (Experimental Section; Tables 1 and 2). However, detailed NMR analysis showed an additional hydroxy group at C-15 (δ_C 67.5 ppm). These data suggested that **2** is a 15-oxygenated derivative of **1**, which was further confirmed by HMBC and NOESY experiments on **2**. Thus, compound **2** (gordonoside J) was elucidated as 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)-

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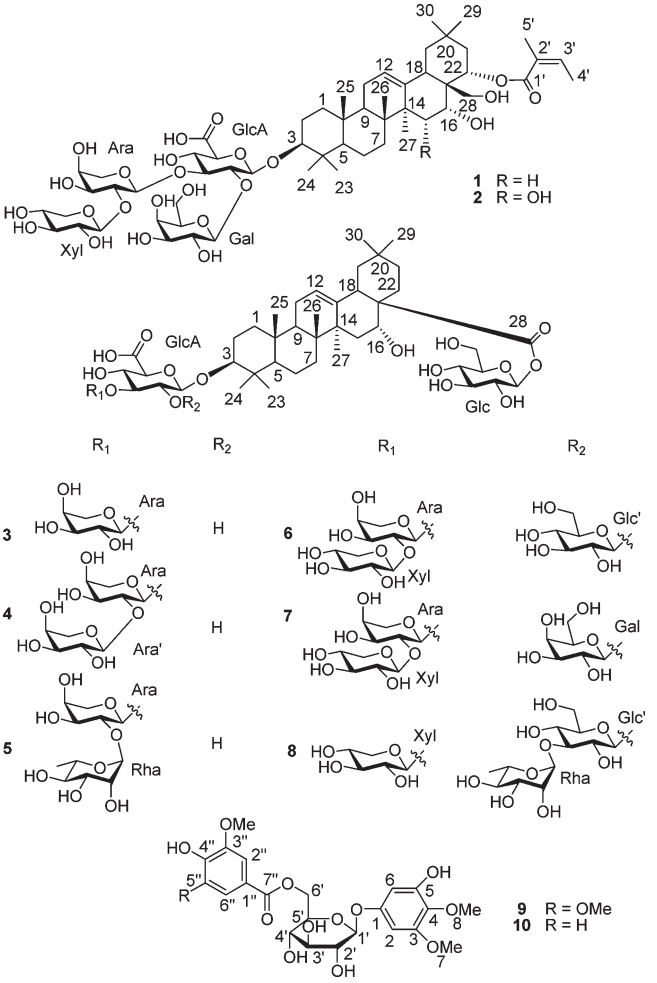

[β-D-galactopyranosyl-(1→2)]-β-D-glucuronopyranosyl-22α-angeloyloxyolean-12-ene-15α,16α,28-triol.

Compound **3** was isolated as a white, amorphous powder. The molecular formula, $C_{47}H_{74}O_{19}$, was determined by HRESIMS (m/z 965.4697 $[M + Na]^+$, calcd for 965.4717). The NMR spectroscopic data of **3** resembled those of **1**, indicating the two compounds have a similar structure (Tables 1 and 2). However, comparison of the NMR data of **3** with those of acacic acid[5] indicated that **3** is a 3,28-bidesmoside of acacic acid. In addition, the ^1H and ^{13}C NMR signals for **3** showed three anomeric protons at δ_H 4.99 (1H, d, J = 6.5 Hz), 5.36 (1H, d, J = 6.0 Hz), and 6.32 (1H, d, J = 8.0 Hz), with the corresponding carbon resonances at δ_C 106.5, 105.6, and 95.7, respectively. The linkages of the sugars and the sugars with the aglycone were established from the following HMBC correlations: GlcA-H-1 (δ_H 4.99)/aglycone-C-3 (δ_C 89.0), Ara-H-1 (δ_H 5.36)/GlcA-C-3 (δ_C 85.5), and Glc-H-1 (δ_H 6.32)/aglycone-C-28 (δ_C 175.7). Thus, compound **3** (gordonoside K) was elucidated as 3-*O*-α-L-arabinopyranosyl(1→3)-β-D-glucuronopyranosyl acacic acid 28-*O*-β-D-glucopyranoside.

Compound **4** was found to possess the molecular formula $C_{52}H_{82}O_{23}$ according to the HRESIMS peak at m/z 1097.5125 $[M + Na]^+$. The NMR spectroscopic data were almost identical to those of **3**, except for an additional set of α-L-arabinose resonances with an anomeric proton signal at δ_H 4.90 (1H, d, J = 7.0 Hz) and a downfield shift of C-2 (from δ_C 74.5 to δ_C 81.4) of the L-arabinopyranosyl unit due to a glycosidation shift (Tables 1 and 2). The linkage position of the sugar units with the aglycone was established from the following HMBC correlations: H-1 (δ_H 4.90) of Ara′ with C-2 (δ_C 81.4) of Ara, H-1 (δ_H 4.97) of GlcA with C-3 (δ_C 88.9) of the aglycone, H-1 (δ_H 5.49) of Ara with C-3 (δ_C 86.1) of GlcA, and H-1 (δ_H 6.32) of Glc with C-28 (δ_C 175.7) of the aglycone. Thus, compound **4** (gordonoside L) was elucidated as 3-*O*-α-L-arabinopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)-β-D-glucuronopyranosyl acacic acid 28-*O*-β-D-glucopyranoside.

The molecular formula of **5**, $C_{53}H_{84}O_{23}$, was indicated from the HRESIMS peak at m/z 1111.5297 $[M + Na]^+$. The NMR spectroscopic data of compound **5** were almost identical with those of **4** except that the arabinose in **4** was replaced by a rhamnose moiety in **5** (Tables 1 and 2). The connectivity for the sugar residues was further confirmed from the following HMBC correlations: GlcA-H-1 (δ_H 4.98)/aglycone-C-3 (δ_C 89.2), Ara-H-1 (δ_H 5.21)/GlcA-C-3 (δ_C 86.5), Rha-H-1 (δ_H 5.99)/Ara-C-2 (δ_C 80.5), and Glc-H-1 (δ_H 6.33)/aglycone-C-28 (δ_C 175.9). Thus, compound **5** (gordonoside M) was determined as 3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)-β-D-glucuronopyranosylacacic acid 28-*O*-β-D-glucopyranoside.

Compound **6** had the molecular formula $C_{58}H_{92}O_{28}$, as indicated from the HRESIMS (m/z 1259.5672 $[M + Na]^+$, calcd for 1259.5667). The NMR spectroscopic data of compound **6** resembled those of **3**, except that **6** has one additional xylose and glucose units when compared with **3** (Tables 1 and 2). The linkages of the sugars and the sugars with the aglycone were established from the following HMBC correlations: GlcA-H-1 (δ_H 4.90)/aglycone-C-3 (δ_C 89.6), Xyl-H-1 (δ_H 4.99)/Ara-C-2 (δ_C 82.2), Ara-H-1 (δ_H 5.77)/GlcA-C-3 (δ_C 83.8), Glc′-H-1 (δ_H 5.90)/GlcA-C-2 (δ_C 78.2), and Glc-H-1 (δ_H 6.32)/aglycone-C-28 (δ_C 175.9). Thus, compound **6** (gordonoside N) was determined as 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucuronopyranosylacacic acid 28-*O*-β-D-glucopyranoside.

Compound **7** gave the same molecular formula as **6**, namely, $C_{58}H_{92}O_{28}$, as deduced by HRESIMS (m/z 1259.5672 $[M + Na]^+$, calcd for 1259.5667). The NMR spectroscopic data of **7** matched quite well with those for **6**. Further analysis indicated that the glucose in **6** was replaced by a galactose in **7** (Tables 1 and 2). In addition, the D-galactose in **7** was assigned to C-2 of GlcA from the HMBC correlation between the H-1 (δ_H 5.74) of galactose and C-2 (δ_C 83.9) of GlcA. Thus, compound **7** (gordonoside O) was determined as 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-glucuronopyranosylacacic acid 28-*O*-β-D-glucopyranoside.

Compound **8** was obtained as a white, amorphous powder, with the molecular formula $C_{59}H_{94}O_{28}$, as deduced from the $[M + Na]^+$ peak at m/z 1273.5827 by HRESIMS. The NMR spectroscopic data of compounds **8** and **6** were almost identical, except that the arabinose in **6** was replaced by a rhamnose in **8** (Tables 1 and 2). In addition, the connectivity for the sugar residues was confirmed from the following HMBC correlations: GlcA-H-1 (δ_H 4.94)/aglycone-C-3 (δ_C 89.6), Glc′-H-1 (δ_H 5.29)/GlcA-C-2 (δ_C 78.8), Xyl-H-1 (δ_H 5.61)/GlcA-C-3 (δ_C 86.1), and Rha-H-1 (δ_H 5.88)/Glc′-C-3 (δ_C 81.3). Thus, compound **8** (gordonoside P) was determined as 3-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylacacic acid 28-*O*-β-D-glucopyranoside.

Compound **9** was isolated as a white, amorphous powder. The IR spectrum of **9** indicated the presence of hydroxy (3423 cm^{-1}), carbonyl (1670 cm^{-1}), and aromatic ring signals (1611 and 1510 cm^{-1}). The HRESIMS of **9** showed a quasimolecular ion

Table 1. ^{1}H NMR Spectroscopic Data for Compounds 1−8[a]

position	1	2	3	4	5	6	7	8
3	3.59 dd (11.5, 4.0)	3.25 m	3.35 brd (11.0)	3.34 brd (13.0)	3.36 dd (11.0, 4.0)	3.22 dd (11.5, 4.5)	3.24 dd (10.5, 4.0)	3.25 dd (12.0, 4.0)
5	1.05 m	0.77 m	0.77 m	0.72 m	0.82 m	0.74 m	0.74 m	0.75 m
9	2.04 m	1.71 m	1.79 m	1.40 m	1.76 m	1.76 m	1.76 m	1.74 m
11	2.12 m	1.82 m	1.92 m	2.00 m	1.93 m	1.91 m	1.91 m	1.91 m
12	5.69 brs	5.45 brs	5.57 brs	5.57 brs	5.58 brs	5.56 brs	5.56 brs	5.56 brs
15	1.93 m	4.25 m	1.77 m	1.24 m	1.23 m	1.25 m	1.24 m	1.25 m
	2.25 m		2.42 m		2.39 m			2.39 m
16	4.82 m	4.58 m	5.31 brs	5.30 brs	5.31 brs	5.56 brs	5.29 brs	5.29 brs
18	3.37 m	3.00 brd (12.0)	3.49 m	3.44 brd (10.5)	3.49 brd (11.0)	3.46 m	3.47 m	3.46 m
19a	2.92 m	2.77 m	2.79 m	2.76 m	2.79 m	2.78 m	2.78 m	2.78 m
19b	1.74 m	2.03 m	1.34 m	1.37 m	1.37 m	1.42 m	1.35 m	1.36 m
21	3.23 m	2.86 m	1.79 m	2.55 m	2.57 m	1.76 m	1.79, m	1.76 m
	2.05 m	1.29 m	1.25 m	1.79 m	1.77 m	2.54 m	2.54, m	2.54 m
22	6.55 m	6.19 dd (12.0, 6.5)	2.39 m	2.42 m	2.36 m	2.39 m	2.10 m	2.08 m
			2.10 m	2.12 m	2.06 m	2.09 m	2.39 m	2.39 m
23	1.61 s	1.25 s	1.25 s	1.24 s	1.27 s	1.17 s	1.24 s	1.16 s
24	1.42 s	1.11 s	0.95 s	0.95 s	0.96 s	1.03 s	1.09 s	1.05 s
25	1.13 s	0.79 s	0.80 s	0.83 s	0.81 s	0.81 s	0.81 s	0.80 s
26	1.19 s	1.00 s	1.10 s	1.10 s	1.07 s	1.10 s	1.09 s	1.09 s
27	2.20 s	1.86 s	1.85 s	1.85 s	1.86 s	1.85 s	1.82 s	1.83 s
28a	4.02 d (10.5)	3.72 d (10.5)						
28b	3.88 d (10.5)	3.62 d (10.5)						
29	1.37 s	1.05 s	0.98 s	0.99 s	0.99 s	0.98 s	0.98 s	0.98 s
30	1.61 s	1.27 s	1.02 s	1.02 s	1.02 s	1.02 s	1.02 s	1.02 s
3′	6.24 q (7.0)	5.83 q (7.0)						
4′	2.41 d (7.0)	2.02 d (7.5)						
5′	2.25 s	1.82 s						
Sugar (C-28)								
Glc 1			6.32 d (8.0)	6.32 d (8.0)	6.33 d (8.0)	6.32 d (8.0)	6.32 d (8.0)	6.32 d (8.0)
2			4.17 m	4.13 m	4.17 m	4.14 m	4.15 m	4.13 m
3			4.02 m	4.02 m	4.03 m	4.09 m	4.01 m	4.01 m
4			4.30 m	4.27 m	4.26 m	4.57 m	4.57 m	4.33 m
5			4.26 m	4.25 m	4.28 m	4.26 m	4.64 m	4.17 m
6			4.38 m	4.38 m	4.35 m	4.33 m	4.34 m	4.37 m
			4.43 m	4.43 m	4.46 m	4.37 m	4.45 m	4.54 m
Sugar (C-3)								
GlcA 1	5.27 d (7.5)	4.93 d (7.5)	4.99 d (6.5)	4.97 d (7.5)	4.98 d (7.5)	4.90 d (7.5)	4.93 d (7.0)	4.94 d (7.0)
2	4.96 m	4.66 m	4.18 m	4.12 m	4.06 m	4.34 m	3.99 m	4.41 m
3	4.75 m	4.43 m	4.38 m	4.22 m	4.32 m	4.40 m	4.43 m	4.36 m
4	4.85 m	4.33 m	4.46 m	4.51 m	4.40 m	4.30 m	4.30 m	4.41 m
5	4.87 m	4.58 m	4.26 m	4.62 m	4.68 m	4.20 m	4.55 m	4.39 m
6								
Ara (1→3) GlcA								
1	6.11 d (7.0)	5.75 d (6.0)	5.36 d (6.0)	5.49 d (5.0)	5.21 d (7.5)	5.77 d (6.5)	5.77 d (5.5)	
2	4.89 m	4.58 m	4.13 m	4.56 m	4.26 m	4.56 m	4.55 m	
3	4.83 m	4.32 m	4.46 m	4.49 m	4.68 m	4.46 m	4.39 m	
4	4.58 m	4.22 m	4.30 m	4.36 m	4.60 m	4.32 m	4.30 m	
5	4.05 m	3.59 t (13.5)	3.79 d (11.5)	3.64 d (12.0)	3.77 d (7.5)	3.76 m	3.72 m	
	4.66 m	4.33 m	4.38 m	4.37 m	4.26 m	4.35 m	4.43 m	
Xyl (1→2) Ara								
1	5.37 d (8.0)	5.04 d (7.0)				4.99 d (8.0)	5.02 d (7.5)	5.61 d (7.5)
2	4.48 m	4.17 m				4.24 m	4.15 m	4.05 m
3	4.36 m	4.01 m				4.67 m	4.25 m	3.83 m
4	4.44 m	4.15 m				4.26 m	4.24 m	4.13 m
5	3.82 m	3.48 t (9.5)				3.46 m	3.24 m	3.75 m
	4.65 m	4.32 m				4.37 m	4.30 m	4.21 m
Gal (1→2) GlcA								
1	6.09 d (7.5)	5.74 d (7.5)					5.74 d (8.0)	
2	4.85 m	4.49 m					4.48 m	
3	4.65 m	4.32 m					4.32 m	
4	4.96 m	4.52 m					4.55 m	
5	4.60 m	4.25 m					4.25 m	
6	4.04 m	3.76 m					4.34 m	
	4.74 m	4.43 m					4.45 m	

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Table 1. Continued

position	1	2	3	4	5	6	7	8
Ara′ (1→2) Ara								
1				4.90 d (7.0)				
2				4.13 m				
3				4.62 m				
4				4.22 m				
5				3.73 t (11.0)				
				4.38 m				
Rha (1→2) Ara								
1					5.99 brs			
2					4.71 m			
3					4.54 m			
4					4.28 m			
5					4.39 m			
6					1.65 d (6.0)			
Glc′ (1→2) GlcA								
1						5.90 d (7.5)		5.29 d (7.0)
2						4.13 m		4.43 m
3						4.57 m		4.39 m
4						4.11 m		4.45 m
5						4.01 m		4.26 m
6						4.32 m		4.30 m
						4.38 m		4.45 m
Rha (1→2) Glc′								
1								5.88 brs
2								4.61 m
3								4.51 m
4								4.30 m
5								4.59 m
6								1.63 d (6.0)

[a] ^{1}H NMR data (δ) were measured in C_5D_5N at 500 MHz for proton. Coupling constants (J) in Hz are given in parentheses. The assignments were based on TOCSY, HSQC, and HMBC experiments.

peak at m/z 511.1461 $[M - H]^-$, supporting a molecular formula of $C_{23}H_{28}O_{13}$ (calcd for $C_{23}H_{27}O_{13}$, 511.1457). The ^{1}H NMR spectrum of **9** in DMSO-d_6 showed a singlet at δ_H 7.18 (2H, s), two aromatic protons at δ_H 6.12 (1H, d, J = 2.0 Hz) and 6.15 (1H, d, J = 2.0 Hz), four aromatic methoxy singlets at δ_H 3.56, 3.59, 3.76, and 3.76, and an anomeric signal of a sugar unit at δ_H 4.82 (1H, d, J = 7.5 Hz). The above information, together with the observation of an ester carbonyl signal at δ_C 165.6, indicated the presence of a 3,5-*O*-dimethoxygalloyl unit in the molecule.[6,7] Acid hydrolysis of **9** afforded D-glucose, which was determined by measuring its specific rotation.[3] A series of HMBC correlations from H-1′ to C-1 and from H-6′ to C-7″ indicated that C-1 and C-7″ are attached to the glucose C-1′ and C-6′, respectively. Also, HMBC correlations from H-7 to C-3; H-8 to C-4; H-2 to C-1, C-3, C-4, and C-6; and H-6 to C-1, C-2, C-4, and C-5 demonstrated that a hydroxy group was present at C-5. Thus, compound **9** was determined as 1-*O*-3, 4-dimethoxy-5-hydroxyphenyl-(6-*O*-3,5-dimethoxygalloyl)-β-D-glucopyranoside.

Compound **10** exhibited a quasimolecular ion peak at m/z 481 $[M - H]^-$ in its ESIMS. The molecular formula $C_{22}H_{26}O_{12}$ was indicated by HRESIMS (m/z 481.1354 $[M - H]^-$, calcd for 481.1351). The IR and NMR spectra of **10** resembled those of **9** except that the NMR signals of the symmetrically tetrasubstituted benzene moiety and two methoxy groups of **9** were replaced by the signals attributed to a 1,2,4-trisubstituted benzene moiety and one methoxy group in **10** (Table 3). These data indicated that **10** is a demethoxy derivative of **9**, which was confirmed by HMBC analysis of **10**. Thus, compound **10** was determined as 1-*O*-3,4-dimethoxy-5-hydroxyphenyl-(6-*O*-vanilloyl)-β-D-glucopyranoside.

In an in vitro bioassay, compounds **1**−**10** at 5 μM inhibited nitric oxide production in rat polymorphonuclear leukocytes (PMNS) induced by the LPS by 96.6 ± 3.7, 9.1 ± 2.7, 6.4 ± 3.6, 14.0 ± 2.3, 2.9 ± 2.2, 8.9 ± 2.5, 12.6 ± 0.5, 5.9 ± 1.2, 4.7 ± 2.0, and 6.5 ± 2.5% (n = 3), respectively. Dexamethasone, the positive control, gave a 74.7 ± 2.9% (n = 3) inhibition at the same concentration. The most active compound, **1**, showed a strong inhibitory effect on nitric oxide production in LPS-activated macrophages with an IC_{50} value of 0.14 μM, suggesting that **1** may serve as a potential anti-inflammatory lead.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 automatic digital polarimeter. UV spectra were recorded using a Shimadzu UV-300 spectrophotometer. IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer by a transmission microscope method. 1D and 2D NMR spectra were obtained at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, on an INOVA 500 MHz spectrometer in DMSO or pyridine-d_5 with solvent peaks as references. ESIMS and HRESIMS were obtained using an Agilent 1100 series LC/MSD Trap SL mass spectrometer. GC was conducted using an Agilent Technologies 7890A instrument (Agilent). Preparative HPLC was carried out on a Shimadzu LC-6AD instrument with a SPD-20A detector, using a YMC-Pack ODS-A column (250 × 20 mm, 5 μm). Column chromatography was performed with silica gel (200−300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China) and ODS (50 μm, YMC, Japan). TLC was carried out with glass precoated silica gel GF_{254} plates. Spots were visualized under UV light or by spraying with 10% sulfuric acid in EtOH followed by heating.

Table 2. ^{13}C NMR Spectroscopic Data for Compounds 1−8[a]

position	1	2	3	4	5	6	7	8
1	38.5	38.9	38.4	38.5	38.7	38.7	38.7	38.7
2	26.3	26.6	26.3	26.3	26.6	26.5	26.5	26.5
3	89.4	89.5	89.0	88.9	89.2	89.6	89.6	89.6
4	39.4	39.5	39.3	39.3	39.5	39.5	39.6	39.6
5	55.5	55.5	55.5	55.6	55.8	55.8	55.9	55.8
6	18.2	18.8	18.2	18.3	18.6	18.5	18.5	18.5
7	32.9	36.7	33.2	33.2	33.4	33.4	33.5	33.4
8	39.9	41.5	39.8	39.9	40.1	40.1	40.1	40.0
9	46.7	47.0	46.9	46.9	47.1	47.1	47.1	47.1
10	36.6	36.9	36.7	36.8	37.0	36.9	37.0	36.9
11	23.6	23.9	23.5	23.6	23.8	23.8	23.8	23.7
12	123.1[b]	124.8	123.1[b]	122.5[b]	122.7[b]	123.0	123.1[b]	123.1
13	143.5	144.5	144.2	144.2	144.4	144.4	144.4	144.4
14	41.4	47.7	41.8	41.9	42.1	42.1	42.1	42.0
15	34.9	67.5	35.7	35.7	36.0	35.9	36.0	35.9
16	69.9	75.0	74.2	74.3	74.4	74.4	74.4	74.4
17	44.6	45.2	48.9	48.9	49.1	49.1	49.1	49.1
18	40.7	41.7	41.0	41.1	41.3	41.3	41.3	41.3
19	47.2	47.1	46.9	46.9	47.1	47.1	47.1	47.2
20	31.9	32.0	30.6	30.6	30.8	30.8	30.8	30.8
21	41.5	41.5	35.9	35.9	36.2	36.1	36.2	36.1
22	72.8	72.7	32.0	32.1	32.3	32.2	32.2	32.2
23	27.8	28.0	27.8	27.0	28.0	27.9	28.0	27.9
24	16.6	16.8	16.7	16.7	16.9	16.7	16.7	16.7
25	15.4	15.8	15.4	15.4	15.6	15.6	15.6	15.6
26	16.5	17.5	17.3	17.3	17.5	17.5	17.5	17.5
27	27.4	21.3	27.0	27.8	27.3	27.2	27.2	27.2
28	63.4	63.0	175.7	175.7	175.9	175.9	175.9	175.9
29	33.3	33.4	33.0	33.0	33.2	33.2	33.2	33.2
30	25.0	25.1	24.3	24.4	24.6	24.6	24.6	24.5
1′	167.8	167.9						
2′	129.3	129.5						
3′	136.3	136.3						
4′	15.6	15.8						
5′	20.7	20.8						
Sugar (C-28)								
Glc 1			95.7	95.7	95.9	95.9	95.9	95.9
2			73.9	74.1	74.2	74.2	74.2	74.2
3			79.1	79.2	79.4	79.4	79.4	79.4
4			70.9	70.9	71.1	71.1	71.1	71.1
5			78.6	78.7	78.9	78.9	79.1	78.5
6			61.9	62.0	62.2	62.2	62.5	62.1
Sugar (C-3)								
GlcA 1	105.4	105.6	106.5	106.1	106.7	105.6	105.6	105.2
2	78.8	79.1	74.2	74.0	74.6	78.2	78.2	78.8
3	83.7	83.8	85.5	86.1	86.5	83.8	83.9	86.1
4	70.9	71.1	71.2	71.1	71.4	71.1	71.0	69.3
5	77.1	77.3	78.6	77.2	77.5	77.4	77.3	77.2
6	171.9	172.3	172.0	172.0	172.3	172.0	172.1	172.2
Ara (1→3) GlcA								
1	101.5	101.8	105.6	102.7	106.5	101.8	101.8	
2	81.8	81.9	74.5	81.4	80.5	82.2	81.9	
3	73.1	73.4	72.6	73.2	72.3	73.3	73.3	
4	68.1	68.3	69.0	72.8	69.4	68.3	68.3	
5	65.7	65.9	66.9	67.5	68.0	66.0	65.9	
Xyl (1→2) Ara								
1	106.6	106.7				107.0	106.8	103.7
2	75.5	75.6				75.6	75.7	76.3
3	78.0	78.2				78.6	78.9	77.7
4	70.5	70.7				70.7	70.7	71.5
5	67.3	67.5				67.5	67.5	67.9
Gal (1→2) GlcA								
1	103.3	103.5					103.5	
2	73.6	73.8					73.8	
3	74.9	75.0					75.1	
4	69.9	70.1					70.1	
5	76.2	76.4					76.4	
6	61.7	61.9					61.9	
Ara′ (1→2) Ara								
1				106.6				
2				72.0				
3				74.2				
4				69.2				
5				65.2				
Rha (1→2) Ara								
1					103.8			
2					72.3			
3					72.5			
4					74.1			
5					70.1			
6					18.6			
Glc′ (1→2) GlcA								
1						102.7		105.0
2						72.7		72.5
3						77.4		81.3
4						76.4		77.2
5						78.2		78.8
6						63.5		63.4
Rha (1→3) Glc′								
1								104.1
2								71.9
3								72.1
4								74.0
5								70.1
6								18.5

[a] ^{13}C NMR data (δ) were measured in C_5D_5N at 125 MHz for carbon. The assignments were based on TOCSY, HSQC, and HMBC experiments. [b] The data were overlapped by solvent peaks.

Plant Material. The stems of *Gordonia chrysandra* were collected at Xishuangbanna, Yunnan Province, China, in May 2009 and identified by Prof. Jingyun Cui (Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences). A voucher specimen (No. 21790) was deposited at the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing.

Extraction and Isolation. The air-dried stems of *G. chrysandra* (4.8 kg) were extracted with 95% EtOH (3 × 14 L) at reflux for 3 × 3 h, and the extract was evaporated under reduced pressure to yield a dark brown residue (200.5 g). The residue was suspended in water (2000 mL) and then partitioned with $CHCl_3$ (5 × 2000 mL), EtOAc (5 × 2100 mL), and *n*-BuOH (5 × 1800 mL), successively. After removing solvent, the *n*-BuOH-soluble portion (15.1 g) was fractionated via silica gel column chromatography, eluting with $CHCl_3$−MeOH−H_2O (7:3:0.5), to afford 10 fractions, A_1−A_{10}, on the basis of TLC analysis. Fraction A_6 (1.8 g) was subjected to separation over an ODS column (50 μm, 20−70% MeOH−H_2O) to afford seven fractions. Fraction 3 (520 mg) was purified by preparative HPLC (YMC-ODS-A 5 μm, 250 mm × 20 mm, detection at 210 nm) using 36% CH_3CN−H_2O (7 mL/min) containing 0.05% TFA as mobile phase to yield compounds **1** (15 mg), **2** (10 mg), and **3** (30 mg). Fraction 5 (620 mg) was subjected to preparative HPLC (YMC-ODS-A 5 μm, 250 mm × 20 mm, detection at 210 nm) using 38% CH_3CN−H_2O (7 mL/min) containing 0.05% TFA as mobile phase to yield compounds **4** (10 mg), **5** (18 mg), and **6** (60 mg). Fraction 7 (530 mg) was separated by reversed-phase HPLC with 36% CH_3CN−H_2O containing 0.05% TFA as mobile phase to yield compounds **7** (20 mg) and **8** (22 mg). Fraction 2 (140 mg) was purified further by preparative HPLC (YMC-ODS-A 5 μm, 250 mm × 20 mm, detection at 280 nm) using 14% CH_3CN−H_2O (7 mL/min) as mobile phase to yield compounds **9** (22 mg) and **10** (24 mg).

Gordonoside I (***1***): white, amorphous powder; mp 241−242 °C; $[\alpha]^{20}_D$ −2.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.31) nm; IR ν_{max} 3402, 2950, 1783, 1678, 1437, 1375, 1203, 1162, 1080, 1047 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR

Table 3. NMR Spectroscopic Data (500 MHz, DMSO-d_6) for Compounds 9 and 10[a]

	9		10	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	153.7		153.6	
2	92.8	6.12 d (2.0)	92.9	6.13 d (3.0)
3	153.3		153.3	
4	131.6		131.6	
5	150.8		150.8	
6	97.2	6.15 d (2.0)	97.2	6.18 d (3.0)
OMe-7	55.5	3.56 s	55.6	3.57 s
OMe-8	60.1	3.59 s	60.1	3.61 s
1′	100.6	4.82 d (7.5)	100.5	4.82 d (8.0)
2′	73.2	3.23 m	73.2	3.21 m
3′	76.3	3.32 m	76.3	3.30 m
4′	70.2	3.25 m	70.1	3.23 m
5′	73.9	3.71 m	73.9	3.69 m
6′	64.2	4.20 m	64.1	4.15 dd (12.0, 7.0)
		4.62 d (11.0)		4.57 d (12.0)
7′	165.6		165.6	
1″	119.3		120.4	
2″	107.0	7.18 s	112.6	7.40 brs
3″	147.6		147.5	
4″	140.9		151.8	
5″	147.6		115.3	6.85 d (8.0)
6″	107.0	7.18 s	123.7	7.46 dd (8.0, 1.5)
OMe	56.1	3.76 s	55.6	3.77 s
	56.1	3.76 s		

[a] Data assignments were based on HSQC and HMBC experiments.

(pyridine-d_5, 125 MHz) are given in Tables 1 and 2, respectively; (+)-ESIMS m/z 1181 $[M + Na]^+$; (−)-ESIMS m/z 1157 $[M - H]^-$; HRESIMS m/z 1181.5703 $[M + Na]^+$ (calcd for $C_{57}H_{90}O_{24}Na$, 1181.5714).

Gordonoside J (**2**): white, amorphous powder; mp 239−240 °C; $[\alpha]^{20}_D$ −4.8 (c 0.06 MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.28) nm; IR ν_{max} 3389, 2940, 1781, 1727, 1675, 1455, 1365, 1204, 1169, 1078, 1049 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) are given in Tables 1 and 2, respectively; (+)-ESIMS m/z 1197 $[M + Na]^+$; (−)-ESIMS m/z 1173 $[M - H]^-$; HRESIMS m/z 1197.5668 $[M + Na]^+$ (calcd for $C_{57}H_{90}O_{25}Na$, 1197.5663).

Gordonoside K (**3**): white, amorphous powder; mp 226−227 °C; $[\alpha]^{20}_D$ −7.0 (c 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.33) nm; IR ν_{max} 3383, 2939, 1730, 1673, 1445, 1363, 1256, 1204, 1142, 1073, 1047 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) are given in Tables 1 and 2, respectively; (+)-ESIMS m/z 965 $[M + Na]^+$; (−)-ESIMS m/z 941 $[M - H]^-$; HRESIMS m/z 965.4697 $[M + Na]^+$ (calcd for $C_{47}H_{74}O_{19}Na$, 965.4717).

Gordonoside L (**4**): white, amorphous powder; mp 234−235 °C; $[\alpha]^{20}_D$ −9.3 (c 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.27) nm; IR ν_{max} 3404, 2937, 1728, 1674, 1434, 1366, 1202, 1142, 1075, 1026 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) are given in Tables 1 and 2, respectively; (+)-ESIMS m/z 1097 $[M + Na]^+$; HRESIMS m/z 1097.5125 $[M + Na]^+$ (calcd for $C_{52}H_{82}O_{23}Na$, 1097.5139).

Gordonoside M (**5**): white, amorphous powder; mp 235−236 °C; $[\alpha]^{20}_D$ −18.0 (c 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.32) nm; IR ν_{max} 3404, 2943, 1728, 1675, 1435, 1388, 1365, 1203, 1142, 1074, 1049 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) are given in Tables 1 and 2, respectively; (+)-ESIMS m/z 1111 $[M + Na]^+$; HRESIMS m/z 1111.5297 $[M + Na]^+$ (calcd for $C_{53}H_{84}O_{23}Na$, 1111.5296).

Gordonoside N (**6**): white, amorphous powder; mp 238−239 °C; $[\alpha]^{20}_D$ −4.8 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.32) nm; IR ν_{max} 3396, 2939, 1731, 1674, 1434, 1365, 1223, 1160, 1077, 1047 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) are given in Tables 1 and 2, respectively; (+)-ESIMS m/z 1259 $[M + Na]^+$; (−)-ESIMS m/z 1235 $[M - H]^-$; HRESIMS m/z 1259.5672 $[M + Na]^+$ (calcd for $C_{58}H_{92}O_{28}Na$, 1259.5667).

Gordonoside O (**7**): white, amorphous powder; mp 232−233 °C; $[\alpha]^{20}_D$ −7.3 (c 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.28), 276 (2.94) nm; IR ν_{max} 3372, 2936, 1728, 1673, 1430, 1366, 1201, 1141, 1075, 1044 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) are given in Tables 1 and 2, respectively; (+)-ESIMS m/z 1259 $[M + Na]^+$; HRESIMS m/z 1259.5667 $[M + Na]^+$ (calcd for $C_{58}H_{92}O_{28}Na$, 1259.5667).

Gordonoside P (**8**): white, amorphous powder; mp 227−228 °C; $[\alpha]^{20}_D$ −12.5 (c 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.29) nm; IR ν_{max} 3390, 2942, 1727, 1676, 1434, 1365, 1203, 1141, 1075, 1045 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) are given in Tables 1 and 2, respectively; (+)-ESIMS m/z 1273 $[M + Na]^+$; (−)-ESIMS m/z 1249 $[M - H]^-$; HRESIMS m/z 1273.5827 $[M + Na]^+$ (calcd for $C_{59}H_{94}O_{28}$ Na, 1273.5824).

1-O-3,4-Dimethoxy-5-hydroxyphenyl-(6-O-3,5-dimethoxygalloyl)-β-D-glucopyranoside (**9**): white, amorphous powder; mp 132−133 °C; $[\alpha]^{20}_D$ −50.7 (c 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.10), 277 (2.44) nm; IR ν_{max} 3423, 3005, 2942, 2845, 1700, 1611, 1510, 1463, 1427, 1337, 1228, 1188, 1110, 1076, 766 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) are given in Table 3; (+)-ESIMS m/z 535 $[M + Na]^+$; (−)-ESIMS m/z 511 $[M - H]^-$; HRESIMS m/z 511.1461 $[M - H]^-$ (calcd for $C_{23}H_{27}O_{13}$, 511.1457).

1-O-3,4-Dimethoxy-5-hydroxyphenyl-(6-O-vanilloyl)-β-D-glucopyranoside (**10**): white, amorphous powder; mp 127−128 °C; $[\alpha]^{20}_D$ −46.7 (c 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.27), 263 (2.72), 292 (2.41) nm; IR ν_{max} 3405, 3005, 2941, 2845, 1696, 1602, 1510, 1461, 1431, 1286, 1224, 1175, 1105, 1073, 765 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) are given in Table 3; (+)-ESIMS m/z 505 $[M + Na]^+$; (−)-ESIMS m/z 481 $[M - H]^-$; HRESIMS m/z 481.1354 $[M - H]^-$ (calcd for $C_{22}H_{25}O_{12}$, 481.1351).

Acid Hydrolysis and Sugar Analysis. The determination of the absolute configuration of the sugars in compounds **1**−**10** was conducted as described previously.[3,4]

Inhibitory Effects on Nitric Oxide Production in LPS-Activated Macrophages. The procedure for NO determination was based on the Griess reaction.[8] One hundred microliters of culture supernatant or sodium nitrite standard (5.2−103.6 μM) was mixed with an equal volume of Griess reagent [a mixture of 0.1% (w/v) *N*-(1-naphthyl)ethylenediamine dihydrochloride and 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid; the two parts being mixed together within 1 h of use] using a 96-well plate. After 20 min at room temperature, the absorbance at 540 nm was measured by a microtitration plate reader.

■ ASSOCIATED CONTENT

Supporting Information. MS and 1D and 2D NMR spectra of compounds **1**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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