Glycosides from the Bark of *Adina polycephala*

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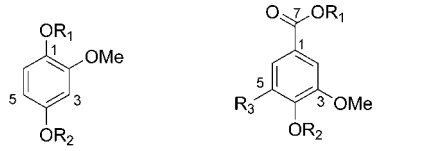
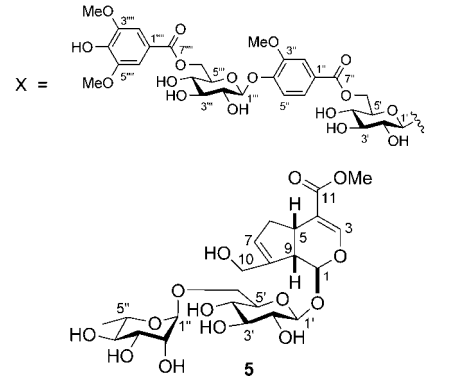
Four new dimeric phenolic glycosides (**1**−**4**), a new iridoid diglycoside (**5**), and 15 known glycosides have been isolated from an ethanolic extract of the bark of *Adina polycephala*. Their structures were determined by spectroscopic and chemical methods. Compounds **1**, **3**, and **5** showed *in vitro* inhibitory activity against the release of β-glucuronidase in rat polymorphonuclear leukocytes induced by platelet-activating factor.

Adina polycephala Benth (Rubiaceae) is distributed widely in southern China.[1,2] Different parts of this plant are used in Chinese traditional medicine for treatment of inflammatory diseases and cattle anthrax.[1,3] Alkaloids, iridoid glycosides, coumarins, flavonoids, triterpenoids, and chromones[4–7] have been reported from several species of the genus *Adina*. However, no investigations of the chemical constituents of *A. polycephala* have been reported. As part of a program to access chemical and biological diversities of several Chinese traditional medicines, we carried out an investigation of *A. polycephala*. In this paper we describe the isolation and structural characterization of four new dimeric phenolic glycosides (**1**−**4**) and a new iridoid diglycoside (**5**) from an ethanolic extract of the bark of *A. polycephala*. Some *in vitro* bioassay results are also included.



1 R_1 = X; R_2 = H **3** R_1 = R_3 = H; R_2 = X
2 R_1 = H; R_2 = X **4** R_1 = X; R_2 = H; R_3 = OMe



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Compound **1** was obtained as a white, amorphous solid, and the IR spectrum indicated the presence of OH (3294 cm^{-1}), conjugated carbonyl (1692 and 1650 cm^{-1}), and aromatic (1620, 1547, and 1516 cm^{-1}) functional groups. Positive and negative ESIMS of **1** gave quasi-molecular ion peaks at *m/z* 833 $[M + K]^+$ and 817 $[M + Na]^+$, and *m/z* 793 $[M - H]^-$. The molecular formula $C_{36}H_{42}O_{20}$ was indicated by HRESIMS (*m/z* 817.2190 $[M + Na]^+$). The ^1H NMR spectrum of **1** in DMSO-d_6 showed two sets of ABX couplings attributed to two 1,3,4-trisubstituted aromatic rings at δ 6.33 (1H, d, *J* = 2.0 Hz), 6.06 (1H, dd, *J* = 8.5 and 2.0 Hz), and 6.82 (1H, d, *J* = 8.5 Hz) and at δ 7.42 (1H, brs), 7.30 (1H, brd, *J* = 8.5 Hz), and 7.16 (1H, d, *J* = 8.5 Hz), respectively. It also showed a singlet assignable to a symmetrical 1,3,4,5-tetrasubstituted aromatic ring at δ 7.13 (2H, s) and four aromatic methoxy singlets at δ 3.77, 3.69, 3.69, and 3.63. Two doublets due to anomeric protons at δ 5.15 (1H, d, *J* = 7.0 Hz, H-1‴) and 4.74 (1H, d, *J* = 7.5 Hz, H-1′), together with partially overlapped signals attributable to oxymethylenes and oxymethines between δ 3.20 and 4.60, as well as signals of exchangeable OH protons between δ 5.10 and 5.45, indicated that there were two β-glycosyl groups in **1**. Acid hydrolysis of **1** produced a glucose that gave a positive optical rotation $[\alpha]^{20}_D$ +35.9 indicating that it was D-glucose.[8] The ^{13}C NMR spectrum of **1** showed carbon signals corresponding to the above structural units (Table 1) and two conjugated ester carbonyls at δ 165.1 and 165.5.

1D TOCOSY and 2D NMR experiments were carried out to determine the connectivity of the three aromatic and two glucose moieties in **1**. Analyses of the 1D TOCOSY and gHSQC spectra of **1** led to unambiguous assignment of proton and corresponding carbon signals in the NMR spectra (Table 1). HMBC correlations of C-1 with H-3, H-5, H-6, and the anomeric proton (H-1′), of C-2 with H-3, H-6, and the methoxy protons at δ 3.63, and of C-4 with H-3, H-5, and H-6, in combination with chemical shifts and coupling patterns of these protons and carbons, provided evidence for the 2-methoxy-*p*-hydroxyquinone 1-*O*-β-D-glucopyranoside moiety in **1**. HMBC correlations of the carbonyl carbon (C-7″) with H-2″ and H-6″, C-3″ with H-2″, H-5″, and the methoxy protons at δ 3.77, and C-4″ with H-2″, H-5″, H-6″, and the remaining anomeric proton (H-1‴), in combination with the chemical shifts and coupling patterns, demonstrated that there was a 4-β-D-glucopyranosyloxy-3-methoxybenzoyl moiety in **1**. Meanwhile, HMBC correlations from H-2⁗ and/or H-6⁗ (overlapped) to C-1⁗, C-3⁗ and/or C-5⁗ (overlapped), and C-4⁗ and from the overlapped methoxy protons at δ 3.69 (6H) to C-3⁗ and C-5⁗ demonstrated that there was a syringyloyl in **1**. Basic hydrolysis of **1** with 0.5 N NaOH yielded isotachioside,[9] vanillic acid 4-*O*-β-D-glucopyranoside,[10] and syringic acid.[11] In addition, HMBC correlations of C-7″ with H-6′a and H-6′b and of C-7⁗ with H-6‴a and H-6‴b, in combination with chemical shifts of these protons and carbons, indicated ester linkages between C-6′ and C-7″ and between C-6‴ and C-7⁗. Therefore, the structure of **1** was determined as 1-*O*-{6-*O*-[4-*O*-(6-*O*-syringyloyl-β-D-glucopyranosyl)vanilloyl]-β-D-glucopyranosyl}-2-methoxy-*p*-hydroxyquinone.

Compound **2** exhibited ESIMS, IR, and NMR spectroscopic data similar to those of **1**. However, comparison of the NMR data between **1** and **2** indicated that H-3 and H-5 of **2** were deshielded 0.20 and 0.34 ppm from those of **1**, respectively, and H-6 was shielded 0.33 ppm. Meanwhile, C-1, C-3, and C-5 of **2** were

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Table 1. NMR Data for Compounds **1**−**4**[a]

	1		**2**		**3**		**4**	
no.	H	C	H	C	H	C	H	C
1		139.1		141.5		123.4		118.2
2		149.9		147.7	7.42 brs	112.9	7.24 s	107.3
3	6.33 d (2.0)	100.9	6.53 d (2.4)	102.4		148.5		147.6
4		152.7		150.3		148.6		140.2
5	6.06 dd (8.5, 2.0)	105.7	6.40 dd (9.0, 2.4)	107.6	6.98 d (8.5)	114.6		147.6
6	6.82 d (8.5)	116.9	6.49 d (9.0)	115.0	7.27 d (8.5)	123.3	7.24 s	107.3
7						165.6		164.5
OMe	3.63 s	55.4	3.56 s	55.3	3.70 s	55.8	3.79 s	56.0
1′	4.74 d (7.5)	101.0	4.75 d (7.8)	101.2	5.02 d (7.5)	99.5	5.56 d (6.6)	94.8
2′	3.30 dd (9.0, 7.5)	73.3	3.21 dd (9.0, 7.8)	73.2	3.36 dd (9.0, 7.5)	73.4	3.2–3.4 m	73.0
3′	3.36 dd (9.0, 9.0)	76.8	3.33 dd (9.0, 9.0)	76.5	3.34 dd (9.0, 9.0)	76.8	3.2–3.4 m	76.5
4′	3.28 dd (9.0, 9.0)	70.0	3.23 dd (9.0, 9.0)	70.2	3.25 dd (9.0, 9.0)	70.7	3.2–3.4 m	70.0
5′	3.61 dd (9.0, 7.0)	73.6	3.67 dd (9.0, 7.8)	73.7	3.74 dd (9.0, 7.5)	74.1	3.66 dd (9.0, 6.6)	74.0
6′	a: 4.15 dd (12.5, 7.0) b: 4.58 brd (12.5)	64.0	a: 4.20 dd (12.0, 7.8) b: 4.57 brd (12.0)	64.2	a: 4.20 dd (11.5, 7.5) b: 4.53 brd (11.5)	64.4	a: 4.23 dd (12.0, 6.0) b: 4.57 brd (12.0)	63.8
1″		123.0		123.1		123.4		123.0
2″	7.42 brs	112.5	7.42 brs	112.5	7.40 brs	112.9	7.44 brs	112.5
3″		148.5		148.5		148.5		148.5
4″		150.4		150.4		150.8		150.4
5″	7.16 d (8.5)	114.1	7.17 d (6.4)	114.2	7.14 d (8.5)	114.6	7.19 d (8.4)	114.1
6″	7.30 brd (8.5)	122.6	7.30 brd (6.4)	122.6	7.27 brd (8.5)	123.3	7.31 brd (8.4)	122.5
7″		165.1		165.1		165.6		165.1
OMe	3.77 s	55.6	3.75 s	55.6	3.74 s	56.1	3.80 s	55.5
1‴	5.15 d (7.0)	99.1	5.15 d (6.6)	99.1	5.19 d (7.5)	99.5	5.12 d (6.6)	99.1
2‴	3.40 dd (9.0, 7.0)	73.0	3.33 dd (9.0, 6.6)	73.0	3.36 dd (9.0, 7.5)	73.4	3.2–3.4 m	72.4
3‴	3.40 dd (9.0, 9.0)	76.5	3.28 dd (9.0, 9.0)	76.4	3.34 dd (9.0, 9.0)	76.7	3.2–3.4 m	76.2
4‴	3.31 dd (9.0, 9.0)	69.9	3.23 dd (9.0, 9.0)	70.0	3.26 dd (9.0, 9.0)	70.7	3.2–3.4 m	69.7
5‴	3.81 dd (9.0, 7.0)	74.1	3.82 dd (9.0, 7.2)	74.0	3.91 dd (9.0, 7.5)	74.1	3.81 dd (9.0, 7.8)	74.6
6‴	a: 4.16 dd (12.5, 7.0) b: 4.58 brd (12.5)	63.8	a: 4.18 dd (12.0, 7.2) b: 4.60 brd (12.0)	64.0	a: 4.11 dd (11.5, 7.5) b: 4.65 brd (11.5)	64.4	a: 4.16 dd (12.0, 7.8) b: 4.59 brd (12.0)	63.8
1⁗		118.1		117.8[b]		119.8		118.2[b]
2⁗	7.13 s	107.4	7.15 s	107.1	7.10 s	107.4	7.16 s	107.3
3⁗		147.9		147.6		147.8		147.8
4⁗		142.3		142.0		141.1		140.2
5⁗		147.9		147.6		147.8		147.8
6⁗	7.13 s	107.4	7.15 s	107.1	7.10 s	107.4	7.16 s	107.3
7⁗		165.5		165.5		166.0		165.4
OMe	3.69 s	56.0	3.71 s	56.0	3.64 s	56.4	3.74 s	56.0
						56.6		

[a] NMR data (δ) were measured in DMSO-d_6 at 500 or 600 MHz for proton and 125 or 150 MHz for carbon. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, ^{1}H−^{1}H COSY, 1D TOCSY, HSQC, and HMBC experiments. [b] Data were obtained from the HMBC spectra.

shielded 2.4, 1.5, and 1.9 ppm from those of **1**, respectively, and C-2, C-4, and C-6 shielded 2.2, 2.4, and 1.9 ppm. These data suggested that **2** was an isomer of **1** in which the substituents at C-1 and C-4 were exchanged. This was confirmed by HSQC and HMBC experiments of **2**. In the HMBC spectrum, long-range correlations of C-1 with H-3, H-5, and H-6, C-2 with H-3, H-6, and the methoxy protons at δ 3.56, and C-4 with H-3, H-5, H-6, and H-1′ indicated unambiguously that the glucopyranosyl moiety of **2** was located at C-4. Basic hydrolysis of **2** yielded tachioside,[9] vanillic acid 4-*O*-β-D-glucopyranoside,[10] and syringic acid.[11] Thus, the structure of **2** was determined to be 4-*O*-{6-*O*-[4-*O*-(6-*O*-syringyloyl-β-D-glucopyranosyl)vanilloyl]-β-D-glucopyranosyl}-2-methoxy-*p*-hydroxyquinone.

Compound **3** was obtained as a white, amorphous powder, and positive and negative ESIMS displayed quasimolecular ion peaks at *m/z* 845 $[M + Na]^+$ and 821 $[M - H]^-$, respectively. The molecular formula was $C_{37}H_{42}O_{21}$, as indicated by HRESIMS (*m/z* 845.2117 $[M + Na]^+$), one CO unit more than that of **1** or **2**. The UV, IR, and NMR spectra of **3** resembled those of **1** and **2** (Experimental Section and Table 1). However, the ^{1}H NMR spectrum of **3** exhibited a group of broad and partially overlapped signals assignable to another 3-methoxy-4-hydroxybenzoyl unit, replacing signals due to the 2-methoxy-*p*-hydroxyquinone unit in the ^{1}H NMR spectrum of **1** or **2**. The ^{13}C NMR spectrum of **3** showed 12 fewer carbons than were expected from the molecular formula. Comparison of the observed carbon resonances with those of **1** or **2** suggested the presence of two repeated 4-*O*-β-D-glucopyranosylvanilloyl groups in **3**. This was confirmed by basic hydrolysis of **3** that produced vanillic acid 4-*O*-β-D-glucopyranoside and syringic acid as the products. The 2D NMR experiments resulted in the assignment of all NMR signals and the linkage of the structural units of **3**. Thus, **3** was determined to be 4-*O*-{6-*O*-[4-*O*-(6-*O*-syringyloyl-β-D-glucopyranosyl)vanilloyl]-β-D-glucopyranosyl}-3-methoxybenzoic acid.

Compound **4**, a white, amorphous powder, exhibited quasimolecular ion peaks at *m/z* 875 $[M + Na]^+$ and 851 $[M - H]^-$ (positive and negative ESIMS, respectively). The molecular formula $C_{38}H_{44}O_{22}$ was indicated by HRESIMS (*m/z* 875.2257 $[M + Na]^+$). The UV, IR, and NMR spectroscopic data of **4** were also similar to those of **1** (Table 1). However, in the NMR spectra of **4**, the data attributed to the 2-methoxy-*p*-hydroxyquinone unit of **1** were replaced by those ascribed to a syringyloyl unit. In addition, H-1′ was significantly deshielded from δ_H 4.74 of **1** to δ_H 5.56 in **4**, whereas C-1′ was shielded from δ_C 101.0 of **1** to δ_C 94.8 in **4**. These data indicated that the additional syringyloyl was located at C-1′ to form an ester glycoside bond in **4**. Basic hydrolysis of **4** with 0.5 N NaOH gave vanillic acid 4-*O*-β-D-glucopyranoside and syringic acid. Therefore, the structure of **4** was determined to be 6-*O*-[4-*O*-(6-*O*-syringyloyl-β-D-glucopyranosyl)vanilloyl]-β-D-glucopyranosyl 4-hydroxy-3,5-dimethoxybenzoate.

Compound **5** was obtained as a colorless gum, and the IR spectrum displayed absorption bands for OH (3358 cm^{-1}) and carbonyl (1704 cm^{-1}) groups. The positive ESIMS of **5** gave quasimolecular ion peaks at *m/z* 535 $[M + H]^+$ and 557 $[M + Na]^+$, and HRESIMS at *m/z* 557.1819 $[M + Na]^+$, indicating the molecular formula to be $C_{23}H_{34}O_{14}$. The ^{1}H NMR spectrum of **5** in D_2O showed signals diagnostic for an iridoid glycoside at δ 5.22 (1H, d, J = 7.6 Hz, H-1), 7.60 (1H, s, H-3), and 4.84 (1H, d, J = 8.4 Hz, H-1′, partially overlapped with the solvent signal) and between δ 3.30 and 4.10 (m, H-2′ to H-6′).[12] Comparison of the NMR data of **5** and the co-occurring geniposide indicated the presence of an additional rhamnopyranosyl unit. In addition, the NMR data of H_2-6′ and C-6′ of **5** were significantly deshielded by comparison with those of geniposide. This indicated that **5** was a geniposide derivative with the rhamnopyranosyl located at C-6′, which was verified by correlations from H-1 to C-1′, from H-1′ to C-1, from H_2-6′ to C-1″, and from H-1″ to C-6′ in the HMBC spectrum of **5**. Acid hydrolysis of **5** afforded glucose and rhamnose, which were identified by TLC comparison with authentic samples. The glucose and rhamnose isolated from the hydrolysate gave optical rotations of $[\alpha]^{20}_D$ +42.3 (*c* 0.90, H_2O) and $[\alpha]^{20}_D$ +6.3 (*c* 1.1, H_2O), respectively, indicating that they were D-glucose and L-rhamnose, respectively. Therefore, **5** was determined to be genipin 1-*O*-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside.

The known compounds were identified by comparison of spectroscopic data with those reported in the literature as clemochinenoside B,[13] kelampayoside A,[14] osmanthuside H,[15] 4-hydroxy-3-methoxyphenol 1-*O*-β-D-[6-*O*-(4-hydroxy-3,5-dimethoxylbenzoyl)]glucopyranoside,[16] syringic acid β-D-glucopyranosyl ester,[9] geniposidic acid,[17] geniposide,[18] 6β-hydroxygeniposide, 6α-hydroxygeniposide, 7β-hydroxysplendoside, gardoside, mussaenosidic acid,[19] ixoside, ixoside 11-methyl ester,[20] and 11-methyl forsythide.[21]

Although compounds with a similar core were previously known,[22] **1**−**4** are not common natural products. Compounds **1**, **3**, and **5** showed potent *in vitro* activities against the release of β-glucuronidase in rat polymorphonuclear leukocytes (PMNs) induced by platelet-activating factor (PAF), with inhibitory rates of 43.6%, 60.2%, and 44.8%, respectively, at a concentration of 10^{-5} M. At the same concentration, the positive control ginkgolide B gave an inhibitory rate of 78.8% (Supporting Information, Table S1). The activities of **1**, **3**, and **5** indicated that they may play a partial rule of this plant in the treatment of inflammatory diseases.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Rudolph Research Autopol III automatic polarimeter. IR spectra were recorded on a Nicolet 5700 FT-IR instrument. UV spectra were measured on a Cary 300 spectrometer. 1D- and 2D-NMR spectra were obtained at 600 or 500 MHz for ^{1}H and 150 or 125 MHz for ^{13}C, respectively, on Inova 600 and 500 MHz spectrometers in DMSO-d_6 or D_2O with solvent peaks (or TMS, in the case of D_2O) used as references. ESIMS data were measured with a Q-Trap LC/MS/MS (Turbo Ionspray Source) spectrometer. HRESIMS data were measured using an AccuToFCS JMS-T100CS spectrometer. Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc. China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala Sweden). HPLC separation was performed on an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector with an Alltima (250 × 10 mm i.d.) preparative column packed with C_{18} (5 μm). TLC was carried out with glass precoated silica gel GF_{254} plates. Spots were visualized under UV light or by spraying with 7% H_2SO_4 in 95% EtOH followed by heating.

Plant Material. The bark of *Adina polycephala* Benth was collected at Dayao Moutain, Guangxi Province, China, in August 2002. The plant was identified by Mr. Guang-Ri Long (Guangxi Forest Administration, Guangxi 545005, China). A voucher specimen (no. 02019) was deposited at the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Beijing, China.

Extraction and Isolation. The air-dried bark of *A. polycephala* (7.7 kg) was powdered and extracted with 95% EtOH (3 × 20 L) at room temperature for 3 × 48 h. The ethanolic extract was evaporated under reduced pressure to yield a dark brown residue (339.3 g). The residue was suspended in H_2O (1500 mL) and then partitioned with EtOAc (5 × 1200 mL) and *n*-BuOH (5 × 1000 mL), successively. After removing solvent, the *n*-BuOH extract (115 g) was subjected to CC over silica gel, eluting with a gradient of increasing MeOH (0−100%) in $CHCl_3$, to afford 10 fractions (A1−A10) on the basis of TLC analysis. Fraction A5 (6.4 g), eluted by 5% MeOH in $CHCl_3$, was chromatographed over Sephadex LH-20 with successive $CHCl_3$−MeOH (1:4) and $CHCl_3$−MeOH (1:1) as mobile phases to give four subfractions (A5-1−A5-4). The subfraction A5-4 (119 mg) was separately purified by reversed-phase preparative HPLC, using a mobile phase of 50% MeOH in H_2O, to yield **1** (11 mg), **2** (9 mg), **3** (8 mg), and **4** (6 mg). Fraction A6 (44.8 g), eluted by 10% MeOH in $CHCl_3$, was further chromatographed over silica gel, eluting with a gradient of increasing MeOH (0−10%) in $CHCl_3$, to give nine subfractions (A6-1−A6-9). Subfraction A6-7 (2.1 g) was subjected to CC over Sephadex LH-20 with MeOH

Table 2. NMR Data for Compound **5**[a]

no.	H	C	no.	H	C
1	5.22 d (7.6)	100.6	1′	4.84 d (8.4)	102.1
3	7.60 s	155.8	2′	3.38 dd (9.2, 8.4)	75.9
4		114.8	3′	3.54 dd (9.2, 9.2)	78.8
5	3.25 q (7.6)	37.6	4′	3.44 dd (9.2, 9.2)	72.8
6	a: 2.16 dd (16.4, 7.6) b: 2.84 dd (16.4, 7.6)	41.2	5′	3.60 dd (9.2, 5.6)	78.2
7	5.91 brs	132.7	6′	a: 3.70 dd (10.8, 5.6) b: 4.01 brd (10.8)	70.0
8		144.5	1″	4.83 brs	103.8
9	2.87 t (7.6)	48.7	2″	3.95 brs	73.2
10	a: 4.28 d (14.0) b: 4.32 d (14.0)	63.0	3″	3.73 m	73.4
11		173.5	4″	3.42 brd (8.8)	75.2
11-OMe	3.77 s	55.0	5″	3.67 dq (8.8, 6.0)	71.9
			6″	1.30 d (6.0)	19.8

[a] NMR data (δ) were measured in D_2O at 500 MHz for proton and 125 MHz for carbon. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, $^1H-^1H$ COSY, HSQC, and HMBC experiments.

as a mobile phase to afford A6-7a (1.6 g). A6-7a was further separated by a reversed-phase flash column, eluting with a gradient of increasing EtOH in H_2O, to yield six fractions. Fraction 4 (0.18 g) was further purified by reversed-phase preparative HPLC using 15% MeOH in H_2O as a mobile phase to obtain **5** (8 mg).

1-*O*-{6-*O*-[4-*O*-(6-*O*-Syringyloyl-*β*-D-glucopyranosyl)vanilloyl]-*β*-D-glucopyranosyl}-2-methoxy-*p*-hydroxyquinone (1): white, amorphous solid; $[\alpha]^{20}_D$ −42.9 (*c* 0.29, DMSO); UV (MeOH) λ_{max} (log ϵ) 215 (4.10), 257 (3.72), 285 (3.69) nm; IR (KBr) ν_{max} 3294, 2919, 1692, 1651, 1620, 1548, 1516, 1456, 1413, 1341, 1275, 1217, 1126, 1073, 1045, 990, 955, 934, 905, 873, 838 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 1; (+)-ESIMS *m/z* 833 $[M + K]^+$, 817 $[M + Na]^+$; (−)-ESIMS *m/z* 793 $[M - H]^-$; (+)-HRESIMS *m/z* 817.2190 $[M + Na]^+$ (calcd for $C_{36}H_{42}O_{20}Na$, 817.2167).

4-*O*-{6-*O*-[4-*O*-(6-*O*-Syringyloyl-*β*-D-glucopyranosyl)vanilloyl]-*β*-D-glucopyranosyl}-2-methoxy-*p*-hydroxyquinone (2): white, amorphous solid; $[\alpha]^{20}_D$ −28.5 (*c* 0.20, DMSO); UV (MeOH) λ_{max} (log ϵ) 215 (4.37), 259 (3.91), 285 (3.89) nm; IR (KBr) ν_{max} 3383, 2924, 1710, 1600, 1512, 1461, 1421, 1339, 1275, 1220, 1188, 1115, 1075, 1027, 989, 943, 828, 829 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 150 MHz) data, see Table 1; (+)-ESIMS *m/z* 817 $[M + Na]^+$; (−)-ESIMS *m/z* 793 $[M - H]^-$; (+)-HRESIMS *m/z* 817.2172 $[M + Na]^+$ (calcd for $C_{36}H_{42}O_{20}Na$, 817.2167).

4-*O*-{6-*O*-[4-*O*-(6-*O*-Syringyloyl-*β*-D-glucopyranosyl)vanilloyl]-*β*-D-glucopyranosyl}-3-methoxybenzoic acid (3): white, amorphous solid; $[\alpha]^{20}_D$ –36.2 (*c* 0.23, DMSO); UV (MeOH) λ_{max} (log ϵ) 212 (4.31), 253 (3.89), 284 (3.75) nm; IR (KBr) ν_{max} 3359, 2940, 1709, 1603, 1554, 1513, 1461, 1419, 1384, 1336, 1273, 1217, 1185, 1114, 1071, 1025, 986, 874, 824 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 1; (+)-ESIMS *m/z* 845 $[M + Na]^+$; (−)-ESIMS *m/z* 821 $[M - H]^-$; (+)-HRESIMS *m/z* 845.2117 $[M + Na]^+$ (calcd for $C_{37}H_{42}O_{21}Na$, 845.2116).

6-O-[4-O-(6-O-Syringyloyl-β-D-glucopyranosyl)vanilloyl]-β-D-glucopyranosyl 4-hydroxy-3,5-dimethoxybenzoate (4): white, amorphous solid; $[\alpha]^{20}_D$ −4.3 (*c* 0.12, DMSO); UV (MeOH) λ_{max} (log ϵ) 213 (4.25), 262 (3.80), 285 (3.83) nm; IR (KBr) ν_{max} 3525, 3470, 3397, 2940, 2844, 1710, 1599, 1515, 1461, 1424, 1340, 1275, 1219, 1185, 1078, 1023, 991, 929, 875, 820 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 150 MHz) data, see Table 1; (+)-ESIMS *m/z* 875 $[M + Na]^+$; (−)-ESIMS *m/z* 851 $[M - H]^-$; (+)-HRESIMS *m/z* 875.2257 $[M + Na]^+$ (calcd for $C_{38}H_{44}O_{22}Na$, 875.2222).

Genipin 1-*O*-α-L-rhamnopyranosyl(1→6)-*β*-D-glucopyranoside (5): colorless gum; $[\alpha]^{20}_D$ +25.7 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 234 (3.45) nm; IR (KBr) ν_{max} 3358, 2951, 1704, 1629, 1589, 1408, 1282, 1248, 1157, 1074, 985, 942, 896, 842 cm^{-1}; ^1H NMR (D_2O, 500 MHz) data, see Table 2; ^{13}C NMR (D_2O, 125 MHz) data, see Table 2; (+)-ESIMS *m/z* 557 $[M + Na]^+$; (+)-HRESIMS *m/z* 557.1819 $[M + Na]^+$ (calcd for $C_{23}H_{34}O_{14}Na$, 557.1846).

Basic Hydrolysis of 1–4. Each compound (5 mg) was individually kept in 0.5 N NaOH (5.0 mL) at room temperature for 1 h. The solution was neutralized with 2 N HCl and then passed through a C-18 solid-phase extraction column (1.0 g), which was successively eluted with H_2O (20 mL) and MeOH (20 mL). The MeOH elutate was concentrated to 0.5 mL and separated by reversed-phase semipreparative HPLC using a mobile phase of 20% MeOH in H_2O containing 3% AcOH. Two products, vanillic acid 4-*O*-*β*-D-glucopyranoside[10] and syringic acid,[11] were obtained from both hydrolysates. In addition, isotachioside (1.1 mg) and tachioside[9] (1.0 mg) were obtained from the hydrolysates of **1** and **2**, respectively. The ESIMS and ^1H NMR data of these hydrolysis products were in good agreement with those reported in the literature.

Acid Hydrolysis of 1 and 5. Each compound (5 mg) was individually refluxed in 2 N HCl (5.0 mL) at 80 °C for 3 h. Each reaction mixture was extracted with $CHCl_3$ (3 × 5 mL), and the H_2O phase was dried by using a N_2 stream. The residues were separately subjected to CC over silica gel with MeCN−H_2O (8:1) as the eluent to yield glucose (2.1 mg) from **1**, $[\alpha]^{20}_D$ +35.9 (*c* 1.0, H_2O), and glucose (0.9 mg) and rhamnose (1.1 mg) from **5**, $[\alpha]^{20}_D$ +42.3 (*c* 0.90, H_2O) and $[\alpha]^{20}_D$ +6.3 (*c* 1.1, H_2O), respectively. The solvent system MeCN−H_2O (6:1) was used for TLC identification of glucose and rhamnose.

Anti-inflammatory Activity Assay.[23] Test compounds were dissolved in DMSO with a concentration of 0.1 mol/L and diluted with RPMI-1640 to 10^{-3} mol/L when used. The suspension of rat polymorphonuclear leukocytes (PMNs) (245 μL) at a density of 2.5×10^6 cells mL^{-1} and test samples (2.5 μL) was incubated at 37 °C for 15 min. Then 2.5 μL of 1 mM cytochalasin B was added and incubated for 5 min, followed by addition of 0.2 μM platelet-activating factor (PAF) (2.5 μL). After 10 min, the reaction was terminated in an ice-bath. The supernatant was obtained by centrifugation at 4000 rpm for 5 min. Then, 25 μL of the supernatant and 2.5 mM phenolphthalein glucuronic acid (25 μL) were incubated with 100 μL of 0.1 M acetic acid buffer (pH 4.6) at 37 °C, 5% CO_2, for 18 h. The reaction was stopped by addition of 0.3 M NaOH (150 μL). The absorbance was read at 550 nm. The inhibitory rate (IR) was calculated as follows. IR (%) = $(A_{PAF} - A_t)/(A_{PAF} - A_C) \times 100\%$. A_{PAF}, A_t, and A_C refer to the average absorbance of three wells of PAF, test compound, and control groups, respectively.

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Supporting Information Available: MS and 1D and 2D NMR spectra of compounds **1**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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