Iridoid Glycosides and Grayanane Diterpenoids from the Roots of *Craibiodendron henryi*

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Four new iridoid glycosides, 10-*O*-*trans*-*p*-coumaroylscandoside (**1**), 10-*O*-*cis*-*p*-coumaroylscandoside (**2**), 10-*O*-*trans*-*p*-coumaroyldesacetyl asperulosidic acid (**3**), and 10-*O*-*cis*-*p*-coumaroyldesacetyl asperulosidic acid (**4**), and two new grayanane diterpenoids, 14β-*O*-(2*S*,3*S*-nilyl)-2α,3β,5β,6β,16α-pentahydroxygrayanane (**5**) and 14β-*O*-(2*S*,3*S*-nilyl)-2α,3β,5β,6β,16α-pentahydroxygrayan-10(20)-ene (**6**), have been isolated from *Craibiodendron henryi*. The structures of these compounds were determined by chemical and spectroscopic methods including $^{1}H-^{1}H$ COSY, HMQC, HMBC, and NOESY experiments. Antioxidant activities and vasodilator effects of these compounds were assessed.

Craibiodendron henryi W. W. Smith (Ericaceae) is a well-known toxic plant, distributed mainly in hilly and valley regions of south, central, and northwest Yunnan Province of China. Up to the present, chemical and biological work on this plant has been limited to a report of two grayanane diterpenoids, rhodojaponins II and III.[1] The *n*-BuOH-soluble fraction of an ethanolic extract was found to show antioxidant activity in a microsomal lipid peroxidation induced by ferrous-cysteine model and moderate vasodilator activity in a phenylepherine-induced vasoconstriction assay using rat aortic rings. Chemical investigation of the plant has led to the isolation of four new iridoid glycosides (**1**−**4**) and two new grayanane diterpenoids (**5**, **6**), along with three known iridoid glycosides and two grayanane diterpenoids. In this paper we describe the isolation and structural elucidation of the new compounds and the unambiguous assignment of spectroscopic data by a combination of NMR techniques, including $^{1}H-^{1}H$ COSY, HMQC, HMBC, and NOESY.

Results and Discussion

The *n*-BuOH fraction of the ethanolic extract was subjected to column chromatography on polyamide, silica gel, RP-18, Sephadex LH-20, and HPLC to afford four new iridoid glycosides (**1**−**4**) and two new grayanane diterpenoids (**5**, **6**), along with five known compounds. The known compounds were characterized as vaccinoside,[2] andromedoside,[3] splendoside,[4] rhodojaponin III,[1] and rhodomollein I.[5]

Compound **1** was isolated as an amorphous powder. The molecular formula was determined to be $C_{25}H_{28}O_{13}$ by HRFABMS. Its UV spectrum showed absorption bands characteristic for a conjugated enol-ether system and an aromatic system at 242, 303, and 314 nm. The IR spectrum of **1** indicated hydroxyl (3407 cm^{-1}), conjugated carbonyl (1682 cm^{-1}), and aromatic (1604 and 1516 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectroscopic data of **1** displayed signals characteristic of an iridoid glycoside.[6−9] The ^{13}C NMR spectrum contained 25 carbon signals including six for the glucopyranosyl unit, nine for a *p*-coumaroyl group, a carboxyl group, and the remaining nine for the iridoid skeleton. The ^{13}C NMR chemical shifts of **1** were closely related to those reported for scandoside (**1a**).[9] The only evident difference was that **1** has resonances due to an additional *p*-coumaroyl group, which was assigned an *E*-configuration from the large coupling constant between H-2″ and H-3″ (J = 16.0 Hz) in the ^{1}H NMR spectrum. The C-7 and C-10 downfield shifts (δ 133.7 from δ 129.9; δ 62.3 from δ 61.1) and the C-8 upfield shift (δ 140.6 from δ 147.3) established the attachment of the (*E*)-*p*-coumaroyl group to the C-10 carbon. The assumption was confirmed by the correlations between two proton signals of C-10 at δ 5.33 and 5.06 and the ester carbonyl (δ 167.0) of an (*E*)-*p*-coumaroyl group in the HMBC experiment (Figure 1). The C-6 hydroxyl in **1** was assigned as a β-substituent on the basis of the coupling constant between H-5 and H-6 (J = 6.5 Hz) in the ^{1}H NMR spectrum. The correlation of H-5 to H-9 and the absence of the correlation of H-5 to H-6 in a NOESY experiment (Figure 2) supported the above conclusion. On the basis of the reported procedure,[10] the absolute configuration of the glucose moiety was determined as D-glucose. Therefore the structure of **1** was determined to be 10-*O*-*trans*-*p*-coumaroylscandoside.

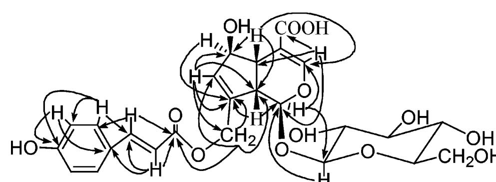

Figure 1. Selected HMBC (H → C) correlations of **1**.

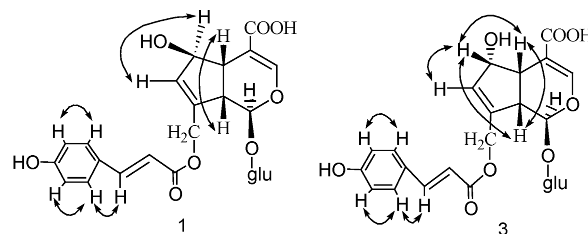

Figure 2. Selected NOESY correlations of **1** and **3**.

Compound **2**, an amorphous powder, was found to have the same molecular formula, $C_{25}H_{28}O_{13}$, as **1** (HRFABMS). The similarity of the ^{1}H and ^{13}C NMR spectroscopic data of **2** to those of compound **1** indicated that they had the same skeleton structure except for the NMR data assign-

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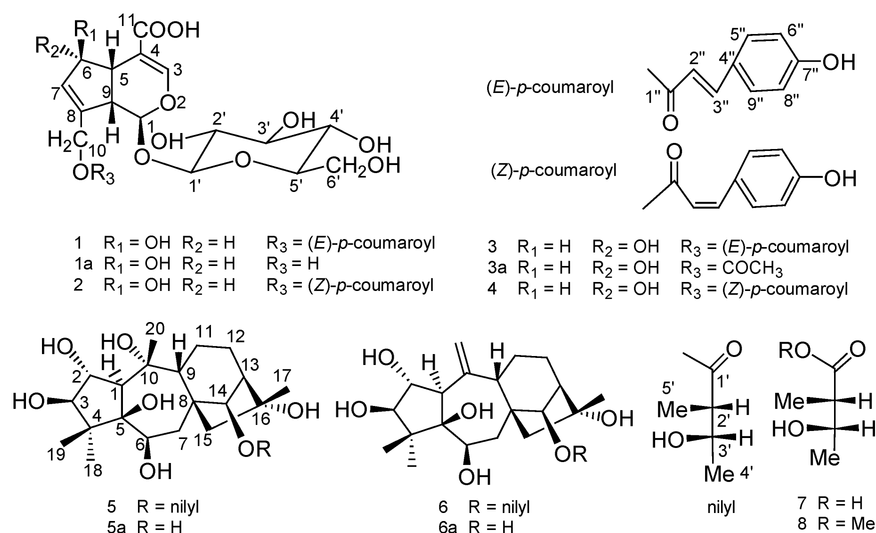
Chart 1



able to the *p*-coumaroyl group. Specifically, a small coupling constant (13.0 Hz) observed for two vicinal olefinic protons suggested the presence of a (*Z*)-*p*-coumaroyl ester unit in **2**, which was further confirmed by the NOESY correlation between two olefinic protons. The HMBC correlations between protons (δ 5.01 and 5.23) of C-10 and the ester carbonyl function (δ 166.2) indicated that this (*Z*)-*p*-coumaroyl group was located at the C-10 position. From these data compound **2** was clearly assigned as 10-*O*-*cis*-*p*-coumaroylscandoside.

Compound **3**, obtained as an amorphous powder, had a molecular formula of $C_{25}H_{28}O_{13}$ as determined by HR-FABMS. The UV spectrum of **3** exhibited absorption maxima at 236, 301, and 314 nm, indicating the presence of a conjugated enol-ether system and a coumaroyl moiety. Its IR spectrum displayed absorptions due to hydroxyl groups at 3410 cm^{-1}, a conjugated carbonyl group at 1682 cm^{-1}, and an aromatic moiety at 1604 and 1516 cm^{-1}. The ^1H and ^{13}C NMR spectroscopic data of **3** revealed expected signals for a β-glucopyranosyl unit, a *p*-coumaroyl group with an *E* double bond (J = 16.0 Hz), and an iridoid skeleton. Compound **3** showed the same substituted hydroxyl group patterns as that in **1**. However, differences in chemical shifts were observed in the ^1H and ^{13}C NMR spectroscopic data, indicating the existence of a different relative configuration. The coupling constant between H-5 and H-6 (J = 5.5 Hz) together with the downfield shifts of C-1 and C-8 and the upfield shifts of C-4, C-5, C-6, C-7, and C-11 indicated that the orientation of the hydroxyl group at C-6 was α. Moreover, H-6 of **3** correlated with H-5 and H-9 in a NOESY experiment (Figure 2), which confirmed that the relative configuration of the hydroxyl group at C-6 was α. On comparing the NMR spectra of **3** with those of asperulosidic acid (**3a**),[9] a close resemblance was observed except for the presence of an (*E*)-*p*-coumaroyl group in **3** instead of an acetyl group in asperulosidic acid. These spectroscopic data also implied that the (*E*)-*p*-coumaroyl group of **3** was attached at the C-10, the same position as the acetyl group of asperulosidic acid, which was further supported by the HMBC correlations of two protons (δ 5.01 and 5.60) of C-10 to the ester carbonyl (δ 167.1) of the (*E*)-*p*-coumaroyl group. Thus, compound **3** was identified as 10-*O*-*trans*-*p*-coumaroyldesacetylasperulosidic acid.

Compound **4**, an amorphous powder, gave the molecular formula $C_{25}H_{28}O_{13}$ as determined by HRFABMS. The ^1H and ^{13}C NMR spectroscopic data for **4** were identical with those of **3**, except for the chemical shifts and J values due to H-2″ and H-3″ of the *p*-coumaroyl unit. A small J value (13.0 Hz) between H-2″ and H-3″ of the *p*-coumaroyl group was observed, indicating the *Z* isomer. Additionally, the *p*-coumaroyl group was determined to be linked at C-10 on the basis of the HMBC correlations between two protons (δ 5.16 and 5.42) of C-10 and the ester carbonyl function (δ 166.3). Thus, compound **4** was assigned as the *Z* isomer of compound **3**.

Compound **5** was isolated as an amorphous powder. The molecular formula was determined to be $C_{25}H_{42}O_9$ by HRFABMS. Its IR spectrum showed the presence of hydroxyl groups (3390 cm^{-1}) and an ester carbonyl group (1724 cm^{-1}). The ^1H NMR spectrum contained signals for four methyl singlets (δ 1.49, 1.60, 1.64, 1.88), two methyl doublets (δ 1.20, 1.26), and five oxygenated methines (δ 4.12, 4.17, 4.42, 5.16, 6.31). The ^{13}C NMR (DEPT) spectrum revealed 25 carbon signals, including six methyls, four methylenes, nine methines (five oxygenated), and six quaternary carbons (one ester carbonyl and three oxygenated). The $^1H-^1H$ COSY spectrum indicated the existence of the following fragments: CH−CH(OH)−CH(OH), CH-(OH)−CH_2, and CH−CH_2−CH_2−CH. These structural features suggested that **5** was a grayanane-type diterpenoid with seven sites of oxygenation. In addition to the signals for a grayanane-type diterpenoid skeleton, there were the resonances due to an extra nilyl group. The assignment of the nilyl group was determined on the basis of the fragment of successive connectivities, CH_3−CH−CH-(OH)−CH_3, in the $^1H-^1H$ COSY spectrum. This assumption was confirmed by protons of C-5′ correlated with C-1′, C-2′, and C-3′, but protons of C-4′ only correlated with C-2′ and C-3′ in the HMBC spectrum of **5**. Alkaline hydrolysis of **5** yielded the aglycon **5a**. The ^1H and ^{13}C NMR and optical rotation data of **5a** were in agreement with those of rhodojaponin VI.[11,12] Thus, the structure of **5a** was established as 2α,3β,5β,6β,14β,16α-hexahydroxygrayanane. The downfield shifts of H-14 (δ 5.08 in the aglycon (**5a**) to δ 6.31 in **5**) and C-14 (δ 80.1 in the aglycon (**5a**) to δ 83.0 in **5**) indicated that the nilyl group of **5** was located at C-14. This deduction was confirmed by the HMBC correlations among H-14/C-15 and H-14/C-16 and the ester carbonyl carbon. The absolute stereochemistry of the nilate moiety was assigned by comparison of the specific rotations of methyl nilate (**8**) with reported values for the stereoisomers of this compound in the literature.[13−16] The specific rotation values ($[\alpha]^{23}_D$ (*c* 5.00, $CHCl_3$)) of methyl nilate obtained by

methylation of nilic acid (**7**) derived from alkaline hydrolysis of **5** were as follows: (λ (nm)) 589, +34.6; 578, +35.2; 546, +37.6; 436, +47.0. These results indicated the nilate moiety present in **5** had the (+)-erythro, i.e., (2*S*, 3*S*), configuration. Therefore the structure of **5** was determined as 14β-*O*-(2*S*,3*S*-nilyl)-2α,3β,5β,6β,16α-pentahydroxygrayanane.

Compound **6**, colorless needles, was assigned a molecular formula of $C_{25}H_{40}O_8$, as deduced from its positive HRFABMS. The IR spectrum showed characteristic absorptions for hydroxyl (3433 cm^{-1}), ester carbonyl (1711 cm^{-1}), and double-bond (1637 cm^{-1}) groups. The ^{1}H NMR spectrum contained three methyl singlets (δ 1.52, 1.56, 1.66), two methyl doublets (δ 1.26, 1.38), five oxygenated methines (δ 4.12, 4.18, 4.30, 4.95, 5.71), and two olefinic protons (δ 5.26, 5.45). The ^{13}C NMR (DEPT) spectrum revealed 25 carbon signals, including five methyls, five methylenes (one olefinic), nine methines (five oxygenated), and six quaternary carbons (one ester carbonyl, one olefinic, and two oxygenated). The ^{1}H−^{1}H COSY spectrum of **6** indicated the existence of the following fragments: CH−CH(OH)−CH(OH), CH(OH)−CH_2, and CH−CH_2−CH_2−CH, the same as those in **5**. These structural features suggested that **6** was a grayanane-type diterpenoid with six sites of oxygenation. The ^{1}H and ^{13}C NMR data of **6** were very similar to those of **5**. By comparison of the ^{13}C NMR data of **6** and **5**, the major difference was that **6** had an exomethylene instead of a methyl and an oxygenated quaternary carbon in **5**. This indicated that **6** was a C-10/20 dehydrated derivative of **5**. The exomethylene at C-10/20 was confirmed by the HMBC correlations between two proton signals of C-20 and C-1 and C-9. The NOESY spectrum and the *J* values for each proton in **6** were comparable with those of **5**, thereby indicating that the relative stereochemistry of **6** was the same as **5**. In addition, **6** was hydrolyzed with KOH to afford the aglycon **6a**. The ^{1}H and ^{13}C NMR data of **6a** were in accordance with those of rhodomollein I,[5] which was established as 2α,3β,5β,6β,14β,16α-hexahydroxygrayan-10(20)-ene. The absolute configuration of the nilate moiety in **6** was assumed to be the same as that of **5**. Thus, the structure of **6** was determined to be 14β-*O*-(2*S*,3*S*-nilyl)-2α,3β,5β,6β,-16α-pentahydroxygrayan-10(20)-ene.

All seven iridoid glycosides showed low-level antioxidant activity in the microsomal lipid peroxidation induced by ferrous-cysteine testing. The inhibition rate of MDA for the seven iridoid glycosides, at a concentration of 1.0×10^{-5} M, was found to be 24.3, 23.9, 17.7, 14.1, 17.9, 17.5, and 27.9%, respectively, compared to 49.7% for vitamin E, a well-known antioxidant.

Vasodilator effects of four grayanane diterpenoids (1.0×10^{-5} M) on phenylepherine-induced vasoconstriction of rat aortic rings in the presence of indomethacin (Indo) and N^{ω}-L-nitroarginine (L-NA) were (33 ± 1.1), (27 ± 2.1), (33 ± 1.8), and (35 ± 2.4)%, respectively, compared to (105 ± 4.7)% for sodium nitroprusside as a positive control. All four compounds exhibited weak vasodilator activities.

Experimental Section

General Experimental Procedures. Melting points were determined on an XT-4 micro melting point apparatus and are uncorrected. IR spectra were recorded as KBr disks on a Nicolet Impact 400 FT-IR spectrophotometer. 1D- and 2D-NMR spectra were obtained at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, on an Inova 500 FT-NMR spectrometer in pyridine-d_5 with solvent peaks as references. ESIMS were measured on an Agilent 1100 Series LC/MSD trap mass spectrometer. HRFABMS data were measured with a Micromass Autospec-Ultima ETOF spectrometer. Column chromatography was performed with polyamide (30−60 mesh), silica gel (200−300 mesh), RP-18 (40−70 μm), and Sephadex LH-20. HPLC was carried out on a Shimadzu LC-6AD, and the detector was a SPD-10A. A reversed-phase C_{18} column (YMC-Pack ODS-A ϕ 20 × 250 mm, 10 μm) was employed. 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 roots of *Craibiodendron henryi* (8.0 kg) were collected from Gejiu City of Yunnan Province in China, in August of 2003. The plant was identified by associate Prof. Lin Ma of the Department of Natural Products Chemistry, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (No. 211) is deposited in the herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College.

Extraction and Isolation. The roots of *C. henryi* (8.0 kg) were air-dried, ground, and extracted with EtOH under reflux. The ethanolic extract was evaporated to almost dryness in vacuo, and the resulting mixture was partitioned successively between H_2O and EtOAc and between H_2O and *n*-BuOH. The *n*-BuOH phase was concentrated to give a black mass (171 g), which was applied to polyamide column chromatography eluting with H_2O, 20% EtOH, 60% EtOH, and 95% EtOH to give Fractions A_1 (5.8 g), A_2 (6.1 g), A_3 (107.5 g), and A_4 (3.8 g), respectively. Fraction A_2 was subjected to a silica gel column eluting with a gradient of increasing MeOH in $CHCl_3$ to yield Fractions B_1−B_7. Fraction B_1 (91 mg) was chromatographed on a silica gel column with $CHCl_3$−MeOH (20:1) and an RP-18 column with MeOH−H_2O (47:53) to yield **8** (51 mg). Fraction B_2 (146 mg) was subjected to an RP-18 silica gel column using MeOH−H_2O (45:55) as a solvent to provide **9** (59 mg). From fraciton B_3 (789 mg), repeated silica gel and Sephadex LH-20 column chromatography led to the isolations of **10** (14 mg) and **11** (17 mg). Compound **7** (129 mg) was obtained by applying fraction B_5 (707 mg) to a silica gel column eluting with $CHCl_3$−MeOH (15:1). Fraction B_6 (1.36 g) was purified by Sephadex LH-20 column chromatography eluted with $CHCl_3$−MeOH (2:1) and then was separated into fractions C_1−C_3 (C_1, 817 mg; C_2, 88 mg; C_3, 107 mg) by RP-18 column chromatography eluted with MeOH−H_2O (29:71). Fraction C_1 was purified by reversed-phase HPLC using 42% MeOH in H_2O as the mobile phase to afford **5** (94 mg) and **6** (25 mg). Fraction C_3 was similarly purified by reversed-phase HPLC using MeOH−H_2O (39:61) to obtain **1** (18 mg), **2** (8 mg), **3** (31 mg), and **4** (7 mg), respectively.

Compound 1: amorphous powder; mp 160−162 °C; $[\alpha]^{23}_D$ −65.4 (*c* 0.54, MeOH); UV (MeOH) λ_{max} (log ϵ) 242 (3.87), 303 (3.56), 314 (4.01) nm; IR (KBr) ν_{max} 3407, 2924, 1682, 1632, 1604, 1516, 1205, 1142, 1076, 839 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 7.91 (1H, d, *J* = 16.0 Hz, H-3″), 7.89 (1H, s, H-3), 7.58 (2H, d, *J* = 8.5 Hz, H-5″, 9″), 7.17 (2H, d, *J* = 8.5 Hz, H-6″, 8″), 6.57 (1H, d, *J* = 16.0 Hz, H-2″), 6.04 (1H, s, H-7), 5.40 (1H, d, *J* = 7.5 Hz, H-1′), 5.37 (1H, d, *J* = 8.0 Hz, H-1), 5.33 (1H, d, *J* = 14.5 Hz, H-10), 5.06 (1H, d, *J* = 14.5 Hz, H-10), 4.92 (1H, br s, H-6), 4.48 (1H, dd, *J* = 12.0, 2.0 Hz, H-6′), 4.33 (1H, dd, *J* = 12.0, 5.5 Hz, H-6′), 4.28 (1H, dd, *J* = 9.0, 8.5 Hz, H-3′), 4.26 (1H, m, H-4′), 4.12 (1H, dd, *J* = 9.0, 7.5 Hz, H-2′), 3.97 (1H, m, H-5′), 3.39 (1H, dd, *J* = 7.0, 6.5 Hz, H-5), 3.12 (1H, dd, *J* = 8.0, 7.0 Hz, H-9); ^{13}C NMR (pyridine-d_5, 125 MHz) δ 173.0 (C, C-11), 167.0 (C, C-1″), 161.5 (C, C-7″), 152.5 (CH, C-3), 145.6 (CH, C-3″), 140.6 (C, C-8), 133.7 (CH, C-7), 130.8 (2CH, C-5″, 9″), 125.9 (C, C-4″), 116.8 (2CH, C-6″, 8″), 114.7 (CH, C-2″), 111.7 (C, C-4), 101.0 (CH, C-1′), 98.9 (CH, C-1), 82.1 (CH, C-6), 78.8 (CH, C-5′), 78.3 (CH, C-3′), 74.8 (CH, C-2′), 71.3 (CH, C-4′), 62.6 (CH_2, C-6′), 62.3 (CH_2, C-10), 47.2 (CH, C-9), 46.3 (CH, C-5); ESIMS (positive-ion mode) *m/z* 559 [M + Na]$^+$; HRFABMS *m/z* 559.1460 [M + Na]$^+$ (calcd for $C_{25}H_{28}O_{13}Na$, 559.1428).

Compound 2: amorphous powder; mp 137−139 °C; $[\alpha]^{23}_D$ −27.6 (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ϵ) 242 (3.99), 302 (3.67), 315 (4.13) nm; IR (KBr) ν_{max} 3407, 2924, 1682, 1633,

1604, 1516, 1205, 1142, 1076, 839 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 8.03 (2H, d, J = 8.5 Hz, H-5″, 9″), 7.87 (1H, s, H-3), 7.16 (2H, d, J = 8.5 Hz, H-6″, 8″), 6.92 (1H, d, J = 13.0 Hz, H-3″), 6.04 (1H, s, H-7), 5.93 (1H, d, J = 13.0 Hz, H-2″), 5.39 (1H, d, J = 7.5 Hz, H-1′), 5.35 (1H, d, J = 8.0 Hz, H-1), 5.23 (1H, d, J = 14.5 Hz, H-10), 5.01 (1H, d, J = 14.5 Hz, H-10), 4.89 (1H, br s, H-6), 4.48 (1H, dd, J = 12.0, 2.5 Hz, H-6′), 4.31 (1H, dd, J = 12.0, 5.5 Hz, H-6′), 4.27 (1H, t, J = 9.0 Hz, H-3′), 4.21 (1H, t, J = 9.0 Hz, H-4′), 4.10 (1H, dd, J = 9.0, 7.5 Hz, H-2′), 3.97 (1H, m, H-5′), 3.35 (1H, dd, J = 7.0, 6.5 Hz, H-5), 3.08 (1H, dd, J = 8.0, 7.0 Hz, H-9); ^{13}C NMR (pyridine-d_5, 125 MHz) δ 173.0 (C, C-11), 166.2 (C, C-1″), 160.7 (C, C-7″), 152.6 (CH, C-3), 144.7 (CH, C-3″), 140.3 (C, C-8), 133.8 (2CH, C-5″, 9″), 132.9 (CH, C-7), 126.4 (C, C-4″), 116.0 (2CH, C-6″, 8″), 115.6 (CH, C-2″), 111.8 (C, C-4), 100.9 (CH, C-1′), 98.8 (CH, C-1), 82.1 (CH, C-6), 78.9 (CH, C-5′), 78.3 (CH, C-3′), 74.9 (CH, C-2′), 71.5 (CH, C-4′), 62.7 (CH$_2$, C-10), 62.3 (CH$_2$, C-6′), 47.3 (CH, C-9), 46.4 (CH, C-5); ESIMS (positive-ion mode) *m/z* 559 [M + Na]$^+$; HRFABMS *m/z* 559.1449 [M + Na]$^+$ (calcd for $C_{25}H_{28}O_{13}Na$, 559.1428).

Compound 3: amorphous powder; mp 173−175 °C; $[\alpha]^{23}_D$ −19.3 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 236 (3.70), 301 (3.67), 314 (3.88) nm; IR (KBr) ν_{max} 3410, 2929, 1682, 1633, 1604, 1516, 1203, 1076, 839 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 8.15 (1H, d, J = 1.0 Hz, H-3), 7.90 (1H, d, J = 16.0 Hz, H-3″), 7.56 (2H, d, J = 9.0 Hz, H-5″, 9″), 7.16 (2H, d, J = 9.0 Hz, H-6″, 8″), 6.60 (1H, d, J = 16.0 Hz, H-2″), 6.34 (1H, s, H-7), 5.85 (1H, d, J = 8.5 Hz, H-1), 5.60 (1H, d, J = 15.0 Hz, H-10), 5.47 (1H, dd, J = 5.5, 2.0 Hz, H-6), 5.34 (1H, d, J = 7.5 Hz, H-1′), 5.20 (1H, d, J = 15.0 Hz, H-10), 4.36 (1H, m, H-6′), 4.31 (1H, m, H-4′), 4.30 (1H, m, H-6′), 4.25 (1H, dd, J = 9.0, 8.5 Hz, H-3′), 4.16 (1H, dd, J = 8.5, 7.5 Hz, H-2′), 3.82 (1H, m, H-5′), 3.47 (1H, dd, J = 8.0, 5.5 Hz, H-5), 2.88 (1H, dd, J = 8.5, 8.0 Hz, H-9); ^{13}C NMR (pyridine-d_5, 125 MHz) δ 169.8 (C, C-11), 167.1 (C, C-1″), 161.6 (C, C-7″), 153.8 (CH, C-3), 145.6 (CH, C-3″), 144.5 (C, C-8), 132.3 (CH, C-7), 130.8 (2CH, C-5″, 9″), 126.0 (C, C-4″), 116.8 (2CH, C-6″, 8″), 114.8 (CH, C-2″), 109.2 (C, C-4), 101.7 (CH, C-1), 101.6 (CH, C-1′), 78.3 (CH, C-3′), 78.3 (CH, C-5′), 74.9 (CH, C-2′), 74.5 (CH, C-6), 71.2 (CH, C-4′), 63.0 (CH$_2$, C-10), 62.5 (CH$_2$, C-6′), 46.1 (CH, C-9), 42.7 (CH, C-5); ESIMS (positive-ion mode) *m/z* 559 [M + Na]$^+$; HRFABMS *m/z* 559.1443 [M + Na]$^+$ (calcd for $C_{25}H_{28}O_{13}Na$, 559.1428).

Compound 4: amorphous powder; mp 146−148 °C; $[\alpha]^{23}_D$ −55.6 (*c* 0.53, MeOH); UV (MeOH) λ_{max} (log ϵ) 237 (4.00), 301 (3.87), 314 (4.07) nm; IR (KBr) ν_{max} 3406, 2927, 1685, 1633, 1604, 1516, 1203, 1140, 839 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 8.13 (1H, s, H-3), 8.04 (2H, d, J = 8.5 Hz, H-5″, 9″), 7.15 (2H, d, J = 8.5 Hz, H-6″, 8″), 6.92 (1H, d, J = 13.0 Hz, H-3″), 6.29 (1H, s, H-7), 5.94 (1H, d, J = 13.0 Hz, H-2″), 5.82 (1H, d, J = 8.5 Hz, H-1), 5.45 (1H, dd, J = 5.5, 2.0 Hz, H-6), 5.42 (1H, d, J = 14.0 Hz, H-10), 5.31 (1H, d, J = 7.5 Hz, H-1′), 5.16 (1H, d, J = 14.0 Hz, H-10), 4.35 (1H, m, H-6′), 4.29 (1H, m, H-4′), 4.26 (1H, dd, J = 9.0, 8.5 Hz, H-3′), 4.25 (1H, m, H-6′), 4.13 (1H, dd, J = 8.5, 7.5 Hz, H-2′), 3.80 (1H, m, H-5′), 3.41 (1H, dd, J = 8.0, 5.5 Hz, H-5), 2.83 (1H, dd, J = 8.5, 8.0 Hz, H-9); ^{13}C NMR (pyridine-d_5, 125 MHz) δ 169.7 (C, C-11), 166.3 (C, C-1″), 160.7 (C, C-7″), 153.7 (CH, C-3), 144.6 (CH, C-3″), 144.2 (C, C-8), 133.8 (2CH, C-5″, 9″), 132.8 (CH, C-7), 126.4 (C, C-4″), 116.0 (2CH, C-6″, 8″), 115.7 (CH, C-2″), 109.3 (C, C-4), 101.5 (CH, C-1), 101.4 (CH, C-1′), 78.3 (CH, C-5′), 78.2 (CH, C-3′), 74.9 (CH, C-2′), 74.5 (CH, C-6), 71.4 (CH, C-4′), 62.9 (CH$_2$, C-10), 62.5 (CH$_2$, C-6′), 46.1 (CH, C-9), 42.6 (CH, C-5); ESIMS (positive-ion mode) *m/z* 559 [M + Na]$^+$; HRFABMS *m/z* 559.1402 [M + Na]$^+$ (calcd for $C_{25}H_{28}O_{13}Na$, 559.1428).

Compound 5: amorphous powder; mp 103−105 °C; $[\alpha]^{23}_D$ −12.3 (*c* 0.13, EtOH); UV (MeOH) λ_{max} (log ϵ) 274 (0.86) nm; IR (KBr) ν_{max} 3390, 2964, 1724, 1458, 1377, 1265, 1038, 997, 796 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 6.31 (1H, s, H-14), 5.16 (1H, dd, J = 7.5, 4.5 Hz, H-2), 4.42 (1H, dd, J = 9.5, 3.5 Hz, H-6), 4.17 (1H, m, H-3′), 4.12 (1H, d, J = 4.5 Hz, H-3), 3.02 (1H, d, J = 7.5 Hz, H-1), 2.70 (1H, dd, J = 14.5, 3.5 Hz, H-7α), 2.69 (1H, m, H-12α), 2.66 (1H, dd, J = 14.5, 9.5 Hz, H-7β), 2.66 (1H, m, H-2′), 2.52 (1H, m, H-13), 2.37 (1H, d, J = 14.5 Hz, H-15α), 2.31 (1H, d, J = 14.5 Hz, H-15β), 2.20 (1H, d, J = 7.5 Hz, H-9), 2.16 (1H, m, H-11α), 1.88 (3H, s, H-20), 1.76 (1H, m, H-12β), 1.64 (1H, m, H-11β), 1.64 (3H, s, H-18), 1.60 (3H, s, H-19), 1.49 (3H, s, H-17), 1.26 (3H, d, J = 6.0 Hz, H-4′), 1.20 (3H, d, J = 7.0 Hz, H-5′); ^{13}C NMR (pyridine-d_5, 125 MHz) δ 175.3 (C, C-1′), 86.7 (CH, C-3), 83.0 (CH, C-14), 82.8 (C, C-5), 80.9 (CH, C-2), 78.8 (C, C-16), 78.2 (C, C-10), 71.8 (CH, C-6), 69.7 (CH, C-3′), 61.8 (CH$_2$, C-15), 56.8 (CH, C-1), 55.3 (CH, C-9), 54.6 (CH, C-13), 50.4 (C, C-8), 48.9 (C, C-4), 48.8 (CH, C-2′), 44.5 (CH$_2$, C-7), 29.4 (CH$_3$, C-20), 27.2 (CH$_2$, C-12), 26.8 (CH$_3$, C-18), 24.1 (CH$_3$, C-17), 21.8 (CH$_2$, C-11), 21.7 (CH$_3$, C-4′), 20.2 (CH$_3$, C-19), 14.2 (CH$_3$, C-5′); ESIMS (positive-ion mode) *m/z* 509 [M + Na]$^+$; HRFABMS *m/z* 509.2748 [M + Na]$^+$ (calcd for $C_{25}H_{42}O_9Na$, 509.2727).

Compound 5a: amorphous powder; mp 257−259 °C; $[\alpha]^{23}_D$ −27.5 (*c* 0.38, EtOH); IR (KBr) ν_{max} 3464, 2925, 1385, 1105, 795 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 5.15 (1H, dd, J = 7.5, 4.5 Hz, H-2), 5.08 (1H, s, H-14), 4.73 (1H, dd, J = 9.5, 3.5 Hz, H-6), 4.05 (1H, d, J = 4.5 Hz, H-3), 2.97 (1H, d, J = 7.5 Hz, H-1), 2.90 (1H, dd, J = 14.5, 3.5 Hz, H-7α), 2.62 (1H, dd, J = 14.5, 9.5 Hz, H-7β), 2.62 (1H, m, H-12α), 2.53 (1H, m, H-13), 2.35 (1H, d, J = 14.5 Hz, H-15α), 2.31 (1H, d, J = 14.5 Hz, H-15β), 2.23 (1H, d, J = 7.5 Hz, H-9), 2.18 (1H, m, H-11α), 1.90 (3H, s, H-20), 1.72 (1H, m, H-12β), 1.66 (1H, m, H-11β), 1.63 (3H, s, H-18), 1.57 (3H, s, H-19), 1.53 (3H, s, H-17); ^{13}C NMR (pyridine-d_5, 125 MHz) δ 86.9 (CH, C-3), 83.4 (C, C-5), 80.8 (CH, C-2), 80.1 (CH, C-14), 79.8 (C, C-16), 78.2 (C, C-10), 72.7 (CH, C-6), 60.8 (CH$_2$, C-15), 58.0 (CH, C-1), 56.2 (CH, C-9), 54.9 (CH, C-13), 52.3 (C, C-8), 49.2 (C, C-4), 44.9 (CH$_2$, C-7), 29.5 (CH$_3$, C-20), 27.1 (CH$_2$, C-12), 26.2 (CH$_3$, C-18), 24.0 (CH$_3$, C-17), 21.9 (CH$_2$, C-11), 20.4 (CH$_3$, C-19); ESIMS (positive-ion mode) *m/z* 409 [M + Na]$^+$.

Compound 6: colorless needles; mp 210−212 °C; $[\alpha]^{23}_D$ −23.6 (*c* 0.19, EtOH); UV (MeOH) λ_{max} (log ϵ) 213 (1.54) nm; IR (KBr) ν_{max} 3433, 2968, 1711, 1637, 1452, 1383, 1284, 1039, 906 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 5.71 (1H, s, H-14), 5.45 (1H, s, H-20α), 5.26 (1H, s, H-20β), 4.95 (1H, dd, J = 8.0, 5.0 Hz, H-2), 4.30 (1H, dd, J = 8.5, 3.5 Hz, H-6), 4.18 (1H, d, J = 5.0 Hz, H-3), 4.12 (1H, m, H-3′), 3.23 (1H, d, J = 8.0 Hz, H-1), 2.95 (1H, br s, H-13), 2.73 (1H, m, H-2′), 2.66 (1H, dd, J = 13.0, 8.5 Hz, H-7α), 2.56 (1H, dd, J = 13.0, 3.5 Hz, H-7β), 2.38 (1H, d, J = 13.5 Hz, H-15α), 2.20 (1H, d, J = 7.0 Hz, H-9), 2.14 (1H, d, J = 13.5 Hz, H-15β), 2.03 (1H, m, H-12α), 1.92 (1H, m, H-11α), 1.81 (1H, m, H-12β), 1.66 (3H, s, H-19), 1.64 (1H, m, H-11β), 1.56 (3H, s, H-18), 1.52 (3H, s, H-17), 1.38 (3H, d, J = 7.0 Hz, H-5′), 1.26 (3H, d, J = 6.5 Hz, H-4′); ^{13}C NMR (pyridine-d_5, 125 MHz) δ 174.7 (C, C-1′), 149.0 (C, C-10), 113.5 (CH$_2$, C-20), 88.2 (CH, C-3), 82.9 (CH, C-14), 82.5 (C, C-5), 82.0 (CH, C-2), 79.1 (C, C-16), 70.9 (CH, C-6), 69.1 (CH, C-3′), 62.9 (CH$_2$, C-15), 55.8 (CH, C-13), 52.0 (CH, C-9), 51.8 (CH, C-1), 51.0 (CH, C-2′), 48.0 (C, C-8), 47.9 (C, C-4), 40.4 (CH$_2$, C-7), 27.5 (CH$_3$, C-17), 26.7 (CH$_3$, C-18), 24.1 (CH$_2$, C-12), 23.4 (CH$_2$, C-11), 21.1 (CH$_3$, C-4′), 20.2 (CH$_3$, C-19), 15.2 (CH$_3$, C-5′); ESIMS (positive-ion mode) *m/z* 491 [M + Na]$^+$; HRFABMS *m/z* 491.2650 [M + Na]$^+$ (calcd for $C_{25}H_{40}O_8Na$, 491.2621).

Compound 6a: amorphous powder; mp 225−227 °C; $[\alpha]^{23}_D$ −34.8 (*c* 0.39, EtOH); IR (KBr) ν_{max} 3448, 2925, 1637, 1385, 1078, 804 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 5.67 (1H, s, H-20α), 5.26 (1H, s, H-20β), 4.94 (1H, dd, J = 8.0, 5.0 Hz, H-2), 4.69 (1H, dd, J = 8.5, 3.5 Hz, H-6), 4.66 (1H, s, H-14), 4.13 (1H, d, J = 5.0 Hz, H-3), 3.32 (1H, d, J = 8.0 Hz, H-1), 3.10 (1H, br s, H-13), 2.61 (1H, dd, J = 13.0, 8.5 Hz, H-7α), 2.55 (1H, dd, J = 13.0, 3.5 Hz, H-7β), 2.52 (1H, d, J = 13.5 Hz, H-15α), 2.34 (1H, d, J = 13.5 Hz, H-15β), 2.26 (1H, d, J = 7.0 Hz, H-9), 2.00 (1H, m, H-12α), 1.91 (1H, m, H-11α), 1.84 (1H, m, H-12β), 1.66 (1H, m, H-11β), 1.56 (3H, s, H-19), 1.53 (3H, s, H-18), 1.40 (3H, s, H-17); ^{13}C NMR (pyridine-d_5, 125 MHz) δ 150.9 (C, C-10), 112.4 (CH$_2$, C-20), 88.8 (CH, C-3), 84.2 (C, C-5), 83.9 (CH, C-2), 81.3 (CH, C-14), 80.6 (C, C-16), 69.4 (CH, C-6), 61.5 (CH$_2$, C-15), 54.0 (CH, C-13), 53.7 (CH, C-9), 52.5 (CH, C-1), 50.3 (C, C-8), 48.1 (C, C-4), 42.0 (CH$_2$, C-7), 25.3 (CH$_3$, C-17), 25.1 (CH$_3$, C-18), 24.5 (CH$_2$, C-12), 23.9 (CH$_2$, C-11), 19.2 (CH$_3$, C-19); ESIMS (positive-ion mode) *m/z* 391 [M + Na]$^+$.

Alkaline Hydrolysis of 5 and 6. Compounds **5** (47 mg) and **6** (50 mg) were dissolved in 5% KOH in EtOH−H_2O (1:1), and the solution was refluxed at 80 °C for 3 h, respectively. Then the solution was removed, acidified, saturated with NaCl, and extracted with EtOAc to yield **5a** (26 mg) and **6a** (30 mg). The aqueous phase was acidified and extracted with EtOAc to give nilic acid (**7**) (7 and 9 mg), respectively.

Methylation of 7. Compound **7** (7 mg) was methylated using CH_2N_2 in Et_2O at 0 °C. The reaction mixture was kept in ice for 30 min and then allowed to stand overnight at room temperature. This procedure resulted in **8** (6 mg), which was identified as methyl (2*S*,3*S*)-nilate.

Methyl (2*S*,3*S*)-nilate (8): colorless oil; $[\alpha]^{23}_D$ +34.6 (*c* 5.00, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.88 (1H, m, H-3′), 3.71 (3H, s, OCH_3), 2.45 (1H, m, H-2′), 1.18 (3H, d, J = 6.0 Hz, H-4′), 1.15 (3H, d, J = 7.0 Hz, H-5′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.2 (C, C-1′), 69.3 (CH, C-3′), 51.6 (CH_3, OCH_3), 46.9 (CH, C-2′), 20.6 (CH_3, C-4′), 13.9 (CH_3, C-5′); ESIMS *m/z* 155 $[M + Na]^+$.

Absolute Configuration of Glucose Moieties in 1−4. Compounds **1**−**4** (5 mg) were each refluxed with 4% HCl (5 mL) at 95 °C for 1 h. The reaction mixture was extracted with EtOAc. The H_2O layer was neutralized with 1 N NaOH, then concentrated in vacuo and dried to give a glucose residue. According to the reported procedure,[10] the absolute configuration of glucose was confirmed by comparison of the retention times of glucose derivatives obtained from the above residues with those of standard samples using HPLC (t_R (min): D-glucose (40.7), L-glucose (38.5)). HPLC was performed with an Inertsil sil-100A column (250 × 4.6 mm, 5 μm, Dikma) eluting with *n*-hexane−ethanol (95:5); flow rate 1.2 mL/min; detection at 230 nm.

Antioxidant Activity. The antioxidant activity of compounds was determined by measuring the content of MDA, a compound that was produced during microsomal lipid peroxidation induced by ferrous-cysteine.[17] MDA was detected by using the thiobarbituric acid (TAB) method. Briefly, 1 mg/mL microsomal protein, different concentrations of compound or vehicle, and 0.2 mM cysteine in 0.1 M PBS were incubated for 15 min at 37 °C. After the addition of 0.5 mM ferrous sulfate, the mixture was incubated for 15 min at 37 °C again. An equal volume of 20% TCA was added to terminate the reaction, and the mixture was centrifuged for 10 min at 3000 rpm. The supernatant was reacted with 0.67% TBA for 10 min at 100 °C. After cooling, the MDA was quantified by determining the absorbance at 532 nm and then calculated in the inhibition rate.

Preliminary Vasodilator Assays. According to ref 18 Wistar rats (6−7 weeks old, 200−250 g) were killed by a blow on the heads. The aortas were cut in rings 3−5 mm in length and balanced in PSS solution. An MP100WSW system on a PC-586 was used to record the relaxations and contractions. The presence of functional endothelium was assessed in all preparations by determining the ability of acetylcholine (Ach, 10^{-5} M) to induce more than 80% relaxation of rings precontracted with phenylepherine (Phe, 10^{-7} M). The prepared endothelium-intact aortic rings were balanced in an organ bath in 15 mL of PSS solution for 30 min, during which the solution was replaced every 15 min. Cumulative concentrations of Phe (1×10^{-8} to 3×10^{-7} M) were added to make the aorta precontract. After the contracted value responded to 80% of the peak value (1×10^{-5} M Phe value), cumulative concentrations of Ach were added to the above organ bath. The relaxations caused by Ach were recorded as the control group. Then, the ring was washed with PSS solution and balanced for 30 min, during which the solution was replaced every 5 min. Indomethacin (Indo 10^{-5} M, COX inhibitor) and N^{ω}-L-nitroarginine (L-NA 3×10^{-7} M, NOS inhibitor) were added to the organ bath and thermoregulated at 37 °C for 15 min, after which the same processes as above were performed to obtain the L-NA + Indo group relaxations. The ring was washed again, and then the above processes were carried out after the isolated compound solution in DMSO (10^{-5} M) was added 8 min before the L-NA and Indo to record the L-NA + Indo + compound group relaxations.

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