New Diarylheptanoids and Diarylheptanoid Glucosides from the Rhizomes of *Tacca chantrieri* and Their Cytotoxic Activity

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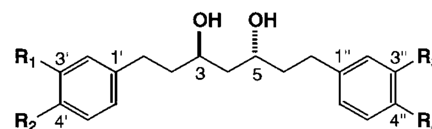
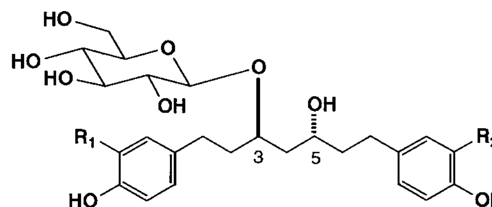
Two new diarylheptanoids (**1**, **2**) and seven new diarylheptanoid glucosides (**3**−**9**) were isolated from the rhizomes of *Tacca chantrieri*. Their structures were determined by spectroscopic analysis, including 2D NMR data, and the results of hydrolytic cleavage. The absolute configurations of the 3,5-dihydroxyheptane moieties of the new diarylheptanoids were determined to be 3*R* and 5*R* by the application of the CD exciton chirality method to the corresponding 3,5-bis-*p*-bromobenzoyl derivatives. The cytotoxic activities of the isolated compounds and some derivatives against HL-60 human promyelocytic leukemia cells, HSC-2 human oral squamous carcinoma cells, and normal human gingival fibroblasts (HGF) are reported.

The family Taccaceae is composed of two genera, *Tacca* and *Schizocapsa*, and about 10 species, with most distributed in tropical regions of Asia, The Pacific Islands, and Australia.[1] *Tacca chantrieri* André, the subject of this report, is a perennial plant that occurs in the southeast area of mainland China, and its rhizomes have been used for the treatment of gastric ulcers, enteritis, and hepatitis in Chinese folk medicine. According to a Chinese herbal dictionary, *T. plantaginea* has also been used for the same purpose as *T. chantrieri*.[2] Although the chemical constituents of *T. plantaginea* were examined extensively and a series of five-cyclic highly oxygenated steroids with a γ-enol-lactone ring, named taccalonolids, were isolated as its characteristic components,[3] there has been only one report of the secondary metabolites of *T. chantrieri*, in which only a few sterols, such as stigmasterol and daucosterol, and a diosgenin glycoside were present.[4] Therefore, our attention was directed to the constituents of *T. chantrieri* rhizomes, on which a detailed phytochemical investigation has been carried out. As a result, two new diarylheptanoids (**1**, **2**) and seven new diarylheptanoid glucosides (**3**−**9**) were isolated. In this paper, we describe the structure elucidation of the new diarylheptanoids (**1**−**9**) and the cytotoxic activities of **1**−**9** and some derivatives against HL-60 human promyelocytic leukemia cells, HSC-2 human oral squamous carcinoma cells, and normal human gingival fibroblasts (HGF).



	R1	R2	R3	R4
1	OH	OH	H	OH
1a	OMe	OMe	H	OMe
2	OH	OH	OH	OH
2a	OMe	OMe	OMe	OMe
5a	OMe	OH	H	OH
7a	OMe	OH	OH	OH
8a	OMe	OH	OMe	OH
9a	H	OH	H	OH
9b	H	OMe	H	OMe



	R1	R2
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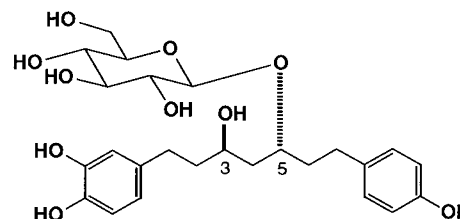
5	OMe	H
6	OH	OH
7	OMe	OH
8	OMe	OMe
9	H	H



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Results and Discussion

The rhizomes of *T. chantrieri* (dry wt. of 7.3 kg) were extracted with hot MeOH twice. After removal of solvent, the extract was passed through a porous-polymer polystyrene resin (Diaion HP-20) column, eluting with MeOH−H_2O gradients, EtOH, and EtOAc. The 50% MeOH eluate portion was subjected to Si gel and octadecylsilanized (ODS) Si gel column chromatography to afford compounds **1**−**9**.

Compound **1** was isolated as a viscous syrup, $[\alpha]_D$ +1.7° (MeOH). The HREIMS of **1** showed an $[M]^+$ peak at *m*/*z* 332.1623, corresponding to the empirical molecular formula of $C_{19}H_{24}O_5$, which was also deduced by analysis of its ^{13}C NMR and DEPT spectral data. The IR spectrum suggested the presence of hydroxyl groups (3347 cm^{-1}) and aromatic

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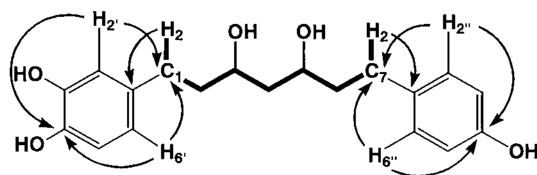

Figure 1. HMBC correlations of **1**.

rings (1611 and 1515 cm^{-1}). The UV spectrum showed an absorption maximum due to substituted aromatic rings (281.4 nm). The ^{1}H NMR spectrum contained signals for 1,3,4-trisubstituted aromatic protons [δ 6.65 (1H, d, J = 8.4 Hz), 6.62 (1H, d, J = 2.0 Hz), and 6.50 (1H, dd, J = 8.4, 2.0 Hz)] and 1,4-disubstituted aromatic protons [δ 6.99 (2H, d, J = 8.5 Hz) and 6.67 (2H, d, J = 8.5 Hz)]. In the ^{13}C NMR spectrum, three oxygenated sp^2 carbons were observed at δ 156.3, 146.1, and 144.2, and treatment of **1** with CH_2N_2 in MeOH gave a trimethyl derivative (**1a**). Thus, the presence of both a 3,4-dihydroxyphenyl group and a 4-hydroxyphenyl group in **1** was evident. In addition, the ^{13}C NMR spectrum showed the presence of a seven sp^3 carbon atom units in **1**, with these carbons being five methylenes and two oxymethines. Signals in the ^{1}H NMR spectrum that could be ascribed to the C_7 unit included spin systems due to two pairs of adjacent methylenes, an isolated methylene, and two hydroxymethine groups. Each structural fragment constituting the C_7 unit was connected by analysis of the ^{1}H$-$^{1}H COSY and 2D TOCSY spectra, allowing the construction of the symmetrical structural unit $-C_{(1)}H_2C_{(2)}H_2-C_{(3)}H(OH)-C_{(4)}H_2-C_{(5)}H(OH)-C_{(6)}$-$H_2C_{(7)}H_2-$. The two aromatic rings were consequently placed at the terminal methylene groups. This was ascertained by long-range correlations from δ_H 6.99 (H-2″ and H-6″) to δ_C 32.1 (C-7), δ_H 6.62 (H-2′) to δ_C 32.4 (C-1), and 6.50 (1H, H-6′) to δ_C 32.4 (C-1) in the HMBC spectrum (Figure 1). The planar structure of **1** was assigned as 3,5-dihydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)-heptane.

The absolute configurations of the 3,5-dihydroxyl groups were determined by the application of the CD exciton chirality method to acyclic 1,3-dibenzoates.[5] The trimethyl derivative (**1a**) was converted to the corresponding 3,5-bis-(*p*-bromobenzoate) (**1b**), and its CD spectrum exhibited positive (237.4 nm, $\Delta\epsilon$ +29.9) and negative (253.3 nm, $\Delta\epsilon$ −20.0) Cotton effects, which was consistent with a negative chirality. Thus, the absolute configurations were determined as 3*R* and 5*R*. The structure of **1** was shown to be (3*R*,5*R*)-3,5-dihydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane.

Compound **2**, $[\alpha]_D$ +4.0° (MeOH), was isolated as a viscous syrup. Its molecular formula was deduced as $C_{19}H_{24}O_6$ by HREIMS, ^{13}C NMR, and DEPT spectral data. The spectral properties of **2** were suggestive of a 3,5-dihydroxydiarylheptanoid whose planar structure was completely symmetrical. Each of the aromatic proton signals at δ 6.65 (d, J = 8.1 Hz), 6.62 (d, J = 2.0 Hz), and 6.50 (dd, J = 8.1, 2.0 Hz) was integrated into two protons, being consistent with the presence of two 3,4-dihydroxyphenyl groups. These data and analysis of the ^{1}H$-$^{1}H COSY, HMQC and HMBC spectra identified the planar structure of **2** as 3,5-dihydroxy-1,7-bis(3,4-dihydroxyphenyl)heptane. Compound **2** was methylated to protect the phenolic hydroxyl groups (**2a**) and then converted to the 3,5-bis(*p*-bromobenzoate) derivative (**2b**) as performed for **1**. The CD spectrum of **2b** exhibited positive and negative Cotton effects, similar to **1b**. The structure of **2** was therefore formulated as (3*R*,5*R*)-3,5-dihydroxy-1,7-bis-(3,4-dihydroxyphenyl)heptane.

Compound **3** was obtained as a pale yellow amorphous solid. Its molecular formula was determined to be $C_{25}H_{34}O_{10}$ by positive-ion FABMS, which showed a pseudomolecular ion peak at *m*/*z* 517 [M + Na]$^+$, and by elemental analysis. The deduced molecular formula was higher by $C_6H_{10}O_5$ than that of **1**. The ^{1}H NMR spectrum showed signals for a 1,3,4-trisubstituted aromatic group [δ 6.65 (1H, d, J = 8.2 Hz), 6.64 (1H, d, J = 1.8 Hz), and 6.52 (1H, dd, J = 8.2, 1.8 Hz)], a 1,4-disubstituted aromatic group [δ 7.00 (2H, d, J = 8.5 Hz) and 6.67 (2H, d, J = 8.5 Hz)], and a 3,5-dihydroxyheptane moiety. In addition, a signal due to an anomeric proton was observed at δ 4.29 (d, J = 7.8 Hz). Enzymatic hydrolysis of **3** with naringinase gave **1** and D-glucose. Identification of D-glucose, including its absolute configuration, was carried out by direct HPLC analysis of the hydrolysate. Thus, **3** was considered to be a monoglucoside of **1**. A long-range correlation was observed from the anomeric proton to the C-3 carbon (δ 76.8) in the HMBC spectrum. Furthermore, a downfield shift of C-3 (8.1 ppm) and upfield shifts of C-2 (2.3 ppm) and C-4 (1.2 ppm) were detected when the ^{13}C NMR spectrum of **3** was compared with that of **1**. The structure of **3** was elucidated as (3*R*,5*R*)-3,5-dihydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)-heptane 3-*O*-β-D-glucopyranoside.

Compound **4** was shown to have a molecular formula of $C_{25}H_{34}O_{10}$ from its positive-ion FABMS, elemental analysis, and ^{13}C NMR spectral data, which was the same as that of **3**. However, slight differences between the two compounds were recognized in the ^{1}H and ^{13}C NMR signals arising from the 3,5-dihydroxyheptane moiety. Enzymatic hydrolysis of **4** with β-D-glucosidase gave **1** and D-glucose. In the HMBC spectrum, a long-range correlation was observed from the anomeric proton signal (δ 4.30) of the D-glucose to C-5 (δ 76.9) of the heptane moiety. Thus, **4** was revealed to be an isomer of **3** with regard to the D-glucose linkage position, and the structure was formulated as (3*R*,5*R*)-3,5-dihydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane 5-*O*-β-D-glucopyranoside.

Compound **5** ($C_{26}H_{34}O_{10}$) was obtained as an amorphous solid. The ^{1}H and ^{13}C NMR spectral features of **5** were quite similar to those of **3**, except for the presence of a signal for one methoxyl group [δ_H 3.82 (3H, s); δ_C 56.4]. The HMBC spectrum showed a correlation between the methoxyl proton and the C-3′ carbon (δ 148.8). Enzymatic hydrolysis of **5** with naringinase gave an aglycon (**5a**) and D-glucose, and consecutive methylation of **5a** with CH_2N_2 afforded **1a**. Thus, **5** was shown to be (3*R*,5*R*)-3,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)heptane 3-*O*-β-D-glucopyranoside.

Compound **6** showed an accurate [M + Na]$^+$ ion peak at *m*/*z* 533.1975 in the HRFABMS, corresponding to a molecular formula $C_{25}H_{34}O_{11}$ (Δ −2.5 mmu of calcd). The ^{1}H and ^{13}C NMR spectra of **6** were similar to those of **3** with the exceptions of the signals for one phenolic part. Enzymatic hydrolysis of **6** with β-D-glucosidase gave **3** and D-glucose. The linkage position of D-glucose was shown to be at C-3 of the aglycon by detecting a correlation from the anomeric proton at δ 4.25 to the C-3 carbon at δ 76.8 in the HMBC spectrum. All of these data were consistent with the structure (3*R*,5*R*)-3,5-dihydroxy-1,7-bis(3,4-dihydroxyphenyl)heptane 3-*O*-β-D-glucopyranoside, which was assigned to compound **6**.

Compound **7** was obtained as an amorphous solid with a molecular formula of $C_{26}H_{36}O_{11}$. Comparison of the ^{1}H and ^{13}C NMR data of **7** with **6** revealed that **7** was closely related to **6** but contained a methoxyl group [δ_H 3.82 (3H, s); δ_C 56.4]. By detecting a correlation from the methoxyl

proton signal to the C-3′ carbon in the HMBC spectrum, the methoxyl group was placed at C-3′. Enzymatic hydrolysis of **7** with β-D-glucosidase followed by methylation with CH_2N_2 afforded **2a**. The structure of **7** was concluded to be (3*R*,5*R*)-3,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(3,4-dihydroxyphenyl)heptane 3-*O*-β-D-glucopyranoside.

Compound **8** ($C_{27}H_{38}O_{11}$) was assigned as a diarylheptanoid glucoside structurally related to **6**. The ^{1}H NMR spectrum showed signals for two methoxyl groups at δ 3.82 (3H × 2, s) and two 1,3,4-trisubstituted aromatic rings at δ 6.79 (1H, d, J = 1.7 Hz), 6.76 (1H, d, J = 1.7 Hz), 6.68 (2H, d, J = 8.0 Hz), 6.64 (1H, dd, J = 8.0, 1.7 Hz), and 6.62 (1H, dd, J = 8.0, 1.7 Hz). An HMBC correlation was observed from the δ 3.82 resonance to C-3′ and C-3″, which appeared at the same position. Enzymatic hydrolysis of **8** with β-D-glucosidase followed by methylation with CH_2N_2 furnished **2a**. Thus, the structure of **8** was established as (3*R*,5*R*)-3,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-heptane 3-*O*-β-D-glucopyranoside.

Compound **9** ($C_{25}H_{34}O_9$) exhibited eight aromatic proton signals at δ 7.02 (2H, d, J = 8.6 Hz), 7.00 (2H, d, J = 8.6 Hz), and 6.67 (4H, d, J = 8.6 Hz) in the ^{1}H NMR spectrum, suggesting the presence of two 4-hydroxyphenyl groups. Enzymatic hydrolysis of **9** with naringinase gave an aglycon (**9a**) and D-glucose. The planar structure of **9a** was identified as 3,5-dihydroxy-1,7-bis(4-hydroxyphenyl)heptane by comparison of its NMR data with literature values.[6] The positive specific rotation of **9a**, $[\alpha]_D$ +4.0° (MeOH), confirmed the 3*R* and 5*R* configurations, since the corresponding 3*S* and 5*S* form was reported to show an opposite $[\alpha]_D$ value.[7] The structure of **9** was established as (3*R*,5*R*)-3,5-dihydroxy-1,7-bis(4-hydroxyphenyl)heptane 3-*O*-β-D-glucopyranoside.

Diarylheptanoids are known to occur only in a limited number species of higher plants belonging to the families Zingiberaceae,[8-11] Betulaceae,[12] and Aceraceae.[13] This is the first isolation of diarylheptanoids from the plant family Taccaceae.

The isolated compounds and some derivatives, in which **9b** prepared by treatment of **9** with CH_2N_2 was included, were evaluated for their cytotoxic activities against HL-60 leukemia cells, HSC-2 cells, and normal HGF, using a modified MTT assay method (Table 2).[14] The diarylheptanoids **1**, **2**, and **7a**, and the diarylheptanoid glucosides **3**, **4**, **6**, and **7**, which each have three or four phenolic hydroxyl groups, showed moderate cytotoxic activity against HL-60 cells with IC_{50} values ranging from 1.8 to 6.4 μg/mL, while those possessing two phenolic hydroxyl groups (**5**, **5a**, **8**, **8a**, **9**, **9a**) did not exhibit apparent cytotoxic activity even at a sample concentration of 10 μg/mL. It is noteworthy that the compounds whose phenolic hydroxyl groups were all masked with methyl groups (**1a**, **2a**, **9b**) were also cytotoxic. These observations suggest that the number of phenolic hydroxyl groups contributes to the resultant cytotoxicity. As for activity against HSC-2 cells, **1a**, **2a**, and **9b** showed considerable cytotoxicity. They showed much higher cytotoxic activities against HSC-2 cells than against normal HGF.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-360 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer, UV spectra on a JASCO V-520 spectrophotometer, and CD spectra on a JASCO J-720 instrument. NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^{1}H NMR, Karlsruhe, Germany) spectrophotometer using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as internal standard. MS were recorded on a Finnigan MAT TSQ-700 (San Jose, CA) mass spectrometer. Elemental analysis was carried out using an Elementar Vario EL elemental analyzer (Hanau, Germany). Diaion HP-20 (Mitsubishi-Kasei, Tokyo, Japan), Si gel (Fuji-Silysia Chemical, Aichi, Japan), and ODS Si gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck, Darmstadt, Germany) and RP_{18} F_{254} S plates (0.25 mm thick, Merck), and spots were visualized by UV irradiation (254 nm) and spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed using a system composed of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), a RI-8010 (Tosoh) or a Shodex OR-2 (Showa-Denko, Tokyo, Japan) detector, and Rheodyne injection port. A Kaseisorb LC-ODS-120-5 column (10 mm i.d. × 250 mm, ODS 5 μm, Tokyo-Kasei, Tokyo, Japan) was employed for preparative HPLC. The following reagents were obtained from the indicated companies: RPMI 1640 medium and DMEM (GIBCO BRL, Gland Island, NY); FBS (JRH Biosciences, Lenexa, KS); penicillin, streptomycin, MTT, and α-MEM (Sigma, St. Louis, MO). All other chemicals used were of biochemical reagent grade.

Plant Material. The rhizomes of *T. chantrieri* were collected in SiMao City, Yunnan Province, People's Republic of China, in October 1996, and identified by one of the authors (Y.S.). A voucher of the plant is on file in our laboratory (voucher no. TC-96-003, Laboratory of Medicinal Plant Science).

Extraction and Isolation. The plant material (dry wt, 7.3 kg) was extracted with hot MeOH twice (each 3 L). The MeOH extract was concentrated under reduced pressure and then passed through a Diaion HP-20 column, eluting with 30% MeOH, 50% MeOH, MeOH, EtOH, and EtOAc. Column chromatography of the 50% MeOH eluate portion on Si gel and elution with a stepwise gradient mixture of $CHCl_3$−MeOH (9:1; 4:1; 3:1; 2:1; 1:1), and finally with MeOH alone, gave four fractions (I−IV). Fraction I was chromatographed on Si gel eluting with $CHCl_3$−MeOH−H_2O (60:10:1) and $CHCl_3$−EtOAc (1:1; 1:2; 1:3; 1:4) and ODS Si gel with CH_3CN−H_2O (2:7) to give **1** (382 mg). Fraction II was subjected to column chromatography on ODS Si gel eluting with MeOH−H_2O (1:2) and Si gel with $CHCl_3$−MeOH−H_2O (60:10:1; 40:10:1) to give **2** (1.46 g), **5** (145 mg), and **8** (52.9 mg). Fraction III was subjected to column chromatography on Si gel eluting with $CHCl_3$−MeOH−H_2O (60:10:1; 50:10:1; 40:10:1; 30:10:1) and ODS Si gel with MeOH−H_2O (1:2; 2:3) to give **7** (91.3 mg) and **9** (190 mg) in pure form and a mixture of **3** and **4**. The mixture was separated by preparative HPLC using MeOH−H_2O (2:3) to furnish **3** (105 mg) and **4** (19.9 mg). Fraction IV was chromatographed on Si gel eluting with $CHCl_3$−MeOH−H_2O (40:10:1; 30:10:1; 20:10:1) and ODS Si gel with MeOH−H_2O (1:3; 2:5) to give **6** (158 mg).

Compound 1: viscous syrup; $[\alpha]^{23}_D$ +1.7° (*c* 0.12, MeOH); UV (MeOH) λ_{max} 281.4 nm (log ϵ 3.63); UV (MeOH + 1 M NaOH) λ_{max} 290.6 nm; IR (film) ν_{max} 3347 (OH), 2941 (CH), 1611 and 1515 (aromatic rings), 1447, 1366, 1282, 1236, 1173, 1153, 1113, 1091, 1059, 957, 826 cm^{-1}; ^{1}H NMR (CD_3OD) δ 6.99 (2H, d, J = 8.5 Hz, H-2″, H-6″), 6.67 (2H, d, J = 8.5 Hz, H-3″, H-5″), 6.65 (1H, d, J = 8.4 Hz, H-5′), 6.62 (1H, d, J = 2.0 Hz, H-2′), 6.50 (1H, dd, J = 8.4, 2.0 Hz, H-6′), 3.80 (2H, m, H-3, H-5), 2.68−2.45 (4H, m, H_2-1, H_2-7), 1.70−1.63 (4H, m, H_2-2, H_2-6), 1.53 (2H, dd, J = 6.6, 5.9 Hz, H_2-4); ^{13}C NMR, see Table 1; HREIMS *m/z* 332.1623 [M]$^+$ (calcd for $C_{19}H_{24}O_5$, 332.1624).

Methylation of 1. Compound **1** (66.4 mg) was dissolved in MeOH (5 mL) and cooled at 0 °C. A large excess of CH_2N_2 in Et_2O was added to the sample solution, and it was allowed to stand at room temperature for 12 h. The reaction mixture was chromatographed on Si gel eluting with hexane−Me_2CO (2:1) to yield **1a** (40.0 mg).

Table 1. ^{13}C NMR Data for Compounds **1**−**9** and Their Derivatives (**1a**, **4a**, **5a**, **7a**−**9a**, and **9b**) in CD_3OD

	carbon	**1**	**1a**	**2**	**2a**	**3**	**4**	**5**	**5a**
	1	32.4	32.6	32.4	32.6	31.7	32.5	32.0	32.6
	2	41.3	41.2	41.3	41.2	39.0	40.9	39.2	41.3
	3	68.7	68.7	68.8	68.6	76.8	67.6	76.9	68.7
	4	45.6	45.6	45.6	45.6	44.4	44.3	44.4	45.6
	5	68.7	68.7	68.8	68.6	67.4	76.9	67.5	68.7
	6	41.4	41.2	41.3	41.2	41.0	39.1	41.0	41.4
	7	32.1	32.1	32.4	32.6	32.3	31.5	32.3	32.1
	1′	135.3	136.8	135.3	136.7	135.3	135.5	135.3	135.3
	2′	116.6	113.7	116.3	116.3	116.6	116.7	113.2	113.2
	3′	146.1	150.4	146.1	146.1	146.1	146.0	148.8	148.8
	4′	144.2	148.6	144.1	144.1	144.1	144.1	145.4	145.5
	5′	116.3	113.3	116.6	116.6	116.3	116.2	116.1	116.1
	6′	120.6	121.7	120.7	121.7	120.7	120.8	121.8	121.8
	1″	134.5	135.7	135.3	136.7	134.6	134.5	134.6	134.5
	2″	130.3	130.3	116.3	116.3	116.0	130.3	130.4	130.3
	3″	116.1	114.8	146.1	146.1	116.1	116.1	116.0	116.1
	4″	156.3	159.3	144.1	144.1	156.2	156.3	156.2	156.3
	5″	116.1	114.8	116.6	116.6	116.0	116.1	116.0	116.1
	6″	130.3	130.3	120.7	121.7	130.4	130.3	130.4	130.3
Glc	1					103.7	103.7	103.8	
	2					75.2	75.2	75.3	
	3					78.1	78.2	78.2	
	4					72.4	72.4	72.4	
	5					77.6	77.7	77.7	
	6					63.3	63.3	63.3	
	3′-OMe		56.4		56.4			56.4	56.4
	4′-OMe		56.6		56.6				
	3″-OMe				56.4				
	4″-OMe		55.6		56.6				

	carbon	**6**	**7**	**7a**	**8**	**8a**	**9**	**9a**	**9b**
	1	31.7	32.0	32.6	32.0	32.6	31.5	32.2	32.1
	2	39.0	39.1	41.4	39.1	41.3	39.2	41.4	41.3
	3	76.8	76.9	68.7	76.9	68.7	76.9	68.7	68.7
	4	44.3	44.3	45.6	44.4	45.6	44.4	45.7	45.6
	5	67.6	67.6	68.8	67.5	68.7	67.5	68.7	68.7
	6	40.9	40.9	41.4	41.0	41.3	41.0	41.4	41.3
	7	32.5	32.5	32.4	32.8	32.6	32.3	32.2	32.1
	1′	135.3	135.5	135.3	135.3	135.3	134.5	134.5	135.7
	2′	116.6	113.3	113.2	113.2	113.2	130.3	130.3	130.3
	3′	146.0	148.8	148.8	148.8	148.8	116.0	116.1	114.8
	4′	144.1	145.4	145.5	145.4	145.5	156.3	156.3	159.3
	5′	116.2	116.1	116.1	116.0	116.1	116.1	116.1	114.8
	6′	120.7	121.8	121.8	121.8	121.8	130.3	130.3	130.3
	1″	135.5	135.3	135.3	135.4	135.3	134.4	134.5	135.7
	2″	116.7	116.7	116.6	113.3	113.3	130.4	130.3	130.3
	3″	146.1	146.1	146.1	148.8	148.8	116.1	116.1	114.8
	4″	144.2	144.1	144.2	145.4	145.5	156.3	156.3	159.3
	5″	116.3	116.2	116.3	116.1	116.1	116.1	116.1	114.8
	6″	120.8	120.8	120.6	121.9	121.8	130.4	130.3	130.3
Glc	1	103.7	103.8		103.8		103.8		
	2	75.2	75.3		75.3		75.3		
	3	78.2	78.2		78.2		78.2		
	4	72.4	72.4		72.4		72.4		
	5	77.7	77.7		77.7		77.7		
	6	63.3	63.3		63.3		63.3		
	3′-OMe		56.4	56.4	56.4	56.4			
	4′-OMe								55.6
	3″-OMe				56.4	56.4			
	4″-OMe								55.6

Compound 1a: amorphous solid; $[\alpha]^{25}_{D}$ +2.7° (*c* 0.07, MeOH); UV (EtOH) λ_{max} 278.6 nm (log ϵ 3.59); IR (film) ν_{max} 3407 (OH), 2936 (CH), 1611, 1590 and 1514 (aromatic rings), 1454, 1246, 1178, 1155, 1064, 1030, 848, 812, 764 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.08 (2H, d, J = 8.6 Hz, H-2″, H-6″), 6.80 (2H, d, J = 8.6 Hz, H-3″, H-5″), 6.83 (1H, d, J = 8.2 Hz, H-5′), 6.80 (1H, d, J = 2.0 Hz, H-2′), 6.73 (1H, dd, J = 8.2, 2.0 Hz, H-6′), 3.81 (2H, m, H-3, H-5), 3.80 (3H, s, MeO-3′), 3.78 (3H, s, MeO-4′), 3.74 (3H, s, MeO-4″), 2.74−2.53 (4H, m, H_2-1, H_2-7), 1.73−1.65 (4H, m, H_2-2, H_2-6), 1.54 (2H, dd, J = 6.6, 5.7 Hz, H_2-4); ^{13}C NMR, see Table 1; HREIMS *m/z* 374.2104 [M]$^+$ (calcd for $C_{22}H_{30}O_5$, 374.2093).

Bis-*p*-bromobenzoate (1b) of 1a. To a solution of **1a** (15.3 mg) in pyridine (1 mL) containing DMAP (19 mg) as catalyst was added dropwise *p*-bromobenzoyl chloride (40 mg) dissolved in pyridine (3 mL), and the mixture was stirred at 70 °C for 9 h. The reaction mixture was diluted with H_2O (20 mL) and extracted with $CHCl_3$ (10 mL × 3). After concentration of the $CHCl_3$-soluble phase, it was chromatographed on Si gel eluting with hexane−Me_2CO (4:1) to yield **1b** (8.0 mg).

Compound 1b: amorphous solid; $[\alpha]^{24}_{D}$ −28.8° (*c* 0.16, EtOH); UV (EtOH) λ_{max} 243.8 nm (log ϵ 4.64); CD (EtOH) λ_{max} ($\Delta\epsilon$) 237.4 (+29.9), 253.3 (−20.0); IR (film) ν_{max} 2930, 2854 and 2834 (CH), 1716 (C=O), 1589, 1513 and 1483 (aromatic rings), 1464, 1454, 1418, 1397, 1360, 1270, 1173, 1156, 1141, 1116, 1102, 1068, 1032, 1011, 848, 809, 755, 682 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.65 (2H, d, J = 8.5 Hz), 7.64 (2H, d, J = 8.5 Hz), 7.50 (2H × 2, d, J = 8.5 Hz), 7.04 (2H, d, J = 8.6 Hz, H-2″, H-6″),

Table 2. Cytotoxic Activities of Compounds **1**–**9** and Their Derivatives (**1a**, **4a**, **5a**, **7a**–**9a**, and **9a**) and Etoposide against HL-60, HSC-2, and HGF[a]

compound	IC$_{50}$ (μg/mL)		
	HL-60	HSC-2	HGF
1	2.1	54.0	162
1a	5.5	3.9	176
2	1.8	54.0	>250
2a	4.9	6.6	174
3	6.2	158	220
4	5.5	155	>250
5	>10	160	>250
5a	>10	*b*	*b*
6	3.0	92.0	189
7	4.5	209	>250
7a	4.1	*b*	*b*
8	>10	198	>250
8a	>10	*b*	*b*
9	>10	157	213
9a	>10	231	177
9b	6.4	23.0	173
etoposide	0.2	24.0	>200

[a] Key: HL-60 (human promyelocytic leukemia cells); HSC-2 (human oral squamous carcinoma cells); and HGF (normal human gingival fibroblasts). [b] Not determined.

6.76 (1H, d, J = 1.8 Hz, H-2′), 6.74 (1H, d, J = 8.2 Hz, H-5′), 6.73 (2H, d, J = 8.6 Hz, H-3″, H-5″), 6.70 (1H, dd, J = 8.2, 1.8 Hz, H-6′), 5.19 (2H, m, H-3, H-5), 3.73 (3H × 2, s, OMe × 2), 3.70 (3H, s, OMe), 2.65–2.56 (4H, m, H$_2$-1, H$_2$-7), 2.18–1.88 (6H, m, H$_2$-2, H$_2$-4, H$_2$-6); EIMS m/z 740 [M]$^+$.

Compound 2: viscous syrup; $[\alpha]^{23}_{D}$ +4.0° (c 0.10, MeOH); UV (MeOH) λ_{max} 283.4 nm (log ϵ 3.79); UV (MeOH + 1 M NaOH) λ_{max} 292.4 nm; IR (film) ν_{max} 3444 (OH), 2941 (CH), 1605 and 1519 (aromatic rings), 1455, 1372, 1283, 1114, 1060, 957, 866, 815, 788 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 6.65 (2H, d, J = 8.1 Hz, H-5′, H-5″), 6.62 (1H, d, J = 2.0 Hz, H-2′, H-2″), 6.50 (1H, dd, J = 8.1, 2.0 Hz, H-6′, H-6″), 3.83–3.78 (2H, m, H-3, H-5), 2.63–2.44 (4H, m, H$_2$-1, H$_2$-7), 1.69–1.63 (4H, m, H$_2$-2, H$_2$-6), 1.52 (2H, t-like, J = 5.8 Hz, H$_2$-4); ^{13}C NMR, see Table 1; HREIMS m/z 348.1570 [M]$^+$ (calcd for $C_{19}H_{24}O_6$, 348.1573).

Methylation of 2. Compound **2** (56.7 mg) was methylated by the same procedure as described for **1** to yield **2a** (32.8 mg).

Compound 2a: amorphous solid; $[\alpha]^{26}_{D}$ +18.0° (c 0.10, CHCl$_3$); UV (EtOH) λ_{max} 277 nm (log ϵ 3.77); IR (film) ν_{max} 3289 (OH), 2936 and 2840 (CH), 1604, 1588 and 1518 (aromatic rings), 1465, 1451, 1419, 1335, 1276, 1260, 1238, 1194, 1155, 1140, 1108, 1066, 1025, 938, 904, 853, 835, 817, 803, 787, 765 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 6.83 (2H, d, J = 8.2 Hz, H-5′, H-5″), 6.80 (2H, d, J = 1.9 Hz, H-2′, H-2″), 6.72 (2H, dd, J = 8.2, 1.9 Hz, H-6′, H-6″), 3.80 (3H × 2, s, OMe × 2), 3.77 (3H × 2, s, OMe × 2), 3.83–3.77 (2H, m, H-3, H-5), 2.72–2.54 (4H, m, H$_2$-1, H$_2$-7), 1.74–1.67 (4H, m, H$_2$-2, H$_2$-6), 1.55 (2H, t-like, J = 5.7 Hz, H$_2$-4); ^{13}C NMR, see Table 1; EIMS m/z 404 [M]$^+$.

Bis-*p*-bromobenzoate (2b) of 2a. Compound **2a** (10.0 mg) was treated with *p*-bromobenzoyl chloride (40 mg) in pyridine to yield **2b** (4.8 mg).

Compound 2b: amorphous solid; $[\alpha]^{23}_{D}$ −37.5° (c 0.03, EtOH); UV (EtOH) λ_{max} 242.2 nm (log ϵ 4.45); CD (EtOH) λ_{max} ($\Delta\epsilon$) 236.9 (+15.0), 252.8 (−16.7); IR (film) ν_{max} 2926 and 2854 (CH), 1714 (C=O), 1590, 1516 and 1483 (aromatic rings), 1464, 1454, 1418, 1397, 1360, 1264, 1237, 1173, 1156, 1141, 1117, 1103, 1068, 1030, 1011, 848, 805, 756, 682 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 7.63 (2H × 2, d, J = 8.6 Hz), 7.50 (2H × 2, d, J = 8.6 Hz), 6.76 (2H, d, J = 1.8 Hz, H-2′, H-2″), 6.73 (2H, d, J = 8.2 Hz, H-5′, H-5″), 6.67 (2H, dd, J = 8.2, 1.8 Hz, H-6′, H-6″), 3.73 (3H × 4, s, OMe × 4), 5.20 (2H, m, H-3, H-5), 2.67–2.58 (4H, m, H$_2$-1, H$_2$-7), 2.15–1.90 (6H, m, H$_2$-2, H$_2$-4, H$_2$-6); EIMS m/z 768 [M]$^+$.

Compound 3: amorphous solid; $[\alpha]^{25}_{D}$ −12.0° (c 0.10, MeOH); UV (MeOH) λ_{max} 281.6 nm (log ϵ 3.55); UV (MeOH + 1 M NaOH) λ_{max} 294.4 nm; IR (film) ν_{max} 3357 (OH), 2924 (CH), 1612 and 1516 (aromatic rings), 1444, 1365, 1282, 1232, 1172, 1078, 1055, 1033, 825 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 7.00 (2H, d, J = 8.5 Hz, H-2″, H-6″), 6.67 (2H, d, J = 8.5 Hz, H-3″, H-5″), 6.65 (1H, d, J = 8.2 Hz, H-5′), 6.64 (1H, d, J = 1.8 Hz, H-2′), 6.52 (1H, dd, J = 8.2, 1.8 Hz, H-6′), 4.29 (1H, d, J = 7.8 Hz, Glc-1), 3.93 (1H, m, H-3), 3.92 (1H, m, H-5), 3.89 (1H, dd, J = 11.5, 2.2 Hz, Glc-6a), 3.56 (1H, dd, J = 11.5, 7.6 Hz, Glc-6b), 3.35 (1H, dd, J = 9.3, 9.0 Hz, Glc-3), 3.25 (1H, ddd, J = 9.3, 7.6, 2.2 Hz, Glc-5), 3.17 (1H, dd, J = 9.3, 9.3 Hz, Glc-4), 3.16 (1H, dd, J = 9.0, 7.8 Hz, Glc-2), 2.69–2.52 (4H, m, H$_2$-1, H$_2$-7), 1.88–1.68 (2H, m, H$_2$-2), 1.68–1.55 (3H, m, H-4a, H$_2$-6), 1.49 (1H, ddd, J = 14.1, 10.3, 2.3 Hz, H-4b); ^{13}C NMR, see Table 1; FABMS (positive mode) m/z 517 [M + Na]$^+$; *anal.* C 57.25%, H 7.05% (calcd for $C_{25}H_{34}O_{10}\cdot 3/2H_2O$, C 57.57%, H 7.15%).

Enzymatic Hydrolysis of 3. Compound **3** (10.5 mg) was treated with naringinase (Sigma, 23.5 mg) in HOAc/KOAc buffer (pH 4.3, 10 mL) at room temperature for 12 h. The reaction mixture was chromatographed on Si gel eluting with hexane–Me$_2$CO (1:1) followed by MeOH to yield **1** (3.4 mg) and a sugar fraction (2.0 mg). The sugar fraction was passed through a Sep-Pak C$_{18}$ cartridge (Waters, Milford, MA) and a Toyopak IC-SP M cartridge (Tosoh), which was then analyzed by HPLC under the following conditions: column, Capcell Pak NH2 UG80 (4.6 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan); solvent, MeCN–H$_2$O (17:3); flow rate, 0.8 mL/min; detection, RI and OR. Identification of D-glucose present in the sugar fraction was carried out by comparison of its retention time and optical rotation with those of an authentic sample; t_R (min) 17.10 (D-glucose, positive optical rotation).

Compound 4: amorphous solid; $[\alpha]^{25}_{D}$ −12.0° (c 0.10, MeOH); UV (MeOH) λ_{max} 280.6 nm (log ϵ 3.21); UV (MeOH + 1 M NaOH) λ_{max} 294.6 nm; IR (film) ν_{max} 3347 (OH), 2924 (CH), 1612 and 1515 (aromatic rings), 1444, 1363, 1281, 1231, 1171, 1077, 1055, 1034, 825 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 7.02 (2H, d, J = 8.4 Hz, H-2′, H-6′), 6.67 (2H, d, J = 8.4 Hz, H-3′, H-5′), 6.65 (1H, d, J = 8.1 Hz, H-5″), 6.63 (1H, d, J = 2.0 Hz, H-2″), 6.50 (1H, dd, J = 8.1, 2.0 Hz, H-6″), 4.29 (1H, d, J = 7.8 Hz, Glc-1), 3.94 (1H, m, H-3), 3.93 (1H, m, H-5), 3.89 (1H, dd, J = 11.6, 2.2 Hz, Glc-6a), 3.58 (1H, dd, J = 11.6, 7.4 Hz, Glc-6b), 3.35 (1H, dd, J = 9.1, 8.9 Hz, Glc-3), 3.25 (1H, ddd, J = 9.5, 7.4, 2.2 Hz, Glc-5), 3.19 (1H, dd, J = 9.5, 8.9 Hz, Glc-4), 3.17 (1H, dd, J = 9.1, 7.8 Hz, Glc-2), 2.72–2.46 (4H, m, H$_2$-1, H$_2$-7), 1.88–1.70 (2H, m, H$_2$-2), 1.70–1.56 (3H, m, H-4a, H$_2$-6), 1.49 (1H, ddd, J = 14.2, 10.2, 2.5 Hz, H-4b); ^{13}C NMR, see Table 1; FABMS (positive mode) m/z 517 [M + Na]$^+$; *anal.* C 57.69%, H 7.24% (calcd for $C_{25}H_{34}O_{10}\cdot 3/2H_2O$, C 57.57%, H 7.15%).

Enzymatic Hydrolysis of 4. Compound **4** (4.7 mg) was treated with naringinase (24.2 mg) in HOAc/KOAc buffer (pH 4.3, 10 mL) at room temperature for 16 h to yield **1** (1.0 mg) and a sugar fraction (1.0 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **3** showed the presence of D-glucose; t_R (min) 17.24 (D-glucose, positive optical rotation).

Compound 5: amorphous solid; $[\alpha]^{25}_{D}$ −2.0° (c 0.10, MeOH); UV (MeOH) λ_{max} 281.4 nm (log ϵ 3.54); UV (MeOH + 1 M NaOH) λ_{max} 289.8 nm; IR (film) ν_{max} 3374 (OH), 2925 (CH), 1613, 1600 and 1516 (aromatic rings), 1454, 1433, 1366, 1261, 1233, 1154, 1080, 1033, 821 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 7.00 (2H, d, J = 8.6 Hz, H-2″, H-6″), 6.79 (1H, d, J = 1.7 Hz, H-2′), 6.68 (1H, d, J = 8.0 Hz, H-6′), 6.67 (2H, d, J = 8.5 Hz, H-3″, H-5″), 6.64 (1H, dd, J = 8.1, 1.7 Hz, H-5′), 4.30 (1H, d, J = 7.8 Hz, Glc-1), 3.92 (1H, m, H-3), 3.91 (1H, m, H-5), 3.89 (1H, dd, J = 11.5, 2.3 Hz, Glc-6a), 3.82 (3H, s, OMe), 3.55 (1H, dd, J = 11.5, 7.5 Hz, Glc-6b), 3.35 (1H, dd, J = 9.3, 9.0 Hz, Glc-3), 3.24 (1H, ddd, J = 9.1, 7.5, 2.3 Hz, Glc-5), 3.17 (1H, dd, J = 9.1, 9.0 Hz, Glc-4), 3.17 (1H, dd, J = 9.3, 7.8 Hz, Glc-2), 2.73–2.51 (4H, m, H$_2$-1, H$_2$-7), 1.91–1.72 (2H, m, H$_2$-2), 1.71–1.57 (3H, m, H-4a, H$_2$-6), 1.50 (1H, ddd, J = 14.3, 10.3, 2.6 Hz, H-4b); ^{13}C NMR, see Table 1; FABMS (positive mode) m/z 531 [M + Na]$^+$; *anal.* C 59.08%, H 7.44% (calcd for $C_{26}H_{34}O_{10}\cdot H_2O$, C 59.30%, H 7.27%).

Enzymatic Hydrolysis of 5. Compound **5** (19 mg) was treated with naringinase (34.3 mg) in HOAc/KOAc buffer (pH 4.3, 10 mL) at room temperature for 8 h to yield **5a** (6.7 mg) and a sugar fraction (4.2 mg). HPLC analysis of the sugar

fraction under the same conditions as in the case of **3** showed the presence of D-glucose; t_R (min) 16.92 (D-glucose, positive optical rotation).

Compound 5a: amorphous solid; $[\alpha]^{25}_D$ +2.2° (*c* 0.09, MeOH); UV (MeOH) λ_{max} 279.8 nm (log ϵ 3.49); UV (MeOH + 1 M NaOH) λ_{max} 288.8 nm; IR (film) ν_{max} 3347 (OH), 2938 (CH), 1613, 1601 and 1516 (aromatic rings), 1454, 1433, 1368, 1262, 1233, 1152, 1059, 1035, 824, 797 cm^{-1}; ^{1}H NMR (CD_3OD) δ 6.99 (2H, d, *J* = 8.4 Hz, H-2″, H-6″), 6.76 (1H, d, *J* = 1.8 Hz, H-2′), 6.68 (1H, d, *J* = 8.0 Hz, H-5′), 6.67 (2H, d, *J* = 8.4 Hz, H-3″, H-5″), 6.61 (1H, dd, *J* = 8.0, 1.7 Hz, H-6′), 3.81 (2H, m, H-3, H-5), 3.81 (3H, s, OMe), 2.69−2.50 (4H, m, H_2-1, H_2-7), 1.72−1.64 (4H, m, H_2-2, H_2-6), 1.54 (2H, dd, *J* = 6.5, 5.7 Hz, H_2-4); ^{13}C NMR, see Table 1; HREIMS *m/z* 346.1764 [M]$^+$ (calcd for $C_{20}H_{26}O_5$, 346.1780).

Methylation of 5a. Compound **5a** (4.0 mg) was methylated by the same procedure as described for **1** to yield **1a** (2.5 mg).

Compound 6: amorphous solid; $[\alpha]^{23}_D$ −8.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 283.6 nm (log ϵ 3.70); UV (MeOH + 1 M NaOH) λ_{max} 293.2 nm; IR (film) ν_{max} 3347 (OH), 2942 (CH), 1605 and 1520 (aromatic rings), 1445, 1369, 1284, 1113, 1077, 1024, 813 cm^{-1}; ^{1}H NMR (CD_3OD) δ 6.60 (2H, d, *J* = 7.9 Hz, H-5′, H-5″), 6.60 (1H, d, *J* = 1.8 Hz, H-2′), 6.58 (1H, d, *J* = 1.9 Hz, H-2″), 6.48 (1H, dd, *J* = 7.9, 1.8 Hz, H-6′), 6.46 (1H, dd, *J* = 7.9, 1.9 Hz, H-6″), 4.25 (1H, d, *J* = 7.8 Hz, Glc-1), 3.89 (1H, m, H-3), 3.87 (1H, m, H-5), 3.84 (1H, dd, *J* = 11.6, 2.3 Hz, Glc-6a), 3.53 (1H, dd, *J* = 11.6, 7.4 Hz, Glc-6b), 3.31 (1H, dd, *J* = 9.0, 8.9 Hz, Glc-3), 3.20 (1H, ddd, *J* = 9.3, 7.4, 2.3 Hz, Glc-5), 3.14 (1H, dd, *J* = 9.3, 8.9 Hz, Glc-4), 3.12 (1H, dd, *J* = 9.0, 7.8 Hz, Glc-2), 2.60−2.42 (4H, m, H_2-1, H_2-7), 1.83−1.65 (2H, m, H_2-2), 1.64−1.52 (3H, m, H-4a, H_2-6), 1.47 (1H, ddd, *J* = 14.2, 10.2, 2.4 Hz, H-4b); ^{13}C NMR, see Table 1; HR-FABMS (positive mode) *m/z* 533.1975 [M + Na]$^+$ (calcd for $C_{25}H_{34}O_{11}$·Na, 533.2000).

Enzymatic Hydrolysis of 6. Compound **6** (27.5 mg) was treated with naringinase (50 mg) in HOAc/KOAc buffer (pH 4.3, 10 mL) at room temperature for 12 h to yield **2** (18.0 mg) and a sugar fraction (6.5 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **3** showed the presence of D-glucose; t_R (min) 16.97 (D-glucose, positive optical rotation).

Compound 7: amorphous solid; $[\alpha]^{25}_D$ −16.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 282.2 nm (log ϵ 3.45); UV (MeOH + 1 M NaOH) λ_{max} 289.2 nm; IR (film) ν_{max} 3388 (OH), 2924 (CH), 1604 and 1516 (aromatic rings), 1451, 1432, 1364, 1271, 1232, 1078, 1033, 815 cm^{-1}; ^{1}H NMR (CD_3OD) δ 6.79 (1H, d, *J* = 1.7 Hz, H-2′), 6.68 (1H, d, *J* = 8.1 Hz, H-5′), 6.65 (1H, d, *J* = 7.9 Hz, H-5″), 6.64 (1H, dd, *J* = 8.1, 1.7 Hz, H-6′), 6.63 (1H, d, *J* = 1.8 Hz, H-2″), 6.50 (1H, dd, *J* = 7.9, 1.8 Hz, H-6″), 4.30 (1H, d, *J* = 7.8 Hz, Glc-1), 3.94 (1H, m, H-3), 3.93 (1H, m, H-5), 3.88 (1H, dd, *J* = 11.6, 2.3 Hz, Glc-6a), 3.82 (3H, s, OMe), 3.58 (1H, dd, *J* = 11.6, 7.3 Hz, Glc-6b), 3.34 (1H, dd, *J* = 9.0, 8.8 Hz, Glc-3), 3.24 (1H, ddd, *J* = 9.6, 7.3, 2.3 Hz, Glc-5), 3.18 (1H, dd, *J* = 9.6, 9.0 Hz, Glc-4), 3.17 (1H, dd, *J* = 8.8, 7.8 Hz, Glc-2), 2.73−2.46 (4H, m, H_2-1, H_2-7), 1.90−1.73 (2H, m, H_2-2), 1.71−1.58 (3H, m, H-4a, H_2-6), 1.50 (1H, ddd, *J* = 14.2, 10.4, 2.5 Hz, H-4b); ^{13}C NMR, see Table 1; HRFABMS (positive mode) *m/z* 547.2139 [M + Na]$^+$ (calcd for $C_{26}H_{36}O_{11}$·Na, 547.2155).

Enzymatic Hydrolysis of 7. Compound **7** (19.8 mg) was treated with β-D-glucosidase (Sigma, 94 mg) in HOAc/NaOAc buffer (pH 5.0, 10 mL) at room temperature for 18 h to yield **7a** (6.0 mg) and D-glucose.

Compound 7a: amorphous solid; $[\alpha]^{24}_D$ +3.2° (*c* 0.06, MeOH); UV (MeOH) λ_{max} 282.6 nm (log ϵ 3.71); UV (MeOH + 1 M NaOH) λ_{max} 293.2 nm; IR (film) ν_{max} 3376 (OH), 2936 (CH), 1604 and 1516 (aromatic rings), 1454, 1367, 1261, 1151, 1033, 797 cm^{-1}; ^{1}H NMR (CD_3OD) δ 6.76 (1H, d, *J* = 1.8 Hz, H-2′), 6.68 (1H, d, *J* = 8.1 Hz, H-5′), 6.64 (1H, d, *J* = 8.1 Hz, H-5″), 6.62 (1H, d, *J* = 2.0 Hz, H-2″), 6.61 (1H, dd, *J* = 8.1, 1.8 Hz, H-6′), 6.50 (1H, dd, *J* = 8.1, 2.0 Hz, H-6″), 3.81 (2H, m, H-3, H-5), 3.82 (3H, s, OMe), 2.70−2.45 (4H, m, H_2-1, H_2-7), 1.72−1.61 (4H, m, H_2-2, H_2-6), 1.53 (2H, t-like, *J* = 5.6 Hz, H_2-4); ^{13}C NMR, see Table 1; HREIMS *m/z* 362.1724 [M]$^+$ (calcd for $C_{20}H_{26}O_6$, 362.1729).

Methylation of 7a. Compound **7a** (4.0 mg) was methylated by the same procedure as described for **1** to yield **2a** (2.8 mg).

Compound 8: amorphous solid; $[\alpha]^{23}_D$ −18.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 282.0 nm (log ϵ 3.70); UV (MeOH + 1 M NaOH) λ_{max} 290.0 nm; IR (film) ν_{max} 3388 (OH), 2936 (CH), 1602 and 1516 (aromatic rings), 1455, 1429, 1365, 1270, 1234, 1154, 1123, 1079, 1034, 818 cm^{-1}; ^{1}H NMR (CD_3OD) δ 6.79 (1H, d, *J* = 1.7 Hz, H-2′), 6.76 (1H, d, *J* = 1.7 Hz, H-2″), 6.68 (2H, d, *J* = 8.0 Hz, H-5′, H-5″), 6.64 (1H, dd, *J* = 8.0, 1.7 Hz, H-6′), 6.62 (1H, dd, *J* = 8.0, 1.7 Hz, H-6″), 4.30 (1H, d, *J* = 7.8 Hz, Glc-1), 3.94 (1H, m, H-3), 3.94 (1H, m, H-5), 3.88 (1H, dd, *J* = 11.5, 2.3 Hz, Glc-6a), 3.82 (3H × 2, s, OMe × 2), 3.54 (1H, dd, *J* = 11.5, 7.6 Hz, Glc-6b), 3.35 (1H, dd, *J* = 9.2, 9.0 Hz, Glc-3), 3.24 (1H, ddd, *J* = 9.5, 7.6, 2.3 Hz, Glc-5), 3.17 (1H, dd, *J* = 9.2, 7.8 Hz, Glc-2), 3.16 (1H, dd, *J* = 9.5, 9.0 Hz, Glc-4), 2.74−2.52 (4H, m, H_2-1, H_2-7), 1.90−1.73 (2H, m, H_2-2), 1.73−1.58 (3H, m, H-4a, H_2-6), 1.51 (1H, ddd, *J* = 14.2, 10.2, 2.4 Hz, H-4b); ^{13}C NMR, see Table 1; FABMS (positive mode) *m/z* 561 [M + Na]$^+$; *anal.* C 57.87%, H 7.40% (calcd for $C_{27}H_{38}O_{11}$·H_2O, C 58.26%, H 7.24%).

Enzymatic Hydrolysis of 8. Compound **8** (11.8 mg) was treated with β-D-glucosidase (39 mg) in HOAc/NaOAc buffer (pH 5.0, 10 mL) at room temperature for 18 h to yield **8a** (5.5 mg) and D-glucose.

Compound 8a: amorphous solid; $[\alpha]^{25}_D$ +4.7° (*c* 0.09, MeOH); UV (MeOH) λ_{max} 280.1 nm (log ϵ 3.49); UV (MeOH + 1 M NaOH) λ_{max} 288.6 nm; IR (film) ν_{max} 3333 (OH), 2932 (CH), 1600 and 1517 (aromatic rings), 1456, 1427, 1264, 1152, 1035, 796 cm^{-1}; ^{1}H NMR (CD_3OD) δ 6.76 (2H, d, *J* = 1.7 Hz, H-2′, H-2″), 6.68 (2H, d, *J* = 7.9 Hz, H-5′, H-5″), 6.61 (2H, dd, *J* = 7.9, 1.7 Hz, H-6′, H-6″), 3.86−3.79 (2H, m, H-3, H-5), 3.81 (3H × 2, s, OMe × 2), 2.70−2.52 (4H, m, H_2-1, H_2-7), 1.72−1.67 (4H, m, H_2-2, H_2-6), 1.54 (2H, t-like, *J* = 5.6 Hz, H_2-4); ^{13}C NMR, see Table 1; EIMS *m/z* 376 [M]$^+$.

Methylation of 8a. Compound **8a** (4.0 mg) was methylated by the same procedure as described for **1** to yield **2a** (2.1 mg).

Compound 9: amorphous solid; $[\alpha]^{23}_D$ −8.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 279.4 nm (log ϵ 3.44); UV (MeOH + 1 M NaOH) λ_{max} 281.6 nm; IR (film) ν_{max} 3357 (OH), 2926 (CH), 1613 and 1515 (aromatic rings), 1454, 1371, 1237, 1172, 1078, 1031, 825 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.02 (2H, d, *J* = 8.6 Hz, H-2′, H-6′), 7.00 (2H, d, *J* = 8.6 Hz, H-2″, H-6″), 6.67 (4H, d, *J* = 8.6 Hz, H-3′, H-5′, H-3″, H-5″), 4.29 (1H, d, *J* = 7.8 Hz, Glc-1), 3.94 (1H, m, H-3), 3.92 (1H, m, H-5), 3.89 (1H, dd, *J* = 11.6, 2.4 Hz, Glc-6a), 3.58 (1H, dd, *J* = 11.6, 7.5 Hz, Glc-6b), 3.35 (1H, dd, *J* = 9.1, 9.0 Hz, Glc-3), 3.25 (1H, ddd, *J* = 9.3, 7.5, 2.3 Hz, Glc-5), 3.17 (1H, dd, *J* = 9.3, 9.0 Hz, Glc-4), 3.16 (1H, dd, *J* = 9.1, 7.8 Hz, Glc-2), 2.71−2.52 (4H, m, H_2-1, H_2-7), 1.87−1.71 (2H, m, H_2-2), 1.70−1.57 (3H, m, H-4a, H_2-6), 1.50 (1H, ddd, *J* = 14.2, 10.2, 2.5 Hz, H-4b); ^{13}C NMR, see Table 1; FABMS (positive mode) *m/z* 501 [M + Na]$^+$; *anal.* C 59.44%, H 7.38% (calcd for $C_{25}H_{34}O_9$·3/2H_2O, C 59.39%, H 7.38%).

Enzymatic Hydrolysis of 9. Compound **9** (94.0 mg) was treated with naringinase (140 mg) in HOAc/KOAc buffer (pH 4.3, 15 mL) at room temperature for 18 h to yield **9a** (50.0 mg) and a sugar fraction (15 mg). HPLC analysis of the sugar fraction under the same conditions as in the case of **3** showed the presence of D-glucose; t_R (min) 16.97 (D-glucose, positive optical rotation).

Compound 9a: amorphous solid; $[\alpha]^{26}_D$ +4.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 279.8 nm (log ϵ 3.50); UV (MeOH + 1 M NaOH) λ_{max} 289.2 nm; IR (film) ν_{max} 3279 (OH), 2933 (CH), 1613, 1598 and 1514 (aromatic rings), 1454, 1365, 1239, 1173, 1056, 826 cm^{-1}; ^{1}H NMR (CD_3OD) δ 6.99 (4H, d, *J* = 8.4 Hz, H-2′, H-6′, H-2″, H-6″), 6.67 (4H, d, *J* = 8.4 Hz, H-3′, H-5′, H-3″, H-5″), 3.78 (2H, m, H-3, H-5), 2.68−2.50 (4H, m, H_2-1, H_2-7), 1.69−1.63 (4H, m, H_2-2, H_2-6), 1.53 (2H, dd, *J* = 6.5, 5.8 Hz, H_2-4); ^{13}C NMR, see Table 1; EIMS *m/z* 298 [M]$^+$.

Methylation of 9a. Compound **9a** (66.4 mg) was methylated by the same procedure as described for **1** to yield **9b** (40.0 mg).

Compound 9b: amorphous solid; $[\alpha]^{28}_D$ +6.9° (*c* 0.14, EtOH); UV (MeOH) λ_{max} 278.0 nm (log ϵ 3.47); IR (film) ν_{max} 3301 (OH), 2937 and 2838 (CH), 1612, 1582 and 1514

(aromatic rings), 1462, 1442, 1428, 1336, 1250, 1178, 1095, 1066, 1032, 908, 819, 810 cm^{-1}; ^1H NMR (CD_3OD) δ 7.08 (4H, d, $J = 8.6$ Hz, H-2′, H-6′, H-2″, H-6″), 6.80 (4H, d, $J = 8.6$ Hz, H-3′, H-5′, H-3″, H-5″), 3.78 (2H, m, H-3, H-5), 3.74 (6H, s, OMe × 2), 2.71−2.53 (4H, m, H_2-1, H_2-7), 1.70−1.65 (4H, m, H_2-2, H_2-6), 1.54 (2H, dd, $J = 6.5$, 5.8 Hz, H_2-4); ^{13}C NMR, see Table 1; EIMS m/z 344 $[M]^+$.

Cell Culture and Assay for Cytotoxic Activity against HL-60 Cells. HL-60 cells, which were obtained from Human Science Research Resources Bank (JCRB 0085, Osaka, Japan), were maintained in RPMI 1640 medium containing 10% heat-inactivated FBS and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin) in a 5% CO_2 humidified incubator at 37 °C. The cells were washed and resuspended in the medium to 4×10^4 cells/mL, and 196 μL of this cell suspension was divided into 96-well flat bottom plates (Iwaki Glass, Chiba, Japan). The cells were incubated in 5% CO_2/air for 24 h at 37 °C. After incubation, 4 μL of EtOH−H_2O (1:1) solution containing the sample was added to give the final concentrations of 0.1−10 μg/mL, and 4 μL of EtOH−H_2O (1:1) was added into control wells. The cells were further incubated for 72 h in the presence of each agent, and then cell growth was evaluated using a modified MTT reduction assay.[11] At the end of incubation, 10 μL of 5 mg/mL MTT in phosphate-buffered saline was added to every well, and the plate was further incubated in 5% CO_2/air for 4 h at 37 °C. Then the plate was centrifuged at 1500g for 5 min to precipitate MTT formazan. An aliquot of 150 μL of supernatant was removed from every well, and 175 μL of DMSO was added to dissolve the MTT formazan crystals. The plate was mixed on a microplate mixer for 10 min and then read on a microplate reader (Spectra Classic, Tecan, Salzburg, Austria) at 550 nm. Each assay was done in triplicate, and cytotoxicity was expressed as IC_{50} value, which reduces the viable cell number by 50%.

Cell Culture and Assay for Cytotoxic Activity against HSC-2 Cells and HGF. HSC-2 cells were maintained as monolayer cultures at 37 °C in DMEM supplemented with 10% heat-inactivated FBS in a humidified 5% CO_2 atmosphere. HGF were isolated, as described previously.[15] Briefly, gingival tissues were obtained from healthy gingival biopsies from a 10-year-old girl, undergoing periodontal surgery. The tissue were cut into 1 to 2 mm^3 pieces, washed twice with phosphate-buffered saline (PBS, 0.01 M phosphate buffer, 0.15 M NaCl, pH 7.4) supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin, and placed into 25 cm^2 tissue culture flasks. The explants were incubated in α-MEM supplemented with 30% FBS and antibiotics. When outgrowth of the cells was observed, the medium was replaced twice until the cells reached confluence. The cells were detached from the monolayer by trypsinization and recultured in 100 cm^2 tissue culture flasks until confluent monolayers were again obtained. Cells between the fifth and seventh passages were used. Cells were trypsinized and inoculated at 6×10^3 per each 96-microwell plate (Falcon, flat bottom, treated polystyrene, Becton Dickinson, San Jose, CA) and incubated for 24 h. After washing once with PBS, they were treated for 24 h without or with test compounds. They were washed once with PBS and incubated for 4 h with 0.2 mg/mL MTT in DMEM supplemented with 10% FBS. After the medium was removed, the cells were lysed with 0.1 mL DMSO and the relative viable cell number was determined by measuring the absorbance at 540 nm of the cell lysate, using Labsystems Multiskan (Biochromatic, Helsinki, Finland) connected to a Star/DOT Matrix printer JL-10.

References and Notes

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