

Inhibitors of Osteoclast Differentiation from *Cephalotaxus koreana*

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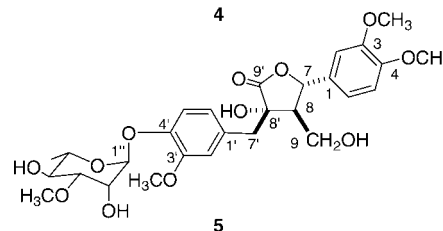
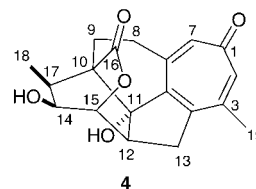
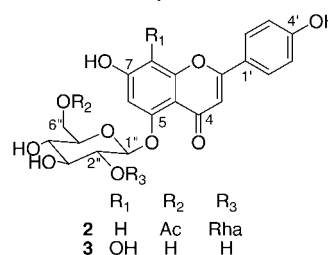
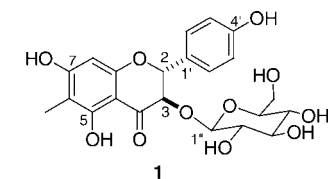
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Three new flavonoid glycosides (**1–3**), 11-hydroxyhainanolidol (**4**), and a new dibenzylbutyrolactone lignan glycoside (**5**) were isolated from the aerial parts of *Cephalotaxus koreana* Nakai, along with 19 known flavonoids. The structures of the new compounds were elucidated using spectroscopic evidence, primarily NMR and MS. Twenty-four compounds were isolated, and among these isoscutellarein 5-*O*- β -D-glucopyranoside (**3**), apigenin (**6**), kaempferol 3-*O*- α -L-rhamnopyranosyl(1'' \rightarrow 6'')- β -D-glucopyranoside (**7**), tamarixetin 3-*O*- α -L-rhamnopyranosyl(1'' \rightarrow 6'')- β -D-glucopyranoside (**8**), quercetin 3-*O*-[6''-*O*-acetyl]- β -D-glucopyranoside (**9**), and quercetin 3-*O*- α -L-rhamnopyranoside (**10**) showed significant inhibitory activities against osteoclast differentiation at concentrations of 0.1 and 1.0 μ g/mL.

Osteoclasts are formed by fusion of mononucleated cells originating from hematopoietic tissue and play a critical role in bone homeostasis along with osteoblasts. Osteoclastogenesis takes place through multiple steps such as differentiation, fusion, and activation of mature osteoclasts by cell-to-cell contact with osteoblast lineages that express the factors regulating osteoclast differentiation.¹ Since osteoclasts have a relatively short life span and they undergo rapid apoptotic cell death at the end of the bone resorption process, the regulation of osteoclast apoptosis has provided a strategy for controlling bone resorption and enabled us to treat and prevent bone disease due to high bone resorption rate.² Recently, some papers have reported that natural phenolics showed inhibitory activity against osteoclastogenesis.^{3–7}

Cephalotaxus koreana Nakai (Cephalotaxaceae) is distributed in the southern parts of Korea, and the fruits of this plant have been used for the treatment of wounds caused by insects.⁸ The genus *Cephalotaxus* is well-known for its alkaloids such as cephalotaxine, harringtonine, and their derivatives,^{9–11} but few compounds have been reported from *C. koreana*.^{12,13} Amentoflavone-type biflavonoids from the EtOAc-soluble fraction of *C. koreana* may have therapeutic potential for bone disease.¹³ The present study describes the isolation and structure elucidation of five new compounds (**1–5**) from *C. koreana*, together with 19 known compounds, and their inhibitory activities toward osteoclast differentiation.

Compound **1** was isolated as a yellowish powder and the molecular formula deduced as C₂₂H₂₄O₁₁ by HRFABMS. The ¹H NMR spectrum of **1** displayed two doublet signals at δ 5.23 (1H, d, *J* = 10.1 Hz, H-2) and 4.96 (1H, d, *J* = 10.1 Hz, H-3), characteristic of a dihydroflavonol. Two doublets at δ 7.35 (2H, d, *J* = 8.6 Hz, H-2', H-6') and 6.80 (2H, d, *J* = 8.6 Hz, H-3', H-5') suggested an A₂B₂ spin system in the B ring. Singlets at δ 5.91 (1H, s, H-8) and 1.94 (3H, s, 6-CH₃) suggested that the methyl group was attached to the A ring. These signals, together with ¹³C NMR resonances, were similar to the reported values for 6-methylaromadendrin aglycone.¹⁴ An anomeric proton resonance at δ 3.78 (1H, d, *J* = 7.7 Hz, H-1'') indicated that **1** was a glycoside, and the sugar moiety was identified as D-glucose. The HMBC correlation between δ _H 3.78 (H-1'') and δ _C 78.1 (C-3) indicated that the glucosyl moiety was attached at C-3 of 6-methylaromadendrin, and the CD spectrum showed positive Cotton effects at 257 and 345 nm and a negative Cotton effect at 294 nm, demonstrating **1** to be in the 2*R*,3*R* configuration.¹⁵ On the basis



of these data and the remaining spectroscopic evidence, **1** was determined as (2*R*,3*R*)-6-methylaromadendrin 3-*O*- β -D-glucopyranoside.

Spectroscopic data of **2** showed patterns similar to those of apigenin 5-*O*- α -L-rhamnopyranosyl(1'' \rightarrow 2'')- β -D-glucopyranoside except that signals for an additional acetyl group were observed in NMR spectra and by an increase of 42 amu by HRFABMS. HMBC correlations of the H-6'' protons at δ 4.28 and 4.00 to a carbonyl carbon at δ 170.0 suggested that the acetyl group was linked to the oxygen at C-6. Hence, the structure of **2** was assigned as apigenin 5-*O*- α -L-rhamnopyranosyl(1'' \rightarrow 2'')-[6''-*O*-acetyl]- β -D-glucopyranoside.

Compound **3** was isolated as a yellowish powder with a molecular formula of C₂₁H₂₀O₁₁, as determined by HRFABMS. ¹H and ¹³C NMR data of **3** suggested that it could be an isoscutellarein glycoside. The attached sugar was determined to be glucose by

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analysis of its NMR data and GC analysis of its hydrolysate. The H-1'' signal at δ 4.55 (1H, d, $J = 6.8$ Hz, H-1'') showed an HMBC correlation to δ 149.6 (C-5), which revealed the site of glycosidation. Thus, **3** was determined to be isoscutellarein 5-*O*- β -D-glucopyranoside.

The spectroscopic data of **4** were characteristic of a hainanolid. Positive ion HRFABMS and NMR spectra revealed the molecular formula to be $C_{19}H_{20}O_5$, 16 amu greater than that of hainanolidol, suggesting the presence of an additional OH group. The hydroxylated quaternary carbon resonance of **4** at δ 88.2 (C-11) showed long-range correlations with H-9 (δ 1.84), H-12 (δ 2.78), H-15 (δ 4.67), and H-17 (δ 1.36) in the HMBC experiment. These results suggested that the OH group was attached to C-11. The NOESY spectrum revealed the relative configuration of **4** (H-8_a/H-9_a; H-8_b/H-9_b; H-12/H-13,15; H-14/H-15,17,18; H-17/H-18). The most stable confirmation was established by calculating the minimum energy (24 kcal/mol) with SYBYL software. Therefore, **4** was determined to be 11-hydroxyhainanolidol.

The molecular formula of **5** was assigned as $C_{28}H_{36}O_{12}$ by HRFABMS. 1H and ^{13}C NMR spectra of **5** indicated two trisubstituted benzene units. Extensive 2D NMR evidence indicated that **5** was a dibenzylbutyrolactone lignan.¹⁷ An anomeric proton at δ 5.32 (d, $J = 1.4$ Hz, H-1'') indicated that **5** was present as a glycoside, and the ^{13}C NMR chemical shifts of the sugar moiety were in agreement with those of 3-*O*-methylrhamnopyranoside.¹⁸ The sugar residue was shown to be connected to C-4' by HMBC correlation of H-1'' (δ 5.32) to C-4' (δ 146.7). The absolute configuration of **5** was revealed by comparison of its CD spectrum with those of (–)-olivil and (–)-berchemol. Compound **5** showed negative Cotton effects at 228 and 284 nm, which were almost identical to those of (–)-olivil and (–)-berchemol.¹⁹ Thus **5** was determined to be (8*R*,8'*S*,7*S*)-4'-(3''-methoxy-rhamnopyranosyl)oxy-8'-hydroxy-3,3',4'-trimethoxy-8-hydroxymethyl-lign-7-9'-lactone.

Compounds **1–5** are reported for the first time as natural products, and the structures of the known compounds (see the Supporting Information) were determined by comparison of their spectroscopic data (optical rotation, 1H NMR, ^{13}C NMR, and MS) with those of literature values.^{20–27}

The 24 compounds from *C. koreana* were tested for their inhibitory activity on osteoclast differentiation at concentrations of 0.1 and 1.0 $\mu g/mL$. Compounds **3** and **6–10** showed significant inhibitory activity against osteoclast differentiation, and the number of TRAP(+) MNCs was suppressed by more than 50% inhibition (Table 2). It has been reported that apigenin (**6**) induced apoptosis of mature osteoclasts obtained from rabbit long bone at a concentration range of 5–20 μM .²⁸ In addition, kaempferol and quercetin inhibit osteoclast differentiation by antagonizing RANKL action in RAW 264.7 cells and promote osteoclast apoptosis with IC₅₀ values of 1.6 and 5.3 μM , respectively.²⁹ The present study suggested that flavonoid aglycones and their glycosides have inhibitory activity against osteoclastogenesis.

Experimental Section

General Experimental Procedures. Melting points were measured on a Fisher-Johns melting point apparatus. 1H NMR and ^{13}C NMR data were obtained on Bruker AVANCE 400 and 500 spectrometers. Mass spectra (FABMS and HRFABMS) were recorded on a JEOL JMS 700 spectrometer. Optical rotations were recorded with a JASCO DIP-1000 polarimeter. UV spectra were measured on a Beckman DU 650 spectrophotometer, and CD spectra were recorded on a JASCO J-715 spectropolarimeter. IR spectra were obtained on a JASCO FT/IR-300E spectrophotometer, and GC data were obtained with a GL Science GC 353B. A Gilson HPLC system (Gilson, USA) was used for isolation of compounds and was equipped with two pumps (321 pump), a UV/vis detector (UV/vis-151), and an autosampler (234 autoinjector).

Plant Material. The aerial parts (leaves and twigs) of *C. koreana* Nakai (Cephalotaxaceae) were collected in the Kwanak Arboretum, Seoul National University, located in Anyang-Si in August 2004, and

Table 1. ^{13}C NMR Data for Compounds **1–3** (δ_C , mult)

position	1	2	3
2	84.4, CH	160.1, qC	161.3, qC
3	78.1, CH	105.7, CH	105.1, CH
4	196.9, qC	175.6, qC	177.7, qC
5	162.6, qC	157.0, qC	149.6, qC
6	103.1, qC	99.1, CH	105.6, CH
7	168.2, qC	162.5, qC	150.8, qC
8	96.3, CH	96.3, CH	129.4, qC
9	163.4, qC	158.7, qC	146.8, qC
10	106.6, qC	107.1, qC	108.5, qC
1'	129.5, qC	121.3, qC	121.4, qC
2'	131.3, CH	127.8, CH	129.4, CH
3'	117.0, CH	115.8, CH	115.8, CH
4'	160.1, qC	160.5, qC	160.8, qC
5'	117.0, CH	115.8, CH	115.8, CH
6'	131.3, CH	127.8, CH	129.4, CH
6-CH ₃	7.8, CH ₃		
1''	103.4, CH	97.0, CH	105.1, CH
2''	75.4, CH	76.7, CH	73.7, CH
3''	79.1, CH	76.5, CH	75.7, CH
4''	72.0, CH	69.9, CH	69.4, CH
5''	78.4, CH	73.3, CH	77.4, CH
6''	63.4, CH ₂	62.8, CH ₂	60.9, CH ₂
1'''		99.5, CH	
2'''		70.4, CH	
3'''		70.3, CH	
4'''		72.1, CH	
5'''		68.6, CH	
6'''		17.8, CH ₃	
OCOCH ₃		170.0, qC	
OCOCH ₃		20.3, CH ₃	

Table 2. Inhibitory Activity of Compounds **3** and **6–10** on Osteoclast Differentiation

compound ^a	number of TRAP (+) MNCs/well		inhibitory activity (%)	
	0.1 $\mu g/mL$	1.0 $\mu g/mL$	0.1 $\mu g/mL$	1.0 $\mu g/mL$
3	54 \pm 6.7	21 \pm 9.2	63 \pm 4.6	86 \pm 6.3
6	80 \pm 3.5	51 \pm 3.2	45 \pm 2.4	65 \pm 2.2
7	46 \pm 4.9	23 \pm 2.1	68 \pm 3.4	85 \pm 1.5
8	87 \pm 2.8	54 \pm 3.5	40 \pm 1.9	63 \pm 2.4
9	71 \pm 12.1	51 \pm 2.6	51 \pm 8.3	65 \pm 1.8
10	65 \pm 0.6	48 \pm 7.5	56 \pm 0.4	67 \pm 5.1
control		146 \pm 9.2		0

^a The structures of compounds are as follows: isoscutellarein 5-*O*- β -D-glucopyranoside (**3**), apigenin (**6**), kaempferol 3-*O*- α -L-rhamnopyranosyl (1''' \rightarrow 6'')- β -D-glucopyranoside (**7**), tamarixetin 3-*O*- α -L-rhamnopyranosyl (1''' \rightarrow 6'')- β -D-glucopyranoside (**8**), quercetin 3-*O*-[6''-*O*-acetyl]- β -D-glucopyranoside (**9**), and quercetin 3-*O*- α -L-rhamnopyranoside (**10**). Inhibitory activities were expressed as mean \pm SD of three replicates. All samples were significantly different ($p < 0.01$) from the control.

identified by Prof. Jong Hee Park, College of Pharmacy, Pusan National University. Voucher specimens (SNUPH-0821) have been deposited in the Medicinal Herb Garden, Seoul National University.

Extraction and Isolation. The air-dried and milled aerial parts of *C. koreana* (1.2 kg) were extracted with MeOH, giving a crude extract (128 g). The MeOH extract was partitioned with *n*-hexane, CH_2Cl_2 , EtOAc, and *n*-BuOH. The EtOAc-soluble fraction (24 g) was subjected to silica gel column chromatography (CC) (230–400 mesh, 40 cm \times 8 cm) using CH_2Cl_2 –MeOH–H₂O (50:5:1 \rightarrow 5:5:1) and afforded five fractions (F1–F5). F1 (6.2 g) was subjected to CC on Sephadex LH-20 with MeOH to give 10 subfractions (A1–A10). Subfractions A7–A10 (470 mg) were combined and subjected to reversed-phase HPLC (J'sphere ODS H80, 10 \times 250 mm, 5 μm , 2 mL/min, MeCN–H₂O, 10:90 \rightarrow 30:70) to give 11-hydroxyhainanolidol (**4**, 5.2 mg), (+)-catechin (14 mg), and (–)-epicatechin (7.3 mg). F2–F4 (12.2 g) were combined and chromatographed on a Sephadex LH-20 column (50 cm \times 2.5 cm) with MeOH to afford six subfractions (B1–B6). Subfraction B2 (1.9 g) was subjected to HPLC (J'sphere ODS H80, 10 \times 250 mm, 5 μm , 2 mL/min, MeCN–H₂O, 10:90 \rightarrow 30:70) to yield apigenin 5-*O*- α -L-rhamnopyranosyl(1''' \rightarrow 2'')- β -D-glucopyranoside (24 mg), apigenin 5-*O*- α -L-rhamnopyranosyl(1''' \rightarrow 2'')-[6''-*O*-acetyl]- β -D-glucopyranoside (**2**, 17 mg), apigenin 7-*O*- α -L-rhamnopyrano-

syl(1''''→2'')-β-D-glucopyranoside (5.5 mg), luteolin 5-O-α-L-rhamnopyranosyl(1''''→2'')-β-D-glucopyranoside (8.1 mg), kaempferol 3-O-α-L-rhamnopyranosyl(1''''→6'')-β-D-glucopyranoside (**7**, 8.6 mg), and tamarixetin 3-O-α-L-rhamnopyranosyl(1''''→6'')-β-D-glucopyranoside (**8**, 13.2 mg). Subfraction B3 (2.6 g) was chromatographed on the C₁₈ HPLC column (MeCN–H₂O, 10:90 → 50:50) to afford apigenin 5-O-β-D-glucopyranoside (10 mg), luteolin 5-O-β-D-glucopyranoside (11.6 mg), quercetin 3-O-[6''-O-acetyl]-β-D-glucopyranoside (**9**, 15 mg), and quercetin 3-O-α-L-rhamnopyranosyl(1''''→6'')-β-D-glucopyranoside (9.1 mg). Subfraction B4 (3.1 g) was applied to a C₁₈ HPLC column (YMC-Pack Ph, 10 × 250 mm, 5 μm, 2 mL/min, MeCN–H₂O, 10:90 → 40:60) to give (8R,8'S,7S)-4'-(3''-methoxyrhamnopyranosyl)oxy-8'-hydroxy-3,3',4-trimethoxy-8-hydroxymethyl-lign-7-9'-lactone (**5**) (6.8 mg), (2R,3R)-6-methylaromadendrin 3-O-β-D-glucopyranoside (**1**, 21 mg), isoscutellarein 8-O-β-D-glucopyranoside (12 mg), isoscutellarein 5-O-β-D-glucopyranoside (**3**, 8 mg), quercetin 3-O-β-D-glucopyranoside (15 mg), quercetin 3-O-α-L-rhamnopyranoside (**10**, 9.1 mg), and puerarin (4.8 mg). Subfraction B5 (870 mg) was separated on Sephadex LH-20 using MeOH to yield catechin 7-O-β-D-glucopyranoside (12.7 mg). Subfraction B6 (2.2 g) was subjected to C₁₈ HPLC (YMC-Pack Ph, 10 × 250 mm, 5 μm, 2 mL/min, MeCN–H₂O, 20:80 → 50:50) to give aromadendrin (9 mg), apigenin (**6**, 24 mg), and isoscutellarein (7.9 mg).

(2R,3R)-6-Methylaromadendrin 3-O-β-D-glucopyranoside (1): yellowish powder; mp 166–168 °C; [α]_D²⁴ –19.3 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.94), 256 (4.79), 355 (sh, 4.66) nm; CD (c 0.0005, MeOH) [θ]₂₅₇ +2798, [θ]₂₉₄ –14787, [θ]₃₄₅ +3835; IR (KBr) ν_{max} 3364, 1653, 1604, 1504, 1359, 1299, 1199 cm^{–1}; ¹H NMR (CD₃OD, 400 MHz) δ 7.35 (2H, d, *J* = 8.6 Hz, H-2', H-6'), 6.80 (2H, d, *J* = 8.6 Hz, H-3', H-5'), 5.91 (1H, s, H-8), 5.23 (1H, d, *J* = 10.1 Hz, H-2), 4.96 (1H, d, *J* = 10.1 Hz, H-3), 3.78 (1H, d, *J* = 7.7 Hz, H-1''), 2.90–3.80 (5H, m, glucosyl protons), 1.94 (3H, s, 6-CH₃); ¹³C NMR (CD₃OD, 100 MHz) see Table 1; HMBC H-2/C-3, 4, 9, 1', 2', 6'; H-3/C-4, 10, 1'; 6-CH₃/C-5, 7; H-8/C-6, 7, 9; H-2', 6'/C-1', 4'; H-3', 5'/C-1', 2', 4', 6'; H-1''/C-5; positive ion FABMS *m/z* 487 [M + Na]⁺, 465 [M + H]⁺; HRFABMS *m/z* 487.1216 (calcd for C₂₂H₂₄O₁₁Na [M + Na]⁺, 487.1217).

Apigenin 5-O-α-L-rhamnopyranosyl(1''''→2'')-[6''-O-acetyl]-β-D-glucopyranoside (2): yellowish powder; mp 117–120 °C; [α]_D²⁴ –25.2 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 209 (2.80), 261 (2.33), 327 (sh, 2.45) nm; IR (KBr) ν_{max} 3363, 1636, 1510, 1452, 1357, 1255 cm^{–1}; ¹H NMR (DMSO-*d*₆, 500 MHz) δ 7.84 (2H, d, *J* = 8.7 Hz, H-2', H-6'), 6.91 (2H, d, *J* = 8.7 Hz, H-3', H-5'), 6.58 (1H, d, *J* = 1.6 Hz, H-8), 6.51 (1H, s, H-3), 6.47 (1H, d, *J* = 1.6 Hz, H-6), 5.37 (1H, d, *J* = 6.0 Hz, H-1''), 5.16 (1H, br s, H-1''), 3.10–3.80 (4H, m, H-2'' to H-5''), 3.00–3.80 (6H, m, H-2'' to H-6''), 1.85 (3H, s, OCOCH₃), 1.02 (3H, d, *J* = 6.0 Hz, H-6''); ¹³C NMR (DMSO-*d*₆, 125 MHz) see Table 1; HMBC H-3/C-2, 4, 10, 1'; H-6/C-5, 7, 8, 10; H-8/C-7, 10; H-2', 6'/C-3', 4', 5'; H-3', 5'/C-1', 2', 4', 6'; H-1''/C-5; 6''-OCOCH₃/C-6''; H-1''/C-2''; positive ion FABMS *m/z* 643 [M + Na]⁺, 621 [M + H]⁺; HRFABMS *m/z* 643.1639 (calcd for C₂₉H₃₂O₁₅Na [M + Na]⁺, 643.1640).

Isoscutellarein 5-O-β-D-glucopyranoside (3): yellowish powder; mp 153–156 °C; [α]_D²⁴ –20.3 (c 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 222 (2.61), 272 (2.50), 300 (2.49) nm; IR (KBr) ν_{max} 3365, 1632, 1513, 1360, 1253 cm^{–1}; ¹H NMR (DMSO-*d*₆, 400 MHz) δ 7.98 (2H, d, *J* = 8.4 Hz, H-2', H-6'), 6.93 (1H, s, H-6), 6.91 (2H, d, *J* = 8.4 Hz, H-3', H-5'), 6.67 (1H, s, H-3), 4.55 (1H, d, *J* = 6.8 Hz, H-1''), 3.75 (1H, br d, *J* = 11.1 Hz, H-6'a), 3.56 (1H, dd, *J* = 11.1, 4.6 Hz, H-6''b), 3.11–3.28 (4H, m, H-2'' to H-5''); ¹³C NMR (DMSO-*d*₆, 100 MHz) see Table 1; HMBC H-3/C-2, 4, 10, 1'; H-6/C-5, 7, 8, 10; H-2', 6'/C-2, 4'; H-3', 5'/C-1', 2', 4', 6'; H-1''/C-5; positive ion FABMS *m/z* 471 [M + Na]⁺, 449 [M + H]⁺; HRFABMS *m/z* 449.1090 (calcd for C₂₁H₂₁O₁₁ [M + H]⁺, 449.1084).

11-Hydroxyhainanolidol (4): colorless, amorphous solid; [α]_D²⁴ –11.6 (c 0.28, MeOH); UV (MeOH) λ_{max} (log ε) 240 (2.27), 324 (1.68) nm; IR (KBr) ν_{max} 3396, 2920, 1742, 1618, 1536, 1087 cm^{–1}; ¹H NMR (CD₃OD, 500 MHz) δ 7.00 (1H, brs, H-2), 6.92 (1H, brs, H-7), 4.67 (1H, t, *J* = 4.2 Hz, H-15), 3.60 (1H, dd, *J* = 7.0, 4.0 Hz, H-14), 3.51 (1H, dd, *J* = 19.0, 3.3 Hz, H-13a), 3.26 (1H, dd, *J* = 19.0, 10.1 Hz, H-13b), 3.17 (1H, m, H-8a), 2.78 (1H, m, H-12), 2.69 (1H, m, H-8b), 2.55 (1H, m, H-9a), 2.28 (1H, s, H-19), 1.84 (1H, m, 9b), 1.36 (1H, q, *J* = 7.0 Hz, H-17), 1.00 (1H, d, *J* = 7.0 Hz, H-18); ¹³C NMR (CD₃OD, 125 MHz) δ 189.0 (C, C-1), 176.9 (C, C-16), 151.3 (C, C-3), 150.4 (C, C-6), 149.8 (C, C-4), 145.7 (C, C-5), 142.0 (CH, C-2), 140.0 (CH,

C-7), 88.2 (C, C-11), 80.1 (CH, C-15), 76.6 (CH, C-14), 51.1 (C, C-10), 46.9 (CH, C-12), 40.3 (CH, C-17), 36.6 (CH₂, C-13), 30.1 (CH₂, C-8), 25.6 (CH₃, C-19), 19.5 (CH₂, C-9), 17.8 (CH₃, C-18); HMBC: H-2/C-3, 4, 7, 19; H-7/C-2, 5, 6, 8; H-8b/C-5, 6, 7, 9; H-9a/C-10, 16; H-9b/C-6, 9, 10, 11; H-12/C-11, 14, 15; H-15/C-11, 14, 16, 17; H-17/C-9, 10, 11, 14, 16; H-18/C-10, 14, 17; H-19/C-2, 3, 4; NOESY: H-8a/H-9a; H-8b/H-9b; H-12/H-13, 15; H-14/H-15, 17, 18; H-17/H-18; positive ion FABMS *m/z* 329 [M + H]⁺; HRFABMS *m/z* 329.1381 (calcd for C₁₉H₂₁O₅ [M + H]⁺, 329.1389).

(8R,8'S,7S)-4'-(3''-methoxyrhamnopyranosyl)oxy-8'-hydroxy-3,3',4-trimethoxy-8-hydroxymethyl-lign-7-9'-lactone (5): white, amorphous solid; [α]_D²⁴ –4.8 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 227 (2.12), 277 (1.68) nm; CD (c 0.00013, MeOH) [θ]₂₈₄ –3514, [θ]₂₃₂ –33726; IR (KBr) ν_{max} 3463, 2921, 1768, 1515, 1459, 1267, 1137, 1024 cm^{–1}; ¹H NMR (CD₃OD, 400 MHz) δ 7.05 (1H, d, *J* = 8.2 Hz, H-5'), 6.96 (1H, d, *J* = 1.7 Hz, H-2'), 6.84 (1H, d, *J* = 8.2 Hz, H-5), 6.81 (1H, dd, *J* = 8.2, 1.7 Hz, H-6'), 6.58 (1H, dd, *J* = 8.2, 1.8 Hz, H-6), 6.38 (1H, d, *J* = 1.8 Hz, H-2), 5.32 (1H, d, *J* = 1.4 Hz, H-1''), 5.13 (1H, d, *J* = 9.1 Hz, H-7), 3.93 (1H, dd, *J* = 11.3, 7.3 Hz, H-9a), 3.80 (3H, s, 3'-OCH₃), 3.78 (3H, s, 4-OCH₃), 3.62 (3H, s, 3-OCH₃), 3.58 (1H, dd, *J* = 11.4, 4.8 Hz, H-9b), 3.51 (3H, s, 3''-OCH₃), 3.32 (1H, d, 13.1 Hz, H-7'a), 3.05 (1H, d, *J* = 13.1 Hz, H-7'b), 2.44 (1H, ddd, *J* = 9.1, 7.3, 4.8 Hz, H-8), 1.21 (3H, d, *J* = 6.2 Hz, H-6''), 3.82–4.27 (4H, m, H-2'' to H-5''); ¹³C NMR (CD₃OD, 100 MHz) δ 180.3 (C, C-9'), 152.8 (C, C-3'), 151.7 (C, C-3), 151.4 (C, C-4), 146.7 (C, C-4'), 133.5 (C, C-1'), 133.1 (C, C-1), 125.2 (CH, C-6'), 121.5 (CH, C-6), 120.9 (CH, C-5'), 117.0 (CH, C-2'), 113.3 (CH, C-5), 111.2 (CH, C-2), 102.4 (CH, C-1''), 83.5 (CH, C-7), 82.7 (CH, C-3'), 80.1 (C, C-8'), 73.5 (CH, C-4''), 71.7 (CH, C-5''), 68.8 (CH, C-2''), 59.4 (CH₂, C-9), 58.2 (CH₃, 3''-OCH₃), 57.3 (CH₃, 3'-OCH₃), 57.2 (CH₃, 4-OCH₃), 57.2 (CH₃, 3-OCH₃), 52.5 (CH, C-8), 43.3 (CH₂, C-7'), 18.9 (CH, C-6''); HMBC H-2/C-1, 3, 4, 6, 7; H-5/C-1, 3, 4; H-6/C-2, 4, 7; H-7/C-2, 6, 8, 8', 9'; H-9/C-8, 8'; H-2'/1', 3', 4', 6', 7'; H-5'/C-1', 3', 4'; H-6'/C-1', 2', 4', 7'; H-7'/H-1', 2', 6', 8', 9'; 3-OCH₃/C-3; 4-OCH₃/C-4; 3'-OCH₃/C-3'; 3''-OCH₃/C-3''; positive ion FABMS *m/z* 587 [M + Na]⁺; HRFABMS *m/z* 565.2289 (calcd for C₂₈H₃₇O₁₂ [M + H]⁺ 565.2285).

Sugar Analysis. Acidic hydrolysis of compounds was performed according to the literature.³⁰ Hydrolysates were analyzed by GC; column: BPX50 (0.25 mm × 30 m), detector: FID, column temperature: 210 °C, injector temperature: 270 °C, detection temperature: 300 °C, carrier gas: He (2.0 kg/cm). Under this condition, authentic sugars gave peaks at *t*_R 8.41 (L-rhamnose), 11.31 (D-glucose), 12.23 (L-glucose), 12.68 (D-galactose), 14.10 (L-galactose), 8.49 (D-arabinose), and 7.57 min (L-arabinose). The retention times of sugars obtained by acid hydrolysis were 11.36 min for compound **1**, 8.40 and 11.35 min for compound **2**, and 11.32 min for compound **3**.

Osteoclast Formation Assay. Test compounds were dissolved in DMSO at the concentration of 2 mg/mL and filtered using a PIFE filter (0.2 μm). These sterilized stock solutions were treated with concentrations of 0.1 and 1.0 μg/mL in cocultured cells.

Osteoclast formation was determined by counting tartrate-resistant acid phosphatase-positive multinucleated osteoclasts (TRAP(+) MNCs).³¹ To investigate the effect of compounds on osteoclast differentiation, coculture between bone marrow cells (2.5 × 10⁵ cells/cm²) and calvaria-derived osteoblastic cells (4 × 10⁴ cells/cm²) was conducted in the presence of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) (1 × 10^{–8} M) and dexamethasone (1 × 10^{–7} M) with α-MEM containing 10% FBS. Bone marrow cells were isolated from the femora of 6-week-old ddY mice, and osteoblastic cells were from calvariae of newborn ICR mice by sequential digestion with 0.2% collagenase. The medium was changed every two days with fresh osteoclastic medium. On day 6, the cells were fixed with 10% formaldehyde and stained for TRAP. The number of TRAP(+) MNCs containing more than 6 to 7 nuclei was counted under a microscope. The results are shown as an inhibitory activity (% inhibitory activity) compared to the control group. All values are expressed as mean ± SD of three replicates. Statistical significance was evaluated between each treated group and control by the Student's *t*-test.

Supporting Information Available: Physical constant data and references for known compounds (**6–24**) isolated in this study. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

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