Inhibitors of Osteoclast Differentiation from *Cephalotaxus koreana*

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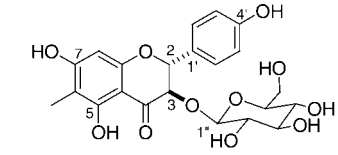
Three new flavonoid glycosides (**1**–**3**), 11-hydroxyhainanolidol (**4**), and a new dibenzylbutyrolactone lignan glycoside (**5**) were isolated from the aerial parts of *Cephalotaxus koreana* Nakai, along with 19 known flavonoids. The structures of the new compounds were elucidated using spectroscopic 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‴→6″)-*β*-D-glucopyranoside (**7**), tamarixetin 3-*O*-α-L-rhamnopyranosyl(1‴→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 hematopoetic 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 $C_{22}H_{24}O_{11}$ by HRFABMS. The ^{1}H 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 A_2B_2 spin system in the B ring. Singlets at *δ* 5.91 (1H, s, H-8) and 1.94 (3H, s, 6-C*H*$_3$) suggested that the methyl group was attached to the A ring. These signals, together with ^{13}C 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.



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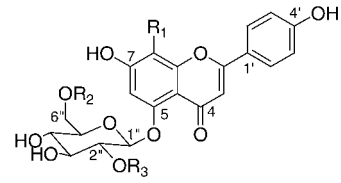
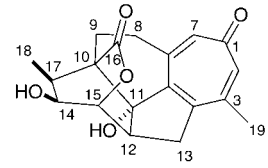


	R_1	R_2	R_3
2	H	Ac	Rha
3	OH	H	H



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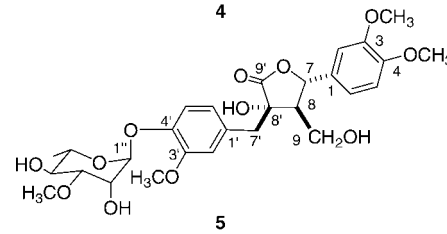

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Spectroscopic data of **2** showed patterns similar to those of apigenin 5-*O*-α-L-rhamnopyranosyl(1‴→2″)-*β*-D-glucopyranoside except that signals for an additional acetyl group were observed in NMR spectra and by an increase of 42 amu by HRFABMS. 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‴→2″)[6″-*O*-acetyl]-*β*-D-glucopyranoside.

Compound **3** was isolated as a yellowish powder with a molecular formula of $C_{21}H_{20}O_{11}$, as determined by HRFABMS. ^{1}H and ^{13}C 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 hainanolide.[16] 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. ^{1}H 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, ^{1}H 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 μg/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 IC_{50} 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.

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-*C*H$_3$	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$_3$	
O*C*OCH$_3$		170.0, qC	
OCO*C*H$_3$		20.3, CH$_3$	

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

	number of TRAP (+) MNCs/well		inhibitory activity (%)	
compound[a]	0.1 μg/mL	1.0 μg/mL	0.1 μg/mL	1.0 μg/mL
3	54 ± 6.7	21 ± 9.2	63 ± 4.6	86 ± 6.3
6	80 ± 3.5	51 ± 3.2	45 ± 2.4	65 ± 2.2
7	46 ± 4.9	23 ± 2.1	68 ± 3.4	85 ± 1.5
8	87 ± 2.8	54 ± 3.5	40 ± 1.9	63 ± 2.4
9	71 ± 12.1	51 ± 2.6	51 ± 8.3	65 ± 1.8
10	65 ± 0.6	48 ± 7.5	56 ± 0.4	67 ± 5.1
control	146 ± 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‴→6″)-β-D-glucopyranoside (**7**), tamarixetin 3-*O*-α-L-rhamnopyranosyl(1‴→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 ± SD of three replicates. All samples were significantly different ($p < 0.01$) from the control.

Experimental Section

General Experimental Procedures. Melting points were measured on a Fisher-Johns melting point apparatus. ^{1}H 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 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 × 8 cm) using CH_2Cl_2−MeOH−H_2O (50:5:1 → 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 × 250 mm, 5 μm, 2 mL/min, MeCN−H_2O, 10:90 → 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 × 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 × 250 mm, 5 μm, 2 mL/min, MeCN−H_2O, 10:90 → 30:70) to yield apigenin 5-*O*-α-L-rhamnopyranosyl(1‴→2″)-β-D-glucopyranoside (24 mg), apigenin 5-*O*-α-L-rhamnopyranosyl(1‴→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 C_{18} HPLC column (MeCN−H_2O, 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 C_{18} HPLC column (YMC-Pack Ph, 10 × 250 mm, 5 μm, 2 mL/min, MeCN−H_2O, 10:90 → 40:60) to give (8*R*,8′*S*,7*S*)-4′-(3″-methoxyrhamnopyranosyl)oxy-8′-hydroxy-3,3′,4-trimethoxy-8-hydroxymethyl-lign-7–9′-lactone (**5**) (6.8 mg), (2*R*,3*R*)-6-methylaromadendrin 3-*O*-β-D-glucopyranoside (**1**, 21 mg), isoscuerllarein 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 C_{18} HPLC (YMC-Pack Ph, 10 × 250 mm, 5 μm, 2 mL/min, MeCN−H_2O, 20:80 → 50:50) to give aromadendrin (9 mg), apigenin (**6**, 24 mg), and isoscutellarein (7.9 mg).

(2*R*,3*R*)-6-Methylaromadendrin 3-*O*-β-D-glucopyranoside (1): yellowish powder; mp 166–168 °C; $[\alpha]^{24}_D$ −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) $[\theta]_{257}$ +2798, $[\theta]_{294}$ −14787, $[\theta]_{345}$ +3835; IR (KBr) ν_{max} 3364, 1653, 1604, 1504, 1359, 1299, 1199 cm^{-1}; ^1H NMR (CD_3OD, 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-C*H*$_3$); ^{13}C NMR (CD_3OD, 100 MHz) see Table 1; HMBC H-2/C-3, 4, 9, 1′, 2′, 6′; H-3/C-4, 10, 1′; 6-C*H*$_3$/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-3; positive ion FABMS *m/z* 487 $[M + Na]^+$, 465 $[M + H]^+$; HRFABMS *m/z* 487.1216 (calcd for $C_{22}H_{24}O_{11}Na$ $[M + Na]^+$, 487.1217).

Apigenin 5-*O*-α-L-rhamnopyranosyl(1‴→2″)[6″-*O*-acetyl]-β-D-glucopyranoside (2): yellowish powder; mp 117–120 °C; $[\alpha]^{24}_D$ −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, OCOC*H*$_3$), 1.02 (3H, d, *J* = 6.0 Hz, H-6‴); ^{13}C 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″-OCOC*H*$_3$/C-6″; H-1‴/C-2″; positive ion FABMS *m/z* 643 $[M + Na]^+$, 621 $[M + H]^+$; HRFABMS *m/z* 643.1639 (calcd for $C_{29}H_{32}O_{15}Na$ $[M + Na]^+$, 643.1640).

Isoscutellarein 5-*O*-β-D-glucopyranoside (3): yellowish powder; mp 153–156 °C; $[\alpha]^{24}_D$ −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″); ^{13}C 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 $C_{21}H_{21}O_{11}$ $[M + H]^+$, 449.1084).

11-Hydroxyhainanolidol (4): colorless, amorphous solid; $[\alpha]^{24}_D$: −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 (CD_3OD, 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); ^{13}C NMR (CD_3OD, 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 (CH_2, C-13), 30.1 (CH_2, C-8), 25.6 (CH_3, C-19), 19.5 (CH_2, C-9), 17.8 (CH_3, 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 $C_{19}H_{21}O_5$ $[M + H]^+$, 329.1389).

(8*R*,8′*S*,7*S*)-4′-(3″-methoxyrhamnopyranosyl)oxy-8′-hydroxy-3,3′,4-trimethoxy-8-hydroxymethyl-lign-7–9′-lactone (5): white, amorphous solid; $[\alpha]^{24}_D$ −4.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 227 (2.12), 277 (1.68) nm; CD (*c* 0.00013, MeOH) $[\theta]_{284}$ −3514, $[\theta]_{232}$ −33726; IR (KBr) ν_{max} 3463, 2921, 1768, 1515, 1459, 1267, 1137, 1024 cm^{-1}; ^1H NMR (CD_3OD, 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′-OC*H*$_3$), 3.78 (3H, s, 4-OC*H*$_3$), 3.62 (3H, s, 3-OC*H*$_3$), 3.58 (1H, dd, *J* = 11.4, 4.8 Hz, H-9b), 3.51 (3H, s, 3″-OC*H*$_3$), 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″); ^{13}C NMR (CD_3OD, 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 (CH_2, C-9), 58.2 (CH_3, 3″-O*C*H$_3$), 57.3 (CH_3, 3′-O*C*H$_3$), 57.2 (CH_3, 4-O*C*H$_3$), 57.2 (CH_3, 3-O*C*H$_3$), 52.5 (CH, C-8), 43.3 (CH_2, 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-OC*H*$_3$/C-3; 4-OC*H*$_3$/C-4; 3′-OC*H*$_3$/C-3′; 3″-OC*H*$_3$/C-3″; positive ion FABMS *m/z* 587 $[M + Na]^+$; HRFABMS *m/z* 565.2289 (calcd for $C_{28}H_{37}O_{12}$ $[M + H]^+$ 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 (2.5×10^5 cells/cm^2) and calvaria-derived osteoblastic cells (4×10^4 cells/cm^2) was conducted in the presence of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) (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.

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