New Diarylheptanoids from *Alnus japonica* and Their Antioxidative Activity

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In the course of research on the bioactive constituents of woody plants in the Cyugoku area of Japan, a methanol extract of the leaves of *Alnus japonica* were found to have strong antioxidative activity. Ethyl acetate soluble and *n*-buthanol soluble fractions of the methanol extract had a potent antioxidative effect. Both fractions were purified by silica gel column chromatography and HPLC using an ODS column to give four new diarylheptanoids along with known diarylheptanoids and flavonoids. These new compounds were elucidated to be 7-(3,4-dihydroxyphenyl)-5-hydroxy-1-(4-hydroxyphenyl)-3-heptanone-5-*O*-β-D-xylopyranoside (1), 1-(3,4-dihydroxyphenyl)-5-hydroxy-7-(4-hydroxyphenyl)-3-heptanone-5-*O*-β-D-xylopyranoside (2), 1,7-bis-(3,4-dihydroxyphenyl)-5-hydroxy-3-heptanone-5-*O*-[2-(2-methylbutenoyl)]-β-D-xylopyranoside (3) and 1,7-bis-(3,4-dihydroxyphenyl)-5-methoxy-3-heptanone (4) using spectral methods and especially ^{1}H-, ^{13}C-NMR and 2D-NMR measurements. The isolated compounds including their main constituent, oregonin (5), were tested for antioxidative activity. Some of these compounds having two catechol structures showed potent antioxidative activity. Compounds having one catechol structure showed moderate antioxidative activity, but a peracetate of 5 having no catechol structure exhibited no antioxidative activity. Thus the catechol structure of the diarylheptanoids is indispensable for antioxidative activity.

Key words *Alnus japonica*; Betulaceae; diarylheptanoid; antioxidant; oregonin

Phytochemical, constitutional and biological studies on plant constituents are less popular in woody plants than herbal plants. But woody plants have a similar potential for bioresources as herbal plants, having already yield useful bioactive compounds such as taxol.[1] In a bioassay-guided study of woody plants in Japan using biological tests for antioxidative activity, anti-tumor promoting activity, nerve cell elongation activity and cell differentiation activity, we isolated antibacterial compounds from *Abies sachalinensis*[2] and *Chaenocyparis pisifera*,[3] and anti-tumor promoting compounds from *Chaenomeles sinensis*.[4] *Alnus japonica* provided one of the most potent antioxidative samples. A variety of pathologies including cancer and cardiovascular disease have been linked to the generation of reactive oxygen species.[5] Antioxidants may prevent oxidative damage by scavenging reactive oxygen species. Phenolic compounds are recognized to have antioxidative properties. Various phenolic compounds including flavonoids, lignoids, stylbenoids, tannnins and diarylheptanoids are distributed in the plant kingdom. So the phenolic constituents of plants are of imterest as potential chemopreventive agents. *A. japonica* (Betulaceae, Japanese name; Hannoki) is a common tree in low mountainous areas of Japan. Compounds isolated from *Alnus* plants include numerous diarylheptanoids along with triterpenoids.[6—10] Many kinds of diarylheptanoids have also been isolated from Zingiberaceae[11—13] and Betulaceae plants, including some which showed inhibitiory activity toward cyclooxygenase-2,[14] and antiemetic activity.[15] Curcumin, one of the best known diarylheptanoids, has various biological properties including anticancer,[16] antiinflammatory[17] and antioxidative activities.[18] In the present study, leaves of *A. japonica* were treated with methanol to obtain an extract which showed strong antioxidative activity. Ethyl acetate (EtOAc) soluble and *n*-buthanol (*n*-BuOH) soluble fractions of the methanol extract were then fractionated by silica gel column chromatography and HPLC using a reversed phase column according to the biological activity to give five known diarylheptanoids and four new diarylheptanoids along with three known flavone derivatives. The structures of these compounds were elucidated using spectral methods including ^{1}H-, ^{13}C-NMR and 2D-NMR. The antioxidative activity of these compounds is discussed.

Results and Discussion

Compounds **5**—**11** isolated from the AcOEt soluble fraction were identified as oregonin (**5**),[19] hirsutanonol-5-*O*-β-D-glucopyranoside (**6**),[19] hirsutanonol (**7**),[19] platyphyllonol-5-*O*-β-D-xylopyranoside (**8**),[20] afzelin (**9**), quercitrin (**10**) and isohyperoside (**11**), based on comparisons of the NMR data of the compounds with reported data. The optical rotation ($[\alpha]_D$ −16.9°) of the key compound (**5**) was the same as that reported optical rotation ($[\alpha]_D$ −18.6°).[7] This indicated that **5** was identical in structure including stereochemistry to the authentic oregonin. Oregonin (**5**) was also isolated from the *n*-BuOH soluble fraction of the plant.

Compound **1** was found to have the molecular formula $C_{24}H_{30}O_9$ by high resolution fast atom bombardment mass spectrometry (HR-FAB-MS), *m/z* 463.1965 $[M+H]^+$. The ^{1}H-NMR spectrum showed the presence of a 4-oxyphenyl group [δ 6.67 (2H, d, J=8.5 Hz), 6.99 (2H, d, J=8.5 Hz)], a 3,4-dioxyphenyl group [δ 6.47 (1H, dd, J=7.5, 2.0 Hz), 6.60 (1H, d, J=2.0 Hz), 6.64 (1H, d, J=7.5 Hz)], the same C_7-moiety [δ 2.74 (4H, br s), 2.79 (1H, dd, J=16.0, 6.5 Hz), 2.57 (1H, dd, J=16.0, 5.0 Hz), 4.10 (1H, br qui, J=5.0 Hz), 1.72 (1H, m), 1.77 (1H, m), 2.50 (2H, m)] as that of oregonin (**5**), and a xylopyranosyl moiety [δ 4.21 (1H, d, J=8.0 Hz),

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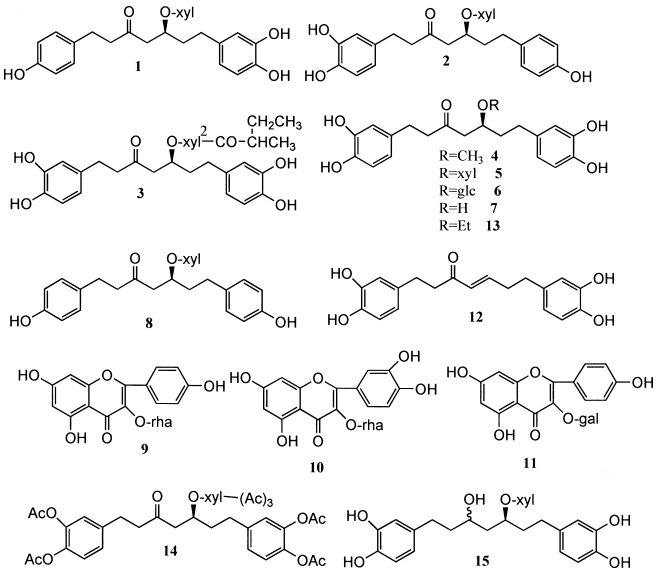

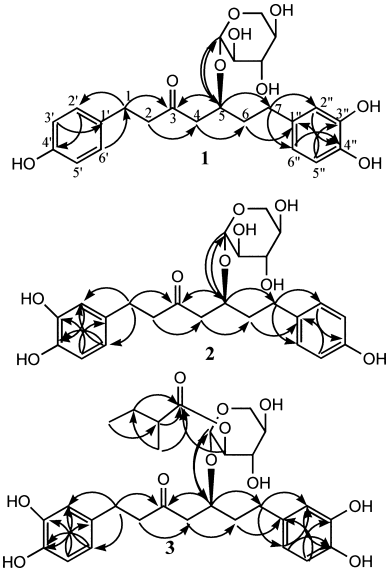

Fig. 1. Selected HMBC Correlations of **1**, **2**, **3**

3.12 (1H, dd, J=9.0, 7.5 Hz), 3.29 (1H, t, J=9.0 Hz), 3.41 (1H, ddd, J=10.5, 8.5, 5.5 Hz), 3.86 (1H, dd, J=11.5, 5.5 Hz), 3.16 (1H, dd, J=11.5, 10.0 Hz)]. The ^{13}C-NMR data also showed the presence of 4-oxyphenyl (δ 156.6, 134.3, 130.3×2, 116.2×2) and 3,4-dioxyphenyl (δ 146.1, 144.2, 134.0, 120.7, 116.6, 116.3) moieties, a carbonyl group (δ 211.8), a secondary carbinyl group (δ 76.2), five methylene groups (δ 48.7, 46.5, 38.6, 31.8, 29.8) and a xylopyranosyl moiety (δ 104.3, 77.9, 75.1, 71.3, 67.9) the same as **5**. These results and the FAB-MS data indicated that **1** had the structure of a diarylheptanoid with a 4-hydroxyphenyl, a 3,4-dihydroxyphenyl and the same C_7-moiety as oregonin (**5**). The positions of the two phenyl groups were determined in a heteronuclear multiple bond connectivity (HMBC) experiment (Fig. 1) in which H-1 showed a correlation with C-3, C-2′ and C-6′; H-2′ and H-6′ showed a correlation with C-1 and C-4′; H-7 showed a correlation with C-5, C-2″ and C-6″; anomeric H showed a correlation with C-5; H-5 showed a correlation with the anomeric carbon and C-3; and H-6″ showed a correlation with C-7, C-2″ and C-4″. The absolute configuration at C-5 was taken to be the same with that of **5** from a optical rotation ($[\alpha]_D$ −19.5°), which was identical with the reported data ($[\alpha]_D$ −18.6°)[7] of **5**, and the ^{13}C-chemical shifts of the xylopyranoside and carbons around C-5 were almost the same as those of **5** as shown in Table 1. Thus the structure of **1** was determined to be (5*S*)-7-(3,4-dihydroxyphenyl)-5-hydroxy-1-(4-hydroxyphenyl)-3-heptanone-5-*O*-β-D-xylopyranoside. Compound **1** was named alnuside A.

Compound **2** was found to have the molecular formula $C_{24}H_{30}O_9$ by HR-FAB-MS, *m/z* 463.1978 $[M+H]^+$. The ^{1}H-NMR spectrum had almost the same signal pattern as that of **1**. The ^{13}C-NMR spectrum also showed almost the same signal pattern as that of **1**. These findings indicated that **2** was an isomer of **1** in terms of the position of the two phenyl groups, a 4-hydroxyphenyl and a 3,4-dihydroxyphenyl group. Positions of the phenyl groups were determined in a HMBC experiment as shown in Fig. 1. H-1 showed a correlation with C-2′, C-6′ and C-3; H-2′ showed a correlation with C-3′, C-4′ and C-1; H-6′ showed a correlation with C-2′, C-4′ and C-1; H-5 showed a correlation with the anomeric carbon, C-3 and C-7; H-6 showed a correlation with C-7, C-5 and C-1″; the anomeric H showed a correlation with C-5; and H-2″ and H-6″ showed a correlation with C-7 and C-4″. The $[\alpha]_D$ and ^{13}C-NMR values around xylopyranosyl and C-5 were identical with those of **5**. Thus **2** was determined to be (5*S*)-1-(3,4-dihydroxyphenyl)-5-hydroxy-7-(4-hydroxyphenyl)-3-heptanone-5-*O*-β-D-xylopyranoside. Compound **2** was named alnuside B.

1 and **2** were isolated from *A. serrulatoides* as a mixture and then purified as trimethyl ethers. No spectral data of **1** and **2** were obtained. Structures of the trimethyl ethers were determined from the 1D-NMR data and MS fragmentation without 2D-NMR data.[21] Thus the structure of neither **1** nor **2** was clearly elucidated. We determined the structures of the compounds separately based on spectral data including 2D-NMR measurements.

Compound **3** was found to have the molecular formula $C_{29}H_{38}O_{11}$ by HR-FAB-MS, *m/z* 563.2490 $[M+H]^+$. The ^{1}H-NMR spectrum showed the presence of two 3,4-dihydroxyphenyl groups, the same C_7-moiety as that of **5**, and a xylopyranoside to which an acyl group was linked. The acyl part attached to the xylopyranosyl moiety was elucidated from the ^{1}H-, ^{13}C-NMR and HMBC experiments. The ^{1}H-NMR spectrum of **3** showed an extra ethyl [δ 0.93 (3H, t, J=7.5 Hz), 1.48 (1H, m), 1.66 (1H, m)], a doublet methyl group [δ 1.11 (3H, d, J=7.0 Hz)] and a methine group [δ 2.36 (1H, six, J=7.0 Hz)]. The ^{13}C-NMR spectrum showed the presence of two methyl groups (δ 11.8, 16.9), a methylene group (δ 27.8), a methine group (δ 42.2) and an ester carbonyl group (δ 177.4). The data indicated that the acyl group should be a 2-methylbutanoyl (MeBu) group. The acyl structure and position of the acyl group were confirmed by performing HMBC experiments, in which the anomeric H of xylopyranosyl moiety showed a correlation with C-5, xyl-2, xyl-3 and xyl-5 carbon; the xyl-2 proton showed the correlation with the acyl carbonyl carbon; the triplet methyl group showed a correlation with at C-2 and C-3 of 2-methylbutanoyl moiety; the multiplet methin carbon of MeBu moiety showed a correlation with C-1, C-3, C-4 and C-5 of MeBu

Table 1. ^{13}C-NMR Data for **1**—**5** Isolated from *A. japonica* (125 MHz, in CD_3OD)[a)]

Carbon	**1**	**2**	**3**	**4**	**5**
C-1	29.8	30.1	30.7	30.5	30.1
2	46.5	46.5	46.1	46.2	46.5
3	211.8	211.8	210.7	211.5	211.8
4	48.7	48.8	48.8	48.2	48.8
5	76.2	76.2	75.5	77.8	76.3
6	38.6	38.7	38.5	36.9	38.6
7	31.8	31.5	31.6	31.5	31.7
1′	134.3	134.0	133.8	134.7	134.0
2′	130.3	116.5	116.5	116.4	116.5
3′	116.2	146.2	146.2	146.1	146.1
4′	156.6	144.5	144.5	144.4[b)]	144.5
5′	116.2	116.4	116.2	116.3	116.2
6′	130.3	120.6	120.5	120.5	120.5
1″	134.0	134.3	135.1	133.9	135.1
2″	116.3	130.3	116.6	116.4	116.6
3″	146.1	116.1	146.0	146.1	146.0
4″	144.2	156.3	144.1	144.2[b)]	144.1
5″	116.6	116.1	116.4	116.3	116.3
6″	120.7	130.3	120.6	120.5	120.6
Xyl-1	104.3	104.3	101.8		104.3
2	75.1	75.1	74.8		75.1
3	77.9	77.9	76.2		77.9
4	71.3	71.3	71.5		71.2
5	67.0	67.0	66.8		66.9
MeBu-1			177.4		
2			42.2		
3			27.8		
4			11.8		
5			16.9		
OMe				57.0	

a) Assignments were made by HMQC and HMBC spectra. *b*) Signals in same column may be interchanged. Xyl is β-D-xylopyranosyl group. MeBu is 2-methylbutanoyl group.

Table 2. Radical Scavenging Activities of Compounds Isolated from *Alnus japonica*

Compound	Superoxide radical scavenging activity (%) at 3.125 μg/ml	DPPH radical scavenging activity (%) at 6.25 μg/ml
1	14.0	29.0
2	14.5	31.1
3	19.2	31.5
5	33.0	41.9
6	3.4	3.9
7	36.9	40.5
8	1.4	2.0
9	5.5	9.4
10	15.6	11.6
Curcumin[a)]	16.1	50.0

Each value represents the mean of triplicate assays. *a*) Use as a positive control.

Table 3. Radical Scavenging Activities of Oregonin and Its Derivatives

Compound	IC_{50} (μg/ml)	
	Superoxide radical scavenging activity	DPPH radical scavenging activity
4	2.8	2.9
5	2.2	4.6
7	3.0	3.1
12	1.2	2.4
13	2.9	4.3
14	31.0	n.d. (at 10 μg/ml)
15a	2.8	4.1
15b	4.2	7.3

n.d.; activity was not detectable at the concentration indicated.

moiety; the methylene protons showed a correlation with C-1, C-2, C-4 and C-5 of MeBu moiety; and the doublet methyl protons showed a correlation with C-1, C-2 and C-3 of MeBu moiety. The ^{13}C-NMR spectrum of carbons around C-5 exhibited almost the same chemical shifts as those of **5** (shown in Table 1). Thus **3** was elucidated to be (5*S*)-1,7-bis-(3,4-dihydroxyphenyl)-5-hydroxy-3-heptanone-5-*O*-[2-(2-methylbutenoyl)]-β-D-xylopyranoside, but the absolute configuration at C-2 of MeBu moiety could not be determined. Compound **3** was named alnuside C.

Compound **4** was found to have the molecular formula $C_{20}H_{24}O_6$ by FAB-MS, *m/z* 361 $[M+H]^+$. The ^{1}H-NMR spectrum showed the presence of two 3,4-dihydroxyphenyl groups, the same C7-moiety as that of hirsutanonol, and an alcoholic methoxyl group [δ 3.25 (3H, s) in the ^{1}H-NMR spectrum and δ 57.0 in the ^{13}C-NMR spectrum]. The data indicated **4** to be 5-*O*-methylhirustanonol. It was identified as a product derived from the methanolysis of **5** in an acidic methanol solution. The optical rotation of **4** ($[\alpha]_D$ +3.8°) was identical with that the product from **5** ($[\alpha]_D$ +3.6°). These results suggested that **4** might be an artifact derived from **5** by extraction of *A. japonica* with methanol under reflux. **4** was obtained as an optically active compound, so the configuration at C-5 must be the opposite of that of **5**, because **4** should be transformed from **5** through the *SN*2 reaction process at C-5.

The main constituent, oregonin (**5**), was hydrolyzed by acidic methanol to give 5-*O*-methylhirsutanonol (**4**) and dehydrohirsutanonol (**12**). Oregonin (**5**) gave **12** and 5-*O*-ethylhirsutanonol (**13**) when treated with acidic ethanol. Acetylation of **5** gave oregonin peracetate (**14**). Oregonin (**5**) gave a mixture of epimer alcohols (**15a**, **15b**) at C-3 following its reduction with $NaBH_4$.

The constituents and some of these derivatives were tested for anti-oxidative activity in a superoxide radical scavenging test[21)] and a DPPH radical scavenging test[22)] (see Tables 2, 3). Compounds **4**, **5**, **6**, **7**, **8**, **12**, **13** and **15** having two 3,4-dihydroxyphenyl moieties showed potent activity. Compounds **1** and **2** having a 3,4-dihydroxyphenyl and a 4-hydroxyphenyl moieties showed moderate activity, but **8** having two 4-hydroxyphenyl moieties and **14** having no free catechol structure showed no activity. Hirusutanonol glucoside (**6**) showed weak activity. These results indicated that the catechol structure was important for the potent anti-oxidative effect of biarrylheptanoids from *A. japonica* and the type of sugar moiety at C-5 was important for the activity.

Experimental

^{1}H- and ^{13}C-NMR data were measured on a JEOL α-500 NMR spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR). Chemical shifts are shown as a δ-value (ppm) with tetramethyl silane (TMS) as an internal standard, including NOE, HMQC and HMBC. HR-FAB-MS data were measured on a JEOL HX110 mass spectrometer. $[\alpha]_D$ was recorded on a JASCO P-1010 polarimeter at 20 °C. Analytical and preparative HPLCs were carried out using a reverse phase column (mightysil RP-18, Kanto Chemical Co. Ltd.) with a CH_3CN–H_2O solvent system. TLC was performed using precoated silica gel $60F_{254}$ (Merck) 200×200×0.25 mm plates for the analysis and 200×200×0.5 mm plates for the preparation of the constituents.

Plant Materials *Alnus japonica* trees were identified by Mr. T. Sano of

the Hiroshima Prefectural Forestry Research Center. Leaves of *A. japonica* were collected in September 2001 in the mountainous northern part of Hiroshima prefecture, Japan.

Extraction and Isolation Powdered leaves (1.0 kg) of *A. japonica* were extracted with methanol under reflux to give a methanol extract, which showed strong antioxidative activity. The MeOH extract was poured into water and partitioned with ethyl acetate (AcOEt) and then *n*-butanol (*n*-BuOH), to give an AcOEt layer, a *n*-BuOH layer and a water layer. The AcOEt layer and the *n*-BuOH layer showed antioxidative activity. The *n*-BuOH layer (35 g) was chromatographed on a SiO_2 column using a gradient $CHCl_3$–MeOH solvent system to give nine fractions, AJMB-1—AJMB-9. Of these fractions, AJMB-2 (6.1 g), AJMB-4 (3.7 g) and AJMB-4 (1.1 g) showed potent activity. AJMB-2 and AJMB-3 were successively purified by silica gel column chromatography and HPLC using a ODS column and 45% CH_3CN solvent to give **5** (2.9 g) and **11** (180 mg).

The AcOEt soluble fraction (90 g) was chromatographed on a silica gel column using a gradient $CHCl_3$–MeOH solvent system to give seven fractions. Fr. 3 (6.2 g) was purified by repeated HPLC using a 30% CH_3CN solvent system to give **4** (180 mg). Fr. 4 (8.4 g) was purified by HPLC using an ODS column and preparative TLC, successively to give **3** (88 mg), **6** (10 mg), **7** (43 mg), **8** (74 mg) and **9** (7 mg). Fr. 5 (14.7 g) was purified by repeated HPLC using an ODS column and preparative TLC, successively to give **1** (38 mg), **2** (35 mg), **5** (390 mg), **9** (25 mg), **10** (170 mg) and **11** (45 mg). Fr. 6 (16.2 g) was purified by HPLC using an ODS column to give **5** (1.1 g).

Alnuside A (**1**): Colorless viscous liquid, HR-FAB-MS: *m/z* 463.1965 $[M+H]^+$ (Calcd 463.1968 for $C_{24}H_{31}O_9$. $[\alpha]_D$ −19.5° (*c*=0.03, MeOH). ^{1}H-NMR (CD_3OD): δ 1.72 (1H, m, H-6), 1.77 (1H, m, H-6), 2.50 (2H, m, H-7), 2.57 (1H, dd, *J*=17.0, 5.5 Hz, H-4), 2.74 (4H, s, H-1, H-2), 2.79 (1H, dd, *J*=17.0, 7.0 Hz, H-4), 3.12 (1H, dd, *J*=9.0, 7.5 Hz, xyl-2), 3.16 (1H, dd, *J*=11.5, 10.0 Hz, xylH-5), 3.29 (1H, t, *J*=9.0 Hz, xyl-3), 3.47 (1H, ddd, *J*=10.5, 8.5, 5.5 Hz, xyl-4), 3.86 (1H, dd, *J*=11.5, 5.5 Hz, xyl-5), 4.10 (1H, br qui, *J*=5.0 Hz, H-5), 4.21 (1H, d, *J*=8.0 Hz, xyl-1), 6.47 (1H, dd, *J*=7.5, 2.0 Hz, H-6″), 6.60 (1H, d, *J*=2.0 Hz, H-2″), 6.64 (1H, d, *J*=7.5 Hz, H-5″), 6.67 (2H, d, *J*=8.5 Hz, H-3′, 5′), 6.99 (2H, d, *J*=8.5 Hz, H-2′, 6′). ^{13}C-NMR data are shown in Table 1.

Alnuside B (**2**): Colorless viscous liquid. HR-FAB-MS: *m/z* 463.1978 $[M+H]^+$ (Calcd 463.1968 for $C_{24}H_{31}O_9$). $[\alpha]_D$ −15.5° (*c*=0.04, MeOH). ^{1}H-NMR (CD_3OD): δ 1.72 (1H, m, H-6), 1.77 (1H, m, H-6), 2.5 (2H, m, H-7), 2.57 (1H, dd, *J*=17.0, 5.5 Hz, H-4), 2.69 (2H, br t, *J*=5.5 Hz, H-2), 2.73 (2H, br t, *J*=5.5 Hz, H-1), 2.79 (1H, dd, *J*=17.0, 7.0 Hz, H-4), 3.12 (1H, dd, *J*=9.0, 8.0 Hz, xyl-2), 3.16 (1H, dd, *J*=11.5, 10.0 Hz, xyl-5), 3.29 (1H, t, *J*=9.0 Hz, xyl-3), 3.47 (1H, ddd, *J*=10.5, 8.5, 5.5 Hz, xyl-4), 3.84 (1H, dd, *J*=11.5, 5.5 Hz, xyl-5), 4.10 (1H, br qui, *J*=5.0 Hz, H-5), 4.21 (1H, d, *J*=8.0 Hz, xyl-1), 6.47 (1H, dd, *J*=7.5, 2.0 Hz, H-6′), 6.60 (1H, d, *J*=2.0 Hz, H-2′), 6.64 (1H, d, *J*=7.5 Hz, H-4′), 6.67 (2H, d, *J*=8.5 Hz, H-3″, 5″), 6.99 (2H, d, *J*=8.6 Hz, H-2″, 6″). ^{13}C-NMR data are shown in Table 1.

Alnuside C (**3**): Colorless viscous liquid. HR-FAB-MS: *m/z* 563.2490 $[M+H]^+$ (Calcd 563.2493 for $C_{29}H_{39}O_{11}$). $[\alpha]_D$ −15.6° (*c*=0.04, MeOH). ^{1}H-NMR (CD_3OD): δ 0.93 (3H, t, *J*=7.5 Hz, MeBu-4), 1.11 (3H, d, *J*=7.0 Hz, MeBu-5), 1.48 (1H, m, MeBu-3), 1.55 (1H, m, H-6), 1.66 (1H, m, MeBu-3), 1.68 (1H, m, H-6), 2.36 (1H, six, *J*=7.0 Hz, MeBu-2), 2.47 (3H, overlap, H-2, 4), 2.5 (2H, m, H-7), 2.64 (3H, overlap, H-1, 4), 3.19 (1H, dd, *J*=11.5, 10.0 Hz, xyl-5), 3.40 (1H, t, *J*=9.5 Hz, xyl-3), 3.53 (1H, ddd, *J*=10.8, 8.5, 5.5 Hz, xyl-4), 3.88 (1H, dd, *J*=11.5, 5.5 Hz, xyl-5), 4.07 (1H, br qui, *J*=5.5 Hz, H-5), 4.39 (1H, d, *J*=7.2 Hz, xyl-1), 4.63 (1H, dd, *J*=9.5, 7.5 Hz, xyl-2), 6.46 (1H, dd, *J*=8.5, 2.5 Hz, H-6″), 6.48 (1H, dd, *J*=7.5, 2.5 Hz, H-6′), 6.59 (1H, d, *J*=2.5 Hz, H-2″), 6.60 (1H, d, *J*=2.5 Hz, H-2′), 6.64 (1H, d, *J*=8.5 Hz, H-5″), 6.66 (1H, d, *J*=7.5 Hz, H-5′). ^{13}C-NMR data are shown in Table 1.

5-*O*-Methylhirusutanonol (**4**): Colorless viscous liquid. FAB-MS: *m/z* 361 $[M+H]^+$ $C_{20}H_{25}O_6$. $[\alpha]_D$ +3.8° (*c*=0.07, MeOH). ^{1}H-NMR (CD_3OD): δ 1.65 (1H, m, H-6), 1.72 (1H, m, H-6), 2.45 (2H, m, H-7), 2.48 (1H, dd, *J*=16.2, 5.4 Hz, H-4), 2.65 (1H, dd, *J*=16.2, 7.2 Hz, H-4), 2.68 (4H, br s, H-1, 2), 3.25 (3H, s, OMe), 3.62 (1H, qui, *J*=6.6 Hz, H-5), 6.46 (1H, dd, *J*=8.4, 1.8 Hz, H-6″), 6.48 (1H, dd, *J*=8.4, 1.8 Hz, H-6′), 6.59 (1H, *J*=1.8 Hz, H-2″), 6.60 (1H, *J*=1.8 Hz, H-2′), 6.64 (1H, d, *J*=8.4 Hz, H-5′), 6.65 (1H, d, *J*=8.4 Hz, H-5″). ^{13}C-NMR data are shown in Table 1.

Oregonin (**5**): Pale yellow viscous liquid. FAB-MS: *m/z* 479 $[M+H]^+$ $C_{24}H_{31}O_{10}$. $[\alpha]_D$ −16.9° (*c*=0.12, MeOH). ^{1}H-NMR (CD_3OD): δ 1.71 (1H, m, H-6), 1.76 (1H, m, H-6′), 2.46 (1H, ddd, *J*=13.8, 9.6, 6.2 Hz, H-7), 2.53 (1H, ddd, *J*=13.8, 10.0, 5.3 Hz, H-7), 2.56 (1H, dd, *J*=16.8, 5.2 Hz, H-4), 2.70 (2H, overlap, H-1), 2.70 (2H, overlap, H-2), 2.78 (1H, dd, *J*=16.8, 6.8 Hz, H-4), 3.11 (1H, dd, *J*=9.2, 7.9 Hz, xyl-2), 3.16 (1H, dd, *J*=11.3, 10.6 Hz, xyl-5), 3.28 (1H, t, *J*=9.2 Hz, xyl-3), 3.46 (1H, ddd, *J*=10.3, 8.9, 5.5 Hz, xyl-4), 3.84 (1H, dd, *J*=11.3, 5.5 Hz, xyl-5), 4.09 (1H, qui, *J*=6.6 Hz, H-5), 6.47 (1H, dd, *J*=7.9, 2.0 Hz, H-6″), 6.48 (H, dd, *J*=7.9, 2.0 Hz, H-6′), 6.60 (1H, d, *J*=2.0 Hz, H-2″), 6.61 (1H, d, *J*=2.0 Hz, H-2′), 6.63 (1H, d, *J*=7.9 Hz, H-5″), 6.64 (1H, d, *J*=7.9 Hz, H-5′). ^{13}C-NMR data are shown in Table 1.

Treatment of 5 with Acidic Methanol Fifty milligrams of **5** was dissolved in 5% HCl–MeOH (10 ml) and stood for 1 h. The reaction solution was poured into 100 ml of ice-water and passed through a HP 20 column, thoroughly washed with water, and eluted with methanol to give 35 mg of product. The crude product was purified by preparative HPLC to give two compounds, **4** (15 mg) and **12** (5 mg).

12: HR-FAB-MS: *m/z* 329.1379 $[M+H]^+$ (Calcd 329.1389, $C_{19}H_{21}O_5$). ^{1}H-NMR (CD_3OD): δ 2.46 (2H, br q, *J*=7.5 Hz, H-6), 2.61 (2H, t, *J*=7.5 Hz, H-7), 2.71 (2H, t, *J*=7.0 Hz, H-2), 2.79 (2H, t, *J*=7.0 Hz, H-1), 6.06 (1H, d, *J*=15.5 Hz, H-4), 6.48, 6.49 (each 1H, dd, *J*=8.0, 1.5 Hz, H-6′, 6″), 6.60 (each 2H, d, *J*=1.5 Hz, H-2′, 2″), 6.65, 6.66 (each 1H, d, *J*=8.0 Hz, H-5′, 5″), 6.87 (1H, dt, *J*=15.5, 7.5 Hz, H-5). ^{13}C-NMR ($CDCl_3$): δ 30.9 (C-6), 34.8 (C-7), 36.3 (C-2), 45.4 (C-1), 116.3×2, 116.5×2 (C-2′, 5′, 2″, 5″), 120.5, 120.6 (C-6′, 6″), 131.5 (C-4), 133.8, 134.0 (C-1′, 1″), 144.4, 144.5 (C-3′, 3″), 146.1, 146.2 (C-4′, 4″), 149.2 (C-5), 202.8 (C-3).

Treatment of 5 with Acidic Ethanol Fifty milligrams of **5** was dissolved in 3% HCl–EtOH (10 ml) and stood for 1 h. The reaction solution was poured into 100 ml of ice-water and passed through a HP 20 column, thoroughly washed with water, and eluted with methanol to give 35 mg of product. The crude product was purified by preparative HPLC (ODS column, 35% CH_3CN) to give **12** (8 mg) and **13** (15 mg).

13: HR-FAB-MS: *m/z* 375.1777 $[M+H]^+$ (Calcd 375.1785, $C_{21}H_{27}O_6$). ^{1}H-NMR ($CDCl_3$): δ 1.11 (3H, t, *J*=7.0 Hz, CH_3 of ethoxyl), 1.67 (2H, m, H-6), 2.47 (2H, overlap, H-7), 2.49 (1H, overlap, H-4), 2.64 (1H, dd, *J*=16.0, 6.0 Hz, H-4) 2.69 (4H, overlap, H-1, 2), 3.33 (2H, m, CH_2 of ethoxyl), 6.60, 6.61 (1H each, d, *J*=1.5 Hz, H-2′, 2″), 6.47, 6.48 (1H each, dd, *J*=8.0, 1.5 Hz, H-6′, 6″), 6.65, 6.66 (1H each, d, *J*=8.0 Hz, H-5′, 5″). ^{13}C-NMR ($CDCl_3$): δ 15.8 (CH_3 of ethoxyl), 30.1 (C-1), 31.8 (C-7), 37.5 (C-6), 46.8 (C-2), 48.8 (C-4), 65.6 (CH_2 of ethoxyl), 76.4 (C-5), 116.3, 116.4 (C-5′, 5″), 116.5×2 (C-2′, 2″), 120.6×2 (C-6′, 6″), 134.1, 134.9 (C-1′, 1″), 144.3, 144.5 (C-3′, 3″), 146.2×2 (C-4′, 4″), 211.7 (C-3).

Acetylation of 5 Fifty-four milligrams of **5** was dissolved in pyridine (1 ml) and acetic anhydride (1 ml) and stood for 4 h at room temperature. The reaction solution was poured into ice-water and extracted with $CHCl_3$. The $CHCl_3$ solution was washed successively with dil-HCl aq. soln, sat-$NaHCO_3$ aq. soln and water. The $CHCl_3$ solution was evaporated to give 90 mg of crude product. The crude product was purified by preparative TLC to give a per acetate (**14**) (60 mg).

14: MS: *m/z* 773 $[M+H]^+$ $C_{38}H_{44}O_{17}$, ^{1}H-NMR ($CDCl_3$): δ 1.83 (2H, m, H-7), 2.01, 2.02, 2.04 (3H each, s, Ac on xyl), 2.27, 2.28 (6H each, s, Ac on benzene rings), 2.40 (1H, dd, *J*=16.5, 4.0 Hz, H-4), 2.66 (1H, overlap, H-4), 2.67 (4H, overlap, H-1, 2), 3.32 (1H, dd, *J*=12.0, 9.5 Hz, xyl-5), 4.08 (1H, dd, *J*=12.0, 5.0 Hz, xyl-5), 4.19 (1H, m, H-5), 4.55 (1H, d, *J*=7.0 Hz, xyl-1), 4.86 (1H, dd, *J*=9.5, 7.0 Hz, xyl-2), 4.95 (1H, dt, *J*=5.0, 9.5 Hz, xyl-4), 5.17 (1H, t, *J*=9.5 Hz, xyl-3), 6.99 (1H, *J*=2.0 Hz, H-2′ or 2″), 7.01 (1H, d, *J*=2.0 Hz, H-2″ or 2′), 7.02—7.10 (4H, overlap, H-5′, 6′, 5″, 6″). ^{13}C-NMR ($CDCl_3$): δ 20.6×3 (Mes of acetyl groups), 20.7×4 (Mes of acetyl groups), 28.6 (C-1), 30.3 (C-17), 36.4 (C-16), 45.0 (C-2), 47.5 (C-4), 62.2 (xyl-5), 69.1, 71.3, 71.9 (xyl-2, 3, 4), 75.3 (C-5), 168.2, 168.3×2, 168.4, 168.5, 168.8, 170.0 (COs of acetyl groups), 206.9 (C-3).

$NaBH_4$ Reduction of 5 Sixty milligrams of **5** was dissolved in 10 ml of MeOH, and 20 mg of $NaBH_4$ was added and stirred for 1 h at room temperature. The reaction solution was poured into water and passed through a HP 20 column, washed with water and eluted with MeOH to give a MeOH eluate (42 mg). The MeOH eluate was purified by HPLC (ODS column, 40% CH_3CN) to give two compounds **15a** (9 mg) and **15b** (7 mg). **15a** and **15b** should be epimers at C-3 each other, but their configurations at C-3 of these compound have not been determined.

15a: FAB-MS: *m/z* 503 $[M+H]^+$ $C_{24}H_{33}O_{10}$. ^{1}H-NMR (CD_3OD): δ 1.61—1.82 (5H, m, overlap H-2, H-4, H-6), 2.46—2.61 (4H, m, overlap, H-1, H-7), 3.15 (1H, dd, *J*=9.5, 7.5 Hz, xyl-2), 3.17 (1H, dd, *J*=11.5, 10.0 Hz, xyl-5), 3.29 (1H, t, *J*=9.5 Hz, xyl-3), 3.49 (1H, ddd, *J*=10.5, 9.5, 5.5 Hz, xyl-4), 3.67 (1H, m, H-3), 3.80 (1H, quin, *J*=6.5 Hz, H-5), 3.86 (1H, dd, *J*=11.5, 5.5 Hz, xyl-5), 4.22 (1H, d, *J*=7.5 Hz, xyl-1), 6.48 (1H, dd, *J*=8.0, 2.0 Hz, H-6′ or H-6″), 6.50 (1H, dd, *J*=8.0, 2.0 Hz, H-6″ or H-67), 6.61 (1H, d, *J*=2.0 Hz, H-2′ or H-2″), 6.63 (1H, d, *J*=2.0 Hz, H-2″ or H-2′), 6.64 (1H, d, *J*=8.0 Hz, H-5′ or H-5″), 6.66 (1H, d, *J*=8.0 Hz, H-5″ or H-5′). ^{13}C-NMR (CD_3OD): δ 31.7, 32.2 (C-1, C-7), 38.7 (C-6), 41.2 (C-2), 43.0 (C-4), 67.0

(xyl-5), 69.9 (C-3), 71.3 (xyl-4), 75.1 (xyl-2), 78.1 (xyl-3), 79.9 (C-5), 104.7 (C-1′), 116,3, 116.4, 116.6, 116.7 (C-2′, C-5′, C-2″, C-5″), 120.7×2 (C-6′, C-6″), 135.2, 135.5 (C-1′, C-1″), 114.1, 114.2 (C-4′, C-4″), 146.0, 146.1 (C-3′, C-3″).

Superoxide Radical Scavenging Activity Superoxide radical (O_2^-) scavenging activity was determined with the hypoxanthine–xanthine oxidase O_2^--generating system by the nitrite method modified by Ooyanagui.[21] One hundred microliters of 65 mM KH_2PO_4–35 mM Borax–0.5 mM EDTA buffer (pH 8.2), 100 μl of 0.5 mM hypoxanthine, 50 μl of 10 mM hydroxylamine hydrochloride–1 mg/ml hydroxylamine-*O*-sulfonic acid solution, 100 μl of distilled water, 50 μl of sample solution containing test compounds, and 100 μl of xanthine oxidase (14.5 mU/ml) were added successively into a 1.5 ml microtube. After the reaction mixture was incubated at 37 °C for 30 min, 1 ml of 30 μM *N*-1-naphthylethylene diamine–3 mM sulfanilic acid–25% acetic acid mixture was added. The absorbance at 550 nm was determined in a spectrophotometer (JASCO 550). Compounds tested were dissolved in methanol and diluted with water at appropriate concentrations. The final concentration of methanol in the reaction mixture was less than 1%. O_2^--Scavenging activity was evaluated as the percentage (%) of the reduction of color development.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity The free radical, DPPH, was used as an analytical reagent for radical scavengers. DPPH radical scavenging activity was determined by the method of Ahnfelt-Bønne and Nielsen[22] with a modification. A test sample (20 μl) was mixed with 980 μl of an ethanolic solution of 100 μM DPPH. After 20 min, the absorbance at 516 nm was measured in a spectrophotometer (JASCO 550). All test compounds were dissolved in methanol and diluted at appropriate concentrations. DPPH radical scavenging activity was evaluated as the percentage (%) of the reduction of absorbance.

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