New Flavonol Oligoglycosides and Polyacylated Sucroses with Inhibitory Effects on Aldose Reductase and Platelet Aggregation from the Flowers of *Prunus mume*[1]

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The methanolic extract from the fresh flowers of *Prunus mume* exhibited inhibitory effects against aldose reductase and platelet aggregation. From the methanolic extract, two new flavonol oligoglycosides, 2″-*O*-acetylrutin and 2″-*O*-acetyl-3′-*O*-methylrutin, and two new polyacylated sucroses, prunoses I and II, were isolated together with 11 known constituents. The structures of 2″-*O*-acetylrutin, 2″-*O*-acetyl-3′-*O*-methylrutin, and prunoses I and II were determined on the basis of chemical and physicochemical evidence as quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-2″-*O*-acetyl-*β*-D-glucopyranoside, 3′-*O*-methylquercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-2″-*O*-acetyl-*β*-D-glucopyranoside, 1,4,3′,4′,6′-penta-*O*-acetyl-6-*O*-*p*-coumaroylsucrose, and 1,3′,4′,6′-tetra-*O*-acetyl-6-*O*-*p*-coumaroylsucrose, respectively. The flavonol glycosides and prunose I were found to inhibit aldose reductase, while prunoses I and II inhibited platelet aggregation induced by thrombin.

Prunus (*P.*) *mume* SIEB. et ZUCC. (Rosaceae) has been widely cultivated as an ornamental plant, and its fruit is used as a food garnish (pickled ume) and drink (ume brandy) in Japan. In Chinese traditional medicine, various parts of this plant (i.e., immature fruit, leaves, branches, seeds, and roots) have been used as herbal medicines. In particular, the flowers of *P. mume* have been prescribed for detoxification, stomachic, expectorant, and sedative purposes and for the treatment of eye pain, dipsia, and skin disorders in Chinese traditional preparations. Some triterpenes, amygdalin, and prunasin have been isolated from the immature seeds of *P. mume*,[2] but the chemical constituents as well as the pharmacological properties of the flowers have yet to be characterized. In the course of our studies on the bioactive principles of medicinal flowers,[1,3] we found that the methanolic extract of the fresh flowers of *P. mume* inhibited rat lens aldose reductase and rabbit platelet aggregation induced by thrombin.

From the methanolic extract, we have isolated two new flavonol oligoglycosides, 2″-*O*-acetylrutin (**1**) and 2″-*O*-acetyl-3′-*O*-methylrutin (**2**), and two new polyacylated sucroses, prunoses I (**3**) and II (**4**), together with quercetin 3-*O*-(2″,6″-α-L-dirhamnopyranosyl)-*β*-D-galactopyranoside,[4] quercetin 3-*O*-rhamnosyl(1→6)galactoside,[5] rutin,[6] quercetin 3-*O*-neohesperidoside,[7] isorhamnetin 3-*O*-rhamnoside,[8] chlorogenic acid,[3a,9] benzyl-*β*-D-glucopyranoside,[10] benzyl alcohol xylopyranosyl(1→6)glucopyranoside,[11] chavicol *β*-D-glucoside,[12] eugenylglucoside,[13] and phytol.[14] This paper describes the isolation and structural elucidation of the new glycosides (**1**−**4**). In addition, we describe the aldose reductase inhibitory activity of the flavonol glycosides and platelet aggregation inhibitory activity of the polyacylated sucroses.

Results and Discussion

The methanolic extract from *P. mume* cultivated in Wakayama Prefecture, Japan, was partitioned into an ethyl acetate (AcOEt)−water mixture to furnish the AcOEt-soluble portion and aqueous phase. The aqueous phase was further extracted with 1-butanol (1-BuOH) to give the 1-BuOH-soluble and H_2O-soluble portions. The methanolic extract exhibited potent inhibitory activity against rat lens aldose reductase (IC_{50} 3.0 μg/mL), and its AcOEt-soluble, 1-BuOH-soluble, and H_2O-soluble portions also showed inhibitory activity (IC_{50} 1.6, 2.1, 3.2 μg/mL, respectively). In addition, the methanolic extract and 1-BuOH-soluble and H_2O-soluble portions inhibited rabbit platelet aggregation induced by thrombin (28%, 96%, and 89% inhibition at 100 μg/mL, respectively), while the AcOEt-soluble fraction showed no such activity (5% inhibition at 100 μg/mL).

The AcOEt-soluble and 1-BuOH-soluble portions were subjected to normal-phase and reversed-phase silica gel column chromatography and repeated HPLC to give **1** (0.0039%), **2** (0.00079%), **3** (0.016%), and **4** (0.0084%)

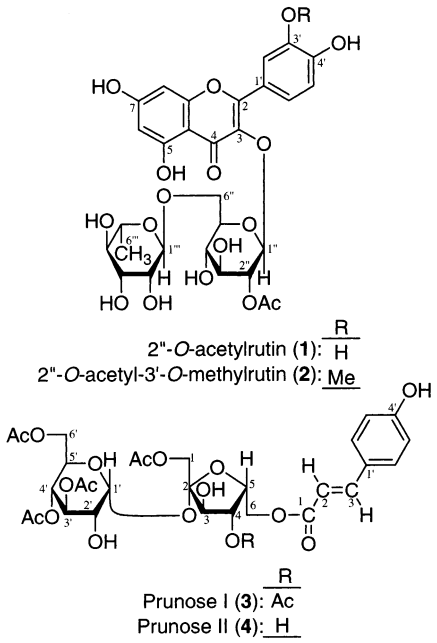

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Table 1. ^{13}C NMR Data for **1**−**4** (68 MHz in DMSO-d_6)

	1	2		3	4
C-2	156.7	156.4	sucrose-1	63.9	64.6
C-3	132.9	132.3	2	104.5	103.7
C-4	176.9	176.5	3	77.3	79.4
C-5	161.1	160.7	4	74.6	72.6
C-6	98.6	98.4	5	74.2	76.3
C-7	164.4	163.8	6	62.0	62.6
C-8	93.5	93.6	1′	91.1	90.7
C-9	156.3	156.0	2′	68.3	68.5
C-10	103.8	103.7	3′	72.2	72.4
C-1′	120.8	120.5	4′	68.2	68.7
C-2′	116.1	112.9	5′	67.6	67.6
C-3′	144.7	149.1	6′	62.1	62.6
C-4′	148.4	146.4	coumaroyl-1	164.7	165.2
C-5′	115.1	114.9	2	112.8	113.5
C-6′	121.5	122.0	3	145.6	145.3
C-1″	98.9	98.6	1′	124.6	124.9
C-2″	74.0	73.8	2′, 6′	130.2	130.2
C-3″	73.8	73.5	3′, 5′	115.5	115.6
C-4″	70.0	70.0	4′	159.7	159.8
C-5″	75.7	75.6	*C*H$_3$CO	20.6	20.7
C-6″	67.1	66.8		20.4	20.6
C-1‴	100.8	100.7		20.4	20.5
C-2‴	70.2	70.3		20.2	20.3
C-3‴	70.4	70.3		20.1	
C-4‴	71.7	71.5	CH$_3$*C*O	169.8	171.1
C-5‴	68.1	68.1		169.7	170.0
C-6‴	17.6	17.6		169.5	169.5
*C*H$_3$CO	20.9	20.8		169.3	169.2
CH$_3$*C*O	169.3	169.1		169.0	
OCH$_3$		55.3			

together with quercetin 3-*O*-(2″,6″-α-L-dirhamnopyranosyl)-β-D-galactopyranoside[4] (0.010%), quercetin 3-*O*-rhamnosyl(1→6)galactoside[5] (0.0016%), rutin[6] (0.0007%), quercetin 3-*O*-neohesperidoside[7] (0.0024%), isorhamnetin 3-*O*-rhamnoside[8] (0.0013%), chlorogenic acid[3a,9] (0.0006%), benzyl-β-D-glucopyranoside[10] (0.050%), benzyl alcohol xylopyranosyl(1→6)glucopyranoside[11] (0.0005%), chavicol β-D-glucoside[12] (0.0014%), eugenylglucoside[13] (0.050%), and phytol[14] (0.0009%).

2″-*O*-Acetylrutin (**1**) was isolated as a yellow powder with optical rotation $[\alpha]_D^{28}$ −28.9°. The IR spectrum of **1** showed absorption bands at 1655 and 1611 cm^{-1} ascribable to carbonyl functions and strong absorption bands at 3432 and 1073 cm^{-1} suggestive of an oligoglycosidic structure. In the negative and positive FABMS of **1**, quasimolecular ion peaks were observed at *m/z* 675 [M + Na]$^+$, *m/z* 653 [M + H]$^+$, and *m/z* 651 [M − H]$^-$, and the molecular formula $C_{29}H_{32}O_{17}$ was determined by HRFABMS measurement of the quasimolecular ion peak [M + Na]$^+$. The ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (Table 1) spectra of **1**, which were assigned by various NMR analytical methods,[15] showed the presence of an acetyl group [δ 2.03 (s, C*H*$_3$-CO)] and a rutin moiety [δ 0.99 (d, *J* = 6.3 Hz, H$_3$-6‴), 4.36 (br s, H-1‴), 4.77 (dd-like, H-2″), 5.40 (d, *J* = 7.9 Hz, H-1″), 6.19, 6.37 (br s, H-6, 8), 6.84 (d, *J* = 7.9 Hz, H-5′), 7.49 (d-like, H-2′), 7.52 (dd-like, H-6′), 12.60 (br s, O*H*-5)]. In the HMBC experiment of **1**, a long-range correlation was observed between the 2″-proton of the β-D-glucopyranosyl moiety and the acetyl carbonyl carbon. Alkaline treatment of **1** with 0.1% NaOMe−MeOH liberated rutin.[6] On the basis of these observations, the structure of **1** was elucidated to be quercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-2″-*O*-acetyl-β-D-glucopyranoside (2″-*O*-acetylrutin).

2″-*O*-Acetyl-3′-*O*-methylrutin (**2**), isolated as a yellow powder with optical rotation $[\alpha]_D^{27}$ −13.0°, gave quasimolecular ion peaks at *m/z* 689 [M + Na]$^+$, *m/z* 667 [M + H]$^+$, and *m/z* 665 [M − H]$^-$ in the negative and positive FABMS, and the molecular formula was defined as $C_{30}H_{34}O_{17}$ from the HRFABMS analysis. The proton and carbon signals in the ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (Table 1) spectra[15] of **2** were found to be superimposable on those of **1**, except for the signals due to the 3′-position. The ^{1}H NMR spectrum of **2** showed signals assignable to a 3′-*O*-methylrutin moiety [δ 0.97 (d, *J* = 6.1 Hz, H$_3$-6‴), 3.90 (s, OC*H*$_3$), 4.37 (br s, H-1‴), 4.75 (dd-like, H-2″), 5.50 (d, *J* = 8.1 Hz, H-1″), 6.20, 6.43 (d, *J* = 1.8 Hz, H-6, 8), 6.90 (d, *J* = 8.4 Hz, H-5′), 7.50 (dd, *J* = 1.6, 8.4 Hz, H-6′), 7.80 (d, *J* = 1.6 Hz, H-2′), 12.60 (br s, O*H*-5)] and an acetyl group [δ 2.04 (s, C*H*$_3$CO)]. Furthermore, in the HMBC experiment of **2**, a long-range correlation was observed between the 2″-proton of the glucopyranosyl moiety and the carbonyl carbon of the acetyl moiety. Upon alkaline treatment with 0.1% NaOMe−MeOH, **2** liberated 3′-*O*-methylrutin.[16] Consequently, the structure of **2** was elucidated to be 3′-*O*-methylquercetin 3-*O*-α-L-rhamnopyranosyl(1→6)-2″-*O*-acetyl-β-D-glucopyranoside (2″-*O*-acetyl-3′-*O*-methylrutin).

Prunose I (**3**) was isolated as a white powder with optical rotation $[\alpha]_D^{27}$ +26.9°. The molecular formula $C_{31}H_{38}O_{18}$ of **3** was determined from the quasimolecular ion peak in the positive FABMS *m/z* 721 [M + Na]$^+$ and by HRFABMS measurement. The ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (Table 1) spectra[15] of **3** showed the presence of five acetyl groups [δ 1.86, 1.97, 2.00, 2.04, 2.05 (s, C*H*$_3$CO × 5)], a *p*-coumaric acid moiety [δ 6.36, 7.63 (d, *J* = 16.0 Hz, H-2, 3), 6.80, 7.58 (d, *J* = 8.6 Hz, H-3′, 5′, H-2′, 6′)], and a sucrose moiety [δ 3.46 (m, H$_2$-6), 3.73 (m, H-2′), 4.12 (m, H$_2$-6′), 4.25, 4.35 (m, H$_2$-1), 4.28 (m, H-3), 4.32 (m, H-5′), 4.85 (t-like, H-4′), 5.15 (t-like, H-3′), 5.37 (d, *J* = 3.3 Hz, H-1′), 5.42 (t-like, H-4), 5.68 (br d, *J* = ca. 7 Hz, H-5)]. In addition, alkaline treatment with 0.1% NaOMe−MeOH of **3** liberated methyl *p*-coumarate and sucrose. The positions of the *p*-coumaroyl and five acetyl groups were determined on the basis of ^{1}H−^{1}H COSY and HMBC. Thus, the ^{1}H−^{1}H COSY experiment for **3** indicated the partial structures of C-3−C-6 and C-1′−C-6′ of the sucrose moiety. In the HMBC experiment of **3**, long-range correlations were observed between the 6-protons of the sucrose moiety and the *p*-coumaroyl carbonyl carbon and between the 1, 4, 3′, 4′, 6′-protons of the sucrose moiety and five acetyl carbonyl carbons. Consequently, the structure of prunose I was elucidated to be 1,4,3′,4′,6′-penta-*O*-acetyl-6-*O*-*p*-coumaroylsucrose (**3**).

Prunoses II (**4**) was isolated as a white powder with optical rotation $[\alpha]_D^{28}$ +18.7°. In the negative and positive FABMS of **4**, quasimolecular ion peaks were observed at *m/z* 679 [M + Na]$^+$ and *m/z* 655 [M − H]$^-$, and HRFABMS analysis revealed the molecular formula of **4** to be $C_{29}H_{36}O_{17}$. The ^{1}H NMR (DMSO-d_6) and ^{13}C NMR (Table 1) spectra[15] of **4** showed signals due to a *p*-coumaric acid moiety [δ 6.39, 7.63 (d, *J* = 15.9 Hz, H-2, 3), 6.80, 7.58 (d, *J* = 8.6 Hz, H-3′, 5′, H-2′, 6′)] and a sucrose moiety [δ 3.40 (m, H$_2$-6), 3.65 (m, H-2′), 3.99 (m, H-3), 4.10 (m, H-4, H$_2$-6′), 4.16, 4.34 (m, H$_2$-1), 4.25 (m, H-5′), 4.79 (t-like, H-4′), 5.10 (t-like, H-3′), 5.32 (d, *J* = 3.5 Hz, H-1′), 5.48 (br d, *J* = ca. 8 Hz, H-5)] together with four acetyl moieties [δ 1.88, 1.94, 2.04, 2.05 (s, C*H*$_3$CO × 4)]. The proton and carbon signals in the ^{1}H and ^{13}C NMR data of **4** were found to be superimposable on those of **3**, except for the signals due to the 4-position. Alkaline treatment of **4** with 0.1% NaOMe−MeOH liberated sucrose and methyl *p*-coumarate. The bonding positions of acyl groups in **4** were clarified by HMBC experiment, which showed long-range correlations between the following protons and carbons: the 6-proton and the *p*-coumaroyl carbonyl carbon, the 1, 3′, 4′, 6′-protons of the sucrose moiety, and four acetyl carbonyl

Table 2. Inhibitory Effects of Constituents of *P. mume* against Rat Lens Aldose Reductase

compound	IC_{50} (μM)[a]
2″-*O*-acetylrutin (**1**)	18
2″-*O*-acetyl-3′-*O*-methylrutin (**2**)	9.8
prunose I (**3**)	58
prunose II (**4**)	>100 (21)
quercetin 3-*O*-(2″,6″-α-L-dirhamnopyranosyl)-β-D-galactopyranoside	>30 (40)
rutin	13
quercetin 3-*O*-neohesperidoside	18
isorhamnetin 3-*O*-rhamnoside	19
epalrestat	0.072

[a] Values in parentheses represent the (%) inhibition at 30 or 100 μM.

Table 3. Inhibitory Effects of Prunoses I (**3**) and II (**4**) on Platelet Aggregation Induced by Thrombin[a]

compound	conc (mM)	inhibition (%)
prunose I (**3**)	0.1	30.5 ± 4.5
	0.3	48.1 ± 4.4
prunose II (**4**)	0.1	27.9 ± 4.9
	0.3	44.0 ± 3.4
aspirin	0.1	15.8 ± 1.9
	0.5	39.4 ± 4.6
	1.0	53.5 ± 3.5

[a] Each value represents the means± SD of four experiments.

carbons. Consequently, the structure of prunoses II was elucidated to be 1,3′,4′,6′-tetra-*O*-acetyl-6-*O*-*p*-coumaroylsucrose (**4**).

As a key enzyme in the polyol pathway, aldose reductase has been reported to catalyze the reduction of glucose to sorbitol. Sorbitol does not readily diffuse across cell membranes, and the intracellular accumulation of sorbitol has been implicated in the chronic complications of diabetes such as cataract. Previously, we reported various flavonoids with inhibitory activities against rat lens aldose reductase from several natural medicines and medicinal food.[3a,f,17] As shown in Table 2, flavonol glycosides exhibited inhibitory effects against rat lens aldose reductase. Among them, 2″-*O*-acetyl-3′-*O*-methylrutin (**2**), which was isolated as a new compound, was found to show potent inhibitory activity. Next, we examined the inhibitory effects of two polyacylated sucroses, prunoses I (**3**) and II (**4**), on rabbit platelet aggregation in vitro. As a result, **3** and **4** inhibited the aggregation of platelets induced by thrombin at concentrations of 0.1 and 0.3 mM (Table 3), and their inhibitory activities at 0.1 mM were stronger than that of aspirin. This is the first report of the inhibitory activity of polyacylated sucroses against platelet aggregation. These results indicated that flavonoids are active principles of this natural medicine for aldose reductase inhibitory activity. On the other hand, polyacylated sucroses were obtained as platelet aggregation inhibitors from this natural medicine.

Experimental Section

General Experimental Procedures. The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l = 5 cm); UV spectra, Shimadzu UV-1200 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EIMS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; FABMS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H NMR spectra, JEOL EX-270 (270 MHz) spectrometer; ^{13}C NMR spectra, JEOL EX-270 (68 MHz) spectrometer with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-6A refractive index detector.

The following experimental conditions were used for chromatography: normal-phase silica gel column chromatography, silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150−350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100−200 mesh); TLC, precoated TLC plates with silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, precoated TLC plates with silica gel RP-18 WF_{254S} (Merck, 0.25 mm); detection was achieved by spraying with 1% $Ce(SO_4)_2$−10% aqueous H_2SO_4 followed by heating.

Plant Material. The flowers of *P. mume* were cultivated by Azumanoen Co., Ltd., Wakayama, Japan, and collected in February 1999. The plant was identified by one of the authors, Y.H., president of Azumanoen Co., Ltd. A voucher of the plant is on file in our laboratory.

Extraction and Isolation. Fresh flowers of *P. mume* (3 kg) were cut and extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (250 g, 8.3%). The MeOH extract (230 g) was partitioned in a mixture of AcOEt−H_2O (1:1, v/v). The aqueous layer was extracted with 1-BuOH, and removal of the solvent in vacuo from the AcOEt-soluble and 1-BuOH-soluble portions yielded 39.2 g (1.4%) and 51.3 g (1.8%) of residue, respectively.

Normal-phase silica gel column chromatography [BW-200 (Fuji Silysia Co., Ltd., 1 kg), *n*-hexane−AcOEt (5:1−1:1, v/v)−$CHCl_3$−MeOH (10:1−1:1, v/v)−MeOH] of the AcOEt-soluble portion (35.0 g) gave eight fractions [fractions 1 (6.5 g), 2 (1.1 g), 3 (5.0 g), 4 (5.2 g), 5 (3.9 g), 6 (4.4 g), 7 (1.2 g), 8 (4.5 g)]. Fraction 2 (1.1 g) was subjected to reversed-phase silica gel column chromatography [Chromatorex ODS DM1020T (Fuji Silysia Co., Ltd., 30 g), MeOH−H_2O (70:30−90:10, v/v)] and HPLC [YMC-Pack ODS-A (YMC Co., Ltd., 250 × 20 mm i.d.), MeOH−H_2O (95:5, v/v)] to furnish phytol (22 mg, 0.0009%). Fraction 4 (5.2 g) was purified by reversed-phase silica gel column chromatography [150 g, MeOH−H_2O (65:35, v/v)] and HPLC [MeOH−H_2O (55:45, v/v)] to provide prunoses I (**3**, 391 mg, 0.016%) and II (**4**, 209 mg, 0.0084%). Fraction 6 (4.4 g) was separated by reversed-phase silica gel column chromatography [130 g, MeOH−H_2O (50:50, v/v)] and HPLC [MeOH−H_2O (45:55, v/v)] to give chavicol β-D-glucoside (36 mg, 0.0014%). Fraction 8 (4.5 g) was purified by reversed-phase silica gel column chromatography [140 g, MeOH−H_2O (50:50, v/v)] and HPLC [MeOH−H_2O (60:40, v/v)] to give 2″-*O*-acetylrutin (**1**, 40 mg, 0.0016%), 2″-*O*-acetyl-3′-*O*-methylrutin (**2**, 20 mg, 0.0008%), and chlorogenic acid (138 mg, 0.0006%).

Normal-phase silica gel column chromatography [2.0 kg, $CHCl_3$−MeOH (10:1−5:1, v/v)−$CHCl_3$−MeOH−H_2O (6:4:1, v/v)−MeOH] of the 1-BuOH-soluble portion (37.0 g) gave eight fractions [fractions 1 (2.7 g), 2 (8.5 g), 3 (7.5 g), 4 (2.5 g), 5 (3.3 g), 6 (1.1 g), 7 (3.7 g), 8 (3.5 g), 9 (5.6 g)]. Fraction 2 (843 mg) was separated by reversed-phase silica gel column chromatography [25 g, MeOH−H_2O (30:70−40:60, v/v)] and HPLC [(1) MeOH−H_2O (30:70, v/v); (2) MeOH−H_2O (50:50, v/v)] to give benzyl-β-D-glucopyranoside (142 mg, 0.050%) and eugenylglucoside (141 mg, 0.050%). Fraction 4 (2.5 g) was separated by reversed-phase silica gel column chromatography [75 g, MeOH−H_2O (30:70−50:50, v/v)] and HPLC [(1) MeOH−H_2O (55:45, v/v); (2) MeOH−H_2O (50:50, v/v); (3) MeOH−H_2O (30:70, v/v)] to give **1** (65 mg, 0.0023%), quercetin 3-*O*-rhamnosyl(1→6)-galactoside (47 mg, 0.0016%), isorhamnetin 3-*O*-rhamnoside (37 mg, 0.0013%), and benzyl alcohol xylopyranosyl(1→6)-glucopyranoside (14 mg, 0.0005%). Fraction 6 (1.0 g) was separated by reversed-phase silica gel column chromatography [30 g, MeOH−H_2O (30:70−50:50, v/v)] and HPLC [MeOH−H_2O (50:50, v/v)] to give rutin (20 mg, 0.0007%) and quercetin 3-*O*-neohesperidoside (70 mg, 0.0023%). Fraction 8 (100 mg) was separated by reversed-phase silica gel column chromatography [3 g, MeOH−H_2O (30:70, v/v)] and HPLC [MeOH−H_2O (40:60, v/v)] to give quercetin 3-*O*-(2″,6″-α-L-dirhamnopyranosyl)-β-D-galactopyranoside (52 mg, 0.010%).

The known compounds were identified by comparison of their physical data ([α]$_D$, IR, ^{1}H NMR, ^{13}C NMR) with reported values.[3−12]

2″-*O*-Acetylrutin (1): yellow powder; $[\alpha]_D^{28}$ −28.9° (*c* 0.80, MeOH); UV (MeOH) λ_{max} (log ϵ) 258 (4.47), 270 (sh) (4.40), 354 (4.36); IR (KBr) ν_{max} 3432, 1655, 1611, 1073, cm^{-1}; ^1H NMR (DMSO-d_6, 270 MHz) δ 0.99 (3H, d, J = 6.3 Hz, H_3-6‴), 2.03 (3H, s, CH_3CO), 4.36 (1H, br s, H-1‴), 4.77 (1H, dd-like, H-2″), 5.40 (1H, d, J = 7.9 Hz, H-1″), 6.19, 6.37 (1H each, both br s, H-6, 8), 6.84 (1H, d, J = 7.9 Hz, H-5′), 7.49 (1H, d-like, H-2′), 7.52 (1H, dd-like, H-6′), 12.60 (1H, br s, O*H*-5); ^{13}C NMR data, see Table 1; positive FABMS *m*/*z* 675 $[M + Na]^+$, 653 $[M + H]^+$; negative FABMS *m*/*z* 651 $[M - H]^-$; HRFABMS *m*/*z* 675.1530 (calcd for $C_{29}H_{32}O_{17}Na$, 675.1538).

2″-*O*-Acetyl-3′-*O*-methylrutin (2): yellow powder; $[\alpha]_D^{27}$ −13.0° (*c* 1.00, MeOH); UV (MeOH) λ_{max} (log ϵ) 254 (4.12), 269 (sh) (4.04), 354 (4.03); IR (KBr) ν_{max} 3410, 1655, 1609, 1067 cm^{-1}; ^1H NMR (DMSO-d_6, 270 MHz) δ 0.97 (3H, d, J = 6.1 Hz, H_3-6‴), 2.04 (3H, s, CH_3CO), 3.90 (3H, s, OCH_3), 4.37 (1H, br s, H-1‴), 4.75 (1H, dd-like, H-2″), 5.50 (1H, d, J = 8.1 Hz, H-1″), 6.20, 6.43 (1H each, both d, J = 1.8 Hz, H-6, 8), 6.90 (1H, d, J = 8.4 Hz, H-5′), 7.50 (1H, dd, J = 1.6, 8.4 Hz, H-6′), 7.80 (1H, d, J = 1.6 Hz, H-2′), 12.60 (1H, br s, O*H*-5); ^{13}C NMR data, see Table 1; positive FABMS *m*/*z* 689 $[M + Na]^+$, 667 $[M + H]^+$; negative FABMS *m*/*z* 665 $[M - H]^-$, 315 $[M - C_{14}H_{23}O_{10}]^-$; HRFABMS *m*/*z* 689.1700 (calcd for $C_{30}H_{34}O_{17}Na$, 689.1693).

Prunose I (3): white powder; $[\alpha]_D^{27}$ +26.9° (*c* 1.00, MeOH); UV (MeOH) λ_{max} (log ϵ) 229 (4.10), 316 (4.41); IR (KBr) ν_{max} 3432, 1748, 1717, 1042 cm^{-1}; ^1H NMR (DMSO-d_6, 270 MHz) δ 1.86, 1.97, 2.00, 2.04, 2.05 (3H each, all s, CH_3CO × 5), 3.46 (2H, m, H_2-6), 3.73 (1H, m, H-2′), 4.12 (2H, m, H_2-6′), 4.25, 4.35 (1H each, both m, H_2-1), 4.28 (1H, m, H-3), 4.32 (1H, m, H-5′), 4.85 (1H, t-like, H-4′), 5.15 (1H, t-like, H-3′), 5.37 (1H, d, J = 3.3 Hz, H-1′), 5.42 (1H, t-like, H-4), 5.68 (1H, br d, J = ca. 7 Hz, H-5), 6.36, 7.63 (1H each, both d, J = 16.0 Hz, coumaroyl-H-2, 3), 6.80, 7.58 (2H each, both d, J = 8.6 Hz, coumaroyl-H-3′, 5′, coumaroyl-H-2′, 6′); ^{13}C NMR data, see Table 1; positive FABMS *m*/*z* 721 $[M + Na]^+$; HRFABMS *m*/*z* 721.1952 (calcd for $C_{31}H_{38}O_{18}Na$, 721.1956).

Prunose II (4): white powder; $[\alpha]_D^{28}$ +18.7° (*c* 1.00, MeOH); UV (MeOH) λ_{max} (log ϵ) 228 (3.84), 315 (4.12); IR (KBr) ν_{max} 3432, 1734, 1717, 1047 cm^{-1}; ^1H NMR (DMSO-d_6, 270 MHz) δ 1.88, 1.94, 2.04, 2.05 (3H each all s, CH_3CO × 4), 3.40 (2H, m, H_2-6), 3.65 (1H, m, H-2′), 3.99 (1H, m, H-3), 4.10 (3H, m, H-4, H_2-6′), 4.16, 4.34 (1H each, both m, H_2-1), 4.25 (1H, m, H-5′), 4.79 (1H, t-like, H-4′), 5.10 (1H, t-like, H-3′), 5.32 (1H, d, J = 3.5 Hz, H-1′), 5.48 (1H, br d, J = ca. 8 Hz, H-5), 6.39, 7.63 (1H each, both d, J = 15.9 Hz, coumaroyl-H-2, 3), 6.80, 7.58 (2H each, both d, J = 8.6 Hz, coumaroyl-H-3′, 5′, coumaroyl-H-2′, 6′-H); ^{13}C NMR data, see Table 1; positive FABMS *m*/*z* 679 $[M + Na]^+$; negative FABMS *m*/*z* 655 $[M - H]^-$; HRFABMS *m*/*z* 679.1846 (calcd for $C_{29}H_{36}O_{17}Na$, 679.1850).

Alkaline Hydrolysis of 2″-*O*-Acetylrutin (1) and 2″-*O*-Acetyl-3′-*O*-methylrutin (2). A solution of **1** (5.2 mg) in 0.1% NaOMe−MeOH (1 mL) was stirred at room temperature (25 °C) for 1 h. The reaction mixture was neutralized with Dowex HCR W2 (H^+ form), and the residue was removed by filtration. After removal of the solvent from the filtrate in vacuo, the residue was separated by normal-phase silica gel column chromatography [500 mg, $CHCl_3$−MeOH−H_2O (65:35:10, lower layer−6:4:1, v/v)] to give rutin (3.9 mg, 75%). By a similar procedure, a solution of **2** (4.5 mg) in 0.1% NaOMe−MeOH (1 mL) was stirred at room temperature (25 °C) for 1 h. The reaction mixture was neutralized with Dowex HCR W2 (H^+ form), and the residue was removed by filtration. After removal of the solvent from the filtrate in vacuo, the residue was separated by normal-phase silica gel column chromatography [500 mg, $CHCl_3$−MeOH (3:1, v/v)] to give 3′-*O*-methylrutin (3.4 mg, 86%). Rutin and 3′-*O*-methylrutin were identified by comparison of physical data ($[\alpha]_D$, UV, IR, ^1H NMR, ^{13}C NMR, MS) with reported values.[6,16]

Alkaline Hydrolysis of Prunoses I (3) and II (4). A solution of prunoses (**3**, 11.4 mg; **4**, 11.3 mg) in 0.1% NaOMe−MeOH (1 mL) was stirred at room temperature (25 °C) for 15 min. The reaction mixture was neutralized with Dowex HCR W2 (H^+ form), and the residue was removed by filtration. After removal of the solvent from the filtrate in vacuo, the residue was separated by normal-phase silica gel column chromatography [500 mg, $CHCl_3$−MeOH−H_2O (10:3:1, lower layer−6:4:1, v/v)] to give methyl *p*-coumarate (1.8 mg, 62% from **3**; 1.9 mg, 62% from **4**) and sucrose (4.9 mg, 88% from **3**; 4.4 mg, 75% from **4**), which were identified by comparison of physical data ($[\alpha]_D$, UV, IR, ^1H NMR, MS) with authentic samples.

Aldose Reductase Assay. Aldose reductase activity was assayed by the method described previously.[3a,f,17] The supernatant fluid of rat lens homogenate was used as the crude enzyme. The incubation mixture contained 135 mM Na, K-phosphate buffer (pH 7.0), 100 mM Li_2SO_4, 0.03 mM NADPH, 1 mM DL-glyceraldehyde as a substrate, and 100 μL of enzyme fraction, with or without 25 μL of sample solution, in a total volume of 0.5 mL. The reaction was initiated by the addition of NADPH at 30 °C. After 30 min, the reaction was stopped by the addition of 150 μL of 0.5 M HCl. Then, 0.5 mL of 6 M NaOH containing 10 mM imidazole was added, and the solution was heated at 60 °C for 10 min to convert NADP to a fluorescent product. Fluorescence was measured using a fluorophotometer (luminescence spectrometer LS50B, Perkin-Elmer, UK) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

Platelet Aggregation Assay. Washed rabbit platelets were prepared as described previously.[18,19] The platelet suspension (10^7 cells/mL) in 225 μL of assay buffer (137 mM NaCl, 2.7 mM KCl, 3.8 mM HEPES, pH 7.4) and 25 μL of 12 mM $CaCl_2$−saline were mixed and incubated at 37 °C for 2 min. Ten microliters of test sample dissolved in DMSO was added to 490 μL of the assay buffer, and 25 μL of the solution was added to the platelet suspension with stirring. Two minutes later, 25 μL of thrombin (0.5 U/mL saline) was then added to the platelet suspension with stirring. Aggregation responses were recorded using an aggregometer (Hema Tracer 1, Nikko Bioscience, Japan) for 5 min. The aggregation (%) was calibrated with the platelet suspension (0% transmission) and assay buffer (100% transmission). The extent of inhibition of platelet aggregation is expressed as percent inhibition using the following equation:

$$\text{Inhibition (\%)} = [(C - T)/C] \times 100$$

where C is the maximal aggregation of control and T is the maximal aggregation of test sample-treated platelets. Values were expressed as the means ± SD of four experiments.

References and Notes

(1) This paper is Medicinal Flowers. VII. in our serial studies.
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