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Studies on the Antihemorrhagic Substances in Herbs Classified as Hemostatics in Chinese Medicine. IX. On the Antihemorrhagic Principles in *Typha lactifolia* L.[1)]

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By following the antihemorrhagic activity according to Tajima's method,[2)] two antihemorrhagic principles, a new flavonol glucoside (**1**) and an unknown compound (**2**), were isolated from the dried pollen of *Typha lactifolia* L., which is one of the most important herbs for treatment of stagnant blood and bloody stools in traditional Chinese medicine. The structure of **1** was established as isorhamnetin 3-rutinoside-7-rhamnoside (3-[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy-5-hydroxy-7-[β-L-mannopyranosyl]oxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one), based on chemical transformations and spectral data.

Keywords——hemostatic; antihemorrhagic principle; *Typha lactifolia*; isorhamnetin; rutinoside; isorhamnetin 3-rutinoside-7-rhamnoside

In traditional Chinese medicine, the parched pollen of *Typha lactifolia* L. has been used mainly as a remedy for bloody stools due to the inflammation of urinary tract and chronic colonic phlegmasia, whereas the untreated herb is used as both an anticoagulant and a hemostatic, for example, for treatment of "stagnant blood" after childbirth or bloody stools with "stagnant blood."[3)] We have examined this herb as a part of our series of studies on the chemical elucidation of antihemorrhagic principles in herbs classified as hemostatics in Chinese medicine.[1)] In addition, the significance of parching the herb was investigated.

As chemical constituents of dried pollen of *Typha lactifolia* L., fatty acids such as palmitic, stearic and linoleic acids,[4a)] steroids,[4b)] sugars (glucose, rhamnose, raffinose, polysaccharides, *etc.*),[4c)] flavonoids,[4d)] amino acids [4e)] and others have been reported. There have been a few pharmacological studies on the anticoagulative properties of polysaccharides,[5a)] the effect of flavonoids on myocardial c-adenosine 3′,5′-cyclic monophosphate (AMP)[5b)] and so on, but to our knowledge, no pharmacological study on the antihemorrhagic and anticoagulative principles in the herb has been reported. Our result on the antihemorrhagic principles in the herb are presented here.

We have isolated two antihemorrhagic principles, a new flavonol glucoside (**1**) and an unknown compound (**2**), and determined the structure of **1** on the basis of spectral data and some chemical examinations. The antihemorrhagic activities of the some compounds related to **1** and the effect of heat treatment of the herb on its biological action are also reported.

Tajima's method using mice was employed for determining the antihemorrhagic activity of the material, as reported previously.[6)] When the antihemorrhagic activity of the herb was examined by this method, it was found that the parched and unparched herbs showed almost equal activity, as described in detail later. From both samples, the same two active principles were isolated by successive procedures of extraction, partition, and column chromatographies (HP-20, Sephadex LH-20 and silica gel), by following the antihemorrhagic activity of the material. The isolation processes are summarized in Chart 1.

pollen of *Typha lactifolia* L. (200 g)
↓ extracted with H_2O under reflux
H_2O-ext (42.79 g) {18.36 g} [1.5 g/kg · · · 6.9 min]
↓ partitioned between *n*-butanol and H_2O
active fraction I (5.38 g) {2.88 g} [0.19 g/kg · · · 6.5 min]
↓ Diaion HP-20 c.c. with H_2O, 20% MeOH, 50% MeOH and MeOH

active fraction II_1 (274.7 mg) {122 mg} [29 mg · · · 5.1 min]
↓ silica gel c.c. with lower layer of $CHCl_3$–MeOH–H_2O (65 : 35 : 10)
active fraction V (47.8 mg) {122 mg} [8 mg/kg · · · 4.1 min]
↓ 1) Sephadex LH-20 c.c. with MeOH 2) recrystallized from dil. MeOH
light yellow needles (14.1 mg) {10.4 mg} [6 mg/kg · · · 2.3 min]

active fraction II_2 (1.112 g) {320 mg} [29 mg · · · 5.1 min]
↓ Sephadex LH-20 c.c. with MeOH
active fraction III (346 mg) {170.4 mg} [12 mg/kg · · · 5.8 min]
↓ silica gel c.c. with lower layer of $CHCl_3$–MeOH–H_2O (65 : 35 : 10)
active fraction IV (198 mg) {170.4 mg} [7 mg/kg · · · 4.8 min]
↓ 1) Sephadex LH-20 c.c. with MeOH 2) recrystallized from dil. MeOH
light yellow needles (80.4 mg) {69.6 mg} [6 mg/kg · · · 4.6 min]

( ) and { } indicate yields of each fraction obtained from unparched and parched herbs, respectively. [ ] indicates dose and activity (shortening of bleeding time).
Silica gel c.c., silica gel column chromatography.

Chart 1. Isolation of the Active Principle

In the case of the unparched herb, as shown in Chart 1, the dried pollen of *Typha lactifolia* L. was extracted with water. The extract was partitioned between *n*-butanol and water. As only the organic layer, fraction I, showed the activity, it was further applied to a HP-20 column. Inactive material of fraction I was eluted with H_2O, and then two active fractions, fraction II_1 and II_2, were obtained by elution with 20% and 50% MeOH, respectively. Firstly, we dealt with fraction II_2, which contained the bulk of the activity of fraction I.

Column chromatography of active fraction II_2 over Sephadex LH-20 gave the active fraction III (K_d value = 0.62—1.26). This fraction was chromatographed over silica gel to afford the active fraction IV. After removal of a small amount of impurity from this fraction by gel filtration through Sephadex LH-20, the product was recrystallized from diluted MeOH to obtain the active compound **1** as light yellow needles.

Another active compound was obtained from fraction II_1 as follows. The fraction II_1 was chromatographed over silica gel to yield the active fraction V, which was gel-filtered through Sephadex LH-20. The product was recrystallized from $CHCl_3$ and MeOH to obtain an active principle **2**.

The dose response curves of the water extracts of unparched and parched herbs, and compounds **1** and **2** are shown in Fig. 1. This pharmacological study showed that compounds **1** and **2** are responsible for the antihemorrhagic activity of the herb and the activity is mostly accounted for by compound **1**.

From the chemical transformations and spectral (nuclear magnetic resonance (NMR), infrared (IR), mass (MS) and ultraviolet (UV)) data of **1** the structure could be determined as isorhamnetin 3-rutinoside-7-rhamnoside, as depicted in Table I. The structural elucidation of **2** has not yet been completed.

On acid hydrolysis, **1** afforded 1 mol of D-glucose and 2 mol of L-rhamnose as sugar

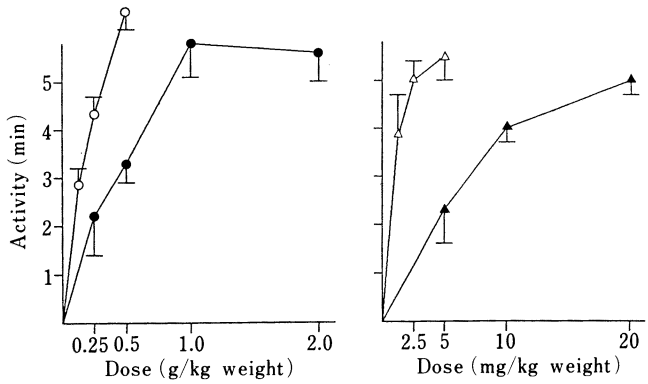
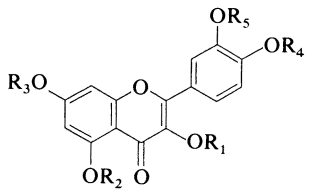


Fig. 1. Dose–Response Relationships for Antihemorrhagic Activity of Water Extracts of Parched Herb and Unparched Herb, and Active Principles **1** and **2**

●—●, unparched herb (ED_{50} = 0.38 [0.11—1.33], LD_{50} = 6.70 [6.09—7.32]); ○—○, parched herb (ED_{50} = 0.15 [0.06—0.36], LD_{50} = 2.70 [2.45—3.00]); △—△, compound **1** (ED_{50} = 1.45 [0.58—3.63]); ▲—▲, compound **2** (ED_{50} = 6.80 [4.68—10.2]).
Each point represents the mean of the antihemorrhagic activity in five different experiments, with the S.E.

TABLE I. Antihemorrhagic Activities of **1** and Isorhamnetin, and Related Compounds



	R_1	R_2	R_3	R_4	R_5	Dose (mg/kg)	Activity (min)
1	Glu-Rham	H	Rham	H	CH_3	5	5.0[a]
3	Glu-Rham	CH_3	Rham	CH_3	CH_3	10	4.4[a]
4	Glu	H	Rham	H	CH_3	10	1.0[a]
5	Glu	H	H	H	CH_3	10	1.6[a]
Rutin	Glu-Rham	H	H	H	H	10	−0.3
3′-*O*-MQ[8]	H	H	H	H	CH_3	10	0.4
7-*O*-MQ[14]	H	H	CH_3	H	H	10	0.6
3′,4′-Di-*O*-MQ[15]	H	H	H	CH_3	CH_3	10	3.0[b]
5,3′,4′-Tri-*O*-MQ[9]	H	CH_3	H	CH_3	CH_3	10	2.5[a]
5,7,3′-Tri-*O*-MQ[9]	H	CH_3	CH_3	H	CH_3	10	0.6
7,3′-Di-*O*-MQ[13]	H	H	CH_3	H	CH_3	10	0.3
5,3′-Di-*O*-MQ[16]	H	CH_3	H	H	CH_3	10	1.5

MQ = methyl quercetin. *a*) $p < 0.001$, *b*) $p < 0.02$.

moiety, and 1 mol of isorhamnetin [3,5,7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one] as its aglycone. The absolute configurations of the monosaccharides were identified according to Oshima *et al.*[7] The identification of the aglycone as isorhamnetin was carried out by mixed melting point determination and direct comparison of physical data (UV, IR, MS and NMR) with those of an authentic sample, which was prepared by Heap and Robinson's method.[8]

The positive fast atom bombardment MS (FAB-MS) of **1** showed a molecular ion peak at *m/z* 771 in agreement with the molecular formula (M+1[M = $C_{34}H_{42}O_{22}$]) and other peaks at 625, 479 and 317 in agreement with (M+H−Rha), (M+H−2×Rha) and (M+H−

2 × Rha − Glu), respectively. The ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** as well as anomeric proton signals at 100.8 (d), 100.7 (d) and 98.7 (d) ppm showed the presence of a α-D-glucopyranoside unit and two β-L-arabinopyranoside units in **1**. A clear ^{13}C-NMR glycosylation shift (+5.2 ppm) was observed at C-6 of D-glucose in **1**, as shown in Table II.[9] These data indicated that compound **1**, which contains rhamnose and rutinose, is a bisdesmoside of "isorhamnetin" with glycosyl linkages at 3-OH and 4′-OH, 5-OH or 7-OH.

The positions of these sugar groups were determined in the following ways. On diazomethane treatment of **1** it afforded a di-*O*-methyl derivative **3**, and acid hydrolysis of **3** gave di-*O*-methylisorhamnetin. The latter was identified as 5,3′,4′-tri-*O*-methylquercetin by comparing the physical data (mp, IR and NMR) with those of an authentic sample prepared by Kuhn's method.[10] On selective cleavage of the glycoside glycosidic linkage of flavonol rutinoside **1** with crude β-rhamnosidase, which was prepared from crude hesperidinase by refering to Okada *et al.*'s report,[11] compound **1** afforded a flavonol glucoside **4**, together with an equimolar amount of rhamnose. Further selective cleavage of another glycosidic linkage with crude hesperidinase in 0.05 M McIlvaine buffer solution containing 4% glucose[12] afforded an flavonol glucoside **5** and an equimolar amount of rhamnose. On comparing the ^{13}C-NMR spectrum of **5** with that of isorhamnetin, it was observed that the signal of flavonol C-3 is shifted about 2.9 ppm up field. Therefore, we assumed that **5** was 3′-*O*-methylisoquercitrin, 3-(β-D-glucofuranosyloxy)-5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one. Its identification was carried out by transforming this compound to tetra-*O*-methyl quercitrin, as described below. Methylation of **5** with diazomethane afforded 3′,4′5,7-*tetra*-*O*-methylquercitrin, which was identified by direct comparison of physicochemical data (mp, IR, UV, MS and NMR) with those of an authentic sample prepared from isoquercitrin by following Attree and Perkin's method.[13]

Based on the above results, the chemical structure of **1** was established as isorhamnetin 3-rutinoside-4′-rhamnoside (3-[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy-5-hydroxy-7-[β-L-mannopyranosyl]oxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzo-pyran-4-one). Thus, the structures of compounds **3** and **4** are determined as isorhamnetin 3-

TABLE II. Chemical Shifts of Sugar Groups of Isoquercitrin (I), Quercitrin (II) and Compound **1**

		I	II	Compound **1**
Glucose	C-1	101.4		100.8 (100.7)
	C-2	74.3		75.7
	C-3	76.8		76.9
	C-4	70.3		70.2
	C-5	77.5		77.5
	C-6	61.5		66.7
Rhamnose	C-1		101.9	100.7 (100.8)
	C-2		70.4	70.4
	C-3		70.6	70.5
	C-4		71.5	71.7
	C-5		70.1	68.2
	C-6		17.3	17.6
Rhamnose	C-1			98.7
	C-2			70.4
	C-3			70.5
	C-4			71.7
	C-5			70.2
	C-6			17.2

glucoside-4′-rhamnoside and isorhamnetin 3-glucoside, respectively. These structures are illustrated in Table I.

Finally we examined the significance of parching the herb and the relationship between the structures and antihemorrhagic activities of **1** and related compounds.

As shown in Chart 1 and Fig. 1, the values of ED_{50} (mg/kg) and LD_{50} (mg/kg) and the weight (g) of the water extract of the unparched herb are about 2.4 times (0.38, 0.15; 6.7, 2.7 and 42.8; 18.4) of those of the parched herb. This shows that the total antihemorrhagic activities and toxicities of both water extracts are almost equal. As shown in Chart 1, the two water extracts contained similar weights of compounds **1** and **2**. Thus, parching of the herb did not appear to have any medicinal or chemical significance under our examinations.

The antihemorrhagic activities of **1** and related compounds shown in Table I were tested. It appeared that 1) activity depends on the presence of both rutinose and rhamnose groups at the 3 and 7 positions of isorhamnetin; **1** and its 4′,5-di-*O*-methyl derivative **3** exhibit activity, while no significant activity was observed for **4**, **5** or rutin, and 2) in the case of compounds related to isorhamnetin[8,10,14-17] an antihemorrhagic activity depends on the presence and locations of *O*-methyl and free hydroxyl groups on the molecule; 2-(3′,4′-dimethoxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one and 2-(3′,4′-dimethoxyphenyl)-5-methoxy-7-hydroxy-4*H*-1-benzopyran-4-one have the activity, whereas the other compounds showed no significant activity.

In summary, a new flavonol glucoside, isorhamnetin 3-rutinoside-7-rhamnoside **1**, together with an unknown compound **2**, was isolated as the antihemorrhagic principle from the pollen of *Typha lactifolia* L. The antihemorrhagic activity of the herb is accounted for by compounds **1** and **2**.

Experimental

Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. UV and IR spectra were taken on Shimadzu UV-360 and JASCO IRA-2 grating infrared spectrometers, respectively. Low- and high-resolution mass spectra were recorded on Hitachi M-80A and JEOL JMS D-100 instruments. Positive and negative FAB-MS were taken on a JEOL JMS-DX303 by a direct inlet method. NMR spectra were recorded on JEOL FX-90 and JEOL GX-270 spectrometers using tetramethylsilane (TMS) as an internal standard. Assignments of the signals in the ^{13}C-NMR spectra of compounds **1**, **4**, **5** and isorhamnetin were carried out on the basis of their noise-decoupled, off-resonance-decoupled, 2D-COSY and C–H-shift COSY NMR spectra, with reference to the Markham *et al.*'s report on flavonoids.[9] For column chromatography, Kieselgel 60 (70—230 mesh, Merck) and Diaion HP-20 (Nippon Rensui Co.) were used.

Material——The herb used in this study was a commercial product available in China, and was identified as *Typha lactifolia* L. by an expert.

Extraction——Pollen of *Typha lactifolia* L. (200 g) was extracted with 1.2 l of H_2O under reflux for 0.5 h. The mixture was centrifuged at 2500 rpm for 20 min and the supernatant was lyophilized to give a yellow powder (42.7 g). From 124 g of parched herb prepared by heating 200 g of the herb on a hot plate at 200 °C for 1 h, 18.36 g of H_2O extract was obtained.

Partition between *n*-Butanol and H_2O——The extract (42.8 g) was dissolved in 500 ml of H_2O and extracted with 500 ml of *n*-butanol three times. The combined organic layer was concentrated under reduced pressure to afford 5.38 g of active fraction I, as a brown gum.

Column Chromatography of Active Fraction I on Diaion HP-20——The active fraction I (5.38 g) was dissolved in 600 ml of H_2O, and applied to a Diaion HP-20 column (3.1 × 27.2 cm). It was eluted with H_2O, 20% MeOH, 50% MeOH and MeOH, successively. Two active fractions, 20% MeOH and 50% MeOH eluates (active fractions II_1 and II_2) were obtained.

Gel Filtration of Active Fraction II_2 through Sephadex LH-20——The active fraction II_2 (1.112 g) was dissolved in 15 ml of MeOH and chromatographed through Sephadex LH-20 (3.1 × 42.5 cm) with MeOH to yield active fraction III (346 mg) (K_d value = 0.62—1.26) as a light yellow gum.

Silica Gel Column Chromatography of Active Fraction III——The active fraction III (346 mg) was chromatographed over silica gel (3.1 × 42.5 cm) to yield 346 mg of active fraction IV, using the lower layer of $CHCl_3$–MeOH–H_2O (65 : 35 : 10) as an eluent.

Recrystallization of Compound 1——After removal of impurities from active fraction IV by gel filtration through

Sephadex LH-20 (2.9 × 24 cm) with MeOH, the product was recrystallized from diluted MeOH to afford an active compound **1** (80.5 mg) as light yellow needles. **1**: mp 196—200 °C. MS *m/z*: 316, 286, 245, 128. *Anal.* Calcd for $C_{34}H_{42}O_{20}\cdot 3H_2O$: C, 49.51; H, 5.83. Found: C, 49.25; H, 5.67. High-resolution MS: 316.0584 (error +0.2 mMU) for $C_{16}H_{12}O_7$. Positive FAB-MS *m/z*: 771 [M+H], 625 [M+1−Rha+H], 497 [M−2×Rha+H], 317 [M−2×Rha−Glu+H]. Negative FAB-MS *m/z*: 769 [M−H], 623 [M+1−Rha−H], 495 [M−2×Rha−H], 315 [M− + ×Rha−Glu−H]. ^{1}H-NMR (in DMSO-d_6) δ: 12.64 (1H, s), 11.0—9.0 (1H, br), 7.87 (1H, d, J=2.0 Hz), 7.52 (1H, dd, J=8.5, 2.0 Hz), 6.94 (1H, d, J=8.5 Hz), 6.45 (1H, d, J=2.0 Hz), 6.43 (1H, d, J=2.0 Hz), 5.65 (1H, d, J=7.0 Hz), 5.40—5.30 (1H, br), 5.30—5.12 (1H, br), 5.10 (1H, d, J=7.0 Hz), 4.80—4.30 (6H, br), 4.45 (1H, s), 3.88 (3H, s), 3.8—3.0 (21H, m), 0.99 (3H, d, J=6.0 Hz), 0.74 (3H, d, J=6.3 Hz). ^{13}C-NMR (in DMSO-d_6) δ: 177.2 (s) (C-4), 164.2 (s) (C-7), 161.2 (s) (C-5), 156.4 (s) (C-2, C-4′), 149.3 (s) (C-9), 146.9 (s) (C-3′), 132.5 (s) (C-3), 122.1 (s) (C-1′), 121.1 (d) (C-6′), 115.2 (d) (C-5′), 113.3 (d) (C-2′), 104.0 (s) (C-10), 100.8 (d), 100.7 (d), 98.7 (d) (C-6), 98.7 (d), 93.8 (d) (C-8), 77.5 (d), 76.9 (d), 75.7 (d), 71.7 (d), 70.5 (d), 70.4 (d), 70.2 (d), 68.2 (d), 66.7 (d), 55.5 (q) ($O\underline{C}H_3$), 17.6 (q), 17.2 (q). IR ν_{max}^{KBr} cm^{-1}: 3430, 3050, 1670, 1590, 1415, 1360, 745, 525. UV λ_{max}^{MeOH} nm (log ε): 352 (4.20), 265 (4.19), 254 (4.26).

Silica Gel Column Chromatography of Active Fraction II_1——The fraction II_1 was subjected to silica gel column chromatography (3.3 × 18.0 cm), using the lower layer of $CHCl_3$–MeOH–H_2O (65 : 35 : 10) as an eluent. Active fraction V (47.8 mg) was obtained by this chromatography.

Recrystallization of Compound 2——The active fraction V (47.8 mg) was gel-filtered through Sephadex LH-20 (1.6 × 28 cm) to remove inorganic material, and then the product was recrystallized from $CHCl_3$ and MeOH to afford an active principle 2 (14.1 mg) as white needles, mp 237—239 °C.

Acid Hydrolysis of 1——**1** (30 mg) was dissolved in 2.5 ml of 5% H_2SO_4 and heated at 90 °C for 30 min. After cooling, the precipitate was filtered off and recrystallized from EtOH to give an aglycone, isorhamnetin (11 mg), as light yellow needles. The filtrate was neutralized with Amberlite MB-3 ion exchange resin, and evaporated to dryness after removal of the resin by filtration. Identification of the resulting monosaccharides, including the absolute configuration, was carried out according to the method reported by Oshima *et al.*[7] Identification of the aglycone as isorhamnetin was described in earlier. Aglycone (isorhamnetin): mp 303 °C (dec.) [lit.,[8] 305 °C (dec.)]. MS *m/z*: 316, 286, 245. *Anal.* Calcd for $C_{16}H_{12}O_7$: C, 60.76; H, 3.82. Found: C, 60.85; H, 3.80. ^{1}H-NMR (in DMSO-d_6) δ: 12.45 (1H, s), 12.00—9.50 (3H, br), 7.76 (1H, d, J=1.8 Hz), 7.16 (1H, dd, J=7.9, 1.8 Hz), 6.90 (1H, d, J=7.9 Hz), 6.47 (1H, d, J=1.8 Hz), 6.21 (1H, d, J=1.8 Hz), 3.88 (3H, s). ^{13}C-NMR (in DMSO-d_6) δ: 175.8 (s) (C-4), 163.9 (s) (C-7), 160.7 (s) (C-5), 156.1 (s) (C-2), 148.8 (s) (C-9), 147.3 (s) (C-4′), 146.5 (s) (C-3′), 135.8 (s) (C-3), 122.0 (s) (C-1′), 121. 7 (d) (C-6′), 115.5 (d) (C-5′), 111.9 (d) (C-2′), 103.1 (s) (C-10), 98.2 (d) (C-6), 93.5 (d) (C-8), 55.8 (q) (OCH_3).

Methylation of 1——Compound **1** (30 mg) was methylated with excess (about 10 times) diazomethane in anhydrous MeOH at 5 °C for 5 h. The product **3** was purified by silica gel column chromatography, using the lower part of $CHCl_3$–MeOH–H_2O (65 : 35 : 10) as an eluent, and recrystallized from diluted MeOH to yield the dimethylated glucoside **3** (31 mg) as light yellow needles. A solution of **3** (31 mg) in 1 ml of 5% H_2SO_4 was heated on a water bath for 30 min. The reaction mixture was diluted with H_2O and then extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$ and evaporated to dryness. The residue was recrystallized from MeOH to afford the di-*O*-methylated aglycone (8 mg) as yellow needles, mp 289—291 °C (lit.,[9] 292—294 °C). Identification of this compound as 5,3′,4′-tri-*O*-methylquercetin was carried out by mixed melting point determination and direct comparison of spectral data (IR, MS and NMR) with those of an authentic sample, which was prepared by Kuhn's method.[8] **3**: mp 143—146 °C. *Anal.* Calcd for $C_{36}H_{46}O_{20}$: C, 54.13; H, 5.80. Found: C, 54.05; H, 5.88. MS *m/z*: 344, 329, 301, 285, 213. ^{1}H-NMR (in MeOH-d_4) δ: 7.95 (1H, d, J=1.8 Hz), 7.71 (1H, dd, J=8.8, 1.8 Hz), 7.05 (1H, d, J=8.8 Hz), 6.67 (1H, d, J=2.2 Hz), 6.45 (1H, d, J=8.8 Hz), 5.69 (1H, d, J=7.5 Hz), 5.16 (1H, br s), 4.51 (1H, br s), 3.94 (3H, s), 3.90 (6H, s), 4.20—3.00 (13H, m), 1.04 (3H, d, J=5.9 Hz), 0.97 (3H, d, J=6.2 Hz). IR ν_{max}^{KBr} cm^{-1}: 3400, 2925, 1610, 1515, 1325, 1270, 1215, 1060, 810. UV λ_{max}^{MeOH} nm (log ε): 351 (4.26), 263 (4.17), 250 (4.19). Di-*O*-methyl derivative, 5,3′,4′-tri-*O*-methylquercetin: mp 189—191 °C. MS *m/z*: 344, 329, 301, 285, 213, 122. *Anal.* Calcd for $C_{18}H_{16}O_7$: C, 62.78; H, 4.68. Found: C, 62.67; H, 4.72. ^{1}H-NMR (in $CDCl_3$) δ: 11.70 (1H, s), 11.5—8.9 (1H, br), 7.83 (1H, dd, J=8.4, 2.0 Hz), 7.77 (1H, d, J=2.0 Hz), 7.00 (1H, d, J=8.4 Hz), 6.49 (1H, d, J=2.2 Hz), 6.38 (1H, d, J=2.2 Hz), 3.98 (3H, s), 3.97 (3H, s), 3.89 (3H, s). IR ν_{max}^{KBr} cm^{-1}: 3360, 1650, 1630, 1595, 1510, 1250, 1210, 1155, 1140, 1010, 820.

Selective Hydrolysis of the D-Glu^6-^{1}L-Rha Linkage of 1 with Hesperidinase——A solution of crude hesperidinase (50 mg) in 50 ml of citrate-phosphate buffer (pH 4.6) was heated at 74 °C for 15 min, then **1** (50 mg) in 5 ml of dimethyl sulfoxide (DMSO) was added and the mixture was stirred for 5 min at this temperature. After cooling of the reaction mixture, 60 ml of *n*-BuOH was added, and the whole was refluxed for 20 min. After cooling, the precipitate was filtered off with the aid of Celite 535. The organic layer was evaporated to dryness. The residue was chromatographed on silica gel with use of the lower layer of $CHCl_3$–MeOH–H_2O (65 : 35 : 10) and the product was recrystallized from MeOH to give compound **4** (32 mg) as yellow needles. The residue after evaporation in a rotary evaporator was subjected to silica gel chromatography, using the lower part of $CHCl_3$–MeOH–H_2O (65 : 35 : 10) as an eluent, to afford rhamnose. The latter was identified by direct comparison of the physical data (GLC, mp and IR) with those of an authentic sample. **4**: mp 235—237 °C (dec.). MS *m/z*: 316, 386, 245, 217, 129. *Anal.* Calcd for $C_{28}H_{32}O_{16}\cdot 2H_2O$: C, 51.22; H, 5.48. Found: C, 51.19; H, 5.29. Positive FAB-MS *m/z*: 625 [M+H], 479

[M − Rha + H], 317 [M − Rha − Glu + H]. ^{1}H-NMR (in DMSO-d_6) δ: 12.60 (1H, s), 12.0—10.7 (1H, br), 7.94 (1H, d, J = 1.8 Hz), 7.49 (1H, dd, J = 8.3, 1.8 Hz), 6.90 (1H, d, J = 8.3 Hz), 6.42 (1H, d, J = 1.8 Hz), 6.18 (1H, d, J = 1.8 Hz), 5.88 (1H, br s), 5.30 (1H, br s), 4.60—4.25 (4H, m), 3.85 (3H, s), 3.8—2.9 (10H, m), 0.65 (3H, d, J = 6.1 Hz). ^{13}C-NMR (in DMSO-d_6) δ: 177.1 (s) (C-4), 164.2 (s) (C-7), 161.2 (s) (C-5), 156.3 (s) (C-2), 155.9 (s) (C-4′), 149.3 (s) (C-9), 146.8 (s) (C-3′), 132.5 (s) (C-3), 121.4 (d) (C-1′, C-6′), 115.0 (d) (C-5′), 113.5 (d) (C-2′), 104.1 (s) (C-10), 101.0 (d), 98.8 (d), 98.4 (d) (C-6), 93.7 (d) (C-8), 77.8 (d), 77.7 (d), 71.1 (d), 70.5 (d), 70.4 (d), 68.2 (d), 60.4 (d), 55.5 (q) (OCH_3), 17.0 (q). IR ν_{max}^{KBr} cm^{-1}: 3450, 1665, 1610, 1510, 1360, 1210, 1070, 810. UV λ_{max}^{MeOH} nm (log ε): 353 (4.20), 264 (4.20), 254 (4.25).

Selective Hydrolysis of the *O*-α-L-Rha Linkage of 4 with Hesperidinase——A solution of **4** (32 mg) and crude hesperidinase (32 mg) in 33 ml of 0.05 M McIlvaine buffer containing 4% β-D-glucose–DMSO (10 : 1) was stirred at 40 °C for 5 min. Then 30 ml of *n*-BuOH was added to the resultant mixture as quickly as possible and the whole was refluxed for 20 min. After cooling, the resultant mixture was filtered with the aid of Celite 535 and the organic layer of the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column with the lower layer of $CHCl_3$–MeOH–H_2O (65 : 35 : 10) as an eluent and the product was recrystallized from MeOH to give **5** (24 mg) as yellow needles. From the water layer, rhamnose was isolated by the method described above. **5**: mp 162—168 °C. MS *m/z*: 316, 301, 286, 217, 195, 121. *Anal.* Calcd for $C_{22}H_{22}O_{12}$: C, 55.23; H, 4.64. Found: C, 55.18; H, 4.60. Positive FAB-MS: 471 [M + H], 317 [M + H − Glu]. ^{1}H-NMR (in DMSO-d_6) δ: 12.59 (1H, s), 11.3—10.5 (1H, br), 10.0—9.5 (1H, br), 7.93 (1H, d, J = 1.8 Hz), 7.49 (1H, dd, J = 8.4, 1.8 Hz), 6.90 (1H, d, J = 8.4 Hz), 6.43 (1H, d, J = 1.8 Hz), 6.19 (1H, d, J = 1.8 Hz), 5.55 (1H, br s), 5.50—5.34 (1H, m), 5.12—5.00 (1H, m), 5.00—4.94 (1H, m), 4.4—4.0 (2H, m), 3.83 (3H, s), 3.8—3.1 (4H, m). ^{13}C-NMR (in DMSO-d_6) δ: 176.4 (s) (C-4), 170.4 (s) (C-7), 161.0 (s) (C-5), 157.6 (s) (C-2), 155.2 (s) (C-4′), 150.5 (s) (C-9), 147.9 (s) (C-3′), 132.9 (s) (C-3), 121.9 (d) (C-6′), 120.1 (s) (C-1′), 115.2 (d) (C-5′), 113.3 (d) (C-2′), 101.8 (s) (C-10), 101.8 (d), 100.2 (d) (C-6), 94.4 (d) (C-8). 77.2 (d), 76.6 (d), 74.2 (d), 69.7 (d), 60.7 (t), 55.6 (q) (OCH_3). IR ν_{max}^{KBr} cm^{-1}: 3400, 1655, 1610, 1560, 1355, 1295, 1061, 815. UV λ_{max}^{MeOH} nm (log ε): 353 (4.94), 264 (4.95), 255 (4.96).

Methylation of 5——Compound **5** (24 mg) was methylated in the same way as **1** to give the tetra-*O*-methylated derivative in a quantitative yield. This product, which was recrystailized from MeOH to afford yellow needles, mp 148—150 °C (lit.,[12] mp 150—152 °C), was identified as 3′,4′,5,7-tetra-*O*-methylisoquercitrin by a direct comparison of physical data (mp, MS, IR and NMR) with those of an authentic sample, which was prepared according to Attree and Perkin,[13] from isoquercitrin, mp 223—224 °C (lit.,[18] 225—227 °C), itself obtained from rutin by the reported method.[19] Tetra-*O*-methylquercitrin: MS *m/z*: 358, 312, 282, 179. ^{1}H-NMR (in DMSO-d_6) δ: 7.84 (1H, d, J = 1.8 Hz), 7.81 (1H, dd, J = 8.4, 1.8 Hz), 7.15 (1H, d, J = 8.4 Hz), 6.54 (1H, d, J = 1.9 Hz), 6.35 (1H, d, J = 1.9 Hz), 5.45 (1H, br s), 4.60—4.50 (1H, m), 3.99 (3H, s), 3.93 (3H, s), 3.88 (3H, s), 3.76—3.40 (4H, m). Other signals of the compound overlapped with those of the solvents.

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