Studies on the Constituents of the Japanese Mistletoe, *Viscum album* L. var. *coloratum* OHWI Grown on Different Host Trees

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The chemical constituents of the Japanese mistletoe, *Viscum album* L. var. *coloratum* OHWI, grown on 12 different kinds of host trees, were examined. Two new flavonoid glycosides, rhamnazin-3,4′-di-*O*-glucoside and (2*S*)-homoeriodictyol-7-*O*-[apiosyl(1→2)]glucoside, were isolated from the *n*-BuOH extract. Two known flavonoid glycosides, flavoyadorinin-B and homo-flavoyadorinin-B, were also isolated. Moreover, comparative studies of the contents of three flavonoid glycosides in the Japanese mistletoe grown on the different host trees were carried out by high performance liquid chromatography. Flavonoid glycosides common to Japanese and European mistletoes were not found.

Keywords *Viscum album* var. *coloratum*; mistletoe; Loranthaceae; rhamnazin-3,4′-di-*O*-glucoside; (2*S*)-homoeriodictyol-7-*O*-[apiosyl(1→2)]glucoside; flavoyadorinin-B; homo-flavoyadorinin-B

Viscum album L. (Loranthaceae) has been used as a folk medicine in Europe from ancient times,[1,2] and it has been used under the name "Sohkisei" in China.[3] In our previous studies, we reported the constituents of the European mistletoe, *V. album* L.[4,5] As regards the constituents of *V. album* L. var. *coloratum* OHWI (Japanese mistletoe), flavonoid glycosides, flavoyadorinin-A, flavoyadorinin-B and homo-flavoyadorinin-B, were reported by Ohta[6] and a peptide, viscumamide, by Sakurai and Okumura.[7] In the present paper, we report on the chemical elucidation of two new flavonoid glycosides, rhamnazin-3,4-di-*O*-glucoside (IX) and (2*S*)-homoeriodictyol-7-*O*-[apiosyl(1→2)]glucoside (X), isolated from the Japanese mistletoe. We also report the results of comparative studies of the constituents and the contents of flavonoid glycosides in Japanese mistletoe grown on different host trees, using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

We collected Japanese mistletoe grown on 12 kinds of host trees as listed in Table I. Samples grown on *Quercus mongollica* FISHER var. *grosseserrata*, *Prunus jamasakura* SIEB., *Acer palmatum* THUNB. var. *palmatum*, *Prunus mume* SIEB., *Pyrus pyrifolia* L. var. *culta* and *Celtis sinensis* PERS. var. *japonica* were extracted with MeOH in the usual way. The MeOH extracts were partitioned with *n*-hexane, $CHCl_3$ and *n*-BuOH successively. Each extract was concentrated and purified by chromatographies with silica gel, Sephadex LH-20 and Amberlite XAD-2 and by HPLC. β-Amyrin acetate (I), phytosterol (II) and fatty acids (III) were isolated from the *n*-hexane extract and oleanolic acid (IV) and phytosterol-glucoside (V) from the $CHCl_3$ extract. We also isolated flavoyadorinin-B (3′,7-dimethoxyluteolin-4′-*O*-glucoside) (VII),[6] homo-flavoyadorinin-B (3′,7-dimethoxyluteolin-4′-*O*-[apiosyl(1→2)] glucoside (VIII),[6] syringin (VI)[4] and two new flavonoid glycosides (IX, X) from the *n*-BuOH extract.

Compound VII was obtained as a yellowish powder, mp 239—241 °C. Upon hydrolysis of VII, glucose was detected by TLC. On the basis of the ultraviolet (UV) absorption spectral data showing the bathochromic shifts with diagnostic reagents, as well as the proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectral data, compound VII was confirmed to be flavoyadorinin-B.[6]

Compound VIII was obtained as a yellow powder, mp 217—220 °C. The UV, ^{1}H-NMR and ^{13}C-NMR spectral data of the aglycone moiety of VIII were very similar to those of compound VII (Table II). After hydrolysis of VIII, glucose and apiose were detected as sugar components of VIII by TLC. In ^{13}C-NMR spectrum of the sugar moiety of VIII, the C-2 signal of glucose showed a downfield shift (glycosylation shift) in comparison with that of compound VII (see Table II). Therefore, the sugar moiety of VIII was confirmed as apiosyl(1→2)glucose. On the basis of the above results, compound VIII was identified as homo-flavoyadorinin-B.[6]

Compound IX was obtained as a yellow powder, mp 245—247 °C. The UV spectrum of IX showed a bathochromic shift of band I with $AlCl_3$ ($\Delta\lambda_{max} = +50$ nm), but showed no bathochromic shift of band I with NaOEt and band II with AcONa. The above bathochromic shifts suggested the presence of a hydroxyl group at C-5.[8] The ^{1}H-NMR spectral data gave the signals of two methoxyl groups and the absence of the signal of 3-H suggested compound IX to be a flavonol. In the ^{13}C-NMR spectrum, the chemical shifts of the aglycone moiety were identical to those of rhamnazin and the presence of two molecules of glucose was suggested. After hydrolysis of IX, glucose was detected by TLC. By comparison of the chemical shifts with those of VII, the positions of attachment of the two glucoses were proved to be C-3 and C-4′. On the basis of the above results, the structure of compound IX was established to be rhamnazin-3,4′-di-*O*-glucoside.

Compound X was obtained as a yellow powder, mp 143—146 °C. The UV spectrum of X showed bathochromic shifts of band I with NaOEt ($\Delta\lambda_{max} = +120$ nm) and with $AlCl_3$ ($\Delta\lambda_{max} = +56$ nm). Besides, the absorption maxima at 324 (sh) and 282 nm in the UV spectrum and the signals of 2-H at δ 5.50 (1H, dd, J=2.8, 12.9 Hz), 3-H at δ 2.74 (1H, dd, J=2.8, 17.2 Hz) and 3-H at δ 3.17 (1H, m) in the ^{1}H-NMR spectrum suggested X to be a flavanone.[8] In the ^{13}C-NMR spectrum of X, the chemical shifts of the aglycone conformed to those of the aglycone of (2*S*)-homoeriodictyol-7-*O*-glucoside, which was isolated from European mistletoe[5] (see Table II). Therefore, the aglycone of X was confirmed to be homoeriodictyol. After hydrolysis of X, glucose and apiose were detected as the sugar components of X by TLC. In the ^{13}C-NMR spectrum, the

C-2 signal of glucose showed a downfield shift (glycosylation shift) in comparison with that of (2*S*)-homoeriodictyol-7-*O*-glucoside. Therefore, the sugar moiety of X was proved to be apiosyl(1→2)glucose. The circular dichroism (CD) spectrum of X exhibited a positive Cotton effect at 332 nm and a negative Cotton effect at 287 nm. Therefore, C-2 was assigned the *S* configuration.[9] On the basis of the above results, the structure of compound X was established to be (2*S*)-homoeriodictyol-7-*O*-[apiosyl(1→2)]glucoside.

Flavoyadorinin-A, which was isolated from Japanese mistletoe by Ohta,[6] was not isolated from the same mistletoe grown on any of the kinds of host trees examined here.

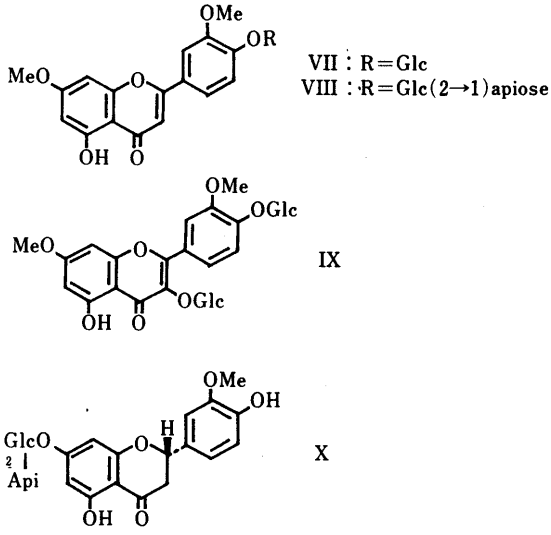

Chart 1

A comparison of the constituents isolated from the Japanese mistletoe epiphyting to the different host trees by TLC showed no remarkable variation. No significant variations related to collection area and time were seen, either. Some variation in the contents of syringin and compound X ((2*S*)-homoeriodictyol-7-*O*-[apiosyl(1→2)]-glucoside) was seen (Table I). A comparative quantitative analysis of flavonoid glycosides (compounds VII—IX) by HPLC (Table III) revealed that the leaves contain more of the flavonoid glycosides than the twigs in three kinds of host trees. The content ratio of compounds VII—IX was about 2 : 15 : 3.

A comparison of the constituents between Japanese and European mistletoes revealed remarkable variations in the flavonoid glycosides (Table IV). Chalcones and flavanones with a methoxyl group at C-5 (A-ring) were isolated only from the European mistletoe. (2*S*)-Homoeriodictyol is the only common aglycone in both mistletoes. Differences were also seen in triterpene and phenylpropanes. The Japanese and European mistletoe contain some compounds with apiose as the sugar component. The European mistletoe contains more phytosterol, phytosterol glucoside and specially syringin than the Japanese mistletoe. In Europe, *V. album* L. has been used as a folk medicine because it has hypotensive, vasodilator, cardiac depressive, sedative, antispasmodic and anticancer activities.[1,2,10] However, in Japan, *V. album* L. is seldom used as medicine. This may be because of the difference in the amount of some compounds or in the constituents.

TABLE I. Comparative Study on the Constituents of *V. album* L. var. *coloratum* OHWI Grown on Different Host Trees by TLC

Host tree	Collection area	Collection time	I	II	III	IV	V	VI	VII	VIII	IX	X
Quercus mongollica FISHER var. *grosseserrata* (mizunara)	Nagano Karuizawa	1986.5	+	+	+	+	+	−	+	+	+	−
Quercus mongollica FISHER var. *grosseserrata* (mizunara)	Aomori Yakeyama	1987.4	+	+	+	+	+	−	+	+	+	+
Prunus jamasakura SIEB. (yamazakura)	Nagano Karuizawa	1986.5	+	+	+	+	+	+	+	+	+	+
Quercus serrara THUNB. (konara)	Nagano Karuizawa	1986.6	+	+	+	+	+	−	+	+	+	+
Fagus crenata BLUME (buna)	Nagano Karuizawa	1986.6	+	+	+	+	+	−	+	+	+	+
Acer palmatum THUNB. var. *palmatum* (irohamomiji)	Nagano Karuizawa	1987.6	+	+	+	+	+	+	+	+	+	+
Fagus japonica MAXIM. (inubuna)	Nagano Karuizawa	1987.6	+	+	+	+	+	−	+	+	+	+
Quercus acutissima CARRUTH. (kunugi)	Nagano Karuizawa	1987.6	+	+	+	+	+	−	+	+	+	+
Prunus mume SIEB. (ume)	Nagano Okaya	1987.5	+	+	+	+	+	+	+	+	+	+
Pyrus pyrifolia·L. var. *culta* (nashi)	Fukushima Minamiaizu	1986.6	+	+	+	+	+	−	+	+	+	+
Alnus japonica STEUD. (hannoki)	Fukushima Minamiaizu	1986.6	+	+	+	+	+	−	+	+	+	+
Celtis sinensis PERS. var. *japonica* (enoki)	Saitama Ohmiya	1987.7	+	+	+	+	+	+	+	+	+	+
Celtis sinensis PERS. var. *japonica* (enoki)	Tokushima Itano	1988.8	+	+	+	+	+	−	+	+	+	+
Celtis sinensis PERS. var. *japonica* (enoki)	Ehime Matsuyama	1988.8	+	+	+	+	+	−	+	+	+	+
Populus nigra L. var. *italica* (seiyohakoyanagi)	Ehime Matsuyama	1988.8	+	+	+	+	+	−	+	+	+	+

TABLE II. ^{13}C-NMR Chemical Shifts[a]

Compd. No.	VII	VIII	IX	A[b]	X	B[c]
C-2	163.3	163.4	156.3	156.4	78.8	78.9
C-3	104.2	104.2	133.7	133.0	42.2	42.1
C-4	181.9	181.9	177.6	177.3	197.1	197.1
C-5	161.0	161.1	160.9	161.1	162.8	162.9
C-6	97.9	98.0	97.9	98.7	96.4	96.5
C-7	165.1	165.2	165.2	164.1	162.7	162.7
C-8	92.7	92.7	92.4	93.7	95.2	95.4
C-9	157.2	157.2	156.0	156.4	164.9	165.3
C-10	104.7	104.7	105.1	103.9	103.2	103.2
C-1′	123.8	123.9	123.5	121.0	129.1	129.1
C-2′	110.3	110.1	113.5	113.3	111.2	111.3
C-3′	149.8	149.6	148.6	149.3	147.5	147.5
C-4′	149.1	149.0	148.1	146.8	147.0	147.0
C-5′	115.0	114.8	114.5	115.2	115.1	115.2
C-6′	119.8	119.7	121.5	122.2	119.7	119.7
7-OMe	55.9	55.9	55.7			
3′-OMe	56.0	56.0	56.1	55.6	55.6	55.7
(Glucose)						
C-1	99.5	98.0	99.5		97.7	99.6
C-2	73.0	77.0	73.1		76.8	73.0
C-3	76.7	74.8	76.8		75.7	76.3
C-4	69.9	69.9	69.6		69.7	69.5
C-5	77.1	77.1	77.1		76.6	77.1
C-6	60.6	60.5	60.6		60.4	60.5
(Glucose)						
C-1			100.7			
C-2			74.3			
C-3			76.4			
C-4			69.8			
C-5			77.4			
C-6			60.6			
(Apiose)						
C-1		108.3			108.6	
C-2		76.0			76.0	
C-3		79.3			79.1	
C-4		73.9			73.8	
C-5		64.4			64.1	

a) Spectra run at 100 Hz in DMSO-d_6. *b*) Isorhamnetin-3-*O*-rutinoside-(quercetin-3′-OMe-3-*O*-rutinoside).[5] *c*) (2*S*)-Homoeriodictyol-7-*O*-glucoside.[5]

TABLE III. Comparative Study on the Quantitative Analysis of Flavonoid Glycosides by HPLC

Host tress	Leaves			Twigs		
	VII	VIII	IX	VII	VIII	IX
	(mg/5 g dry weight)					
Q. mongollica FISHER (Nagano)	1.2	21.4	3.7	0.3	5.3	1.0
Q. mongollica FISHER (Aomori)	3.2	30.5	7.1	0.8	12.9	1.8
Q. serrara THUNB.	1.0	6.0	2.5	0.4	6.3	0.5
F. crenata BLUME	0.9	7.5	1.4	0.5	5.0	—
A. palmatum THUNB.	1.0	10.0	1.7	0.5	5.2	0.4
F. japonica MAXIM.	1.0	10.7	1.6	0.3	7.4	0.9
Q. acutissima CARRUTH.	0.7	8.7	2.1	—	4.7	0.4
P. mume SIEB.	2.1	11.6	4.5	—	1.9	—
P. pyrifolia L.	0.6	4.1	0.2	0.2	3.1	0.2
A. japonica STEUND.	1.0	16.6	2.0	0.6	9.3	—
C. sinensis PERS. (Saitama)	0.1	6.6	1.9	—	2.8	0.6
C. sinensis PERS. (Tokushima)	0.1	0.8	0.5			
C. sinensis PERS. (Ehime)	0.2	4.0	2.4			
Populus nigra L. (Ehime)	0.1	4.0	1.3			

—: not more than 0.1 mg/5 g (dry weight).

TABLE IV. Comparison of the Constituents of Japanese and European *V. album* L.

Compound	Japanese	European
β-Amyrin acetate	○	○
Phytosterol	○	○[a]
Phytosterol-glucoside	○	○[a]
Ceryl alcohol		○
Oleanolic acid	○	○
Butulinic acid		○
Syringin	○	○[a]
Coniferylalcohol-4′-[apiosyl(1→2)]glucoside		○
2′-Hydroxy-4′,6-dimethoxychalcone-4-*O*-glucoside		○
2′-Hydroxy-3,4′,6′-trimethoxychalcone-4-*O*-glucoside		○
2′-Hydroxy-4′,6′-dimethoxychalcone-4-*O*-[apiosyl(1→2)]glucoside		○
(2*R*)-5,7-Dimethoxyflavanone-4′-*O*-glucoside		○
(2*S*)-3′,5,7-Trimethoxyflavanone-4′-*O*-glucoside		○
Isorhamnetin-3-*O*-rutinoside		○
Isorhamnetin-3-*O*-[apiosyl(1→6)]glucosyl-7-*O*-rhamnoside		○
(2*S*)-Homoeriodictyol-7-*O*-glucoside		○
(2*S*)-Homoeriodictyol-7-*O*-[apiosyl(1→2)]-glucoside	○	
Rhamnazin-3,4′-di-*O*-glucoside	○	
3′,7-Dimethoxyluteolin-4′-*O*-glucoside	○	
3′,7-Dimethoxyluteolin-4′-*O*-[apiosyl(1→2)]-glucoside	○	

a) European contains more than Japanese.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Spectral data were obtained with the following apparatus; UV on a Hitachi 220A, infrared (IR) on a JASCO IR-810 and CD on a JASCO J-500C. ^{1}H-NMR and ^{13}C-NMR spectra were taken on a Bruker AM-400 and chemical shifts are given as δ values (ppm) with tetramethylsilane (TMS) as an internal standard (s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet). Gas liquid chromatography (GLC) was run on a Shimadzu GC-15A with a flame ionization detector, using a glass column (2 m × 3 mm i.d.) packed with 5% Unisole 400 on Uniport S (60—80 mesh), column temperature 260 °C. TLC was carried out on precoated 0.25 mm Kieselgel 60 F_{254} (Merck) plates. Column chromatography was carried out with Wakogel C-200 (Wako Pure Chemical Ind., Ltd.) and Sephadex LH-20 (Pharmacia Fine Chemicals) and Amberlite XAD-2 (Tokyo Organic Chemical Ind., Ltd.). HPLC was carried out on the CIG column system (30 μm ODS column, 15 i.d. × 300 mm, Kusano Scientific Co.) and JASCO TWINCLE apparatus (TSK Gel ODS-80TM column 4.6 i.d. × 150 mm, Toyo Soda Manufacturing Co., Ltd.).

Extraction and Isolation Dried leaves and twigs of *V. album* L. var. *coloratum* OHWI grown on *Quercus mongollica* FISHER var. *grosseserrata*, *Prunus jamasakura* SIEB., *Acer palmatum* THUNB. var. *palmatus*, *Prunus mume* SIEB., *Pyrus pyrifolia* L. var. *clulta* and *Celtis sinensis* PERS. var. *japonica* were extracted three times with MeOH in the usual ways. The MeOH extracts were partitioned between water and *n*-hexane with a separatory funnel and then between water and $CHCl_3$, and further between water and water-saturated *n*-BuOH. The *n*-hexane, $CHCl_3$ and *n*-BuOH extracts were subjected to column chromatography on silica gel by eluting with *n*-hexane–EtOAc (95:5—0:100), $CHCl_3$–MeOH (98:2—1:1) and $CHCl_3$–MeOH (8:2—0:10), respectively. β-Amyrin acetate (I) was eluted with *n*-hexane–EtOAc (95:5) and phytosterol (II) and fatty acids (III) with *n*-hexane–EtOAc (7:3) from the *n*-hexane extract. Oleanolic acid (IV) was eluted with $CHCl_3$–MeOH (95:5) and phytosterol-glucoside (V) with $CHCl_3$–MeOH (9:1) from the $CHCl_3$ extract. Flavoyadorinin-B was eluted with $CHCl_3$–MeOH (9:1), homoflavoyadorinin-B with $CHCl_3$–MeOH (8:2) and compound IX with $CHCl_3$–MeOH (7:3) from the *n*-BuOH extract. Further separation of the $CHCl_3$–MeOH (8:2) fraction was achieved by elution over Sephadex LH-20 with MeOH and by HPLC employing an ODS column that was eluted with MeOH–H_2O (1:1). The eluate yielded compound X.

β-Amyrin Acetate (I) Colorless needles, mp 236—239 °C. IR ν_{max}^{KBr} cm^{-1}: 2940, 1735, 1465, 1380, 1365, 1250, 1025, 1000. I was identified as β-amyrin acetate by comparison of the melting point, TLC behavior

and IR spectral data with those of an authentic sample.

Phytosterol (II) Colorless powder, mp 131—134 °C. II was identified as phytosterol by comparison with an authentic sample of β-sitosterol by GLC. GLC: $t_R = 11.9$ (β-sitosterol).

Fatty Acids (III) Colorless powder. IR ν_{max}^{KBr} cm^{-1}:2910, 2840, 1710, 1475, 1460, 1060, 730, 720. III was analyzed by GLC (He 50 ml/min.) and identified as a mixture of stearic, arachidic, behenic, lignoceric, cerotic, hepracosanic, and montanic acid (24, 13, 4, 10, 24, 5, 7%). The composition was determined from the GLC peak areas. GLC: $t_R = 2.2$ (stearic), 3.5 (arachidic), 5.6 (behenic), 9.2 (lignoceric), 15.1 (cerotic), 24.7 (hepracosanic), 34.1 (montanic).

Oleanolic Acid (IV) Colorless powder, mp 306—309 °C. IR ν_{max}^{KBr} cm^{-1}: 3430, 2490, 1685, 1640, 1450, 1390, 1365, 1265, 1180, 1025, 995. IV was identified as oleanolic acid by melting point, TLC behavior and IR spectral data with those of an authentic sample.

Phytosterol-glucoside (V) Colorless powder, mp 284—290 °C (dec.).

Hydrolysis of V V (2 mg) was dissolved in EtOH–5% HCl (1 : 1) (20 ml) and refluxed for 1.5 h. After removal of the EtOH under reduced pressure, phytosterol and glucose were detected by GLC.

Syringin (VI) Colorless needles, mp 187—190 °C. IR ν_{max}^{KBr} cm^{-1}: 3560, 3380, 2900, 1650, 1590, 1510, 1460, 1420, 1350, 1325, 1240, 1130, 1090, 1025, 985, 965. ^{1}H-NMR (DMSO-d_6) δ: 3.77 (6H, s, $-OCH_3 \times 2$), 4.10 (2H, d, $J = 4.9$ Hz, $-CH_2$), 4.88 (1H, d, $J = 5.1$ Hz, anomeric proton of glucose, tentative), 6.33 (1H, dt, $J = 4.9$, 15.9 Hz, =CH–), 6.46 (1H, d, $J = 15.9$ Hz, –CH=), 6.72 (2H, s). ^{13}C-NMR (DMSO-d_6) δ: 56.3 ($-OCH_3$), 60.9 (G-6), 61.4 (C-3), 69.9 (G-4), 74.1 (G-2), 76.5 (G-3), 77.1 (G-5), 102.5 (G-1), 104.5 (C-2′,6′), 128.4 (C-2), 130.1 (C-1), 132.5 (C-1′), 133.9 (C-4′), 152.6 (C-3′,5′).

Flavoyadorinin-B (VII) Pale yellowish powder, mp 239—241 °C. IR ν_{max}^{KBr} cm^{-1}: 3480, 3405, 2895, 1665, 1603, 1592, 1504, 1354, 1262, 1082, 848, 804. UV λ_{max}^{EtOH} nm (log ε): 334 (4.27), 269 (4.24), 240 (4.20). UV $\lambda_{max}^{EtOH+EtONa}$: 286. UV $\lambda_{max}^{EtOH+AlCl_3}$: 385, 349, 278. UV $\lambda_{max}^{EtOH+AlCl_3+HCl}$: 386, 345, 280. UV $\lambda_{max}^{EtOH+AcONa}$: 334, 269, 240. ^{1}H-NMR (DMSO-d_6) δ: 3.88, 3.92 (each 3H, each s, $-OCH_3$), 5.34 (1H, br s, anomeric proton of glucose), 6.37 (1H, d, $J = 2.2$ Hz, 6-H), 6.82 (1H, d, $J = 2.2$ Hz, 8-H), 7.04 (1H, s, 3-H), 7.26 (1H, d, $J = 8.7$ Hz, 5′-H), 7.63 (1H, d, $J = 2.0$ Hz, 2′-H), 7.68 (1H, dd, $J = 2.0$, 8.7 Hz, 6′-H).

Homo-flavoyadorinin-B (VIII) Yellowish powder, mp 217—220 °C. IR ν_{max}^{KBr} cm^{-1}: 3380, 2925, 1659, 1613, 1502, 1160, 1072, 1038, 821. UV λ_{max}^{EtOH} nm (log ε): 337 (4.33), 269 (4.27), 242 (4.25). UV $\lambda_{max}^{EtOH+EtONa}$: 329, 288, 270. UV $\lambda_{max}^{EtOH+AlCl_3}$: 387, 348, 279. UV $\lambda_{max}^{EtOH+AlCl_3+HCl}$: 387, 348, 279. UV $\lambda_{max}^{EtOH+AcONa}$: 336, 269, 241. ^{1}H-NMR (DMSO-d_6) δ: 3.88, 3.91 (each 3H, each s, $-OCH_3$), 5.31 (1H, d, $J = 5.5$ Hz, anomeric proton of glucose), 5.44 (1H, s, anomeric proton of apiose), 6.38 (1H, d, $J = 2.2$ Hz, 6-H), 6.83 (1H, d, $J = 2.2$ Hz, 8-H), 7.04 (1H, s, 3-H), 7.23 (1H, d, $J = 8.7$ Hz, 5′-H), 7.63 (1H, d, $J = 2.1$ Hz, 2′-H), 7.67 (1H, dd, $J = 2.1$, 8.7 Hz, 6′-H).

Rhamnazin-3,4′-di-*O*-glucoside (IX) Yellowish powder, mp 245—247 °C. IR ν_{max}^{KBr} cm^{-1}: 3320, 2900, 1658, 1600, 1499, 1070, 800. UV λ_{max}^{EtOH} nm (log ε): 347 (3.94), 267 (3.98), 252 (4.01). UV $\lambda_{max}^{EtOH+EtONa}$: 283. UV $\lambda_{max}^{EtOH+AlCl_3}$: 397, 350, 275. UV $\lambda_{max}^{EtOH+AlCl_3+HCl}$: 396, 348, 277. UV $\lambda_{max}^{EtOH+AcONa}$: 346, 266, 251. ^{1}H-NMR (DMSO-d_6) δ: 3.86, 3.87 (each 3H, each s, $-OCH_3$), 5.34 (1H, d, $J = 4.8$ Hz, anomeric proton of glucose, tentative), 5.60 (1H, d, $J = 7.4$ Hz, anomeric proton of glucose), 6.40 (1H, d, $J = 2.2$ Hz, 6-H), 6.79 (1H, d, $J = 2.2$ Hz, 8-H), 7.25 (1H, d, $J = 8.8$ Hz, 5′-H), 7.61 (1H, dd, $J = 2.1$, 8.8 Hz, 6′-H), 7.99 (1H, $J = 2.1$ Hz, 2′-H).

(2*S*)-Homoeriodictyol-7-*O*-[apiosyl(1→2)]glucoside (X) Pale yellowish powder, mp 143—146 °C. CD ($c = 4.7 \times 10^{-4}$, MeOH) $[\theta]^{25}$ (nm): +6140 (332) (positive maximum), −18900(287) (negative maximum). IR ν_{max}^{KBr} cm^{-1}: 3350, 2930, 1645, 1605, 1580, 1525, 1460, 1300, 1275, 1200, 1185, 1090, 1050, 825. UV λ_{max}^{EtOH} nm (log ε): 324 (sh), 282 (4.22). UV $\lambda_{max}^{EtOH+EtONa}$: 444, 282. UV $\lambda_{max}^{EtOH+AlCl_3}$: 380, 306. UV $\lambda_{max}^{EtOH+AlCl_3+HCl}$: 380, 304. UV $\lambda_{max}^{EtOH+AcONa}$: 324 (sh), 282, ^{1}H-NMR (DMSO-d_6) δ: 2.74 (1H, dd, $J = 2.8$, 17.2 Hz, 3-H *cis*), 3.17 (1H, m, 3-H *trans*), 3.79 (3H, s, $-OCH_3$), 5.29 (1H, d, $J = 4.7$ Hz, anomeric proton of glucose), 5.32 (1H, s, anomeric proton of apiose), 5.50 (1H, dd, $J = 2.8$, 12.9 Hz, 2-H), 6.11 (1H, d, $J = 1.6$ Hz, 6-H), 6.16 (1H, d, $J = 1.6$ Hz, 8-H), 6.80 (1H, d, $J = 8.2$ Hz, 5′-H), 6.92 (1H, d, $J = 8.2$ Hz, 6′-H), 7.11 (1H, s, 2′-H).

Hydrolysis of VII—X The samples (1 mg) in 2 ml of EtOH–5% H_2SO_4 (1 : 1) were refluxed for 3 h on a water bath and then neutralized with $BaCO_3$. The precipitate was filtered off and the filtrate was used for detection of the sugar components by TLC (*n*-BuOH–AcOH–H_2O, 6 : 3 : 1, anisaldehyde reagent).

Comparison of the Constituents by TLC Dried leaves and twigs (5 g) of *V. album* L. var. *coloratam* Ohwi were extracted with MeOH (80 ml). The MeOH extracts were analyzed by TLC [I. *n*-hexane–EtOAc (7 : 3), II. $CHCl_3$–MeOH (9 : 1), III. $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower layer)], which indicated the presence of compounds I—X.

Quantitative Analysis of Flavonoid Glycosides (VII—IX) by HPLC Dried leaves and twigs (each 5 g) were extracted with MeOH (80 ml). The MeOH extracts were weighed, dissolved in MeOH and passed through a Sep-Pak C_{18} cartridge (Waters Associates). The filtrates were made up to 100 ml with MeOH. Column, TSK Gel ODS 80 TM (4.6 mm i.d. × 150 mm); eluents, 5 mM KH_2PO_4–H_3PO_4 (pH 4.0)/MeOH (45 : 55); flow rate, 1.0 ml/min; detection, UV 335 nm; injection volume, 20 μl.

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