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Studies on the Constituents of the European Mistletoe, *Viscum album* L. II[1)]

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A new flavonol glycoside, isorhamnetin-3-*O*-[apiosyl(1→6)]glucosyl-7-*O*-rhamnoside, and a new phenylpropane glycoside, coniferylalcohol-4′-*O*-[apiosyl(1→2)]glucoside, were isolated from the *n*-BuOH extract of *Viscum album* L. (Loranthaceae). The structures were established based on the spectral and chemical data. Two known flavonoid glycosides, isorhamnetin-3-*O*-rutinoside and (2*S*)-homoeriodictyol-7-*O*-glucoside, were also isolated.

Keywords——*Viscum album*; mistletoe; Loranthaceae; flavonoid; isorhamnetin-3-*O*-[apiosyl-(1→6)]glucosyl-7-*O*-rhamnoside; coniferylalcohol-4′-*O*-[apiosyl(1→2)]-glucoside; isorhamnetin-3-*O*-rutinoside; (2*S*)-homoeriodictyol-7-*O*-glucoside

The European mistletoe, *Viscum album* L. (Loranthaceae), is an evergreen parasitic plant widely distributed throughout Europe, except in northern areas. In our previous studies on the constituents of *V. album* L.,[1)] we reported on the isolation and the structural elucidation of four new flavonoid glycosides, 2′-hydroxy-3,4′,6′-trimethoxychalcone-4-*O*-glucoside, 2′-hydroxy-4′, 6′-dimethoxychalcone-4-*O*-[apiosyl (1→2)]glucoside, (2*R*)-5,7-dimethoxy-flavanone-4′-*O*-glucoside and (2*S*)-3′,5,7-trimethoxy-flavanone-4′-*O*-glucoside. In our further studies on this plant, a new flavonol glycoside, isorhamnetin-3-*O*-[apiosyl(1→6)]glucosyl-7-*O*-rhamnoside (II), and a new phenylpropane glycoside, coniferylalcohol-4′-*O*-[apiosyl-(1→2)]glucoside (IV), were isolated from the *n*-BuOH extract of *V. album* L.

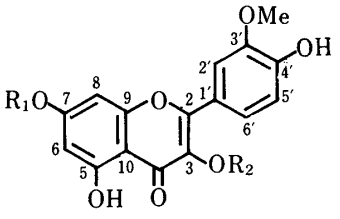
The MeOH extract of *V. album* L. (dried commercial product was extracted with *n*-hexane, $CHCl_3$ and *n*-BuOH, successively. The *n*-BuOH extract was concentrated and subjected to separation by the use of silica gel, Amberlite XAD-2, Sephadex LH-20 and high performance liquid chromatography (HPLC). Two known flavonoid glycosides, isorhamnetin-3-*O*-rutinoside (I)[2)] and (2*S*)-homoeriodictyol-7-*O*-glucoside (III),[3)] were also isolated.

Compound I was obtained as a yellowish powder, mp 178—180 °C. Upon hydrolysis of I, glucose and rhamnose were detected. On the basis of the bathochromic shifts with diagnostic reagents, the proton nuclear magnetic resonance (^{1}H-NMR) and the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectral data, compound I was confirmed to be isorhamnetin-3-*O*-rhamnoside.[2)]

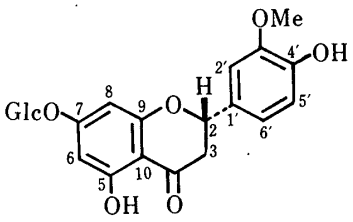
Compound II was obtained as a yellowish powder, mp 162—163 °C. The ^{1}H-NMR, ultraviolet (UV) and ^{13}C-NMR spectral data of the aglycone moiety were very similar to those of compound I (Table I). The UV absorption of II showed bathochromic shifts of band I with NaOEt ($\Delta\lambda_{max} = +63$ nm) and with $AlCl_3$ ($\Delta\lambda_{max} = +53$ nm), but showed no bathochromic shift of band II with NaOAc. The above bathochromic shifts in UV absorption and the ion peaks at *m/z* 151 (B_2^+) and 153 ($A_1 + H^+$) in the electron impact-mass (EI-MS)

spectrum suggested the presence of two hydroxyl groups at C-5 and C-4′ and a methoxyl group at C-3′.[4,5] Thus, the aglycone structure was assumed to be same as that of I. In addition, after hydrolysis of II, glucose, rhamnose and apiose were detected as the sugar components of II by thin layer chromatography (TLC). In the ^{13}C-NMR spectrum of the sugar moiety of II, the C-6 signal of glucose showed a downfield shift (glycosylation shift) in comparison with that of 2′-hydroxy-4′,6′-dimethoxychalcone-4-*O*-[apiosyl(1→2)]glucoside[1] (see Table II). The anomeric carbon signal of rhamnose showed an upfield shift in comparison with that of compound I and the chemical shifts for rhamnose of II were in good agreement with those of kaempferol-3-*O*-glucosyl-7-*O*-rhamnoside[2] (Table II). Therefore, the position of attachment of rhamnose was proved to be at C-7, and apiosyl(1→6)glucose was attached to C-3. On the basis of the above results, the structure of compound II was established as isorhamnetin-3-*O*-[apiosyl(1→6)]glucosyl-7-*O*-rhamnoside.

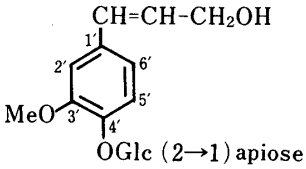
Compound III was obtained as a pale yellowish powder, mp 142—144 °C. After hydrolysis of III, glucose was detected. The bathochromic shifts with diagnostic reagents in the UV spectrum, and the ^{1}H-NMR and the EI-MS data suggested the aglycone of III to be homoeriodictyol.[2] The circular dichroism (CD) spectrum of III exhibited a positive Cotton effect at 333 nm and a negative Cotton effect at 287 nm. Therefore, C-2 was assigned the *S* configuration.[6] On the basis of the above results and ^{13}C-NMR spectral data, compound III was identified as (2*S*)-homoeriodictyol-7-*O*-glucoside, which has been isolated from Chinese mistletoe.[3]



I: R_1 = H, R_2 = Glc(6→1)rham
II: R_1 = Rham, R_2 = Glc(6→1)apiose



III



IV

Chart 1

TABLE I. ^{13}C-NMR Chemical Shifts[a] (Aglycone Moieties)

Compd. No.	A[b]	I	II	III
C-2	156.2[c]	156.4[c]	156.4[c]	78.9
C-3	133.3	133.0	132.7	42.1
C-4	177.3	177.3	177.2	197.1
C-5	161.2	161.1	161.2	162.9
C-6	98.7	98.7	98.6	96.5
C-7	164.0	164.1	164.0	165.3
C-8	93.7	93.7	93.7	95.4
C-9	156.4[c]	156.4[c]	156.4[c]	162.7
C-10	104.1	103.9	104.0	103.2
C-1′	121.2[d]	121.0[d]	121.2[d]	129.1
C-2′	113.9[e]	113.3[e]	113.2[e]	111.3
C-3′	149.5	149.3	149.3	147.5
C-4′	147.0	146.8	146.9	147.0
C-5′	115.3[e]	115.2[e]	115.2[e]	115.2
C-6′	122.4[d]	122.2[d]	122.3[d]	119.7
3′-OMe	56.0	55.6	55.6	55.7

a) Spectra run at 100 Hz in DMSO-d_6. *b*) Isorhamnetin-3-*O*-rutinoside (quercetin-3′-OMe-3-*O*-rhamnosyl(1→6)glucoside).[2] *c*—*e*) Assignments may be interchanged in each column.

Table II. ^{13}C-NMR Chemical Shifts[a] (Sugar Moieties)

Compd. No.	B[b]	I	II	C[c]	D[d]	III
Glucose						
C-1	101.5	101.1	100.8	101.5	98.3	99.6
C-2	74.4	74.2	75.7	74.3	76.9[f]	73.0
C-3	76.7	76.3	76.8	76.5	75.8	76.3
C-4	70.2[e]	70.0[e]	70.3[e]	70.0[e]	69.8	69.5
C-5	76.1	75.9	76.0	77.1	76.8[f]	77.1
C-6	67.0	66.8	66.8	61.1	60.5	60.5
Rhamnose						
C-1	100.8	100.8	98.8	98.9		
C-2	70.4[e]	70.2[e]	70.5[e]	70.3[e]		
C-3	70.8[e]	70.5[e]	70.6[e]	70.6[e]		
C-4	72.1	71.7	71.8	71.8		
C-5	68.2	68.2	68.2	70.0[e]		
C-6	17.6	17.6	17.6	17.5		
Apiose						
C-1			108.6		108.7	
C-2			76.8		76.0	
C-3			79.2		79.2	
C-4			74.0		73.9	
C-5			64.4		64.2	

a) Spectra run at 100 Hz in DMSO-d_6. *b*) Isorhamnetin-3-*O*-rhamnosyl(1→6)glucoside.[2] *c*) Kaempferol-3-*O*-glucosyl-7-*O*-rhamnoside.[2] *d*) 2′-Hydroxy-4′,6′-dimethoxychalcone-4-*O*-[apiosyl(1→2)]glucoside.[1] *e*, *f*) Assignments may be interchanged in each column.

Table III. ^{13}C-NMR Chemical Shifts[a]

Compd. No.	Syringin	IV	E[b]
C-1	61.4	61.5	—
C-2	128.4	128.3	—
C-3	130.1	128.9	—
C-1′	132.5	130.9	132.5
C-2′	104.5	109.6	111.2
C-3′	152.6	148.8	148.8
C-4′	133.9	145.8	146.5
C-5′	152.6	115.0	115.1
C-6′	104.5	118.8	118.9
OMe	56.3, 56.3	55.5	55.7
Glucose			
C-1	102.5	98.4	99.9
C-2	74.1	76.8[c]	73.1
C-3	76.5	74.9	76.8[c]
C-4	69.9	69.9	69.6
C-5	77.1	77.1[c]	77.0[c]
C-6	60.9	60.5	60.6
Apiose			
C-1		108.2	
C-2		76.0[c]	
C-3		79.2	
C-4		73.8	
C-5		64.4	

a) Spectra run at 100 Hz in DMSO-d_6. *b*) B-ring and sugar moiety of (2*R*)-5,7-dimethoxyflavanone-4′-*O*-glucoside.[1] *c*) Assignments may be interchanged in each column.

Compound IV was obtained as colorless needles, mp 119—122 °C. The ^{1}H-NMR spectrum of IV exhibited signals at δ 3.77 (s) due to a methoxyl group and at δ 6.98 (1H, d, J=8.4 Hz), 7.05 (1H, d, J=1.8 Hz) and 6.87 (1H, dd, J=1.8, 8.4 Hz) due to *ortho* and *meta* coupling arising from three protons of the aromatic ring. Three signals at δ 6.27 (1H, d, J=5.2, 15.9 Hz), 6.47 (1H, d, J=15.9 Hz) and 4.09 (2H, ABq, J=5.2 Hz) suggested the presence of two olefinic protons and a hydroxymethylene moiety (CH=CH–CH$_2$–OH). After hydrolysis of IV, glucose and apiose were detected as the sugar components of IV by TLC. The UV spectrum of the aglycone showed a bathochromic shift ($\Delta\lambda_{max}$=+16 nm) with NaOEt, which suggested the presence of the phenolic hydroxyl group. Therefore, the sugar was attached to the aromatic ring. The ^{13}C-NMR spectral data suggested that the sugar moiety of IV was apiosyl(1→2)glucose and the position of attachment of the sugar was proved to be at C-4′ by comparing the chemical shifts with the B-ring carbon signals of (2*R*)-5,7-dimethoxyflavanone-4′-*O*-glucoside[1] (Table III). On the basis of the above results, the structure of compound IV was established as coniferylalcohol-4′-*O*-[apiosyl(1→2)]glucoside. Syringin has been isolated from $CHCl_3$ extract of *V. album* L.[1] Syringenin-4′-*O*-[apiosyl(1→2)]glucoside, which has a similar structure to compound IV, has also been isolated from *V. album* L.[7]

Experimental

All melting points were recorded on a Yanagimoto micro melting point apparatus and are uncorrected. Spectral data were obtained with the following instruments; UV on a Hitachi 220A, infrared (IR) on a JASCO IR-810, CD on a JASCO J-500C, and MS on a Hitachi M80. ^{1}H-NMR and ^{13}C-NMR spectra were taken on a Bruker AM400 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; q, quartet; m, mutiplet). TLC was carried out on precoated 0.25 mm Kieselgel 60 F_{254} (Merck) plates. Spots were detected by exposure to UV light (254, 365 nm) and spraying 10% H_2SO_4 and anisaldehyde sulfate reagent followed by heating. Column chromatography was carried out with Wakogel C-200 (Wako Pure Chemical Ind. Ltd.), Amberlite XAD-2 (Tokyo Organic Chemical Ind. Ltd.) and Sephadex LH-20 (Pharmacia Fine Chemicals). HPLC was carried out on the CIG column system (30 μm ODS column, 15 i.d. × 300 mm, Kusano Scientific Co.).

Extraction and Isolation——The twigs and leaves, cut into pieces, of *Viscum album* L. (10 kg) from West Germany were purchased from Iwase-Kenjiro Shoten and were extracted with MeOH, three times. The MeOH extract (2.75 kg) was 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, yielding 749 g of *n*-BuOH extract. The *n*-BuOH extract (310 g) was subjected to column chromatography on silica gel (1.8 kg) by eluting with $CHCl_3$–MeOH (8:2—0:10). The eluate with $CHCl_3$–MeOH (7:3) gave a new flavonol glycoside (II), a new phenylpropane glycoside (IV) and two known flavonoid glycosides, isorhamnetin-3-*O*-rutinoside (I) and (2*S*)-homoeriodictyol-7-*O*-glucoside (III).

Isorhamnetin-3-*O*-rutinoside (I)——Yellowish powder, 107 mg. mp 178—180 °C. UV λ_{max}^{EtOH} nm (log ε): 359 (4.23), 266 sh, 254 (4.30), $\lambda_{max}^{EtOH+NaOEt}$ nm: 418, 332, 273, $\lambda_{max}^{EtOH+NaOAc}$ nm: 402, 321, 274, $\lambda_{max}^{EtOH+AlCl_3}$ nm: 405, 370 sh, 300 sh, 269, $\lambda_{max}^{EtOH+AlCl_3/HCl}$ nm: 400, 360, 302, 275 sh, 267. IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 1660, 1610, 1500, 1360, 1290, 1205, 1060. ^{1}H-NMR(DMSO-d_6) δ: 0.99 (3H, d, J=6.2 Hz, –CH$_3$ of rhamnose), 3.85 (3H, s, –OCH$_3$), 4.43 (1H, s, anomeric proton of rhamnose), 5.45 (1H, d, J=7.3 Hz, anomeric proton of glucose), 6.21, 6.44 (each 1H, d, J=2.0 Hz, 6-H and 8-H), 6.92 (1H, d, J=8.4 Hz, 5′-H), 7.53 (1H, dd, J=2.0, 8.4 Hz, 6′-H), 7.86 (1H, d, J=2.0 Hz, 2′-H).

Isorhamnetin-3-*O*-[apiosyl(1→6)]glucosyl-7-*O*-rhamnoside (II)——Yellowish powder, 172 mg. mp 162—163 °C. UV λ_{max}^{EtOH} nm (log ε): 352 (4.16), 265 sh, 253 (4.23), $\lambda_{max}^{EtOH+NaOEt}$ nm: 415, 333, 271, $\lambda_{max}^{EtOH+NaOAc}$ nm: 358, 268, 254, $\lambda_{max}^{EtOH+AlCl_3}$ nm: 405, 359 sh, 303, 275 sh, 268, $\lambda_{max}^{EtOH+AlCl_3/HCl}$ nm: 401, 360, 300 sh, 275 sh, 268. IR ν_{max}^{KBr} cm^{-1}: 3390, 2930, 1660, 1610, 1510, 1360, 1290, 1205, 1070. EI-MS *m/z* (%): 350 (35), 316 (100), 153 (8), 151 (8). ^{1}H-NMR (DMSO-d_6) δ:0.97 (3H, d, J=6.2 Hz, –CH$_3$ of rhamnose), 3.86 (3H, s, –OCH$_3$), 4.36 (1H, s, anomeric proton of rhamnose), 5.33 (1H, d, J=0.9 Hz, anomeric proton of apiose), 5.54 (1H, d, J=7.4 Hz, anomeric proton of glucose), 6.21, 6.44 (each 1H, d, J=2.0 Hz, 6-H and 8-H), 6.92 (1H, d, J=8.4 Hz, 5′-H), 7.56 (1H, dd, J=2.0, 8.4 Hz, 6′-H), 7.82 (1H, d, J=2.0 Hz, 2′-H).

(2*S*)-Homoeriodictyol-7-*O*-glucoside (III)——Pale yellowish powder, 98 mg. mp 141—144 °C. CD (c=1.1 × 10^{-4}, MeOH) $[\theta]^{25}$ (nm): +4910 (333) (positive maximum), –24400 (287) (negative maximum). UV λ_{max}^{EtOH} nm (log ε): 327 sh, 283 (4.34), $\lambda_{max}^{EtOH+NaOEt}$ nm: 439, 285, $\lambda_{max}^{EtOH+NaOAc}$ nm: 327 sh, 283, $\lambda_{max}^{EtOH+AlCl_3}$ nm: 383, 305, $\lambda_{max}^{EtOH+AlCl_3/HCl}$ nm: 383, 307. IR ν_{max}^{KBr} cm^{-1}: 3380, 2920, 1650, 1580, 1520, 1300, 1200, 1175, 1080. EI-MS *m/z* (%): 302

(100), 179 (23), 153 (79), 150 (91). ^{1}H-NMR (DMSO-d_6) δ: 2.75 (1H, dd, J=2.8, 17.2 Hz, 3-H *cis*), 3.16 (1H, m, 3-H *trans*), 3.79 (3H, s, $-OCH_3$), 5.35 (1H, d, J=4.9 Hz, anomeric proton of glucose), 5.49 (1H, dd, J=2.8, 12.9 Hz, 2-H), 6.14, 6.18 (each 1H, d, J=2.1 Hz, 6-H, 8-H), 6.80 (1H, d, J=8.2 Hz, 5′-H), 6.92 (1H, dd, J=1.9, 8.2 Hz, 6′-H), 7.11 (1H, d, J=1.9 Hz, 2′-H).

Coniferylalcohol-4′-*O*-[apiosyl(1→2)]glucoside (IV)——Colorless needles, 265 mg. mp 119—122 °C. UV λ_{max}^{EtOH} nm (log ε): 258 (4.14), $\lambda_{max}^{EtOH+NaOEt}$ nm: 258 [after hydrolysis] UV λ_{max}^{EtOH} nm: 280, $\lambda_{max}^{EtOH+NaOEt}$ nm: 296, 247. IR ν_{max}^{KBr} cm^{-1}: 3370, 2920, 1590, 1515, 1260, 1225, 1125, 1085. ^{1}H-NMR (DMSO-d_6) δ: 3.77 (3H, s, $-OCH_3$), 4.09 (2H, ABq, J=5.2 Hz, $-CH_2$), 4.93 (1H, d, J=7.6 Hz, anomeric proton of glucose, tentative), 5.41 (1H, s, anomeric proton of apiose), 6.27 (1H, dt, J=5.2, 15.9 Hz, =CH–), 6.47 (1H, d, J=15.9 Hz, –CH=), 6.87 (1H, dd, J=1.8, 8.4 Hz, 6′-H), 6.98 (1H, d, J=8.4 Hz, 5′-H), 7.05 (1H, d, J=1.8 Hz, 2′-H).

Hydrolysis of I—IV——A sample (1 mg) in 2 ml of EtOH–5% H_2SO_4 (1 : 1) was heated under reflux for 3 h, and then extracted with ethyl ether several times. The aqueous layer was neutralized with $BaCO_3$. The precipitate was filtered off and the filtrate was used for detection of the sugar component. Glucose (*Rf* 0.43), apiose (*Rf* 0.50) and rhamnose (*Rf* 0.58) were detected by TLC (*n*-BuOH–AcOH–H_2O=6 : 3 : 1, anisaldehyde reagent).

References and Notes

1) T. Fukunaga, I. Kajikawa, K. Nishiya, Y. Watanabe, K. Takeya and H. Itokawa, *Chem. Pharm. Bull.*, **35**, 3292 (1987).
2) K. R. Markham, B. Ternai, R. Stanley, H. Geiger and T. J. Mabry, *Tetrahedron*, **34**, 1389 (1978).
3) J. H. Lin, *Kou Li Chung-Kuo I Yao Yen Chiu So Yen Chiu Pao Kao*, July, 131 (1983).
4) T. J. Mabry, K. R. Markham and M. B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, New York, 1970.
5) J. B. Harborne, T. J. Mabry and H. Mabry, "The Flavonoids," Chapman and Hall, London, 1975.
6) *a*) W. Gaffield and A. C. Waiss, *Chem. Commun.*, **1968**, 29; *b*) W. Gaffield, *Tetrahedron*, **26**, 4093 (1970).
7) H. Wagner, B. Feli and S. Blade, *Dtsch. Apoth. Ztg.*, **29**, 1429 (1984).