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Chemical Studies on the Constituents of *Hyphear Tanakae* HOSOKAWA from Different Host Trees

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The chemical constituents of *Hyphear Tanakae* HOSOKAWA epiphyting to four different host trees were examined. A new triterpene fatty acid ester (III) and four known flavonoid glycosides, rhamnocitrin-3-*O*-rhamnoside (V), kaempferol-3-*O*-rhamnoside (VI), rhamnetin-3-*O*-rhamnoside (VII), and quercetin-3-*O*-rhamnoside (VIII) have been isolated from the leaves and twigs of this plant epiphyting to *Castanea crenata* SIEB., together with fatty acids (I), phytosterol (II), and phytosterol-glucoside (IV). The present investigation has revealed that compounds II—V, VII, and VIII are contained in this plant irrespective of the host, among the four host trees, examined.

Keywords—*Hyphear Tanakae*; mistletoe; Loranthaceae; triterpene; lup-20(29)-ene-3 β ,7 β ,15 α -triol; flavonoid; rhamnocitrin-3-*O*-rhamnoside; kaempferol-3-*O*-rhamnoside; rhamnetin-3-*O*-rhamnoside; quercetin-3-*O*-rhamnoside

Japanese mistletoe, *Hyphear Tanakae* HOSOKAWA (hozakiyadorigi in Japanese) is a deciduous shrub of Loranthaceae. The present paper describes the isolation and the structural elucidation of the constituents in leaves and twigs of *H. Tanakae* HOSOKAWA epiphyting to *Castanea crenata* SIEB. and presents a comparison of the constituents from this plant epiphyting to different host trees: *Prunus Mume* SIEB., *Prunus Armeniaca* L. var. *ansu* MAXIM., and *Zelkova serrata* MAKINO.

The methanolic extract of *H. Tanakae* HOSOKAWA epiphyting to *Castanea crenata* SIEB. was partitioned between chloroform and water. The aqueous layer was further extracted with *n*-butanol. The chloroform extract was subjected to silica gel column chromatography. We isolated a new triterpene fatty acid ester (III), together with fatty acids (I), phytosterol (II), and phytosterol-glucoside (IV) from the chloroform extract.

Compound III was obtained as a colorless powder. The infrared (IR) spectrum of III showed *endo*-methylene (3065, 1640, 880 cm⁻¹) and ester carbonyl (1733 cm⁻¹) absorption bands. Compound III was hydrolyzed with EtOH-5% KOH to give an aglycone (IIIa), and fatty acids, *i.e.*, palmitic, stearic, arachidic, behenic and lignoceric acids.

Compound IIIa was obtained as a colorless powder, mp 263—266 °C, [α]_D + 17°. The electron impact-mass (EI-MS) spectrum of IIIa showed the molecular ion peak at *m/z* 458 (M⁺). The proton nuclear magnetic resonance (¹H-NMR) spectrum of IIIa showed one olefinic methyl signal, six tertiary methyl signals and signals due to the three hydroxymethine protons at δ 3.19 (dd, *J* = 4.7, 11.4 Hz), 3.79 (dd, *J* = 5.0, 11.1 Hz), and 4.14 (dd, *J* = 5.2, 11.2 Hz). On the basis of a comparison of the carbon¹³ nuclear magnetic resonance (¹³C-NMR) data for IIIa with those of lupeol,¹⁾ IIIa exhibited substituents shifts of +38.4, +10.0, +7.0, -5.1 ppm at C-7, C-6, C-8, C-26 and +40.3, +6.2, +10.0, -6.1 ppm at C-15, C-14, C-16, C-27, respectively, suggesting the presence of hydroxyl groups at C-7, and C-15 in addition to C-3. Therefore, IIIa was deduced to be lup-20(29)-ene-3 β ,7 β ,15 α -triol (Table I).²⁾

A comparison of the ^{13}C -NMR data of III with those of IIIa showed that the C-2 and C-3 signals of III are shifted upfield by 3.7 ppm, and downfield by 1.6 ppm, respectively, on going from IIIa to III. Therefore the fatty acid ester part of III was determined to be linked at the C-3 hydroxyl group.²⁾

Based on the above-mentioned evidence, the structure of III has been determined as a mixture of lup-20(29)-ene-7 β ,15 α -diol-3 β -palmitate, stearate, arachidate, behenate and

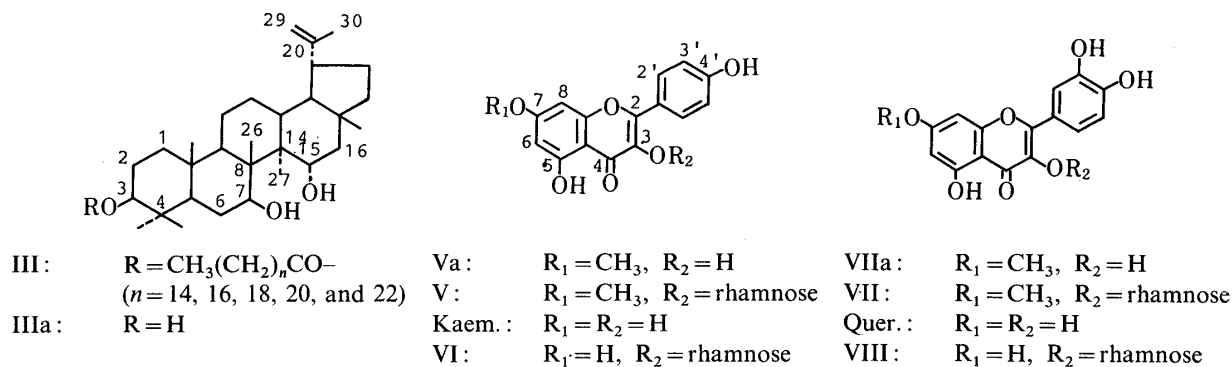


Chart 1

TABLE I. ^{13}C -NMR Spectral Data for III and Related Compounds in CDCl_3

	Lupeol ¹⁾	IIIa	III	
C-1	38.7	38.8	38.5	
C-2	27.5	27.5	23.8	
C-3	79.0	78.7	80.3	
C-4	38.9	38.6	37.6	
C-5	55.3	52.1	52.2	
C-6	18.3	28.3	28.1	
C-7	34.3	72.7	72.5	
C-8	40.9	47.9	47.9	
C-9	50.5	50.4	50.3	
C-10	37.2	37.4	37.3	
C-11	21.0	20.7	20.7	
C-12	25.2	25.0	25.2	
C-13	38.1	37.6	37.5	
C-14	42.9 ^{a)}	49.1	49.0	Fatty acid
C-15	27.5	67.8	67.9	Ester part of III
C-16	35.6	45.6	45.6	CH ₃ 14.1
C-17	43.0 ^{a)}	42.6	42.6	CH ₂ 22.7
C-18	48.0 ^{b)}	47.6 ^{b)}	47.6 ^{b)}	24.9
C-19	48.3 ^{b)}	48.2 ^{b)}	48.2 ^{b)}	29.2
C-20	150.9	150.4	150.3	29.4
C-21	29.9	30.2	30.2	29.5
C-22	40.0	39.9	39.8	29.6
C-23	28.0	28.0	27.9	29.7
C-24	15.3	15.3	15.6	31.9
C-25	16.1 ^{c)}	15.9	16.5	34.8
C-26	16.0 ^{c)}	10.9	10.9	C 173.7
C-27	14.6	8.5	8.4	
C-28	18.0	18.9	18.9	
C-29	109.3	109.6	109.7	
C-30	19.3	19.5	19.4	

a—c) Assignments bearing the same superscript in any column may be reversed.

lignocerate.

The butanol extract was subjected to silica gel column chromatography, Diaion HP-20 column chromatography, and high-performance liquid chromatography (HPLC). We isolated four known flavonoid glycosides, V—VIII, from the butanol extract. On the basis of the results of hydrolysis, the bathochromic shifts with diagnostic reagents (EtONa, AlCl_3 , AlCl_3/HCl , and AcONa) in the ultraviolet (UV) spectra, and the IR, $^1\text{H-NMR}$, and $^{13}\text{C-NMR}$ (Table II) spectral data, compounds V-VIII were identified as rhamnocitrin-3-*O*-rhamnoside, kaempferol-3-*O*-rhamnoside, rhamnetin-3-*O*-rhamnoside, and quercetin-3-*O*-rhamnoside, respectively.³⁻⁵⁾

Harvala *et al.*⁶⁾ isolated rhamnocitrin-3-*O*-rhamnoside, rhamnetin-3-*O*-glucoside, and rhamnetin-3-*O*-rhamnoside from leaves of *Loranthus europaeus* L. (Loranthaceae).

A comparison of the constituents from *Hyphear Tanakae* HOSOKAWA epiphyting to different host trees by thin layer chromatography (TLC) showed that compounds II—V, VII,

TABLE II. $^{13}\text{C-NMR}$ Spectral Data for Flavonoid Glycosides and Related Compounds in $\text{DMSO-}d_6$

	Kaem. ⁵⁾	VI	Va	V	Quer. ⁵⁾	VIII	VIIa	VII
C-2	146.8	157.1	147.2	157.4	146.9	157.2	147.2	157.6
C-3	135.6	134.2	135.9	134.4	135.5	134.2	135.9	134.4
C-4	175.9	177.6	175.9	177.8	175.8	177.7	175.8	177.8
C-5	160.7	161.2	160.3	160.9	160.7	161.3	160.3	160.9
C-6	98.2	98.7	97.3	97.8	98.2	98.6	97.3	97.8
C-7	163.9	164.2	164.8	165.1	163.9	164.1	164.8	165.1
C-8	93.5	93.7	91.9	92.3	93.3	93.6	91.8	92.2
C-9	156.2	156.4	156.0	156.3	156.2	156.4	156.0	156.3
C-10	103.1	104.1	103.9	105.0	103.1	104.0	103.9	105.0
C-1'	121.7	120.5	121.5	120.3	122.1	120.7	121.8	120.6
C-2'	129.5	130.5	129.5	130.5	115.3 ^{b)}	115.4 ^{b)}	115.2 ^{b)}	115.4 ^{b)}
C-3'	115.4	115.3	115.3	115.3	145.0	145.1	145.0	145.2
C-4'	159.2	159.9	159.2	160.0	147.6	148.4	147.7	148.5
C-5'	115.4	115.3	115.3	115.3	115.6 ^{b)}	115.6 ^{b)}	115.5 ^{b)}	115.7 ^{b)}
C-6'	129.5	130.5	129.5	130.5	120.0	121.1	119.9	121.1
OMe			55.9	56.0			55.9	56.0
3- <i>O</i> -Rhamnose								
C-1		101.7		101.7		101.8		101.8
C-2		70.3 ^{a)}		70.3 ^{a)}		70.3 ^{a)}		70.3 ^{a)}
C-3		70.5 ^{a)}		70.6 ^{a)}		70.5 ^{a)}		70.5 ^{a)}
C-4		71.1		71.1		71.2		71.2
C-5		70.0 ^{a)}		70.0 ^{a)}		70.0 ^{a)}		70.0 ^{a)}
C-6		17.4		17.4		17.4		17.4

Kaem., kaempferol; Quer., quercetin. a, b) Assignments bearing the same superscript in any column may be reversed.

TABLE III. Contents of Flavonoid Compounds in the Leaves and Twigs of *Hyphear Tanakae* HOSOKAWA (mg/g)

Host species		V	VII	VIII
<i>Prunus Mume</i> SIEB.				
	Leaves	6.12	28.7	8.59
	Twigs	2.57	19.8	5.34
<i>Prunus Armeniaca</i> L.				
	Leaves	3.17	19.5	5.59
	Twigs	2.10	16.3	3.95
<i>Zelkova serrata</i> MAKINO				
	Leaves	4.27	19.8	5.17
	Twigs	2.55	18.4	5.00

and VIII are contained in this plant irrespective of the host tree. The contents of flavonoid compounds in the leaves were higher than those in the twigs, especially in the plant epiphyting to *Prunus Mume* SIEB., but there was no marked variation among the plants on different host trees (Table III).

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were obtained with a JASCO IR-810 spectrometer. UV spectra were recorded on a Hitachi 220A spectrophotometer. Gas liquid chromatography (GLC) was run on a Hewlett Packard 5730 with a flame ionization detector, using glass columns (1.8 m \times 4 mm i.d.) packed with 5% Unisole 400 on Uniport S (60–80 mesh) (GLC-1) or with 2% SE-30 on Uniport HP (60–80 mesh) (GLC-2); column temperature, 260 °C (GLC-1,2). MS were recorded on a Hitachi M-80 mass spectrometer. ^1H -NMR spectra were taken at 90 MHz with a JEOL FX90Q or at 400 MHz with a Bruker AM400 spectrometer and ^{13}C -NMR spectra were taken at 100 MHz with a Bruker AM400 spectrometer; chemical shifts are given as δ (ppm) with tetramethylsilane (TMS) as an internal standard (s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet). TLC was carried out on precoated Kieselgel 60F₂₅₄ plates (Merck). Spots were detected on the basis of UV absorbance (254, 365 nm) and by spraying 10% H_2SO_4 followed by heating, or by a Shimadzu CS-920 high-speed TLC scanner. Column chromatography was carried out with Wakogel C-200 (Wako Pure Chemical Ind. Ltd.), and Diaion HP-20 (Mitsubishi Chemical Industry Ltd.). HPLC was carried out on the CIG column system (50 μm silica gel column, 22 i.d. \times 300 mm, or 30 μm ODS column, 15 i.d. \times 300 mm, Kusano Scientific Co., Tokyo).

Isolation of Constituents—Dried leaves and twigs (2.2 kg) of *Hyphear Tanakae* HOSOKAWA epiphyting to *Castanea crenata* SIEB., collected at Tateiwa, Fukushima Prefecture in June 1986, were extracted three times with methanol (ca. 11 l). The methanolic extract was evaporated to dryness (269 g) and then partitioned between CHCl_3 and water to give the chloroform extract (93 g). The aqueous layer was further extracted with *n*-BuOH to give the butanol extract (124 g).

The chloroform extract (50 g) was placed on a column of silica gel (400 g). The column was eluted with *n*-hexane–EtOAc (4:1, 7:3), EtOAc, and EtOAc–MeOH (1:1) successively. Fatty acids (I), phytosterol (II), and compound III were isolated from the *n*-hexane–EtOAc fractions. Phytosterol-glucoside (IV) was isolated from the EtOAc–MeOH fractions.

The butanol extract (124 g) was placed on a column of silica gel (1.2 kg). The column was eluted with CHCl_3 –MeOH (9:1, 4:1, 7:3, 1:1) successively. The fractions were subjected to Diaion HP-20 column chromatography (MeOH– H_2O) and HPLC (ODS column, MeOH– H_2O). Compound V was isolated from the CHCl_3 –MeOH (9:1) fractions. Compounds VI–VIII were isolated from the CHCl_3 –MeOH (4:1) fractions.

Fatty Acids (I)—Colorless needles from EtOAc (1.18 g), mp 71–72 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 2915, 2845, 2650, 1710. This was analyzed by GLC-1 (He 50 ml/min) and identified as a mixture of arachidic, behenic, tricosanoic, lignoceric and heptacosanoic acids (5, 52, 3, 34 and 3%, respectively). The composition was determined from the GLC peak areas.

Phytosterol (II)—Colorless needles from EtOAc–MeOH (584 mg), mp 136–137 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3415, 2925, 1465, 1380, 1050. This was analyzed by GLC-2 (He 50 ml/min) and identified as a mixture of β -sitosterol and stigmasterol (93 and 7%, respectively).

III—Colorless powder from EtOAc (313 mg), mp 105–108 °C. $[\alpha]_{\text{D}}^{25} + 16^\circ$ ($c = 0.52$, CHCl_3). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3170, 3065, 2915, 2840, 1733, 1640, 1470, 1165, 1040, 980, 880. ^1H -NMR (CDCl_3) δ : 0.63, 0.65, 0.65, 0.67, 0.99, 1.08, 1.69 (each 3H, each s, *tert*-Me \times 7), 0.68 (3H, t, $J = 6.8$ Hz, CH_3 part of ester), 1.25 (CH_2 part of ester), 2.28 (2H, t, $J = 7.5$ Hz, $-\text{CH}_2\text{CO}-$), 3.80 (1H, dd, $J = 5.0, 11.0$ Hz), 4.14 (1H, dd, $J = 5.2, 11.2$ Hz), 4.47 (1H, dd, $J = 4.8, 11.4$ Hz, $3\alpha\text{H}$), 4.60 (1H, s), 4.68 (1H, s).

Hydrolysis of III—A solution of III (100 mg) in 40 ml of EtOH–5% KOH was heated under reflux for 2 h on a water bath and then the reaction mixture was extracted with ether. The ether layer was evaporated to dryness and crystallized from EtOAc to give IIIa (36 mg) as a colorless powder. The aqueous layer was acidified with HCl and then extracted with ether. The ether extract was evaporated to dryness and crystallized from MeOH to give IIIb (16 mg) as a colorless powder.

IIIa—mp 263–266 °C. $[\alpha]_{\text{D}}^{25} + 17^\circ$ ($c = 0.50$, CHCl_3). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3350, 3065, 2940, 1641, 1562, 1464, 1385, 1041, 1019, 881. EI-MS m/z (%): 458 (M^+ , 16). ^1H -NMR (CDCl_3) δ : 0.77, 0.82, 0.83, 0.99, 0.99, 1.08, 1.68 (each 3H, each s, *tert*-Me \times 7), 3.19 (1H, dd, $J = 4.7, 11.4$ Hz, $3\alpha\text{H}$), 3.79 (1H, dd, $J = 5.0, 11.1$ Hz), 4.14 (1H, dd, $J = 5.2, 11.2$ Hz), 4.59 (1H, d, $J = 2.1$ Hz), 4.68 (1H, d, $J = 2.1$ Hz).

IIIb—mp 69–70 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 2910, 2845, 2650, 1705. This was analyzed by GLC-1 (He 50 ml/min) and identified as a mixture of palmitic, stearic, arachidic, behenic, lignoceric acids (17, 6, 38, 24 and 7%, respectively).

Phytosterol-glucoside (IV)—Colorless needles from CHCl_3 –MeOH (88 mg), mp 279–283 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3390, 2925, 1072, 1022. IV (1 mg) was hydrolyzed by heating in 4 ml of EtOH–5% HCl (1:1) for 4 h and

then the reaction mixture was extracted with benzene. The benzene extract was analyzed by GLC-2 (He 50 ml/min) and identified as a mixture consisting mainly of β -sitosterol (95%). The aqueous layer was evaporated to dryness and analyzed by TLC (*n*-BuOH–AcOH–H₂O = 6 : 3 : 1, anisaldehyde reagent), which indicated the presence of glucose. IV was identical with an authentic sample on the basis of TLC comparisons.

Rhamnocitrin-3-O-rhamnoside (V)—Pale yellowish powder (1.51 g), mp 142–144 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3400, 1661, 1601, 1501, 1353, 1216, 1170, 974, 841, 810. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 343 (4.20), 264 (4.31), $\lambda_{\text{max}}^{\text{EtOH} + \text{EtONa}}$ nm: 394, 264, 235 sh, $\lambda_{\text{max}}^{\text{EtOH} + \text{AlCl}_3}$ nm: 395, 345, 302, 274, 232 sh, $\lambda_{\text{max}}^{\text{EtOH} + \text{AlCl}_3/\text{HCl}}$ nm: 396, 344, 301, 274, 232 sh, $\lambda_{\text{max}}^{\text{EtOH} + \text{AcONa}}$ nm: 410 sh, 345, 264. ¹H-NMR (DMSO-*d*₆) δ : 0.82 (3H, d, *J* = 5.7 Hz), 3.86 (3H, s, –OCH₃), 5.34 (1H, d, *J* = 1.4 Hz, anomeric proton), 6.38 (1H, d, *J* = 2.2 Hz), 6.69 (1H, d, *J* = 2.2 Hz), 6.94 (2H, d, *J* = 8.8 Hz), 7.80 (2H, d, *J* = 8.8 Hz).

Va—V (200 mg) in 20 ml of EtOH–5% HCl was heated under reflux for 1 h on a water bath. The precipitated yellowish needles were filtered off, washed with water, and dried (124 mg). Va was identified as rhamnocitrin on the basis of IR, MS, UV, and NMR spectral data.

Kaempferol-3-O-rhamnoside, Afzelin (VI)—Pale yellowish powder (31 mg), mp 172–175 °C (sublm.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3280, 1657, 1611, 1591, 1498, 1363, 1173, 1060, 974, 942, 829. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 342 (4.15), 263 (4.29), $\lambda_{\text{max}}^{\text{EtOH} + \text{EtONa}}$ nm: 394, 326, 273, 227 sh, $\lambda_{\text{max}}^{\text{EtOH} + \text{AlCl}_3}$ nm: 396, 345, 302, 273, 229 sh, $\lambda_{\text{max}}^{\text{EtOH} + \text{AlCl}_3/\text{HCl}}$ nm: 396, 342, 300, 273, 229 sh, $\lambda_{\text{max}}^{\text{EtOH} + \text{AcONa}}$ nm: 357, 300 sh, 271. ¹H-NMR (DMSO-*d*₆) δ : 0.80 (3H, d, *J* = 5.8 Hz), 5.30 (1H, d, *J* = 1.5 Hz, anomeric proton), 6.22 (1H, d, *J* = 2.0 Hz), 6.42 (1H, d, *J* = 2.0 Hz), 6.92 (2H, d, *J* = 8.8 Hz), 7.76 (2H, d, *J* = 8.8 Hz).

VIa—VI (1 mg) in 2 ml of EtOH–5% HCl was heated under reflux for 1 h on a water bath and then the reaction mixture was extracted with EtOAc. VIa was identical with an authentic sample of kaempferol on the basis of IR and TLC comparisons.

Rhamnetin-3-O-rhamnoside (VII)—Yellowish powder (13.1 g), mp 151–155 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3380, 1658, 1597, 1498, 1443, 1342, 1212, 1162, 996, 964, 810. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 349 (4.20), 299 sh (3.89), 254 (4.33), $\lambda_{\text{max}}^{\text{EtOH} + \text{EtONa}}$ nm: 402, 262, $\lambda_{\text{max}}^{\text{EtOH} + \text{AlCl}_3}$ nm: 435, 302 sh, 273, $\lambda_{\text{max}}^{\text{EtOH} + \text{AlCl}_3/\text{HCl}}$ nm: 400, 360, 296 sh, 269, $\lambda_{\text{max}}^{\text{EtOH} + \text{AcONa}}$ nm: 412 sh, 355, 295 sh, 256. ¹H-NMR (DMSO-*d*₆) δ : 0.85 (3H, d, *J* = 6.0 Hz), 3.87 (3H, s, –OCH₃), 5.31 (1H, d, *J* = 1.3 Hz, anomeric proton), 6.38 (1H, d, *J* = 2.2 Hz), 6.67 (1H, d, *J* = 2.2 Hz), 6.90 (1H, d, *J* = 8.3 Hz), 7.30 (1H, dd, *J* = 2.1, 8.3 Hz), 7.36 (1H, d, *J* = 2.1 Hz).

VIIa—VII (200 mg) in 20 ml of EtOH–5% HCl was heated under reflux for 1 h on a water bath. The precipitated yellowish needles were filtered off, washed with water, and dried (112 mg). VIIa was identified as rhamnetin on the basis of IR, MS, UV, and NMR spectral data.

Quercetin-3-O-rhamnoside, Quercitrin (VIII)—Pale yellowish powder (6.22 g), mp 174–177 °C (sublm.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3280, 1660, 1608, 1500, 1202, 1170, 1360, 998, 966, 823. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 349 (4.23), 265 sh (4.31), 255 (4.36), $\lambda_{\text{max}}^{\text{EtOH} + \text{EtONa}}$ nm: 395, 323, 271, $\lambda_{\text{max}}^{\text{EtOH} + \text{AlCl}_3}$ nm: 428, 273, $\lambda_{\text{max}}^{\text{EtOH} + \text{AlCl}_3/\text{HCl}}$ nm: 400, 355, 301 sh, 270, $\lambda_{\text{max}}^{\text{EtOH} + \text{AcONa}}$ nm: 361, 266. ¹H-NMR (DMSO-*d*₆) δ : 0.84 (3H, d, *J* = 6.0 Hz), 5.28 (1H, d, *J* = 1.4 Hz, anomeric proton), 6.23 (1H, d, *J* = 2.0 Hz), 6.41 (1H, d, *J* = 2.0 Hz), 6.89 (1H, d, *J* = 8.3 Hz), 7.28 (1H, dd, *J* = 2.1, 8.3 Hz), 7.32 (1H, d, *J* = 2.1 Hz).

VIIIa—VIII (200 mg) in 20 ml of EtOH–5% HCl was heated under reflux for 1 h on a water bath. The precipitated yellowish needles were filtered off, washed with water, and dried (122 mg). VIIIa was identical with an authentic sample of quercetin on the basis of IR, UV, NMR and TLC comparisons.

Hydrolysis of V–VIII—A sample (1 mg) in 2 ml of EtOH–5% H₂SO₄ (1 : 1) was heated under reflux for 3 h on a water bath and then neutralized with BaCO₃. The precipitate was filtered off and the filtrate was concentrated and analyzed by TLC (*n*-BuOH–AcOH–H₂O = 6 : 3 : 1, anisaldehyde reagent), which indicated the presence of rhamnose in each case.

Comparison of the Constituents—Dried leaves and twigs (each 5 g) of *H. Tanakae* HOSOKAWA epiphyting to *Prunus Mume* SIEB., *Prunus Armeniaca* L. var. *ansu* MAXIM., and *Zelkova serrata* MAKINO, collected at Nagano Prefecture in June 1987, were extracted with methanol (100 ml). Each methanolic extract was analyzed by TLC (I, hexane–EtOAc (7 : 3); II, CHCl₃–MeOH (9 : 1); III, CHCl₃–MeOH–H₂O (65 : 35 : 10, lower layer)), which indicated the presence of compounds II–IV and major flavonoid glycosides V, VII, and VIII. The contents of these flavonoid compounds in the leaves and twigs were determined by a Shimadzu CS-920 high-speed TLC scanner (apparatus conditions: UV 254 nm, LINEARIZER 1, AZS OFF; solvent, CHCl₃–EtOAc–formic acid (2 : 1 : 1)).

References and Notes

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