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Studies on the Constituents of Apocynaceae Plants
Flavonoids from *Trachelospermum jasminoides* var. *pubescens* and *T. difforme*[1]

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Five known flavone glycosides, i.e., cosmosiin (I), luteolin-7-O-β-glucoside (II), luteolin-4′-O-β-glucoside (III), rhoifolin (IV), and apigenin-7-O-β-gentiobioside (V), one new flavone glycoside, i.e., luteolin-7-O-β-gentiobioside (VI), and one C-glycosylflavone, i.e., vicenin-2 (VII) were isolated from the leaves of *Trachelospermum jasminoides* Lemaire var. *pubescens* Makino (Apocynaceae) of Japan origin.

Two flavonol glycosides, i.e., isoquercitrin (VIII) and rutin (IX) were isolated from the leaves of *T. difforme* A. Gray of North American origin.

In our previous papers,[3,4] we reported the isolation of flavone glycosides from *Trachelospermum asiaticum* Nakai var. *intermedium* Nakai.

This paper describes the structural determination of flavonoids isolated from leaves of *T. jasminoides* Lemaire var. *pubescens* Makino of Japan origin and *T. difforme* A. Gray of North American origin.

Extraction was carried out as described in the previous paper.[5] Each of their *n*-butanol extract was subjected to silica gel column chromatography using methanol-chloroform gradient as eluent to afford compounds I, II, III, IV, V, VI, and VII from *T. jasminoides* var. *pubescens*, and compounds VIII and IX from *T. difforme*.

Compounds I (yellow needles, $C_{21}H_{20}O_{10}\cdot H_2O$, mp 195–198°), II (yellow needles, $C_{21}H_{20}O_{11}\cdot 1^1/_2H_2O$, mp 227–231°), III (yellow needles, $C_{21}H_{20}O_{11}\cdot 1^1/_2H_2O$, mp 174–178°), IV (pale yellow needles, $C_{27}H_{30}O_{14}$, mp 260–264° (dec.)) and V (pale yellow needles, $C_{27}H_{30}O_{15}\cdot 2H_2O$, mp 226–228°) were identified as cosmosiin(I), luteolin-7-O-β-glucoside(II), luteolin-4′-O-β-glucoside(III), rhoifolin(IV) and apigenin-7-O-β-gentiobioside(V) respectively by direct comparison with authentic samples.

Compound VI was recrystallized from aqueous methanol to give pale yellow needles, $C_{27}H_{30}O_{16}\cdot 3H_2O$, mp 203–204°, which is positive to Mg-HCl and Zn-HCl tests, and to ferric chloride reaction. The infrared (IR) spectral absorption bands and ultraviolet (UV) spectral absorption maxima suggested the presence of hydroxyl (3400 cm^{-1}) and carbonyl (1655 cm^{-1}) groups and an aromatic ring (1600 cm^{-1}) and of a flavone skeleton.

Profile of bathochromic shift of absorption maxima produced by the addition of aluminum chloride and sodium acetate resembles that of II. The mass spectrum (MS) data of its peracetate(VIa) showed fragmental ions derived from terminal hexose (*m/z* 331), disaccharide (*m/z* 619) and aglycone (*m/z* 412).

The product of acid hydrolysis of the permethylate(VIb) was suggested by gas chromatography-mass spectrometry (GC-MS) to be 3′,4′,5-tri-O-methylluteolin, which was confirmed by direct comparison with

1) Part of this work was presented at the 25th Annual Meeting of Japanese Society of Pharmacognosy, Fukuoka, October, 1978. Abstract of Papers, p. 17.
2) Location: *Ishikari-Tobetsu, Hokkaido, 061-02, Japan.*
3) I. Inagaki, S. Hisada, S. Nishibe and A. Sakushima, *Yakugaku Zasshi*, **93**, 1231 (1973).
4) A. Sakushima, S. Hisada, S. Nishibe and I. Inagaki, *Yakugaku Zasshi*, **93**, 1127 (1973).
5) A. Sakushima, S. Nishibe, S. Hisada, Y. Noro and Y. Hisada, *Yakugaku Zasshi*, **96**, 1046 (1976).

an authentic sample.

The hydrolysis of VI with 5% sulfuric acid solution or with β-glucosidase gave luteolin and D-glucose. Photohydrolysis thereof under irradiation of U.V. light gave gentiobiose, which was identified by direct comparison with an authentic sample. Consequently, the structure of VI was determined to be luteolin-7-O-β-gentiobioside, which is a new flavone glycoside.

Compound VII was recrystallized from ethanol to give pale yellow needles, $C_{27}H_{30}O_{15}\cdot 2H_2O$, mp 247–251.5°, which was positive to Mg-HCl and Zn-HCl tests, and to ferric chloride reaction.

UV spectral absorption maxima were at 273 and 332 nm. Profile of bathochromic shift of the absorption maxima produced by the addition of aluminum chloride and sodium acetate was similar to that of violantin.[6]

VII was not hydrolyzed by 5% sulfuric acid solution; oxidation of VII with ferric chloride produced glucose and arabinose.[7]

Figure 1 shows MS data of peracetate (VIIa). VII was suggested by proton nuclear magnetic resonance (^{1}H-NMR) spectrum of VIIa to be vicenin-2.[8] The carbon -13 nuclear magnetic resonance (^{13}C-NMR) and IR spectral data of VII and MS data of permethylate (VIIb) were in good agreement with those of vicenin-2 and of its permethylate reported in literature.[9]

No C-glycosylflavone has been, so far, isolated from Apocynaceae. This is the first C-glycosylflavone from Apocynaceae plants.

Compound VIII was identified as isoquercitrin by direct comparison with an authentic sample.

Compound IX was identified as rutin by comparison of its IR, UV and GC-MS spectral data with those of an authentic sample.

Thus flavonoids in *T. differme* of North American origin were shown to be flavonol glycosides, whereas those in *T. jasminoides* var. *pubescens* and *T. asiaticum* var. *intermedium* of Japan origin were shown to be flavone glycosides.

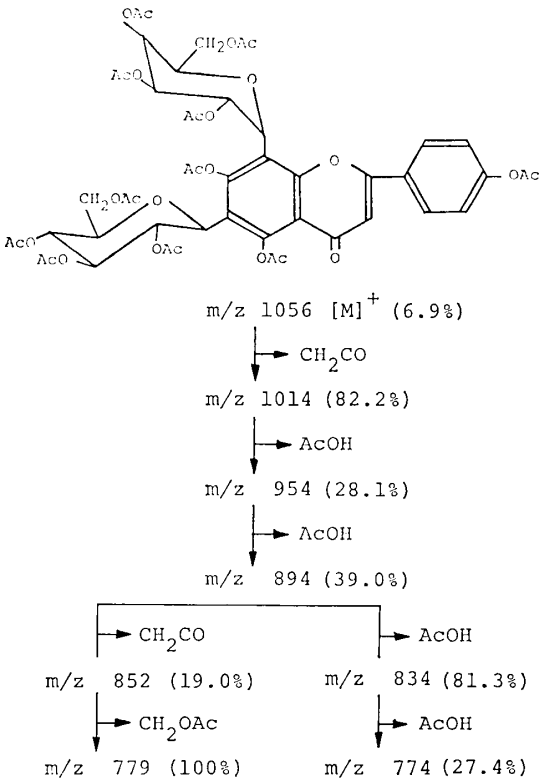
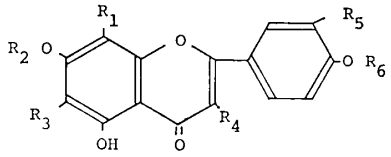


Fig. 1. Mass Fragmentation of Vicenin-2 Peracetate (VIIa)

6) Y. J. Mabry, K. R. Markham and M. B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, Berlin, Heidelberg, New York, 1970, p. 89.
7) J. B. Harbone, T. J. Mabry and H. Mabry, "The Flavonoids," Chapman and Hall, London, 1975, p. 649.
8) B. Gentili and R. M. Horowitz, *J. Org. Chem.*, **33**, 1571 (1968).
9) B.-G. Österdahl, *Acta Chem. Scand.*, **B.33**, 400 (1979).



I	R_1=	R_3=	R_4=	R_5=	R_6= H,	R_2= glucose	
II	R_1=	R_3=	R_4=	R_6= H,	R_2= glucose,	R_5= OH	
III	R_1=	R_2=	R_3=	R_4= H,	R_5= OH,	R_6= glucose	
IV	R_1=	R_3=	R_4=	R_5=	R_6= H,	R_2= neohesperidose	
V	R_1=	R_3=	R_4=	R_5=	R_6= H,	R_2= gentiobiose	
VI	R_1=	R_3=	R_4=	R_6= H,	R_2= gentiobiose,	R_5= OH	
VII	R_1=	R_3= glucose,	R_2=	R_4=	R_5=	R_6= H	
VIII	R_1=	R_2=	R_3=	R_6= H,	R_4= O-glucose,	R_5= OH	
IX	R_1=	R_2=	R_3=	R_6= H,	R_4= O-rutinose,	R_5= OH	

Chart 1.

Experimental

All melting points were determined with a Yanagimoto micro melting point apparatus and are recorded uncorrected. IR spectra were obtained with Shimadzu IR-400 machine and ^{1}H-NMR spectra were taken with a Hitachi R-40 high resolution NMR spectrometer and chemical shifts are given in the δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. ^{13}C-NMR spectra were taken with a JEOL FX 60 spectrometer. GC was carried out on Shimadzu GC-6 AM machine equipped with a hydrogen flame ionization detector. Mass spectra were measured on a Shimadzu LKB-9000 (Computer, Shimadzu GC-MSPAC-300M) with direct inlet of the probe into the ion source. Conditions were as follows: ionizing voltage 20 eV or 70 eV; ionizing current 60 μA; ion accelerating voltage 3.5 kV. Column chromatography was carried out using silica gel (100 mesh, Mallinckrodt). TLC on Kiesel gel 60_{F-254} was performed with following solvent systems: AcOEt–MeCOEt–H_2O–HCOOH–C_6H_6=4:3:1:1:2 (upper layer) (TLC-1), AcOEt–MeCOEt–H_2O–HCOOH=5:3:1:1 (TLC-2). The spots were detected with $FeCl_3$ reagent or by spraying of 10% H_2SO_4 solution followed by heating.

Extraction and isolation—Dried leaves (1.5 kg) of *Trachelospermum jasminoides* var. *pubescens* collected at Kamikumamoto (Kumamoto Pref.) in August 1970, were crushed and treated as described in the previous paper.[5] The *n*-BuOH extract was subjected to silica gel column chromatography using successively 20%, 23%, 25%, and 28% MeOH in $CHCl_3$: the eluted fractions were tested for the presence of flavonoids by TLC developed with TLC-1. Rechromatography of each of the fractions containing flavonoids over silica gel gave 22 mg of I, 30 mg of II, 60 mg of III, 79 mg of IV, 45 mg of V, 40 mg of VI and 34 mg of VII.

Dried leaves (110 g) of *T. difforme* collected at Mississippi (U.S.A.) in August 1970, were treated according to the method described above for *T. jasminoides* var. *pubescens* to give 5 mg of VIII and 16 mg of IX.

Cosmosiin (I)—Pale yellow needles from aq. EtOH, mp 195–198°. Brown colour in $FeCl_3$ reaction, reddish-pink colour in Mg-HCl and Zn-HCl tests. *Rf* 0.45 (TLC-1). UV λ_{max}^{MeOH} nm: 269, 339. UV $\lambda_{max}^{MeOH+NaOAc}$ nm: 269, 349. UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 275, 305, 324, 364. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650 (C=O), 1600 (C=C), 1075 (C—O). MS (acetate of I) *m/z*: 354, 331, 312, 270, 169. *Anal.* Calcd. for $C_{21}H_{20}O_{10}\cdot H_2O$: C, 56.00; H, 4.92. Found: C, 55.64; H, 4.65. GC-MS [glass column (1 m×3 mm) packed with 0.5% OV-17, isothermal 260°, He at 30 ml/min]; t_R (min): 49.0 (per-trimethylsilyl (TMS) derivatives of I). MS *m/z*: 450, 414, 399, 361, 217, 207, 204, 191, 147. This compound was identified as cosmosiin.

Luteolin-7-O-β-glucoside (II)—Pale yellow needles from aq. EtOH, mp 227–231°. Dark green colour in $FeCl_3$ reaction, reddish-pink colour in Mg-HCl and Zn-HCl tests. *Rf* 0.37 (TLC-1). UV λ_{max}^{MeOH} nm: 257, 269 (sh), 351. UV $\lambda_{max}^{MeOH+NaOAc}$ nm: 257, 268, 360, 410 (sh). UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 275, 296, 365,

390 (sh). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650 (C=O), 1600 (C=C), 1060 (C—O). MS (acetate of II) *m/z*: 700, 412, 370, 331, 328, 286, 169. *Anal.* Calcd. for $C_{21}H_{20}O_{11}\cdot 1^1/_2H_2O$: C, 53.04; H, 4.87. Found: C, 53.13; H, 4.42. GC-MS [conditions described above]. t_R (min): 57.0 (per-TMS derivative of II). MS *m/z*: 502, 487, 450, 361, 271, 215, 204, 191, 147. This compound was identified as luteolin-7-O-β-glucoside.

Luteolin-4′-O-β-glucoside (III)—Pale yellow needles from aq. EtOH, mp 174–178°. Brown colour in $FeCl_3$ reaction, reddish-pink colour in Mg-HCl and Zn-HCl tests. *Rf* 0.41 (TLC-1). UV λ_{max}^{MeOH} nm: 243, 272, 336. UV $\lambda_{max}^{MeOH+NaOAc}$ nm: 278, 360. UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 270, 368. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1645 (C=O), 1605 (C=C), 1065 (C—O). MS (acetate of III) *m/z*: 700, 412, 370, 331, 328, 286, 169. *Anal.* Calcd. for $C_{21}H_{20}O_{11}\cdot 1^1/_2H_2O$: C, 53.04; H, 4.87. Found C, 53.16; H, 4.66. GC-MS [condition described above]. t_R (min): 52.1 (per-TMS derivative of III). MS *m/z*: 502, 487, 450, 361, 271, 204, 191, 147. This compound was identified as luteolin-4′-O-β-glucoside.

Rhoifolin (IV)—Pale yellow needles from aq. C_5H_5N (or aq. EtOH). mp 260–264° (dec.). Brown colour in $FeCl_3$ reaction, reddish-pink colour in Mg-HCl and Zn-HCl tests. *Rf* 0.15 (TLC-1). UV λ_{max}^{MeOH} nm: 269, 339. UV $\lambda_{max}^{MeOH+NaOAc}$ nm: 269, 348. UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 278, 312, 352. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1660 (C=O), 1605 (C=C), 1500 (C=C), 1080 (C—O). MS (acetate of IV) *m/z*: 561, 354, 312, 273, 171, 169, 153. *Anal.* Calcd. for $C_{27}H_{30}O_{14}$: C, 56.05; H, 5.23. Found: C, 55.53; H, 5.38. This compound was identified as rhoifolin (apigenin-7-O-β-neohesperidoside).

Apigenin-7-O-β-gentiobioside (V)—Pale yellow needles from aq. C_5H_5N (or aq. EtOH). mp 226–228°. Brown colour in $FeCl_3$ reaction, reddish-pink colour in Mg-HCl and Zn-HCl tests. *Rf* 0.05 (TLC-1), *Rf* 0.25 (TLC-2). UV λ_{max}^{MeOH} nm: 269, 338. UV $\lambda_{max}^{MeOH+NaOAc}$ nm: 269, 345. UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 277, 302, 351. IR ν_{max}^{KBr} cm^{-1}: 3370 (OH), 1655 (C=O), 1605 (C=C), 1070 (C—O). MS (acetate of V) *m/z*: 619, 354, 331, 312, 270, 169. *Anal.* Calcd. for $C_{27}H_{30}O_{15}\cdot 2H_2O$: C, 51.41; H, 5.44. Found: C, 51.35; H, 5.61. This compound was identified as apigenin-7-O-β-gentiobioside.

Luteolin-7-O-β-gentiobioside (VI)—Pale yellow needles from aq. MeOH, mp 203–204°. Dark green colour in $FeCl_3$ reaction, reddish-pink colour in Mg-HCl and Zn-HCl tests. *Rf* 0.02 (TLC-1), *Rf* 0.22 (TLC-2). UV λ_{max}^{MeOH} nm(log ε): 257.5 (4.27), 267.5 (4.26), 346.5 (4.37). UV $\lambda_{max}^{MeOH+NaOAc}$ nm: 258, 265, 350, 398. UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 273, 296, 350, 396. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1655 (C=O), 1600 (C=C), 1060 (C—O). *Anal.* Calcd. for $C_{27}H_{30}O_{16}\cdot 3H_2O$: C, 48.78; H, 5.46. Found: C, 48.53; H, 5.28.

Enzymatic hydrolysis of VI—β-glucosidase (BETA Glucosidase from Almonds, Miles Laboratories) was added to VI (15 mg) in H_2O. The mixture was stirred at room temperature for 5 days, and then treated with AcOEt. The AcOEt layer was washed with water and evaporated to dryness *in vacuo*. The residue was identified as luteolin by the comparison of its IR and UV data with those of authentic sample.

Photohydrolysis of VI—VI (5.0 mg) was photohydrolyzed with 0.5 N H_2SO_4 in 50% aq. EtOH under U.V. light (15 W, National GL-15) for 4 days, and then the mixture was treated in the usual way. GC [glass column (1 m×3 mm) packed with 1.5% OV-1, temperature programmed 180° to 280° at 4°/min, N_2 at 30 ml/min] of the residue gave peaks at t_R (min): 16.5, 18.7 (TMS derivatives of gentiobiose).

Identification of 3′,4′,5-tri-O-methylluteolin (VIb)—VI was methylated with CH_2N_2. The reaction mixture was hydrolyzed with 5% H_2SO_4 without any purification, and then treated with AcOEt. The AcOEt layer was washed with water, and evaporated to dryness *in vacuo*. GC-MS [glass column (2m× 3 mm) packed with 2% OV-17, isothermal 260°, He at 30 ml/min] of the residue gave peak at t_R (min): 46.0 (TMS derivative of 3′,4′,5-tri-O-methylluteolin). MS *m/z* (%): 400 (100) [M^+], 399 (45.8), 385 (11.3), 371 (26.1), 370 (7.6), 369 (3.0), 355 (18.2), 354 (22.7), 165 (3.1), 162 (7.0).
This compound was identified with authentic sample by GC-MS.

Luteolin-7-O-β-gentiobioside peracetate (VIa)—VI (20 mg) was acetylated with Ac_2O and C_5H_5N. The crude acetate was subjected to column chromatography with $CHCl_3$-MeOH as eluent to afford luteolin-7-O-β-gentiobioside peracetate, mp 220–223°, which was negative to $FeCl_3$ reaction. IR ν_{max}^{KBr} cm^{-1}:

2900 (CH), 1760 (C=O), 1655 (C=O). ^{1}H-NMR (in $CDCl_3$) δ (ppm): 1.80–2.10 (21 H, m, 7×aliphatic C$\underline{H}_3$CO), 2.33–2.46 (9 H, each s, 3×aromatic C$\underline{H}_3$CO), 3.50–5.40 (14 H, m, aliphatic H), 6.60 (1 H, s, aglycone 3-H), 6.71 (1 H, d, J=2.5 Hz, arcmatic 6-H), 7.05 (1 H, d, J=2.5 Hz, aromatic 8-H), 7.40 (1 H, d, J=9.0 Hz, aromatic 5′-H), 7.75–7.95 (2 H, m, aromatic 2′, 6′-H). MS m/z (%): 619 (4.8) [$C_{26}H_{35}O_{17}^+$], 412 (0.8) [$C_{21}H_{16}O_9^+$], 370 (2.2) [$C_{19}H_{14}O_8^+$], 331 (60.6) [$C_{14}H_{19}O_9^+$], 328 (2.5) [$C_{17}H_{12}O_7^+$], 286 (6.5) [$C_{15}H_{10}O_6^+$], 169 (100) [$C_8H_9O_4^+$].

Vicenin-2 (VII)—Pale yellow powder from MeOH, mp 247–251.5°. Greenish-brown colour in $FeCl_3$ reaction, orange colour in Mg-HCl test and violet in Zn-HCl test. Rf 0.18 (TLC-1). UV λ_{max}^{MeOH} nm (log ε): 273 (4.31), 332 (4.33). UV $\lambda_{max}^{MeOH+NaOAc}$ nm: 283, 310 (sh), 340 (sh), 390. UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 280, 303, 344, 380 (sh). ^{13}C-NMR (in DMSO-d_6) δ (ppm): 182.3 (C-4), 164.1 (C-2), 161.7 (C-7), 161.2 (C-4′), 160.8 (C-5), 158.6 (C-8a), 129.0 (C-2′, 6′), 121.5 (C-1′), 115.8 (C-3′, 5′), 107.5 (C-6), 103.9 (C-8), 103.0 (C-3), 102.7 (C-4a), 81.7, 80.7 (glu. C-5″, 5‴), 78.8, 77.8 (glu. C-3″, 3‴), 73.9, 73.3 (glu. C-1″, 1‴), 71.2, 70.9 (glu. C-2″, 2‴), 70.6, 69.1 (glu. C-4″, 4‴), 61.2, 59.4 (glu. C-6″, 6‴). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1650 (C=O), 1620 (C=C), 1580 (C=C), 1080 (C−O). *Anal.* Calcd. for $C_{27}H_{30}O_{15}\cdot 2H_2O$: C, 51.41; H, 5.43. Found C, 51.19; H, 5.18.

Vicenin-2 peracetate (VIIa)—VII (10 mg) was treated with Ac_2O and C_5H_5N. The crude acetate was subjected to column chromatography to afford vicenin-2 peracetate (VIIa), mp 157–161°, which was negative to $FeCl_3$ reaction. IR ν_{max}^{KBr} cm^{-1}: 2920 (CH), 1760 (C=O), 1650 (C=O), 1610 (C=C), 1090 (C−O). ^{1}H-NMR (in $CDCl_3$) δ(ppm): 1.76–2.10 (24 H, m, 8×aliphatic C$\underline{H}_3$CO), 2.39, 2.51, 2.55 (9 H, each s, 3×aromatic C$\underline{H}_3$CO), 3.50–6.10 (14 H, m, aliphatic H), 6.71 (1 H, s, aglycone 3-H), 7.40 (2H, d, J=8.0 Hz, aromatic 3′,5′-H), 8.10 (2 H, d, J=8.0 Hz, aromatic 2′, 6′-H). MS m/z (%): 1056(6.9) [M^+=$C_{49}H_{52}O_{26}$], 1014(82.2)[M^+-C_2H_2O], 954(28.1)[M^+-$C_4H_6O_3$], 894(39.0)[M^+-$C_6H_{10}O_5$], 852 (19.0) [M^+-$C_8H_{12}O_6$], 834(81.3)[M^+-$C_8H_{14}O_7$], 779(100)[M^+-$C_{11}H_{17}O_8$], 774(27.4)[M^+-$C_{10}H_{18}O_9$].

Ferric chloride oxidation of VII—VII (5.0 mg) was heated in 30% $FeCl_3$ soln. under reflux at 115° for 20 min and then at 125° for 6 h. The pH of the reaction mixture was adjusted with NaOH solution to pH 8, then with HCl to pH 3, and finally with NH_4OH to pH 7. The filtrate was evaporated to dryness *in vacuo*. The residue was subjected to GC [glass column (1 m×3 mm) packed with 1.5% OV-1, temp. programmed 150° to 200° at 3°/min, N_2 at 20 ml/min] to give peaks at t_R (min): 12.0, 13.1 (TMS derivatives of D-glucose), and 5.1 (TMS derivative of arabinose).

Vicenin-2 permethylate (VIIb)—VII (3.0 mg) was treated according to Hakomori's method, and the reaction mixture was subjected to preparative TLC. The spot on TLC was detected by fluorecence (blue colour) under U.V. light. MS m/z (%): 748 (5.5)[M^+=$C_{38}H_{52}O_{15}$], 733(15.5)[M^+-15], 731(4.1) [M^+-17], 717(61.8)[M^+-31], 701(6.7)[M^+-47], 685(5.4)[M^+-63], 645(7.8)[M^+-103], 615(3.5)[M^+-133], 585(18.6)[M^+-163], 573(31.6)[M^+-175], 559(7.0)[M^+-189], 543(10.7)[M^+-205], 541(6.6)[M^+-207], 529 (2.4)[M^+-219].

Isoquercitrin (VIII)—Yellow needles from aq. EtOH, mp 236–239°. Dark-green colour in $FeCl_3$ reaction, red colour in Mg-HCl test. Rf 0.44 (TLC-1). UV λ_{max}^{MeOH} nm: 259, 305 (sh), 362. UV $\lambda_{max}^{MeOH+NaOAc}$ nm: 273, 325, 388. UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 268, 300 (sh), 360 (sh), 400. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1650 (C=O), 1600 (C=C), 1090 (C−O). GC; t_R (min): 41.8 (TMS derivative of VII). MS m/z: 590, 575, 559, 450, 305, 271, 217, 204, 191, 147. This compound was identified as isoquercitrin.

Rutin (IX)—Yellow needles from aq. EtOH, mp 182–193°. Dark green colour in $FeCl_3$ reaction, red colour in Mg-HCl and Zn-HCl tests. Rf 0.18 (TLC-1), Rf 0.37 (TLC-2). UV λ_{max}^{MeOH} nm: 261, 365. UV $\lambda_{max}^{MeOH+NaOAc}$ nm: 275, 405. UV $\lambda_{max}^{MeOH+AlCl_3}$ nm: 275, 440. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1655 (C=O), 1605 (C=C), 1065 (C−O). *Anal.* Calcd. for $C_{27}H_{30}O_{16}\cdot 3H_2O$: C, 48.79; H, 5.46; Found: C, 48.76; H, 5.30. This compound was identified as rutin.

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