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Constituents of Pollen. XI.[1] Constituents of *Cryptomeria japonica* D. Don

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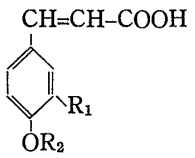
A new biflavone, 5‴-hydroxyamentoflavone, was isolated, together with naringenin, apigenin, luteolin, 2,3-dihydroamentoflavone, amentoflavone, afzelin, cosmosiin, quercitrin, and 4-β-D-glucopyranosyloxyferulic acid, from pollen grains of *Cryptomeria japonica* D. Don.

Keywords——*Cryptomeria japonica*; Taxodiaceae; pollen grains; 2,3-dihydroamentoflavone; amentoflavone; 5‴-hydroxyamentoflavone

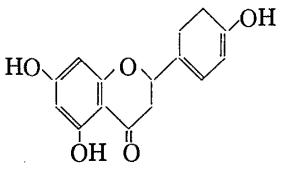
There are a number of reports on chemical constituents of the leaves of *Cryptomeria japonica* D. Don (Japanese name "Sugi"); seven biflavonoids [hinokiflavone,[2] cryptomerin A and cryptomerin B,[3] isocryptomerin,[4] neocryptomerin,[4] kayaflavone,[5] and sciadopitysin[6]] and two quercetin glycosides [quercimeritrin[7] and isoquercitrin[8]] have been isolated. The present paper deals with the isolation and characterization of nine flavonoids and three aromatic acids from the pollen grains of *C. japonica*.

The pollen grains of *C. japonica* were extracted with ether and fractionated into acidic and phenolic fractions. The acidic compounds were identified as *p*-coumaric acid (I) and ferulic acid (II). The phenolic fraction was further fractionated by preparative thin-layer chromatography (TLC) and six flavonoids, III, IV, V, VI, VII, and VIII, were isolated.

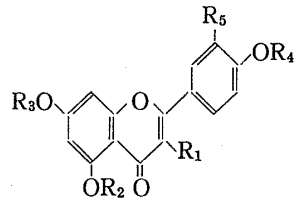
Compounds III, IV, V, and VII were identified as naringenin (III), apigenin (IV), luteolin



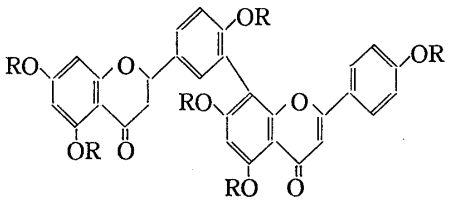
I: $R_1=R_2=H$
II: $R_1=OCH_3$, $R_2=H$
XII: $R_1=OCH_3$, $R_2=$glucose
XIIa: $R_1=OCH_3$, $R_2=$glucose$(Ac)_4$



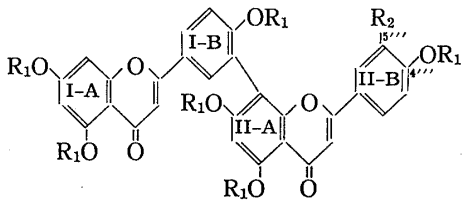
III



IV: $R_1=R_2=R_3=R_4=R_5=H$
IVa: $R_1=R_5=H$, $R_2=R_3=R_4=Ac$
V: $R_1=R_2=R_3=R_4=H$, $R_5=OAc$
Va: $R_1=H$, $R_2=R_3=R_4=Ac$, $R_5=OAc$
IX: $R_1=O$-rhamnose, $R_2=R_3=R_4=R_5=H$
X: $R_1=R_2=R_4=R_5=H$, $R_3=$glucose
XI: $R_1=O$-rhamnose, $R_2=R_3=R_4=H$, $R_5=OH$
XIa: $R_1=O$-rhamnose$(Ac)_3$, $R_2=R_3=R_4=Ac$, $R_5=OAc$



VI: R=H
VIa: R=Ac



VII: $R_1=R_2=H$
VIIa: $R_1=Ac$, $R_2=H$
VIII: $R_1=H$, $R_2=OH$
VIIIa: $R_1=Ac$, $R_2=OAc$

Chart 1

(V) and amentoflavone (VII) from their spectral data, and by comparison with authentic samples.

Compound VI was obtained as yellow granular crystals (mp 208—210°C) with a positive magnesium-hydrochloric acid reaction for flavonoids. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of VI gave signals due to C_2–H and C_3–H of the flavanone skeleton as a 1H double doublet at δ 5.36 and as a 2H multiplet at δ 2.80—3.12. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of VI gave signals due to C-2 and C-3 of the flavanone skeleton at δ 80.55 and δ 46.19, and in addition, carbonyl carbons of flavanone and flavone at δ 197.74 and δ 184.30, respectively. Therefore, VI was inferred to be a biflavonoid containing flavanone and flavone systems.

Acetylation of VI gave an acetate (VIa) as colorless granular crystals with mp 208—210°C. The ^{1}H-NMR spectrum and mass spectrum (MS) of VIa revealed the presence of six acetyl groups. These data suggested the presence of a biflavonoid structure for VI as shown in Chart 1, and VI was identified as 2,3-dihydroamentoflavone[9] by mixed melting point determination as well as infrared (IR) spectral and TLC comparisons with an authentic sample.

Compound VIII was obtained as yelow granular crystals (mp 298—300°C) with a positive magnesium-hydrochloric acid reaction. The ultraviolet (UV) absorption spectrum of VIII

Table I. ^{1}H-NMR Spectral Data for Biflavonoids[a]

Proton	VII[b]	VIIa[c]	VIII[b]	VIIIa[c]
3	6.50 s	6.66 s	6.50 s	6.63 s
5		2.46 s ($-OCOCH_3$)		2.46 s ($-OCOCH_3$)
6	6.15 d (J=2 Hz)	6.84 d (J=2 Hz)	6.16 d (J=2 Hz)	6.84 d (J=2 Hz)
7		2.06 s ($-OCOCH_3$)		2.06 s ($-OCOCH_3$)
8	6.38 d (J=2 Hz)	7.27 d (J=2 Hz)	6.38 d (J=2 Hz)	7.34 d (J=2 Hz)
2′	7.91 d (J=2 Hz)	8.04 d (J=2 Hz)	7.91 d (J=2 Hz)	8.05 d (J=2 Hz)
4′		2.33 s ($-OCOCH_3$)		2.22 s ($-OCOCH_3$)
5′	7.03 d (J=9 Hz)	7.52 d (J=9 Hz)	7.01 d (J=9 Hz)	7.23 d (J=9 Hz)
6′	7.75 dd (J=2, 9 Hz)	7.97 dd (J=2, 9 Hz)	7.82 dd (J=2, 9 Hz)	7.96 dd (J=2, 9 Hz)
3″	6.50 s	6.68 s	6.55 s	6.69 s
5″		2.41 s ($-OCOCH_3$)		2.42 s ($-OCOCH_3$)
6″	6.31 s	7.02 s	6.35 s	7.00 s
7″		2.02 s ($-OCOCH_3$)		2.03 s ($-OCOCH_3$)
2‴	7.43 d (J=9 Hz)	7.48 d (J=9 Hz)	7.09 dd (J=2, 9 Hz)	7.46 dd (J=2, 9 Hz)
3‴	6.66 d (J=9 Hz)	7.05 d (J=9 Hz)	6.66 d (J=9 Hz)	7.05 d (J=9 Hz)
4‴		2.28 s ($-OCOCH_3$)		2.28 s ($-OCOCH_3$)
5‴	6.66 d (J=9 Hz)	7.05 d (J=9 Hz)		2.12 s ($-OCOCH_3$)
6‴	7.43 d (J=9 Hz)	7.48 d (J=9 Hz)	7.07 d (J=2 Hz)	7.42 d (J=2 Hz)

a) Chemical shifts in ppm units.
b) Measured in CD_3OD.
c) Measured in $CDCl_3$.

was very similar to that of amentoflavone (VII), and the field desorption mass spectrum (FD–MS) of VIII gave the molecular formula $C_{30}H_{18}O_{11}$, which also suggested the presence of a biflavonoid structure for VIII (Chart 1).

Acetylation of VIII gave an acetate (VIIIa) as colorless granular crystals, mp 183—185°C. The ^{1}H-NMR spectrum of VIIIa indicated the presence of seven acetyl groups (Table I). The ^{13}C-NMR spectrum of VIII was closely similar to that of amentoflavone (VII) except for the chemical shifts due to II-B ring carbons (Table II). Therefore, VIII was inferred to be a biflavone which possessed one additional hydroxyl group on the II-B ring of amentoflavone (VII). As for the II-B ring, the ^{13}C-NMR spectrum of VIII showed signals at δ 146.70 and δ 150.90, upfield as compared to δ 162.34 observed in amentoflavone (VII), which has a hydroxyl group on C-4‴. On the other hand, it is known that the chemical shifts of carbons in an aromatic ring substituted with two hydroxyl groups are observed at *ca.* δ 160, *ca.* δ 150 and *ca.* δ 145 for *meta*-,[10] *para*-,[11] and *ortho*-[10] substitution, respectively. These data suggested that the two hydroxyl groups of VIII were located at an *ortho*-position, either C-4‴ and C-5‴ or C-5‴ and C-6‴. The ^{1}H-NMR spectrum of VIII was similar to that of amentoflavone (VII) except for chemical shifts due to II-B ring protons (Table I). In amentoflavone (VII), four protons at C-2‴, C-3‴, V-5‴ and C-6‴ appeared in two pairs, both indicating *ortho* couplings, while in VIII, three protons of the II-B ring appeared in two pairs, one indicating *ortho* and the other *meta* couplings. The 1H at δ 7.07 (d, J=2 Hz) observed in VIII was assigned to C_6‴–H. It was concluded from the splitting pattern that two hydroxyl groups of VIII were substituted on C-4‴ and C-5‴. This conclusion was supported by the fact that the splitting pattern of VIII was identical with that of 5′, 5‴-dihydroxyamentoflavone.[12] The structure of VIII was, therefore, determined to be 5‴-hydroxyamentoflavone.

Table II. ^{13}C-NMR Spectral Data for Biflavonoids[a]

Carbon	VII[b]	VIII[b]	Carbon	VII	VIII
2	165.81	165.85	2″	165.81	165.96
3	103.97	103.93	3″	103.32	103.51
4	183.57	183.84	4″	184.03	184.26
5	162.34	163.15	5″	162.34	162.42
6	100.00	100.04	6″	100.27	100.23
7	163.26	163.38	7″	162.99	163.23
8	95.23	95.38	8″	105.24	105.28
9	159.14	160.80	9″	156.33	159.37
10	105.24	105.28	10″	105.24	105.28
1′	123.12	123.31	1‴	121.35	121.50
2′	128.94	129.09	2‴	129.24	120.34
3′	123.12	123.74	3‴	116.84	116.53
4′	160.76	160.91	4‴	162.34	150.90
5′	117.26	117.45	5‴	116.84	146.70
6′	132.79	132.91	6‴	129.24	114.37

a) Chemical shifts in ppm units.
b) Measured in CD_3OD.

The *n*-BuOH extract of the aqueous layer was fractionated by preparative TLC and compounds IX, X, XI, and XII were isolated. Compounds IX, X, and XI were identified as afzelin (IX), cosmosiin (X) and quercitrin (XI) by direct comparison with authentic samples. Compound XII was recrystallized from EtOH to give pale yellow granular crystals, mp 245—247°C, which were hydrolyzed with acid to yield ferulic acid (II) and D-glucose. As XII was not hydrolyzed by alkali, the binding position of the sugar moiety was determined to be a phenolic hydroxyl group, not an acidic moiety. Acetylation of XII gave an acetate (XIIa) as colorless granular crystals, mp 183—184°C. The ^{1}H-NMR spectrum of XIIa indicated the

presence of four acetyl groups. Accordingly, XII was concluded to be a ferulic acid monoglucoside. The configuration of the linkage between the sugar and the aglycone deduced from the anomeric proton signal at δ 5.33 (d, J=7 Hz) was β. XII was therefore determined to be 4-β-D-glucopyranosyloxyferulic acid.

Twelve compounds (I, II, III, IV, V, VI, VII, VIII, IX, X, XI, and XII, of which VIII is a new compound) were isolated from the pollen grains of *C. japonica* for the first time in the present investigation. All of the biflavonoids of these pollen grains (VI, VII, and VIII) belonged to the amentoflavone family, while none of these biflavonoids was isolated from the leaves of *C. japonica*, which yielded only hinokiflavone family biflavonoids. These findings are of interest from a phytophysiological point of view.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. UV, IR, ^{1}H-NMR and ^{13}C-NMR spectra and MS and FD-MS were taken on Hitachi 340, Hitachi 295, JEOL JNM-4H-100, Hitachi R-900, JEOL JMS-01-SG-2 and JEOL JMS-D-300 spectrometers, respectively. TLC and preparative TLC were carried out on precoated silica gel plates (Merck silica gel 60) and silica gel (Wako, B-5), and spots were detected with 5% $FeCl_3$ and under UV illumination. S-1 [flavonoids and aromatic acids, $CHCl_3$–MeOH (5: 1)], S-2 [flavonoid glycosides, $CHCl_3$–MeOH (3: 1)] and S-3 [aromatic acid glycoside, AcOEt–MeOH–AcOH (2: 1: 0.2)] were used as the developing solvents. Gas–liquid chromatography (GLC) was carried out on a Hitachi 063 gas–liquid chromatograph using a stainless steel column (3 mm×1 m) packed with 10% SE-30 on Chromosorb W (60—80 mesh) with N_2 carrier gas at a flow rate of 30 ml/min.

Extraction and Fractionation of Components——Pollen grains (3435 g) collected in March, 1980, at Yachiyo City, Chiba Prefecture, were extracted with ether in a Soxhlet apparatus. The ether solution was shaken with H_2O, 5% $NaHCO_3$ and 5% NaOH successively, and the water layer was shaken with *n*-BuOH.

Isolation of Aromatic Acids——The 5% $NaHCO_3$ extract was acidified with dil. HCl and extracted with ether. The ether extract was dried over anhydrous sulfate, and the ether was evaporated off. The residue (2.2 g) was separated by preparative TLC (S-1), and gave two aromatic acids.

***p*-Coumaric Acid (I)**——A crystalline substance (64 mg) obtained by preparative TLC was recrystallized from H_2O, yielding colorless needles (44 mg), mp 214°C. IR ν_{max}^{KBr} cm^{-1}: 3350, 1675, 1630, 1600, 1515, 1450, 1310, 1245, 1210. The melting point on admixture of I with an authentic sample of *p*-coumaric acid showed no depression, and the IR spectra and TLC properties of the two samples were identical.

Ferulic Acid (II)——A crystalline substance (84 mg) obtained by preparative TLC was recrystallized from $CHCl_3$, yielding colorless plates (52 mg), mp 172—173°C. IR ν_{max}^{KBr} cm^{-1}: 3460, 1700, 1675, 1620, 1597, 1515, 1433, 1325, 1275, 1205. The melting point on admixture of II with an authentic sample of ferulic acid showed no depression, and the IR spectra and TLC properties of the two samples were identical.

Isolation of Flavonoids——The 5% NaOH extract was acidified with dil. HCl and extracted with ether. The ether extract was dried over anhydrous sulfate, and the ether was evaporated off. The residue (5.7 g) was separated by preparative TLC (S-1), and gave six flavonoids.

Naringenin (III)——A crystalline substance (30 mg) obtained by preparative TLC was recrystallized from 80% EtOH, yielding yellow needles (18 mg), mp 249—250°C. IR ν_{max}^{KBr} cm^{-1}: 3240, 1705, 1640, 1600, 1250, 835. The melting point on admixture of III with an authentic sample of naringenin showed no depression, and the IR spectra and TLC properties of the two samples were identical.

Apigenin (IV)——A crystalline substance (80 mg) obtained by preparative TLC was recrystallized from 80% EtOH, yielding pale yellow needles (54 mg), mp >300°C. IR ν_{max}^{KBr} cm^{-1}: 3300, 1648, 1605, 1582, 1545, 1491.

Acetylation of Apigenin (IV)——Compound IV (25 mg) was acetylated with Ac_2O (0.3 ml) and pyridine (1.0 ml). The product was crystallized from AcOEt to give the triacetate (IVa, 26 mg) as colorless granular crystals, mp 177—179°C. IR ν_{max}^{KBr} cm^{-1}: 1766, 1650, 1630, 1612, 1376, 1210, 1180, 1137, 1084. ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.31 (3H×2, s), 2.40 (3H, s), 6.59 (1H, s, C_3–H), 6.80 (1H, d, J=2 Hz, C_6–H), 7.20 (2H, d, J=9 Hz, $C_{3'}$, $C_{5'}$–H), 7.30 (1H, d, J=2 Hz, C_8–H), 7.86 (2H, d, J=9 Hz, $C_{2'}$, $C_{6'}$–H). The melting point on admixture of IV with an authentic sample of 5,7,4′-tri-*O*-acetylapigenin showed no depression, and the IR and ^{1}H-NMR spectra and TLC properties of the two samples were identical.

Luteolin (V)——A crystalline substance (41 mg) obtained by preparative TLC was recrystallized from 80% EtOH, yielding yellow granular crystals (28 mg), mp >300°C.

Acetylation of Luteolin (V)——Compound V (25 mg) was acetylated in the same manner as IV. The product was crystallized from AcOEt to give the tetraacetate (Va, 26 mg) as colorless granular crystals, mp 220—222°C. IR ν_{max}^{KBr} cm^{-1}: 1776, 1640, 1612, 1505, 1432, 1371, 1202, 1140. ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.31 (3H, s), 2.33 (3H×2, s), 2.42 (3H, s), 6.59 (1H, s, C_3–H), 6.82 (1H, d, J=2 Hz, C_6–H), 7.32 (1H, d, J=

9 Hz, $C_{3'}$–H), 7.33 (1H, d, J=2 Hz, C_8–H), 7.34 (1H, dd, J=2, 9 Hz, $C_{2'}$–H), 7.68 (1H, d, J=2 Hz, $C_{6'}$–H).

2,3-Dihydroamentoflavone (VI)——A crystalline substance (32 mg) obtained by preparative TLC was recrystallized from 80% EtOH, yielding yellow granular crystals (20 mg), mp 208—210°C. UV λ_{max}^{EtOH} nm (log ε): 223 (4.20), 288 (4.17), 339 (4.05). IR ν_{max}^{KBr} cm^{-1}: 3370, 1645, 1600, 1500, 1442, 1350, 1273, 1232, 1170, 1081, 830. ^{1}H-NMR (CD_3OD) δ (ppm): 2.80—3.12 (2H, m, C_3–H), 5.36 (1H, dd, J=4, 13 Hz, C_2–H), 5.86 (2H, d, J=2 Hz, C_6, C_8–H), 6.35 (1H, s, $C_{6''}$–H), 6.54 (1H, s, $C_{3''}$–H), 6.74 (2H, d, J=9 Hz, $C_{3'''}$, $C_{5'''}$–H), 7.02 (1H, d, J=9 Hz, $C_{5'}$–H), 7.41 (2H, d, J=9 Hz, $C_{2'''}$, $C_{6'''}$–H), 7.49 (1H, dd, J=2, 9 Hz, $C_{6'}$–H), 7.54 (1H, d, J=2 Hz, $C_{2'}$–H). ^{13}C-NMR (CD_3OD) δ (ppm): 197.74 (C-4), 184.30 (C-4″), 168.35 (C-7), 166.12 (C-2″), 165.46 (C-5), 164.92 (C-7″), 163.34 (C-5″), 162.57 (C-4′), 162.30 (C-4‴), 157.25 (C-9), 156.44 (C-9″), 132.40 (C-6′), 131.10 (C-1′), 129.48 (C-2‴, C-6‴), 128.70 (C-2′), 123.31 (C-1‴), 120.58 (C-3′), 116.88 (C-3‴, C-5″), 116.15 (C-5′), 106.09 (C-8″, C-10″), 105.55 (C-10), 103.28 (C-3″), 100.04 (C-6‴), 97.15 (C-6), 96.31 (C-8), 80.55 (C-2), 46.19 (C-3). The melting point of VI on admixture with an authentic sample of 2,3-dihydroamentoflavone showed no depression and the IR and ^{1}H-NMR spectra and TLC properties of the two samples were identical.

Acetylation of 2,3-Dihydroamentoflavone (VI)——A solution of VI (18 mg) in pyridine (0.5 ml) and Ac_2O (0.5 ml) was heated for 3 h at 100°C, then poured into ice water. The product was crystallized from AcOEt to give the hexaacetate (VIa, 15 mg), as colorless granular crystals, mp 208—210°C. IR ν_{max}^{KBr} cm^{-1}: 1774, 1692, 1642, 1620, 1370, 1192, 1133, 1088. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.93 (3H, s), 2.06 (3H, s), 2.25 (3H, s), 2.29 (3H, s), 2.34 (3H, s), 2.44 (3H, s), 2.87—3.10 (2H, m, C_3–H), 5.53 (1H, dd, J=4, 13 Hz, C_2–H), 6.48 (1H, d, J=2 Hz, C_6–H), 6.61 (1H, s, $C_{3''}$–H), 6.72 (1H, d, J=2 Hz, C_8–H), 6.95 (1H, s, $C_{8''}$–H), 7.07 (2H, d, J=9 Hz, $C_{3'''}$, $C_{5'''}$–H), 7.41 (1H, d, J=9 Hz, $C_{5'}$–H), 7.43 (1H, dd, J=2, 9 Hz, $C_{6'}$–H), 7.46 (2H, d, J=9 Hz, $C_{2'''}$, $C_{6'''}$–H), 7.58 (1H, d, J=2 Hz, $C_{2'}$–H).

Amentoflavone (VII)——A crystalline substance (62 mg) obtained by preparative TLC was recrystallized from 80% EtOH, yielding yellow granular crystals (48 mg), mp >300°C. UV λ_{max}^{EtOH} nm (log ε): 227 (4.18), 267 (4.18), 290 (4.18), 337 (4.16). IR ν_{max}^{KBr} cm^{-1}: 3200, 1660, 1605, 1350, 1160, 830. ^{1}H-NMR and ^{13}C-NMR: The results are shown in Tables I and II.

Acetylation of Amentoflavone (VII)——Compound VII (30 mg) was acetylated in the same manner as VI. The product was crystallized from MeOH to give the hexaacetate (VIIa, 22 mg), as colorless granular crystals, mp 245—247°C. IR ν_{max}^{KBr} cm^{-1}: 1770, 1650, 1365, 1183, 1092, 1080, 1045, 1028, 1010, 906, 812. ^{1}H-NMR: The data are shown in Table I. MS m/z: 706 ($M^+-COCH_2\times2$), 664 ($M^+-COCH_2\times3$), 538 ($M^+-COCH_2\times6$). The melting point on admixture of VII with an authentic sample of 5,7,4′,5″,7″,4‴-hexa-*O*-acetylamentoflavone showed no depression and the IR and ^{1}H-NMR spectra and TLC properties of the two samples were identical.

5‴-Hydroxyamentoflavone (VIII)——A crystalline substance (45 mg) obtained by preparative TLC was recrystallized from 80% EtOH, yielding yellow granular crystals (31 mg), mp 298—300°C. UV λ_{max}^{EtOH} nm (log ε): 227 (4.15), 267 (4.14), 290 (4.11), 339 (4.10). IR ν_{max}^{KBr} cm^{-1}: 3400, 1660, 1605, 1560, 1495, 1420, 1348, 1280, 1238, 1155, 1125, 830. ^{1}H-NMR and ^{13}C-NMR: The results are shown in Tables I and II. FD-MS m/z: 555 (M^++1), 539, 491.

Acetylation of 5‴-Hydroxyamentoflavone (VIII)——Compound VIII (25 mg) was acetylated in the same manner as VI. The product was crystallized from AcOEt to give the heptaacetate (VIIIa, 20 mg), as colorless granular crystals, mp 183—185°C. IR ν_{max}^{KBr} cm^{-1}: 1775, 1649, 1506, 1478, 1430, 1370, 1260, 1190, 1085, 1046, 1016, 906. ^{1}H-NMR: The data are shown in Table I. FD-MS m/z: 849 (M^++1), 848 (M^+), 806 (M^+-COCH_2), 764 ($M^+-COCH_2\times2$), 722 ($M^+-COCH_2\times3$), 554 ($M^+-COCH_2\times7$).

Isolation of Flavonoid Glycosides——The *n*-BuOH extract (17.0 g) was separated by preparative TLC (S-2), and gave three flavonoid glycosides.

Afzelin (IX)——A crystalline substance (48 mg) obtained by preparative TLC was recrystallized from EtOH, yielding yellow granular crystals (32 mg), mp 165—168°C. IR ν_{max}^{KBr} cm^{-1}: 3280, 1664, 1618, 1595, 1577, 1506, 1455, 1370. The melting point on admixture of IX with an authentic sample of afzelin showed no depression, and the IR spectra and TLC properties of the two samples were identical.

Cosmosiin (X)——A crystalline substance (53 mg) obtained by preparative TLC was recrystallized from MeOH, yielding yellow granular crystals (34 mg), mp 248—250°C. IR ν_{max}^{KBr} cm^{-1}: 3400, 1665, 1600, 1493, 1445, 1337, 1242, 1174, 1070, 832. The melting point on admixture of X with an authentic sample of cosmosiin showed no depression, and IR spectra and TLC properties of the two samples were identical.

Quercitrin (XI)——A crystalline substance (33 mg) obtained by preparative TLC was recrystallized from MeOH, yielding yellow granular crystals (21 mg), mp 180—184°C. IR ν_{max}^{KBr} cm^{-1}: 3410, 1667, 1605, 1496, 1440, 1352, 1194. ^{1}H-NMR (CD_3OD) δ (ppm): 0.95 (3H, d, J=6 Hz, rhamnose-CH_3), 5.36 (1H, d, J=2 Hz, anomeric H), 6.20 (1H, d, J=2 Hz, C_6–H), 6.37 (1H, d, J=2 Hz, C_8–H), 6.92 (1H, d, J=9 Hz, $C_{5'}$–H), 7.26 (1H, d, J=2 Hz, $C_{2'}$–H), 7.35 (1H, t, J=4 Hz, $C_{6'}$–H).

Acetylation of Quercitrin (XI)——Compound XI (19 mg) was acetylated in the same manner as VI. The product was crystallized from AcOEt to give the heptaacetate (XIa, 16 mg), as colorless plates, mp 95—98°C. ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.88 (3H, d, J=6 Hz, rhamnose-CH_3), 1.99 (3H, ×2, s), 2.12 (3H, s), 2.21 (3H, s), 2.33 (3H×2, s), 2.43 (3H, s), 5.66 (1H, d, J=2 Hz, anomeric H), 6.82 (1H, d, J=2 Hz, C_6–H), 7.28 (1H, d, J=2 Hz, C_8–H), 7.38 (1H, d, J=9 Hz, $C_{5'}$–H), 7.73 (1H, d, J=2 Hz, $C_{2'}$–H), 7.85 (1H,

dd, $J=2$, 9 Hz, $C_{6'}$–H).

Isolation of Aromatic Acid Glycoside——A crystalline substance (350 mg) obtained by preparative TLC of the *n*-BuOH extract was separated by repeated preparative TLC (S-3), and gave aromatic acid glycoside.

4-β-D-Glucopyranosyloxyferulic Acid (XII)——A crystalline substance (64 mg) obtained by preparative TLC was recrystallized from EtOH, yielding pale yellow granular crystals (51 mg), mp 245—247°C. IR ν_{max}^{KBr} cm^{-1}: 3400, 1645, 1550, 1511, 1383, 1266, 1072.

Acetylation of 4-β-D-Glucopyranosyloxyferulic Acid (XII)——A solution of XII (20 mg) in pyridine (0.5 ml), Ac_2O (0.5 ml) and AcOH (0.2 ml) was heated for 3 h at 100°C, then poured into ice water. The product was crystallized from benzene to give the tetraacetate (XIIa, 16.6 mg), as colorless granular crystals, mp 183—184°C. ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.05 (3H×2, s), 2.09 (3H×2, s), 3.88 (3H, s, $-OCH_3$), 4.25 (2H, m, glucose C_6–H), 4.98—5.32 (4H, m, glucose C_2, C_3, C_4, C_5–H), 5.33 (1H, d, $J=7$ Hz, anomeric H), 6.36 (1H, d, $J=16$ Hz, C_8–H), 7.12 (2H, br s, C_2, C_6–H), 7.33 (1H, d, $J=9$ Hz, C_5–H), 7.77 (1H, d, $J=16$ Hz, C_7–H).

Hydrolysis of 4-β-D-Glucopyranosyloxyferulic Acid (XII)——A solution of XII (30 mg) in EtOH (3 ml) containing 2% H_2SO_4 (6 ml) was refluxed for 3 h. The reaction mixture was poured into ice water and extracted with ether. The ether extract was recrystallized from $CHCl_3$, yielding colorless plates (21 mg), mp 171—172°C. IR ν_{max}^{KBr} cm^{-1}: 3460, 1700, 1675, 1620, 1597, 1515, 1433, 1325, 1275, 1205. The melting point on admixture of XII with an authentic sample of ferulic acid showed no depression, and the IR spectra and TLC properties of the two samples were identical. The aqueous layer was neutralized with anion exchange resin and concentrated to dryness *in vacuo*. The sugar moiety of the glycoside was identified as D-glucose by TLC and GLC (retention time of the TMS ether) comparisons with an authentic sample.

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