Chem. Pharm. Bull. 30(7)2342—2348(1982)

Studies on the Constituents of *Iris florentina* L. II. *C*-Glucosides of Xanthones and Flavones from the Leaves

Masao Fujita and Takao Inoue*

Hoshi College of Pharmacy, Ebara 2-4-41, Shinagawa-ku, Tokyo, 142, Japan

(Received December 11, 1981)

Four *C*-glucosylxanthones [Mangiferin (**3**), isomangiferin (**2**), **4** and **5**] and three *C*-glucosylflavones [swertisin (**6**), isoswertisin (**7**) and isoswertiajaponin (**8**)] were isolated from the fresh leaves of *Iris florentina* L. (Iridaceae). The structures of **4** and **5** were shown to be 7-*O*-methylmangiferin and 7-*O*-methylisomangiferin, respectively, by chemical and spectroscopic methods. In addition, the ^{13}C nuclear magnetic resonance chemical shifts of aryl carbons in these *C*-glucosylxanthones (**2**—**5**) were assigned. On the basis of the co-occurrence of several types of xanthone and flavone *C*-glucosides, the biosynthetic relationship between both *C*-glucosides is briefly discussed.

Keywords——*Iris florentina* L.; Iridaceae; *C*-glucosylxanthones; mangiferin; isomangiferin; 7-*O*-methylmangiferin; 7-*O*-methylisomangiferin; *C*-glucosylflavones; ^{13}C NMR; biosynthesis

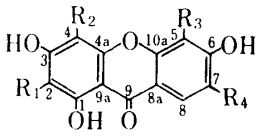
Previously we reported the isolation of 1-hydroxy-3,5,6-trimethoxyxanthone-2-*C*-glucoside, 1,3,5,6-tetrahydroxyxanthone-2-*C*-glucoside (**1**) and isomangiferin (**2**)[1] as well as mangiferin (**3**) and irisxanthone (5-methyl ether of **1**) from the underground parts of *Iris florentina* L. (Iridaceae).[2] This paper deals with the isolation and characterization of the *C*-glucosides of xanthones and flavones from the leaves of this plant.

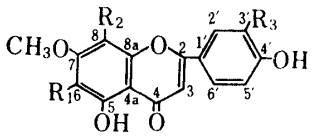
The fresh leaves were extracted with methanol, and from the water-soluble fraction of the extracts four *C*-glucosylxanthones (**2**—**5**) and three *C*-glucosylflavones (**6**—**8**) were isolated, together with vanillic acid (**9**) and protocatechuic acid (**10**), according to the procedure described in "Experimental."

Compounds **6**—**8** exhibited positive $FeCl_3$ and Mg–HCl tests, and their ^{13}C nuclear magnetic resonance (^{13}C NMR) spectra are characteristic of flavone *C*-glucosides.[3] Compound **6**, pale yellow crystalline powder, mp 243°C (dec.), gave a positive Gibbs test.[4] The ultraviolet (UV) absorption spectrum of **6** is very similar to the spectra reported for apigenin-type flavones.[5] Acetylation of **6** yielded a hexaacetate (**6a**), colorless prisms, mp 153—155°C, which gave a negative $FeCl_3$ test. The proton nuclear magnetic resonance (^1H NMR) spectrum of **6a** showed the signals of a methoxyl group [δ 4.04 (3H, s)], a methine proton at C-3 [δ 6.55 (1H, s)] and five aromatic protons [δ 6.82 (1H, br s, C_8–H), 7.25 (2H, d, J=8.8 Hz, $C_{3',5'}$–H) and 7.87 (2H, d, J=8.8 Hz, $C_{2',6'}$–H)]. The UV absorption maxima of **6** exhibited a bathochromic shift on addition of aluminum chloride, while the maxima were unaffected by sodium acetate. These observations show the presence of a C-5 hydroxyl group and the absence of a free C-7 hydroxyl group in the molecule.[5] Thus **6** was identified as swertisin[6] by direct comparison with an authentic specimen.

Compound **7**, pale yellow crystalline powder, mp 285—286°C, gave a negative Gibbs test. The UV absorption maxima of **7** and the changes of the maxima in the presence of the above diagnostic shift reagents showed a striking resemblance to those of **6**. Acetylation of **7** yielded a hexaacetate (**7a**), colorless crystalline powder, mp 131—132°C, which gave a negative $FeCl_3$ test. The ^1H NMR spectrum of **7a** showed the signals of a methoxyl group [δ 3.95 (3H, s)], a methine proton at C-3 and five aromatic protons [δ 6.61 (1H, s, C_3 or C_6–H), 6.63 (1H, s, C_3 or C_6–H), 7.37 (2H, d, J=8.8 Hz, $C_{3',5'}$–H) and 8.07 (2H, d, J=8.8 Hz, $C_{2',6'}$–H)]. In addition, **7** gave an equilibrium mixture of **6** and **7** in boiling 2 N hydrochloric acid. Consequently, **7** was identified as isoswertisin[6] by direct comparison with an authentic specimen.

Compound **8**, pale yellow crystalline powder, mp 252°C (dec.), exhibited UV absorption maxima at 258 (sh), 268 and 351 nm. The ^{1}H NMR spectrum of **8** showed the signals of a methoxyl group [δ 3.88 (3H, s)], a methine proton at C-3 [δ 6.68 (1H, s)] and four aromatic protons [δ 6.51 (1H, s, C_6–H), 6.86 (1H, d, J=8.8 Hz, $C_{5'}$–H) and 7.50—7.65 (2H, m, $C_{2',6'}$–H)]. A bathochromic shift of the UV absorption maxima of **8** on addition of boric acid–sodium acetate and aluminum chloride showed the presence of a C-5 hydroxyl group and *ortho*-dihydroxyl groups in the molecule.[5] In addition, **8** gave an equilibrium mixture of **8** and swertiajaponin (**11**)[7] in boiling 2 N hydrochloric acid. Accordingly, **8** was identified as isoswertiajaponin[7] by direct comparison with an authentic specimen. Isoswertisin (**7**) has been reported to be present in *Mollugo distica* (Aizoaceae),[8] *Gnetum gnemon* (Gnetaceae)[9] and *Avena sativa* (Gramineae),[10] and isoswertiajaponin (**8**) in *Gnetum gnemon*[9] and *Phragmites australis* (Gramineae).[11]





1: R_1=Glc, R_2=R_4=H, R_3=OH
2: R_1=R_3=H, R_2=Glc, R_4=OH
3: R_1=Glc, R_2=R_3=H, R_4=OH
4: R_1=Glc, R_2=R_3=H, R_4=OCH_3
5: R_1=R_3=H, R_2=Glc, R_4=OCH_3

6: R_1=Glc, R_2=R_3=H
7: R_1=R_3=H, R_2=Glc
8: R_1=H, R_2=Glc, R_3=OH
11: R_1=Glc, R_2=H, R_3=OH

Chart 1

Compounds **2**—**5** gave orange-red colors in the Mg–HCl test. Their ^{13}C NMR and UV absorption spectra are characteristic of 1,3,6,7-tetraoxygenated xangthone *C*-glucosides.[3a,12] Compound **2**, pale yellow needles, mp 255°C (dec.) and compound **3**, pale yellow needles, mp 272—273°C (dec.) were identified as isomangiferin and mangiferin, respectively, by direct comparison with authentic specimens.

Compound **4** was obtained as pale yellow needles, $C_{20}H_{20}O_{11}$, mp 237—238°C (dec.), $[\alpha]_D^{31}$ +31.7° (pyridine). Compound **4** gave a positive Gibbs test, indicating that an aromatic proton is located *para* to a phenolic hydroxyl group and that glucose is linked at C-2. The ^{1}H NMR spectrum of **4** showed the signals of a methoxyl group [δ 3.88 (3H, s)] and three aromatic protons [δ 6.39, 6.91 and 7.45 (each 1H, s)] ascribable to the C-4, C-5 and C-8 protons of the xanthone nucleus, respectively, on the basis of known data.[12,13] Methylation of **4** with diazomethane afforded a trimethyl ether, colorless needles, mp 229°C which was identified as the tetramethyl ether of mangiferin (**3**). These data indicate that **4** is a monomethyl ether of **3**. Compound **4** also gave a negative *ortho*-diphenol test,[14] and the UV absorption maxima of **4** showed a characteristic bathochromic shift on addition of aluminum chloride.[12] These observations indicate the presence of a C-1 hydroxyl group and the absence of *ortho*-dihydroxyl groups in **4**, and therefore reveal that the methoxyl group exists at either C-6 or C-7. The position of the methoxyl group in **4** was clarified by measurement of nuclear Overhauser effect (NOE) in dimethyl sulfoxide (DMSO)-d_6 solution. Irradiation of the methoxyl signal of **4** caused a 21% increase in the integrated intensity of the C-8 proton signal, while the signal intensity of the other aromatic protons was unaffected. Accordingly, the methoxyl group of **4** must be located at C-7, and **4** is suggested to be 1,3,6-trihydroxy-7-methoxyxanthone-2-*C*-β-D-glucopyranoside, namely 7-*O*-methylmangiferin which has been synthesized by Aritomi and Kawasaki.[12] Compound **4** was shown to be identical with a synthetic sample of 7-*O*-methylmangiferin by direct comparison. This is the first report of the natural occurrence of **4**.

Compound **5** was obtained as pale yellow needles, $C_{20}H_{20}O_{11}$, mp 247—248°C (dec.), $[\alpha]_D^{20}$ +11.4° (pyridine), exhibiting a negative Gibbs test. The changes of the UV absorption

maxima of **5** in the presence of diagnostic shift reagents [12] have a striking resemblance to those of **4**. The ^{1}H NMR of **5** showed the signals of a methoxyl group [δ 3.89 (3H, s)] and three aromatic protons, among which the one at δ 6.24 (1H, s) could be assigned to the C-2 proton and the other two [δ 6.88 and 7.42 (each 1H, s)] to the C-5 and C-8 protons of the xanthone nucleus respectively.[12,13] Methylation of **5** with diazomethane yielded a trimethyl ether, colorless needles, mp 286—287°C. This was identical with the tetramethyl ether of isomangiferin (**2**). These data indicate that **5** is a monomethyl ether of **2**. The position of the methoxyl group in **5** was demonstrated by measurement of the NOE in DMSO-d_6 solution. Irradiation of the methoxyl signal of **5** caused a 20% increase in the integrated intensity of the C-8 proton signal, while the signal intensity of the other aromatic protons was unaffected. Therefore, the methoxyl group of **5** must be located at C-7, and **5** is indicated to be 1,3,6-trihydroxy-7-methoxyxanthone-4-*C*-β-D-glucopyranoside, namely 7-*O*-methylisomangiferin. In order to confirm the above structure, 7-*O*-methylisomangiferin was synthesized from **2** by the synthetic method described for **4**.[12] Compound **5** was identical with the synthetic 7-*O*-methylisomangiferin on direct comparison. Although isomangiferin (**2**) has been isolated from several species,[13a,15] no methyl ether of **2** has been reported. 7-*O*-Methylisomangiferin (**5**) is the first example of the natural occurrence of isomangiferin derivatives.

Recently we reported the *O*-methylation effect on the ^{13}C NMR chemical shifts of the aryl carbons of *ortho*-substituted phenols.[16] In the above report, the *O*-methylation of a hydroxyl group of *ortho*-monosubstituted phenols including *o*-dihydroxybenzene caused an upfield shift by about 4 ppm for the *ortho*-methine carbon, whereas it caused a downfield shift by about 1 ppm for the substituted *ortho*-carbon. This regularity is very useful for interpretation of the spectra of *ortho*-substituted phenols and their methyl ethers. We assigned the chemical shifts of aryl carbons in *C*-glucosylxanthones (**2**—**5**) on the basis of the signal multiplicity in off-resonance decoupled spectra, the above *O*-methylation shifts of *ortho*-substituted phenols, the reported ^{13}C NMR data for xanthones,[17] and the additivity rule of substituent effects,[18] and by comparison of the chemical shifts of these *C*-glucosylxanthones with each other. The results are listed in Table I together with the chemical shifts of the sugar carbons.[3a]

Table I. ^{13}C Chemical Shifts[a] of *C*-Glucosylxanthones (**2**—**5**) in DMSO-d_6

Compound	**3**	**4**	**2**	**5**
C-1	161.6	161.6	161.5	161.4
C-2	107.3	107.6	97.4	97.5
C-3	163.6	163.7	163.2	163.4
C-4	93.3	93.3	103.8	103.9
C-4a	156.1	156.0	155.7	155.7
C-5	102.5	102.6	102.6	102.7
C-6	153.6	154.4	153.7	154.6
C-7	143.7	145.8	143.6	146.3
C-8	108.1	104.7	107.9	104.3
C-8a	111.7	111.3	111.4	111.0
C-9	179.0	178.8	179.1	178.9
C-9a	101.2	101.2	101.7	101.7
C-10a	150.7	151.5	150.7	151.5
OCH_3		55.7		55.7
C-1′	73.0	72.9	73.2	73.2
C-2′	70.5[b]	70.5[b]	70.9[b]	70.9[b]
C-3′	78.8	78.8	78.6	78.6
C-4′	70.3[b]	70.2[b]	70.5[b]	70.6[b]
C-5′	81.3	81.3	81.1	81.2
C-6′	61.4	61.4	61.4	61.4

a) Chemical shifts are given in δ (ppm) with tetramethylsilane as an internal standard.
b) Assignments may be reversed.

Flavanone synthase has been isolated from several plants.[19] Recently it has been shown that not the flavanone but the isomeric chalcone is the immediate product in this enzyme reaction,[20] and that chalcone isomerase catalyzes the formation of flavanone from chalcone.[20a] We reported that *C*-glucosylation takes place at the chalcone stage in the biosynthesis of the isoflavone puerarin in Pueraria root.[21] Wallace *et al.* observed that labelled flavanone was efficiently incorporated into *C*-glucosylflavones in *Spirodela* and *Lemna* species, but labelled aglycone was not.[22] We also obtained similar results for the biosynthesis of *C*-glucosylflavones in *Swertia japonica* and showed that *C*-glucosylflavone is biosynthesized *via* the corresponding *C*-glucosyflavanone.[23] These labelling experiments were supported by an enzymatic study on oat shoots.[24] However, it has not been concluded whether *C*-glucosylation of flavones takes place at the flavanone stage or at the corresponding chalcone stage, because flavanone and chalcone are interconvertible in plants.[20a,25] It is suggested from the co-occurrence of several types of xanthone and flavone *C*-glucosides in *I. florentina* that both *C*-glucosides are closely related to each other in their biosynthesis. Recently we reported that the aglycones of these *C*-glucosylxanthones are derived from the condensation of *p*-coumaroyl CoA with two malonyl units, instead of three malonly units in flavonoid biosynthesis, and that *C*-glucosylation of these xanthones takes place at the benzophenone stage rather than after ring closure to the xanthone.[1,26] These findings suggest that *C*-glucosylation of flavone probably occurs at the chalcone stage before ring closure to flavanone. The above suggestion is also supported by the co-occurrence of a *C*-glucosylchalcone and the corresponding *C*-glucosylflavaone in *Cladrastis platycarpa* (Leguminosae)[27] and by the existence of *C*-glycosyldihydrochalcones in *Aspalathus acuminatus* (Leguminosae)[28] and in *Nothofagus fusca* (Fagaceae).[29] A probable biosynthetic relationship between xanthone and flavone *C*-glucosides in *Iris florentina* is proposed in Chart 2.

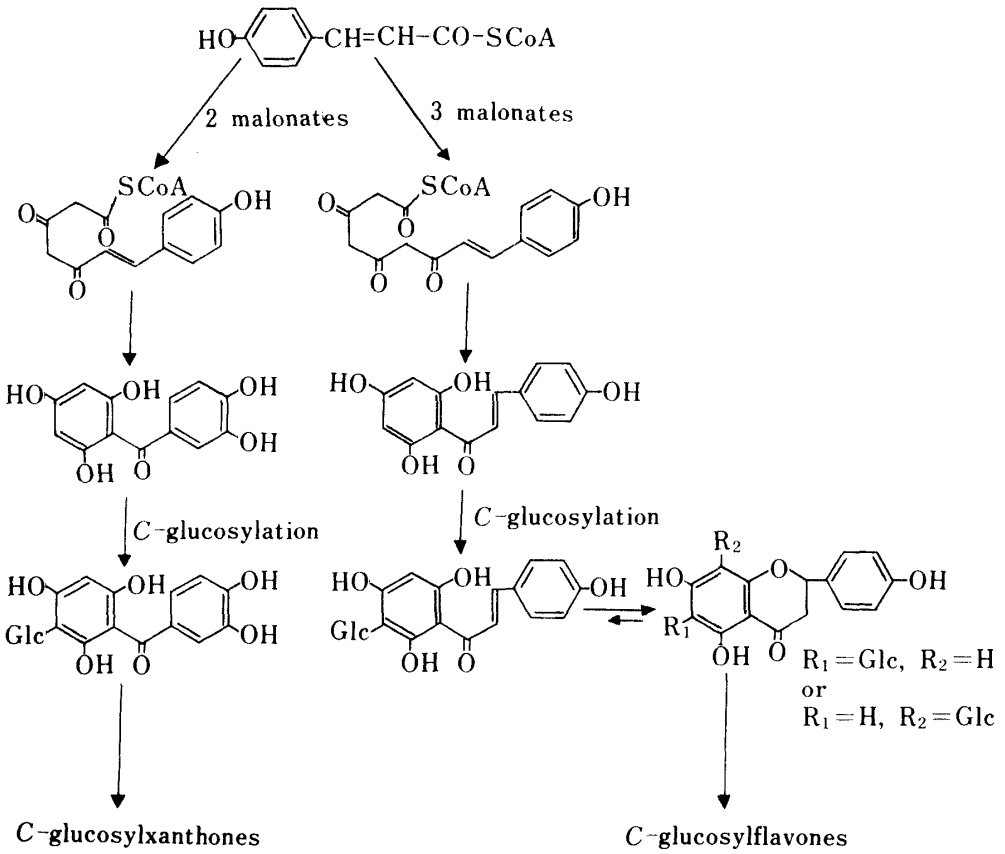


Chart 2. Proposed Biosynthetic Relationship between *C*-Glucosylxanthones and *C*-Glucosylflavones in *Iris florentina*

Experimental

All melting points were taken on a Shimadzu micro melting point determination apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-181 automatic polarimeter in a 1 dm tube. UV spectra were recorded on a Shimadzu UV-200 machine and infrared (IR) spectra were obtained with a Hitachi IR-215 spectrometer. NMR spectra were recorded on a JEOL JNM FX-100 spectrometer with tetramethylsilane as the internal standard. Chemical shifts are given on the δ scale (ppm). Column chromatography was performed on polyamide (Wako C-200, Wako Pure Chemical Ind., Ltd.), silica gel (100 mesh, Kanto Chemical Co., Ltd.) and cellulose powder (Avicel, Asahi Kasei Co., Ltd.). Thin-layer chromatography (TLC) was conducted on cellulose F_{254} precoated plates (Merck) unless otherwise stated, and the spots were detected under UV light. $FeCl_3$ test was performed using a mixture of 1% $FeCl_3$ and 1% $K_3Fe(CN)_6$ solutions.

Extraction and Separation——The fresh leaves of *I. florentina* (88 kg) were collected in the Medicinal Plants Garden of Hoshi College of Pharmacy in May, 1977 and extracted repeatedly with MeOH at room temperature. The extracts were concentrated to dryness, the residue was dissolved in hot H_2O and insoluble material was filtered off. The filtrate was extracted with Et_2O, and the extracts were chromatographed on silica gel using $CHCl_3$–MeOH (10: 1) as the eluent to give **9** (130 mg) and **10** (52 mg). The aqueous layer was passed through a column of polyamide and separated into the adsorbed portion (A) and non-adsorbed portion (B). The adsorbed portion (A) was eluted with MeOH, and the eluate was concentrated to a small volume. The resulting precipitates were recrystallized from aq. MeOH to give **3** (60.5 g), pale yellow needles, mp 272—273°C (dec.), which was identical with authentic mangiferin by mixed mp, TLC and IR comparisons. The filtrate after removal of crude **3** was chromatographed on cellulose and the eluate with 10% AcOH was recrystallized from aq. MeOH to give **2** (5.8 g), pale yellow needles, mp 255°C (dec.). This was identical with authentic isomangiferin (mixed mp, TLC and IR). The non-adsorbed portion (B) was concentrated to a small volume and extracted with *n*-BuOH, and then the extracts were chromatographed on silica gel. The fractions eluted with $CHCl_3$–MeOH (6: 1) were combined and concentrated, affording **6** (7.2 g) as a pale yellow powder. The fractions eluted with $CHCl_3$–MeOH (4: 1) were combined and concentrated, affording **5** (210 mg) as a pale yellow crystalline powder. The filtrate after removal of crude **6** was chromatographed on cellulose. The fractions eluted with 15% AcOH were rechromatographed on polyamide using aq. MeOH as the eluent to give **7** (89 mg) and **8** (13 mg). The filtrate of crude **5** was rechromatographed on cellulose using 10% AcOH as the eluent to give **4** (52 mg).

Swertisin (**6**)——Pale yellow crystalline powder from MeOH, mp 243°C (dec.), $[\alpha]_D^{24}$ −10.3° (c=1.0, pyridine). $FeCl_3$ (+), Mg–HCl (+), Gibbs test (+). TLC *Rf*: 0.61 (10% AcOH) (solv. 1); 0.76 (30% AcOH) (solv. 2). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1660 (C=O). UV λ_{max}^{EtOH} nm (log ε): 270 (4.22), 335 (4.32). UV $\lambda_{max}^{EtOH\text{-}AlCl_3}$ nm: 276, 302, 350, 386 (sh). UV $\lambda_{max}^{EtOH\text{-}NaOAc}$ nm: 270, 335, 400 (sh). ^{13}C NMR (DMSO-d_6) δ: 181.8 (C-4), 164.2 (C-7), 163.7 (C-2), 161.0 (C-4′), 160.0 (C-5), 156.4 (C-8a), 128.3 (C-2′, 6′), 120.9 (C-1′), 115.9 (C-3′, 5′), 109.4 (C-6), 104.2 (C-4a), 102.9 (C-3), 90.5 (C-8), 81.3 (C-5″), 79.1 (C-3″), 72.8 (C-1″), 70.9 (C-2″ or 4″), 70.0 (C-2″ or 4″), 61.8 (C-6″), 56.2 (OCH_3). **6** was shown to be identical with authentic swertisin (mixed mp, IR and TLC).

Swertisin Hexaacetate (**6a**): A solution of **6** in dry pyridine and acetic anhydride was heated on a boiling water bath for 3 h, then the reaction mixture was worked up in the usual manner. The crude acetate was recrystallized from hexane–$CHCl_3$ to give **6a** as colorless needles, mp 153—155°C, exhibiting a negative $FeCl_3$ test. ^{1}H NMR ($CDCl_3$) δ: 1.78, 2.02, 2.07 (total 12H, 4× aliphatic OAc), 2.34, 2.50 (each 3H, s, 2× aromatic OAc), 4.04 (3H, s, OCH_3), 6.55 (1H, s, C_3–H), 6.82 (1H, s, C_8–H), 7.25 (2H, d, J=8.8 Hz, $C_{3',5'}$–H), 7.87 (2H, d, J=8.8 Hz, $C_{2',6'}$–H). **6a** was shown to be identical with authentic swertisin hexaacetate (mixed mp, IR and TLC).

Isoswertisin (**7**)——Pale yellow crystalline powder from acetone, mp 285—286°C (dec.), $[\alpha]_D^{22}$ −42.6° (c=1.0, pyridine). $FeCl_3$(+), Mg–HCl(+), Gibbs test(−). TLC *Rf*: 0.14 (solv. 1), 0.43 (solv. 2). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650 (C=O). UV λ_{max}^{EtOH} nm (log ε): 268 (4.24), 340 (4.30). UV $\lambda_{max}^{EtOH\text{-}AlCl_3}$ nm: 273, 301, 346, 385. UV $\lambda_{max}^{EtOH\text{-}NaOAc}$ nm: 268, 340, 398 (sh). ^{1}H NMR (pyridine-d_5) δ: 3.78 (3H, s, OCH_3). ^{13}C NMR (DMSO-d_6) δ: 181.8 (C-4), 164.0 (C-2 or 7), 163.1 (C-2 or 7), 161.0 (C-5, 4′), 155.3 (C-8a), 128.8 (C-2′, 6′), 121.3 (C-1′), 115.8 (C-3′, 5′), 105.2 (C-4a or 8), 104.6 (C-4a or 8), 102.4 (C-3), 95.4 (C-6), 81.7 (C-5″), 78.7 (C-3″), 73.1 (C-1″), 70.7 (C-2″ or 4″), 70.4 (C-2″ or 4″), 61.2 (C-6″), 56.2 (OCH_3). A solution of **7** (20 mg) in 2 N HCl (40 ml) was refluxed for 3 h, then cooled. The resulting precipitates were filtered off, and the filtrate was diluted with H_2O then passed through a column of polyamide. The column was washed with H_2O and the adsorbed material was eluted with MeOH to give a mixture of two flavonoids which were identified as **7** and swertisin (**6**) by TLC (solv. 1 and 2). **7** was shown to be identical with authentic isoswertisin (mixed mp, IR and TLC).

Isoswertisin Hexaacetate (**7a**): A solution of **7** in dry pyridine and acetic anhydride was allowed to stand at room temperature for 48 h, and the reaction mixture was worked up in the usual manner. The crude acetate was purified by silica gel chromatography and recrystallized from hexane–$CHCl_3$ to give **7a** as colorless needles, mp 131—132°C, exhibiting a negative $FeCl_3$ test. ^{1}H NMR ($CDCl_3$) δ: 1.73, 1.91, 1.99,

2.10 (each 3H, s, 4×aliphatic OAc), 2.35, 2.44 (each 3H, s, 2×aromatic OAc), 3.95 (3H, s, OCH_3), 6.61 (1H, s, C_3 or C_6–H), 6.63 (1H, s, C_3 or C_6–H), 7.37 (2H, d, J=8.8 Hz, $C_{3',5'}$–H), 8.07 (2H, d, J=8.8 Hz, $C_{2',6'}$–H). **7a** was shown to be identical with authentic isoswertisin hexaacetate (mixed mp and IR).

Isoswertiajaponin (8)——Pale yellow crystalline powder from acetone–MeOH, mp 252°C (dec.). $FeCl_3$ (+), Mg–HCl (+). TLC *Rf*: 0.11 (solv. 1), 0.33 (solv. 2). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1640 (C=O). UV λ_{max}^{EtOH} nm (log ε): 258 (sh) (4.26), 268 (4.28), 351 (4.35). UV $\lambda_{max}^{EtOH\text{-}AlCl_3}$ nm: 276 (sh), 335, 407. UV $\lambda_{max}^{EtOH\text{-}H_3BO_3\text{-}NaOAc}$ nm: 266, 372. UV $\lambda_{max}^{EtOH\text{-}NaOAc}$ nm: 259 (sh), 268, 354 (sh), 409. ^1H NMR (DMSO-d_6) δ: 3.88 (3H, s, OCH_3), 4.77 (1H, d, J=9.9 Hz, anomeric H), 6.51 (1H, s, C_6–H), 6.68 (1H, s, C_3–H), 6.86 (1H, d, J=8.8 Hz, $C_{5'}$–H), 7.50—7.65 (2H, m, $C_{2',6'}$–H), 13.33 (1H, s, C_5–OH). ^{13}C NMR (DMSO-d_6) δ: 182.0 (C-4), 164.3 (C-2), 163.1 (C-7), 161.1 (C-5), 155.0 (C-8a), 149.5 (C-4′), 145.6 (C-3′), 121.8 (C-1′), 119.3 (C-6′), 115.6 (C-5′), 113.9 (C-2′), 105.5 (C-8), 104.3 (C-4a), 102.2 (C-3), 94.7 (C-6), 81.8 (C-5″), 78.7 (C-3″), 73.0 (C-1″), 70.7 (C-2″ or 4″), 70.5 (C-2″ or 4″), 61.4 (C-6″), 56.4 (OCH_3). Treatment of **8** (5 mg) with boiling 2 N HCl (10 ml) as described in the case of **7** afforded a mixture of two flavonoids which were identified as **8** and swertiajaponin (**11**) by TLC (solv. 1 and 2). **8** was shown to be identical with authentic isoswertiajaponin (mixed mp, IR and TLC).

7-*O*-Methylmangiferin (4)——Pale yellow needles from dioxane–H_2O, mp 237—238°C, $[\alpha]_D^{31}$ +31.7° (c=0.7, pyridine). *Anal.* Calcd for $C_{20}H_{20}O_{11}$: C, 55.05; H, 4.62. Found: C, 55.41; H, 4.50. $FeCl_3$ (+), Mg–HCl (orange-red), *ortho*-diphenol (−), Gibbs test (+). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1640 (C=O). UV λ_{max}^{EtOH} nm (log ε): 239 (4.06), 256 (3.93), 318 (3.52), 372 (3.92). UV $\lambda_{max}^{EtOH\text{-}AlCl_3}$ nm: 231, 265, 338, 396. UV $\lambda^{EtOH\text{-}AlCl_3\text{-}HCl}$ nm: 229, 265, 336, 397. UV $\lambda_{max}^{EtOH\text{-}H_3BO_3\text{-}NaOAc}$ nm: 238, 261, 377. UV $\lambda_{max}^{EtOH\text{-}NaOAc}$ nm: 237, 267, 384. ^1H NMR (DMSO-d_6) δ: 3.88 (3H, s, OCH_3), 6.39 (1H, s, C_4–H), 6.91 (1H, s, C_5–H), 7.45 (1H, s, C_8–H), 13.71 (1H, s, C_1–OH). ^{13}C NMR (DMSO-d_6): see Table I. **4** was shown to be identical with the synthetic 7-*O*-methylmangiferin[12] (mixed mp, IR, ^{13}C NMR and TLC).

Methylation of 4——An ethereal solution of CH_2N_2 was added to a solution of **4** (30 mg) in dimethylformamide (0.5 ml) and MeOH (8 ml), and the mixture was left to stand overnight. After removal of the solvent by evaporation, the residue was chromatographed on silica gel using EtOAc–EtOH (9 : 1) and recrystallized from MeOH to give **4a** as colorless needles, mp 229°C, exhibiting a negative $FeCl_3$ test. UV λ_{max}^{EtOH} nm (log ε): 255 (4.61), 356 (4.10). ^1H NMR (pyridine-d_5) δ: 3.62—4.32 (total 12H, 4×OCH_3). **4a** was shown to be identical with authentic tetra-*O*-methylmangiferin[12] by mixed mp determination and IR and TLC (Kieselgel 60F_{254}, Merck) comparisons.

7-*O*-Methylisomangiferin (5)——Pale yellow needles from MeOH, mp 247—248°C, $[\alpha]_D^{20}$ +11.4° (c=1.0, pyridine). *Anal.* Calcd for $C_{20}H_{20}O_{11}$: C, 55.05; H, 4.62. Found: C, 55.23; H, 4.68. $FeCl_3$ (+), Mg–HCl (orange red), *ortho*-diphenol (−), Gibbs test (−). IR ν_{max}^{KBr} cm^{-1}: 3320 (OH), 1640 (C=O). UV λ_{max}^{EtOH} nm (log ε): 238 (4.22), 255 (4.19), 314 (3.84), 370 (4.04). UV $\lambda_{max}^{EtOH\text{-}AlCl_3}$ nm: 229, 265, 335, 401. UV $\lambda_{max}^{EtOH\text{-}AlCl_3\text{-}HCl}$ nm: 228, 264, 334, 398. UV $\lambda_{max}^{EtOH\text{-}H_3BO_3\text{-}NaOAc}$ nm: 237, 261, 377. UV $\lambda_{max}^{EtOH\text{-}NaOAc}$ nm: 236, 268, 381. ^1H NMR (DMSO-d_6) δ: 3.89 (3H, s, OCH_3), 6.24 (1H, s, C_2–H), 6.88 (1H, s, C_5–H), 7.42 (1H, s, C_8–H), 13.29 (1H, s, C_1–OH). ^{13}C NMR (DMSO-d_6): see Table I.

Methylation of 5——**5** (60 mg) was methylated with CH_2N_2 in the manner described for the methylation of **4**. The crude methyl ether was chromatographed on silica gel using EtOAc–EtOH (9: 1) and recrystallized from MeOH to give **5a** as colorless needles, mp 286—287°C, exhibiting a negative $FeCl_3$ test. UV λ_{max}^{EtOH} nm (log ε): 256 (4.49), 358 (4.09). ^1H NMR (pyridine-d_5) δ: 3.65—4.20 (total 12H, 4×OCH_3). **5a** was shown to be identical with authentic tetra-*O*-methylisomangiferin[13a] by mixed mp determination and IR and TLC (Kieselgel 60 F_{254}, Merck) comparisons.

Synthesis of 5——Octa-*O*-acetylisomangiferin[13a] (1.2 g) was refluxed with benzyl chloride (1.2 ml), K_2CO_3 (4.8 g) and KI (0.15 g) in dry acetone (30 ml) for 33 h. After cooling, the reaction mixture was filtered, and the filtrate was poured into a large amount of hexane. The resulting precipitates were collected and recrystallized from MeOH to give 3,6-di-*O*-benzyl-1,2′,3′,4′,6′,7-hexa-*O*-acetylisomangiferin (**12**) as colorless needles (840 mg), mp 189—190°C. ^1H NMR ($CDCl_3$) δ: 1.70, 1.95, 2.01, 2.06 (each 3H ,s, 4×aliphatic OAc), 2.28, 2.45 (each 3H, s, 2×aromatic OAc), 5.16 (4H, s, 2×$OC\underline{H}_2Ph$), 7.43 (10H, br s, 2×$OCH_2\underline{Ph}$). **12** (0.8 g) was refluxed with MeOH (160 ml) and 10% HCl (80 ml) for 7 h. After cooling, the reaction mixture was diluted with H_2O (80 ml), and the resulting precipitates were collected, washed with H_2O and recrystallized from MeOH to give 3,6-di-*O*-benzylisomangiferin (**13**) as pale yellow needles (570 mg), mp 258—260°C, exhibiting a negative *ortho*-diphenol test. UV λ_{max}^{EtOH} nm (log ε): 237 (4.33), 257 (4.63), 308 (4.27), 368 (4.08). UV $\lambda_{max}^{EtOH\text{-}AlCl_3}$ nm: 228, 262, 326, 412. UV $\lambda_{max}^{EtOH\text{-}AlCl_3\text{-}HCl}$ nm: 229, 263, 327, 411. UV $\lambda_{max}^{EtOH\text{-}NaOAc}$ nm: 236, 257, 309, 369. ^1H NMR (DMSO-d_6) δ: 5.34 (4H, s, 2×$OC\underline{H}_2Ph$), 7.53 (10H, br. s, 2×$OCH_2\underline{Ph}$). A solution of **13** (0.5 g) in acetone (600 ml) was treated with an ethereal solution of CH_2N_2. After standing for 3 d, the reaction mixture was evaporated to dryness and the residue was chromatographed on silica gel using $CHCl_3$–MeOH (20: 1). The product was recrystallized from MeOH to give 7-*O*-methyl-3,6-di-*O*-benzylisomangiferin (**14**) as pale yellow needles (152 mg), mp 171—172°C. *Anal.* Calcd for $C_{34}H_{32}O_{11}\cdot H_2O$: C, 64.35; H, 5.40. Found: C, 64.03; H, 5.31. $FeCl_3$ (+). UV λ_{max}^{EtOH} nm (log ε): 238 (4.30), 256 (4.59), 308 (4.25), 364 (4.07). UV $\lambda_{max}^{EtOH\text{-}AlCl_3}$ nm: 228, 263, 329, 408. UV $\lambda_{max}^{EtOH\text{-}NaOAc}$ nm: 238, 257, 309, 365. ^1H NMR (DMSO-d_6) δ: 5.30 (4H, s, 2×$OC\underline{H}_2Ph$), 7.49 (10H, br s, 2×$OCH_2\underline{Ph}$), 13.55 (1H, s, C_1–OH, disappeared with D_2O). ^1H NMR (pyridine-d_5) δ: 3.78 (3H, s, OCH_3). **14** (150 mg) in AcOH (15 ml) was shaken with Pd/C (10%) (150 mg) under an H_2 stream. The catalysts were filtered off and washed with hot pyridine. The filtrate and

the washings were combined and evaporated to dryness. The residue was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (7: 4: 1) and the product was recrystallized from MeOH to give 7-*O*-methylisomangiferin (**5**) as pale yellow needles (49 mg), mp 247—248°C (dec.). The synthetic 7-*O*-methylisomangiferin was identified with the natural product (mixed mp, IR, ^{13}C NMR and TLC).

Vanillic Acid (**9**)——Colorless needles from H_2O, mp 206°C. $FeCl_3$ (+). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1670 (C=O). ^1H NMR (DMSO-d_6) δ: 3.82 (3H, s, OCH_3), 6.84 (1H, d, J=9.0 Hz, C_5–H), 7.39—7.48 (2H, m, $C_{2,6}$–H). **9** was shown to be identical with authentic vanillic acid by mixed mp determination and IR and TLC comparisons.

Protocatechuic Acid (**10**)——Pale brown crystalline powder, mp 198—199°C. $FeCl_3$ (+). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1680 (C=O). **10** was identical with authentic protocatechuic acid (mixed mp, IR and TLC).

Acknowledgement The authors are indebted to Prof. M. Aritomi, Faculty of Education, Kumamoto University, for his kind gift of 7-*O*-methylmangiferin.

References and Notes

1) Part I: M. Fujita and T. Inoue, *Yakugaku Zasshi*, **101**, 1118 (1981).
2) M. Arisawa, N. Morita, Y. Kondo, and T. Takemoto, *Chem. Pharm. Bull.*, **21**, 2562 (1973).
3) *a*) V.M. Chari, H. Wagner, G. Shilling, and A. Nesmelyi, Symposium Papers, 11th IUPAC International Symposium on the Chemistry of Natural Products, Golden Sands, Bulg., Sept. 1978, Vol. 2, p. 279; *b*) K.R. Markham, B. Ternai, R. Stanley, H. Geiger, and T.J. Mabry, *Tetrahedron*, **34**, 1389 (1978), and references cited therein.
4) H.D. Gibbs, *J. Biol. Chem.*, **72**, 649 (1927).
5) T.J. Mabry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, New York, 1970, p. 33.
6) M. Komatsu, T. Tomimori, and M. Ito, *Chem. Pharm. Bull.*, **15**, 263 (1967).
7) M. Komatsu, T. Tomimori, and Y. Makiguchi, *Chem. Pharm. Bull.*, **15**, 1567 (1967).
8) J. Chopin, M.L. Bouillant, A.G.R. Nair, P. Ramesh, and T.J. Mabry, *Phytochemistry*, **17**, 299 (1978).
9) J.W. Wallace and G. Morris, *Phytochemistry*, **17**, 1809 (1978).
10) J. Chopin, G. Dellamonica, M.L. Bouillant, A. Besset, G. Popovici, and G. Weissenböck, *Phytochemistry*, **16**, 2041 (1977).
11) M.A.M. Nawwar, H.I. El-Sissi, and H.H. Baracat, *Phytochemistry*, **19**, 1854 (1980).
12) M. Aritomi and T. Kawasaki, *Chem. Pharm. Bull.*, **18**, 2224 (1970).
13) *a*) M. Aritomi and T. Kawasaki, *Chem. Pharm. Bull.*, **18**, 2327 (1970); *b*) L.J. Haynes and D.R. Taylor, *J. Chem. Soc.* (*C*), **1966**, 1685.
14) M. Shimokoriyama, "Plants Pigments," ed. by K. Hayashi, Yokendo, Ltd., Tokyo, 1980, p. 179.
15) D.M. Smith and J.B. Harbone, *Phytochemistry*, **10**, 2117 (1971); V.I. Glyzin, A.I. Ban'kovskii, M.G. Primenov, and K.I. Boryaev, *Khim. Prir. Soedin.*, **9**, 434 (1973) [*Chem. Abstr.*, **79**, 79119 (1973)]; B.A. Bohm, *Phytochemistry*, **14**, 287 (1975); N.A.M. Saleh and M.A.I. El-Ansori, *Planta Medica*, **28**, 124 (1975); M. Arisawa, H. Kizu, and N. Morita, *Chem. Pharm. Bull.*, **24**, 1609 (1976).
16) M. Fujita, M. Nagai, and T. Inoue, *Chem. Pharm. Bull.*, **30**, 1151 (1982).
17) P.W. Westerman, S.P. Gunasekera, M. Uvais, S. Sultanbawa, and R. Kazlauskas, *Org. Magn. Reson.*, **9**, 631 (1977); A.K. Sen, K.K. Sarkar, P.C. Mazumder, N. Banerji, R. Unsvuori, and T.A. Hase, *Phytochemistry*, **19**, 2223 (1980), and references cited therein.
18) G.C. Levy, R.L. Lichter, and G.L. Nelson, "Carbon-13 Nuclear Magnetic Resonance Spectroscopy," 2nd ed., John Wiley and Sons, Inc., New York, 1980, pp. 102—135.
19) G. Hrazdina and L.L. Creasy, *Phytochemistry*, **18**, 581 (1979), and references cited therein.
20) *a*) B. Kuhn, G. Forkmann, and W. Seyffert, *Planta*, **138**, 199 (1978); G. Forkmann and B. Kuhn, *ibid.*, **144**, 189 (1979); *b*) W. Heller and K. Hahlbrock, *Arch. Biochem. Biophys.*, **200**, 617 (1980).
21) T. Inoue and M. Fujita, *Chem. Pharm. Bull.*, **25**, 3226 (1977).
22) J.W. Wallace, T.J. Mabry, and R.E. Alston, *Phytochemistry*, **8**, 93 (1969); J.W. Wallace, *ibid.*, **14**, 1765 (1975); J.W. Wallace and H. Grisebach, *Biochim. Biophys. Acta*, **304**, 837 (1973).
23) M. Fujita and T. Inoue, *Yakugaku Zasshi*, **99**, 165 (1979).
24) G. Weissenböck, *Z. Pflanzenphysiol.*, **74**, 226 (1974).
25) K. Hahlbrock and H. Grisebach, "The Flavonoids," ed. by J.B. Harbone, T.J. Mabry, and H. Mabry, Chapman and Hall, Ltd., London, 1975, pp. 866—915; W.R. Raymond and V.P. Maier, *Phytochemistry*, **16**, 1535 (1977), and references cited therein.
26) M. Fujita and T. Inoue, *Tetrahedron Lett.*, **1977**, 4503; *idem*, *Chem. Pharm. Bull.*, **28**, 2476 (1980); *idem*, *ibid.*, **28**, 2482 (1980); *idem*, *Phytochemistry*, **20**, 2183 (1981).
27) H. Ohashi, M. Goto, and H. Imamura, *Phytochemistry*, **16**, 1106 (1977).
28) B.H. Koeppen and D.G. Roux, *Tetrahedron Lett.*, **1965**, 3497.
29) W.E. Hillis and T. Inoue, *Phytochemistry*, **6**, 59 (1967).