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THREE HIGHLY OXYGENATED FLAVONE GLUCURONIDES IN LEAVES OF *SPINACIA OLERACEA*

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Key Word Index—*Spinacia oleracea*; Chenopodiaceae; spinach, jaceidin 4′-glucuronide; 5,3′,4′-trihydroxy-3-methoxy-6·7-methylenedioxyflavone 4′-glucuronide; 5,4′-dihydroxy-3,3′-dimethoxy-6·7-methylenedioxyflavone 4′-glucuronide, ^{1}H NMR; ^{13}C NMR; FABMS.

Abstract—Droplet counter-current chromatographic separation and subsequent TLC demonstrated the existence of at least 14 phenolics in the leaves of *Spinacia oleracea*. Three have now been isolated and identified, respectively, as the 4′-glucuronides of 5,7,4′-trihydroxy-3,6,3′-trimethoxyflavone (jaceidin), 5,3′,4′-trihydroxy-3-methoxy-6:7-methylenedioxyflavone and 5,4′-dihydroxy-3,3′-dimethoxy-6:7-methylenedioxyflavone.

INTRODUCTION

Spinach (*Spinacia oleracea* L., annual herb of the Chenopodiaceae; Japanese name, Hôren-sô) is native to West Asia and, at present, is widely cultivated in the world as one of the most popular vegetables. So far, one 3,5,6,7,4′-penta- and four 3,5,6,7,3′,4′-hexa-oxygenated flavones, i.e. 6-methoxykaempferol [1], patuletin [1–3], patulitrin [2], spinacetin [1–3] and spinatoside [4], have been isolated from its leaves [2–4] and pollen [1]. The presence of *p*-coumaric acid derivatives has also been reported by Tadera and Mitsuda [5].

In continuation of our chemical studies on the constituents of edible plants, we now report the isolation and characterization of three new flavone glucuronides.

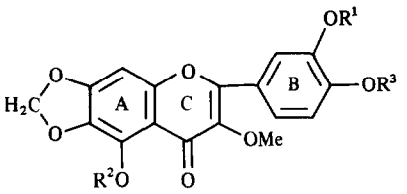
RESULTS AND DISCUSSION

Droplet counter-current chromatographic (DCCC) separation of the methanolic extract of fresh leaves of *S. oleracea* gave 13 fractions which showed on TLC the presence of at least 14 phenolics. Fraction 10 crystallized from methanol to give compound **1** as yellow needles (yield 0.01% of fresh leaves). The flavonoid structure was evident from its UV spectrum and colour reactions. On the basis of elementary analysis and the fast-atom bombardment mass spectrum (FABMS) (*m/z* 521 $[M+1]^+$, 345 $[M+1-176]^+$), it was formulated as $C_{23}H_{20}O_{14}\cdot 4H_2O$. The peak $[M+1-176]^+$ in the FABMS could be ascribed to the loss of a sugar moiety as was reported in FDMS [6], and suggested, in harmony with two carbonyl absorptions in the IR spectrum (1740 and 1690 cm^{-1}), the existence of a hexuronic acid residue in the molecule.

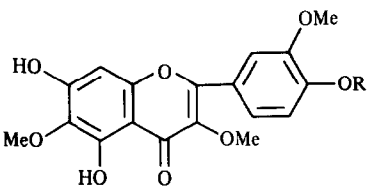
Acid hydrolysis of **1** gave glucuronic acid (identified by TLC) and an aglycone (**2**). **2** afforded a triacetate (**3**) and a trimethyl ether (**4**). **1** was methylated with diazomethane to give a dimethyl ether methyl ester (**5**), which was hydrolysed to a partially methylated aglycone (**6**).

The ^{1}H NMR spectra of **1** and its derivatives (**2**–**6**) revealed signals due to one methoxyl and one methylenedioxy group. The doublet (5.82 and 5.87 ppm, 1H) and multiplet (around 4–5 ppm, 4H) in the spectra of **1** and **5** were ascribed to the protons of the glucuronic acid moiety, and the *J* value (8 Hz) of the doublet suggested a β-configuration of the anomeric centre. Aromatic protons were found in the lowest field of each spectrum as a multiplet (3H) and a sharp singlet (1H), the former of which was assigned to the protons on the B-ring having *O*-functions at C-3′ and C-4′. The latter singlet was ascribed to an isolated proton on the A-ring (C-6 or C-8) or on the C-ring (C-3), whose location at C-8 was determined by the fact that, in $Eu(FOD)_3$, the singlet in the spectrum of **4** showed a slight downfield shift (from 6.60 to 6.88 ppm, 10% of the shift of MeO-5) in contrast to the corresponding singlet in the spectra of 3,5,7,8,3′,4′-hexamethoxyflavone (downfield shift from 6.41 to 7.73 ppm, 40% of that of MeO-5) and of 5,6,7,8-tetramethoxy-3′:4′-methylenedioxylflavone (upfield shift from 6.51 to 4.86 ppm) [7–9].

From these findings, **1** was assumed to be a β-glucuronide of a 3,5,6,7,3′,4′-hexa-oxygenated flavone having methoxyl and methylenedioxy groups. The sites of linkage of methoxyl, methylenedioxy and glycosyloxy groups, respectively, at C-3, C-6:C-7 and C-4′ were proved on the basis of UV spectral data [10]. The absorption maximum at 348 nm of **2** in methanol was shifted by 25 nm in boric acid–sodium acetate solution, while **1** gave practically the same spectra in both solutions. This indicates the presence of an *ortho*-diphenol system in the molecule of **2**, one hydroxyl group of which is linked with glucuronic acid to form **1**. The addition of sodium methoxide caused a bathochromic shift of 61 nm from 336 nm of **6** accompanied with an increase in intensity, characteristic of 4′-hydroxyflavones. The absorptions at 336 nm in **1** and 348 nm in **2** were shifted on the addition of aluminium chloride–hydrochloric acid by 19 and 32 nm, respectively, indicating that the free hydroxyl group was at C-5, not C-3. The fact that **2** was not decomposed in methanol on the addition of sodium



1 $R^1 = R^2 = H$, R^3 = Glur
2 $R^1 = R^2 = R^3 = H$
3 $R^1 = R^2 = R^3$ = Ac
4 (10) $R^1 = R^2 = R^3$ = Me
5 (11) $R^1 = R^2$ = Me, R^3 = Glur Me ester
6 $R^1 = R^2$ = Me, $R^3 = H$
7 R^1 = Me, $R^2 = H$, R^3 = Glur
8 R^1 = Me, $R^2 = R^3 = H$
9 R^1 = Me, $R^2 = R^3$ = Ac
12 $R^1 = R^2 = H$, R^3 = Glur Me ester



R
13 Glur
14 H

methoxide and showed an intense absorption at 402 nm due to the shift of the maximum from 348 nm supported the above findings.

In the ^{13}C NMR spectra, two aromatic quaternary carbon signals of **1** (140.3 and 138.2 ppm) and **2** (140.4 and 137.5 ppm) were respectively ascribed to C-2 and C-3 of 3-oxygenated flavones [9, 11–13]. The spectrum of **1** showed the signals due to a glucuronic acid moiety [14], and those assignable to C-3, C-7, C-1′ and C-3′ were found, respectively, at lower field by +0.7, +0.6, +3.5 and +1.4 ppm than the corresponding signals of **2**, while C-9 and C-4′ appeared at higher field (−0.7 and −1.5 ppm, respectively). This is in good agreement with the 4′-*O*-glycosylation effect reported by Markham *et al.* [13].

Only the D-isomer of glucuronic acid has been reported in nature and, as mentioned before, the β-anomeric centre is evident from ^1H NMR. Thus, compound **1** is 5,3′,4′-trihydroxy-3-methoxy-6:7-methylenedioxyflavone 4′-β-D-glucuronide.

Crystallization of fraction 12 from methanol gave compound **7**, which analysed for $C_{24}H_{22}O_{14} \cdot 2H_2O$ and was also regarded as a flavonoid hexuronide on the basis of its colour reactions, UV and IR spectra and FABMS (*m/z* 535 $[M+1]^+$, 359 $[M+1-176]^+$). It was hydrolysed to give glucuronic acid (by TLC) and an aglycone (**8**), which gave an acetate (**9**) and a methyl ether (**10**). Methylation of **7** with diazomethane provided a methylated glycoside (**11**). **10** and **11** were respectively identical to the derivatives of compounds **1**, **4** and **5**, indicating that **7** is a monomethyl ether of **1**. The ^1H NMR spectra of **7** and **8** were almost superimposable with those of **1** and **2**, respectively, except for an additional signal due to one methoxyl group. The ^{13}C NMR spectrum of **7** exhibited signals at 59.7 and 55.7 ppm assignable to methoxyl carbons. On comparison with the spectrum of **1**, a noticeable shift was observed in the signals of the B-ring carbons (C-1′, −0.6; C-2′, −3.5; C-3′, +1.8; C-4′, +1.4; C-5′, −1.0; C-6′, +1.4 ppm). Considering the O-methylation effect of *ortho*-substituted phenols [12], this is well accounted for by methylation of the hydroxyl group at C-3′ of **1**. The UV spectra of **7** and **8** in solutions of aluminium chloride–hydrochloric acid and boric acid–sodium acetate indicated the presence of a free hydroxyl group at C-5 and the absence of an *ortho*-diphenol system in both molecules [10]. Therefore, compound **7** is considered to be the 3′-methyl ether of **1**.

Fraction 13 crystallized from methanol to give compound **12** in 0.002% yield. It was identified as a methyl ester of **1** on the basis of spectral data. **12** is thought to be an artefact formed during the extraction and isolation procedures.

On treatment with aqueous methanol, fraction 9 yielded compound **13**. Its FABMS (*m/z* 537 $[M+1]^+$, 361 $[M+1-176]^+$) and IR spectrum (1750, 1650 cm^{-1}) suggested the presence of a hexuronic acid residue. Acid hydrolysis of **13** afforded glucuronic acid (by TLC) and an aglycone (**14**), $C_{18}H_{16}O_8 \cdot H_2O$, mp 92–93°. The ^1H NMR spectra of **13** and **14** showed signals ascribable to three methoxyl groups but nothing due to a methylenedioxy group. The aromatic and aliphatic signals of both compounds closely resembled those of glycosides **1** and **7**, and their aglycones **2** and **8**, respectively, suggesting that **13** is a 3,5,6,7,3′,4′-hexa-oxygenated flavone β-glucuronide. The other two probable structures, 3,5,7,8,3′,4′- and 5,6,7,8,3′,4′-, were excluded by a positive Gibbs reaction [15]. The UV spectral data demonstrated the presence of a free hydroxyl group at C-5 and the absence of an *ortho*-diphenol system in both **13** and **14**. A free 4′-hydroxyl group was detected in **14** but not in **13**. Comparison of the ^{13}C NMR spectrum of **13** with that of **7** showed that both compounds have the same B-ring structure [11]. Three methoxy carbon signals (55.8, 59.9 and 60.1 ppm) were accounted for by the location of methoxyl groups at C-3′, C-3 and C-6 [9, 11]. Finally, the aglycone, **14**, was identified as jaceidin [16] (5,7,4′-trihydroxy-3,6,3′-trimethoxyflavone) by comparison with an authentic specimen. Thus, compound **13** is jaceidin 4′-β-D-glucuronide.

EXPERIMENTAL

Extraction and fractionation. S. oleracea cv Atoras, cultivated in Co Kumamoto, was used. The fresh leaves (3 kg) were homogenated with 6 l. MeOH After standing overnight, the homogenate was centrifuged, and the supernatant evapd to dryness *in vacuo*. The residue was extracted with hot H_2O and the aq soln filtered through a column of Amberlite XAD-2. The column was successively washed with 1% HOAc, H_2O and MeOH. The MeOH washings were collected, evapd to dryness and subjected to DCCC (ascending method; $CHCl_3$–*n*-BuOH–MeOH–H_2O, 9:2:12:8), giving 13 fractions (fractions 1–13 in elution order).

5,3′,4′-Trihydroxy-3-methoxy-6,7-methylenedioxyflavone 4′-β-D-glucuronide (**1**). Fraction 10 (yield 0.36 g) gave only one spot (R_f 0.35) on TLC (microcrystalline cellulose, 15% HOAc), and

crystallized from MeOH to give **1** (yield 0.34 g). The compound was still contaminated with a trace amount of mineral, and was purified through the Pb-salt (yellow ppt.). It was recrystallized from 50% dioxane to give yellow needles, mp 197–198°, $[\alpha]_D^{22}$ −74.9° (50% dioxane; *c* 0 14). It gave positive colour reactions with Mg–HCl (red), Zn–HCl (red), $FeCl_3$ (dark green) and Gibbs reagent (green). (Found: C, 46 79; H, 4.60. $C_{23}H_{20}O_{14} \cdot 4H_2O$ requires: C, 46.62; H, 4.76%.) FABMS (accelerating voltage, 2 kV; solvent, DMSO–glycerol) *m/z* (rel. int.): 521 $[M+1]^+$ (100), 345 $[M+1-176]^+$ (100). IR ν_{max}^{KBr} cm^{-1}: 1740 (COOH), 1690 (C=O). ^1H NMR (60 MHz, C_5D_5N): δ3.89 (3H, *s*, MeO), 4.2–4.9 (4H, *m*, H on sugar moiety), 5.82 (1H, *d*, *J* = 8 Hz, anomeric H), 6.10 (2H, *s*, –O–CH_2–O–), 6.60 (1H, *s*, H-8), 7.65, 8.00 (3H, H-2′, 5′, 6′). UV λ_{max}^{MeOH} nm (log ε): 250 (4.21), 277 (4.29), 336 (4.41); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 246 (4.20), 274 (4.29), 337 (4.35); $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 250 (4.20), 276 (4.28), 338 (4.40); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 240 sh (4.23), 263 (4.17), 291 (4.32), 367 (4.43); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 240 sh (4.21), 265 sh (4.17), 282 (4.25), 355 (4.34); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 270 (4.35), 327 (4.19), *ca* 370 sh (4.09). ^{13}C NMR (25 MHz, DMSO-d_6)· δ140.3 (*s*, C-2), 138.2 (*s*, C-3), 178.4 (*s*, C-4), 151.7 (*s*, C-5), 129.2 (*s*, C-6), 153.9 (*s*, C-7), 89.5 (*d*, C-8), 155.2 (*s*, C-9), 107.2 (*s*, C-10), 124.0 (*s*, C-1′), 115.5 (*d*, C-2′ or C-5′), 146.5 (*s*, C-3′ or C-4′), 147.2 (*s*, C-4′ or C-3′), 115.7 (*d*, C-5′ or C-2′), 120.1 (*d*, C-6′), 102.8 (*t*, –O–CH_2–O–), 59.7 (*q*, MeO-3), 100.5 (*d*, C-1″), 73.0 (*d*, C-2″), 75.4 (*d*, C-3″), 71.3 (*d*, C-4″), 75.2 (*d*, C-5″), 169.9 (*s*, C-6″).

Acid hydrolysis of **1**. **1** (106.4 mg) was refluxed with 10% HCl in dioxane for 3 hr. The reaction mixture was diluted with H_2O, allowed to stand overnight and filtered. The filtrate was taken to dryness *in vacuo*. The residue showed two spots [R_f 0.69 (major) and 0.07 (trace)] on TLC (microcrystalline cellulose, *n*-BuOH–C_5H_5N–H_2O, 3:2:1; *p*-anisidine · HCl), identical to those of glucuronolactone and glucuronic acid, respectively. The ppt. (67.8 mg) was subjected to CC over Sephadex LH-20 (solvent EtOH) and recrystallized from 50% dioxane to give **2** as yellow needles, mp 241–243° (decomp.). (Found. C, 59.13; H, 3.53 $C_{17}H_{12}O_8$ requires: C, 59.31; H, 3.51%.) ^1H NMR (60 MHz, C_5D_5N): δ3.87 (3H, *s*, MeO), 6.07 (2H, *s*, –O–CH_2–O–), 6.58 (1H, *s*, H-8), 7.27 (1H, *d*, *J* = 8 Hz, H-5′), 7.66 (1H, *dd*, *J* = 2 and 8 Hz, H-6′), 8 11 (1H, *d*, *J* = 2 Hz, H-2′). UV λ_{max}^{MeOH} nm (log ε): 248–257 (4.16), 281 (4.16), 348 (4.37); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 243 (4.15), 262 (4.16), 270 (4.17), 353 (4.27), *ca* 405 sh (4.01); $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 245 (4.17), 264 (4.30), 373 (4.38), $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 277 (4.24), 433 (4.42); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε). 243 (4.13), 269 (4.16), 283 (4.15), 380 (4 36); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 242–246 (4.13), 274 (4.20), 402 (4.36). ^{13}C NMR (25 MHz, DMSO-d_6): δ140.4 (*s*, C-2), 137.5 (*s*, C-3), 178.3 (*s*, C-4), 151.5 (*s*, C-5), 129.0 (*s*, C-6), 153.3 (*s*, C-7), 89.3 (*d*, C-8), 155 9 (*s*, C-9), 107 0 (*s*, C-10), 120 5 (*s*, C-1′), 115.4 (*d*, C-2′ or C-5′), 145.1 (*s*, C-3′), 148.7 (*s*, C-4′), 115.6 (*d*, C-5′ or C-2′), 120.5 (*d*, C-6′), 102.7 (*t*, –O–CH_2–O–), 59.5 (*q*, MeO-3).

Acetylation of **2** with Ac_2O–C_5H_5N at room temp. provided **3** as colourless needles, mp 175–176° (from MeCN–MeOH) (Found: C, 58.43; H, 3 87 $C_{23}H_{18}O_{11}$ requires: C, 58.73; H, 3.86%.) UV λ_{max}^{MeOH} nm (log ε). 258 (4.24), 317 (4.41). ^1H NMR (60 MHz, $CDCl_3$): δ2.30, 2 44 (6H and 3H, *s*, AcO), 3.78 (3H, *s*, MeO), 6 03 (2H, *s*, –O–CH_2–O–), 6.78 (1H, *s*, H-8), 7 3–8.1 (3H, *m*, H-2′, 5′, 6′)

Trimethyl ether of **2** (**4**). **2** was methylated with CH_2N_2 and the product purified by CC over silica gel (solvent $CHCl_3$–Me_2CO, 9:1) to yield colourless needles (**4**), mp 156–157° (from MeOH). (Found: C, 61.97; H, 4.72 $C_{20}H_{18}O_8$ requires: C, 62.17; H, 4.70%)

^1H *NMR spectra of* **4** *and related compounds*. The spectra of **4**, gossypetin hexamethyl ether and lucidin dimethyl ether were measured in $CDCl_3$ with or without an almost equimolar amount of $Eu(FOD)_3$

Compound **4**. ^1H NMR (60 MHz, $CDCl_3$): δ3.82, 3.91 (3H and 6H, *s*, MeO), 4.10 (3H, *s*, MeO-5), 6.00 (2H, *s*, –O–CH_2–O–), 6.60 (1H, *s*, H-8), 6.90 (1H, *d*, *J* = 9 Hz, H-5′), 7.5–7.8 (2H, *m*, H-2′, 6′). ^1H NMR (60 MHz, $CDCl_3$–$Eu(FOD)_3$): δ3.97, 4.15 (6H and 3H, *s*, MeO), 6.28 (2H, *s*, –O–CH_2–O–), 6.88 (1H, *s*, H-8), *ca* 6.9 (*br s* MeO-5), 7.00 (1H, *d*, *J* = 9 Hz, H-5′), 7.7–8.0 (2H, *m*, H-2′, 6′).

Gossypetin hexamethyl ether, mp 169–170° (lit. [17] mp 170–172°), was prepared by methylation of gossypetin isolated [18] from *Equisetum arvense*. ^1H NMR (60 MHz, $CDCl_3$): δ3 89, 3.93, 3.95 (3H, 3H and 6H, *s*, MeO), 3.97 (6H, *s*, MeO-5 and another one), 6.41 (1H, *s*, H-6), 6 96 (1H, *d*, *J* = 9 Hz, H-5′), 7.6–8.0 (2H, *m*, H-2′, 6′). ^1H NMR (60 MHz, $CDCl_3$–$Eu(FOD)_3$): δ3.91, 3 92, 3.94, 4.02, 4.28 (each 3H, *s*, MeO), 7.31 (3H, *s*, MeO-5), 7.73 (1H, *s*, H-6), 6.98 (1H, *d*, *J* = 9 Hz, H-5′), 7 8–8.0 (2H, *m*, H-2′, 6′).

Lucidin dimethyl ether, mp 167–168° (lit. [19] mp 171–172°), was prepared from authentic lucidin ^1H NMR (60 MHz, $CDCl_3$): δ3.91, 4.00 (6H and 3H, *s*, MeO), 4.08 (3H, *s*, MeO-5), 6.03 (2H, *s*, –O–CH_2–O–), 6.51 (1H, *s*, H-3), 6.87 (1H, *d*, *J* = 8 Hz, H-5′), 7.2–7.6 (2H, *m*, H-2′, 6′). ^1H NMR (60 MHz, $CDCl_3$–$Eu(FOD)_3$): δ4.63, 6.23, 10.96 (each 3H, *s*, MeO), 18.34 (3H, *s*, MeO-5), 5.87 (2H, *s*, –O–CH_2–O–), 4.86 (1H, *s*, H-3), 6.63 (1H, *d*, *J* = 9 Hz, H-5′), 6.8–7.0 (2H, *m*, H-2′, 6′).

Methylation of **1** *to* **5**. A soln of **1** (200 mg) in 50% dioxane (20 ml) and MeOH (100 ml) was treated with an excess amount of CH_2N_2. Purification of the product by CC over silica gel (solvent: $CHCl_3$–EtOH, 9:1) followed by recrystallization from MeOH provided **5** as colourless needles, mp 221–222°. (Found: C, 53 96; H, 4.79. $C_{26}H_{26}O_{14} \cdot H_2O$ requires: C, 53.79, H, 4.86%). UV λ_{max}^{MeOH} nm (log ε): 245 (4.20), 270 (4.17), 328 (4.40). ^1H NMR (60 MHz, C_5D_5N)· δ3 64 (3H, *s*, ester Me), 3.80, 3.95, 4.12 (each 3H, *s*, MeO), 4.2–5.0 (4H, *m*, H on sugar moiety), 5.87 (1H, *d*, *J* = 8 Hz, anomeric H), 6.06 (2H, *s*, –O–CH_2–O–), 6.80 (1H, *s*, H-8), 7 4–8.0 (3H, *m*, H-2′, 5′, 6′). ^{13}C NMR (25 MHz, DMSO-d_6)· δ140.0 (*s*, C-2), 139.6 (*s*, C-3), 172.6 (*s*, C-4), 151.7 (*s*, C-5 or C-7 or C-9), 134 7 (*s*, C-6), 152 7 (*s*, C-7 or C-5 or C-9), 93.2 (*d*, C-8), 152.9 (*s*, C-9 or C-5 or C-7), 112.1 (*s*, C-10), 123.9 (*s*, C-1′), 111.6 (*d*, C-2′), 148.5 (*s*, C-3′ or C-4′), 147 5 (*s*, C-4′ or C-3′), 114.6 (*d*, C-5′), 121 0 (*d*, C-6′), 102.5 (*t*, –O–CH_2–O–), 60.5 (*q*, MeO-5), 59.3 (*q*, MeO-3), 55.7 (*q*, MeO-3′), 52.1 (*q*, ester Me), 99.1 (*d*, C-1″), 72.7 (*d*, C-2″), 75.5 (*d*, C-3″), 71.3 (*d*, C-4″), 75.1 (*d*, C-5″), 169.2 (*s*, C-6″).

Acid hydrolysis of **5** *to* **6**. A solution of **5** in 1 N HCl–MeOH was refluxed for 2 hr The reaction mixture was diluted with H_2O and the ppt. run on prep TLC (Kieselgel 60 PF_{254}; $CHCl_3$–Me_2CO, 4:1), and recrystallized from 50% dioxane to colourless needles (**6**), mp 238–240° (Found: C, 61.11; H, 4 31. $C_{19}H_{16}O_8$ requires: C, 61.29; H, 4.33%.) UV λ_{max}^{MeOH} nm (log ε): 245 (4 28), 270 sh (4 17), 336 (4.46), unchanged in solns of H_3BO_3–NaOAc and $AlCl_3$; $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 245 (4.28), 270 sh (4 17), 336 (4.39), 400 sh (3.81); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 245 (4.28), 255–260 (4.24), 335 sh (4.03), 397 (4.49). ^1H NMR (60 MHz, C_5D_5N): δ3.88, 4.02, 4.14 (each 3H, *s*, MeO), 6.06 (2H, *s*, –O–CH_2–O–), 6 84 (1H, *s*, H-8), 7.28 (1H, *d*, *J* = 9 Hz, H-5′), 7 7–8.0 (2H, *m*, H-2′, 6′).

5,4′-Dihydroxy-3,3′-dimethoxy-6:7-methylenedioxyflavone 4′-β-D-glucuronide (**7**). Fraction 12 (yield 0.21 g, R_f 0 44) crystallized from MeOH to give **7** (yield 0 10 g) which was purified through Pb-salt (yellow ppt.). Yellow needles (from 50% dioxane), mp 165–168° with the foregoing softening at *ca* 140–145°, $[\alpha]_D^{22}$ −74.5° (50% dioxane; *c* 0.10). It gave colour reactions with Mg–HCl (red), Zn–HCl (red), $FeCl_3$ (dark green) and Gibbs reagent (olive green with red tint). (Found: C, 50.50; H, 4.47. $C_{24}H_{22}O_{14} \cdot 2H_2O$ requires: C, 50.52; H, 4.59 %) FABMS (accelerating voltage, 2 kV, solvent, DMSO–glycerol) *m/z* (rel. int.): 535 $[M+1]^+$ (38), 359 $[M+1-176]^+$ (100). IR ν_{max}^{KBr} cm^{-1} 1740 (COOH), 1690 (C=O). ^1H NMR (60 MHz,

C_5D_5N): δ3.82, 3.93 (each 3H, *s*, MeO), 4.2–4.9 (4H, *m*, H on sugar moiety), 5.92 (1H, *d*, *J* = 7 Hz, anomeric H), 6.10 (2H, *s*, –O–CH_2–O–), 6.70 (1H, *s*, H-8), 7.5–7.9 (3H, *m*, H-2′, 5′, 6′). ^{13}C NMR (25 MHz, DMSO-d_6): δ140.2 (*s*, C-2), 138.1 (*s*, C-3), 178.4 (*s*, C-4), 151.6 (*s*, C-5), 129.1 (*s*, C-6), 153.9 (*s*, C-7), 89.6 (*d*, C-8), 155.0 (*s*, C-9), 107 1 (*s*, C-10), 123.4 (*s*, C-1′), 112.0 (*d*, C-2′), 148.3 (*s*, C-3′ or C-4′), 148.6 (*s*, C-4′ or C-3′), 114.7 (*d*, C-5′), 121.5 (*d*, C-6′), 102.8 (*t*, –O–CH_2–O–), 59.7 (*q*, MeO-3), 55.7 (*q*, MeO-3′), 99.0 (*d*, C-1″), 72.7 (*d*, C-2″), 75.9 (*d*, C-3″), 71.2 (*d*, C-4″), 75.4 (*d*, C-5″), 169.9 (*s*, C-6″). UV λ_{max}^{MeOH} nm (log ε): 246 (4.14), 278 (4.22), 338 (4.39), unchanged in solutions of NaOAc and H_3BO_3–NaOAc; $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 262 (4.06), 292 (4.23), 367 (4.38), unchanged on addition of HCl; $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 283 (4.22), 337 (4.33).

Acid hydrolysis of **7**. **7** (101.4 mg) was treated with 10% HCl in dioxane as in the hydrolysis of **1**. The sugar fraction showed two spots on TLC due to glucuronic acid (minor) and its lactone (major). The ppt. (65 3 mg) was subjected to CC over Sephadex LH-20 (solvent EtOH) and recrystallized from 50% dioxane to give **8** as yellow needles, mp 198–200°. (Found: C, 60.08; H, 3.94. $C_{18}H_{14}O_8$ requires: C, 60.34; H, 3.94%.) UV λ_{max}^{MeOH} nm (log ε): 240 (4 22), 255 (4.19), 276 (4.19), 347 (4.44), unchanged in solution of H_3BO_3–NaOAc; $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 244 (4.17), 255–260 (4.16), 274 (4.17), 350 (4.30), 406 (4.07); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 244 (4.18), 266 (4.17), 292 (4.21), 381 (4.48), unchanged on addition of HCl; $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 249 (4.20), 269 (4.21), 398 (4.43). ^{1}H NMR (60 MHz, C_5D_5N): δ3.83, 3.95 (each 3H, *s*, MeO), 6.05 (2H, *s*, –O–CH_2–O–), 6.69 (1H, *s*, H-8), 7.23 (1H, *d*, *J* = 9 Hz, H-5′), 7.6–8.0 (2H, *m*, H-2′, 6′). Acetylation of **8** gave an acetate (**9**) as colourless needles (from $CHCl_3$–MeOH), mp 200–203°. ^{1}H NMR (60 MHz, $CDCl_3$): δ2.31, 2.43 (each 3H, *s*, AcO), 3.80, 3.89 (each 3H, *s*, MeO), 6.10 (2H, *s*, –O–CH_2–O–), 6.81 (1H, *s*, H-8), 7.0–7.8 (3H, *m*, H-2′, 5′, 6′). Methylation of **8** with CH_2N_2 gave **10** as colourless needles (from MeOH), mp 158–159°, no mp depression on admixture with **4**. IR, UV and ^{1}H NMR were superimposable with those of **4**.

Methylation of **7** *to* **11** (**5**). Methylation of **7** gave **11** as colourless needless (from MeOH), mp 222–224°, undepressed on admixture with **5** IR, UV and ^{1}H NMR were identical to those of **5**.

5,3′,4′-Trihydroxy-3-methoxy-6:7-methylenedioxyflavone 4′-β-D-glucuronide methyl ester (**12**) On treatment of fraction 13 [yield 0.23 g, R_f 0 33 (**12**) and 0.36] with MeOH, only **12** crystallized. It was recrystallized from 50% dioxane as pale yellow needles, mp 233–235° (Found: C, 49.63; H, 4.61. $C_{24}H_{22}O_{14}$ 2.5 H_2O requires: C, 49.74; H, 4.70%.) FABMS (accelerating voltage, 3 kV; solvent, DMSO–glycerol) *m/z* (rel. int.): 535 $[M+1]^+$ (23), 345 $[M+1-190]^+$ (25). UV spectra in MeOH with or without reagents were similar to those of **1**. ^{1}H NMR (60 MHz, C_5D_5N) δ3.71 (3H, *s*, ester Me), 3.88 (3H, *s*, MeO), 4 2–5.0 (4H, *m*, H on sugar moiety), 5.84 (1H, *d*, *J* = 8 Hz, anomeric H), 6.11 (2H, *s*, –O–CH_2–O–), 6.63 (1H, *s*, H-8), 7.67, 8.02 (3H, H-2′, 5′, 6′). ^{13}C NMR (25 MHz, DMSO-d_6): δ140.1 (*s*, C-2), 138.0 (*s*, C-3), 178.2 (*s*, C-4), 151.4 (*s*, C-5), 129.0 (*s*, C-6), 153 8 (*s*, C-7), 89 2 (*d*, C-8), 154.9 (*s*, C-9), 106.9 (*s*, C-10), 124.0 (*s*, C-1′), 115.7 (*d*, C-2′ or C-5′), 146.2 (*s*, C-3′ or C-4′), 147.0 (*s*, C-4′ or C-3′), 115.1 (*d*, C-5′ or C-2′), 120.2 (*d*, C-6′), 102.8 (*t*, –O–CH_2–O–), 59 7 (*q*, MeO-3), 52.3 (*q*, ester Me), 100.3 (*d*, C-1″), 72.8 (*d*, C-2″), 75 2 (*d*, C-3″), 71 4 (*d*, C-4″), 75.2 (*d*, C-5″), 169.3 (*s*, C-6″).

5,7,4′-Trihydroxy-3,6,3′-trimethoxyflavone 4′-β-D-glucuronide (**13**). Fraction 9 [yield 0.8 g from 9 kg leaves, R_f 0.56 (**13**) and 0.99] crystallized from MeOH–H_2O to **13** (yield 40 mg). It was purified through Pb-salt and recrystallized from H_2O Yellow needles, mp 136–137°, $[\alpha]_D^{20}$ −68.9° (MeOH; *c* 0.08), it gave colour reactions with Mg–HCl (red), Zn–HCl (red), $FeCl_3$ (dark green) and Gibbs reagent (olive green with red tint). (Found: C, 50.43; H, 4.72. $C_{24}H_{24}O_{14} \cdot 2H_2O$ requires: C, 50.35; H, 4.93%.) FABMS (accelerating voltage, 3 kV; solvent, DMSO–glycerol) *m/z* (rel. int.): 537 $[M+1]^+$ (11), 361 $[M+1-176]^+$ (50). IR ν_{max}^{KBr} cm^{-1}. 1750 (COOH), 1655 (C=O). UV λ_{max}^{MeOH} nm (log ε): 250 (4.18), 271 (4.23), 337 (4.29), unchanged on addition of H_3BO_3–NaOAc; $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 273 (4.38), 308 (4.19), 370 (4.19); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 260–265 (4.30), 280 (4.32), 358 (4.36); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 261–264 (4.29), 274 (4.30), 354 (4.34); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 273 (4.39), 300–310 (4.05), 374 (4.21). ^{1}H NMR (60 MHz, C_5D_5N): δ3.82, 3.97 (3H and 6H, *s*, MeO), 4.2–5.0 (4H, *m*, H on sugar moiety), 5.87 (1H, *d*, *J* = 9 Hz, anomeric H), 6.85 (1H, *s*, H-8), 7.67, 7.79 (3H, H-2′, 5′, 6′). ^{13}C NMR (25 MHz, DMSO-d_6): δ151.6 (*s*, C-2 or C-5), 137.8 (*s*, C-3), 178.2 (*s*, C-4), 152.1 (*s*, C-5 or C-2), 131.1 (*s*, C-6), 155.0 (*s*, C-7), 94.2 (*d*, C-8), 157.3 (*s*, C-9), 104.7 (*s*, C-10), 123.9 (*s*, C-1′), 112.0 (*d*, C-2′), 148.0 (*s*, C-3′ or C-4′), 148.5 (*s*, C-4′ or C-3′), 114.7 (*d*, C-5′), 121.6 (*d*, C-6′), 60.1 (*q*, MeO-6), 59.9 (*q*, MeO-3), 55.8 (*q*, MeO-3′), 99.2 (*d*, C-1″), 72.8 (*d*, C-2″), 75.7, (*d*, C-3″), 71.3 (*d*, C-4″), 75.3 (*d*, C-5″), 170.2 (*s*, C-6″).

Acid hydrolysis of **13**. **13** was refluxed with 10% HCl–MeOH for 3 hr. The reaction mixture was treated in the same manner as the hydrolyses of **1** and **7**. Glucuronic acid (minor) and its lactone (major) were detected on TLC The aglycone was recrystallized from MeOH–H_2O to give jaceidin (**14**) as yellow needles, mp 92–93° (lit. [16] mp 127–133°). (Found: C, 57.60; H, 4.98. Calc. for $C_{18}H_{16}O_8 \cdot H_2O$: C, 57.14, H, 4.80%.) The IR spectrum of **14** was identical to that of authentic jaceidin.

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