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FLAVONOL GLYCOSIDES IN LEAVES OF *SPINACIA OLERACEA**

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Key Word Index—*Spinacia oleracea*; Chenopodiaceae; spinach; 6-methoxy-3,5,7,3',4'-pentahydroxyflavone 3-*O*-β-D-glucosyl-(1 → 6)-[β-D-apiosyl-(1 → 2)]-β-D-glucoside; 6-methoxy-3,5,7,3',4'-pentahydroxyflavone 3-*O*-β-gentiobioside; 6,3'-dimethoxy-3,5,7,4'-tetrahydroxyflavone 3-*O*-β-gentiobioside; spinatoside.

Abstract—Besides spinatoside (3,6-dimethoxy-5,7,3',4'-tetrahydroxyflavone 4'-*O*-β-D-glucopyranuronide), three new flavonol glycosides have now been isolated from the polar fractions of the methanolic extract of *Spinacia oleracea*. They have been identified as patuletin 3-*O*-β-D-glucopyranosyl-(1 → 6)-[β-D-apiofuranosyl-(1 → 2)]-β-D-glucopyranoside, patuletin 3-*O*-β-gentiobioside and spinacetin 3-*O*-β-gentiobioside, respectively.

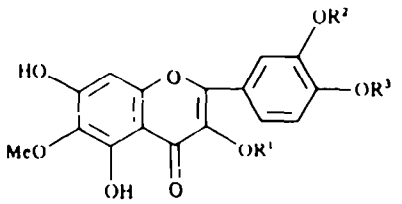
INTRODUCTION

In the previous paper [1] of this series, we reported the presence of three 3,5,6,7,3',4'-hexa-oxygenated flavon glucuronides in spinach leaves. This paper deals with the isolation and identification of four additional flavonol glycosides from the same source.

RESULTS AND DISCUSSION

As described in the previous paper [1], thirteen fractions were obtained by DCCC of the methanolic extract of spinach leaves. From the most polar fraction compound **1** was yielded as yellow powder (yield 0.004% of the fresh leaves). Column chromatographic purification of the fractions 3, 4 and 8 respectively provided compounds **2** (0.003%), **3** (0.002%) and **4** (0.006%) in crystalline state. The compounds were all positive to flavonoid colour reactions and the IR and UV spectra suggested they were flavonoid glycosides.

FABMS of **1** showed peaks at *m/z* 811, 789, 657, 495 and 333, which were respectively ascribed to $[M+23]^+$, $[M+1]^+$, $[M+1-132]^+$, $[M+1-132-162]^+$ and $[M+1-132-2\times162]^+$ ions, suggesting the existence of one pentose and two hexose moieties in the molecule of **1**. Acid hydrolysis of **1** afforded an aglycone (**5**), which was methylated to 3,5,6,7,3',4'-hexamethoxyflavone and identified as petuletin [2] on the basis of its UV [3–5], ^{1}H NMR [3, 4] and ^{13}C NMR [6–8] spectra. As sugar component glucose and apiose were detected by TLC and GC from the hydrolysate. **1** was partially hydrolysed with the crude hesperidinase and gave glucose and a new glycoside (**6**), whose FABMS exhibited the peaks at *m/z* 627 $[M+1]^+$, 495 $[M+1-132]^+$ and 333 $[M+1-132-162]^+$). UV spectra of **1**, **5** and **6** were diagnostically shifted on addition of shift reagents and indicated the absence of a free hydroxyl group at C-3 from **1** and **6** [3–5]. Methylation of **1** and subsequent hydrolysis yielded 3-hydroxy-5,6,7,3',4'-pentamethoxyflavone [9]. Thus **1** is a patuletin 3-triglycoside, where the trisaccharide contains one apiose and two glucose moieties, while **6** is the corresponding 3-diglycoside containing apiose and glucose. This was verified from the ^{13}C NMR spectra of **1**, **5** and **6** (Table 1) [6–8]. The signals due to the flavone nucleus in the spectra of **1** and **6** were superimposable each other, while on comparison with those of **5** the signal assignable to C-3 was shifted upfield by *ca* −3 ppm accompanying the downfield shift of C-2 (*ca* +9 ppm) and C-4 (+1.5 ppm), characteristic of 3-glycosylation of 3-hydroxyflavones. The disaccharide residue in **6** was



1 R^1 = β-D-Glcpyr (6→1 β-D-Glcpyr; 2→1 β-D-Apifur), $R^2 = R^3 = H$

2 R^1 = β-D-Glcpyr (6→1 β-D-Glcpyr), $R^2 = R^3 = H$

3 R^1 = β-D-Glcpyr (6→1 β-D-Glcpyr), R^2 = Me, R^3 = H

4 R^1 = Me, R^2 = H, R^3 = β-D-GlcpyrU

5 $R^1 = R^2 = R^3 = H$

6 R^1 = β-D-Glcpyr (2→1 β-D-Apifur), $R^2 = R^3 = H$

7 $R^1 = R^3 = H$, R^2 = Me

8 R^1 = Me, $R^2 = R^3 = H$

*Part 5 in the series "Chemical Studies on the Edible Plants". For Part 4 see *Phytochemistry* (1984) **24**, 2438.

Table 1. ^{13}C NMR spectra of flavonoids 1–8*

C	1	6	5	2	3	7	4	8
2	156.1	155.8	146.9	156.3	156.3	147.3	151.5[a]	151.5[a]
3	132.6	132.7	135.4	132.9	132.6	135.4	137.9	137.3
4	177.4	177.5	175.9	177.5	177.5	176.0	178.2	178.1
5	152.1[a]	152.3[a]	151.7[a]	152.3[a]	152.3[a]	151.6[a]	152.3[a]	152.3[a]
6	131.2	131.1	130.7	131.1	131.2	130.8	131.1	131.1
7	151.6[a]	151.3[a]	151.3[a]	151.5[a]	151.5[a]	151.3[a]	154.9[a]	155.6[a]
8	93.7	93.6	93.6	93.7	93.9	93.8	93.9	93.8
9	157.6[a]	157.2[a]	157.1[a]	157.2[a]	157.2[a]	157.1[a]	157.4[a]	157.2[a]
10	104.3	104.3	103.3	104.3	104.4	103.4	104.7	104.5
1'	122.0[b]	121.9[b]	121.9[b]	121.6[b]	121.0[b]	122.0[b]	124.2	120.8[b]
2'	116.0[c]	115.8[c]	115.5[c]	116.2[c]	113.3	111.8	115.7[b]	115.7[c]
3'	144.7	144.8	145.0	144.6	146.8	145.7	146.6[c]	145.2
4'	148.3	148.3	147.6	148.3	149.3	148.8	147.2[c]	148.6
5'	115.0[c]	115.1[c]	115.0[c]	115.1[c]	115.2	115.5	115.6[b]	115.4[c]
6'	121.2[b]	121.2[b]	120.0[b]	121.1[b]	122.0[b]	121.7[b]	120.1	120.6[b]
OMe	60.0	59.9	59.9	59.9	59.9	60.0	59.9	59.9
					55.8	55.7	59.8	59.7
Hexose attached to aglycone								
1	98.6†	98.4		100.8	100.9		100.6	
2	77.2[d]	77.0[d]		73.3[d]	73.3[c]		72.9	
3	76.2[d]	76.1[d]		76.4[e]	76.3[d]		75.4[d]	
4	69.6	70.1		69.6[f]	69.7		71.3	
5	76.2[d]	77.4[d]		76.3[e]	76.2[d]		75.3[d]	
6	67.8	60.3		68.0	67.7		169.9	
Terminal glucose								
1	102.8‡			103.0	103.0			
2	73.2[e]			73.9[d]	74.1[c]			
3	76.2[d]			76.3[e]	76.4[d]			
4	69.6			69.7[f]	69.7			
5	76.2[d]			76.4[e]	76.6[d]			
6	60.6			60.6	60.7			
Apiose								
1	108.6§	108.5						
2	76.2[d]	76.4[d]						
3	79.2	79.1						
4	73.8[e]	73.8						
5	64.1	64.2						

*Measured at 68 MHz (except 1, which was determined at 25 MHz) in DMSO-d_6 with TMS as internal reference. The values with the same superscript may be interchangeable in the vertical column.

†$J_{C'\text{-}H'}$ = 164 Hz.

‡$^1J_{C'\text{-}H'}$ = 161 Hz.

§$^1J_{C'\text{-}H'}$ = 171 Hz.

assumed to be β-apiofuranosyl-(1 → 2)-β-glucopyranosyl because of the good agreement of its carbon signals with those of apiin (5,7,4'-trihydroxyflavone 7-*O*-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside [10]) reported in the literature [6, 8]. The coupling constants (J = 8 Hz and singlet) of the anomeric proton signals in the ^1H NMR spectrum of **6** also supported the β-configuration of the glucose and apiose [10] moieties. On passing from **6** to **1** the carbon signals due to the apiose moiety remained unchanged while the one ascribable to C-6 of the glucose was replaced from 60.3 ppm to 67.8 ppm, showing the 1 → 6 linkage between two glucose moieties of **1** [6, 8]. The anomeric configuration of the terminal glucose residue could not be established from the ^1H NMR spectrum of **1** (100 MHz in DMSO-d_6 and in pyridine-d_5) because of the overlap of the signals due to anomeric and methine protons of the sugar moiety. In the ^{13}C NMR spectrum of **1** (Table 1) the anomeric carbon signal of the terminal glucose was at 102.8 ppm and had a coupling constant, $^1J_{C'\text{-}H'}$ = 160 Hz, the values agreeing well with those of β-D-glucopyranosides [11]. Thus **1** is patuletin 3-*O*-β-D-glucopyranosyl-(1→6)-[β-D-apiofuranosyl-(1 → 2)]-β-D-glucopyranoside.

Compound **2** was formulated as $C_{28}H_{32}O_{18}\cdot H_2O$ from elemental analysis and FDMS [12]. An assumption that **2** is a glycoside of monomethoxypentahydroxyflavone having two hexose moieties was due to the fragment ions in FDMS ($[M+1-162]^+$ and $[M-2\times162]^+$) and to the signals ascribable to one methoxyl, four aromatic and fourteen sugar protons in the ^1H NMR spectrum [δ 3.95 (*s*); 6.73 (*s*), 7.30 (*d*), 8.12 (*dd*) and 8.40 (*d*); 4.06 (*d*), 5.46 (*d*) and 2.5–3.9 (*m*)]. Compound **2** was hydrolysed to patuletin and glucose. On the basis of UV and ^{13}C NMR spectra, the glycosidic linkage was

positioned at C-3. The sugar carbon signals were in good agreement with the reported values for glucopyranosides except for the one at 68.0 ppm, which can be ascribed to C-6 of one glucose moiety where the terminal glucose is linked [6, 8]. Two anomeric protons were detected as two doublets (J = 7 Hz) at δ4.06 and 5.46 in the ^{1}H NMR spectrum of **2**. Compound **2** is thus petuletin 3-*O*-β-gentiobioside.

Compound **3**, $C_{29}H_{34}O_{18}\cdot H_2O$, was assumed to be monomethyl ether of **2** from the analytical values, FDMS, ^{1}H NMR and ^{13}C NMR spectra. The sugar carbon signals in the ^{13}C NMR spectrum were hardly distinguishable from those of **2**, indicating that it contained the same disaccharide. Compound **3** was hydrolysed to glucose and an aglycone (**7**), which showed two methoxyl proton signals and was methylated to 3,5,6,7,3',4'-hexamethoxyflavone. UV and ^{13}C NMR spectra proved the location of two methoxyl groups in **3** and **7** at C-6 and C-3' and of the glycosidic linkage of **3** at C-3. Compound **3** was thus identified as spinacetin 3-*O*-β-gentiobioside.

Compound **4** exhibited two carbonyl absorptions at 1730 and 1640 cm^{-1} in its IR spectrum and the FABMS revealed the peaks assignable to $[M+1]^+$ and $[M+1-176]^+$ ions, indicating the presence of hexuronic acid residue. Acid hydrolysis of **4** provided 3,6-dimethoxy-5,7,3',4'-tetrahydroxyflavone [13] and glucuronic acid. Thus **4** is 3,6-dimethoxy-5,7,3',4'-tetrahydroxyflavone 4'-β-D-glucopyranuronide (spinatioside) [13].

In combination with the results of the previous paper [1], it is clear that all the flavonols reported so far in spinach leaves, i.e. patuletin [2], spinacetin [2] and 3-methoxy-5,3',4'-trihydroxy-6,7-methylenedioxyflavone* [14], occur in glycosidic form.

EXPERIMENTAL

Compound **1**. The MeOH extract of the fresh leaves of *S. oleracea* cv Atoras was treated as described in the previous paper [1]. The fastest elution of DCCC revealed a spot at R_f 0.14 [silica gel, $CHCl_3$–MeOH (7:3) satd with H_2O]. It was subjected to CC over polyamide with gradient concentration of MeOH ($H_2O \rightarrow$ MeOH). The fractions showing one spot of **1** were combined and again subjected to CC of Diaion HP20AG (a high porous copolymer of styrene and divinylbenzene) ($H_2O \rightarrow$ 50% MeOH). The fractions of **1** were collected and evaporated to dryness in vacuo to give **1** as yellow powder, $[\alpha]_D^{25}$ −77.3° (MeOH; *c* 0.14). It gave positive colour reactions with $FeCl_3$, Mg–HCl and Zn–HCl. ν_{max}^{KBr} cm^{-1}: 1650, 1610, 1100–1000. FABMS *m/z* (rel. int.): 811 $[M+23]^+$ (0.9), 789 $[M+1]^+$ (1), 657 $[M+1-132]^+$ (0.4), 495 $[M+1-132-162]^+$ (0.4), 333 $[M+1-132-2\times162]^+$ (23). UV λ_{max}^{MeOH} nm (log ε): 256 (4.25), *ca* 270 sh (4.19), 290–298 (3.92), 351 (4.28). $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 270 (4.33), *ca* 330 sh (4.03), 380 (4.23). $\lambda_{max}^{MeOH\ H_3BO_3-NaOAc}$ nm (log ε): 261 (4.34), 374 (4.28). $\lambda_{max}^{MeOH\ NaOMe}$ nm (log ε): 269 (4.32), 333 (3.92), 400 (4.36). $\lambda_{max}^{MeOH\ AlCl_3}$ nm (log ε): 268 (4.37), *ca* 310 sh (3.86), 340–358 (3.80), 438 (4.38). $\lambda_{max}^{MeOH\ AlCl_3-HCl}$ nm (log ε): 270 (4.26), *ca* 300 sh (3.93), 385 (4.23), *ca* 410 inflec [5] (4.20). ^{1}H NMR (100 MHz, DMSO-d_6): δ3.79 (OMe), 2.6–5.0 (*m*), 5.37 (*s*, H-1 of apiose), 5.57 (*d*, J = 7 Hz, H-1 of internal glucose), 6.56 (*s*, H-8), 6.91 (*d*, J = 8 Hz, H-5'), 7.6–7.7 (*m*, H-2', 6'). ^{1}H NMR (100 MHz, pyridine-d_5): δ3.93 (OMe), 3.6–5.0 (*m*), 6.21 (*d*, J = 8 Hz, H-1 of internal glucose), 6.51 (*s*, H-1 of apiose), 6.71 (*s*, H-8), 7.36 (*d*, J = 9 Hz, H-5'), 8.26 (*dd*, J = 2, 9 Hz, H-6'), 8.38 (*d*, J = 2 Hz, H-2').

Acid hydrolysis of **1**. A soln of **1** in 5% HCl was heated on a water bath for 1 hr. After a night the ppt that separated were collected and recrystallized from MeOH–H_2O to give **5**, mp 244–247°. UV λ_{max}^{MeOH} nm (log ε): 256 (4.28), *ca* 270 sh (4.11), 293 (3.81), 370 (4.32). $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 261 (4.23), *ca* 270 sh (4.22), 322 (4.02), 388 (4.29). $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 261 (4.34), 386 (4.37). $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): *ca* 240 sh (4.12), 334 (4.34). $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 274 (4.33), *ca* 340 sh (3.63), 454 (4.48). $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 268 (4.33), *ca* 305 (3.66), *ca* 370 sh (3.98), 430 (4.39). ^{1}H NMR (60 MHz, pyridine-d_5): δ3.96 (3H, *s*, OMe), 6.78 (1H, *s*, H-8), 7.38 (1H, *d*, J = 9 Hz, H-5'), 8.09 (1H, *dd*, J = 2, 9 Hz, H-6'), 8.54 (1H, *d*, J = 2 Hz, H-2'). It was methylated with CH_2N_2. The product exhibited one spot at R_f 0.30 (light blue fluorescens) on TLC (silica gel, C_6H_6–Me_2CO, 8:2), identical with that of 3,5,6,7,3',4'-hexamethoxyflavone (3,5,7,8,3',4'-hexamethoxyflavone, R_f 0.06, dark yellow fluorescence).

The hydrolysate free from aglycone was filtered through a column of Amberlite IR 45 (OH^-), evaporated to dryness and subjected to TLC (microcrystalline cellulose, *n*-BuOH–pyridine–H_2O, 3:2:1, *p*-anisidine·HCl). Two spots were detected at R_f 0.28 (brown) and 0.51 (pale yellow). An authentic glucose had R_f 0.28 (brown) and the hydrolysate of apiin showed two spots at R_f 0.28 (brown) and 0.51 (pale yellow). The sugar fraction was acetylated with Ac_2O–pyridine and subjected to GC [column, 2.5 mm × 2 m, 2% diethyleneglycol adipate on Chromosorb W HP (100–200 mesh); carrier gas, N_2; temp., 220–250° (5°/min)]. Four peaks were detected at R_t 5.6, 5.9, 10.1 and 11.1 (acetate of glucose; R_t 10.1, 11.1: acetate of the hydrolysate of apiin; R_t 5.5, 5.8, 10.1, 11.1).

Enzymic hydrolysis of **1**. A soln of **1** (138 mg) and the crude hesperidinase (10–20 mg) in 0.05 M acetate buffer (pH 4.75) was incubated at 30° for 24 hr. The reaction mixture was filtered through a column of Diaion HP20AG. The filtrate was desalted with a column of Amberlite IR 120 (H^+), concd *in vacuo* and subjected to TLC (microcrystalline cellulose, *n*-BuOH–pyridine–H_2O, 3:2:1, *p*-anisidine·HCl). Only one spot of glucose was detected. The column of Diaion HP20AG was washed with MeOH. The MeOH soln was evaporated and the residue was crystallized from H_2O to give **6** as yellow needles, mp 180–200°, $[\alpha]_D^{25}$ −98.2° (MeOH; *c* 0.12). FABMS *m/z* (rel. int.): 627 $[M+1]^+$ (6), 495 $[M+1-132]^+$ (3), 333 $[M+1-132-162]^+$ (33). UV λ_{max}^{MeOH} nm (log ε): 256 (4.24), *ca* 267 sh (4.18), 290–300 (3.90), 350 (4.25). $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 270 (4.31), *ca* 330 sh (4.02), 382 (4.21). $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 261 (4.32), 373 (4.26). $\lambda_{max}^{MeOH\ NaOMe}$ nm (log ε): 269 (4.31), 335 (4.01), 401 (4.33). $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 277 (4.35), *ca* 305 sh (3.90), 340–355 (3.75), 434 (4.35). $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 270 (4.26), *ca* 300 sh (3.94), 383 (4.22), *ca* 410 inflec [5] (4.18). ^{1}H NMR (270 MHz, DMSO-d_6): δ2.5–5.4 (H on sugar except two anomeric H), 3.76 (*s*, OMe), 5.36 (*s*, H-1 of apiose), 5.65 (*d*, J = 8 Hz, H-1 of glucose), 6.84 (*d*, J = 9 Hz, H-5'), 7.54 (*d*, J = 2 Hz, H-2'), 7.68 (*dd*, J = 2, 9 Hz, H-6').

Under the same conditions, β-glucosidase (from almond) did not hydrolyse **1**.

Methylation of **1** *followed by hydrolysis*. Compound **1** was methylated with CH_2N_2 and the product was hydrolyzed with 1 N HCl in MeOH for 1 hr. The hydrolysate was diluted with H_2O and the ppt that separated was collected and crystallized from 50% dioxane to give 3-hydroxy-5,6,7,3',4'-pentamethoxyflavone as pale yellow needles, mp 183–185° (lit. [9] mp 190–191°). UV λ_{max}^{MeOH} nm (log ε): 252 (4.35), 356 (4.39),

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unchanged on addition of NaOAc and H_3BO_3–NaOAc. $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 265 (4.39), 421 (4.49), unchanged on addition of HCl. ^{1}H NMR (60 MHz, $CDCl_3$): δ3.89, 3.92, 3.95, 4.01 (15H, OMe), 7.72 (1H, *s*, H-8), 6.94 (1H, *d*, *J* = 10 Hz, H-5'), 7.6–7.8 (2H, *m*, H-2', 6').

Compound **2**. Fraction 3 exhibited a spot of R_f 0.18 [silica gel, $CHCl_3$–MeOH (7:3) satd with H_2O]. It was subjected to CC over Diaion HP20AG ($H_2O \rightarrow$ MeOH). The TLC homogeneous **2** crystallized from 50% MeOH to yellow needles, mp 220–222°, $[\alpha]_D^{27}$ – 22.3° (MeOH; *c* 0.10). It gave positive colour reactions with $FeCl_3$, Mg–HCl and Zn–HCl. (Found: C, 49.53; H, 5.16. $C_{28}H_{32}O_{18} \cdot H_2O$ requires: C, 49.85; H, 5.08%.) FDMS *m/z* (rel. int.): 679 $[M+23]^+$ (10), 657 $[M+1]^+$ (100), 495 $[M+1-162]^+$ (10), 332 $[M-2\times162]^+$ (85). IR ν_{max}^{KBr} cm^{-1}: 1660, 1610, 1100–1000. UV λ_{max}^{MeOH} nm (log ε): 257 (4.31), *ca* 270 sh (4.25), *ca* 300 sh (3.97), 355 (4.33). $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 270 (4.16), *ca* 330 sh (3.84), 390 (4.09). $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 262 (4.19), 377 (4.13). $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 270 (4.17), 337 (3.87), 409 (4.22). $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 276 (4.43), *ca* 310 sh (3.89), 336 (3.81), 437 (4.44). $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 270 (4.30), *ca* 300 (3.92), 390 (4.28), *ca* 400 inflec [5] (4.28). ^{1}H NMR (270 MHz, DMSO-d_6): δ2.5–3.9 (*m*, H on sugar except two anomeric H), 3.76 (*s*, OMe), 4.06 (*d*, *J* = 7 Hz, H-1 of terminal glucose), 5.41 (*d*, *J* = 7 Hz, H-1 of inner glucose), 6.50 (*s*, H-8), 6.85 (*d*, *J* = 9 Hz, H-5'), 7.56–7.59 (*m*, H-2', 6').

Acid hydrolysis of **2**. Compound **2** was hydrolysed with 5% HCl in 50% MeOH for 3 hr. The hydrolysate was treated just in the same manner as in the case of **1**. The aglycone, mp 240–243°, was identical with **5** in every respects. TLC of the sugar fraction exhibited the spot of glucose.

Compound **3**. Fraction 4 was chromatographed over Diaion HP20AG ($H_2O \rightarrow$ MeOH). The TLC homogeneous **3** (R_f 0.37) crystallized from MeOH to yellow needles, mp 192–194°, $[\alpha]_D^{26}$ – 19.0° (MeOH; *c* 0.19). It gave positive colour reactions with $FeCl_3$, Mg–HCl and Zn–HCl. (Found: C, 50.50; H, 5.40. $C_{29}H_{34}O_{18} \cdot H_2O$ requires; C, 50.58; H, 5.27%.) FDMS *m/z* (rel. int.): 693 $[M+23]^+$ (6), 671 $[M+1]^+$ (100), 509 $[M+1-162]^+$ (3), 346 $[M-2\times162]^+$ (18). IR ν_{max}^{KBr} cm^{-1}: 1640, 1600, 1100–1000. UV λ_{max}^{MeOH} nm (log ε): 254 (4.28), *ca* 270 sh (4.21), *ca* 300 sh (3.97), 350 (4.33). $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 272 (4.35), 322 (3.79), 390 (4.27). $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 255 (4.27), 268 (4.25), 355 (4.29). $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 270 (4.33), 334 (4.05), 415 (4.48). $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 267 (4.29), *ca* 280 sh (4.25), *ca* 305 sh (3.95), 382 (4.33), *ca* 400 inflec [5] (4.30). $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 267 (4.28), *ca* 280 inflec [5] (4.24), *ca* 300 sh (3.97), 382 (4.32), *ca* 410 inflec [5] (4.25). ^{1}H NMR (270 MHz, DMSO-d_6): δ2.6–5.4 (H on sugar except two anomeric H), 3.76, 3.85 (*s*, OMe), 4.08 (*d*, *J* = 7 Hz, H-1 of terminal glucose), 5.52 (*d*, *J* = 7 Hz, H-1 of inner glucose), 6.55 (*s*, H-8), 6.92 (*d*, *J* = 9 Hz, H-5'), 7.51 (*dd*, *J* = 2, 9 Hz, H-6'), 7.94 (*d*, *J* = 2 Hz, H-2').

Hydrolysis of **3**. A soln of **3** in 5% HCl–MeOH was refluxed for 1 hr. The reaction mixture was diluted with H_2O and the ppt that separated was collected and crystallized from MeOH–H_2O to give **7**, mp 227–230° (lit. [2] mp 235–236°). UV λ_{max}^{MeOH} nm (log ε): 255 (4.29), *ca* 270 sh (4.13), 295 (3.86), *ca* 340 inflec [5] (4.22), 367 (4.35). $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 270 (4.25), *ca* 275 sh (4.25), 320 (4.04), 390 (4.32). $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 255 (4.28), *ca* 270 sh (4.14), 370 (4.33). $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): *ca* 240 sh (4.22), 333 (4.32). $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 266 (4.35), *ca* 300 sh (3.78), *ca* 380 sh (4.17), 430 (4.41). $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 266 (4.36), *ca* 305 (3.75), *ca* 370 sh (4.10), 430 (4.34). ^{1}H NMR (60 MHz, pyridine-d_5): δ3.97, 4.05 (each 3H, OMe), 6.90 (1H, *s*, H-8), 7.35 (1H, *d*, *J* = 9 Hz, H-5'), 8.0–8.4 (2H, *m*, H-2', 6'). It gave an acetate, mp 168–169° (MeOH), and its methyl ether showed the same behaviour on TLC with those of 3,5,6,7,3',4'-hexamethoxyflavone.

Compound **4**. Fraction **8** was purified by CC over Diaion HP20AG ($H_2O \rightarrow$ 70% MeOH). The fractions of **4** (R_f 0.27) were collected and crystallized from MeOH–H_2O, mp 153° (lit. [13] mp 159°), $[\alpha]_D^{25}$ – 80.0° (MeOH; *c* 0.11). IR ν_{max}^{KBr}: 1730, 1640, 1610, 1100–1000, identical with that of authentic spinatoside. UV spectra in MeOH and in MeOH-reagents were identical with the reported values [13]. FABMS *m/z* (rel. int.): 523 $[M+1]^+$ (12), 347 $[M+1-176]^+$ (12). ^{1}H NMR (270 MHz, DMSO-d_6): δ3.4–4.0 (*m*, H on sugar except anomeric H), 3.77, 3.82 (*s*, OMe), 5.12 (*d*, *J* = 7 Hz, anomeric H), 6.57 (*s*, H-8), 7.22 (*d*, *J* = 9 Hz, H-5'), 7.5–7.6 (*m*, H-2', 6'). Compound **4** was hydrolysed with 10% HCl to **8**, mp 207–209° (lit. [13] mp 205–207°) and glucuronic acid (by TLC). UV spectra of **8** (in MeOH, MeOH–$AlCl_3$ and MeOH–$AlCl_3$–HCl) were identical with the reported values [5, 13]. The IR spectrum was identical with that of authentic axillarin. Compound **8** was methylated to 3,5,6,7,3',4'-hexamethoxyflavone, mp 138–139° (MeOH–H_2O) (lit. [2] mp 143–144°).

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