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ACYLATED FLAVONOL GLUCOSIDES OF *PINUS CONTORTA* NEEDLES

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Key Word Index—*Pinus contorta*; Pinaceae; mono- and di-acyl flavonol glucosides; 6-methylkaempferol; isorhamnetin.

Abstract—From fresh *Pinus contorta* Doug (Coastal) needles four flavonol acylated glucosides and 6-methylkaempferol 3-β-D-glucoside were isolated. The three monoacylated glucosides were kaempferol-3-β-D(6-*O*-*p*-coumaryl)glucoside, isorhamnetin-3-β-D-(6-*O*-acetyl)glucoside, quercetin-3-β-D-(*p*-coumaryl)glucoside and the diacyl compound was kaempferol-3-β-D-(di-*p*-coumaryl)glucoside.

INTRODUCTION

A major study on the polyphenol content of pine needles was a survey of 35 species analysed by Takahashi *et al.* in 1960 [1]. The presence of flavonoid glycosides was reported in most species; in particular quercetin, dihydroquercetin and kaempferol, but myricetin glycoside was detected only in three pines, *Pinus halepensis*, *P. muricata* and *P. sabiniana*.

During our investigation of the needle constituents of *P. contorta* in a search for phenylpropanes [2, 3], compounds thought to exert an allelochemic influence in the nutritional and metabolic aspect of insect host–plant specificity [4], a series of acylated flavonol glucosides were identified. This paper describes their chemistry.

Most acylated flavonol glycosides so far reported have a single acyl group attached to one of the sugar hydroxyls. The first to be characterised was petunoside, isolated from *Petunia* petals [5]; the acyl group, ferulic acid, was attached to the terminal glucose at position-2. A series of acylated kaempferol derivatives were found in the fern *Asplenium rhizophyllum* [6] and acylated quercetin derivatives in the common pea, *Pisum sativum* [7]; helichryoside, also a quercetin derivative, was reported recently [8].

RESULTS AND DISCUSSION

The procedure for isolation of the four acyl flavonol glucosides, tentatively listed (in order of increasing polarity) as compounds **1**–**4** and the 6-methylkaempferol glucoside (**5**) is described in detail in the Experimental and only data relevant to the structural assignments will be referred to here.

Kaempferol was the flavonol in both optically active esters **1** and **3**. Each had UV spectra and chromatographic behaviour characteristic of acylated flavonol glycosides. Mild alkaline hydrolysis (Na_2CO_3 in MeOH) [9] gave methyl *p*-coumarate and a crystalline glycoside which on acid hydrolysis afforded kaempferol and D-glucose. The UV spectrum of the glucoside and the PMR spectrum of its permethyl ether indicated that it was kaempferol-3-monoglucoside [10]. This structure assignment was confirmed by isolation of kaempferol 5,7,4′-tri-*O*-methyl ether and the methyl pyranosides of 2,3,4,6-tetra-*O*-methyl-α- and β-glucose. In order to locate the position of attachment of the acid group in **3**, an analysis of the PMR spectrum of its tetramethyl ether was undertaken (Fig. 1).

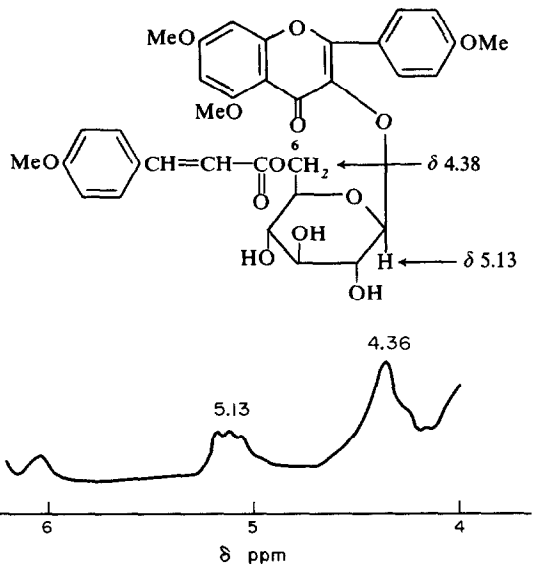


Fig. 1.

The ester bearing methylene (C-6) proton signal was at δ 4.36 and only one methine signal appears at the downfield region (δ 4.9–5.6) and is assigned to the anomeric proton (δ 5.13). The likely structure for **3** is kaempferol-3-β-D-(6-*O*-*p*-coumarylglucopyranoside). This structure was assigned to tribuloside found in the leaves and fruit of *Tribulus terrestris* [10] and to tiliroside (from *Tilea argentea*) [11, 12] but in the latter natural

* Part 3 in the series 'Pines' by DMXD. For Part 2, see Higuchi, R. and Donnelly, D. M. X. (1977) *Phytochemistry* **16**, 1587.

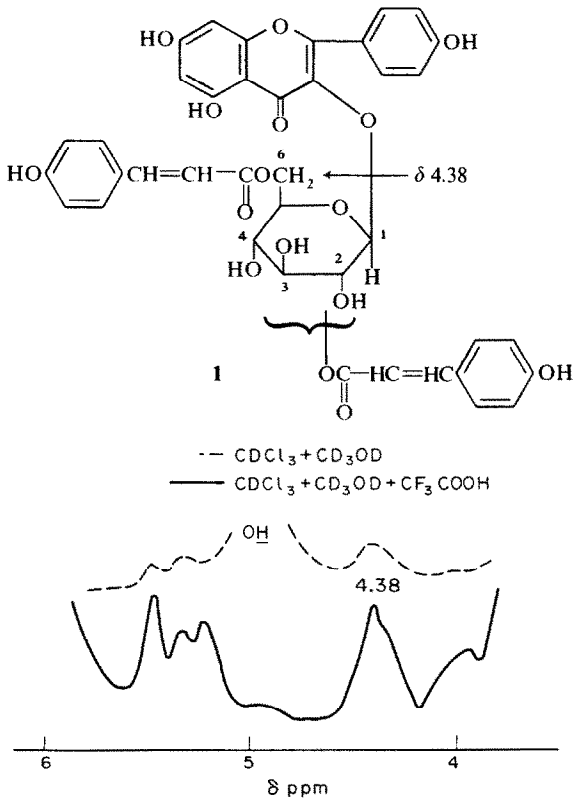


Fig 2

product, the exact point of attachment of the acid unit was not assigned. The position of attachment of the acid group in tribuloside was determined from a periodate oxidation giving formic acid which could only be obtained if the acid group was attached to the 6-hydroxyl of a pyranose glucose. The comparison of the reported data on physical measurements (mp, $[\alpha]_D$) for the acyl flavonol glycosides is complicated because of the presence of water of crystallisation. Samples of tribuloside and tiliroside were unfortunately not available for direct comparison, but from the above considerations is presumably identical to tribuloside.

1 had the same components as **3**. The PMR spectrum of the acetate of **1** showed five aromatic and two alcoholic acetoxyl signals in the range δ 2.0–2.49 and signals for two α-protons of the *p*-coumaryl residue (ϕ—CH=CH. CO) at δ 6.35 and 6.41. This observation indicates that **1** is composed of 1 mole of kaempferol-3-β-D-glucopyranoside and 2 moles of *p*-coumaric acid. The acids are on the sugar moiety as the product from methylation and subsequent acetylation of **1** gave a pentamethylether-diacetate (five aromatic methoxyl signals and two alcoholic acetoxyl signals in its PMR spectrum). A β-linkage of the sugar unit was inferred from the anomeric proton signal at δ 5.57 (*m*, $W^{h/2}$ = 9.0 Hz) in the PMR spectrum of the heptamethyl ether of kaempferol-3-monoglucoside. A partial structure (**1**) is proposed. The PMR spectrum of (**1**) (solvent $CDCl_3$ + CD_3OD + CF_3CO_2H) [13] had the signals of the methylene protons adjacent to an ester link at δ 4.38 and two methine protons at δ 5.0–5.6. One of the *p*-coumaryl residues must be linked to the C-6 hydroxyl group in glucose (Fig. 2). This is the first reported example of a diacyl flavonol glucoside.

The partial structure of **2** is isorhamnetin-3-β-D-(*O*-acetyl) glucopyranoside. This structure assignment is based on isolation of quercetin-3'-methyl ether and D-glucose from the acid hydrolysis demethylation of the aglycone (isorhamnetin) gave quercetin. **2** showed an absorption at 1730 cm^{-1} and a signal at δ 1.9 in its IR and PMR spectra respectively and gave a heptaacetate on acetylation. Based on this data, **2** is mono(monoacetyl)glucoside of isorhamnetin. The placement of the acetyl sugar residue at the 3-hydroxyl of the aglycone fitted the UV spectra and was confirmed by the methanolysis of the deacetyl methyl ether obtained by methylation with CH_2N_2 and the Kuhn method [13]. The methanolysate yielded quercetin-5,7,3',4'-tetra-*O*-methyl ether and the methyl pyranosides of 2,3,4,6-tetra-*O*-methyl-α- and β-glucose. The β-glucopyranoside linkage was identified from the PMR spectrum of (a) the heptaacetate which contained a doublet at δ 5.67 (J = 8.0 Hz) and (b) a doublet at δ 5.7 in the octamethyl ether of the glucoside. The location of the acetyl residue was deduced as follows. The PMR spectrum of **2** when taken in $CDCl_3$ + CD_3OD had a broad OH signal overlapping the region for the ester-bearing methine or methylene proton signal. By addition of CF_3COOH [14] to the solvent, a simplified spectrum with a signal at δ 4.2 (2H, *m*) due to the C-6 methylene protons (Fig. 3) was observed. In the region δ 4.9–5.5 which is characteristic of methine protons (1,2,3,4-H) of acetylated glucose, only the anomeric proton signal was observed δ 5.1 (1H, *br d*, J = 7.0 Hz). Thus the acetyl group in **2** must be linked to the C-6 hydroxyl group in the glucose moiety. The isolate is therefore isorhamnetin-3-β-D-(6-*O*acetyl)-glucopyranoside.

An acyl flavonol glycoside structure was assigned to **4** on the basis of its UV, IR spectra and on the analysis

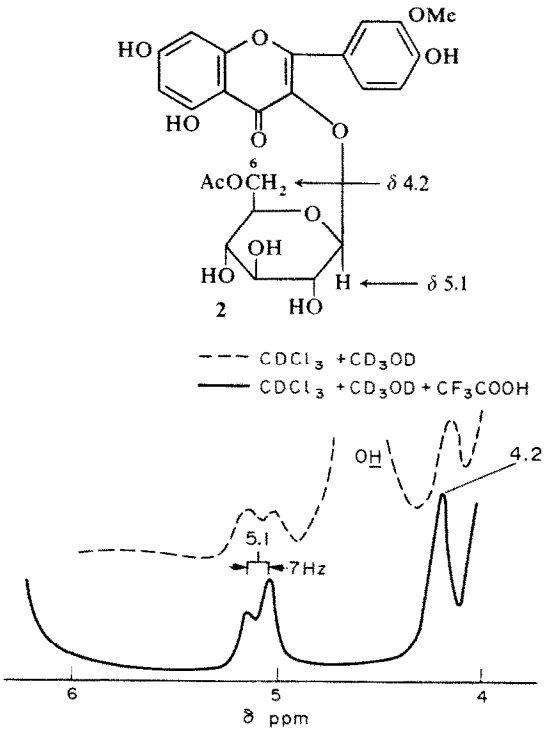
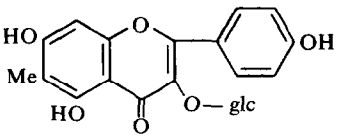


Fig. 3.

of the products of alkaline and acid hydrolysis. The degradation compounds were methyl *p*-coumarate, glucose and quercetin. Acetylation afforded an octa-acetate which showed signals for three alcoholic and five aromatic acetoxyl groups. The UV spectrum of the flavonol glucoside obtained after alkaline hydrolysis of **4** indicated that the glucose unit is attached to the 3-hydroxyl group of quercetin. The pyranoside form and the β-linkage of the glucose were evident from the PMR spectrum of the octaacetate. **4** is therefore quercetin-3-D-(*p*-coumaryl)glycopyranoside. From an analysis of the chemical shift of the sugar acetoxyl groups, the linkage of the *p*-coumaric acid is apparently not at the 6-position. A series of acetylated glycosides were examined (Table 1) for the chemical shift values of the acetoxyl groups. The acetate group when attached at position-6-registers in the region δ 1.95. The remaining acetyl groups at positions 2, 3 or 4 have values in the region δ 2.0–2.16. This observation would suggest that **4** is different from helichrysoside (quercetin-3-β-D-(6-*p*-coumarylglucoside) found in *Helichrysum kraussii*, the structure of which was proposed from an X-ray analysis [8] and which contains $5\frac{1}{2}$ molecules of water per mole of glycoside.

The fifth flavonol derivative, **5** was considered to have a glycoside structure from examination of its UV spectrum. Acid hydrolysis afforded D-glucose and an aglycone ($C_{16}H_{12}O_6$). The PMR spectrum of the acetate derivative integrated for five aromatic ring protons, four acetoxyl groups and one aromatic C-Me (δ 2.13) group. Among the five aromatic ring protons four (δ 7.94, 7.33 *dd* J = 9.0 Hz) were assigned as A_2B_2 system, and the remaining aromatic proton (δ 7.37) was presumed to be 8-H of a 5,6,7-trisubstituted flavonoid [15]. One acetoxyl group (δ 2.5) was assigned as a 5-acetoxyl of flavonoid. Coupled with the UV spectrum, the mp and the known co-occurrence of kaempferol glycoside, the aglycone moiety of **5** was assigned as 6-methyl-kaempferol. Interestingly, the 6-*C*-methyl derivatives pinoquercetin and pinomyricetin have been found in bark extracts of *Pinus ponderosa* [16]. The presence of only one sugar unit in the glycoside was evident from the PMR spectrum of its peracetate. The sugar was attached at the 3-hydroxyl position as noted from UV spectrum of **5** on addition of reagents. The isolation of 5,7,4-tri-*O*-methyl-6-methylflavonol and the methyl pyranoside of 2,3,4,6-tetra-*O*-methyl-α-D-glucose from



5

the methanolysis of the permethyl ether of **5** confirmed the 3-hydroxyl linkage. The β-linkage of the D-glucopyranose was assigned from the presence of one anomeric proton doublet at δ 5.67 (8.0 Hz) in the PMR spectrum of the peracetate of **5**. **5** is therefore 6-methyl-kaempferol 3-β-D-glucopyranoside, a new natural product.

EXPERIMENTAL

Mps were uncorr. PMR spectra were determined at 60 MHz from solns in $CDCl_3$ (TMS as int. ref.) unless otherwise specified. Details for the general procedures are as described previously [2].

Isolation of **1–5**. Fraction V (polyamide fraction V [2]) (3.6 g) was chromatographed on Sephadex LH-20 using MeOH as solvent to yield five fractions (i–v). Fractions (i) (0.13 g), (ii) (0.28 g) and (iii) (0.47 g) were each placed on Si gel columns and eluted with $CHCl_3$–MeOH–H_2O (8:2:0.1) to give **2** (0.03 g), **5** (0.10 g) and **3** (0.05 g) respectively. Fraction (iv) (0.20 g) on Si gel (eluent, $CHCl_3$–MeOH–H_2O (43:7:0.35)) afforded **4** (0.05 g). Separation of fraction (v) on Si gel by the same procedure as for fraction (iv) and subsequently on Sephadex LH-20 using MeOH (80%) gave **1** (0.09 g).

Kaempferol 3-β-D-(di-p-coumaryl)glucopyranoside) (**1**). Yellow needles (from MeOH), mp 205–207° $[\alpha]_D^{22}$ −72.5° (c = 0.4, MeOH). ν_{max} (KBr) cm^{-1}: 3300 (OH), 1700 (ester $\rangle$C=O, 1660 flavonoid $\rangle$C=O). λ_{max} nm (ε): MeOH 268 (28120), 300*sh* (46620), 314 (51060), 350*sh* (17640) and MeOH–$AlCl_3$ 278, 309, 398.

Alkali hydrolysis of (**1**). **1** (50 mg) was refluxed with Na_2CO_3 in MeOH for 20 min, worked up by standard procedures to give *p*-coumarate (10 mg) (from MeOH), mp 135–137°. PMR: δ 3.87 ppm (3H, *s*, —COOC$\underline{H}_3$), 6.37 (1H, *d*, J = 16 Hz. ϕ—CH=C$\underline{H}$OCO—), 6.96 (δ_A), 7.51 (δ_B) (4H, *q*, J = 9 Hz, A_2B_2 system), 7.76 (1H, *d*, J = 16 Hz, ϕ—C$\underline{H}$=CH—CO—), identified by direct comparison (mp, TLC and PMR) with an authentic sample. The other product was kaempferol 3-glucoside, yellow needles (7 mg), mp 178–180° (lit. mp 175–177° [10], UV spectrum + shifts as for authentic material. On acid

Table 1. δ values of the sugar acetyls of acylated flavonol glycosides

	2, 3, 4-OAc			6-OAc
Isorhamnetin-3-Glc (heptaacetate)	2.14	2.05	2.03	1.95
6-Mekaempferol-3-Glc (heptaacetate)	2.16	2.06	2.02	1.96
Tri-*o*-kaempferol-3-Glc (tetraacetate)	2.15	2.04	2.04	1.92
Tri-*o*-Me-kaempferol-3-Glc-6-*p*-coumaryl (triacetate)	2.16	2.04	2.04	—
Kaempferol-3-Glc-6-*p*-coumaryl (heptaacetate)	2.12	2.04	2.04	—
Kaempferol-3-Glc-6-*p*-coumaryl (heptaacetate) *p*-coumaryl	2.13	—	2.0	—
Quercetin-3-Glc-*p*-coumaryl (octaacetate)	2.12	—	1.99	1.94

hydrolysis, it gave kaempferol mp 275–278° (identical mp, TLC, UV with authentic specimen and glucose).

Permethylether of kaempferol-3-glucoside. Prepared by the (Kuhn method) and purified by PLC (C_6H_6–Me_2CO, (3:1) to afford an amorphous powder. PMR: δ 3.10 ppm (3H, *s*, glucose OMe), 3.53 (3H, *s*, glucose OMe), 3.68 (6H, *s*, glucose OMe × 2), 3.92 (6H, *s*, ϕ-OMe × 2), 3.98 (3H, *s*, ϕ-OMe), 5.57 (1H, *bd*, $W^{h/2}$ = 9 Hz, anomeric proton of glucose), 6.21 (1H, *d*, J = 2 Hz, 6-H), 6.57 (1H, *d*, J = 2 Hz, 8-H), 7.02 (2H, *d*, J = 9 Hz, 3',5'-H), 8.17 (2H, *d*, J = 9 Hz, 2',6'-H). Subsequent methanolysis by reflux with HCl (8%) in MeOH for 30 min gave a mixture which was treated with Ag_2O and filtered. Evapn of the filtrate and examination of the residue by TLC (C_6H_6–Me_2CO, 4:1) showed the aglycone and the Me pyranoside of 2,3,4,6-tetra-*O*-Me-α- and β-glucose. The residue was subjected to PLC (C_6H_6–Me_2CO, 3:1) to afford 3-hydroxy-5,7,4'-trimethoxyflavone as yellow needles (MeOH), mp 146–147° (identical mp, UV as authentic material [10]. Acetylation of **1** with Ac_2O–Py and PLC of the product using C_6H_6–Me_2CO (6:1) gave a heptaacetate (amorphous powder). PMR: δ 2.0 ppm (3H, *s*, glucose OAc), 2.13 (3H, *s*, glucose OAc), 2.23 (3H, s, ϕ—OAc), 2.32 (9H, ϕ—OAc × 3), 2.49 (3H, *s*, ϕ—OAc), 3.5–4.4 (3H, *m*, glucose 5,6-H), 5.0–5.9 (4H, *m*, glucose 1,2,3,4-H), 6.35 (1H, *d*, J = 16 Hz, ϕ—CH=CH—CO—) 6.41 (1H, *d*, J = 16 Hz, ϕ—CH=CH—CO—), 6.87 (1H, *d*, J = 2 Hz, 6-H), 7.0–8.0 (13H, *m*), 8.16 (2H, *d*, J = 9 Hz, 2',6'-H). Methylation of **1** (30 mg) with CH_2N_2, followed by acetylation with Ac_2O–Py and purification of the reaction product with PLC (solvent C_6H_6–Me_2CO, 4:1) gave a pentamethylether-diacetate (19 mg) as amorphous powder. PMR: δ 2.0 ppm (3H, *s*, glucose OAc), 2.1 (3H, *s*, glucose OAc), 3.7–4.1 (15H, ϕ—OMe × 5).

*Kaempferol-3-*β-D-(p-*coumarylglucopyranoside*) (**3**). Yellow amorphous powder [10] λ_{max} nm: MeOH 267, 300*sh*, 315, 350*sh* and MeOH–$AlCl_3$ 276, 307, 320*sh*, 404. The glucoside was hydrolysed with mild alkali and worked up as above to give kaempferol-3-glucoside and Me-*p*-coumarate (identical mp, TLC with authentic sample). Methylation of **3** with CH_2N_2, followed by purification on PLC (solvent: $CHCl_3$–MeOH–H_2O, 9:1·0.05) gave a tetramethyl ether as an amorphous powder [10]. PMR: δ 3.74 (3H, *s*, ϕ—OMe) 3.86 (9H, ϕ—OMe × 3) 4.36 (2H, *m*, glucose 6-H), 5.13 (1H, *m*, $W^{h/2}$ = 9 Hz, anomeric proton of glucose). Acetylation with Ac_2O–Py afforded the tetramethyl ether-triacetate, (amorphous powder), $[\alpha]_D^{22}$ −69.5° (c = 0.6, $CHCl_3$). λ_{max} nm (ε): (a) 263 (18430), 300*sh* (22.310), 312 (24250). PMR. δ 2.04 ppm (6H, *s*, glucose OAc × 2), 2.16 (3H, *s*, glucose OAc), 3.81 (3H, *s*, ϕ—OMe), 3.92 (6H, *s*, ϕ—OMe × 2), 4.02 (3H, *s*, ϕ—OMe), 5.0–6.0 (4H, *m*, glucose 1,2,3,4-H), 6.21 (1H, *d*, J = 16 Hz, ϕ—CH=CH—CO—), 6.44 (1H, *d*, J = 2 Hz, 6-H), 6.55 (1H, *d*, J = 2 Hz, 8-H), 6.7–7.9 (7H, *m*), 8.13 (2H, *d*, J = 9 Hz, 2',6'-H). Alkaline hydrolysis of the tetramethyl ether-triacetate with Na_2CO_3 in MeOH (heat, 30 min), filtered and the filtrate was evapd, diluted with H_2O, neutralized, and extracted successively with Et_2O and EtOAc-*n*-BuOH (2:1). Me *p*-methoxycinnamate was obtained from the Et_2O layer as plates from MeOH, mp 84.5–85°. PMR: δ 3.83 ppm (3H, *s*, —COOMe), 3.87 (3H, *s*, ϕ—OMe), 6.37 (1H, *d*, J = 16 Hz, ϕ—CH=CH—CO—), 6.98 (δ_A), 7.57 (δ_B) (4H, *q*, J = 9 Hz, A_2B_2 system), 7.75 (1H, *d*, J = 16 Hz, ϕ—CH=CH—CO—). From the EtOAc-*n*-BuOH layer kaempferol-5,7,4'-tri-*O*-methyl ether-3-β-D-glucopyranoside was obtained as needles (from MeOH), mp 140–143°, $[\alpha]_D^{17}$ −10.0° (c = 0.2 MeOH), λ_{max} nm (ε): 262 (12005), 335 (10780). Acetylation (Ac_2O–Py) afforded a tetraacetate as an amorphous powder. PMR: δ 1.92 ppm (3H, *s*, glucose OAc), 2.04 (6H, *s*, glucose OAc × 2), 2.15 (3H, *s*, glucose OAc), 3.96 (6H, *s*, ϕ—OMe × 2), 4.03 (3H, *s*, ϕ—OMe), 5.0–5.5 (3H, *m*, glucose 2,3,4-H), 5.89 (1H, *d*, J = 7 Hz, anomeric proton of glucose), 6.45 (1H, *d*, J = 2 Hz, 6-H), 6.62 (1H, *d*, J = 2 Hz, 8-H), 7.09 (2H, *d*, J = 9 Hz, 3',5',-H), 8.15 (2H, *d*, J = 9 Hz, 2',6'-H). Acid hydrolysis gave kaempferol-5,7,4'-trimethyl ether and glucose.

*Isorhamnetin-3-*β-D(*6-O-acetylglucopyranoside*) (**2**). Isolated as yellow needles (from MeOH), mp 167–170°. λ_{max}(KBr) cm^{-1}: 3400 (OH), 1730 (ester C=O), 1660 (flavonoid (C=O). λ_{max} nm (ε): MeOH 255, (13520), 265*sh* (12064), 305*sh* (6656), 355 (12272); MeOH–$AlCl_3$ 270, 303, 265*sh*, 405; MeOH–$AlCl_3$–HCl 267, 300, 360, 400; NaOMe 271, 327, 413 and NaOAc 274, 320 403. PMR ($CDCl_3$ + CD_3OD) δ 1.90 ppm (3H, *s*, glucose OAc), 3.97 (3H, *s*, ϕ—OMe), 6.33 (1H, *d*, J = 2 Hz, 6-H), 6.47 (1H, *d*, J = 2 Hz, 8-H), 6.6–7.7 (3H, *m*, 2',5',6'-H). Acid hydrolysis gave glucose and an aglycone as yellow needles from MeOH mp 288–292° (mp of isorhamnetin 304–306° [17]; tamarixetin mp 259–262° [18]. λ_{max} nm (ε): MeOH 254 (10712), 267*sh* (8927), 308 (11694); MeOH–$AlCl_3$ 266, 300*sh*, 355*sh*, 426; NaOMe 278, 327, 422 and NaOAc 276, 326, 397. Acetylation gave a tetraacetate, needles from MeOH, mp 203–205° (mp of isorhamnetin tetraacetate, 202–204° [17] (Found: C, 59.24; H, 4.27. Calc. for $C_{24}H_{20}O_{11}$: C, 59.50, H, 4.16%). PMR: δ 2.38 ppm (9H, *s*, ϕ—OAc × 2 and 3-OAc), 2.47 (3H, *s*, ϕ—OAc), 3.94 (3H, *s*, ϕ—OMe), 6.95 (1H, *d*, J = 2 Hz, 6-H), 7.1–7.7 (3H, *m*, 2',5',8-H), 7.92 (1H, *d*, J = 9 Hz, 6'-H). Demethylation of the aglycone gave quercetin (identical TLC, UV with authentic sample). Acetylation of **2** gave an amorphous powder, $[\alpha]_D^{17}$ −53.3° (c = 0.23, $CHCl_3$). λ_{max} nm (ε): 243 (13440), 317 (10752). PMR: δ 1.95, 2.03, 2.05, 2 14 ppm (12H, glucose OAc × 4), 2.36 (6H, *s*, 2.36 (6H, *s*, ϕ—OAc × 2), 2.50 (3H, *s*, ϕ—OAc), 3.7 (1H, *m*, glucose 5-H), 4.02 (3H, *s*, ϕ—OMe), 4.9–5.5 (3H, *m*, glucose 2,3,4-H), 5.67 (1H, *d*, J = 8 Hz, anomeric proton of glucose), 6.95 (1H, *d*, J = 2 Hz, 6-H), 7.1–7.9 (3H, *m*, 2',5',8-H), 8.17 (*d*, J = 9 Hz, 6'-H). Methylation of **2** with CH_2N_2, and the Kuhn method afforded heptamethyl ether as amorphous powder. PMR: δ 3.14, 3.53, 3.67, 3.69 ppm (12H, glucose OMe × 4), 3.94 (3H, *s*, ϕ—OMe), 4 0 (9H, *s*, ϕ—OMe × 3), 5.70 (1H, *d*, J = 6 Hz, anomeric proton of glucose), 6.43 (1H, *d*, J = 2 Hz, 6-H), 6.58 (1H, *d*, J = 2 Hz, 8-H), 7.02 (1H, *d*, J = 9 Hz, 5'-H), 7.75 (1H, *bd*, J = 9 Hz, 6'-H), 8.03 (1H, *d*, J = 2 Hz, 2'-H). Methanolysis of this product followed by treatment with Ag_2O gave quercetin 5,7,3',4'-tetramethylether, yellow needles (from MeOH) mp 184–186° (identical mp, TLC authentic sample), and the methyl pyranoside of 2,3,4,6-tetra-*O*-methyl-α- and β-glucose.

*Quercetin-3-*β-D-(p-*coumaryl*)*glucopyranoside* (**4**). Yellow amorphouse powder, $[\alpha]_D^{18}$ −30 6° (c = 0.35, MeOH). ν_{max} KBr) cm^{-1}: 3300 (OH), 1700 (ester >C=O), 1655 (flavonoid C=O). λ_{max} nm (ε): MeOH 257 (36905), 268 (37515), 300*sh* (47580), 314 (53070), 360*sh* (28975); MeOH–$AlCl_3$ 277, 311, 432 and MeOH–$AlCl_3$–HCl 277, 300*sh*, 312, 400 Alkaline hydrolysis afforded methyl-*p*-coumarate and a glycoside as yellow needles (from MeOH) mp 198–200°. λ_{max} nm: MeOH 256, 265*sh*, 300*sh*, 354; MeOH–$AlCl_3$ 275, 300*sh*, 430; MeOH–$AlCl_3$–HCl 269, 300, 360, 400; + NaOMe 271, 330, 405 and + NaOAc 272, 320, 398 Acid hydrolysis (2N HCl) of the glycoside afforded quercetin and D-glucose. Acetylation of **4** (Ac_2O–Py method) gave octaacetate as amorphous powder. PMR: δ 1.94, 1.99, 2 12 ppm (9H, glucose OAc × 3), 2.35 (12H, *m*, ϕ—OAc × 4), 2.47 (3H, *s*, ϕ—OAc), 3.80 (1H, *m*, glucose 5-H), 4.05 (2H, *m*, glucose 6-H), 5.0–5.9 (3H, *m*, glucose 2,3,4-H), 5.74 (1H, *d*, J = 7 Hz, anomeric proton of glucose), 6.38 (1H, *d*, J = 16 Hz, ϕ—CH=CH—CO—), 6.92 (1H, *d*, J = 2 Hz, 6-H), 7.18 (δ_A), 7.61 (δ_B) (4H, *q*, J = 9 Hz, A_2B_2 system of *p*-coumaryl), 7.37 (1H, *d*, J = 2 Hz, 8-H), 7 42 (1H, *d*, J = 9 Hz, 5'-H), 7.76 (1H, *d*, J = 16 Hz, ϕ—CH=CH—CO—), 7.9–8.2 (2H, *m*, 2',6'-H).

*6-Methylkaempferol-3-*β-D-*glucopyranoside* (**5**). Yellow needles (from Me_2CO), mp 204–207°, $[\alpha]_D^{17}$ −18.0° (c = 0.25, MeOH). (Found: C, 52 86, H, 5 18. $C_{22}H_{22}O_{11}$, $2H_2O$ requires: C, 53.01; H, 5.26%). λ_{max} nm (e): MeOH 270 (12243), 335 (10742); + $AlCl_3$ 278, 306, 357, 390*sh*; + $AlCl_3$–HCl 280, 305*sh*, 354, 390*sh*; +NaOMe 275, 330, 400 and +NaOAc 275, 307, 386 Hydrolysis with 2N HCl at 100° for 1 hr afforded an aglycone and D-glucose, $[\alpha]_D^{17}$ + 46.0° (c = 0.75, H_2O). The aglycone was recrystallised from dil. MeOH to give yellow needles mp 275–278°. (Found: C, 64.48; H, 4.29. $C_{16}H_{12}O_6$ requires: C, 64.00; H, 4.8%). λ_{max} nm(ε): MeOH 254*sh* (9375), 270 (10875), 300 (5438), 333*sh* (9000), 366 (12375), + $AlCl_3$, 272, 305*sh*, 363, 430;

+ NaOMe 278, 320, 417 and NaOAc 275, 340, 390*sh*. Acetylation (Ac_2O–Py) gave a tetraacetate as needles from MeOH, mp 186–188° (Found C, 61.67; H, 3.98. $C_{24}H_{20}O_{10}$ requires: C, 61.54; H, 4.30%). PMR: δ 2.13 ppm (3H, *s*, ϕ—Me), 2.37, 2.42 (9H, ϕ—OAc × 2 and 3-OAc), 2.50 (3H, *s*, ϕ—OAc), 7.33 (2H, *d*, J = 9 Hz, 3′,5′-H), 7.37 (1H, *s*, 8-H), 7.94 (2H, *d*, J = 9 Hz, 2′,6′-H).

Heptaacetate of **5**. Acetylation gave a product which yielded needles from MeOH, mp 205–208°. (Found: C, 57.56; H, 4.66. $C_{36}H_{36}O_{18}$ requires: C, 51.14; H, 4.80%); $[\alpha]_D^{17}$ −80.0° (c = 0.3, $CHCl_3$). PMR: δ 1.96, 2.02, 2.06, 2.16 ppm (12H, glucose OAc × 4), 2.16 (3H, *s*, ϕ—*Me*), 2.39, 2.43, 2.56 (9*H*, ϕ—OAc × 3), 3.66 (1H, *m*, glucose 5-H), 4.07 (2H, *m*, glucose 6-H), 4.9–5.5 (3H, *m*, glucose 2,3,4-H), 5.67 (1H, *d*, J = 8 Hz anomeric proton of glucose), 7.37 (2H, *d*, J = 9 Hz, 3′,5′-H), 7.40 (1H, *s*, 8-H), 8.20 (2H, *d*, J = 9 Hz, 2′,6′-H). The permethyl ether of **5** was obtained as amorphous powder, $[\alpha]_D^{18}$ −30.0° (c = 15, $CHCl_3$). PMR: δ 2.20 ppm (3H, *s*, ϕ—Me), 3.11 (3H, *s*, glucose OMe), 3.53 (3H, *s*, glucose OMe), 3.67 (6H, glucose OMe × 2), 3.92 (9H, *s*, ϕ—OMe × 3), 5.55 (1H, *m*, $W^{h/2}$ = 9 Hz, anomeric proton of glucose), 6.73 (1H, *s*, 8-H), 7.02 (2H, *d*, J = 9 Hz, 3′,5′-H), 8.13 (2H, *d*, J = 9 Hz, 2′,6′-H). Subsequent methanolysis by reflux with HCl (8%) in MeOH for 30 min and chromatography of the product (showed spots of Me pyranoside of 2,3,4,6-tetra-*O*-Me-α- and β-glucose on TLC) over Si gel with *n*-hexane–EtOAc (1:1) gave a syrup $[\alpha]_D^{18}$ + 109.3° (c = 0.1, $CHCl_3$), and yellow crystals. The former was identified as Me pyranoside of 2,3,4,6-tetra-*O*-methyl-α-D-glucose (TLC, $[\alpha]_D$), the latter was recrystallized from MeOH to give yellow prisms of 6-methylkaempferol-5,7,4′-trimethyl ether, mp 181–182°. λ_{max} nm (ε): MeOH 257 (18810), 330*sh* (17100), 353 (21803); + $AlCl_3$ 270, 300*sh*, 340, 417; + NaOMe 265, 320*sh*, 396 and + NaOAc 258, 380*sh*, 355, 400*sh*. PMR: δ 2.24 ppm (3H, *s*, ϕ—Me), 3.96, 3.98, 4′01 (9H, ϕ—OMe × 3), 6.82 (1H, *s*, 8-H), 7.12 (2H, *d*, J = 9 Hz, 3′,5′-H), 8.28 (2H, *d*, J = 9 Hz, 2′,6′-H).

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