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Convenient syntheses of metabolically important quercetin glucuronides and sulfates

Paul W. Needs* and Paul A. Kroon

BBSRC Institute of Food Research, Norwich Research Park, Colney Lane, Norwich, Norfolk NR4 7UA, UK

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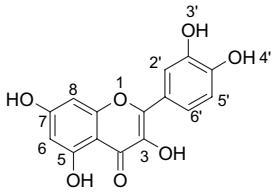
Abstract—Synthetic approaches to the major human plasma metabolites of quercetin, quercetin 3′-sulfate and the β-D-glucopyranosiduronic acid derivatives 3′-methylquercetin 3-glucuronide (isorhamnetin 3-glucuronide), quercetin 3-glucuronide and quercetin 3′-glucuronide are described. This is the first report of the chemical synthesis of quercetin 3′-glucuronide. All procedures start from the same precursor, 4′,7-di-*O*-benzylquercetin, and all are more convenient than existing methods.

1. Introduction

The human diet includes several classes of plant flavonoids; many appear to be protective against coronary heart disease and/or a variety of carcinomas.[1] Quercetin **1** (Scheme 1) is the major flavonol found in plants (though usually in glycosylated forms)[2] and is thus a ubiquitous part of the human diet. In the past, the majority of evidence for the health benefits of quercetin came from in vitro experiments on free quercetin. Recent work has studied the human absorption and metabolism of quercetin derivatives and has shown that quercetin itself is not present in human plasma, but is found instead in various glucuronidated and sulfated forms.[3] In order to better determine the biological effects of quercetin, we required 100 mg quantities of the most abundant circulating forms—the β-D-glucopyranosiduronic acid derivatives quercetin 3-glucuronide **2**, quercetin 3′-glucuronide **3** and 3′-methylquercetin 3-glucuronide (isorhamnetin 3-glucuronide) **4**, together with quercetin 3′-sulfate **5**. Although quercetin glucuronides have been synthesised using liver microsomal preparations,[4,5] this is not convenient if larger quantities of glucuronides are needed; and existing chemical syntheses of both the sulfate and the glucuronides, where available, are either involved and/or low yielding. We describe convenient syntheses from a common easily prepared precursor, 4′,7-di-*O*-benzylquercetin **6** (Scheme 2).



1

Scheme 1. Structure of quercetin **1** (showing ring numbering).

2. Results and discussion

2.1. 4′,7-Di-*O*-benzylquercetin, 6

4′,7-Di-*O*-benzylquercetin, **6**, was prepared as described by Jurd.[6] Although the yields were modest (17–29%), gram quantities of pure **6** were easily obtained without the need for chromatography.

2.2. Quercetin 3-glucuronide, 2

Wagner et al. first reported the synthesis of **2** in 1970.[7] Glucuronidation of **6** with methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide **7** in the presence of silver oxide (Ag_2O), gave **9** in 44% yield (Scheme 2); debenzylation and deacetylation afforded **2** in 24% overall yield. When we attempted this procedure, we failed to obtain any glucuronidated products, or to recover **6**. To ensure anhydrous conditions, we had stirred **6**, Ag_2O and a desiccant (either calcium sulfate, $CaSO_4$, or 3 Å molecular sieves) in pyridine for 2 h before adding **7**. Conversely, when we added **7** immediately, the reaction gave two mono-glucuronidated products, **8a** and **8b**, in 18 and 19% yields, respectively, after

Keywords: Quercetin glucuronide; Quercetin sulfate; Quercetin 3-glucuronide; Quercetin 3′-glucuronide; Isorhamnetin 3-glucuronide; Quercetin 3′-sulfate; Glucuronidation; Sulfation; Synthesis.
* Corresponding author. Tel.: +44 1603 255066; e-mail: needsp@bbsrc.ac.uk

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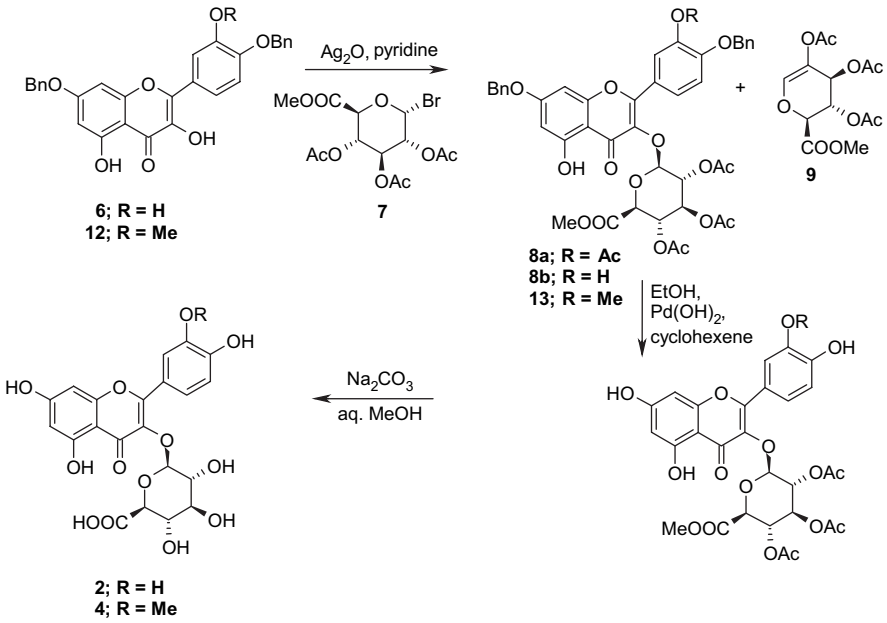


Scheme 2. Synthesis of quercetin 3-glucuronide **2** and isorhamnetin 3-glucuronide **4**.

chromatography. Compound **8a** was contaminated with a trace of the glycal **9** (Scheme 2), a known by-product of glucuronidation of phenols.[8] Both **8a** and **8b** gave only **2** after debenzylation[9] and ester hydrolysis[10] in a combined purified yield of 23% from **6**. Compound **8b** was the expected product; but **8a** was, in addition, 3′-*O*-acetylated. (In contrast, glucosylation of **6** with the corresponding tetraacetylglucosyl bromide gave only the expected 3-glucosylated product—data not shown. We noted the tendency of **7** to transfer an acetyl group to a less acidic, unprotected phenolic hydroxyl during the development of our synthesis of the isoflavone conjugate daizein 7-glucuronide.)[11] When the reaction was repeated at 0 °C, **8a** and **8b** were obtained in an improved combined yield of 52%. Debenzylation and hydrolysis gave, after purification, **2** in 40% overall yield in three steps from **6**. This represents a considerable improvement over both the original report, and a more recent procedure involving selective oxidation of a glucoside derivative.[12]

We briefly investigated why delayed addition of **7** was deleterious to product yield. A ^{1}H NMR spectrum after workup of a mixture of **6**, Ag_2O, pyridine and $CaSO_4$ after 2 h showed no signs of debenzylation. The flavonol signals were greatly broadened, suggesting complexation to silver had occurred, which might account for the failure to react with **7**. Thus the problem might be anticipated in other Ag_2O-catalysed glycosylations where the substrate has a suitable site for complexation, unrelated to the intended reactive site (such as vicinally positioned 5-hydroxy and 4-oxo groups here). No problem occurred in the synthesis of quercetin 7-glucuronide,[13] where all hydroxyls—except the 7—were protected.

2.3. Quercetin 3′-glucuronide, 3

Treatment of **6** with methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate trichloroacetimidate **10** gave **11** and recovered **6** (Scheme 3), which was partially purified by chromatography. No 3-*O*-glucuronidation was observed. Debenzylation and de-esterification gave, after purification, **3** in 11% yield. Although this yield is low, this is the first reported chemical synthesis of this compound. The different regioselectivities of **7** and **10** are noteworthy. Characteristic downfield shifts of the 2′ and 6′-protons were evident in the ^{1}H NMR spectrum (see Table 1 for comparative data). We confirmed the structure of **3** by HMBC, when the expected long range ^{1}H–^{13}C connectivities were observed. Thus H″-1 showed ^3J correlation to C-3′, C-3′ showed ^3J correlation to H-5′ and ^2J correlation to H-2′.

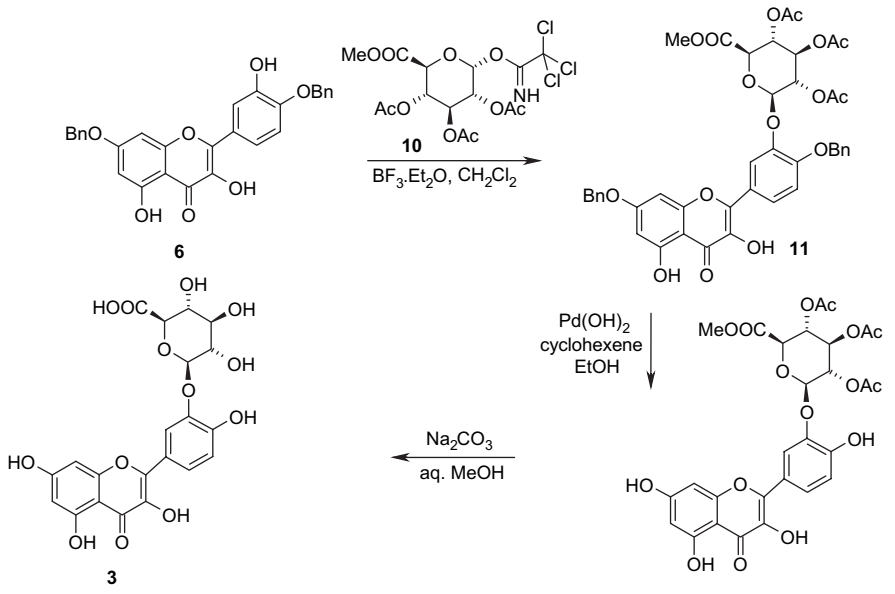
A synthesis of **3** using liver microsomes has been described.[4] Table 2 shows comparative NMR data for this material (the ionic form of the material was not specified) and chemically synthesised **3** (as both free acid and sodium salt).

2.4. 3′-Methylquercetin 3-glucuronide, 4 (isorhamnetin 3-glucuronide)

We reported the first synthesis of this compound, from **6**,[10] but the yield was very low (5%). The procedure was based on the treatment of 3′-*O*-methyl-4′,7-*O*-dibenzylquercetin **12** (derived from **6**) with **10**. Our synthesis of **3** suggested that reaction with **7** would be a better approach, and indeed treatment of **12** with **7** and Ag_2O in pyridine at 0 °C gave crude **13** in 51% yield (Scheme 2). Debenzylation, hydrolysis and purification of **13** gave **4** in 33% yield from **12** (see Table 1). We confirmed the structure of **4** by HMBC, when the expected long range ^{1}H–^{13}C connectivities were observed. Thus H″-1 showed ^3J correlation to C-3 and C-3′ showed ^3J correlation to the protons of the methyl group.

2.5. Quercetin 3′-sulfate 5

Although treatment of **1** with sulfamic acid gives **5**,[3] it is as part of a complex mixture, which is difficult to purify. We thus considered alternative approaches based on sulfation of 3,4′,7-tri-*O*-benzylquercetin **14**. Compound **14** was prepared in 66% yield by treatment of **6** with 1.6 equiv of



Scheme 3. Synthesis of quercetin 3′-glucuronide **3**.

Table 1. ^{1}H NMR shifts/δ (CD_3OD) for quercetin glucuronides (H^+ form)

Proton	Quercetin (**1**)	Quercetin 3-glucuronide (**2**)	Quercetin 3′-glucuronide (**3**)	Isorhamnetin 3-glucuronide (**4**)
2′	7.72	7.61	8.03	7.95
6′	7.62	7.64	7.89	7.54
5′	6.87	6.84	6.95	6.86
8	6.37	6.38	6.39	6.37
6	6.17	6.19	6.15	6.17

Table 2. ^{1}H NMR shifts/δ (DMSO-d_6) for quercetin 3′-glucuronide **3**

Proton	Quercetin (**1**)[a]	**3**		**3**[a,b]
		H^+ form	Na^+ form	
2′	7.67	7.85	7.93	7.98
6′	7.53	7.88	7.85	7.90
5′	6.87	6.99	6.97	6.96
8	6.40	6.48	6.50	6.59
6	6.18	6.20	6.19	6.16

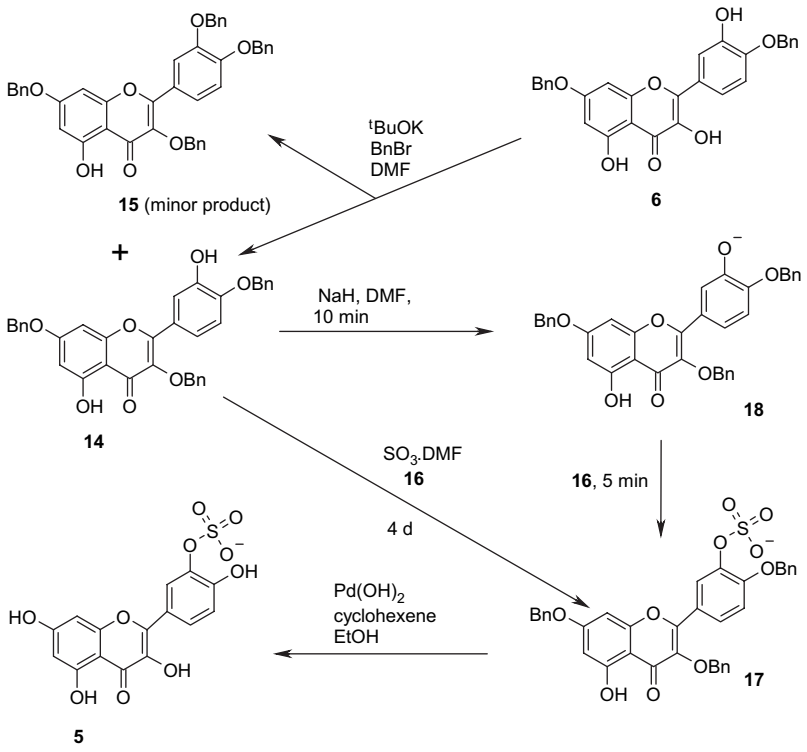
[a] Ref. 4.
[b] Cationic form unspecified.

potassium *tert*-butoxide and benzyl bromide in DMF (Scheme 4; 3,3′,4′,7-tetra-*O*-benzylquercetin **15** was also produced, but these conditions maximised the yield of **14**). Treatment of **14** with a chlorosulfonic acid/pyridine mixture at room temperature—conditions used to sulfate partially protected daidzein derivatives[14]—or overnight at 80 °C was unsuccessful. Treatment of **14** in DMF with sulfur trioxide-*N*,*N*-dimethylformamide complex **16**, a highly reactive sulfating reagent[15] for 4 d, gave **17**, which was debenzylated to give a mixture of **1** and **5** (Scheme 4). The latter were easily separated on a C-18 reverse phase solid phase extraction (SPE) cartridge; elution with water gave pure **5** in 68% yield from **14**. (Alternatively, treatment of **14** with a slight excess of sodium hydride for 10 min, to give the phenoxide anion **18**, followed by addition **16**, also gave, after debenzylation of **18**, a mixture of **1** and **5**, from which **5** was isolated in 45% yield. Though this approach had the advantage of a short reaction time, it was difficult to stoichiometrically control NaH addition on the small scale employed, which lowered the yield). Thus **5** was obtained directly as its sodium salt, avoiding the need for the elaborate purification procedures typically used in sulfate synthesis (see below); but only if the **14** used was pure. We found **14**, prepared by benzylation of **1** as described,[16] contained small amounts of two isomeric tribenzylquercetins, which we were unable to remove; and that these led, after sulfation and deprotection to a mixture of **5** and **1**, together with two other compounds, tentatively identified as quercetin 7-sulfate and quercetin 4′-sulfate (data not shown). Purification of **5** in this case required preparative HPLC (see below); complete separation of the closely running monosulfates was difficult, and the yield of **5** was lowered as a result.

2.6. Purification of quercetin glucuronides and sulfates by preparative HPLC

C-18 reverse phase columns, and gradients of 0.1% aq trifluoroacetic acid (TFA) and acetonitrile, were effective for preparative scale purification of glucuronides. It was necessary, however, to load the crude glucuronides in their acid forms in 50% methanol. Solubility in this solvent was limited; this restricted loading capacity, but this was the best compromise between solubility and solvent polarity. Though sodium salts could be loaded in concentrated aqueous solution, two peaks—one early peak corresponding to the sodium form, and a later one to the acid form—were obtained for each glucuronide, making the approach suited to simple mixtures only.

We also report a substantial improvement in the HPLC purification of sulfates. Although it was not ultimately required for **5** prepared as above, it was used to obtain pure sulfated products from several procedures that were ultimately less efficient and are therefore not reported here.



Scheme 4. Synthesis of quercetin 3′-sulfate **5**.

Aq TFA–acetonitrile gradients were found to be unsuitable. Retention times were highly variable (due presumably to the low pK_a of sulfates) and fractions containing sulfates were prone to acid catalysed sulfate cleavage during subsequent evaporation (data not shown). We found that substituting 50 mM aq ammonium acetate for aq TFA overcame this problem, and products were easily desalted by lyophilisation. Previously reported flavonoid sulfate purifications are generally involved, employ several steps, and use techniques such as size exclusion chromatography, normal phase HPLC, preparative TLC and separation on polyamide.[17–20] As far as we are aware, NH_4OAc–CH_3CN gradients and reverse phase HPLC have not been used before for the isolation of pure flavonoid sulfates.

2.7. Storage and handling of glucuronides and sulfates

We initially stored glucuronides in their acid forms, as protection against possible autooxidative reactions (to which **1** is increasingly susceptible as temperature, pH and/or salt concentrations are raised),[21] in solution in 50% aq methanol at −20 °C; they were indefinitely stable. Solutions were warmed to room temperature and briefly sonicated before use. Although the latter apparently redissolved any material that had precipitated during refrigeration, subsequent filtration through a 0.2 μm membrane led to losses. Methanolic solutions (100%) were filtered without loss, but under these conditions the glucuronic acid moieties were prone to self-catalysed methyl esterification. The addition of water successfully prevented esterification during storage at −20 °C, or, for example, rotary evaporation. Sulfates were stored as solid Na salts, or in solution in aq methanol or methanol, at −20 °C. More recently we have converted glucuronides to their sodium salts by titration, in 50% aq methanol, to pH 6.0 with aq sodium hydroxide, followed by immediate evaporation. The resultant solids were stored at −20 °C, and have again been stable. The salt forms of these materials are freely soluble in water, which is highly convenient for use in cell culture studies, etc. Frozen, millimolar solutions have also been stable. We are unable yet to fully comment on long-term stability; but we have found solutions to be stable for 48 h at 37 °C in culture media.[5]

3. Experimental

3.1. General methods

Solvents were dried over freshly activated 3 Å molecular sieves. Evaporations were performed in vacuo at 50 °C. Solids were dried overnight in vacuo over P_2O_5 before use. TLC was performed on Macherey-Nagel Silica Gel 60/UV254 plates using UV light, or 50% sulfuric acid and charring, for visualisation. MPLC used pre-packed silica cartridges (Isolute Flash Si, Argonaut Technologies) and UV detection. Analytical HPLC and HPLC–ESMS used a 5 μm Lunar column (250×4.4 mm) eluted at 1 mL min^{-1} at 30 °C. Eluant A 80% for 5 min (isocratic); to 10% A at 35 min (gradient). Eluate was monitored by UV detection at 205 and 280 nm. Preparative HPLC of glucuronides used a 5 μm Prodigy ODS3 column (Phenomenex Inc., 250×21.2 mm+60×21.2 mm guard) eluted at 5 mL min^{-1}. Eluant A—0.1% CF_3COOH (TFA); Eluant B—CH_3CN. 80% A for 15 min (isocratic); then to 10% A at 75 min (gradient). Preparative HPLC of sulfates used the same column and flow rate, but different elution conditions: Eluant A—50 mM aq NH_4OAc, pH 6.5; Eluant B—CH_3CN. 80% A for 15 min (isocratic); then to 40% A at 75 min (gradient).

Solid phase extraction (SPE) was performed on TechElut SPE C-18 2000/12 mL cartridges, preconditioned with MeOH and water. Melting points were determined with a Reichert 7905 hot stage microscope, and are uncorrected. ^{1}H and ^{13}C NMR spectra were run on a JEOL EX-270 spectrometer at 21 °C. HMBC NMR spectra were run on a Bruker Avance 600 spectrometer at 27 °C. NMR chemical shifts were referenced to residual solvent absorption. ESMS, APCIMS and HPLC–ESMS analyses were performed on a Micromass Quattro II mass spectrometer. HRMS data were obtained by syringe pump injection of solutions in aq CH_3CN, using either a Micromass LCT MS or a Bruker microTOF, equipped with electrospray sources. IR spectra were measured by Attenuated Total Reflectance (ATR), using a BioRad FTS175C Fourier transform infrared spectrometer with HgCdTe detector and Specac GoldenGate single reflection diamond Horizontal ATR system; 128 scans at 2 cm^{-1} resolution, background spectrum empty crystal. Optical rotation measurements were made on a Perkin Elmer 341 Polarimeter.

3.2. Synthesis of quercetin conjugates

3.2.1. 4′,7-Di-*O*-benzylquercetin, 6. 4′,7-Di-*O*-benzylquercetin, **6**, was prepared from quercetin pentaacetate (25 g) as described by Jurd,[6] except that the product was initially purified by extraction with toluene at 80 °C, rather than with refluxing benzene. Yield of recrystallised **6** (as yellow needles) 4.0–6.8 g, 17–29% (lit.[6] 13%), mp 181–182 °C (lit.[6] 181 °C); δ_H ($CDCl_3$) 11.70 (s, 1H, 5-OH), 7.77 (dd, 1H, *J* 9.6, 2.3 Hz, H-6′), 7.78 (d, 1H, *J* 2.3 Hz, H-2′), 7.29–7.46 (m, 10H, 2×Ph), 7.02 (d, 1H, *J* 9.6 Hz, H-5′), 6.61 (s, 1H, 3-OH), 6.55 (d, 1H, *J* 2.3 Hz, H-8), 6.44 (d, 1H, *J* 2.3 Hz, H-6), 5.77 (s, 1H, 3-OH), 5.18, 5.13 (2×s, 2×2H, 2×C*H*$_2$Ph).

3.2.2. Quercetin 3-glucuronide, 2. Ag_2O (0.27 g, 2.5 equiv), $CaSO_4$ (dried at 120 °C, 0.5 g) and dry pyridine (4 mL) were stirred, in the dark, under Ar at 0 °C for 5 min. Methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide **7** (0.23 g, 580 μmol, 1.25 equiv) was added; and, after a further 5 min, 4′,7-di-*O*-benzylquercetin **6** (0.22 g, 460 μmol). Stirring was continued at 0 °C. After 16 h, aq KCl (10%, 20 mL) and aq AcOH (10%, 100 mL) were added and the mixture was filtered through Celite. The latter was washed with water (2×100 mL), and the crude product eluted with acetone (2×50 mL). The latter was evaporated to give a light brown solid (0.35 g), which was purified by MPLC (20 g silica, 4% acetone/96% toluene isocratic elution) to give two main products **8a** and **8b**.

Compound **8a**: δ_H ($CDCl_3$) 12.42 (s, 1H, 5-OH), 8.01 (dd, 1H, *J* 8.9, 2.3 Hz, H-6′), 7.85 (d, 1H, *J* 2.3 Hz, H-2′), 7.31–7.42 (m, 10H, 2×Ph), 7.10 (d, 1H, *J* 8.9 Hz, H-5′), 6.49 (d, 1H, *J* 2.0 Hz, H-8), 6.42 (d, 1H, *J* 2.0 Hz, H-6), 5.75 (d, 1H, *J* 7.9 Hz, H-1″), 5.1–5.4 (m, 3H, H-2″, H-3″, H-4″), 5.26, 5.11 (2×s, 2×2H, 2×C*H*$_2$Ph), 3.94 (d, 1H, *J* 9.9 Hz, H-5″), 3.58 (s, 3H, OCH_3), 2.33 (s, 3H, 3′-$COCH_3$), 2.10, 2.03, 2.01 (3×s, 3×3H, 3×sugar $COCH_3$); *m*/*z* (APCI, +ve mode) 863 [$(M+Na)^+$].

Compound **8b**: δ_H ($CDCl_3$) 12.43 (s, 1H, 5-OH), 7.68 (dd, 1H, *J* 8.6, 2.3 Hz, H-6′), 7.63 (d, 1H, *J* 2.3 Hz, H-2′), 7.27–7.46 (m, 10H, 2×Ph), 7.02 (d, 1H, *J* 8.6 Hz, H-5′), 6.49 (d, 1H, *J* 2.0 Hz, H-8), 6.42 (d, 1H, *J* 2.0 Hz, H-6), 5.83 (s, 1H, 3′-OH), 5.73 (d, 1H, *J* 7.9 Hz, H-1″), 5.1–5.4 (m, 3H, H-2″, H-3″, H-4″), 5.21, 5.11 (2×s, 2×2H, 2×C*H*$_2$Ph), 4.00 (d, 1H, *J* 10.2 Hz, H-5″), 3.60 (s, 3H, OCH_3), 2.10, 2.02, 2.00 (3×s, 3×3H, 3×$COCH_3$); *m*/*z* (APCI, +ve mode) 821 [$(M+Na)^+$].

Compounds **8a** and **8b** were not further purified or characterised but were debenzylated, de-esterified and purified by HPLC as follows. The products were recombined and suspended in a mixture of EtOH (80 mL) and cyclohexene (20 mL). Twenty percent $Pd(OH)_2$ on charcoal (200 mg) was added. The mixture was refluxed under Ar for 30 min, cooled and filtered through a 0.5 μm filter. The filtrate was evaporated and the residue was suspended in 50% aq MeOH (200 mL). Aq Na_2CO_3 (0.5 M, 6 mL) was added and the mixture was stirred under Ar at room temperature for 2.5 h. After cooling Dowex 50W resin (H^+ form) was added with stirring until the pH fell to below 3.0. The mixture was filtered (0.5 μm), the resin was washed (50% aq MeOH, 1×50 mL; MeOH, 1×50 mL) and filtrate and resin washes were combined and evaporated. The residue was dissolved in 50% aq MeOH (8 mL). Compound **2** was isolated by preparative HPLC (2×4 mL injections) as an orange glass and was >95% pure by analytical HPLC. It was identical to authentic material.[22] Yield 89 mg (41% from **6**).

Compound **2** (H+ form): δ_H (CD_3OD) 7.64 (dd, 1H, *J* 8.9, 2.3 Hz, H-6′), 7.61 (d, 1H, *J* 2.3 Hz, H-2′), 6.84 (d, 1H, *J* 8.9 Hz, H-5′), 6.38 (d, 1H, *J* 2.0 Hz, H-8), 6.19 (d, 1H, *J* 2.0 Hz, H-6), 5.32 (d, 1H, *J* 7.6 Hz, H-1″), 3.74 (d, 1H, *J* 9.6 Hz, H-5″), 3.42–3.62 (m, 3H, H-2″, H-3″, H-4″).

Compound **2** (lit.[22] H+ form): δ_H (CD_3OD) 7.63 (H-6′), 7.63 (H-2′), 6.84 (H-5′), 6.37 (H-8), 6.18 (H-6), 5.31 (H-1″), 3.75 (H-5″), 3.47–3.59 (H-2″, H-3″, H-4″).

3.2.3. Quercetin 3′-glucuronide, 3. Compound **6** (0.36 g, 0.75 mmol), powdered 3 Å molecular sieves (0.5 g) and dry CH_2Cl_2 (5 mL) were stirred at room temperature under Ar for 2 h. The trichloroacetimidate **10** (0.39 g, 1.1 equiv) was added and the mixture was cooled to −15 °C. $BF_3{\cdot}Et_2O$ (freshly distilled, 120 μL, 1.25 equiv) was added drop wise over 10 min. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was filtered through Celite and the latter was washed with CH_2Cl_2, acetone and methanol until the washings were colourless. Filtrate and washings were combined and evaporated to give a bright yellow glass. The crude product was purified by MPLC (20 g silica, 5% acetone/95% toluene isocratic elution) to give recovered **6** (0.21 g, 58%) and crude **11** (0.10 g).

Compound **11**: δ_H ($CDCl_3$) 11.65 (s, 1H, 5-OH), 8.05 (d, 1H, *J* 2.3 Hz, H-2′), 7.94 (dd, 1H, *J* 8.6, 2.3 Hz, H-6′), 7.28–7.46 (m, 10H, 2×Ph), 7.02 (d, 1H, *J* 8.6 Hz, H-5′), 6.69 (s, 1H, 3-OH), 6.58 (d, 1H, *J* 2.0 Hz, H-8), 6.41 (d, 1H, *J* 2.0 Hz, H-6), 5.76 (d, 1H, *J* 7.9 Hz, H-1″), 5.1–5.4 (m, 3H, H-2″, H-3″, H-4″), 5.36, 5.14 (2×s, 2×2H, 2×C*H*$_2$Ph), 4.16 (d, 1H, *J* 10.2 Hz, H-5″), 3.70 (s, 3H, OCH_3), 2.05 (s, 3×3H, 3×$COCH_3$).

Compound **11** was not further characterised or purified but was debenzylated, de-esterified and purified in the same

way as **8a** and **8b** (proportionately scaled down) to give **3** as an orange glass. Yield 39 mg (11% from **6**). The substitution position was confirmed by HMBC (see text); **3** ν_{max} (ATR of solid) 3208br d, 1675, 1652, 1616, 1595, 1570, 1502, 1412, 1357, 1268, 1189, 1161, 1136, 1003; $[\alpha]_D^{25}$ −44 (*c* 1.0, water).

Compound **3** (H^+ form): δ_H (CD_3OD) 8.03 (d, 1H, *J* 2.0 Hz, H-2′), 7.89 (dd, 1H, *J* 8.6, 2.0 Hz, H-6′), 6.95 (d, 1H, *J* 8.6 Hz, H-5′), 6.39 (d, 1H, *J* 2.0 Hz, H-8), 6.15 (d, 1H, *J* 2.0 Hz, H-6), 4.92 (d, 1H, *J* 6.9 Hz, H-1″), 4.04 (d, 1H, *J* 9.6 Hz, H-5″), 3.52–3.70 (m, 3H, H-2″, H-3″, H-4″).

Compound **3** (H^+ form): δ_H (DMSO-d_6) 12.43 (s, 1H, 5-OH), 9.45 (br d s, 1H, COOH), 7.88 (dd, 1H, *J* 8.2, 2.0 Hz, H-6′), 7.85 (d, 1H, *J* 2.0 Hz, H-2′), 6.99 (d, 1H, *J* 8.2 Hz, H-5′), 6.48 (d, 1H, *J* 2.0 Hz, H-8), 6.20 (d, 1H, *J* 2.0 Hz, H-6), 5.2 (br d s, 1H, 3-OH), 4.92 (d, 1H, *J* 6.9 Hz, H-1″), 3.79 (d, 1H, *J* 8.9 Hz, H-5″), 3.3–3.5 (m, 3H, H-2″, H-3″, H-4″).

Compound **3** (Na^+ form, obtained by titration in aq MeOH to pH 6.0 with aq NaOH, and evaporation): δ_H (DMSO-d_6) 12.46 (s, 1H, 5-OH), 7.93 (d, 1H, *J* 2.3 Hz, H-2′), 7.85 (dd, 1H, *J* 8.6, 2.3 Hz, H-6′), 6.97 (d, 1H, *J* 8.6 Hz, H-5′), 6.50 (d, 1H, *J* 1.6 Hz, H-8), 6.19 (d, 1H, *J* 1.6 Hz, H-6), 5.04 (br d s, 1H, 3-OH), 4.73 (d, 1H, *J* 6.9 Hz, H-1″), 3.44 (d, 1H, *J* 8.9 Hz, H-5″), 3.2–3.4 (m, 3H, H-2″, H-3″, H-4″); δ_C (DMSO-d_6) 175.9 (C-4), 171.5 (C-6″), 164.1 (C-7), 160.5 (C-5), 156.1 (C-9), 149.6 (C-4′), 146.3 (C-2), 145.3 (C-3′), 135.9 (C-3), 124.2 (C-6′), 121.6 (C-1′), 117.9 (C-2′), 116.5 (C-5′), 103.3 (C-1″), 102.9 (C-10), 98.2 (C-6), 93.7 (C-8), 74.2 (C-5″), 74.5, 76.0, 73.0 (C-2″, C-3″, C-4″).

HRMS (ESI, −ve mode): $(M-H)^-$, found 477.0686. $C_{21}H_{17}O_{13}$ requires 477.0669.

3.2.4. 3′-*O*-Methyl-4′,7-di-*O*-benzylquercetin, 12. 3′-*O*-Methyl-4′,7-di-*O*-benzylquercetin, **12**, was prepared from 3,3′,5,4′,7-di-*O*-benzylquercetin triacetate (1.66 g) as described by Jurd,[6] except that the crude product after filtration and acidification was purified by MPLC (silica, 2% acetone/toluene). Yield of **12** (as a yellow powder) 0.18 g, 15% (lit.[6] 12%); mp 172–174 °C (lit.[6] 175 °C); δ_H ($CDCl_3$) 11.71 (s, 1H, 5-OH), 7.79 (d, 1H, *J* 2.3 Hz, H-2′), 7.74 (dd, 1H, *J* 8.9, 2.3 Hz, H-6′), 7.33–7.46 (m, 10H, 2×Ph), 7.00 (d, 1H, *J* 8.9 Hz, H-5′), 6.60 (s, 1H, 3-OH), 6.56 (d, 1H, *J* 2.0 Hz, H-8), 6.52 (d, 1H, *J* 2.0 Hz, H-6), 5.24, 5.14 (2×s, 2×2H, 2×CH_2Ph), 3.98 (s, 3H, OCH_3).

3.2.5. 3′-Methylquercetin 3-glucuronide (isorhamnetin 3-glucuronide), 4. Ag_2O (0.71 g, 2.5 equiv), $CaSO_4$ (dried at 120 °C, 0.6 g) and dry pyridine (10 mL) were stirred, in the dark, under Ar at 0 °C. After 5 min, methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide **7** (0.61 g, 580 μmol, 1.25 equiv) was added; and, after a further 5 min, 3′-*O*-methyl-4′,7-di-*O*-benzylquercetin **12** (0.61 g, 1.23 mmol). Stirring was continued at 0 °C. After 16 h, the reaction was worked up as described for **8a** and **8b** (on 2.5×the scale) to give 0.82 g of a light brown solid. MPLC (50 g silica, 5% acetone/95% toluene, isocratic elution) gave 0.42 g (51%) of **13**; δ_H ($CDCl_3$) 12.40 (s, 1H, 5-OH), 7.77 (d, 1H, *J* 2.0 Hz, H-2′), 7.53 (dd, 1H, *J* 8.6, 2.0 Hz, H-6′), 7.29–7.46 (m, 10H, 2×Ph), 6.92 (d, 1H, *J* 8.6 Hz, H-5′), 6.41 (d, 1H, *J* 2.0 Hz, H-8), 6.35 (d, 1H, *J* 2.0 Hz, H-6), 5.80 (d, 1H, *J* 7.9 Hz, H-1″), 5.12–5.41 (m, 3H, H-2″, H-3″, H-4″), 5.21, 5.01 (2×s, 2×2H, 2×CH_2Ph), 4.05 (d, 1H, *J* 9.9 Hz, H-5″), 4.00 (s, 3H, OCH_3), 3.55 (s, 3H, OCH_3), 2.10, 2.02, 1.99 (3×s, 3×3H, 3×$COCH_3$).

Compound **13** was not further characterised or purified but was debenzylated, de-esterified and purified in the same way as **8a** and **8b** (proportionately scaled up) to give **4** as an orange glass, identical in all respects to authentic material,[10] yield 198 mg (33% from **6**). The substitution position was confirmed by HMBC (see text); ν_{max} (ATR of solid) 3223br d (OH), 1680, 1652, 1599, 1563, 1463, 1427, 1353, 1286, 1242, 1198, 1181, 1167, 1124; $[\alpha]_D^{25}$ −13 (*c* 1.0, water).

Compound **4** (H^+ form): δ_H (CD_3OD) 7.95 (d, 1H, *J* 2.4 Hz, H-2′), 7.54 (dd, 1H, *J* 8.2, 2.0 Hz, H-6′), 6.86 (d, 1H, *J* 8.2 Hz, H-5′), 6.37 (d, 1H, *J* 2.0 Hz, H-8), 6.17 (d, 1H, *J* 2.0 Hz, H-6), 5.48 (d, 1H, *J* 7.2 Hz, H-1″), 3.93 (s, 3H, OCH_3), 3.78 (d, 1H, *J* 9.2 Hz, H-5″), 3.46–3.62 (m, 3H, H-2″, H-3″, H-4″); δ_C (CD_3OD) 179.1 (C4), 171.6 (C6″), 166.0 (C7), 163.0 (C5), 158.7 (C2), 158.4 (C9), 150.9 (C3′), 148.4 (C4′), 135.1 (C3), 123.5 (C6′), 122.8 (C1′), 116.0 (C5′), 114.4 (C2′), 105.7 (C10), 104.0 (C1″), 99.9 (C6), 94.8 (C8), 77.5 (C3″), 77.2 (C5″), 75.6 (C2″), 73.0 (C4″), 55.7 (OCH_3).

Compound **4** (Na^+ form): δ_H (CD_3OD) 7.99 (d, 1H, *J* 2.0 Hz, H-2′), 7.56 (dd, 1H, *J* 8.3, 2.0 Hz, H-6′), 6.89 (d, 1H, *J* 8.3 Hz, H-5′), 6.38 (d, 1H, *J* 2.0 Hz, H-8), 6.17 (d, 1H, *J* 2.0 Hz, H-6), 5.38 (m, 1H, H-1″), 3.93 (s, 3H, OCH_3), 3.44–3.57 (m, 4H, H-2″, H-3″, H-4″, H-5″).

HRMS (ESI, +ve mode): $(M+H)^+$, found 537.0642. $C_{22}H_{19}Na_2O_{13}$ requires 537.0616.

3.2.6. 3,4′,7-Tri-*O*-benzylquercetin, 14. Compound **6** (2 g, 4.15 mmol) was dissolved with stirring in dry DMF (35 mL) under Ar at 21 °C. ^{t}BuOK (0.745 g, 1.6 equiv) was added, followed by BnBr (790 μL, 1.6 equiv). Stirring was continued overnight at 21 °C for 17 h. The mixture was poured into stirred 10% aq HCl (375 mL) and cooled to 5 °C for 2 h. The resultant solid was recovered by filtration, washed with water, dissolved in EtOAc (300 mL), washed twice with water (2×100 mL) and dried ($MgSO_4$). Evaporation gave a solid (2.38 g) that was purified by MPLC (50 g silica, 100% toluene for 10 min, then to 5% acetone/95% toluene after another 5 min) to give **14** (1.57 g, 66%) as yellow needles, mp 155 °C (lit.[16] mp not given).

Compound **14**: δ_H (DMSO-d_6) 12.67 (s, 1H, 5-OH), 9.44 (s, 1H, 3′-OH), 7.57 (d, 1H, *J* 2.3 Hz, H-2′), 7.53–7.29 (m, 16H, H-6′, 3×Ph), 7.13 (d, 1H, *J* 8.8 Hz, H-5′), 6.80 (d, 1H, *J* 2.0 Hz, H-8), 6.48 (d, 1H, *J* 2.0 Hz, H-6), 5.23–5.32 (br s, 4H, 2×CH_2Ph), 5.03 (s, 2H, CH_2Ph).

Compound **14** (lit.[16]): δ_H (DMSO-d_6) 12.67 (s, 1H, 5-OH), 9.47 (s, 1H, 3′-OH), 7.56 (d, 1H, *J* 1.5 Hz, H-2′), 7.52–7.29 (m, 16H, H-6′, 3×Ph), 7.13 (d, 1H, *J* 8.8 Hz, H-5′), 6.80 (d, 1H, *J* 1.5 Hz, H-8), 6.47 (d, 1H, *J* 1.5 Hz), 5.23–5.32 (br s, 4H, 2×CH_2Ph), 5.02 (s, 2H, CH_2Ph).

3.2.7. Quercetin 3′-sulfate, 5. Compound **14** (0.252 g, 440 μmol) was dissolved in dry DMF (5 mL) with stirring

under Ar. A solution of sulfur trioxide-*N*,*N*-dimethylformamide complex **16** (0.340 g, 5 equiv, weighed and kept under Ar) in DMF (5 mL) was added. After 4 d, the reaction mixture was evaporated, and satd $NaHCO_3$ (6 mL) was added with stirring (final pH 7.6). The mixture was again evaporated to dryness and the residue was dissolved in acetone (150 mL) and filtered (sinter). The residual solid was washed with acetone (50 mL). (Attempts to isolate more products from the residual undissolved material were unsuccessful.) The acetone washings and filtrate were combined and evaporated to a yellow glass (0.27 g). This material was not further purified but was dissolved in EtOH (50 mL); cyclohexene (25 mL) and $Pd(OH)_2$/C (250 mg) were added, and the mixture was heated at reflux (30 min) and cooled. The mixture was filtered (0.5 μm membrane) and evaporated to give a yellow solid (0.34 g). The latter was redissolved in water (10 mL), loaded onto an SPE cartridge and eluted with water (100 mL) to give **5** (121 mg, 68% from **14**) as a yellow solid, identical to authentic material.[23]

Compound **5** (Na^+ form): δ_H (DMSO-d_6) 12.45 (br s, 1H, 5-OH), 9.47 (br s, 2H, 2×OH), 8.03 (d, 1H, *J* 2.0 Hz, H-2′), 7.85 (dd, 1H, *J* 8.6, 2.0 Hz, H-6′), 6.98 (d, 1H, *J* 8.6 Hz, H-5′), 6.44 (d, 1H, *J* 1.9 Hz, H-8), 6.19 (d, 1H, *J* 1.9 Hz, H-6).

Compound **5** (lit.[23], Na^+ form): δ_H (DMSO-d_6) 7.99 (d, 1H, *J* 2.4 Hz, H-2′), 7.80 (dd, 1H, *J* 7.6, 2.4 Hz, H-6′), 6.93 (d, 1H, *J* 7.6 Hz, H-5′), 6.39 (d, 1H, *J* 1.5 Hz, H-8), 6.14 (d, 1H, *J* 1.5 Hz, H-6).

Compound **5** (Na^+ form): δ_H (CD_3OD) 8.20 (d, 1H, *J* 2.3 Hz, H-2′), 8.01 (dd, 1H, *J* 8.9, 2.3 Hz, H-6′), 7.01 (d, 1H, *J* 8.9 Hz, H-5′), 6.42 (d, 1H, *J* 1.9 Hz, H-8), 6.16 (d, 1H, *J* 1.9 Hz, H-6). *m*/*z* (ESI, −ve mode) 381 ($(M-H)^-$).

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