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Note

Syntheses of daidzein-7-yl β-D-glucopyranosiduronic acid and daidzein-4′,7-yl di-β-D-glucopyranosiduronic acid

Paul W. Needs,* Gary Williamson

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

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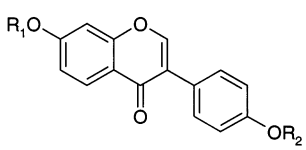
Abstract

Syntheses of the title compounds — commonly known as 'daidzein 7-glucuronide' and 'daidzein 4′,7-diglucuronide' — are described. Selective 7-deacetylation of 4′,7-di-*O*-acetyldaidzein is employed.

Keywords: Daidzein; Glucuronide; Daidzein 7-glucuronide; Daidzein 4′,7-diglucuronide; Synthesis

Several classes of plant flavonoids form part of the human diet. They are of great current interest, as many appear to be protective against coronary heart disease and/or a variety of carcinomas.[1,2] One type, the isoflavones, are additionally suspected of estrogenic activity.[3] Daidzein **1**, occurs as its 7-β-glucopyranoside, daidzin **2**, in soya-based foods. After absorption from the intestinal tract, together with deglucosylation,[4] the resultant daidzein is thought, in common with other absorbed nonnutrients, to be glucuronidated prior to circulation and excretion.[5] We thus required a synthetic route to the title compounds daidzein-7-yl β-D-glucopyranosiduronic acid **3** and daidzein-4′,7-yl di-β-D-glucopyranosiduronic acid **4**, in order to study their possible biological effects.



	R_1	R_2
1	H	H
2	β-D-Glcp	H
3	β-D-GlcpA	H
4	β-D-GlcpA	β-D-GlcpA
5	Ac	Ac
6	H	Ac

Glycosylation of daidzein with protected, simple aldopyranosyl bromides to give 7-glycosides directly has been reported, albeit in low yield.[6] These reactions were performed in aqueous acetone with hydroxide catalysis. More recent work suggests that C-ring cleavage is, in fact, the predominant reaction under these conditions.[7] We thus attempted glucuronidation of daidzein with **7** under non-aqueous conditions, which had successfully provided some other flavonoid derivatives.[8] Thus **1** was treated with 1.1 equiv of **7** and silver carbonate in pyridine, or with 3 equiv of **7** and silver carbonate in pyridine or DMF.

* Corresponding author. Tel.: +44-1603-255000; fax: +44-1603-507723.

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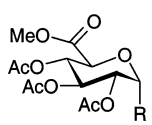
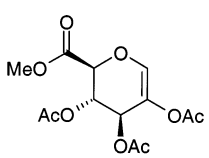
These reactions were unsuccessful; they gave mainly the glycal **9**, recovered **7**, and daidzein. Only minor amounts of glucuronidated products were obtained. In no case was the expected **11** detected. NMR showed that all products were 4′-O-acetylated; some **10** was present, together with variable amounts of some other, unassigned products, apparently bearing 7-substituents other than the expected derivatised glucuronic acid group. A two-phase procedure,[7] which has been reported to give good yields of neutral daizein-7-yl glycosides also failed to give **11**. These results were not entirely unexpected; **9** is often a major product of reactions of **7** with 'complex polyphenolics', and yields of conjugates are often low.[9,10]

Compound **3** was synthesised as follows. Daidzein **1** was diacetylated under acidic conditions[11] to give **5**. Regioselective deacetylation of **5** with imidazole in aqueous THF at pH 7 gave the 4′-*O*-acetyl derivative **6**. Treatment of **6** in dichloromethane with methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide (**7**), silver carbonate and collidine gave the corresponding 7-glucuronic acid derivative **10** in 30% yield, together with the glycal **9**. Compound **10** was converted, with lithium hydroxide in aqueous methanol at 0 °C, to give **3** in 67% yield.



	R
7	Br
8	$OC(NH)CCl_3$



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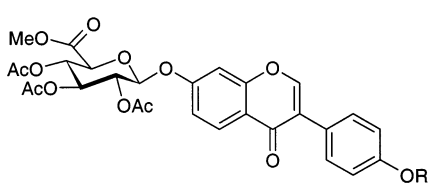
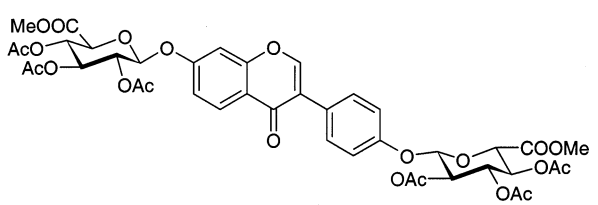


	R
10	OAc
11	H

Compound **12**, the precursor of **4**, was obtained by treatment of daidzein with boron trifluoride and 2 equiv of the trichloroacetimidate **8**, but in only 14% yield. Traces of the 7-substituted product were also detected. Hydrolysis of **12** with LiOH, as above, gave not only **4**, but also the ring-opened product **13**. The decreased susceptibility of **10** to ring opening is presumably a consequence of its conversion, after cleavage of the 4′-*O*-acetyl group, to a phenolic anion which is resistant to nucleophilic attack. However, treatment of **12** with sodium carbonate in aqueous methanol gave **4**, with only traces of **13**, as shown by high-performance liquid chromatography–electrospray mass spectroscopy (HPLC–ESMS). Preparative HPLC gave pure **4**. No attempt has been made to optimise the synthesis of **12**, as the yield of **4** was sufficient for use as an HPLC standard and in biological assays.



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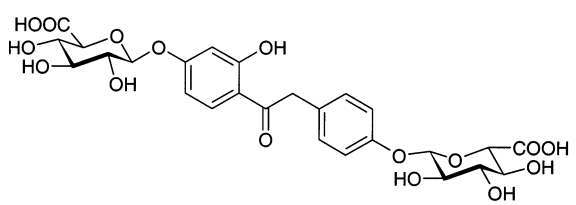

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1. Experimental

General methods.—Solvents were dried over freshly activated 3 Å molecular sieves. Evaporations were performed in vacuo at 40 °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 2 or 9% MeOH in CH_2Cl_2 as eluant, and UV light, or 50% sulphuric acid and charring, for visualisation. Medium pressure column chromatography (MPLC) was performed on a 2.5×60 cm column of Merck 109385 grade silica (40–63 μm mesh), eluted

with 1% MeOH–CH_2Cl_2. Analytical HPLC, and HPLC–ESMS, used a 5 μm Prodigy ODS3 column (250 × 4.4 mm) eluted at 1 mL/min. 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 used a 5 μm Prodigy ODS3 column (Phenomenex Inc., 250 × 21.2 mm + 60 × 21.2 mm guard) eluted at 5 mL/min. 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). NMR spectra were run on a JEOL GX400 spectrometer. Sample temperature was 27 °C and chemical shifts were using the residual solvent's absorption for calibration. Atmospheric pressure chemical-ionisation mass spectroscopy (APCIMS), ESMS, and HPLC–ESMS analyses were performed on a Micromass Quattro II mass spectrometer (Manchester, UK). High resolution fast atom bombardment (FABMS) spectra were obtained on a Kratos MS890 mass spectrometer using glycerol or thioglycerol as a matrix.[13] Elemental analyses were performed on a Carlo Erba 1108 CHN analyser by Satco Ltd. (Hatfield, UK). Melting points were determined on a Reichert Thermopan Koffler hot stage microscope, and are uncorrected.

4′-O-Acetyldaidzein (**6**).—4′,7-Di-*O*-acetyldaidzein **5** (6.0 g, 9.0 mmol) (prepared by treatment of daidzein with concd H_2SO_4 and Ac_2O[11] in 87% yield) was dissolved, with gentle warming, in THF (750 mL). After cooling to 25–30 °C, a solution (750 mL) of imidazole in water (1 M, adjusted to pH 7 with concd HCl) was added, and the mixture stirred for 15 min at 25–30 °C, after which time an initial slight precipitate had redissolved. The mixture was adjusted to pH 4.5 with gl AcOH, water (1 L) was added, and the mixture extracted with CH_2Cl_2 (3 × 400 mL). The combined organic extracts were washed with water (1 × 500 mL), satd Na_2CO_3 (1 × 500 mL) and water (1 × 500 mL), dried ($MgSO_4$), and evaporated to dryness. The resultant solid was recrystallised from EtOH (500 mL). Evaporation of the filtrate, and recrystallisation of the solid from MeOH, yielded a further crop of product. The combined yield of white flakes of **6** was 5.28 g (94%), mp 225.5–226.5 °C. ^{1}H NMR $(CD_3)_2SO$: 8.40 (s, 1 H, H-2), 7.98 (d, 1 H, $J_{5,6}$ 8.8 Hz, H-5), 7.60 (dd, 2 H, AA′BB′ system, $J_{2',3'}$ 8.8 Hz, H-2′, H-6′), 7.18 (AA′BB′ system, 2 H, H-3′, H-5′), 6.94 (dd, 1 H, $J_{6,8}$ 2.0 Hz, H-6), 6.88 (d, 1 H, H-8), 2.28 (s, 3 H, 4′-$COCH_3$). APCIMS: *m/z* 295 $[M-H]^-$. Anal. Calcd for $C_{17}H_{12}O_5$: C, 68.92; H, 4.08. Found C, 68.42; H, 4.12.

Methyl (4′ - O - acetyldaidzein - 7 - yl β - D-2″,3″,4″ - tri - O - acetylglucopyranosid)uronate (**10**).—A suspension of **6** (3 g, 10.1 mmol), **7** (4.43 g, 1.1 equiv), Ag_2CO_3 (3.07 g, 1.1 equiv) and ground 3 Å molecular sieves (2 g) in CH_2Cl_2 (120 mL) was stirred under Ar at rt. Collidine (1.47 mL, 1.1 equiv) was added dropwise over 10 min. The mixture was stirred in the dark for 7 days, and filtered through celite. The latter was washed with 10% MeOH–CH_2Cl_2 (50 mL). The filtrates were combined and washed successively with 10% aq AcOH (2 × 50 mL), water (1 × 100 mL), 0.1 M $Na_2S_2O_3$ (1 × 100 mL), water (1 × 100 mL), satd $NaHCO_3$ (1 × 100 mL), and water (1 × 100 mL), and dried ($MgSO_4$). Evaporation gave 4.89 g of a solid, which was dissolved in 50 mL 1% MeOH–CH_2Cl_2 and cooled to −20 °C overnight. Precipitated **6** (0.27 g) was filtered off. Polar material, and further residual **6** were removed by step elution through a short silica column with 1, 2 and 10% MeOH–CH_2Cl_2, and pooling of appropriate fractions. This gave a crude product (3.7 g) virtually free of **6**. This was used to prepare **3** without further purification.

A sample of the crude product (0.5 g) was purified by MPLC to give 254 mg (30%) of **10**. A portion was recrystallised from MeOH to give white crystals mp 186–188 °C (after drying in vacuo at 70 °C). ^{1}H NMR ($CDCl_3$): 8.25 (d, 1 H, $J_{5,6}$ 8.4 Hz, H-5), 7.97 (s, 1 H, H-2), 7.58 (AA′BB′ system, 2 H, $J_{2',3'}$ 8.4 Hz, H-2′, H-6′), 7.16 (AA′BB′ system, 2 H, H-3′, H-5′), 7.08 (d, 1 H, $J_{8,6}$ 2.4 Hz, H-8), 7.05 (dd, 1 H, H-6), 5.27–5.44 (m, 4 H, H-1″, H-2″, H-3″, H-4″), 4.29 (d, 1 H, $J_{5'',4''}$ 9.2 Hz, H-5″), 3.73 (s, 3 H, $COOCH_3$), 2.32 (s, 3 H, 4′-OAc), 2.08, 2.07, 2.06 (3 s, 3 × 3 H, 3 × $COCH_3$). APCIMS: *m/z* 613 $[M+H]^+$. Anal. Calcd for $C_{30}H_{28}O_{14}$: C, 58.83; H, 4.61. Found C, 58.66; H, 4.50.

Daidzein-7-yl β-D-glucopyranosiduronic acid (**3**).—Crude **10** (0.5 g, containing 254 mg,

0.41 mmol of pure **10**) was sonicated into suspension in MeOH (30 mL), and cooled to 1 °C. Aq LiOH (30 mL, 0.5 M), precooled to 1 °C was added, and the mixture stirred at 1 °C for 16 h. Dowex 50W resin (H^+ form, 20 mL) was added; after stirring for a further 10 min, the solution was eluted through a 5×2 cm column of Dowex, and the latter was washed with MeOH. The combined eluate was evaporated to dryness, and the residue dissolved in MeOH (4 mL). The material (4×1 mL) was purified by preparative HPLC (see above), and purity was checked by analytical HPLC. Yield 120 mg, 67%. ^{1}H NMR (CD_3OD): δ 8.19 (s, 1 H, H-2), 8.15 (d, 1 H, $J_{5,6}$ 9.0 Hz, H-5), 7.38 (AA′BB′ system, 2 H, $J_{2',3'}$ 8.4 Hz, H-2′, H-6′), 7.25 (d, 1 H, $J_{8,6}$ 2.4 Hz, H-8), 7.22 (dd, 1 H, H-6), 6.84 (AA′BB′ system, 2 H, H-3′, H-5′), 5.17 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1″), 4.01 (d, 1 H, $J_{5,4}$ 10.0 Hz, H-5″), 3.4–3.7 (m, 3 H, H-2″, H-3″, H-4″). ESMS: m/z 429 $[M-H]^-$. FABMS (glycerol matrix): m/z $[M-H]^-$. Anal. Calcd 429.0822; Found 429.0899.

*Daidzein-4′,7-yl di-[methyl(2″,3″,4″-tri-*O*-acetyl-β-*D*-glucopyranosiduronic acid)]* (**12**).—The trichloroimidate **8** (0.24 g, 0.50 mmol) and daidzein **1** (62 mg, 0.24 mmol) were suspended with stirring in CH_2Cl_2 (5 mL) at −15 °C under Ar. $BF_3{\cdot}Et_2O$ (8 μL) was added in one portion. The mixture was allowed to warm to rt over 1 h, after which a further portion of $BF_3{\cdot}Et_2O$ (8 μL) was added; stirring was continued for 60 h. EtOAc (100 mL) was added, and the mixture was washed with satd $NaHCO_3$ (2×50 mL), and water (1×50 mL), and dried ($MgSO_4$). The solution was evaporated to dryness, the resulting solid was triturated with CH_2Cl_2, and the insoluble material filtered off to yield recovered daidzein (40 mg, 65%). The solution was evaporated, and MPLC of the crude product, using 1% MeOH–CH_2Cl_2 as eluant, gave **12**, (29 mg, 14%) as a colourless glass. ^{1}H NMR ($CDCl_3$): 8.24 (d, 1 H, $J_{5,6}$ 8.8 Hz, H-5), 7.94 (s, 1 H, H-2), 7.49 (AA′BB′ system, 2 H, $J_{2',3'}$ 8.6 Hz, H-2′, H-6′), 7.07 (AA′BB′ system, 2 H, H-3′, H-5′), 7.07 (d, 1 H, $J_{8,6}$ 2.4 Hz, H-8), 7.05 (dd, 1 H, H-6), 5.27-5.44 (m, 7 H, $1 \times$ H-1″ (7-substituent)), $2 \times$ H-2″, $2 \times$ H-3″, $2 \times$ H-4″), 5.17 (d, 1 H, $J_{1,2}$ 6.8 Hz, H-1″ (4′-substituent)), 4.27 (d, 1 H, $J_{5'',4''}$ 9.2 Hz, H-5″ (7-substituent)), 4.19 (d, 1 H, $J_{5'',4''}$ 9.6 Hz, H-5″ (4′-substituent)), 3.74 and 3.73 ($2 \times$ s, 2×3 H, $2 \times COOCH_3$), 2.10, 2.08, 2.07, 2×2.06, 2.05 (6 s, 6×3 H, $6 \times COCH_3$). APIMS: m/z 887 $[M+H]^+$.

*Daidzein-4′,7-yl di-β-*D*-glucopyranosiduronic acid* (**4**).—Complex **12** (2.34 mg, 2.64 μmol) was sonicated into suspension in MeOH (300 μL) and stirred, and water (100 μL) and aq Na_2CO_3 (30 μL, 0.5 M, 15 μmol) were added at 20 °C. After 90 min, further MeOH (300 μL) and water (100 μL) were added. After 150 min, all solids had dissolved. The solution was desalted (Dowex 50W, H^+) and evaporated. Preparative HPLC gave **4**; purity was checked by analytical HPLC. ^{1}H NMR ($(CD_3)_2SO$): δ 8.42 (s, 1 H, H-2), 8.03 (d, 1 H, $J_{5,6}$ 8.9 Hz, H-5), 7.50 (AA′BB′ system, 2 H, $J_{2',3'}$ 8.4 Hz, H-2′, H-6′), 7.23 (d, 1 H, $J_{8,6}$ 2.4 Hz, H-8), 7.13 (dd, 1 H, H-6), 7.06 (AA′BB′ system, 2 H, H-3′, H-5′), 5.10 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1″), 4.92 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1‴), 4.01 (m, 2 H, H-5″ and H-5‴), 3.3–3.7 (m, 6 H, H-2″, H-2‴, H-3″, H-3‴, H-4″ and H-4‴). ESMS: positive ion mode m/z 607 $[M+H]^+$; negative ion mode m/z 605 $[M-H]^-$, 719 $[M+TFA-H]^-$. FABMS (thioglycerol matrix): m/z $[M-H]^-$. Anal. Calcd 605.1143; Found 605.1105.

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