The phase transfer catalysed synthesis of isoflavone-*O*-glucosides

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1 PERKIN

Daidzin 1, genistin 2, ononin 3 and sissotrin 4 are the major products of the phase transfer catalysed reaction between the isoflavone aglycones daidzein, genistein, formononetin and biochanin A, respectively, and 1-bromo-2,3,4,6-tetra-*O*-acetyl-α-glucopyranose. Complete proton and carbon NMR assignments are presented for compounds 1–4.

Introduction

The isoflavone-*O*-glucosides daidzin (daidzein 7-*O*-β-D-glucopyranoside)[1–8] **1**, genistin (genistein 7-*O*-β-D-glucopyranoside)[1–9] **2**, ononin (formononetin 7-*O*-β-D-glucopyranoside)[8,10,11] **3** and sissotrin (biochanin A 7-*O*-β-D-glucopyranoside)[8,12] **4** have been isolated from numerous plant and food sources. More recently in a metabolism study of orally administered isoflavones, daidzin and partially unhydrolysed daidzin were identified in rat faeces.[13] Structurally related unhydrolysed flavone-*O*-glycosides have been detected in human plasma.[14]

Interest has grown in isoflavone-*O*-glycosides, in particular genistin and daidzin, due to the results of a number of biological studies. Notably daidzin possesses antidipsotropic activity.[15–18] Results show that it is effective in suppressing voluntary alcohol consumption in a pharmacogenetic rat model of alcoholism.[15] Two patents also claim anti-intoxication properties[19] and for use in the treatment of alcohol dependence or alcohol abuse.[20] Daidzin is a potent selective inhibitor of human mitrochondrial aldehyde dehydrogenase *in vitro*.[21] Other *in vitro* work shows daidzin to have antioxidant activity similar in level to that of vitamin E.[22] Daidzin and genistin are added to commercial formulations to act as radical scavengers, dermal angiogenesis inhibitors and antiproliferic agents against melanomas,[23,24] Both also inhibit melanin formation *in vitro*,[23] and show high hypolipidemic activity in rats.[25] Ononin is a principal inhibitor of Epstein–Barr virus early antigen activation *in vitro*.[26,27]

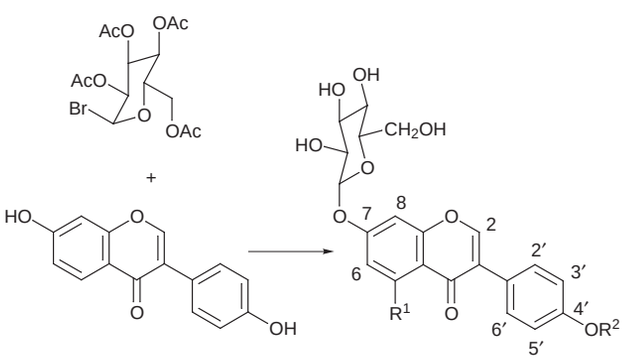
Previous methods for the synthesis of isoflavone-*O*-glucosides are low yielding, *e.g.* daidzin (9%),[28] genistin (17%),[29] sissotrin (13%)[30] and ononin (5–22%).[31,32] In our hands Zemplén's 9% KOH solution used for the base catalysed reaction of α-acetylbromoglucose with unprotected hydroxyisoflavones in acetone to produce genistin[29] and ononin[32] causes mainly isoflavone C-ring cleavage (Waltz showed C-ring cleavage using 5% aqueous KOH)[33] and anomeric hydrolysis of the α-acetylbromoglucose, but no glycosylation. There have also been other attempts[28,30,31] to prepare isoflavone glucosides but none of them involve direct glycosylation on the isoflavone aglycone. Usually, pre-formed deoxybenzoin glycosides, prepared by reaction of the deoxybenzoin with α-acetylbromoglucose in basic aqueous acetone, were cyclized to the corresponding isoflavone glucosides.[28,31] Sissotrin has been prepared by methylation of the free 4′-hydroxy group in genistin and thus lacks the glycosylation step altogether.[30]

We present now a simple method for the direct glycosylation of the unprotected isoflavone aglycone. It is regioselective, providing the more common 7-*O*-glucosides, and stereospecific to yield the naturally occurring β-anomer. In this method of glycosylation, yields are increased significantly and the reaction times reduced. The method is adaptable to other polyhydroxy isoflavones and the sugar moiety is easily varied.

In addition to their synthesis, we have carried out a thorough NMR analysis to allow complete and unambiguous assignments of the proton and carbon spectra for the four isoflavone 7-mono-*O*-glucosides daidzin **1**, genistin **2**, ononin **3** and sissotrin **4**. The results are shown in Tables 1 and 2.

Results and discussion

This is the first report of the synthesis of isoflavone-*O*-glucosides using phase transfer catalysis, involving the tetrabutylammonium bromide catalysed reaction of an isoflavone anion with α-tetraacetylbromoglucose (Scheme 1).[34]



1 $R^1 = R^2 = H$, Daidzin
2 $R^1 = OH$, $R^2 = H$, Genistin
3 $R^1 = H$, $R^2 = Me$, Ononin
4 $R^1 = OH$ $R^2 = Me$, Sissotrin

Scheme 1

The products from this aqueous–organic two phase reaction are the isoflavone-*O*-(tetra-*O*-acetyl-β-D-glycopyranosides). Deprotection of the sugar moiety is carried out using standard procedures (NaOMe–MeOH) to give the isoflavone-*O*-β-D-glucosides **1**–**4**.

This methodology produces ononin **3** and sissotrin **4** in reasonable yields of 48 and 52% respectively. The regioselectivity in the reaction of genistein and daidzein is towards the 7-position. Thus daidzin **1** (40%) and genistin **2** (42%) are obtained preferentially. This correlates well with CAMEO[35] calculations that show the acidity to be much greater at the 7- than the 4′-position. No 1,2-*cis* glucosides were found during routine analysis. Selectivity towards the 1,2-*trans* or β-glucosides suggests either an S_N2 mechanism or reaction of the α-acetylbromo sugar *via* a cyclic acyl oxonium ion, with attack by the phenolate thus being limited to the β-position.

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Table 1 The ^{1}H NMR spectra of daidzin **1**, genistin **2**, ononin **3** and sissotrin **4** (300 MHz, [$^2H_6$]DMSO)

	Daidzin			Genistin			Ononin			Sissotrin		
Proton	δ/ppm	multiplicity	J/Hz	δ/ppm	multiplicity	J/Hz	δ/ppm	multiplicity	J/Hz	δ/ppm	multiplicity	J/Hz
2	8.48	s	—	8.35	s	—	8.50	s	—	8.57	s	—
5	8.15	d	9.0	12.93	s	OH	8.12	d	9.0	13.01	s	OH
6	7.24	dd	9.0, 2.1	6.55	d	2.2	7.21	dd	9.0, 2.4	6.58	d	2.1
8	7.33	d	2.1	6.79	d	2.2	7.30	d	2.4	6.83	d	2.1
2′, 6′	7.51	d	8.4	7.44	d	8.7	7.6	dd	8.7, 2.1	7.63	dd	8.7, 2.1
3′, 5′	6.92	d	8.4	6.91	d	8.7	7.06	dd	8.7, 2.1	7.12	dd	8.7, 2.1
4′	9.64	s	OH	9.95	s	OH	3.85	s	OMe	3.89	s	OMe
1″	5.21	d	7.5	5.09	d	7.14	5.17	d	7.5	5.17	d	7.5
2″	3.41	m	—	3.42	m	—	3.31	m	—	3.38	m	—
3″	3.41	m	—	3.42	m	—	3.31	m	—	3.38	m	—
4″	3.30	m	—	3.31	m	—	3.25	m	—	3.28	m	—
5″	3.58	m	—	3.55	m	—	3.54	m	—	3.57	m	—
6″a	3.58	m	—	3.55	m	—	3.54	m	—	3.57	m	—
6″b	3.83	m	—	3.79	m	—	3.77	m	—	3.80	m	—
2″-OH	5.54	d	4.2	5.52	d	4.6	5.51	br s	—	5.50	d	4.8
3″-OH	5.24	d	4.2	5.24	d	4.3	5.25	br s	—	5.23	d	4.2
4″-OH	5.18	d	5.1	5.15	d	4.3	5.18	br s	—	5.16	d	4.2
6″-OH	4.71	t	5.4	4.70	t	5.4	4.67	br s	—	4.70	t	5.4

Table 2 The ^{13}C NMR spectra of daidzin **1**, genistin **2**, ononin **3** and sissotrin **4** (75 MHz, [$^2H_6$]DMSO)

	δ/ppm			
Carbon	Daidzin	Genistin	Ononin	Sissotrin
2	153.1	155.4	153.7	154.9
3	123.7	121.9	124.0	122.2
4	174.7	182.0	174.7	180.4
5	126.9	162.5	127.0	161.6
6	115.5	100.4	115.7	99.7
7	161.4	163.9	161.5	163.0
8	103.5	95.4	103.4	94.6
4a	118.5	107.0	118.4	106.1
8a	157.0	158.1	157.1	157.2
1′	122.3	123.4	123.4	122.7
2′, 6′	130.0	131.0	130.1	130.2
3′, 5′	115.0	115.9	113.6	113.8
4′	157.2	158.3	159.0	159.2
1″	100.2	100.7	100.0	99.9
2″	73.2	73.9	73.2	73.1
3″	76.5	77.2	76.5	76.5
4″	69.7	70.4	69.6	69.6
5″	77.2	78.1	77.3	77.3
6″	60.7	61.5	60.2	60.7
OMe	—	—	55.2	55.3

Daidzein 7-β-4′-α-di-*O*-D-glycopyranose (12%) and genistein 7-β-4′-α-di-*O*-D-glucopyranose (9%) are formed as minor products.

NMR Spectra

Previously, ^{1}H NMR assignments for daidzin **1**,[36–40] genistin **2**,[37,39–41] ononin **3**[42–46] and sissotrin **4**[47] have been limited mainly to the aglycone region of the compound and assignment of the anomeric proton. ^{13}C Assignments have been more complete, however details of verification by 2D NMR spectroscopy have not been provided. One exception involves the full proton assignment of daidzin **1**.[36] Unfortunately this paper does not report a carbon spectrum or details of how proton assignments were carried out.

In our NMR analysis we have used a number of 2-dimensional NMR techniques including gradient selected heteronuclear single quantum correlation (GHSQC), gradient selected heteronuclear multiple bond correlation (GHMBC), COSY 90-135, TOCSY and NOESY spectra, to obtain complete proton and carbon assignments. Interestingly we have found that with careful sample preparation using rigorously dried [$^2H_6$]DMSO, we have been able to view and assign both the glucosyl and aromatic hydroxy protons. The assignment of these previously unreported exchangeable hydroxy protons has proved to be an easy way to ensure the correct structural assignment for each glucoconjugate.

Although the aromatic part of the ^{1}H NMR spectrum of these compounds can be tentatively assigned based on α-to-oxygen and *ortho*-to-oxygen shifts and integrals, the confirmation of assignments comes from COSY 135 and/or TOCSY spectroscopy. The GHSQC spectrum provides the most effective way to make HC-COSY type correlations. For instance, the C-6″/H-6″α/H-6″β system of daidzin and the associated chemical shifts can be easily recognized in the GHSQC spectrum. Also other glucose methine proton assignments can be obtained from the GHSQC spectrum when the glucose carbon atoms have been assigned (see below). Because the hydroxy protons cannot have correlations in a GHSQC spectrum the anomeric proton of daidzin (δ 5.21) is picked out from the 5-signal pattern in the region δ 5.6–4.7 in the ^{1}H NMR spectrum. As usual, the HC-correlation spectrum optimized for $^3J_{CH}$- and $^2J_{CH}$-couplings (GHMBC in this case) proved to be very helpful for assigning the NMR spectra of this kind of molecules. Not only can the otherwise difficult to assign quaternary carbons (C-3, C-4a, C-7, C-8a, C-1′ and C-4′) be unequivocally recognized but also confirmation of the *O*-7-glucoside structure of daidzin is provided by the clear correlation between H-1″ (δ 5.21) and C-7 (δ 161.4). Carefully prepared samples give also clear correlations for the hydroxy protons in the HMBC spectra which can be used to assign signals from the glucose part of the molecule (see below). The assignment of the glucose hydroxys and carbons of daidzin using OH-correlations in the GHMBC spectrum proceeded as follows: C-1″ (δ 100.2), C-6″ (δ 60.7), H-1″ (δ 5.21), H-6″a (δ 3.58) and H-6″b (δ 3.83) were easily assigned based on chemical shift correlation rules and correlations in the GHSQC spectrum and can be used as entry points for further analysis. Also the OH-6″ proton (δ 4.71) is recognizable by its triplet structure. This hydroxy has two correlations in the GHMBC spectrum, to δ 60.7 due to C-6″ and δ 77.2 which must then be C-5″. The low field OH signal (δ 5.54) has a correlation to C-1″ at δ 100.2; this hydroxy is then OH-2″. The hydroxy at δ 5.18 has a correlation to δ 77.2 (C-5″) and must therefore be OH-4″. The remaining aliphatic hydroxy at δ 5.24 must be OH-3″. The OH-4″ has two additional correlations, to δ 76.5 and 69.7, which are assigned to C-3″ and C-4″ or *vice versa*. However, the OH-2″ proton has a correlation to δ 76.5 which must then be assigned to C-3″. Thus, the signal at δ 69.7 must be due to C-4″. These assignments are corroborated by other appropriate correlations to OH-2″ and OH-3″ in the hydroxy

region of the GHMBC spectrum. The remaining C-13 signal at δ 73.2 is then C-2″ which is confirmed by its correlation to δ 5.54 (OH-2″) and δ 5.24 (OH-3″). This completes the assignment of hydroxy groups and carbon signals of the sugar moiety in the NMR spectra. Table 1 contains the proton assignments for compounds **1**–**4**. Table 2 shows the full carbon data for each of the isoflavone 7-*O*-β-D-glucosides.

Experimental

General experimental procedures

NMR Spectra were recorded on a Varian Gemini 2000 spectrometer or Varian Inova 300 spectrometer, proton and carbon assignments being evaluated from GHMQC, GHMBC, COSY 90–135 TOCSY and NOESY spectra where necessary. *J* Values are given in Hz. Samples of the compounds for NMR spectroscopy were dried in vacuum (under 0.01 mbar) at room temperature overnight. Pre-dried 4 Å molecular sieves were added under Ar to [$^2H_6$]DMSO, 72 hours prior to use. To prevent contamination by moisture, NMR samples were prepared and the tubes sealed under Ar.

Mass spectra were recorded on a Finnigan MAT 8200 mass spectrometer operating in the FAB ionization mode. Xenon was used as the bombardment gas and the ionization matric was glycerol.

Melting points were recorded on an Electrothermal melting point apparatus and are uncorrected. Specific rotation values were recorded on a JASCO Model DIP-1000 digital polarimeter. All solvents used were Lab Scan HPLC or AR grade. Merck RP-18 F_{254} 0.25 mm and RP-18 F_{254} 1 mm precoated glass sheets were used for TLC and preparative thin layer chromatography (PTLC), respectively.

HPLC: delivery system: Waters 600E multisolvent delivery system, detection: Waters 996 photodiode array detector, software: Millenium 2010 chromatography manager, column: Hypersil ODS 5 μ, 250 × 10 mm.

Synthesis of isoflavone-*O*-β-D-glucosides

An isoflavone (1 equiv.) in 2.5% aqueous NaOH was stirred at room temperature for 15 min. To this was added a dichloromethane solution of tetrabutylammonium bromide (1.2 equiv.) and 1-bromo-2,3,4,6-tetra-*O*-acetyl-α-glucopyranose[34] (1.2 equiv.). The two phase mixture was stirred vigorously for 5 h before the reaction mixture was neutralized (1 M H_2SO_4), extracted with ethyl acetate, washed with brine, dried (Na_2SO_4) and evaporated to yield the crude isoflavone-*O*-β-D-(tetra-*O*-acetyl glycopyranosides) as a yellow oil. This was dissolved in a solution of sodium methoxide in methanol (0.54 g 100 ml^{-1}) and left at 4 °C for 12 h. Neutralization with Amberlite resin 120 (H) followed by filtration, evaporation and drying at the oil pump provided the crude isoflavone-*O*-glucosides. Initial purification was carried out using reversed-phase preparative TLC (eluent MeOH–H_2O, 65:35). Further purification was carried out on a Hypersil ODS 5 μ semi-preparative column (eluent MeOH–H_2O, 50:50, injection volume 500 μl, flow rate 5 ml min^{-1}).

Daidzein 7-*O*-β-D-glucopyranoside (daidzin) 1. A white solid (methanol–water), mp 235–236 °C [lit.,[48] 235–237 °C (water)]; $[\alpha]_D^{24}$ −24.1 (*c* 0.001 g ml^{-1} in DMSO) (lit.,[46] $[\alpha]_D^{26}$ −20.2 in DMSO); δ_H(300 MHz, [$^2H_6$]DMSO) Table 1; δ_C(75 MHz, [$^2H_6$]DMSO) Table 2; *m*/*z* (FAB) 417 (M^+).

Genistein 7-*O*-β-D-glucopyranoside (genistin) 2. A white solid (methanol–water), mp 252–254 °C [lit.,[29] 255 °C (methanol–water)]; $[\alpha]_D^{24}$ −32.0 (*c* 0.001 g ml^{-1} in [$^2H_6$]DMSO) (lit.,[37] $[\alpha]_D^{24}$ −36.6 in DMSO); δ_H(300 MHz, [$^2H_6$]DMSO) Table 1; δ_C(75 MHz, [$^2H_6$]DMSO) Table 2; *m*/*z* (FAB) 433 (M^+).

Formononetin 7-*O*-β-D-glucopyranoside (ononin) 3. A white solid (methanol–water), mp 213–215 °C [lit.,[48] 213–214 °C (methanol–water)]; $[\alpha]_D^{24}$ −25.6 (*c* 0.001 g ml^{-1} in DMSO) (lit.,[48] $[\alpha]_D^{20}$ −23.1 in pyridine); δ_H(300 MHz, [$^2H_6$]DMSO) Table 1; δ_C(75 MHz, [$^2H_6$]DMSO) Table 2; *m*/*z* (FAB) 431 (M^+).

Biochanin A 7-*O*-β-D-glucopyranoside (sissotrin) 4. A white solid (methanol–water), mp 212–214 °C [lit.,[30] 214–215 °C (methanol–water)]; $[\alpha]_D^{24}$ −32.7 (*c* 0.001 g ml^{-1} in DMSO) (lit.,[49] $[\alpha]_D^{20}$ −24.4 in methanol); δ_H(300 MHz, [$^2H_6$]DMSO) Table 1; δ_C(75 MHz, [$^2H_6$]DMSO) Table 2; *m*/*z* (FAB) 447 (M^+).

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