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The synthesis of daidzein sulfates

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Abstract—The first syntheses of the urinary isoflavone metabolites, daidzein 4′-sulfate, daidzein 7-sulfate and daidzein 4′,7-disulfate are described. These syntheses employ a key protecting group strategy, allowing regiospecific sulfation.

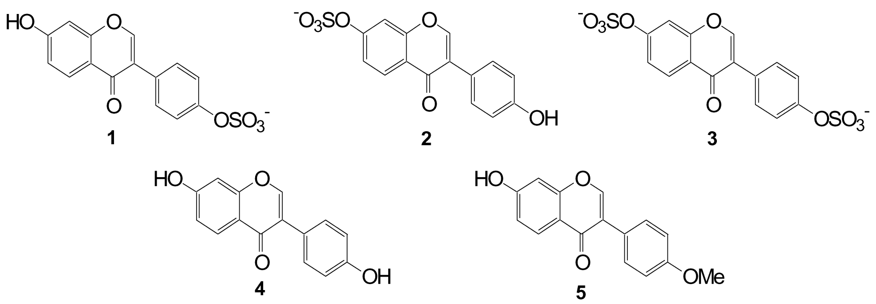
1. Introduction

The isoflavones are phytoestrogens with weak estrogenic activity,[1] which are present in the human diet in soybeans and soy derived products. There are now many studies on the health benefits of these compounds including relief of menopausal symptoms,[2] improvement in blood cholesterol levels[3] and reducing the risk of certain hormone related cancers.[4] However, although many studies have been carried out[5–9] there are still questions to be answered concerning the absorption, metabolism and bioavailability of dietary isoflavones. Also the mechanisms of transport around the body are unclear as most work has been conducted on the free isoflavone aglycons. In the soya plant, and food products, the isoflavones exist as glucosides but following ingestion these are hydrolysed and the aglycons are then converted to glucuronide and sulfate conjugates, mainly in the liver. In order for studies to progress further there is a need for pure standards of the isoflavone conjugates both to allow their accurate determination in biological samples (blood, urine etc.) and for examination of their biological role in vivo. Estradiol is also transported around the body as its sulfate conjugate and it has been proposed that some of the biological effects attributed to isoflavones may result from the effect of isoflavone sulfates on the enzymes that control this transport. Pure samples of the isoflavone sulfates would allow this theory to be addressed.

We wish to report herein the first chemical syntheses of daidzein 4′-sulfate **1**, daidzein 7-sulfate **2** and daidzein 4′,7-disulfate **3** in a pure form for use in analytical and biological studies (Scheme 1).

2. Results and discussion

Several procedures for the sulfation of flavonoids have been reported including sulfation with sulfur trioxide,[10] sulfuric acid/DCC and tetrabutylammonium hydrogen sulfate/DCC.[11] These methods, however, afford complex mixtures of flavonoid mono- and di-sulfates. We have utilised an alternative sulfation procedure for our synthesis, coupled



1 2 3 4 5

Scheme 1.

Keywords: daidzein sulfates; isoflavone; sulfation.

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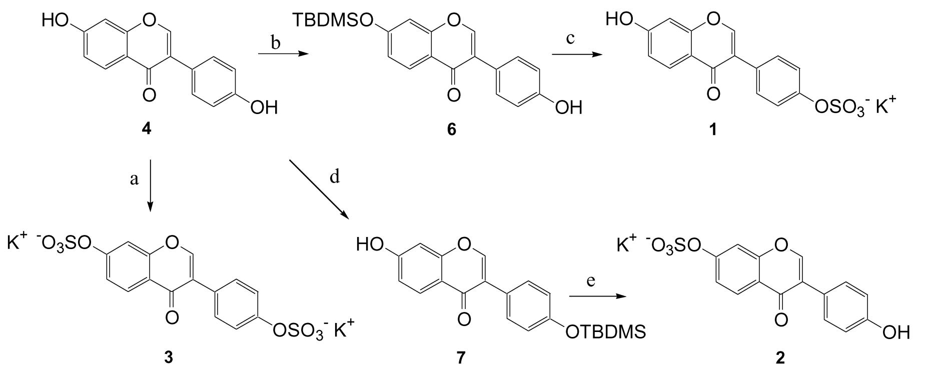

Scheme 2. *Reagents and reaction conditions*: (a) (i) $ClSO_3H$, pyridine, rt 12 h, (ii) K_2CO_3/H_2O, >pH 10, (40%); (b) TBDMSOTf, 2,6-lutidine, DMF, 4 h, rt (74%); (c) (i) $ClSO_3H$, pyridine rt 12 h, (ii) K_2CO_3/H_2O, >pH 10, (iii) TBAF, THF, CH_3CN/H_2O, (45%); (d) (i) $KOBu^t$ (2.1 equiv.), DMF, rt, 2 h, (ii) TBDMSCl, DMF, rt, 12 h (70%); (e) (i) $ClSO_3H$, pyridine rt 12 h, (ii) K_2CO_3/H_2O, >pH 10, (iii) TBAF, THF, CH_3CN/H_2O, (43%).

with a protecting group strategy, to afford the sulfated regioisomers in both good yield and high purity.

An alternative procedure was inspired by other work in our laboratory on the synthesis of glucosinolates, an important group of natural products possessing a sulfated oxime moiety.[12] Synthesis of these compounds employs a sulfation step using a chlorosulfonic acid/pyridine mixture.[13] When daidzein **4** was reacted under these conditions the 7,4′-disulfate **3** (Scheme 2) was obtained in quantitative yield according to the ^{1}H NMR data (Table 1). The aromatic protons adjacent to the sulfate groups at the 6, 8, 3′ and 5′ positions were all shifted downfield as expected, due to the electron withdrawing effect of the sulfates (Table 1). Mass spectral analysis of **3** was most effective in ES −ve mode giving the required mass of 413 [M−H], although no molecular ions could be observed in EI mode for any sulfates. Unfortunately, microanalysis of **3** indicated that large quantities of inorganic material were present. Removal of the inorganic impurities by simple washing was unsuccessful due to the very high polarity of the daidzein disulfate, which meant it was very water soluble. However, purification was eventually achieved using solid phase extraction techniques. Even then, of the Sep-Packs investigated, only a phenyl substituted support[14] was found to give sufficient retention of the isoflavone sulfate to allow removal of the majority of the inorganic salts, although some of the sulfate was still lost on washing. Indeed, the sulfation step appeared to be quantitative based on ^{1}H NMR analysis and the overall yield determined by the losses on purification. Purified **3** was obtained in greater than 95% purity based on microanalysis but in only 40% yield.

It was hoped that sulfation of daidzein **4** with one equivalent of chlorosulfonic acid would give selectivity for the more acidic 7-position. This, however, was not observed. We had previously shown formononetin **5**, to be readily sulfated under our standard conditions to give formononetin 7-sulfate. It was thus concluded that regiospecific sulfation could only be achieved by employing a protecting group strategy, whereby the protecting group could be readily removed after sulfation. This protecting group should be stable to both the harsh sulfation conditions and be easily removable under mild conditions afterwards, leaving the sulfate moiety intact. Of the functionalities investigated, a TBDMS group was found to be most amenable to both these criteria. The more acidic 7-position of daidzein **4** was thus selectively protected with a TBDMS group to afford **6**. Sulfation was then carried out as before, followed by treatment with TBAF to remove the silyl group and to afford daidzein 4′-sulfate **1** (Scheme 2). The signatory downfield shift of the 3′ and 5′ protons in was observed in the ^{1}H NMR spectrum (Table 1).

Table 1. ^{1}H NMR shifts for daidzein and the respective sulfates

Proton	Daidzein (**4**) (ppm)	Daidzein 4′-sulfate (**1**) (ppm)	Daidzein 7-sulfate (**2**) (ppm)	Daidzein 7,4′-disulfate (**3**) (ppm)
2	8.30	8.35	8.40	8.30
5	7.98	7.98	8.03	8.02
6	6.95	6.95	7.25	7.23
8	6.89	6.90	7.45	7.40
2′ and 6′	7.40	7.46	7.40	7.45
3′ and 5	6.89	7.21	6.80	7.16

Daidzein 7-sulfate **2** was prepared in a similar manner. In this case the 4′-position was protected selectively by exploiting the increased reactivity of the di-phenolate anion at the 4′-position. Thus treatment of daidzein **4** with two equivalents of potassium *tert*-butoxide followed by the addition of one equivalent of TBDMSCl gave 4′-*tert*-butyldimethylsilyldaidzein **7** in 60% yield. Interestingly, no selectivity was observed with the more reactive reagent, TBDMSOTf. Finally, sulfation of intermediate **7** and subsequent removal of the protecting group, furnished daidzein 7-sulfate **2** in 43% yield.

In summary we have developed the first efficient syntheses of daidzein 4′-sulfate **1**, daidzein 7-sulfate **2** and daidzein 4′, 7-disulfate **3**. These syntheses employ a key protecting group strategy, allowing regiospecific sulfation. These compounds have recently been identified in human urine using LC-MS-MS methods.[15] A similar strategy is currently being employed in the preparation of the biologically important, equol and genistein sulfates.

3. Experimental

3.1. General procedures

Melting points were determined in open capillary tubes with electrothermal apparatus and are uncorrected. For ^{1}H NMR (300 MHz) spectra the residual peak of $CHCl_3$ (7.26 ppm) and CH_3COCH_3 (2.05 ppm) were used as the internal reference, while for ^{13}C NMR (75 MHz) spectra the central peak of $CDCl_3$ (77.0 ppm) and the central peak CD_3COCD_3 (29.95 ppm) were used as the reference. Chemical shifts are given in δ and J values in Hz.

3.1.1. Daidzein 7,4′-disulfate (3). Daidzein (250 mg, 0.98 mmol) was dissolved in dry pyridine (10 mL) and cooled to 0°C. To this solution was added chlorosulfonic acid (0.80 g, 6.9 mmol) and the mixture allowed to warm to room temperature overnight. Evaporation at reduced pressure afforded a residue which was re-dissolved in water and the pH adjusted to 10 with solid potassium carbonate. The sulfate was then purified by chromatography (C_2-SiO_2, MeCN/H_2O 1:1) to remove organic impurities. Portions of the product (50–100 mg) were then suspended in water and applied to Sep-packs (Strata 50 μ, Tri-Func., Phenyl) and the inorganic salts washed off with water (3 volumes). The sulfate was then removed by washing with acetonitrile (2 volumes) to give the product as a white solid (193 mg, 40%); mp >375 °C; ν_{max}/cm^{-1} (nujol) 1640, 1595, 1176, 814; ^{1}H NMR (300 MHz, d^6-DMSO) 7.16 (2H, d, J=8 Hz, H-3′ and 5′), 7.23 (1H, dd, J=0.6, 8 Hz, H-6), 7.40 (1H, d, J=0.6 Hz, H-8), 7.45 (2H, d, J=8 Hz, H-2′ and 6′), 8.02 (1H, d, J=8 Hz, H-5), 8.30 (1H, s, H-2); ^{13}C NMR (75.4 MHz, d^6-DMSO) 116.6 (CH), 127.5 (CH), 128.6 (C), 129.6 (CH), 133.0 (C), 136.0 (CH), 136.1 (C), 139.0 (CH), 162.9 (C), 163.5 (CH), 166.0 (C), 167.7 (C), 184.3 (C); MS (ES, −ve ion mode) 413 (M−H, 46%), 333 (M−SO_3, 100%), 206 ((M-2H)$^{2-}$); HRMS (ES) calcd for $C_{15}H_8O_{10}S_2$ ((M-2H)$^{2-}$ ion) 205.9786, found 205.9785; Anal. calcd for $C_{15}H_8O_{10}SK_2$: C, 36.73; H, 1.64. Found: C, 36.51; H, 1.50 (data imply 99% purity).

3.1.2. 7-*tert*-Butyldimethylsilyldaidzein (6). To a solution of daidzein (300 mg, 1.18 mmol) in DMF (10 mL) was added 2,6-lutidine (137 μL, 1.18 mmol). After stirring for 15 min, *tert*-butyldimethylsilyl trifluoromethanesulfonate (298 μL, 1.30 mmol) was added and the mixture stirred for a further 8 h. Evaporation under reduced pressure afforded a residue which was purified by chromatography (SiO_2, hexane/diethyl ether 1:1) furnishing **6** as a white crystalline solid (320 mg, 74%); mp 176–178°C; ν_{max}/cm^{-1} (nujol) 3300br (OH), 1624, 1250, 840; ^{1}H NMR (300 MHz, $CDCl_3$) 0.28 (6H, s, 2×CH_3), 1.01 (9H, s, Bu^t), 6.84 (2H, d, J=8 Hz, H-3′and 5′), 6.87 (1H, d, J=0.6 Hz, H-8), 6.94 (1H, dd, J=0.6, 8 Hz, H-5), 7.30 (2H, d, J=8 Hz, H-2′and 6′), 7.78 (1H, s, OH), 7.92 (1H, s, H-2), 8.20 (1H, d, J=8 Hz, H-5); ^{13}C NMR (75.4 MHz, $CDCl_3$) −4.0 (CH_3), 18.7 (C), 26.0 (CH_3), 107.9 (CH), 116.3 (CH), 119.1 (C), 119.8 (CH), 123.4 (C), 125.7 (C), 128.2 (CH), 130.6 (CH), 153.2 (CH), 157.1 (C), 158.3 (C), 161.3 (C), 171.4 (C); MS (EI) 368 (M$^+$, 47%), 311 ((M−Bu^t)$^+$, 100%); HRMS (EI) calcd for $C_{21}H_{24}O_4Si$ 368.1444, found. 368.1450.

3.1.3. Daidzein 4′-sulfate (1). 7-*tert*-Butyldimethlsilyl-daidzein (**6**) (516 mg, 1.4 mmol) was dissolved in dry pyridine (8 mL) and cooled to 0°C. To this solution was added chlorosulfonic acid (1.63 g, 14 mmol) and the mixture allowed to warm to room temperature overnight. Evaporation afforded a residue which was re-dissolved in water and the pH adjusted to 10 with solid potassium carbonate. To the aqueous mixture was added MeOH (5 mL) and TBAF (1 M soln in THF, 1.4 mL, 1.4 mmol) and the solution stirred for a further 2 h. The solvent was removed at reduced pressure, with the crude residue taken up in a 1:1 mixture of acetonitrile/water and the solution acidified to pH 5 with 1 M HCl. Evaporation gave the crude sulfate which was subjected to flash column chromatography (reverse phase silica gel (C_{18}), acetonitrile/water, 1:1) to remove organic impurities. Portions of the product (50–100 mg) were then suspended in water and applied to Sep-packs (Strata 50 μ, Tri-Func., Phenyl) and the inorganic salts washed off with water (3 volumes). The sulfate was then removed by washing with acetonitrile (2 volumes) to give the product as a white solid (210 mg, 45%); mp 296–300°C (dec); ν_{max}/cm^{-1} (nujol) 3210br (OH), 1630, 1590, 1170, 815; ^{1}H NMR (300 MHz, d^6-DMSO) 6.90 (1H, d, J=0.6 Hz, H-8), 6.95 (1H, dd, J=0.6, 8 Hz, H-6), 7.21 (2H, d, J=8 Hz, H-3′ and 5′), 7.46 (2H, d,J=8 Hz, H-2′ and 6′), 7.98 (1H, d, J=8 Hz, H-5), 8.35 (1H, s, H-2); ^{13}C NMR (75.4 MHz, d^6-DMSO) 106.0 (CH), 119.0 (CH), 120.5 (C), 123.9 (CH), 127.2 (CH), 130.4 (C), 131.1 (CH), 133.1 (CH), 157.1 (CH), 161.3 (C), 166.2 (C), 166.4 (C), 177.2 (C); MS (ES, −ve ion mode) 333 (M−H, 100%); HRMS (ES, −ve mode) calcd for $C_{15}H_9O_{15}S$ 333.0069, found 333.0068; Anal. calcd for $C_{15}H_9O_7SK$: C, 48.38; H, 2.44. Found: C, 46.45; H, 2.30 (data imply 96% purity).

3.1.4. 4′-*tert*-Butyldimethylsilyldaidzein (7). To a solution of daidzein (500 mg, 2 mmol) in DMF (15 mL) was added potassium *t*-butoxide (463 mg, 4 mmol) and the mixture left to stir for 2 h. To this was then added *tert*-butyldimethylsilyl chloride (385 mg, 2.6 mmol) and the mixture stirred overnight. Evaporation under reduced pressure afforded a residue which was dissolved in water and acidified to pH 5 with 1 M HCl. The precipitate was then collected by filtration and purified by chromatography (SiO_2, hexane/diethyl ether 1:1), furnishing **6** as a white crystalline solid (438 mg, 60%); mp 242–245°C; ν_{max}/cm^{-1} (nujol) 3300br (OH), 1628, 1250, 835; ^{1}H NMR (300 MHz, d^6-acetone) 0.25 (6H, s, 2×CH_3), 1.01 (9H, s, Bu^t), 6.92 (3H, m, H-8, H-3′and 5′), 7.01 (1H, dd, J=0.6, 8.0 Hz, H-6), 7.53 (2H, d, J=8 Hz, H-2′and 6′), 8.07 (1H, d, J=8 Hz, H-5), 8.19 (1H, s, H-2); ^{13}C NMR (300 MHz, d^6-acetone); ^{13}C NMR (75.4 MHz, d^6-DMSO) 4.2 (CH_3), 18.3 (C), 25.9 (CH_3), 102.5 (CH), 115.5 (CH), 116.9 (C), 119.8 (CH), 123.4 (C),125.5 (C), 127.6 (CH), 130.5 (CH), 153.6 (CH), 155.2 (C), 157.8 (C), 162.9 (C), 174.9 (C); MS (EI) 368 (M$^+$, 47%), 311 ((M−Bu^t)$^+$, 100%); HRMS (ES, −ve mode) calcd for $C_{21}H_{23}O_4Si$ 367.1366, found 367.1359.

3.1.5. Daidzein 7-sulfate (2). This was prepared as for the daidzein 4′-sulfate, to give the product as a white solid in 43% yield; mp 242–246°C (dec); ν_{max}/cm^{-1} (nujol) 3200br (OH), 1636, 1590, 1182, 820; ^{1}H NMR (300 MHz, d^6-DMSO) 6.89 (2H, d, J=8 Hz, H-3′ and 5′), 7.25 (1H, dd, J=0.6, 8 Hz, H-6), 7.40 (2H, d, J=8 Hz, H-2′ and 6′), 7.43 (1H, d, J=0.6 Hz, H-8), 8.03 (1H, d, J=8 Hz, H-5), 8.4 (1H, s, H-2); ^{13}C NMR (75.4 MHz, d^6-DMSO) 116.5 (CH), 124.5 (CH), 127.5 (CH), 128.5 (C), 131.9 (C), 133.2 (C), 135.9 (CH), 139.6 (CH), 162.9 (CH), 166.0 (C), 166.8 (C), 167.7 (C), 184.4 (C); MS (MALDI-TOF, −ve ion mode) 333

(M−H, 100%); HRMS (ES, −ve mode) calcd for $C_{15}H_9O_7S$ 333.0069, found 333.0082; Anal. calcd for $C_{15}H_9O_7SK$: C, 48.38; H, 2.44. Found: C, 46.20; H, 2.31 (data imply 95% purity).

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