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Regiospecific 4'-*O*-β-glucosidation of isoflavones

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Abstract: The first stereospecific synthesis of isoflavone-4'-*O*-β-glucosides from unprotected isoflavone aglycones is presented. The procedure, involving a solid/liquid crown ether catalysed phase transfer system has been used for the synthesis of daidzein 4'-*O*-β-glucoside **3**, genistein 4'-*O*-β-glucoside **4**, and of the isoflavone-7-*O*-β-glucosides genistin and daidzin in improved yields.

Keywords: isoflavones; glycosidation; regioselection; crown ether

Isoflavones, a subgroup of the flavonoid family, occur naturally in legumes and are consumed regularly in the human diet.[1,2] The isolation of isoflavones from human biological fluids[3,4] coupled with their known beneficial role in the prevention of hormone based cancers[5,6] and coronary heart disease,[7,8] as well as being potent anti-oxidant compounds[9,10] has led to increasing interest in this rapidly expanding field.

Two of the most commonly occurring isoflavones are genistein (5,7,4'-trihydroxyisoflavone) **2** and daidzein (7,4'-dihydroxyisoflavone) **1**. It is well documented that these and other isoflavones exist naturally as *O*-glycoside conjugates.[11,12] Both 7- and 4'-*O*-glucosides of genistein and daidzein have been isolated from numerous sources.[13,14] Interest has also grown in these naturally occurring isoflavone glycosides due to the results of a number of biological studies. In general, isoflavone-*O*-glucosides are reported to possess antitumor,[15] antioxidative,[16] antifungal[17] and antihaemolytic[18] activities. Specifically, the 7-*O*-glucosides daidzin and genistin have been found to reduce the levels of known breast cancer risk factors including 17β-estradiol, progesterone and DHEA sulphate (dehydroepiandrosterone sulphate), whilst increasing menstrual cycle length when given orally to premenopausal women.[19] In addition, tests in rats and hamsters have shown that daidzin possesses antidipsotropic activity.[20-22]

In a previous paper we communicated on the synthesis of four isoflavone-7-*O*-β-glucosides, daidzin, genistin, ononin and sissotrin, using phase transfer catalysis, in a liquid/liquid two phase system.[23] Glycosidation yields of the 4'-hydroxy group were poor. In fact, there is no mention in the literature of the synthesis of isoflavone 4'-*O*-glycosides at all.

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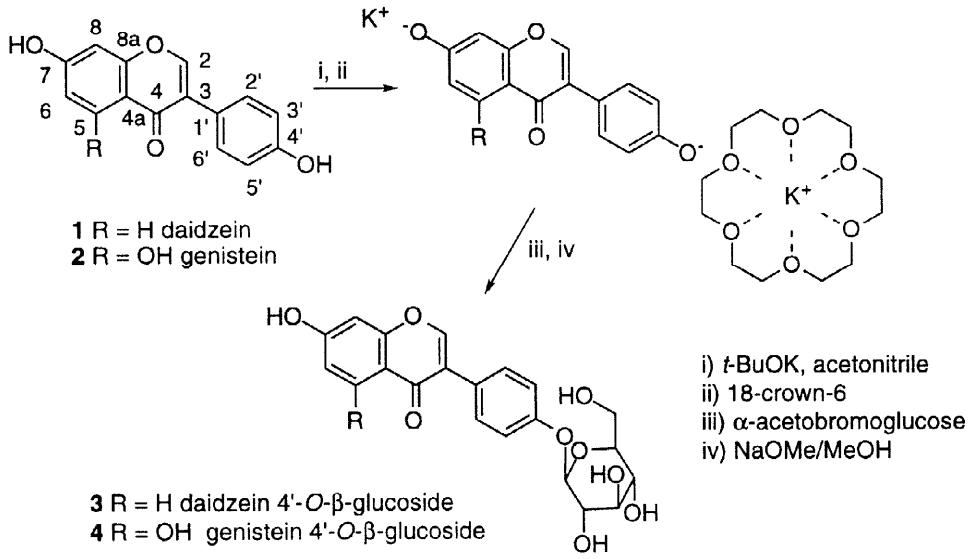
We now report that the use of the 18-crown-6 catalyst in a solid/liquid two phase system leads to either 4'-*O*-glucosidation or 7-*O*-glucosidation, depending upon the reaction stoichiometry. Thus daidzin and genistin are produced in improved yields (>50%) while daidzein 4'-*O*-β-glucoside **3** and genistein 4'-*O*-β-glucoside (sophoricoside) **4** are synthesised selectively for the first time from unprotected aglycones, in moderate yield.[24]

Previously we have had success using phase transfer catalysis in an aqueous/organic two phase system for the synthesis of isoflavonoid glycosides.[23] However, regioselective glycosidation was limited to the 7-hydroxy group of the isoflavone aglycone. Synthesis of the 4'-*O*-β-glucosides was found to be possible only in a low yield and with low regioselectivity.

In our present method, the synthesis of the 4'-*O*-β-glucosides requires the reaction of the aglycone isoflavone with an excess of base, to form the 4',7-diphenolate of daidzein, or the 4',5,7-triphenolate of genistein. Addition of 18-crown-6 brings the precipitated solid into solution and leads to reaction of the more nucleophilic 4'-phenolate with α-acetobromoglucose. Thus, daidzein-4'-*O*-β-glucoside **3** and genistein-4'-*O*-β-glucoside **4** are produced in 37 and 41 % yield, respectively (Scheme 1). Reaction at the 7 position is controlled using one equivalent of base to deprotonate the more acidic 7-hydroxy group, again producing a solid precipitate, presumably the corresponding phenolate. Addition of a catalytic amount of 18-crown-6 resolvates the solid, allowing reaction with α-acetobromoglucose to form the 7-*O*-β-glucosides of daidzein and genistein in 50 and 54% yields, respectively.



Scheme 1 Regiospecific synthesis of daidzein and genistein 4'-*O*-β-glucosides.

These results correlate well with the regioselective mono-*O*-alkylation[4,25] and monoesterification reactions of isoflavones we have reported earlier using similar methodology.[26]

To verify the position of glycosidation, we have carried out a complete analysis of the NMR spectra of the isoflavone monoglycosides. In particular, for the 4'-*O*-glucosides, NOESY, GHMBC and COSY 135 experiments show a clear coupling between the anomeric proton and the 3',5' positions of the isoflavone

skeleton. A complete NMR analysis of the 7-*O*-glucosides has been presented previously[23] and the spectra of compounds produced here are in accord with that data.

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24 Isoflavone (1 eq.), t-BuOK (2.1-3.1 eq.) and freshly distilled acetonitrile were stirred for 12 h at 25°C under Ar. 18-Crown-6 (0.1 eq.) was added followed by α–acetobromoglucose (1.2 eq.). The mixture was stirred for 5 hours, quenched with water, extracted with EtOAc, the organic layer collected, dried and solvent removed under reduced pressure. Deacetylation by treatment with NaOMe/MeOH (0.54g/100ml) followed by purification using Sephadex LH-20 gave the pure products. Further purification by HPLC (Hypersil ODS column, eluent MeOH/H_2O) gave analytically pure compounds. Daidzein 4'-*O*-β-glucoside **3**: *m/z* ES +ve 417 (MH^+), mp 245-246°C from MeOH/H_2O, ^1H NMR (300MHz), DMSO-d_6; 10.89 (1 H, s, 7-OH); 8.43 (1 H, s, 2-H); 8.05 (1 H, d, 5-H, J = 8.7 Hz); 7.58 (2 H, d, H-2', 6', J = 8.7 Hz); 7.16 (2 H, d, H-3', 5', J = 8.7 Hz); 7.02 (1 H, dd, H-6, J = 8.7, 2.1); 6.96 (1-H, d, H-8, J = 2.1 Hz); 4.98 (1 H, d, H-1", J = 7.5 Hz); 3.78 (1 H, m, H-6"a); 3.56 (2 H, m, H-5", 6"b); 3.44 (2 H, m, H-2", 3"); 3.27 (1 H, m, H-4"). ^{13}C NMR (50MHz), DMSO-d_6; 174.5 (C-4); 162.6 (C-7); 157.4 (C-8a); 157.1 (C-4'); 153.1 (C-2); 130.0 (C-2', 6'); 127.3 (C-5); 125.5 (C-1'); 123.1 (C-3); 116.6 (C-4a); 116.0 (C-3', 5'); 115.2 (C-6); 102.2 (C-8); 100.4 (C-1"); 77.1 (C-5"); 76. 7 (C-3"); 73.3 (C-2"); 69.7 (C-4"); 60.8 (C-6"). Genistein 4'-*O*-β-glucoside **4**: *m/z* ES +ve 433 (MH^+), mp 263-265 °C from MeOH/H_2O; ^1H NMR (300MHz), DMSO-d_6; 12.94 (1H, s, 5-OH); 9.60 (1H, s, 7-OH); 8.43 (1H, s, H-2); 7.40 (2H, d, H-2', 6', J= 8.7 Hz); 6.83 (2H, d, H-3', 5', J = 8.7 Hz); 6.72 (1H, d, H-8, J = 2.4); 6.47 (1 H, d, H-6, J = 2.4); 5.06 (1 H, d, H-1", J = 7.8 Hz); 3.82 (1 H, m, H-6"a); 3.54 (2 H, m, H-5", 6"b); 3.45 (2 H, m, H-2", 3"); 3.23 (1 H, m, H-4"). ^{13}C NMR (50MHz), DMSO-d_6; 180.5 (C-4); 163.0 (C-7); 161.6 (C-5); 157.5 (C-4'); 157.2 (C-8a); 154.6 (C-2); 130.2 (C-2', 6'); 122.6 (C-3); 121.0 (C-1'); 115.1 (C-3', 5'); 106.1 (C-4a); 99.9 (C-1"); 99.6 (C-6); 94.6 (C-8); 77.3 (C-5"); 76.5 (C-3"); 73.2 (C-2"); 69.6 (C-4"); 60.7 (C-6").

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