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Synthesis of daidzein 7-O-β-D-glucuronide-4′-O-sulfate

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ARTICLE INFO

Article history:
Received 5 October 2006
Received in revised form
15 March 2007
Accepted 6 July 2007
Published on line 13 July 2007

Keywords:
Daidzein
Isoflavone
Glucuronide
Sulfate
Phytoestrogen
Mixed conjugate

ABSTRACT

The first synthesis of daidzein 7-O-β-D-glucuronide-4′-O-sulfate, a mixed conjugate of an important dietary phytoestrogen is described.

1. Introduction

Daidzein (**1**), one of the major dietary isoflavones, is further metabolised in mammals to various glucuronide, sulfate and sulfoglucuronide conjugates [1]. Daidzein 7-O-β-D-glucuronide-4′-O-sulfate (**5**), the major biliary metabolite in rat, has been isolated and identified from rat urine [2]. Recently Tsuchihashi et al. isolated and identified various isoflavone conjugates from human urine, including daidzein 7-O-β-D-glucuronide-4′-O-sulfate (**5**) [3]. They also reported that a daidzein 7-O-glucuronide-4′-O-sulfate isolate showed a stimulatory effect on the growth of MCF-7 cells and exhibited binding activity to human estrogen receptors (hERs) [4]. They also studied the ER-dependent β-galactosidase induction of several isoflavone conjugates, and daidzein 7-O-glucuronide-4′-O-sulfate (**5**) showed potent hER α- and β-dependent induction [4].

To the best of our knowledge there are no published syntheses of the mixed sulfate/glucuronide isoflavones. In general, analytical work on the naturally occurring isoflavone conjugates appears to suffer from the lack of authentic reference samples [5]. To further study the analytics and biological activity of daidzein 7-O-glucuronide-4′-O-sulfate (**5**), synthetic reference is needed. We report here the first synthesis of the sodium salt of daidzein 7-O-β-D-glucuronide-4′-O-sulfate (**5**) by way of selective 4′-O benzyl protection of daidzein (Scheme 1).

2. Experimental

2.1. General

Daidzein (**1**) was prepared according to our published procedure [6]. NMR spectra were measured on a Varian Inova

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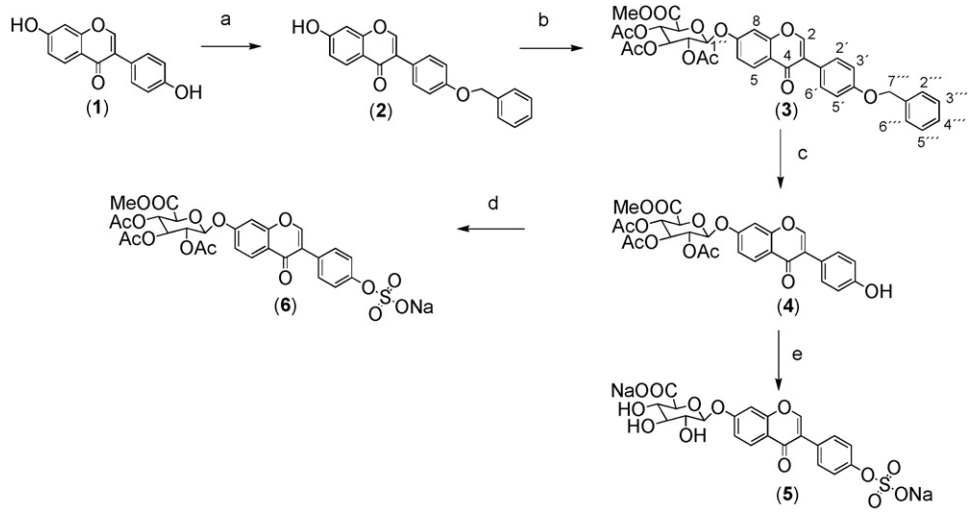
0039-128X/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2007.07.001



Scheme 1 – Synthetic route: (a) (i) KOBu-t (3 equiv.), DMF, rt, 2 h and (ii) BnCl (1.1 equiv.), rt, 16 h (72%); (b) (i) Bu_4NBr (1.2 equiv.), K_2CO_3 (5 equiv.), $CHCl_3$, H_2O, rt, 10 min, (ii) acetobromo-α-D-glucuronic acid, methyl ester (1 equiv.), rt, 2 d and (iii) acetobromo-α-D-glucuronic acid, methyl ester (1 equiv.), rt, 5 d (33%); (c) thioanisole (50 equiv.), TFA, +40 °C, 3 h (70%); (d) (i) $ClSO_3H$ (10 equiv.), pyridine, rt, 14 h and (ii) 5% aq. $NaHCO_3$, pH 8 (74%); (e) (i) $ClSO_3H$ (10 equiv.), pyridine, rt, 14 h and (ii) 0.5 M aq. Na_2CO_3, pH 10, rt, 24 h (41%).

500 spectrometer with TMS as an internal standard. Chemical shifts are in δ values (ppm) and coupling constants in Hz (s, singlet; d, doublet; t, triplet; dd, double doublet; m, multiplet). Mass spectra were obtained with a JEOL JMS SX102 mass spectrometer at 70 eV or Mariner ESI-TOF on negative or positive mode with TuneMix (Agilent Technologies) as the internal standard. Melting points were determined in open capillary tubes with a GWB melting point apparatus, and are uncorrected. TLC was conducted on Merck silica gel 60 F_{254} plates or Merck RP-18 F_{254s} plates. MPLC was performed with Buchi Sepacore using 40 mm × 150 mm silica gel 60 packed columns.

2.2. 4′-O-Benzyldaidzein (2)

A suspension of daidzein (300 mg, 1.18 mmol) and dry potassium *tert*-butoxide (397 mg, 3.5 mmol) in freshly distilled DMF (40 ml) was stirred at room temperature under Ar for 2 h. Freshly distilled BnCl (0.15 ml, 1.3 mmol) was added and the reaction mixture was stirred overnight and then poured into water and neutralised with 10% HCl. The precipitated product was filtered off, washed with water, dried *in vacuo* and purified with MPLC using CH_2Cl_2:EtOAc (8:2) as an eluent. Recrystallisation from EtOH gave white crystals (290 mg, 72%); m.p. 243–245 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.16 (s, 2H, H-7‴), 6.87 (d, 1H, J = 2.5 Hz, H-8), 6.94 (dd, 1H, J = 10.0, 2.5 Hz, H-6), 7.06 (d, 2H, J = 7.0 Hz, H-3′, H-5′), 7.34 (d, 1H, J = 7.0 Hz, H-4‴), 7.40 (t, 2H, J = 7.0 Hz, H-3‴, H-5‴), 7.47 (d, 2H, J = 7.0 Hz, H-2‴, H-6‴), 7.52 (d, 2H, J = 7.0 Hz, H-2′, H-6′), 7.97 (d, 1H, J = 9.0 Hz, H-5), 8.34 (s, 1H, H-2), 10.79 (s, 1H, –OH); ^{13}C NMR (125 MHz, DMSO-d_6): 69.2 (C-7‴), 102.1 (C-8), 114.5 (C-3′, C-5′), 115.1 (C-6), 116.6 (C-4a), 123.1 (C-1′), 124.6 (C-3), 127.3 (C-5), 127.6 (C-2‴, C-6‴), 127.8 (C-4‴), 128.4 (C-3‴, C-5‴), 130.0 (C-2′, C-6′), 137.1 (C-1‴), 153.1 (C-2), 157.4 (C-8a), 158.0 (C-4′), 162.5 (C-7), 174.5 (C-4).

HRMS (EI) calculated for: $C_{22}H_{16}O_4$ 344.1049; found 344.1044.

2.3. 4′-O-Benzyldaidzein 7-O-triacetylglucuronide methyl ester (3)

(**2**) (150 mg, 0.44 mmol), Bu_4NBr (169 mg, 0.52 mmol), K_2CO_3 (301 mg, 2.18 mmol), $CHCl_3$ (13 ml) and H_2O (13 ml) were placed in round bottomed flask and the mixture was stirred at room temperature. After obtaining a clear solution (10 min), acetobromo-α-D-glucuronic acid methyl ester (173 mg, 0.44 mmol) was added. After 2 days another equivalent (173 mg, 0.44 mmol) of acetobromo-α-D-glucuronic acid methyl ester was added. The reaction mixture was stirred for a total of 7 days. $CHCl_3$ (50 ml) was added and the separated organic phase was washed with 10% aq. AcOH (2× 15 ml), water (15 ml), 0.1 M $Na_2S_2O_3$ (15 ml), water (15 ml), sat. $NaHCO_3$ (2× 15 ml) and water (2× 15 ml), and dried with $MgSO_4$. The solvent was removed *in vacuo* to give a solid (490 mg), which was purified by MPLC using CH_2Cl_2:MeOH (95:5) as an eluent. This gave a white solid (368 mg) which was recrystallised from EtOH to give (**3**) (96 mg, 33%); m.p. 223–225 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.01, 2.03 (2s, 9H, 3× $COCH_3$), 3.64 (s, 3H, OCH_3), 4.81 (d, 1H, J = 10.0 Hz, H-5″), 5.12 (t, 1H, J = 10.0 Hz, H-4″), 5.16 (s, 2H, H-7‴), 5.19 (m, 1H, H-2″), 5.48 (t, 1H, J = 10.0 Hz, H-3″), 5.91 (d, 1H, J = 8.0 Hz, H-1″), 7.08 (d, 2H, J = 9.0 Hz, H-3′, H-5′), 7.15 (dd, 1H, J = 9.0 Hz, 2.5 Hz, H-6), 7.29 (d, 1H, J = 2.0 Hz, H-8), 7.34 (d, 1H, J = 7.0 Hz, H-4‴), 7.40 (t, 2H, J = 7.0 Hz, H-3‴, H-5‴), 7.47 (d, 2H, J = 7.5 Hz, H-2‴, H-6‴), 7.53 (d, 2H, J = ca. 9.0 Hz, H-2′, H-6′), 8.10 (d, 1H, J = 9.0 Hz, H-5), 8.46 (s, 1H, H-2); ^{13}C NMR (125 MHz, DMSO-d_6): 20.2, 20.2, 20.3 (3× $COCH_3$), 52.6 (OCH_3), 68.7 (C-4″), 69.2 (C-7‴), 70.3 (C-2″), 71.0 (C-3″), 71.1 (C-5″), 96.3 (C-1″), 103.7 (C-8), 114.5 (C-3′, C-5′), 115.2 (C-6), 119.3 (C-4a), 123.4 (C-1′), 124.1 (C-3), 127.5 (C-5), 127.6 (C-2‴, C-6‴), 127.8 (C-4‴), 128.4 (C-3‴, C-5‴), 130.1 (C-2′, C-6′), 137.0 (C-1‴), 153.8