available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/steroids

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

*Otto Soidinsalo, Kristiina Wähälä**

Laboratory of Organic Chemistry, Department of Chemistry, P.O. Box 55, A.I. Virtasen aukio 1, University of Helsinki, Helsinki 00014, Finland

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].

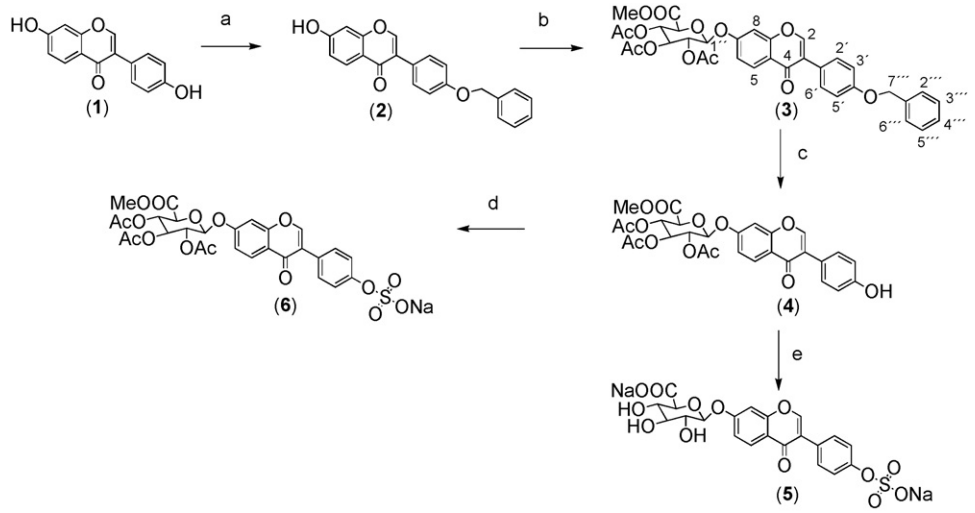
To the best of our knowledge there are no published syntheses of the mixed sulfate/glucuronide isoflavones. In general, analytical work on the naturally occurring isoflavone conjugates appears to suffer from the lack of authentic reference samples [5]. To further study the analytics and biological activity 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

* *Corresponding author*. Fax: +358 9 19150357.
E-mail address: kristiina.wahala@helsinki.fi (K. Wähälä).
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

(C-2), 156.8 (C-8a), 158.1 (C-4′), 159.9 (C-7), 166.9 (C-6″), 169.0 (C=O), 169.3 (C=O), 169.5 (C=O), 174.5 (C-4).

HRMS $(M+H)^+$ calculated for: $C_{35}H_{33}O_{13}$ 661.19157; found 661.19348.

2.4. *Daidzein 7-O-triacetylglucuronide methyl ester (4)*

(**3**) (85 mg, 0.13 mmol) was dissolved in thioanisole (0.76 ml, 6.43 mmol) and TFA (4 ml). The reaction mixture was stirred at 40 °C for 3 h. After completion of the reaction, TFA was evaporated *in vacuo* and the product was precipitated from Et_2O/hexane. The solvents were decanted and the residue washed with Et_2O. Recrystallization from EtOH/H_2O gave a white solid (51 mg, 70%); m.p. 218–220 °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), 4.80 (d, 1H, J = 10.5 Hz, H-5″), 5.12 (t, 1H, J = 9.5 Hz, H-4″), 5.18 (m, 1H, H-2″), 5.47 (t, 1H, J = 10.0 Hz, H-3″), 5.91 (d, 1H, J = 8.0 Hz, H-1″), 6.82 (d, 2H, J = 8.5 Hz, H-3′, H-5′), 7.13 (dd, 1H, J = 9.0, 2.5 Hz, H-6), 7.28 (d, 1H, J = 2.5 Hz, H-8), 7.41 (d, 2H, J = 8.5 Hz, H-2′, H-6′), 8.09 (d, 1H, J = 8.5 Hz, H-5), 8.41 (s, 1H, H-2), 9.55 (s, 1H, 4′-OH); ^{13}C NMR (125 MHz, DMSO-d_6): 20.2, 20.2, 20.3 (3× $COCH_3$), 52.6 (OCH_3), 68.8 (C-4″), 70.2 (C-2″), 71.0 (C-3″), 71.1 (C-5″), 96.3 (C-1″), 103.7 (C-8), 115.0 (C-3′, C-5′), 115.1 (C-6), 119.4 (C-4a), 122.1 (C-1′), 123.8 (C-3), 127.6 (C-5), 130.0 (C-2′, C-6′), 153.5 (C-2), 156.8 (C-8a), 157.3 (C-4′), 159.8 (C-7), 166.9 (C-6″), 169.0 (C=O), 169.3 (C=O), 169.5 (C=O), 174.6 (C-4).

HRMS $(M-H)^-$ calculated for: $C_{28}H_{25}O_{13}$ 569.12897; found 569.13112.

2.5. *Daidzein 7-O-β-D-glucuronide-4′-O-sulfate disodium salt (5)*

Chlorosulfonic acid (58 μl, 0.88 mmol) was added dropwise (*Caution*) into a stirred solution of (**4**) (50 mg, 0.088 mmol) in freshly distilled pyridine (5 ml) at −16 °C under Ar. The reaction was allowed to warm by stirring at room temperature. After 14 h at room temperature, 0.5 M aq. Na_2CO_3 (20 ml) was added until the pH was 10. The reaction mixture was stirred 24 h at room temperature. After completion of the reaction solvents were removed *in vacuo* and the remaining solid was purified with Sephadex LH-20 eluting with water. Further purification was performed with preparative RP-18 PLC plate using MeOH:H_2O (50:50) as an eluent. Recystallisation from H_2O/EtOH gave a white solid (20 mg, 41%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.32–3.40 (m, 3H, H-2″, H-3″, H-4″), 3.98 (d, 1H, J = 9.0 Hz, H-5″), 5.28 (d, 1H, J = 7.0 Hz, H-1″), 7.16 (dd, 1H, J = 9.0 Hz, 2.5 Hz, H-6), 7.22 (d, 2H, J = 9.0 Hz, H-3′, H-5′), 7.28 (d, 1H, J = 2.5 Hz, H-8), 7.49 (d, 2H, J = 9.0 Hz, H-2′, H-6′), 8.08 (d, 1H, J = 9.0 Hz, H-5), 8.45 (s, 1H, H-2); ^{13}C NMR (125 MHz, DMSO-d_6): 71.4 (C-4″), 72.8 (C-2″), 75.0 (C-5″), 75.8 (C-3″), 99.5 (C-1″), 103.4 (C-8), 115.5 (C-6), 118.6 (C-4a), 120.0 (C-3′, C-5′), 123.5 (C-3), 126.4 (C-1′), 127.2 (C-5), 129.4 (C-2′, C-6′), 153.4 (C-2), 153.9 (C-4′), 157.0 (C-8a), 161.1 (C-7), 170.4 (C-6″), 174.5 (C-4). HRMS $(M-2Na)^{2-}$ calculated for: $C_{21}H_{16}O_{13}S$ 254.01504; found 254.01576.

2.6. *Daidzein 7-O-triacetylglucuronide-4′-O-sulfate methyl ester sodium salt (6)*

Chlorosulfonic acid (46 μl, 0.69 mmol) was added dropwise (*Caution*) into a stirred solution of (**4**) (39 mg, 0.069 mmol) in freshly distilled pyridine (4 ml) at −16 °C under argon atmosphere. The reaction was allowed to warm by stirring at room temperature. After 14 h at room temperature 5% aq. $NaHCO_3$ was added until the pH was 8. After evaporation of the solvents, the crude product was purified on Sephadex LH-20 eluting with water. White solid (34 mg, 74%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.01, 2.03, 2.03 (3s, 9H, $COCH_3$), 3.65 (s, 3H, OCH_3), 4.81 (d, 1H, J = 10.5 Hz, H-5″), 5.12 (t, 1H, J = 9.0 Hz, H-4″), 5.17 (m, 1H, H-2″), 5.47 (t, 1H, J = 10.0 Hz, H-3″), 5.92 (d, 1H, J = 8.0 Hz, H-1″), 7.14 (dd, 1H, J = 9.0 Hz, 2.5 Hz, H-6), 7.23 (d, 2H, J = 9.0 Hz, H-3′, H-5′), 7.29 (d, 1H, J = 2.5 Hz, H-8), 7.49 (d, 2H, J = ca. 9.0 Hz, H-2′, H-6′), 8.11 (d, 1H, J = 9.0 Hz, H-5), 8.48 (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), 68.8 (C-4″), 70.3 (C-2″), 71.0 (C-3″), 71.1 (C-5″), 96.3 (C-1″), 103.7 (C-8), 115.2 (C-6), 119.4 (C-4a), 120.0 (C-3′, C-5′), 123.6 (C-3), 126.2 (C-1′), 127.6 (C-5), 129.3 (C-2′, C-6′), 153.5 (C-2), 154.0 (C-4′), 156.9 (C-8a), 159.9 (C-7), 166.9 (C-6″), 169.0 (C=O), 169.3 (C=O), 169.5 (C=O), 174.5 (C-4). HRMS $(M-Na)^-$ calculated for: $C_{28}H_{25}O_{16}S$ 649.08578; found 649.08688.

3. Results and discussion

Glucuronation at position 7-OH required prior selective protection at the 4′-hydroxyl. We have shown that selective 4′-*O*- or 7-*O*-alkylation of daidzein is possible by careful adjustment of the reaction conditions [7]. Thus, 4′-*O*-benzyldaidzein (**2**) was readily prepared by reaction of daidzein dipotassium salt with slightly more than 1 equiv. of benzyl chloride. In our work, glucuronidation at 7-OH using acetobromoglucuronic acid methyl ester was performed by a phase transfer procedure described earlier for phenols [8,9]. An alternative technique employs the Koenigs–Knorr reaction used for the glucuronidation of daidzein [10]. Trichloroacetimidates have also been used for glucuronidation purposes but modest yields or a complete failure have been reported in some cases [8,11]. Attempted debenzylation of 4′-*O*-benzyldaidzein 7-*O*-triacetylglucuronide methyl ester (**3**) with Pd/C and H_2 resulted in partial reduction of the ring C double bond. However, debenzylation with thioanisole/TFA [12] proceeded smoothly, with retention of the acetyl groups, the methyl ester and the ring C enone functionality.

Sulfation of the resulting daidzein 7-*O*-triacetylglucuronide methyl ester (**4**) was successfully performed by our earlier published procedure [13]. *In situ* hydrolysis of the acetyl groups and methyl ester gave daidzein 7-*O*-β-D-glucuronide-4′-*O*-sulfate sodium salt (**5**) in 41% yield. If required, the glucuronide triacetate methyl ester sulfate sodium salt (**6**) may also be prepared by omitting the hydrolysis step.

Products were analyzed by ^{1}H and ^{13}C NMR. 2D (HMQC, HMBC) measurements were run to confirm the identities of ^{1}H and ^{13}C signals in NMR spectra. The presence of the sulfate group is clearly seen by the shifts of the protons 3′ and 5′ and carbons 3′, 4′ and 5′ as reported earlier [2,3,13]. The NMR spectra of (**5**) were virtually identical with the previously published ^{1}H and ^{13}C NMR spectra of natural daidzein 7-*O*-β-D-glucuronide-4′-*O*-sulfate [2,3].

This compound will be useful as an internal standard in LC/MS analysis of the biological samples, as well as in the studies on the metabolism of isoflavones in man.

Acknowledgements

We thank Helsinki University Research Funds and Bioorganic and Medicinal Chemistry Graduate School for financial support.

REFERENCES

[1] Adlercreutz H, van der Wildt J, Kinzel J, Attalla H, Wähälä K, Mäkelä T, et al. Lignan and isoflavonoid conjugates in human urine. J Steroid Mol Biol 1995;52:97–103.
[2] Yasuda T, Kano Y, Saito K, Ohsawa K. Urinary and biliary metabolites of daidzin and daidzein in rats. Biol Pharm Bull 1994;17:1369–74.
[3] Tsuchihashi R, Okawa M, Nohara T, Okabe H, Kinjo J. Soy isoflavone metabolites isolated from human urine. Nat Med 2004;58:71–5.
[4] Kinjo J, Tsuchihashi R, Morito K, Hirose T, Aomori T, Nagao T, et al. Interactions of phytoestrogens with estrogen receptors α and β (III). Estrogenic activities of soy isoflavone aglycones and their metabolites isolated from human urine. Biol Pharm Bull 2004;27:185–8.
[5] Clarke DB, Lloyd AS, Botting NP, Oldfield MF, Needs PW, Wiseman H. Measurement of intact sulfate and glucuronide phytoestrogen conjugates in human urine using isotope dilution liquid chromatography–tandem mass spectrometry with [$^{13}C_3$]isoflavone internal standards. Anal Biochem 2002;309:158–72.
[6] Wähälä K, Hase T. Expedient synthesis of polyhydroxyisoflavones. J Chem Soc, Perkin Trans 1 1991:3005–8.
[7] Al-Maharik NI, Kaltia SA, Wähälä K. Regioselective mono-*O*-carboxymethylation of polyhydroxyisoflavones. Molecular 1999;3:20–4. Online.
[8] Hongu M, Saito K, Tsujihara K. Solid–liquid phase transfer catalyzed novel glycosylation reaction of phenols. Synth Commun 1999;29:2775–81.
[9] Li M, Han X, Yu B. Synthesis of quercetin 3-O-(2″-galloyl)-α-L-arabinopyranoside. Tetrahedron Lett 2002;43:9467–70.
[10] Needs PW, Williamson G. Syntheses of daidzein-7-yl β-D-glucopyranosiduronic acid and daidzein-4′-7-yl di-β-D-glucopyranosiduronic acid. Carbohyd Res 2001;330:511–5.
[11] Needs P, Kroon P. Convenient synthesis of metabolically important quercetin glucuronides and sulfates. Tetrahedron 2006;62:6862–8.
[12] Kiso Y, Ukawa K, Nakamura S, Ito K, Akita T. Efficient removal of protecting groups by a “push-pull” mechanism II. Deprotection of *O*-benzyltyrosine with thioanisole–trifluoroacetic acid system without O-to-C rearrangements. Chem Pharm Bull 1980;28:673–6.
[13] Soidinsalo O, Wähälä K. Synthesis of phytoestrogenic isoflavonoid disulfates. Steroids 2004;69:613–6.