JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd. Radiopharm 2006; **49**: 973–978.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.1114

Research Article

Synthesis of deuterated isoflavone disulfates

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Summary

Efficient synthesis of polydeuterated di-*O*-sulfates of three isoflavones, daidzein (**1**), genistein (**2**), glycitein (**3**), is described. The isoflavones were first deuterated with CF_3COOD under microwave irradiation to yield daidzein-d_6 (**4**), genistein-d_4 (**5**) and glycitein-d_6 (**6**) in 90% yield and in a high isotopic purity. The deuterated isoflavones were then sulfated with chlorosulfonic acid and pyridine to give the title compounds, again in high yield and isotopic purity ($>90\%$). The compounds are useful as internal standards in LC-MS quantitation of isoflavones. Copyright © 2006 John Wiley & Sons, Ltd.

Received 5 July 2006; Revised 11 July 2006; Accepted 12 July 2006

Key Words: isoflavones; sulfation; synthesis; deuteration; phytoestrogen; isoflavonoid

Introduction

Isoflavones are polyphenolic compounds possessing a wide variety of biological activity.[1] They exist in plants mainly as glycosides, and are metabolized in man to a variety of compounds.[2] However, neither the route nor the mechanisms of the metabolic pathways are well known, leading to uncertainty as to the exact structures of the metabolites. Members of the known metabolites include isoflavonoid di-*O*-sulfates which have been detected in human urine.[3] Glucuronide, monosulfate and sulfoglucuronide conjugates are other major metabolites.[3] It has been previously shown that daidzein sulfoconjugates are potent inhibitors of the sterol sulfatase enzyme preventing the production of biologically active estrogenic steroids from their inactive sulfoconjugates.[4]

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Contract/grant sponsor: University of Helsinki
Contract/grant sponsor: Finnish Academy; contract/grant number: 210633

Isotopically labeled di-*O*-sulfated isoflavone derivates are useful for further studies of the biological activity and elucidation of their metabolism. They may also be used as internal standards in LC-MS quantitation of the disulfate conjugates. The conventional methods for deuteration of isoflavonoids involve reactions under acidic or basic conditions.[5,6] Previously daidzein-d_4 has been prepared by refluxing in CF_3COOD for 9 days,[6] while daidzein-d_6 (**4**) was obtained by a $D_3PO_4{\bullet}BF_3/D_2O$ deuteration in an autoclave at 100°C for 7 days.[7] These methods suffer from long reaction times. Herein we report an expedient synthesis in high yields of stable polydeuterated isoflavones and their sulfate conjugates using CF_3COOD as a source of deuterium under microwave irradiation. By this method, CF_3COOD allows H-D exchanges even at the less activated ring sites.

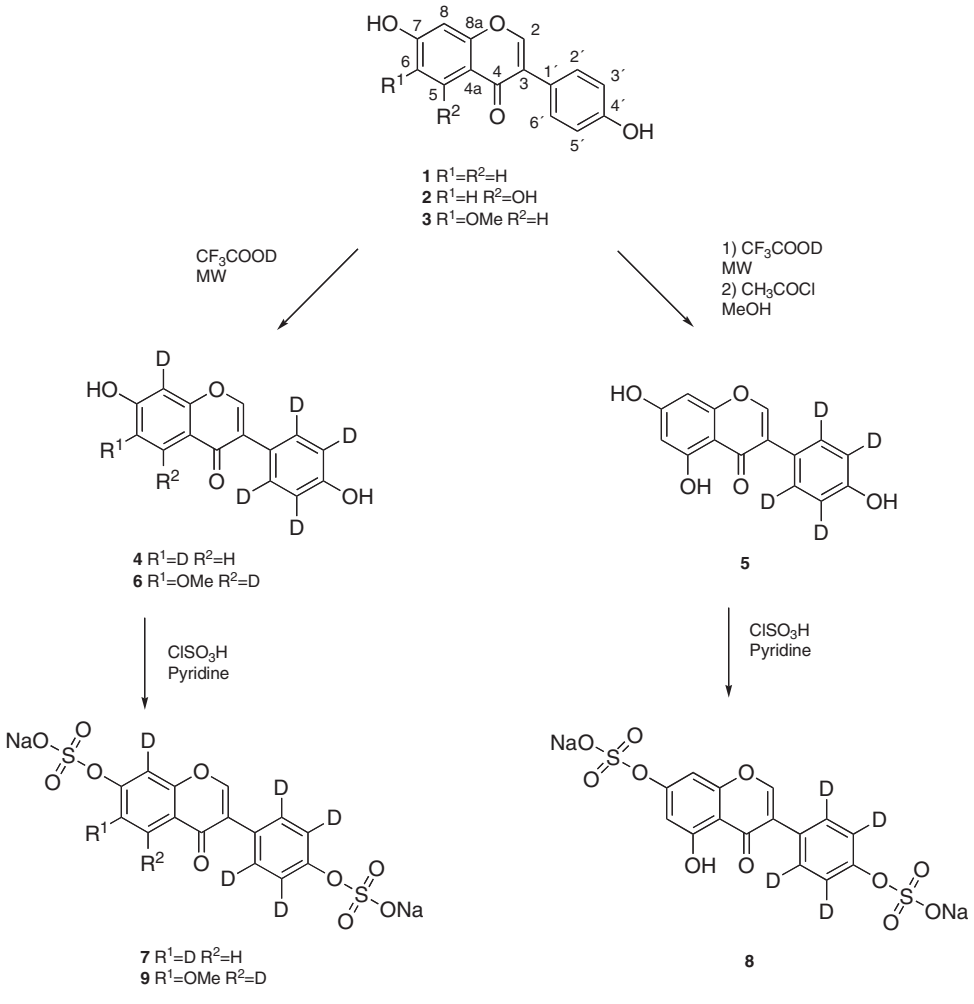
Results and discussion

Isoflavones were first predeuterated by evaporation of their acetone/D_2O solutions to remove the phenolic protons from the ensuing equilibria. The reaction of isoflavones with CF_3COOD was performed under microwave irradiation in pressure vessels. Reaction was followed by LC-MS (ESI+), establishing that the reaction proceeded in the order that first protons *ortho* to the phenolic OH groups were exchanged, followed more slowly by the less activated 2′ and 6′ protons. Repeating the reaction twice, the exchange of protons with deuteriums was achieved in high yield (90%) with all the compounds: daidzein-d_6 (**4**), genistein-d_6 and glycitein-d_6 (**6**) (Scheme 1). According to the ESI-MS and ^{1}H NMR data, the isotopic purity of the deuterated compounds was over 90%. Attempted deuteration with CH_3COOD or DCl/d_6-acetone under microwave irradiation gave no significant H/D exchanges.

In genistein-d_6, the deuterium atoms at C-6 and C-8 are potentially labile,[8] and hence a back exchange was done by refluxing genistein-d_6 in a solution of 0.5% CH_3COCl/MeOH for 10 min to give genistein-d_4 (**5**) (Scheme 1). The deuterium atoms at other positions were stable under this treatment.

The sulfation of the deuterated isoflavones was performed by our previously described procedure for isoflavonoid di-*O*-sulfates,[9] (Scheme 1) giving daidzein-d_6-di-*O*-sulfate (**7**), glycitein-d_6-di-*O*-sulfate (**8**) and genistein-d_4-di-*O*-sulfate (**9**) in high yields. The deuterium labels were stable under these reaction conditions including the work-up and the purification process, giving the products in high isotopic purity. The deuterated di-*O*-sulfates were analyzed by ^{1}H ,^{13}C NMR and ESI(+). In ESI(+) only traces of M-1 (<10%) were detected and the isotopic pattern was the same as for the deuterated isoflavones. Importantly, no undeuterated or other deutero species were observed. The NMR spectra of sulfated polydeuterated isoflavones were compared with our previously published spectra of isoflavone di-*O*-sulfates.[9]

DOI: 10.1002/jlcr



Scheme 1. Synthesis of deuterated isoflavonoid di-*O*-sulfates

The presence of deuterium atoms is clearly seen in the ^{13}C spectrum as the low intensity triplets of the deuterium carrying carbon atoms. Incidentally, a reverse order procedure, i.e. the deuteration of preformed isoflavone sulfates was not applicable as the substrates suffered ready desulfation on treatment with CF_3COOD. Also, the aromatic ring sites of the phenolic sulfate esters are unlikely to have sufficient reactivity for electrophilic H/D exchanges.

Experimental

General

NMR spectra were measured on a Varian Gemini 2000 or a Bruker Avance 500 spectrometer with TMS as an internal standard. Chemical shifts are in δ

DOI: 10.1002/jlcr

values (ppm) and coupling constants in Hz (s, singlet; d, doublet; dd, double doublet; m, multiplet). LC-MS(ESI +) was performed on a HP 1100 equipped with Mariner ESI-TOF. High-resolution mass spectra were measured on a Bruker Mikrotof on the negative mode with Tunemix as the internal standard. TLC was conducted on Merck RP-18 F_{254s} plates. Microwave irradiation was carried out with a CEM Discover® instrument. The starting materials daidzein (**1**) and genistein (**3**) were synthesized according to our published procedures.[10] Glycitein (**2**) was prepared by Houben-Hoesch synthesis from 4-methoxyresorcinol and 4-hydroxyphenylacetonitrile to give 2,4,4′-trihydroxy-5-methoxydeoxybenzoin, followed by the ring closure as previously described.[10]

General procedure for the deuteration of isoflavones

Predeuterated (D_2O/acetone) isoflavone (50 mg) was placed in a 10 ml pressure-proof reaction vial. CF_3COOD (2 ml) was added and the vial was sealed. The vial was irradiated for 5 h with 50 W microwave power. The pressure in the vial during the reaction was 12 bar and temperature 180°C. After 5 h, the solvent was removed *in vacuo* and fresh CF_3COOD was added. This was repeated twice. After completion of the reaction, the solvent was removed *in vacuo* and the residue recrystallized.

[6,8,2′,3′,5′,6′-d_6]-Daidzein (**4**) *[7-hydroxy-3-(4-hydroxyphenyl-2,3,5,6-d_4)-4H-1-benzopyran-4-one-6,8-d_2]*. Recrystallization from EtOH/H_2O gave off white crystals (46 mg, 90%), m.p. 335–336°C.

[2′,3′,5′,6′-d_4]-Genistein (**5**) *[5,7-dihydroxy-3-(4-hydroxyphenyl-2,3,5,6-d_4)-4H-1-benzopyran-4-one]*. The crude product (50 mg) was refluxed in 0.5% CH_3COCl/MeOH (10 ml) for 2 h and poured into ice water. The product was extracted with EtOAc (4 × 20 ml), dried with $MgSO_4$ and the solvent was removed *in vacuo*. Recrystallization from EtOH/H_2O gave off white crystals (46 mg, 90%), m.p. 301–302°C.

[5,8,2′,3′,5′,6′-d_6]-Glycitein (**6**) *[7-hydroxy-3-(4-hydroxyphenyl-2,3,5,6-d_4)-6-methoxy-4H-1-benzopyran-4-one-5,8-d_2]*. Recrystallization from EtOH/H_2O gave off white crystals (45 mg, 89%), m.p. 344–345°C. ^{1}H NMR (200 MHz, DMSO-d_6): δ = 3.88 (s, 3H, OCH_3), 8.28 (s, 1H, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) 55.8 (CH_3), 102.6(C-8)^D, 104.4(C-5)^D, 114.8 (C-3′, C-5′)^D, 116.1 (C-4a), 122.6 (C-1′), 122.9 (C-3), 129.6 (C-2′, C-6′)^D, 146.8 (C-6), 151.6 (C-8a), 152.4 (C-2), 152.8 (C-7), 157.0 (C-4′), 174.3 (C-4).

HRMS calculated for $C_{16}H_6D_6O_5$: 290.1061; found: 290.1069.

DOI: 10.1002/jlcr

General procedure for the synthesis of deuterated isoflavone 4′,7-di-O-sulfates

Chlorosulfonic acid $ClSO_3H$ (10 eq.) was added dropwise and slowly (CAUTION) to a stirred solution of the deuterated isoflavonoid (1 eq.) in freshly distilled pyridine at −16°C under argon atmosphere. After 14 h at room temperature, 5% aq. $NaHCO_3$ was added until the pH was 8. The solvent was removed *in vacuo* and the obtained crude solid product was purified on Sephadex LH-20 using water as an eluent.

[6,8,2′,3′,5′,6′-d_6]-Daidzein 4′,7-di-O-sulfate, disodium salt (**7**) *[7-sulfooxy-3-(4-sulfooxyphenyl-2,3,5,6-d_4)-4H-1-benzopyran-4-one-6,8-d_2, disodium salt]*. White solid: 90%; ^{1}H NMR (200 MHz, DMSO-d_6): δ = 8.05 (s, 1H, H-5), 8.45 (s, 1H, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) 106.9 (C-8)^D, 117.8 (C-6)^D, 118.9 (C-4a), 120.0 (C-3′, C-5′)^D, 123.3 (C-1′), 126.2 (C-5), 126.3 (C-3), 129.3 (C-2′, C-6′)^D, 153.2 (C-4′), 153.9 (C-2), 156.4 (C-8a), 158.0 (C-7), 174.6 (C-4).

m/z (ESI-) 209 $[M\text{-}2Na]^{2-}$

HRMS calculated for $C_{15}H_2D_6O_{10}S_2$: 208.9973; found: 208.9972.

[2′,3′,5′,6′-d_4]-Genistein 4′,7-di-O-sulfate, disodium salt (**8**) *[5-hydroxy-7-sulfooxy-3-(4-sulfooxyphenyl-2,3,5,6-d_4)-4H-1-benzopyran-4-one, disodium salt]*. White solid: 85%; ^{1}H NMR (200 MHz, DMSO-d_6): δ = 6.64 (d, 1H, J = 2.2 Hz, H-6), 6.90 (d, 1H, J = 2.2 Hz, H-8), 8.48 (s, 1H, H-2), 12.80 (s, 1H, H-5); ^{13}C NMR (50 MHz, DMSO-d_6) 97.4 (C-8), 102.1 (C-6), 106.3 (C-4a), 119.8 (C-3′, C-5′)^D, 122.1 (C-1), 124.9 (C-3), 129.2 (C-2′, C-6)^D, 153.4 (C-4′), 155.1 (C-2), 156.6 (C-8a), 159.7 (C-7), 161.0 (C-5), 180.4 (C-4).

m/z (ESI-) 216 $[M\text{-}2Na]^{2-}$

HRMS calculated for $C_{15}H_4D_4O_{11}S_2$: 215.9885; found: 215.9876.

[5,8,2′,3′,5′,6′-d_6]-Glycitein 4′,7-di-O-sulfate, disodium salt (**9**) *[7-sulfooxy-3-(4-sulfooxyphenyl-2,3,5,6-d_4)-6-methoxy-4H-1-benzopyran-4-one-5,8-d_2, disodium salt]*. White solid: 80%; ^{1}H NMR (200 MHz, DMSO-d_6): δ = 3.87 (s, 3H, OCH_3), 8.43 (s, 1H, H-2); ^{13}C NMR (50 MHz, DMSO-d_6) 55.7 (CH_3), 104.6 (C-8)^D, 107.3 (C-5)^D, 118.2 (C-4a), 120.0 (C-3′, C-5′)^D, 122.6 (C-1′), 126.4 (C-3), 128.7 (C-2′, C-6′)^D, 148.2 (C-7), 148.2 (C-6), 150.5 (C-8a), 153.1 (C-4′), 153.6 (C-2), 174.2 (C-4).

m/z (ESI-) 224 $[M\text{-}2Na]^{2-}$

HRMS calculated for $C_{16}H_4D_6O_{11}S_2$: 224.0026; found: 224.0018.

Conclusion

A rapid and efficient method for the preparation of polydeuterated di-*O*-sulfates of three dietary isoflavones has been developed. In addition to use as internal standards, these compounds are useful as monitoring tools during the pretreatment process of biological samples and hence give more understanding about their behavior and chemical stability in such systems.

DOI: 10.1002/jlcr

Acknowledgements

We thank Dr Petri Heinonen for running the HRMS. Research Funds of the University of Helsinki and Finnish Academy (210633) are acknowledged for financial support.

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DOI: 10.1002/jlcr