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Synthesis of D_6-Daidzein

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SUMMARY

Daidzein, an isoflavone abundant in soy, has various interesting biological properties that have been ascribed potential beneficial effects on human health. The study of these properties and effects of daidzein in general requires that an isotopically labeled reference compound is available for quantitative measurements. We report here the preparation of a deuterium labeled daidzein by acid catalyzed H/D exchange of aromatic protons using $D_3PO_4 \cdot BF_3/D_2O$ as a deuterating reagent at 100°C. The product, [6,8,2',3',5',6'-D_6]-daidzein, is isotopically pure with all D atoms chemically stable during the various extraction and purification steps of the ID/GC/MS/SIM quantitation procedure.

Keywords:deuterium, exchange, daidzein, isoflavone, labeling, isotope

INTRODUCTION

The natural isoflavone daidzein 1 is abundant in soy products and is also found in vegetables, beans, peas and other legumes. This dietary diphenolic phytoestrogen has been identified in human urine, plasma and feces, and has many interesting biological properties indicating that it is a possible cancer protective compound. (1) In this context it is significant that health claims of soy foods, rich in daidzein and other isoflavonoids, have recently received FDA authorization.

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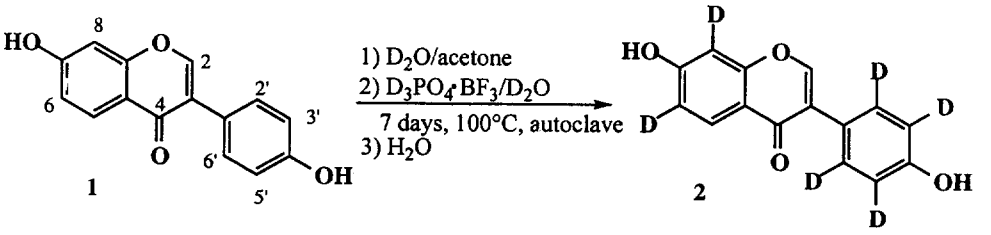
We have previously described the synthesis of stable deuterium labeled diphenolic isoflavones, [6,8,3'4'-D_4]–daidzein (2) and [8,3',5'-D_3]-daidzein (3), for use as internal standards in quantitating daidzein 1 by isotope dilution GC-MS techniques using selected ion monitoring (ID-GC-MS-SIM)(1,4). However, TMS-derivatized phenolic compounds will show quite intense m+1, m+2 etc. ions in the mass spectrum due to the contribution of the heavier isotopes of carbon and silicon. Thus several D atoms are required in the reference compound to avoid peak overlap with those of the analyte.

We now report the deuterium labeling of daidzein 1 to incorporate *six* stable D atoms into the daidzein skeleton. In the ensuing mass spectrum there is a complete separation of peaks from those of unlabeled daidzein.

RESULTS AND DISCUSSION

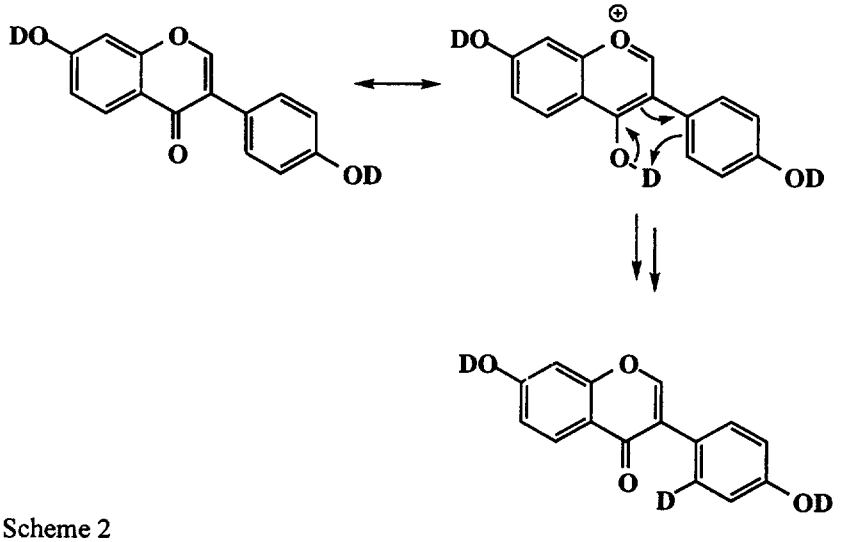
Deuterated phosphoric acid – boron trifluoride complex, used in the synthesis of [8,3',5'-D_3]-daidzein at room temperature (3), will also exchange the 6-, 2'- and 6'-hydrogens (Scheme 1) if used under more severe conditions, 100° C in an autoclave for seven days. Shorter reaction times (2-4 days) or a lower temperature (55°C) gave little or no exchange at those positions even with many repetitions. (3) The D_6 product, obtained in 49% yield, was 86% isotopically pure as calculated from the mass spectrum. In the mass spectrum of TMS-derivatized 2, the ion clusters in the molecular ion region and at M-15 were analogous to those of the undeuterated compound.



Scheme 1: Synthesis of [6,8,2',3',5',6'-D_6]-daidzein 2.

The sites of exchange are readily confirmed by the NMR spectra. In ^{1}H NMR, there are just two 1-H singlets at δ 7.97 and 8.29, known shifts for the protons at C-5 and C-2, respectively, in undeuterated daidzein (5). Similarly in the ^{13}C NMR spectrum, the six low-intensity C-D triplets appear at δ values observed and correlated earlier in the spectrum of undeuterated daidzein.(3)

Mechanistically, it is clear that the hydrogens at C-6, C-8, C-3' and C-5' should exchange very readily (we have also provided an explanation for the somewhat diminished reactivity of the hydrogen at C-6 (ref.3)). The heterocyclic oxygen supplies some degree of activation for exchanges at C-2' and C-6', possibly by way of an aromatic hetero ring with an O-D substituent at C-4 (Scheme 2). Activation by



Scheme 2

the 4'-OH towards reaction at C-2 is opposed by the ether oxygen which is why there is no exchange at this site. Finally, C-5 is completely unactivated.

EXPERIMENTAL

General: The product was characterized by ^{1}H and ^{13}C NMR, LRMS and HRMS and was homogenous by TLC. The melting point was determined in an open capillary tube with an Electrothermal apparatus, and is uncorrected. The NMR spectra were recorded on a Varian GEMINI 2000 spectrometer. $SiMe_4$ was used as an internal

standard. In the ^{13}C NMR spectrum the shifts given for the C-D triplets are those corresponding to the central peaks and are marked "D". Mass spectra were obtained with a JEOL JMS SX102 mass spectrometer operating at 70 eV. Samples were introduced by a direct inlet probe. The isotopic purity is calculated from the negative ion electrospray LC-MS spectrum obtained with a Micromass Quatro II, using 20µl Rheodyne loop and MeOH-H_2O (1:1) eluent. The UV spectrum was recorded with a CARY 5E UV-VIS-NIR spectrophotometer. TLC was conducted with Merck silica gel 60 F_{254} plates and Merck silica gel 60 F_{254} PLC plate was used for preparative thick-layer chromatography (PLC). D_2O (99.9 atom %) was obtained from Sigma and daidzein was synthesized by our one pot procedure (6).

D_6-daidzein 2 *[7-hydroxy-3-(4-hydroxyphenyl-2,3,5,6-D_4)-4H-1-benzopyran-4-one-6,8-D_2]*. Predeuteration of daidzein (100 mg) and the preparation of the $D_3PO_4 \cdot BF_3/D_2O$ (4 mL) deuteration reagent was done as described previously (3). The reaction mixture was stirred in the autoclave at 100°C for 7 days. The cooled reaction mixture was poured into ice water and the product extracted with EtOAc, the extract washed with water, dried (Na_2SO_4) and evaporated. The raw product was purified by PLC. Recrystallization from aqueous EtOH gave white crystals (49%), isotopic purity 86%, mp 335 °C (332 °C for D_3-daidzein)(3); λ_{max} (EtOH)/nm 249 (ε 25 900), 301 (10 900); δ_H [$(CD_3)_2SO$] 7.97 (1H, s, 5-H), 8.29 (1H, s, 2-H); δ_C [$(CD_3)_2SO$] 101.8 (C-8)^D 114.8 (C-6, -3',-5')^D, 116.6 (C-4a), 122.3 (C-1'), 123.4 (C-3), 127.1 (C-5), 129.6 (C-2',-6')^D, 152.8 (C-2), 157.0 (C-4'), 157.4 (C-8a), 162.4 (C-7), 174.7 (C-4); *m/z* (EI) 260 (M^+, 100%), 139 (62), 122 (28) (Found: M^+, 260.0962. $C_{15}H_4D_6O_4$ requires M, 260.0956).

CONCLUSIONS

Six ring protons of daidzein, including those at the less activated C-6, C-2' and C-6' sites, can be exchanged to deuteriums with our modified $D_3PO_4 \cdot BF_3/D_2O$ procedure. The D_6-daidzein obtained can be safely used as a daidzein reference e.g. in metabolic

or quantitative studies since all deuterium atoms are chemically stable and do not re-exchange back to C-H, and there is no peak overlap in the mass spectra of the deuterated and undeuterated material.

ACKNOWLEDGMENT

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