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Synthesis of ^{13}C labelled Daidzein and Formononetin

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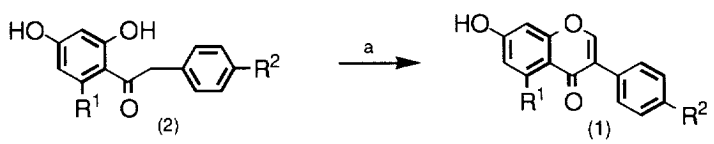
Abstract: Efficient methods are described for the synthesis of daidzein and formononetin labelled with a single ^{13}C atom at the 4-position, to prepare material for metabolic studies.

Recent studies have shown that the phytoestrogens present in soya-based foods may have a considerable impact on human health. In particular the isoflavonoid phytoestrogens daidzein (1; $R^1 = H$, $R^2 = OH$) and genistein (1; $R^1 = OH$, $R^2 = OH$) have been identified as modulators in the growth of hormone dependent cancers,[1] as well as implicated in the prevention of cardiovascular disease,[2] lessening the symptoms of the menopause[3] and protection against oestreoporosis.[4] In addition, recent evidence suggests a role in the central nervous system, stimulating nerve growth, and action as an antioxidant against endogenous toxins that produce free radicals in the CNS which are associated with the development of Alzheimer's disease.[5] Due to the growing interest in the potential for isoflavones as pharmaceuticals there is need for the development of an efficient synthesis of labelled daidzein for use in routine analysis and metabolic studies.

A number of synthetic routes towards the isoflavones have been developed previously and it was proposed that labelled daidzein and formononetin (1; $R^1 = H$, $R^2 = OCH_3$) would be prepared by adapting existing methodologies (Scheme 1). The final step of most isoflavone syntheses involves the formylation and cyclisation of a suitable deoxybenzoin precursor (2). Various reagents have been used to provide the necessary one carbon fragment including dimethyl formamide dimethylacetal in THF,[6] dimethyl formamide followed by mesyl chloride[7] and 1,3,5-triazine with boron trifluoride etherate.[8] Preliminary studies were therefore carried out to investigate the first two methods to examine the possibility of using ^{13}C-labelled dimethyl formamide to introduce the label in the final step. Unfortunately preliminary studies with unlabelled material demonstrated that a large excess of dimethyl formamide, or its dimethyl acetal, were required in order to obtain reasonable yields. This would be feasible if recovery of unreacted labelled starting material was also efficient but this was not the case.



a) DMF dimethylacetal, THF, reflux or DMF then Mesyl Chloride or $BF_3.Et_2O$, 1,3,5-triazine

SCHEME 1

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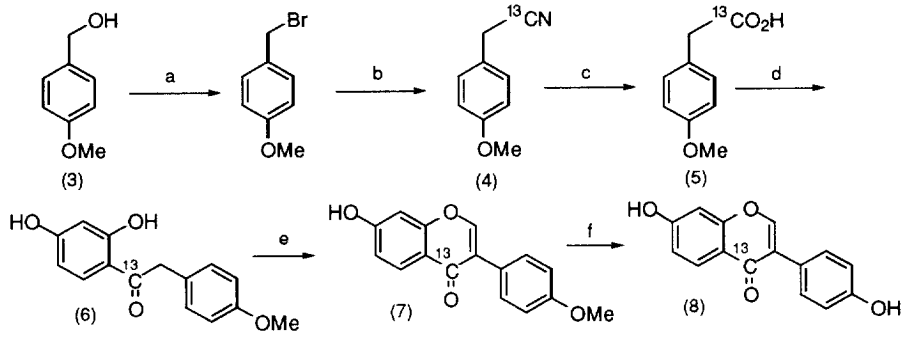
An alternative strategy was therefore sought. The deoxybenzoins are normally prepared *via* condensation of a phenol and either a substituted phenylacetic acid, using boron trifluoride as catalyst, or benzyl nitrile *via* a Hoesch reaction.[9] An alternative route thus involved the use of ^{13}C labelled cyanide to prepare the nitrile, which would lead to labelling at the 4-position of the isoflavone (Scheme 2). The proposed route was first optimised with unlabelled material and then used to prepare both ^{13}C labelled formononetin and daidzein.



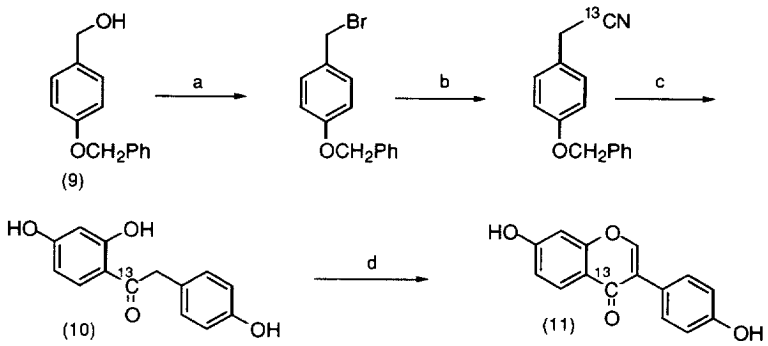
a) TMSBr, Et_2O (98%); b) $K^{13}CN$, 18-Crown-6, MeCN (86%); c) 2M NaOH (aq), reflux (79%); d) Resorcinol, $BF_3.Et_2O$ (66%); e) $DMF(OMe)_2$ (62%); f) BBr_3, CH_2Cl_2 (89%)

SCHEME 2

Commercially available 4-methoxybenzyl alcohol (3) was brominated[10] and then reacted with ^{13}C labelled potassium cyanide in the presence of 18-crown-6 to give the desired nitrile (4) in good yield.[11] The carboxylic acid (5) was obtained by subsequent basic hydrolysis. Formation of the deoxybenzoin (6) was effected by treatment of resorcinol and the ^{13}C labelled acid with boron trifluoride etherate in THF.[12] Dimethylformamide dimethylacetal[6] was then employed for the formylation and cyclisation reaction to give ^{13}C-formononetin (7).[13] This gave identical spectral data to authentic material. The presence of the ^{13}C-label was confirmed by mass spectrometry, showing less than 1% unlabelled material, and by the enhanced signal due to the carbon at the 4-position in the ^{13}C NMR spectrum. Conversion to daidzein then required demethylation of the 4'-methoxy group. The most efficient reagent for this transformation was found to be boron tribromide in dichloromethane,[14] which afforded the ^{13}C-daidzein (8) in 89% yield. Analysis of the ^{13}C-daidzein by GC-MS in comparison with a reference standard of unlabelled material revealed that it was only 54% pure. NMR analysis showed no organic impurities and so it was deduced that the contamination was due to inorganic material, probably boron salts. Unfortunately, attempts to remove the boron salts by extraction, recrystallisation, normal phase column chromatography and reverse phase column chromatography failed. In order to obtain pure (8) it was therefore clear that the best strategy would be to modify the synthetic route to avoid the final demethylation step.

It was decided to protect the 4-hydroxy group as its benzyl ether, which would then be removed under the acidic conditions required for the Hoesch reaction[9] to form the deoxybenzoin. This alternative route is shown in Scheme 3. Thus the nitrile[15] (9) was prepared, as described for the methoxy analogue, from commercially available 4-benzyloxybenzylalcohol. The Hoesch reaction was achieved by treatment

of a solution of resorcinol, nitrile (9) and catalytic zinc chloride in diethyl ether with hydrogen chloride to give the desired deoxybenzoin (10) in good yield. Formylation and cyclisation of (10) afforded the ^{13}C-daidzein (11) which was then successfully purified by flash chromatography. This material was analysed as before and found to be pure by both GC-MS and microanalysis.[16]



a) TMSBr, Et_2O (89%); b) $K^{13}CN$, 18-Crown-6, MeCN (92%);
c) Resorcinol, HCl, cat. $ZnCl_2$, Et_2O (96%); d) $DMF(OMe)_2$ (60%)

SCHEME 3

Work is currently underway to modify the synthesis for the preparation of ^{13}C-genistein and other isoflavonoid phytoestrogens for use in metabolic studies.

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10. All compounds exhibited satisfactory spectral data, consistent with the proposed structures and literature data, where available. NMR spectra were recorded on a Varian Gemini f.t spectrometer (^{1}H 200 MHz; ^{13}C 50.31 MHz) or a Bruker AM-300 f.t. spectrometer (^{1}H, 300MHz; ^{13}C, 74.76 MHz).

11. Data for [^{13}C]-4-methoxybenzyl cyanide: ν_{max} (nujol)/cm^{-1} 2191 (CN); δ_H (200 MHz, $CDCl_3$) 3.60 (2H, d, $J_{C,H}$ 5.3 Hz, C$\underline{H}_2$), 3.72 (3H, s, OC$\underline{H}_3$), 6.84 (2H, d, $J_{2,3}$= $J_{5,6}$ 7.7 Hz, 3, 5-$\underline{H}$), 7.16 (2H, d, $J_{2,3}$= $J_{5,6}$ 7.7 Hz, 2, 6-$\underline{H}$); δ_C (74.76 MHz, $CDCl_3$) 22.9 (d, J 28 Hz, C$\underline{H}_2$), 55.6 (OC$\underline{H}_3$), 114.9 (3-$\underline{C}$ and 5-$\underline{C}$), 119.1 (enhanced, $^{13}\underline{C}$N), 122.6 (1-$\underline{C}$), 129.6 (2-$\underline{C}$ and 6-$\underline{C}$), 159.8 (4-$\underline{C}$); *m/z* (EI) 148 (M^+, 100%).

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13. Data for [4-^{13}C]-formononetin: m.p. 259 °C (Lit.[6] 256-257 °C); (Found: C, 71.09; H, 4.41. $C_{15}{}^{13}C_1H_{12}O_4$ requires C, 71.37; H, 4.49); ν_{max} (nujol)/cm^{-1} 3364 (br, OH), 1732 (C=O), δ_H (200 MHz, d^6-DMSO) 3.81 (3H, s, OC$\underline{H}_3$), 6.9 (2H, m, 6-$\underline{H}$ and 8-$\underline{H}$), 7.0 (2H, d, $J_{2',3'}$ = $J_{5',6'}$ 8.6 Hz, 3', 5'-$\underline{H}$), 7.5 (2H, d, $J_{2',3'}$ = $J_{5',6'}$ 8.6 Hz, 2', 6'-$\underline{H}$), 8.0 (1H, dd, $J_{4,5}$ 2.7 Hz, $J_{5,6}$ 9 Hz, 5-$\underline{H}$), 8.3 (1H, d, $J_{2,4}$ 6.6 Hz, 2-$\underline{H}$); δ_C (50.31 MHz, d^6-DMSO) 55.2 (O$\underline{C}$H3), 102.2 (8-$\underline{C}$), 113.7 (3'-$\underline{C}$ and 5'-$\underline{C}$), 115.2 (6-$\underline{C}$), 116.3 (d, J 57 Hz, 4a-$\underline{C}$), 122.6 (1'-$\underline{C}$), 123.4 (1-$\underline{C}$), 124.2 (d, J 56 Hz, 3-$\underline{C}$), 127.3 (5-$\underline{C}$), 130.1 (2'-$\underline{C}$ and 6'-$\underline{C}$), 153.0 (2-$\underline{C}$), 157.1 (7-$\underline{C}$), 159.0 (8a-$\underline{C}$), 162.6 (4'-$\underline{C}$), 174.7 (enhanced, 4-$\underline{C}$); *m/z* (EI) 269 (M^+, 62%), 254 (14, M^+-CH_3).

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15. Data for [^{13}C]-4-benzyloxybenzyl cyanide: ν_{max} (nujol)/cm^{-1} 2193 (CN); δ_H (200 MHz, $CDCl_3$) 3.51 (2H, d, $J_{C,H}$ 5.4 Hz, C$\underline{H}_2$), 4.92 (3H, s, OC$\underline{H}_2$Ph), 6.84 (2H, d, $J_{2,3}$ = $J_{5,6}$ 7.7 Hz, 3, 5-$\underline{H}$), 7.16 (2H, d, $J_{2,3}$ = $J_{5,6}$ 7.7 Hz, 2, 6-$\underline{H}$); δ_C (50.3 MHz, $CDCl_3$) 21.3 (d, J 28 Hz, $\underline{C}H_2{}^{13}$CN), 70.0 (O$\underline{C}H_2$Ph), 115.5 (3-$\underline{C}$ and 5-$\underline{C}$), 118.2 (enhanced, $^{13}\underline{C}$N), 122.0 (1-$\underline{C}$), 127.5 (2'-$\underline{C}$ and 6'-$\underline{C}$), 128.1 (3'-$\underline{C}$ and 5'-$\underline{C}$) 128.7 (4'-$\underline{C}$), 129.1 (2-$\underline{C}$ and 6-$\underline{C}$), 136.4 (1'-$\underline{C}$), 159.8 (4-$\underline{C}$); *m/z* (EI) 224 (M^+, 14%).

16. Data for [4-^{13}C]-daidzein: m.p. 219 °C (Lit.[6] 212-214 °C); (Found: C, 70.34; H, 4.30. $C_{14}{}^{13}C_1H_{10}O_4$ requires C, 70.59; H, 4.00); ν_{max} (nujol)/cm^{-1} 3360 (br, OH), 1735 (C=O); δ_H (200 MHz, d^6-DMSO) 6.89 (3H, m, 3', 5'-$\underline{H}$ and 8-$\underline{H}$), 6.95 (1H, dd, $J_{6,8}$ 2 Hz, $J_{5,6}$ 9 Hz, 6-$\underline{H}$), 7.4 (2H, d, $J_{2',3'}$ = $J_{5',6'}$ 8.5 Hz, 2', 6'-$\underline{H}$), 7.98 (1H, dd, $J_{4,5}$ 4 Hz, $J_{5,6}$ 9 Hz, 5-$\underline{H}$), 8.3 (1H, d, $J_{2,4}$ 6 Hz, 2-$\underline{H}$); δ_C (50.31 MHz, d^6-DMSO) 102.1 (8-$\underline{C}$), 114.9 (3'-$\underline{C}$ and 5'-$\underline{C}$), 115.1 (6-$\underline{C}$), 116.7 (d, J 46 Hz, 4a-$\underline{C}$), 122.5 (1'-$\underline{C}$), 123.5 (d, J 56 Hz, 3-$\underline{C}$), 127.2 (5-$\underline{C}$), 130.0 (2'-$\underline{C}$ and 6'-$\underline{C}$), 153.1 (2-$\underline{C}$), 157.1 (4-$\underline{C}$), 156.4 (8a-$\underline{C}$), 162.4 (7-$\underline{C}$), 175.0 (enhanced, 4-$\underline{C}$); *m/z* (EI) 255 (M^+, 54 %), 138 (100, $C_7H_4O_3^+$).