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Synthesis of D_4-6'-hydroxy-*O*-demethylangolensin, a deuterium labelled metabolite of genistein

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SUMMARY

6'-Hydroxy-*O*-demethylangolensin *[1-(2,4,6-trihydroxyphenyl-2-(4-hydroxyphenyl)-propan-2-one]* has been recently established as a metabolite of genistein in human urine. We report here the synthesis of 3,3,3",5"-d_4-6'-hydroxy-*O*-demethylangolensin **5** for use as an internal standard in quantitative measurements.

Keywords: deuterium, genistein, 6'-hydroxy-*O*-demethylangolensin, isoflavone, reduction, labeling

INTRODUCTION

6'-Hydroxy-*O*-demethylangolensin **1** was tentatively identified in human urine by GC-MS and was proposed to be a new metabolite of the natural phytoestrogen genistein **2** in man (1,2). More recently, the identity of **1** was secured by direct comparison with a synthetic authentic reference compound (3). 6'-Hydroxy-*O*-demethylangolensin and dihydrogenistein are the only genistein metabolites found in man so far. It is conjectured that, like genistein, the reduction products may have anticancer and other biological activities. Further clarification of the metabolism of isoflavones requires a deuterated internal standard for the quantitative determination of 6'-hydroxy-*O*-demethylangolensin **1** in fluids of human origin. We now report the synthesis of tetradeuterated 6'-hydroxy-*O*-demethylangolensin.

RESULTS AND DISCUSSION

We have previously reported the synthesis of 6'-hydroxy-*O*-demethylangolensin **1** by reduction of genistein **2** with $LiAlH_4$ in refluxing THF in 66% yield (4). We have also reported the deuterium labelling of genistein (5) to give 6,8,3',5'-d_4-genistein **3**. However, in **3** the D atoms at C-6 and C-8 are labile and are easily back-exchanged under acidic conditions (6), for example with methanolic HCl to give 3',5'-d_2-genistein

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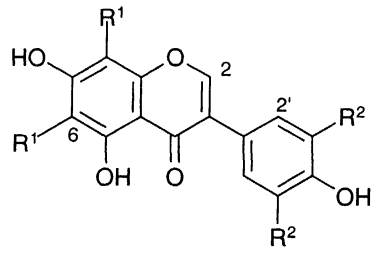
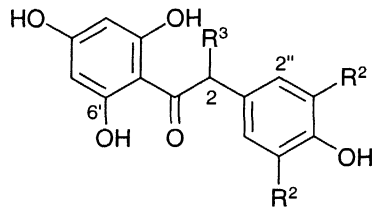
4. The reduction of **4** using the deuterated reducing agent, $LiAlD_4$, introduces two more deuterium atoms by way of a 1,4-addition, hetero ring opening, and another 1,4-addition (7), and gives d_4-6'-hydroxy-*O*-demethylangolensin **5** in 59% yield. The product is 93% isotopically pure d_4 material as measured from the mass spectrum. It should be noted that for use as an internal standard in GC-MS measurements, it is essential that the reference compound contains no d_0 or d_1 species, and that there is a sufficient number of D labels in the reference compound to overcome the overlap in the mass spectrum of M+1, M+2 etc. peaks arising from the high number of carbon and silicon atoms in the derivatized analyte. As for the location of the four D atoms in **5**, it is of course readily apparent in the proton and carbon NMR spectra (see Experimental) which signals have undergone a change compared to the spectra of undeuterated **1**. Specifically, it is seen that the methyl group in **5** contains just one H atom, coupled as a doublet to the adjoining benzylic methine. Furthermore, the remaining *p*-hydroxyphenyl ring protons at C-2" and 6" now appear as a singlet. Correspondingly, in the ^{13}C NMR spectrum the D-carrying carbon atoms C-3" and 5" appear as low-intensity triplets while the methyl carbon is a low-intensity quintuplet.



2 $R^1 = R^2 = H$
3 $R^1 = R^2 = D$
4 $R^1 = H$, $R^2 = D$



1 $R^2 = H$, $R^3 = CH_3$
5 $R^2 = D$, $R^3 = CHD_2$

EXPERIMENTAL

General: The product was characterized by ^1H and ^{13}C NMR, LRMS and HRMS. ^1H and ^{13}C NMR spectra were recorded on a Varian GEMINI-200 FT NMR spectrometer using the standard $^1H/^{13}C$ dual probe (chemical shifts in ppm). *J* values are given in Hz. In the ^{13}C NMR spectrum the chemical shifts given for the C-D triplets are those corresponding to the central peaks and are marked "D". LR and HR mass spectra were obtained with a JEOL JMS SX102 mass spectrometer operating at 70 eV. TLC was conducted on Merck silica gel 60 F_{254} plates and Merck silica gel 60 (0.040-0.063 mm, 230-400 mesh was used for flash chromatography. Tetrahydrofuran (THF) was dried by distilling over CaH_2 before use.

3,3,3",5"-d_4-6'-hydroxy-*O*-demethylangolensin 5 *[1-(2,4,6-trihydroxyphenyl-2-(4-hydroxyphenyl-3,5-D_2)-propan-2-one-3,3-D_2]*

A solution of 6,8,3',5'-d_4-genistein **3** (5) (0.096g, 0.35 mmol) in dry MeOH (10 ml) containing 1 % of AcCl was refluxed for 30 min and poured into ice water. Filtration and drying gave crude 3',5'-d_2-genistein which was added over 30 min to a stirred slurry of $LiAlD_4$ (0.08 g, 1.91 mmol) in refluxing dry THF (5 ml). Refluxing was continued for 2.5 hr and the reaction mixture was poured into icy saturated NH_4Cl solution. The mixture was neutralized with 2M HCl and after dilution with water extracted with EtOAc. The extract was washed with water, dried over Na_2SO_4 and evaporated. The residue (0.096 g, 99%) was purified by flash chromatography, eluting with CH_2Cl_2–EtOAc 1:1 v/v, to give **5** (0.057 g, 59%). ^{1}H-NMR (d_6-acetone): 1.40 (d, 1H, *J* 6.9, H-3), 5.24 (d, 1H, *J* 7.0, H-2), 5.89 (s, 2H, H-3',5'), 7.14 (s, 2H, H-2",6"); ^{13}C-NMR (d_6-acetone): 19.30 (quintuplet, C-3)^D, 49.22 (C-2), 95.92 (C-3',5'), 104.80 (C-1'), 155.85 (t, C-3",5")^D, 129.83 (C-2",6"), 134.06 (C-1"), 156.79 (C-4"), 165.32 (C-2',4',6') and 207.32 (C-1); *m/z* 278 (M^+, 9%), 277 (3), 276 (4), 154 (25), 153 (100) and 125 (16). Found: M^+, 278.1096. $C_{15}H_{10}D_4O_5$ requires M, 278.1092.

CONCLUSIONS

d_4-6'-Hydroxy-*O*-demethylangolensin **5** was synthesized by a one step reduction of 3',5'-d_2-genistein **4** with $LiAlD_4$. The 3',5'-d_2-genistein **4** is available by selective dedeuteration of the d_4-genistein **3**, prepared from genistein by CF_3COOD.

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