Pergamon

Tetrahedron Letters, Vol. 38, No. 41, pp. 7287-7290, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01693-6

Synthesis of D_4-Genistein, a Stable Deutero Labeled Isoflavone, by a Perdeuteration — Selective Dedeuteration Approach

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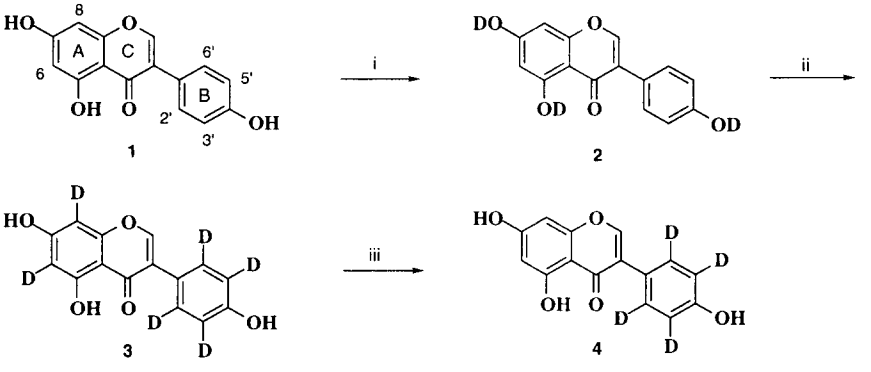
Abstract: Isotopically labile D_6-genistein **3**, obtained by D_3PO_4/BF_3 treatment of genistein **1**, is dedeuterated to the stable D_4-genistein **4** by methanolic HCl. © 1997 Elsevier Science Ltd.

The introduction of several isotopic labels, most often deuterium atoms, into a substrate is occasionally required for reaction mechanistic studies, structural elucidation, or analytical purposes.[1] However, synthetic labeling schemes frequently rely on laborous and inexpedient total syntheses using predeuterated starting materials. We have studied another approach namely the acid or base catalyzed H/D exchange of aromatic protons in natural isoflavonoids,[1-3] lignans[4] and of protons in the steroid ring system to give derivatives such as d_5-epitestosterone.[5] These methods usually afford isotopically homogenous labeled derivatives, without creating a cluster of D_3, D_4 and D_5 species for example. By making use of site-specific activation phenomena one may also direct the introduction of D atoms to give a single polydeuterated isomer which may be a requirement in mechanistic studies particularly. However, problems may arise if a site is very highly activated because the reverse reaction, i.e., the loss of label, becomes then very facile as well. We now describe an example of such a case, namely the 6,8,3',5'-tetradeuterogenistein, and show how this compound, containing a partially labile label arrangement, can be replaced by a fully stable isomer, the 2',3',5',6'-tetradeuterogenistein **4**.

We have previously synthesized 6,8,3',5'-tetradeuterogenistein[1,2] by refluxing D_2O-treated genistein **2** in deuterated trifluoroacetic acid for 2 days. This treatment was repeated three times and after the final H/D-exchange the reaction product was treated with aqueous EtOH to reinstate the protic hydroxy groups. The procedure gives a 75 % yield of D_4-genistein in >90 % isotopic purity, with the hydrogens *ortho* to the hydroxy groups having been changed to deuterium.[2] In the ID-GC-MS quantitation method of

isoflavonoids[1,6] an accurately weighed amount of a deuterated reference compound is added to the biological sample prior to extraction and purification in several ion exchange chromatographic steps. After TMS ether derivatization, the samples are analyzed by capillary column GC/MS in the selective ion monitoring mode, using an intense peak such as that at M-15. However, according to the MS fragmentation pattern the 6,8,3',5'-tetradeuterogenistein standard appeared to have lost a part of the deutero atoms in the ring A. It became clear that the deuterio labels at the highly activated ring positions 6 and 8 do not fully survive the analytical procedure and are lost to some extent. This makes it necessary to monitor the M-15 peak together with the peaks one and two mass units lower. Although the method produced reliable results the need for a stable polydeuterated derivative is obvious.[1,2]



Scheme 1. *Reagents and conditions:* i, D_2O/acetone; ii, D_3PO_4/BF_3, 55°C, 3 days, then H_2O/EtOH; iii, 1 % CH_3COCl in MeOH refl. 30 min.

We have now developed a deuteration method (Scheme 1), based on the use of deuterated phosphoric acid – boron trifluoride complex,[7] where all phenolic ring protons of genistein, m/z 270 ($M^{+\cdot}$, 100%), including those at the less activated C-2' and C-6' sites, can be exchanged to deuteriums.[8] The labile deuterium atoms of the resulting hexadeuterogenistein **3**, m/z 276 ($M^{+\cdot}$, 100%), are then selectively removed by methanolic HCl to give the stable 2',3',5',6'-tetradeutero derivative **4**, m/z 274 ($M^{+\cdot}$, 100%). According to the mass spectrum, all four deutero atoms are located in the B ring [cf. the rDA fragment ions at m/z 122 ($C_8H_2D_4O$) and 152 ($C_7H_4O_4$)]. In the ^{1}H-NMR spectrum all genistein ring B protons (2',3',5',6') are absent while the characteristic ring A AB system (δ 6.29 and 6.41, J = 2.1) remains. In the decoupled ^{13}C-NMR spectrum the ring B signals at δ 114.98 and 130.07 have undergone a change from intensive singlets to low intensity triplets due to C-D coupling.

The 6-, 8-, 3'- and 5'-sites in genistein are obviously highly activated for H-D exchange under electrophilic aromatic substitution conditions, allowing ready deuteration at these sites as mentioned above. Correspondingly, the most highly activated 6- and 8- sites lose their label very easily. The 2'- and 6'-sites are activated to a lesser extent by the heterocyclic ring oxygen, and exchange only under forcing conditions, i.e., by treatment with D_3PO_4/BF_3 at 55°C for 3 days.[8] It is significant that there is very little exchange at these sites when apigenin (5,7,4'-trihydroxyflavone, 5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-

benzopyran-4-one) is heated with D_3PO_4/BF_3. This is presumably due to the inability of the ring oxygen to activate the flavone 2'- and 6'-sites towards electrophilic aromatic substitution.

There has been a great demand for stable deutero labeled genistein **1** (5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) since its isolation and identification in human urine,[1] plasma,[9,10] feces,[6] and breast milk.[11] This dietary isoflavone is a common constituent of various soyfoods,[12] beans and sprouts.[13] Genistein possesses various interesting biological properties[14] and its beneficial role in preventing and treating hormone based cancers,[15] leukemia[16] and cardiovascular disease[17] is of major current interest. The new D_4-genistein **4** can be safely included at the beginning of the analytical procedure, all four deutero atoms being chemically stable and surviving all isolation and purification steps and also derivatizations under acidic or basic conditions. It has been successfully used in quantitating the genistein content of food samples such as soy, crisp bread and tea.[18] The perdeuteration — selective dedeuteration concept will also provide an easy access to other isotopically stable polydeuterated plant polyphenolics.[19]

Acknowledgements: This work was supported by the NIH (USA) grants 1 ROI CA56289-01 and 2 ROI CA56289-04 and the EU research contract FAIR-CT95-0894. KW acknowledges the Foundation of Antti and Jenny Wihuri for a grant and SR the University of Helsinki 350th Anniversary Fund for a scholarship.

REFERENCES AND NOTES

1. The SIM GC/MS technique of quantitation of natural products in complex matrices is greatly facilitated by the use of deuterated internal reference compounds. In the case of genistein **1** and other trihydroxylated compounds, preferably at least four D atoms should be present since the unlabeled TMS-derivatized compound shows fairly intense m+1 and m+2 ions in its mass spectrum owing to the high number of carbon and silicon atoms in the molecule. The incorporation of a sufficient number of D atoms will serve to shift the peaks of the reference compounds to higher m/z values free of interference from the peaks of the analyte. Note that the D atoms must be included in the compound itself and that derivatization using $(CD_3)_3Si$ ethers is not applicable. Adlercreutz, H.; Fotsis, T.; Bannwart, C.; Wähälä, K.; Brunow, G.; Hase, T. *Clin. Chim. Acta* **1991**, *199*, 263.
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8. Synthesis of (2',3',5',6'-D_4)genistein: [5,7-dihydroxy-3-(4-hydroxyphenyl-2,3,5,6-D_4)-4H-1-benzo-pyran-4-one] (**4**). Dry P_2O_5 (1.5 g) was treated with D_2O (1.5 ml) with stirring in an ice bath. This mixture was saturated with BF_3 gas at room temperature. The resulting complex (4 ml) was

added to predeuterated (evaporated from D_2O/acetone) genistein **2** (100 mg) and the reaction mixture was stirred at 55°C under anhydrous conditions for 3 days. The deuteration procedure was repeated twice. After the deuteration, the cooled reaction mixture was poured into ice water and the precipitated product was filtered, washed with water and treated with activated charcoal in 95% EtOH giving a quantitative yield of hexadeuterated genistein **3** [M^+ at m/z 276 (cf. genistein, M^+ at m/z 270); δ 8.16 (s, H-2)]. This material was then refluxed in 1% CH_3COCl/MeOH for 30 min, poured into ice water, filtered and purified by preparative thick-layer chromatography (silica gel, CH_2Cl_2-EtOAc 7:2) giving a 91% yield of 2',3',5',6'-tetradeuterogenistein **4** in >90% isotopic purity; m.p. 301-2 °C from EtOH/H_2O (302-5°C for D_0-genistein)[21]; UV λ_{max} (95% EtOH) 262 nm (log ε 4.52), 210 (4.40); ^{1}H-NMR (D_6-acetone) 6.29 ppm (s, 1H, H-6), 6.41 (s, 1H, H-8), 8.16 (s, 1H, H-2); , ^{13}C-NMR (D_6-acetone) 94.5 ppm (C-8), 99.8 (C-6), 106.2 (C-4a), 115.6 (m, C-3',5')^D, 122.8 (C-1'), 124.0 (C-3), 130.8 (m, C-2',6')^D, 154.3 (C-2), 158.4 (C-4'), 159.1 (C-8a), 163.9 (C-5), 165.0 (C-7), 181.7 (C-4); *m/z* 276 (7%), 275 (36), 274 (M^+, 100%), 273 (16), 272 (9), 245 (11), 153 (26), 137 (8), 122 (23); HRMS: elemental composition $C_{15}H_6D_4O_5$ requires 274.0779, found 274.0772.

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(*Received in UK* 25 *July* 1997; *revised* 14 *August* 1997; *accepted* 15 *August* 1997)