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Tritium Labeling of 7-*Iso*propoxy*iso*flavone

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SUMMARY

Catalytic tritiodehalogenation of 8-bromoipriflavone and 6,8-dibromoipriflavone resulted in [8-^{3}H]ipriflavone (**3**) and [6,8-$^3H_2$]ipriflavone (**7**) with specific activities of 1.08 TBq/mmol (29.2 Ci/mmol) and 1.94 TBq/mmol (52.4 Ci/mmol), respectively. 8-Bromoipriflavone was synthesized by direct bromination of ipriflavone, while 6,8-dibromoipriflavone was formed by *iso*propylation of the phenolic OH group of 6,8-dibromo-7-hydroxy*iso*flavone which itself was prepared from 7-hydroxy*iso*flavone and elemental bromine.

Keywords: tritiation, ipriflavone, isoflavone, bromination, osteopenia

INTRODUCTION

Ipriflavone (**1**, 7-*Iso*propoxy*iso*flavone, Osteochin®) is considered a highly useful agent in the treatment of osteoporosis (1). It is a synthetic flavonoid that decreases the renal excretion of calcium, enhances calcitonin secretion and simulates the effect of oestrogenes to manage bone resorption (2). It decreases bone resorption caused by phenylthiohydantoin, prostaglandin E_2 (3) and improves osteopenia induced by a low calcium and low vitamin D diet (4). For further study of the metabolic fate of ipriflavone and to examine the mechanism of action, the synthesis of ^{3}H labeled ipriflavone with high specific activity was necessary.

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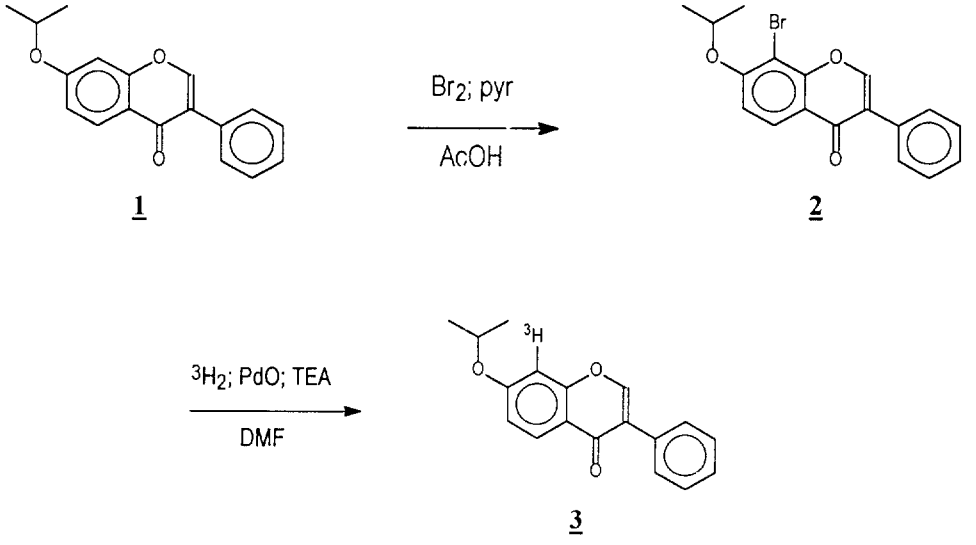
RESULTS AND DISCUSSION

Labeling of 1 was carried out by catalytic tritiodehalogenation of its brominated precursor to obtain high specific activity. This requires the synthesis of a suitable halogenated derivative to be tritiated under reductive conditions.

No satisfactory methods have been available for direct halogenation of 7-alkoxy*iso*flavones. Various methods include the synthesis of phenolic compounds, which were subsequently halogenated and then etherified (5). 7-Hydroxy*iso*flavone (4) and its derivatives with a halogen and/or other functional group(s) can be synthesized either by the reaction of chalcone and phenyl-benzyl-ketone intermediates containing halogen and other functional groups (6, 7, 8) or by direct halogenation of 4 (5).

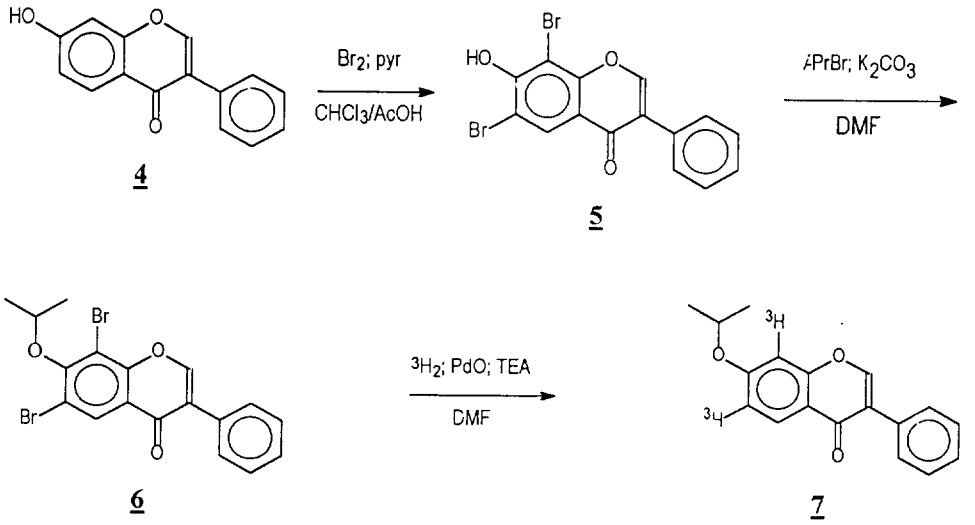
4 was synthesized by the method of *Roy et al.* (8), and converted to 1 by 2-bromopropane in DMF solution (*Scheme* 1). Bromination of 1 with Br_2 was catalyzed by pyridine as described for phenols by *Ingberman et al.* (9). This method, however, resulted in a monobrominated derivative (8-bromoipriflavone, 2), yielding monotritiation only (*Scheme* 1). To reach higher specific activity by dehalogenation, 6,8-dibromoipriflavone (6) was synthesized as a precursor for tritiation (*Scheme* 2). 4 was brominated with Br_2 by the method mentioned earlier (9) to obtain 6,8-dibromo-7-hydroxy*iso*flavone (5),

Scheme 1



which was then etherified with 2-bromopropane resulting in precursor **6** for tritiation. While a poisoned *Lindlar* catalyst was inactive, catalytic dehalogenation using supported Pd catalysts (Pd/C, $Pd/BaSO_4$) was accompanied by the saturation of the 2,3-double bond of **7** and **3**, as described for hydrogenation of other 7-substituted *iso*flavone derivatives by various catalysts such as PtO_2 (10) or Pd/C (11). To avoid this over-saturation, a milder reduction was carried out applying Pd catalyst without support for reduced duration.

Scheme 2



EXPERIMENTAL

General.-. $^3H_2$ Gas was purchased from *Technabexport*, USSR, and contained at least 98% $^3H_2$. All materials were analytical grade. DMF and Et_3N were purified by vacuum distillation and dried over 4 Å molecular sieves prior to use. The amount of tritiated compounds was measured by UV detection on a *Shimadzu-160* spectrophotometer. Tritiated samples were counted in *Liquidfluor* scintillant (*BDH*, England) with a *Searle-Delta-300* liquid scintillation counter. Purity of the compounds were controlled by TLC (silica gel *60* F_{254} (*Merck*, Art. No. 5554) plates developed using $CHCl_3$, and

CH_2Cl_2, and toluene/acetone 95:5 (*v/v*), spots were detected by UV light and iodine vapor). For purification, the plates were previously washed (13) with spectroscopic-grade EtOH and activated at 110° for 1 h. Radiochemical purity was checked with a *Berthold Radiochromatogram Tracemaster*. M.p.: *Kofler* melting-point microscope; uncorrected. ^{1}H-NMR spectra were recorded on a *Bruker-AM-400* spectrometer at the NMR Laboratory of Szeged Regional Center for Scientific Instruments, Szeged, Hungary; d in ppm, *J* (apparent) in Hz. Mass spectra were obtained using a *VG Trio-2* mass spectrometer (EI, 70 eV). All compounds gave satisfactory elemental analysis C ± 0.3 %, H ± 0.2 %, N ± 0.3 %). Tritiation was performed using a glass manifold described earlier (12).

7-hydroxyisoflavone (4). The compound was prepared by the method described by *Chakravarti et al.* (6). M.p. 207-208°. ^{1}H-NMR ($CDCl_3$) 6.86 (d, J = 2.3, H-C(8)); 6.92 (*q*, J_1 = 2.3, J_2 = 8.7, H-C(6)); 7.42 (*m*, 3 H, H-C(3',4',5')); 7.55 (*m*, 2 H, H-C(2',6')); 7.95 (*s*, H-C(2)); 8.18 (*d*, J = 8.7, H-C(5)). MS: 237 (100, M^+), 209 (4), 136 (54), 108 (62) 102 (25). TLC: CH_2Cl_2, R_f 0.02; $CHCl_3$, R_f 0.03; $CHCl_3$/acetone 95:5, R_f 0.35.

7-(2-propoxy)-isoflavone (1, ipriflavone). To a solution of 4 (10.0 g, 41.9 mmol) in DMF (8.0 ml), 2-bromopropane (7.3 g, 59 mmol) was added in the presence of fused K_2CO_3 (7.6 g, 55 mmol). The mixture was stirred for 2 h at temperatures between 75° and 95°, and then for 10 min at 100°. The reaction mixture was triturated with a mixture of 5.5 ml 2-propanol and 35 ml water to crystallize the product. The precipitate was collected by filtration and washed with water and then dried at 60° to constant weight. Yield: 12 g, 93 %. M.p. 118-119°. ^{1}H-NMR ($CDCl_3$) 1.41 (*d*, J = 6.0, 6 H, 1,3-Me_2 of 2-PrO-C(7)); 4.67 (*h*, J = 6.0, *t*-H of 2-PrO-C(7)); 6.84 (*d*, J = 2.3, H-C(8)); 6.96 (*q*, J_1 = 2.3, J_2 = 8.9, H-C(6)); 7.41 (*m*, 3 H, H-C(3',4',5')); 7.56 (*m*, 2 H, H-C(2',6')); 7.94 (*s*, H-C(2)); 8.20 (*d*, J = 8.9, H-C(5)). MS: 280 (27, M^+), 237 (100), 209 (7), 136 (32), 108 (38), 102 (22). UV ($mol^{-1}dm^3cm^{-1}$; 95 % EtOH): e_{299}=14100; $e_{249.8}$=32500; $e_{208.2}$=24900. TLC: CH_2Cl_2, R_f 0.15; $CHCl_3$, R_f 0.36; toluene/acetone 95:5, R_f 0.40).

8-bromo-7-(2-propoxy)-isoflavone (2, 8-bromoipriflavone). To a solution of 1 (100 mg, 0.357 mmol) in glacial acetic acid (5 ml) a solution of 0.15 M Br_2 (10 ml) and 1 ml

solution of pyridine (27 %, m/v) in glacial acetic acid were added. After 3 h at room temperature, the excess of Br_2 was reacted with aqueous KI solution, and the I_2 formed was titrated with $Na_2S_2O_3$, and the product was then extracted from the mixture with $CHCl_3$ (4 x 20 ml). The solvent was evaporated, and the crude product was crystallized from EtOH: 51 mg (14 %). M.p. 163-165°. ^{1}H-NMR ($CDCl_3$) 1.46 (*d*, *J* = 6.0, 6 H, 1,3-Me_2 of 2-PrO-C(7)); 4.79 (*h*, *J* = 6.0, *t*-H of 2-PrO-C(7)); 7.04 (*d*, *J* = 9.1, H-C(6)); 7.43 (*m*, 3 H, H-C(3',4',5')); 7.56 (*m*, 2 H, H-C(2',6')); 8.06 (*s*, H-C(2)); 8.24 (*d*, *J* = 8.9, H-C(5)). MS: 360 (31, M^+), 317 (100), 289 (9), 237 (22), 214 (45), 186 (12), 102 (70). UV ($mol^{-1}dm^3cm^{-1}$; 95 % EtOH): $e_{304.0}$=10900; $e_{248.4}$=35600; $e_{203.0}$=26300. TLC: CH_2Cl_2, R_f 0.22; $CHCl_3$, Rf 0.44; toluene/acetone 95:5, R_f 0.37).

7-(2-propoxy)-[8-^{3}H]isoflavone (3, [8-^{3}H]ipriflavone). To a solution of **2** (3.6 mg, 10 mmol) in DMF (1 ml), PdO catalyst (5.7 mg, *Merck*) and Et_3N (4 *m*l) were added and stirred for 30 min in the presence of $^3H_2$ gas (555 GBq 15 Ci) in a closed vacuum manifold (12). The excess of $^3H_2$ gas was removed by absorption on pyrophoric uranium. The catalyst was filtered off using *Whatman-GF/C* glass-fiber filter. Labile ^{3}H was removed from the compound by repeated evaporation with EtOH/H_2O 3:1. The radioactivity of the crude product was 12.6 GBq (340 mCi), and after purification by TLC (silica gel *60* F_{254} plate, (*Merck*), eluent CH_2Cl_2), 4.92 TBQ (133 mCi, 39 %) were recovered. The purity of **5** was > 97 % by TLC. Specific radioactivity was 1.08 TBq/mmol (29.2 Ci/mmol).

6,8-dibromo-7-hydroxyisoflavone (5). To a solution of **4** (476 mg, 2 mmol) in glacial acetic acid (20 ml) a solution of 0.15 M Br_2 (20 ml) and 1 ml solution of pyridine (27 %, m/v) in glacial acetic acid were added. After 5 min at room temperature the reaction mixture was worked up as for **2**. Yield: 504 mg (1.28 mmol, 64 %). M.p. 216-217° (EtOH). ^{1}H-NMR ($CDCl_3$) 7.44 (*m*, 3 H, H-C(3',4',5')); 7.54 (*m*, 2 H, H-C(2',6')); 8.06 (*s*, H-C(2)); 8.47 (*s*, H-C(5)). MS: 395 (100, M^+), 366 (3), 317 (12), 294 (44), 266 (8), 238 (21), 102 (84). TLC: CH_2Cl_2, R_f 0.04; $CHCl_3$, R_f 0.06; $CHCl_3$/acetone 95:5, R_f 0.42.

6,8-dibromo-7-(2-propoxy)-isoflavone (6, 6,8-dibromoipriflavone). A solution of 5 (400 mg, 1.01 mmol) in 10 ml of 2-bromopropane/$CHCl_3$ (1:1, v/v) was refluxed for 30 min in the presence of K_2CO_3. After cooling the mixture, the inorganic salt was filtered off and the solvent evaporated. The crude product was crystallized from EtOH: 351 mg (79 %). M.p. 148.5-149°. ^{1}H-NMR ($CDCl_3$) 1.45 (*d*, *J* = 6.1, 6 H, 1,3-Me_2 of 2-PrO-C(7)); 4.94 (*h*, *J* = 6.1, *t*-H of 2-PrO-C(7)); 7.44 (*m*, 3 H, H-C(3',4',5')); 7.55 (*m*, 2 H, H-C(2',6')); 8.09 (*s*, H-C(2)); 8.49 (*s*, H-C(5)). MS: 438 (13, M^+), 395 (100), 367 (7), 294 (26), 232 (6), 102 (43). UV ($mol^{-1}dm^3cm^{-1}$; 95 % EtOH): $e_{320.0}$=6880; $e_{256.2}$=35300; $e_{203.0}$=34500. TLC: CH_2Cl_2, R_f 0.37; $CHCl_3$, R_f 0.62; toluene/acetone 95:5, R_f 0.61).

7-(2-propoxy)-[6,8-$^3H_2$]isoflavone (7, [6,8-$^3H_2$]ipriflavone). To a solution of 6 (2.4 mg, 5.5 mmol) in DMF (1 ml), PdO catalyst (5.9 mg, *Merck*) and Et_3N (4 ml) were added and stirred for 30 min in the presence of $^3H_2$ gas (555 TBQ 15 Ci). Further procedures were as for 5. The radioactivity of the crude material was 88.1 MBQ (238 mCi), and after purification by TLC (silica gel *60* F_{254} plate, (*Merck*), eluent CH_2Cl_2), 2.11 TBQ (57.1 mCi, 24 %) were recovered. The purity of 7 was > 96 % by TLC. Specific radioactivity was 1.94 TBq/mmol (52.4 Ci/mmol).

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