SYNTHESIS OF ^{2}H-, ^{3}H- AND ^{14}C-LABELLED CP-45,634 (SORBINIL)

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SUMMARY

The syntheses of ^{3}H- and ^{14}C-labelled *S*-(+)-6-fluoro-2,3-dihydrospiro-[4H-1-benzopyran-4,4'-imidazolidin]-2',5'-dione (sorbinil, CP-45,634), an aldose reductase inhibitor with potential utility in the treatment of chronic diabetic complications, are described. Tritiated sorbinil was prepared by a reductive dehalogenation of the 8-chloro substituted analog with $^{3}H_2$. ^{14}C-labelled CP-45,634 was prepared using a Bucherer-Bergs synthesis of the racemic hydantoin beginning with 2,3-dihydro-6-fluoro-4H-1-benzopyran-4-one and ^{14}C-potassium cyanide, followed by brucine resolution to isolate the pharmacologically active *S*-(+)-enantiomer.

Key Words: sorbinil, CP-45,634, aldose reductase inhibitor, diabetes.

INTRODUCTION

CP-45,634 (sorbinil, *S*-(+)-6-fluoro-2,3-dihydrospiro[4H-1-benzopyran-4, 4'-imidazolidin]-2',5'-dione] 1 was one of the first and most widely studied of a new class of therapeutic agents, the aldose reductase inhibitors, designed to control the chronic complications of diabetes.[1,2] These complications, including neuropathy, retinopathy, nephropathy and cataract formation, often appear in diabetic patients several years after the onset of the disease. In order to better understand the metabolism and organ distribution of this unique agent, it became necessary to prepare derivatives containing ^{3}H and ^{14}C at potentially metabolically stable sites on the molecule. These compounds were obtained by incorporation of the desired isotopes at the final stages of their respective syntheses, providing radiolabelled compounds of high specific activity.

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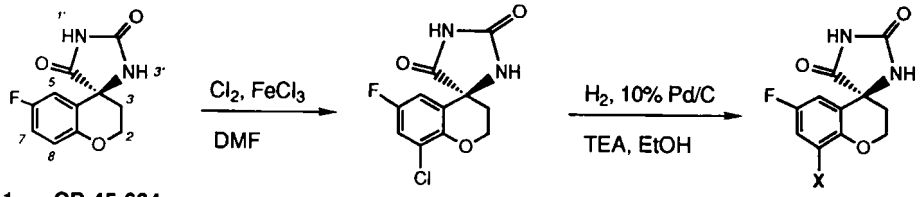
RESULTS AND DISCUSSION

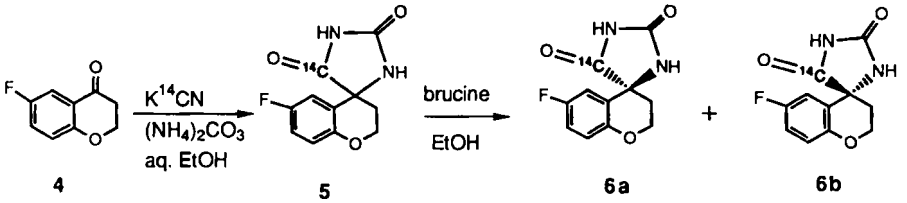
Our prior experiences with CP-45,634 (1) had demonstrated that this molecule was inert under a variety of catalytic hydrogenation conditions. Therefore, it seemed that the most efficient manner for the introduction of a tritium atom was via catalytic dehalogenation of a halogen substituted intermediate. It proved convenient to prepare the 8-chloro analog (2) of CP-45,634 via an $FeCl_3$ catalyzed chlorination of 1 (Scheme 1). Location of the chlorine atom was unambiguously determined by ^{1}H-NMR, and its dechlorination to 3a was initially conducted with deuterium to refine the reaction conditions. In this manner, we were able to achieve a 41% incorporation of ^{2}H in the presence of triethylamine. When compound 2 was treated under similar conditions using tritium gas for 18 hours, the incorporation of ^{3}H at the same position was approximately 35%, with a specific activity for 3b of 10.04 Ci/mmol (Scheme 1).



1, CP-45,634 2 3 a X = ^{2}H b X = ^{3}H

Scheme 1

Since it was conceivable that the tritium in the 8-position of CP-45,634 might be metabolically labile, we were also interested in preparing a ^{14}C-labelled derivative. For this purpose we chose to label the imidazolidinedione ring in the 5'-position, since ^{14}C-labelled potassium cyanide is readily available and since the hydantoin ring formation with KCN is the penultimate step in the synthesis of CP-45,634. Indeed, as shown in Scheme 2, the Bucherer-Bergs reaction proceeded readily in a pressure vessel to give the racemic product which was then resolved with brucine.



Scheme 2

In conclusion, we have prepared the aldose reductase inhibitor CP-45,634 (sorbinil) with ^{3}H and ^{14}C atoms in the 8- and 5'-positions, respectively. These compounds should help to provide a clearer understanding of the disposition and metabolism of this unique therapeutic agent when used in man. The tritium-bearing analog 3b has since been evaluated in two independent studies.[4,5]

EXPERIMENTAL SECTION

Melting points were determined with Pyrex capillary tubes on a Thomas Hoover capillary melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded using a Bruker WM-250 or a Varian XL-300 spectrometer; data are reported in parts per million (δ) with reference to the deuterium lock signal of the solvent. Mass spectral data were obtained using a Finnigan 4510 instrument for low resolution or an AEI30 instrument for high resolution determinations. UV determinations were made in a 2 cm cell with a Cary Model 15 UV Spectrophotometer. Analytical thin-layer chromatography (TLC) was conducted on 0.25 mm thick silica gel 60 F-254 plates manufactured by E. Merck and Co.; plates were visualized with UV light. Elemental analyses were performed by the Pfizer Analytical Department. The reactions employing tritium gas were conducted at New England Nuclear (Dupont), Boston, MA.

Radio-TLC scans were obtained on a Model LB242K Varian Aerograph/Berthold Radio Scanner. Scintillation determinations were made with a Model 6880 Searle Mark III Liquid Scintillation System, using Complete Liquid Scintillation Fluid (ISO Lab Inc.), correcting for counting efficiency using an internal standard technique. Radiochemical purity was

confirmed by HPLC using an 8 mm Nova-Pak C^{18} column and monitoring with a 280 nm UV detector and a Berthold Model LB504 radioactivity monitor. Elution was conducted with MeCN:H_2O:0.003 M H_3PO_4 (22.5:62.5:15) at a flow rate of 2.8 ml/min (the retention time for 1 was 6.5 min under these conditions).

S-(+)-8-Chloro-6-fluoro-2,3-dihydrospiro[benzopyran-4,4'-imidazolidin]-2',5'-dione (2). Through a solution of 1[3] (1.18 g, 5 mmol) and 20 mg of $FeCl_3$ in 10 mL of dry DMF, cooled to -40 °C, Cl_2 gas was slowly bubbled for 20 min. After stirring at -20 °C for 2.5 hr, the light green solution was allowed to warm to room temperature and was treated slowly with 50 mL of H_2O. The diluted mixture was extracted with EtOAc and the organic extracts were washed with saturated NaCl and dried over $MgSO_4$. On concentration in vacuo, a golden yellow oil was obtained which was flash chromatographed on silica gel (230-400 mesh) eluting with EtOAc. The product 2 was isolated as a colorless oil which crystallized to a white solid when triturated with pentane, 0.704 g (55%), mp 100-103 °C dec.

^{1}H-NMR (CD_3OD) δ 2.15-2.45 (m, H(3), 2H), 4.25-4.40 (m, H(2), 1H), 4.65-4.80 (m, H(2), 1H), 6.88 (dd, J=8.6, 3.1 Hz, H(5), 1H), 7.22 (dd, J=8.0, 3.0 Hz, H(7), 1H).

MS (EI): m/e = 272 (32%, M^{+2}), 270 (100%, M^+), 199 (97%, $[M-CONHCO]^+$), 171 (99%).

Anal. calcd for $C_{11}H_8ClFN_2O_3$: C 48.82, H 2.98, N 10.35; found: C 48.00, H 3.15, N 9.93; this analysis is consistent with a partial hydrate: calcd for $C_{11}H_8ClFN_2O_3 \cdot 0.33H_2O$: C 47.75, H 3.16, N 10.13.

[8-^{2}H]-CP-45,634 (3a). A mixture of 2 (60 mg, 0.22 mmol), triethylamine (0.5 mL) and 10% Pd on C (100 mg) in 4 mL of EtOH was treated with deuterium gas at atmospheric pressure. After 20 hr the uptake of $^2H_2$ had ceased and the mixture was filtered through diatomaceous earth and concentrated in vacuo to a pale yellow solid. Chromatography on 5 mL of 230-400 mesh silica gel, eluting with 100% EtOAc, provided pure title compound 3a as a white solid, 32 mg (62%), mp 228-231 °C. The mass spectrum of 3a compared with pure 1 indicated a deuterium incorporation of approximately 41%.

MS (EI): m/e = 237 (53%, M^+_{3a}), 236 (66%, M^+_1), 166 (55%), 165 (82%), 137 (100%).

[8-^{3}H]-CP-45,634 (3b). Using the same quantities of reagents employed in the previous experiment, 60 mg (0.22 mmol) of 2 was treated with $^3H_2$ at 25 °C for 18 hr. After chromatography (as described for 3a), the purified title compound (50.4 mg) was dissolved in 44 mL of a 9:1 MeOH:benzene solution for storage. The specific activity of the solid was determined by liquid scintillation to be 10.04 Ci/mmol. ^{3}H incorporation was estimated to be ca. 35% (based on a theoretical value of 29 Ci/mmol for 100% ^{3}H incorporation). Radiochemical purity, as determined by radio-TLC, was >98%.

[5'-^{14}C]-CP-45,634 (6a). In a steel reaction bomb (approx. volume of 65 mL) were placed 6-fluoro-2,3-dihydro-4H-1-benzopyran-4-one 4[3] (0.83 g, 5 mmol), finely powdered ammonium carbonate (1.92 g, 20 mmol), potassium cyanide (0.300 g, 4.6 mmol) and [^{14}C]-potassium cyanide (K^{14}CN, 0.130 g, 1.9 mmol) in a mixture of 6 mL of H_2O and 15 mL of EtOH. After sealing, the bomb was immersed in an oil bath preheated to 90 ± 2 °C and was heated at this temperature for 72 hr. After cooling to 25 °C, the mixture was washed with H_2O into a 200 mL 3-neck flask and the contents were acidified with 2N HCl to pH 2-3. (NOTE: the acidification was performed under N_2, flushing the generated HCN into a trap containing 2N NaOH). After 30 min., the contents were filtered and the filtrate containing crude product was extracted with EtOAc. After drying with $MgSO_4$, the EtOAc was evaporated in vacuo to a yellow oil, 0.930 g. Chromatography with silica gel (70 g, 70-230 mesh) using EtOAc:Hexanes (60:40) gave 5 as a colorless solid, 0.560 g (47%).

The racemic hydantoin 5 (0.560 g, 2.37 mmol) was dissolved in 10 mL of hot EtOH and was then treated with brucine dihydrate (1.01 g, 2.35 mmol) dissolved in 10 mL of hot EtOH. The mixture was heated at reflux for 10 min and allowed to cool overnight to produce white crystals of the brucine adduct, 0.387 g. This solid was partitioned between 8 mL of 1N HCl and 15 mL of EtOAc, the organic layer was dried with $MgSO_4$ and concentrated to a white solid, 132.5 mg. Recrystallization from EtOH gave 83 mg of fluffy white crystals of the pure *S*-(+) isomer 6a, $[\alpha]_D^{25}$ +53.7 ° (c=1, MeOH)[6]; The specific activity of 6a was determined by liquid scintillation analysis to be 7.84 μCi/mg (1.85 mCi/mmol); radiochemical purity was >99% by HPLC.

From the mother liquor of the original brucine adduct formation, the

R-(-) enantiomer **6b** was similarly isolated as fine white needles (recrystallized from EtOH), 0.049 g, $[\alpha]_D^{25}$ -56.6 ° (c=1, MeOH)[7].

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6. As in ref 3: $[\alpha]_D^{25}$ +54.0 ° (c=1, MeOH).
7. As in ref 3: $[\alpha]_D^{25}$ -54.8 ° (c=1, MeOH).