NOTE

SYNTHESIS OF TRITIUM LABELLED 5,6-DIHYDRO-7-(1H-IMIDAZOL-1-YL) -NAPHTHALENE-2-CARBOXYLIC ACID (FCE 22178), A NEW THROMBOXANE SYNTHETASE INHIBITOR.

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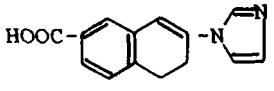
SUMMARY

Synthesis of [^{3}H]FCE 22178, namely 5,6-dihydro-7-(1H-[G-^{3}H]imidazol-1-yl)-naphthalene-2-carboxylic acid 5 was performed in three steps from 5,6,7,8-tetrahydro-8-hydroxy-7-(1H-imidazol-1-yl)-naphtalene-2-carboxylic acid 2 by bromination, catalytic tritiation and final dehydration, in an overall radiochemical yield of 48%. The product was 97% radiochemically pure and had a specific radioactivity of 9.15 GBq/mmol.

Key words : FCE 22178, [^{3}H]FCE 22178, thromboxane-synthetase inhibitor.

INTRODUCTION

Interest in imidazole derivatives as preferential inhibitors of thromboxane A_2 (TxA_2) synthetase [1], without effect on cyclooxygenase, recently led to the preparation of several N-imidazolyl derivatives of the naphthalene and chroman rings [2]. Among these, 5,6-dihydro-7-(1H-imidazol-1-yl)-naphthalene-2-carboxylic acid 1 (FCE 22178) has been shown to exert a strong, long-lasting inhibition of TxA_2 synthetase and an appreciable stimulation of prostacyclin (PGI_2) synthesis [3].



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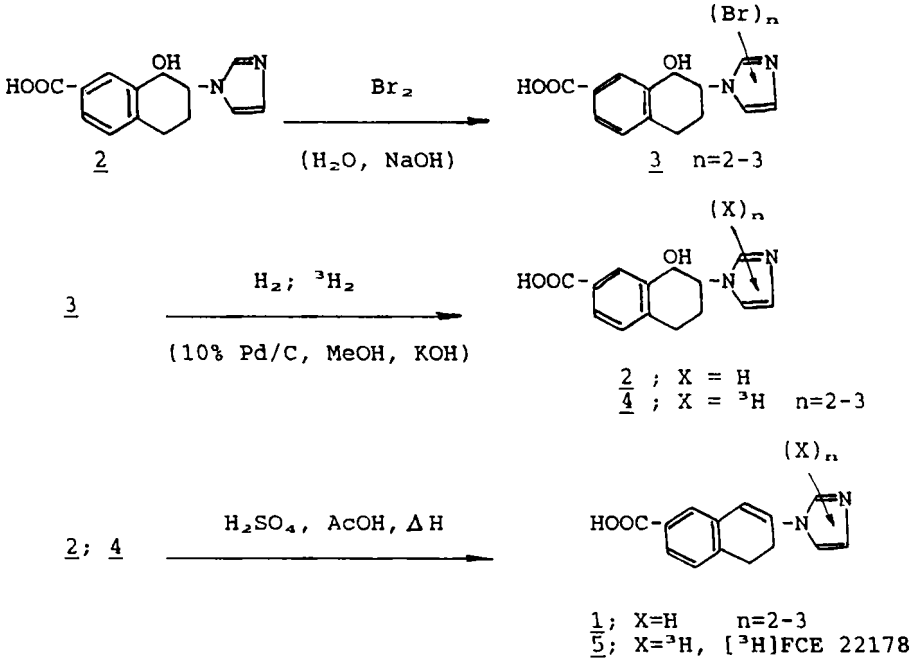
In order to allow subsequent pharmacokinetic and metabolism studies, we investigated possible synthetic routes for the preparation of FCE 22178 labelled with radioisotopes.

RESULTS AND DISCUSSION

Two possible methods of carbon 14 labelling were considered: the introduction of the radiocarbon in the imidazole ring or in the carboxylic group. Neither of these methods were suitable. The first possibility [2] included the use of an excess of [^{14}C]imidazole as commercially available precursor, in a condensation reaction with the appropriate bromoderivative [2]. However the yield of the condensation reaction was not sufficient to produce a satisfactory overall radiochemical yield (about 2-4%) of the final product. The second possibility, using K^{14}CN, was not feasible, at the time of the study, because the required unlabelled precursor, namely 5,6-dihydro-7-(1H-imidazol-1-yl)-2-bromo-naphthalene, was not available.

Incorporation of tritium, rather than ^{14}C, constituted a simpler and more convenient approach to the labelling of FCE 22178, in view of the feasibility of introducing bromine in the imidazole ring and the well known procedure of catalytic dehalogenation by tritium gas. The possibility of an undesired reduction of the 7,8-double bond by tritium, prompted us to perform halogen-tritium replacement on the brominated 8-hydroxy compound 3 and to carry out the dehydration to 5 as a final step (see the scheme).

SCHEME



The hydroxy compound 2 was readily available as an intermediate in the laboratory scale preparation of FCE 22178 [4]. Bromination of the imidazole ring was accomplished with bromine in alkaline medium according to a general method [5]. The elemental and NMR analyses showed that the above reaction yielded a mixture of 5,6,7,8-tetrahydro-8-hydroxy-7-(2,4,5-tribromo-1H-imidazol-1-yl)-naphthalene-2-carboxylic acid and its dibromo analogues.
As it was not important to know the labelling position of tritium for the preliminary drug disposition studies, the hydrogenation of mixture 3 to compound 2 was carried out in alkaline medium (methanolic KOH) employing 10% Pd/C as catalyst. The same procedure was then adopted to obtain the labelled compound 4 (by Amersham Int. p.l.c.), which was purified by preparative TLC and successively submitted to dehydration in AcOH-H_2SO_4 [6]. Crude 5 was purified by preparative TLC giving the expected [^{3}H]FCE 22178, 98% radiochemically pure, with a specific radioactivity of 9.15 GBq/mmol. The overall radiochemical yield from the mixture 3 was 48%.

EXPERIMENTAL

Thin layer chromatography (TLC)

TLC was carried out, where not specified, using Merck silica gel F 254 20x5 cm, 0.25 mm thick plates. The eluting solvent systems were as follows with proportions by volume:

A) acetone:water:acetic acid (45:5:2.5)

B) chloroform:methanol:acetic acid (90:10:5)

C) chloroform:methanol:formic acid (16:4:4)

D) chloroform:methanol:ammonia 30% (14:6:2)

High performance liquid chromatography (HPLC)

Analyses were performed using a Partisil 10 ODS (250 x 4.6 mm ID) column with a mobile phase of CH_3CN : [0.05 M formic acid + 0.02 M acetic acid + 0.02 M n-butyl amine (pH 4.3 with 1N NaOH)] (21 : 79 by volume); flow rate 1.2 ml/min; UV detection 254 nm; radiometric detection with heterogeneous cell (0.36 ml) Yttrium silicate.

Assay methods

Ultraviolet spectra were determined on a Beckman DU50 spectrophotometer. Measurements of radioactivity were carried out with a Packard 300C liquid scintillation counter using Rialuma (Lumac System A.G.) as liquid scintillation cocktail. Radiochemical analyses of TLC plates were performed with a Berthold 3832 automatic linear analyzer.
HPLC analyses were carried out with a Perkin Elmer series 2x2 liquid chromatograph with LC 75 UV/VIS detector and Packard Trace 7130 on line with 512 kRAM 3270 IBM PC as radioactivity flow monitor.
Melting points were determined on a Buchi apparatus and are

uncorrected. ^{1}H-NMR were recorded on a Bruker HX-90 spectrometer (chemical shifts are given in ppm (δ) downfield from tetramethylsilane). Mass spectra were recorded on a Finnigan MAT CH7 mass spectrometer. Elemental analyses were performed on a Carlo Erba 1106 instrument.

The hydrogenation with tritium was carried out by Amersham International plc.

5,6,7,8-Tetrahydro-8-hydroxy-7-(2,4,5-tribromo-1H-imidazol-1-yl)-naphthalene-2-carboxylic acid and its dibromo analogues (3)

To a stirred solution of 5,6,7,8-tetrahydro-8-hydroxy-7-(1H-imidazol-1-yl)-naphthalene-2-carboxylic acid 2 (1.0 g, 3.87 mmoles) in water (30 ml) and 1N NaOH (3.87 ml), bromine (1.85 g, 11.62 mmoles) was added dropwise at room temperature. The reaction mixture was kept at pH 6 by continuous addition of 1N NaOH. At the end of the reaction the mixture was filtered and the pH of the solution was adjusted to 4 with 2N HCl. The precipitate was filtered, washed with water and crystallized from methanol to give 0.7 g of the mixture 3.

m.p.: 240°C (decomposition)

NMR (DMSO-d_6;90 MHz): 1.80-3.0 (4H, m, CH_2-CH_2); 4.5 (1H, m, CH-CHOH); 5.3 (1H, m, CHOH); 6.2 (1H, d, OH); 7.25 (1H, d, phenylH-4); 7.8 (1H, dd, phenylH-3); 8.15 (1H, d phenylH-1).

MS (EI): m/z (% relative intensity) 492 (M , 10), 474 (>1), 413 (17), 395 (1), 302(34), 304 (100).

Br % of mixture 3 : 41.43 %.
Br % calculated for $C_{14}H_{11}Br_3N_2O_3$ and $C_{14}H_{12}Br_2N_2O_3$: 48.45 and 38.46 % respectively.

5,6,7,8-Tetrahydro-8-hydroxy-7-(1H-imidazol-1-yl)-naphthalene--2-carboxylic acid (2)

Mixture 3 (0.1 g), dissolved in methanol (8 ml) containing N/2 methanolic KOH (1.21 ml), was hydrogenated over palladium catalyst (10% Pd/C) for 2 hours at room temperature at normal pressure. The catalyst was filtered off and the solution was evaporated to dryness. The resulting residue was taken up with water and the pH of the solution was adjusted to 5.5 with glacial acetic acid. After filtration, the precipitate was washed with water (2 x 0.5 ml) and dried to give 0.04 g (0.155 mmoles) of compound 2.

TLC: system A R_f = 0.38

m.p.: 240-242°C

NMR (DMSO-d_6; 90 MHz): 1.80-3.0 (4H, m, CH_2-CH_2); 4.21 (1H, dt, CH-CHOH); 4.79 (1H, d, CHOH); 6.93-8.14 (6H, m, benzene-H and imidazole-H).

Elemental Analysis : calculated for $C_{14}H_{14}N_2O_3$: C= 65.10; H= 5.46; N= 10.84. Found: C= 65.51; H= 5.38; N= 10.81.

5,6,7,8-Tetrahydro-8-hydroxy-7-(1H-[G-^{3}H]imidazol-1-yl)-naphthalene-2-carboxylic acid (4)

Compound 3 (10 mg) was treated with tritium gas (370 GBq), in the presence of 10% Pd/C, by Amersham Int. plc. according to the procedure already described for the unlabelled compound, yielding the crude 4 (37 GBq), 80% radiochemically pure (by radio-TLC system A). A portion of the crude 4 (377 MBq) was added to "cold" 4 (10.1 mg) and chromatographed by TLC (silica gel TLC plate Merck F254; 20x20 cm; 0.25 mm thick) using the system A as eluting solvent system. The chromatographic band corresponding to the compound 4 was extracted from silica gel with a mixture methanol:ammonia 30% 100:1 (by volume)(5x5 ml). After solvent evaporation, the hydroxy derivative 4 (268 MBq) was recovered 90% radiochemically pure (by radio-TLC system A: R_f=0.38) and was used without further purification in the next step.

5,6-Dihydro-7-(1H-[G-^{3}H]imidazol-1-yl)-naphthalene-2-carboxylic acid (5)

The compound 4 (268 MBq), dissolved in a mixture of glacial acetic acid (0.4 ml) and 96% H_2SO_4 (0.1 ml), was heated at 100°C with stirring for about 3 hours. At the end of the reaction (checked by radio-TLC; system B: R_f=0.34) the mixture was cooled to 0°C, diluted with water (2 ml) and the pH of the solution was adjusted to > 9 by adding 30% ammonia. The solvent was then evaporated to dryness under vacuum. The solid residue was suspended in a mixture (10 ml) of methanol:30% ammonia ; 100:1 (by volume). The insoluble ammonium salts were separated by filtration through a D_4 sintered glass filter. After solvent evaporation, the compound 5 was obtained 97% radiochemically pure (by radio-TLC ; system B) but spectrometrically impure. The purification was carried out by preparative TLC employing the mixture B as chromatographic eluent. The product 5 was extracted from silica gel with a mixture (5x5 ml) 95°ethanol:30% ammonia; 100:1 (by volume). The combined extracts yielded, after solvent evaporation, [^{3}H]FCE 22178 (180.45 MBq) with a radiochemical purity > 97% (by radio-TLC; system C: R_f=0.39 and system D: R_f=0.24; by radio-HPLC: t_R= 8.1 min) and a specific radioactivity of 9.15 GBq/mmol. The UV spectrum (in 95° ethanol:30% ammonia, 100:1 (by volume); $E^{1\%}_{1cm}$=688 at 288 nm λ_{max}) was concordant to that of a standard sample. The overall radiochemical yield from crude 3 was 48%.

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