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SYNTHESIS OF (R,S)-6H-6-[2-(DIMETHYLAMINO)ETHOXYCARBONYL]--[6-^{14}C]DIBENZO[b,d]PYRAN.HCl ([^{14}C] FCE 20696)

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SUMMARY

A seven steps synthesis of (R,S)-6H-6-[2-(dimethylamino) ethoxycarbonyl]-[6-^{14}C]dibenzo[b,d]pyran.HCl [^{14}C]FCE 20696 employing 9-[9-^{14}C]fluorenone as labelled starting material is reported.The final product [^{14}C] FCE 20696 was obtained in an overall radiochemical yield of 31%, 97% radiochemically pure and with a specific radioactivity of 115 MBq/mmol.

Key words: FCE 20696,(R,S)-6H-[6-^{14}C]dibenzo[b,d]pyran-6-carboxylic acid, immunomodulating agent.

INTRODUCTION

During a screening program for the search of new synthetic immunomodulating agents, FCE 20696, namely (R,S)-6H-6-[2-(dimethylamino)ethoxycarbonyl]-dibenzo[b,d]pyran.HCl, was found to have modulatory properties in a wide number of immune responses and a protective activity in a broad range of viral infections in mice [1][2].

In order to perform "in vivo" and "in vitro" studies with this new immunomodulating agent, a radiolabelled form was required.

The commercial availability of 9-[9-^{14}C]fluorenone 1 and the high yields of each synthetic step, prompted us to prepare [^{14}C] FCE 20696 according to the procedure already employed in our chemical laboratories [3][4], as shown in the scheme.

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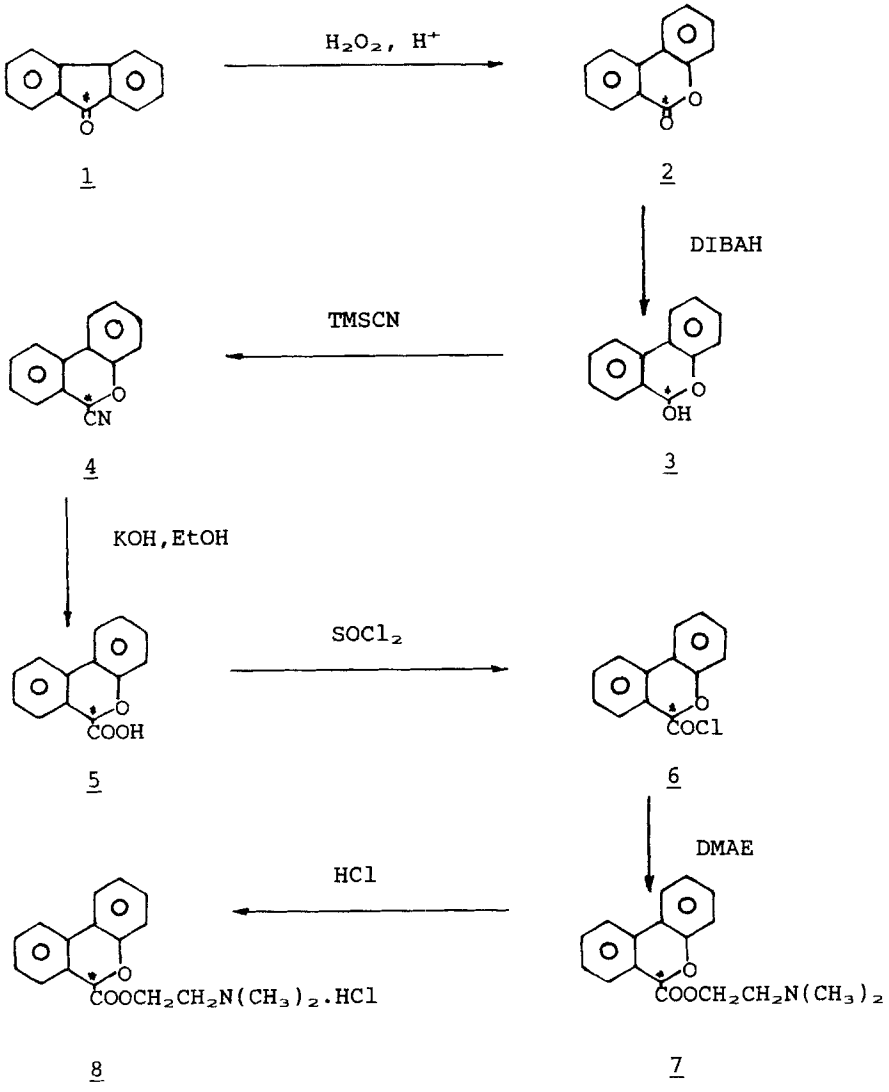
SCHEME



* = ^{14}C

RESULTS AND DISCUSSION

The synthetic sequence as outlined in the scheme suggested two possible positions for labelling with radiocarbon that were likely to be metabolically stable: the carboxyl group and/or any position in the dibenzopyran ring.

The former was discarded because the attempts to convert the lactol 3 into the nitrile 4 by direct one pot conversion with KCN via a trimethylsilyl derivative [5] were unsuccessful. In fact the experimental conditions, because of the low amount of reagents, appeared be very critical giving erratic results and lowering dramatically in some cases the chemical yields.
The latter choice was adopted because of the easy availability of 9-[9-^{14}C]fluorenone 1 and the possibility to transform it quantitatively into a dibenzopyran ring, by Baeyer-Villiger oxidation [6].

According to the scheme, the oxidation of 1 with 30% H_2O_2 in acidic medium afforded the lactone 2 which was then reduced to the corresponding lactol 3 with DIBAH (diisobutylaluminium hydride). Compound 3 was reacted with trimethylsilylcyanide (TMSCN) in the presence of ZnI_2 to yield the nitrile 4. This intermediate, after hydrolysis with 10% alcoholic solution of KOH and purification by preparative TLC, gave the acid 5, 97% radiochemically pure (specific activity 1.68 GBq/mmol) with a radiochemical yield of 59% from 1.

FCE 20696 [7] is readily hydrolysed by atmospheric moisture and consequently it is convenient to store the intermediate 5 at high specific activity. The final steps can be readily carried out when required at the desired specific activity.
The acid chloride 6 is reacted with 2-N,N-dimethylaminoethanol (DMAE) in diethyl ether to yield 7 which is converted to the hydrochloride salt 8 with aqueous HCl.

In the described standard preparation, [^{14}C] FCE 20696 8 was obtained, 97% radiochemically pure with a specific activity of 115 MBq/mmol in an overall radiochemical yield of 31% from 1.

EXPERIMENTAL

Thin layer chromatography (TLC)

TLC was carried out using Merck silica gel F 254 200x50x0.25 mm plates. The eluting solvent systems were:

A) petroleum ether (60-80 °C): acetone (130:70 by volume)
B) chloroform
C) chloroform:methanol:NH_4OH(30%) (190:10:1 ")
D) benzene:ethyl acetate:glacial acetic acid (130:24:12 ")
E) chloroform:methanol:formic acid (99%) (150:20:20 ")

High performance liquid chromatography (HPLC)

HPLC analyses were performed by using a Partisil 10 ODS (25 cm x 4.6 mm ID) column with a mobile phase of CH_3CN : KCl/HCl buffer pH 1.5 (30:70); flow rate 1.5 ml/min; UV detection 254 nm ; radiometric detection with heterogeneous cell (0.36 ml) Yttrium silicate.

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 400 liquid chromatograph with LC 75 UV/VIS detector and Packard Trace II 7150 on line with 512 kRAM 3270 IBM PC as radioactivity flow monitor.

9-[9-^{14}C]Fluorenone was purchased from Amersham International p.l.c.

6H-[6-^{14}C]Dibenzo[b,d]pyran-6-one (2)

To a cooled (0 °C) and stirred solution of 9-[9-^{14}C]fluorenone 1 (925 MBq; 0.57 mmoles) in glacial acetic acid (1.56 ml) was added slowly 96% H_2SO_4 (1.04 ml) followed by 30% H_2O_2 (0.147 ml). The mixture was then stirred at room temperature for about 3 hours. At the end of the reaction (checked by radio-TLC, system A), the solution was carefully added dropwise to cooled (0 °C) water (7 ml). The resulting precipitate was filtered through a D_4 sintered-glass filter and washed with water (3x3 ml). It was dissolved in ethyl acetate (7 ml) and washed with water (3x5 ml) to neutrality in a separating funnel. The organic phase was then separated, dried over Na_2SO_4 and evaporated to dryness to afford compound 2 (815 MBq), 96% radiochemically pure (by radio-TLC; system A; Rf 0.44), which was used in the next step.

(R,S)-6H-[6-^{14}C]Dibenzo[b,d]pyran-6-ol (3)

Diisobutylaluminiumhydride (0.56 ml of a 1.2 M DIBAH in toluene) was added, under nitrogen, to a stirred solution of compound 2 (815 MBq) in anhydrous toluene (1.2 ml) at -60 °C and the mixture stirred at the same temperature for about one hour. At the end of the reaction (checked by radio-TLC; system B), ethyl acetate (0.5 ml), water (0.04 ml) and Na_2SO_4 (44 mg) were successively added. The suspension was stirred at room temperature for about 30 minutes. The resulting gel was filtered through dicalite 478 and washed with ethyl acetate (10x5 ml). The filtrate was dried over anhydrous Na_2SO_4 and evaporated to dryness to yield compound 3 (728 MBq), 85% radiochemically pure (by radio-TLC , system C; Rf 0.46) which was used without purification in the next step.

(R,S)-6-Cyano-6H-[6-^{14}C]dibenzo[b,d]pyran (4)

To a solution of compound 3 (728 MBq) in toluene (1.5 ml), zinc iodide (55 mg) and trimethylsilylcyanide (0.189 ml) were added with stirring and the reaction mixture stirred at room temperature for about 3 hours. When the reaction was complete (checked by radio-TLC, system B), 2N NaOH (1 ml) was added, with stirring. After dilution with ethyl acetate (5 ml), the mixture was transferred into a separating funnel and the aqueous phase was extracted with ethyl acetate (5x3 ml). The combined organic extracts were then washed with water (3x3 ml), dried over Na_2SO_4, and evaporated to dryness under vacuum to yield the intermediate 4, 85% radiochemically pure (by radio-TLC, system B; RF 0.65), which was used without further purification in the next reaction.

(R,S)-6H-[6-^{14}C]Dibenzo[b,d]pyran-6-carboxylic acid (5)

The intermediate 4 (724 MBq) in 10% ethanolic KOH solution (3.5 ml) was stirred and refluxed for about 2 hours. When the

conversion was complete (checked by radio-TLC; system D) the solvent was evaporated to dryness and the residue was dissolved in water (6 ml). The solution was adjusted to pH 2 with 1N HCl giving a precipitate which was dissolved by addition of ethyl acetate (3 ml). The mixture was then transferred into a separating funnel and the aqueous phase was extracted with ethyl acetate (3x4 ml). The organic extracts were combined and, after washing with water (3x3 ml) to neutrality, were dried over Na_2SO_4. The organic solution was evaporated "in vacuo" to give the compound 5 (625.3 MBq), 84% radiochemically pure (by radio-TLC, system D). The crude 5 was purified by preparative TLC using the mixture D as chromatographic eluent.
The chromatographic band corresponding to 5 was removed and the product extracted from silica gel with methanol (25 ml). The combined extracts were filtered and evaporated to dryness to yield the compound 4 (546 MBq) with a radiochemical purity 97% (by radio-TLC, system D, Rf 0.26; by radio-HPLC: retention time 8.4 minutes).

(R,S)-6H-[6-^{14}C]Dibenzo[b,d]pyran-6-carbonyl chloride (6)

A small amount of compound 5 (5.95 MBq) was diluted with unlabelled 5 (10 mg). The mixture was suspended in toluene (0.5 ml) and thionyl chloride (0.05 ml) added and the suspension was refluxed under stirring for about one hour. The solvent and the unreacted thionyl chloride were evaporated under vacuum affording crude 6 which was used in the next step without further purification.

(R,S)-6H-6-[2-(dimethylamino)ethoxycarbonyl]-[6-^{14}C]dibenzo-[b,d]pyran.HCl ([^{14}C]FCE 20696 (8))

2-N,N-Dimethylaminoethanol (0.016 ml) in diethyl ether (0.2 ml) was added to a solution of crude 6 in diethyl ether (0.5 ml). After two hour stirring at room temperature, the conversion was complete (checked by radio-TLC, system E), the precipitate was filtered trough a D_4 sintered-glass filter and the solid was washed with diethyl ether (3x3 ml). The filtrate was then evaporated to a small volume, transferred into a separating funnel and washed with water to neutrality. The organic phase was collected, dried over Na_2SO_4 and evaporated to dryness affording 5.55 MBq of crude 7, 89% radiochemically pure (by radio-TLC , system E). The crude 7 was purified by preparative TLC employing the system E as the eluting solvent system. The chromatographic band corresponding to the required compound, was extracted from silica gel with acetone (25 ml). After solvent evaporation, the recovered free base of FCE 20696 was converted into the salt 8 with the stoichiometric amount of 0.1N HCl. The [^{14}C]FCE 20696 8 (3.11 MBq) had a specific radioactivity of 115 MBq/mmol (344 kBq/mg) and a radiochemical purity of 97% (by radio-TLC, system E: Rf 0.21; by radio-HPLC : retention time 4.1 minutes). The UV/VIS spectrum (in water λ_{max} at 270 nm ; $E^{1\%}_{1cm}$ = 340.13) was in agreement with that of an authentic sample.
The overall radiochemical yield from 1 was 31%.

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