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SYNTHESIS OF 4'-IODO-4'-[14-^{14}C]-DEOXYDOXORUBICIN HYDROCHLORIDE (FCE 21954)

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SUMMARY

The synthesis of 4'-iodo-4'-[14-^{14}C]-deoxydoxorubicin hydrochloride (FCE 21954) in five steps is described. 4'-Epi-N-trifluoroacetyl-[14-^{14}C]-daunorubicin has been employed as starting material. Substitution of C-4'-OH with iodine and subsequent hydroxylation of the side chain, via the 14-bromo derivative, of 4'-iodo-4'-[14-^{14}C]-deoxydaunorubicin afforded the final product, in an overall radiochemical yield of 20%, 96% radiochemically pure and with a specific radioactivity of 252 MBq/mmol (6.8 mCi/mmol).

Key words: anthracyclines, FCE 21954, antitumor antibiotics.

INTRODUCTION

The clinically useful antitumor antibiotic doxorubicin (DXR) is structurally related to the group of glycoside antibiotics which are designed with the generic name anthracyclines [1]. In order to increase the antitumor effectivness, some new compounds of this class have been synthetized [2]. Among those 4'-iodo-4'-deoxydoxorubicin hydrochloride (FCE 21954) was shown to be the most promising DXR analogue for its "in vivo" higher activity against murine P388 leukemia resistant to DXR and against pulmonary metastases from Lewis Lung carcinoma [3].

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This antibiotic differs chemically from DXR for the substitution of the C-4'-OH of the sugar mojety (daunosamine) with an atom of iodine. Because of the interest in the disposition and the fate of FCE 21954 in laboratory animals, a radiolabelled form was required. Previous studies performed with 14-^{14}C-analogues showed the labelling in the 14-position to be stable "in vivo" [4][5][6]. Moreover the easy availability of 4'-epi-N-trifluoroacetyl-[14-^{14}C]-daunorubicin 1 [5][7] allowed us to prepare 4'-iodo-4'-[14-^{14}C]-deoxydoxorubicin hydrochloride, with a good specific radioactivity and in an enough amount for our purposes.

RESULTS AND DISCUSSION

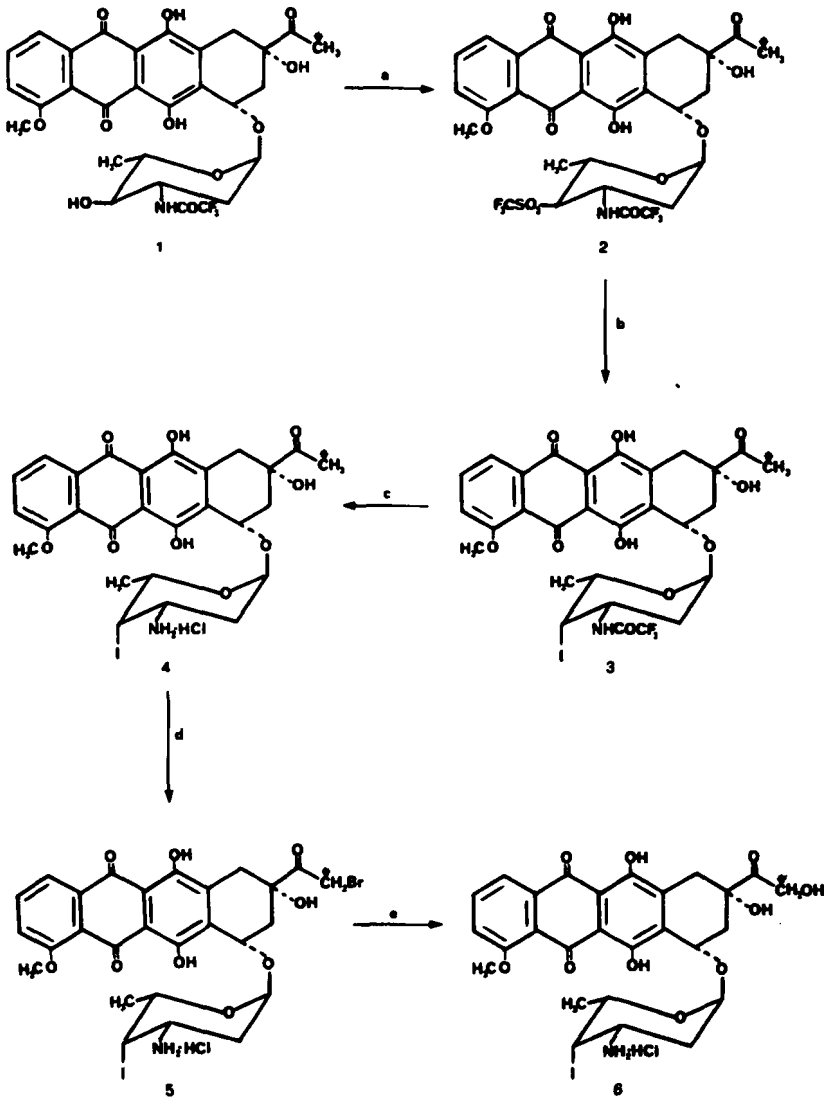
The well known procedure to transform the daunoderivatives into doxoderivatives [8] [9] in mild conditions and with good chemical yields made the intermediate 3 of the Scheme the main compound of interest for the synthesis of labelled FCE 21954.

Two routes are possible to obtain the labelled compound 3. The first one, a condensation of labelled diazomethane with N-trifluoroacetyl-4'-iodo-9-desacetyl-9-formyl daunorubicin (prepared from 13-dihydro derivative of 4'-iodo-4'-deoxydoxorubicin [10] according to [9]) to give 3, was discarded because of the very low and not reproducible yields, due to the instability of the above daunorubicin derivative under the experimental conditions [9].

The second one, outlined in the Scheme, was demonstrated to be more convenient because of the easy feasible replacement of C-4'-OH with iodine as well as the ready availability of the labelled starting material 1. In fact compound 3 was obtained in good yield (≈ 45%) and with the expected steric configuration (axial), via trifluoromethylsulfonic ester by nucleophilic substitution with iodine.

The following three synthetic steps afforded via the 14-bromoderivative 5, 4'-iodo-4'-[14-^{14}C]-deoxydoxorubicin hydrochloride, 96% radiochemically pure (specific radioactivity 252 MBq/mmol) in an overall radiochemical yield of 20% from 1.

SCHEME



* = ^{14}C

Reagents and conditions:
a: $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, -10°C;
b: acetone, NaI;
c: 0.1N NaOH, anhydrous HCl;
d: Br_2, CH_3OH-dioxane, HBr;
e: aqueous HCOONa.

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) chloroform:acetone (9:1 by volume)

B) chloroform:methanol:water:acetic acid (80:20:1.5:3.5 by volume)

Electronic spectra were determined on a Perkin-Elmer 575 UV/VIS spectrophotometer. Measurement of radioactivity was done with a Packard 300C liquid scintillation counter using Rialuma (Lumac System A.G.) as liquid scintillation cocktail.

Radiochemical analysis of TLC plates was performed with a Berthold 2832 automatic TLC linear analyzer.

The crude 4'-epi-N-trifluoroacetyl-[14-^{14}C]-daunorubicin 1 was purchased from Amersham International p.l.c. and purified by preparative TLC as described in [5].

4'-Epi-O-trifluoromethanesulphonyl-N-trifluoroacetyl-[14-^{14}C] daunorubicin (2)

Trifluoromethanesulfonic anhydride (158 µmoles; 26 µl) was added at -10°C to a solution of compound 1 (160 MBq; 72 µmoles) in CH_2Cl_2 (2 ml) and anhydrous pyridine (446 µmoles; 36 µl).

After 10 minutes stirring at -10°C the conversion was complete as showed by radio-TLC (system A). The reaction mixture was washed with with 5% $NaHCO_3$ (1 ml), water (1 ml), 0.1N HCl (2x2 ml) and water (3x3 ml). The organic phase was dried over anhydrous Na_2SO_4 and after evaporation to reduced volume (0.5 ml), was used without further purification in the next step.

4'-Iodo-N-trifluoroacetyl-[14-^{14}C]-daunorubicin (3)

Sodium iodide (121 µmoles, 18.14 mg) in acetone (0.8 ml) was added to compound 2 in CH_2Cl_2. The reaction mixture was stirred for 1 hour and transferred into a separating funnel by dilution with CH_2Cl_2 (10 ml). It was washed with 0.1N HCl (2 ml), water (3x3 ml) and dried over anhydrous Na_2SO_4. The crude product 3 was chromatographed on TLC plate (system A) and the band corresponding to 3 was extracted from silica gel with a mixture of chloroform:methanol (4:1 by volume). The recovered compound 3 (90 MBq) had a radiochemical purity of 80% (by radio-TLC; system A) and was used without further purification in the next step.

4'-Iodo-4'-[14-^{14}C]-deoxydaunorubicin hydrochloride (4)

Compound 3 (90 MBq) and 0.1N NaOH (5 ml) were stirred at 0°C for about 1 hour. At the end of the reaction (checked by radio-TLC; system B) the compound 4 (as free base) was extracted with $CHCl_3$ (5x5 ml). The solvent was then evaporated to small volume and, after dilution with methanol (50 ml) the product 4 (78.4 MBq, 90% radiochemically pure by radio-TLC; system B) was obtained by addition of methanolic 0.1N HCl (0.49 ml).

4'-Iodo-4'-[14-^{14}C]-deoxydoxorubicin hydrochloride (6)

To a small amount of the methanolic solution of compound 4 (8.16 MBq) was added unlabelled 4 (29.4 µmoles) and evaporated to dryness. The residue was brominated by adding bromine (7.62 mg) to a solution (1.5 ml) of 4 in a mixture of anhydrous methanol and dioxane (1:2 by volume) plus ethyl orthoformate (33 µl) and methanolic 10% HBr (26 µl) at 8°C.
At the end of the reaction (checked by radio-TLC; system B) the crude 5 was precipitated by adding 8 ml of a mixture of diethyl ether:n-hexane (1:4 by volume). The precipitate was collected by filtration affording the intermediate 5.

The 14-bromo-derivative 5 was dissolved in 4 ml of aqueous 0.2 N HBr in acetone (1:1 by volume) and stirred at room temperature overnight. Sodium formate (100 mg) was then added and the mixture was kept at room temperature in the dark for three days. As soon as the reaction was completed (checked by radio-TLC, system B), the solution was adjusted to pH 7.2 with 7% $NaHCO_3$ and extracted with $CHCl_3$ (5x3 ml). The organic solvent was evaporated to small volume and diluted with methanol (50 ml), followed by methanolic 0.1N HCl (0.42 ml). The compound 6 was 60% radiochemically pure (by radio-TLC; system B).

It was therefore purified by preparative TLC employing the system B as the eluting solvent system. The chromatographic band corresponding to 6 was removed and the product extracted from silica gel with 50 ml of a mixture chloroform:methanol (1:1 by volume).

The organic solution was transferred into a separating funnel and exhaustivly extracted with aqueous 10^{-4}N HCl (75 ml). The acid aqueous phase was adjusted to pH 7.2 with aqueous 7% $NaHCO_3$ and extracted with chloroform (50 ml).

The concentration of compound 6 (as free base) in the solution was measured by VIS spectrophotometry at 495 nm. The free base was converted to the salt by the addition of methanolic 0.1N HCl (stoichiometric amount +10%).

The [14-^{14}C]FCE 21954 (3.85 MBq) obtained had a specific radioactivity of 252 MBq/mmol (6.8 mCi/mmol) and a radiochemical purity of 96% (by radio-TLC; system B: Rf=0.43). The VIS spectrum (in methanol λ max at 495 nm; $E^{1\%}_{1\ cm}$=182) was in agreement with that of an authentic sample. The overall radiochemical yield from 1 was was 20%.

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