SYNTHESIS OF 1-([1-^{14}C]CYCLOHEXYLOXYCARBONYLOXY)ETHYL 7β-[2-(2-AMINOTHIAZOL-4-YL)ACETAMIDO]-3-[[[1-(2-DIMETHYLAMINOETHYL)-1H-TETRAZOL-5-YL]THIO]METHYL]CEPH-3-EM-4-CARBOXYLATE DIHYDROCHLORIDE ([^{14}C]SCE-2174)

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SUMMARY

1-(Cyclohexyloxycarbonyloxy)ethyl 7β-[2-(2-aminothiazol-4-yl)acetamido]-3-[[[1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylate dihydrochloride (SCE-2174), a new orally active cephalosporin, was labeled with carbon-14 starting from [1-^{14}C]cyclohexanol (1). 1-Chloroethyl[1-^{14}C]cyclohexyl carbonate (3) obtained by acylation of 1 with 1-chloroethyl chloroformate (2) was converted to iodide (4) which was condensed with the potassium salt of Cefotiam (5). [^{14}C]SCE-2174, having a specific activity of 672 MBq/mmol, was obtained in 13% overall radiochemical yield and had a radiochemical purity of 92%.

Key Words: labeled compound, [^{14}C]SCE-2174, orally active cephalosporin, cefotiam

INTRODUCTION

Cefotiam (7β-[2-(2-aminothiazol-4-yl)acetamido]-3-[[[1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylic acid dihydrochloride) is a cephalosporin for injection with broad spectrum activity against Gram-positive and negative bacteria.[1] In an effort to make an orally active derivative of Cefotiam, the 1-cyclohexyloxycarbonyloxyethyl ester (SCE-2174), a mixture of diastereoisomers, was selected as a candidate for clinical trials.[2] SCE-2174 was labeled with

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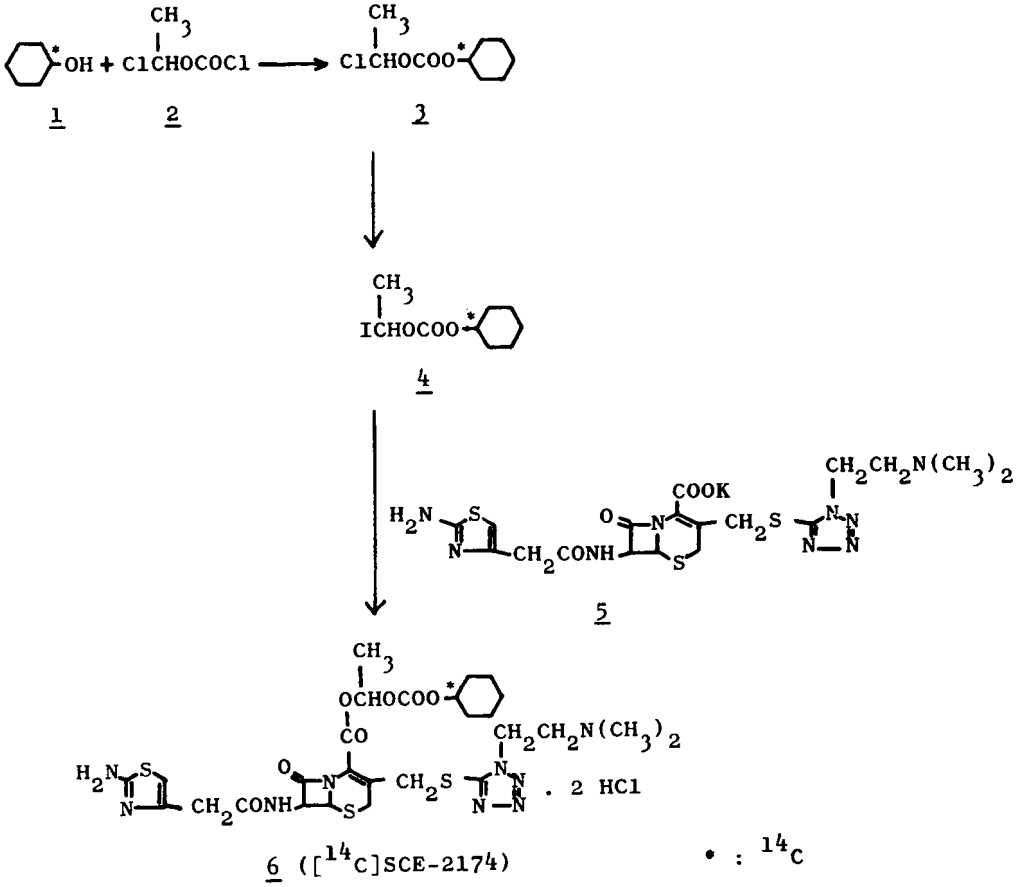
carbon-14 for use in metabolic and pharmacokinetic studies. The carbon-14 was incorporated into the cyclohexyl ring at the 1-position of the 1-cyclohexyloxycarbonyloxyethyl ester using commercially available [1-^{14}C]cyclohexanol (1) as the starting material as shown in Scheme I.



Scheme I

RESULTS

[1-^{14}C]Cyclohexanol (1) was converted to 1-chloroethyl [1-^{14}C]cyclohexyl carbonate (3) with 1-chloroethyl chloroformate (2) and subsequently, to the iodide (4). The potassium salt of Cefotiam

(5), prepared from Cefotiam dihydrochloride and potassium carbonate, was condensed with 4 under cooling in an ice-bath. To avoid isomerization of the double bond at Δ^3 to Δ^2, the reaction was done as fast as possible. The product was isolated as a diastereoisomeric mixture with respect to the 1-cyclohexyloxycarbonyloxyethyl ester moiety.

EXPERIMENTAL

[1-^{14}C]Cyclohexanol (1, 94% radiochemically pure) with a specific activity of 2.22 GBq/mmol was purchased from Amersham Japan. 1-Chloroethyl chloroformate (2) was synthesized in the same division of Takeda Chemical Industries Ltd. High performance liquid chromatography (HPLC) analyses were performed on a Shimadzu LC-1 equipped with a UV-detector (254 nm), a Shimadzu Chromatopac C-RIA integrator and a Nucleosil 5C_{18} column (4x150 mm) using a mobile phase of 0.01 N $(NH_4)_2SO_4$: CH_3CN : AcOH (720 : 280 : 1, v/v). The tR values of a diastereoisomeric mixture of reference compound (SCE-2174) were 9.18 and 11.00 min at a flow rate of 0.8 ml/min. The fractions of eluate (0.4 ml) were collected and the radioactivity was determined by scintillation counting to establish radiochemical purity. Thin layer chromatography (TLC) was done on silica gel plates (Merck 60 F-254, 20x20 cm) and radiochromatogram scans were performed on an Aloka TRM-1B scanner. Liquid scintillation counting was performed with an Aloka LSC-671 liquid scintillation spectrometer.

1-Chloroethyl[1-^{14}C]cyclohexyl carbonate (3)

[1-^{14}C]Cyclohexanol (1, 1.85 GBq, 85 mg) was diluted with a solution of unlabeled cyclohexanol (165 mg) in CH_2Cl_2 (6 ml). A solution of pyridine (0.23 ml, 2.8 mmol) in CH_2Cl_2 (2 ml) was added and the mixture cooled to -70°C. A solution of 1-chloro-

ethyl chloroformate (2, 0.31 ml, 2.8 mmol) in CH_2Cl_2 (2 ml) was added dropwise to the above solution. After the addition was completed, the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was poured into a mixture of ice and aqueous NaCl, and extracted with CH_2Cl_2. The extract was dried over $MgSO_4$ and concentrated in vacuo to yield a colorless oil of 3 (507.7 mg, 98%).

1-Iodoethyl[1-^{14}C]cyclohexyl carbonate (4)

A mixture of 3 (507.7 mg), NaI (1.2 g) and CH_3CN (10 ml) was heated at 60-70°C for 70 min with stirring. The reaction mixture was poured into ice-water, and extracted with Et_2O (30 ml). The extract was washed with 1 N $NaHSO_3$ (5 mlx2) and water, and dried over $MgSO_4$. The solvent was removed in vacuo to afford a pale yellow oil of 4 (474.4 mg, 68%).

1-([1-^{14}C]Cyclohexyloxycarbonyloxy)ethyl 7β-[2-(2-Aminothiazol-4-yl)acetamido]-3-[[[1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylate Dihydrochloride (6, [^{14}C]-SCE-2174)

The potassium salt of Cefotiam (5, 1.51 g, 2.86 mmol), prepared from 1.22 g of Cefotiam dihydrochloride and 0.56 g of K_2CO_3, was dissolved in DMF (5 ml) under cooling in ice with stirring for 20 min. The resulting solution was added at once to the solution of 4 (474.4 mg, 1.59 mmol) in DMF (5 ml) under cooling in an ice-bath with stirring. After stirring for 1.5 min, the reaction mixture was poured into ice-water (80 ml) and extracted with a cold mixture of AcOEt (100 ml) and Et_2O (50 ml). The organic phase was washed with cold water (60 mlx2) and extracted with 0.1 N HCl (10 ml). The aqueous extract was lyophilized and triturated with AcOEt (20 ml). After stirring for 10 min, the resulting powder was filtered, washed with a cold mixture of AcOEt and hexane, and dried in vacuo for 3 h to afford a white powder of 6 (286.5 mg,

245 MBq, 13% radiochemical yield based on 1). The specific activity was 672 MBq/mmol. The radiochemical purity was greater than 92% by HPLC analysis and 90% by TLC analysis (AcOEt : CH_2Cl_2 : MeOH = 30 : 30 : 7, v/v, Rf=0.36 and 0.42). The chemical purity was 95% by HPLC analysis.

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