Synthesis of (6R,7R)-7-[2-(5-amino-1,2,4-[5-^{14}C]thiadiazol-3-yl)-(Z)-2-methoxyiminoacetamido]-3-[(4-carbamoyl-1-quinuclidinio)methyl]ceph-3-em-4-carboxylate ([^{14}C]E1040)

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SUMMARY

(6R,7R)-7-[2-(5-amino-1,2,4-thiadiazol-3-yl)-(Z)-2-methoxyiminoacetamido]-3-[4-(carbamoyl-1-quinuclidinio)methyl]ceph-3-em-4-carboxylate (E1040), a new injectable cephalosporin with potent antipseudomonal activity, was synthesized labelled with carbon-14, starting from potassium [^{14}C]thiocyanate with 2-(N-chloroamidino)-2-(methoxyimino)acetic acid (I) gave (5-amino-1,2,4-[5-^{14}C]thiadiazol-3-yl-2-methoxyimino)acetic acid (II). 7-Amino-3-[(4-carbamoyl-1-quinuclidinio)methyl]ceph-3-em-4-carboxylate hydrochloride (IV) was acylated with the above acid chloride using the PCl_5 method, to afford the title compound (V); [^{14}C]E1040, having a specific activity of 3.74 MBq/mg (as its anhydride) , was obtaind in 47% overall radiochemical yield, with a radiochemical purity of more than 98.1%.

INTRODUCTION

E1040 is a new parenteral cephalosporin with a broad spectrum of activity both in vivo and in vitro. When compared with ceftazidime (1) or cefmenoxime in vitro, against Pseudomonas aeruginosa, Enterobacter cloacae and Citrobacter freundii, it has

Key Words: [^{14}C]E1040, injectable cephalosporin, Pseudomonas aeruginosa, (5-amino-1,2,4-[5-^{14}C]thiadiazol-3-yl- 2-methoxyimino)acetic acid

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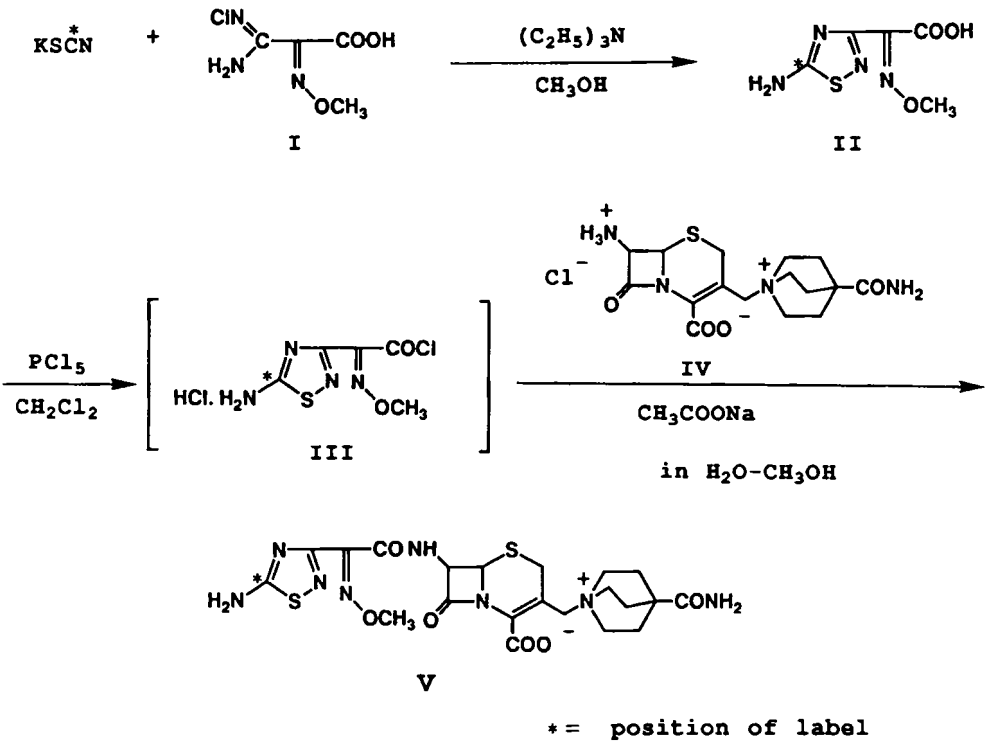
greater activity (2). The comparative therapeutic efficacy of the compound in experimental infections was consistent with its relative activity in vitro (3). This report describes the synthesis of the ^{14}C-labelled cephalosporin for metabolism and pharmacokinetic studies.

RESULTS AND DISCUSSION

The reaction of potassium [^{14}C]thiocyanate with 2-(N-chloroamidino)-2-(methoxyimino)acetic acid (I) produced (5-amino-1,2,4-[5-^{14}C]thiadiazol-3-yl-2-methoxyimino)acetic acid (II). The acid chloride (III), formed using PCl_5, was condensed with amine (IV), and the product passed through an ODS column and then crystalized with EtOH, to produce a crystalline product. The overall chemical yield from potassium thiocyanate was 47%. All experimental conditions were optimized using non-radioactive materials.

SYNTHETIC PATHWAY



EXPERIMENTAL

Potassium [^{14}C]thiocyanate with a specific activity of 2.18 GBq/mmol was purchased from Amersham Japan. All chemicals used in the synthesis were purchased, and used without purification. All other solvents were either distilled or of analytical reagent quality.

High performance liquid chromatography (HPLC) analysis was performed on a Waters Model 590 equipped with a UV-detecter (254 nm, JASCO UVIDEC 100-III), a Shimadzu Chromatopac C-RIA injector and an ODS column (YMC-A312, 6 x 150 mm) using a mobile phase of H_2O:CH_3OH:$AcONH_4$ (850:150:1, v/v/w). The retention time value was 4.0 min at a flow rate of 1.5 mL/min.

Thin layer chromatography (TLC) was carried out silica gel plates (Merck 60F-254, 20 x 20 cm) using with either of two developing solvents: A. CH_3COCH_3:H_2O:AcOH:NH_4OH (50:20:20:2.5, v/v); and B. CH_3CN:AcOEt:H_2O:AcOH (60:40:40:20, v/v). Liquid scintillation counting was performed with an Aloka Model LSC-900 liquid scintillation spectrometer.

(5-amino-1,2,4-[5-^{14}C]thiadiazol-3-yl-2-methoxyimino)acetic acid (II)

In a 100 ml round-bottom flask, potassium [^{14}C]thiocyanate (3.61 GBq, 164 mg) was dissolved in CH_3OH (7.0 mL) and Et_3N (0.59 mL). This mixture was then cooled in an ice bath and 2-(N-chloroamidino)-2-(methoxyimino)acetic acid (I, 304 mg) added. After being allowed to warm to room temperature, the mixture was stirred overnight. After removal of the solvent in vacuo, the residue was triturated with 1N-HCl (4 mL) while cooling in an ice bath. The mixture was extracted with AcOEt (10 mL x 5). The separated organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated

in vacuo, and the residue was triturated with MEK (methyl ethyl ketone). The resulting solid was collected by filtration, washed with MEK:IPE (diisopropyl ether) (1:1), then IPE, and dried over P_2O_5 in vacuo (239.3 mg, 71%).

(6R,7R)-7-[2-(5-Amino-1,2,4-[5-^{14}C]thiadiazol-3-yl)-(Z)-2-methoxyiminoacetamido]-3-[4-(carbamoyl-1-quinuclidinio)methyl]ceph-3-em-4-carboxylate (V, [^{14}C]E1040)

CH_2Cl_2 (4.0 mL) , dried over 4A Moleculer Sieves, was placed in a 100 mL round-bottom flask. PCl_5 (319 mg) and (5-amino-1,2,4-[5-^{14}C]thiadiazol-3-yl-2-methoxyimino)acetic acid (II, 239.3 mg) were added to the CH_2Cl_2 mixture at -40°C.

After being allowed to warm to -10°C, the mixture was stirred at this temperature for 40 min, to prepare the acyl chloride (III). To this above mixture was added a solution of 7-Amino-3-[(4-carbamoyl-1-quinuclidinio)methyl]ceph-3-em-4-carboxylate hydrochloride (IV, 477 mg) and anhydrous AcONa (485 mg) in H_2O-CH_3OH (2.4 mL-14.3 mL), and the mixture was stirred, with ice cooling, for 1.5h.

The reaction mixture was evaporated in vacuo. The residue was dissolved in H_2O (10 mL), and placed on an ODS column containing 100 g of ODS in water. The column was first eluted with H_2O (250 mL), and then with 1% CH_3OH-H_2O (200 mL), 1.5% CH_3OH-H_2O (400 mL) and 2% CH_3OH-H_2O (200 mL). The fraction containing the desired compound was concentrated at reduced pressure to a volume of 5 mL. C_2H_5OH (10 mL) was added to the solution, and the resulting white suspension was then stirred, with ice cooling. An additional portion of C_2H_5OH (10 mL) was added to the mixture and stirred for 30 min at 0-5°C.

The solid produced was collected by filtration, washed with cold 90%

C_2H_5OH-H_2O (10 mL), then C_2H_5OH (10 mL), and then was air-dried at room temperature overnight to afford the amide V, (394.3 mg) as a white powder, water content 20.4%, which was based on water content of non labelled compound prepared by the same procedure, 1.71 GBq, 47% radiochemical yield based on potassium [^{14}C]thiocyanate. The specific activity was 3.74 MBq/mg (57 mCi/mmol) . The radiochemical purity was more than 98.2% by HPLC analysis and 98.1% by TLC analysis (A: Rf=0.53, B: Rf=0.31). The chemical purity was 99.0% by HPLC analysis.

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