SYNTHESIS OF (5-METHYL-2-OXO-1,3-DIOXOL-4-YL)METHYL 7-[D-O-(L-ALANYL)[β-^{14}C]MANDELAMIDO]-3-[[(5-METHYL-1,3,4-THIADIAZOL-2-YL)THIO]METHYL]-3-CEPHEM-4-CARBOXYLATE AND ITS ACTIVE COMPOUND

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SUMMARY

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 7-[D-O-(L-alanyl)[β-^{14}C]-mandelamido]-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylate (^{14}C-KY-109) and its parent drug (^{14}C-KY-087) were synthesized from D-(-)-mandelic-β-^{14}C acid prepared by optical resolution of DL-(±)-mandelic-β-^{14}C acid. The overall radiochemical yields were 16.2% for ^{14}C-KY-087 and 49.6% for ^{14}C-KY-109, based on D-(-)-mandelic-β-^{14}C acid. Both the products had radiochemical purities greater than 97% as measured by TLC.

Key Words: (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 7-[D-O-(L-alanyl)[β-^{14}C]-mandelamido]-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylate, 7-(D-[β-^{14}C]mandelamido)-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)-thio]methyl]-3-cephem-4-carboxylic acid, [^{14}C]cephalosporin analogue.

INTRODUCTION

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 7-[D-O-(L-alanyl)mandelamido]-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylate hydrochloride (KY-109)[1] is an orally active cephalosporin pro-drug. Its active compound is 7-(D-mandelamido)-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)-

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thio]methyl]-3-cephem-4-carboxylic acid (KY-087), which is a cephalosporin with broad spectrum antibacterial activity.

KY-087 is poorly absorbed from the gastrointestinal tract, probably because of its low lipophilicity. A (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl group was introduced at the 4-position in the cephalosporin nucleus in order to improve the lipophilicity of the drug. In addition, L-alanine was incorporated into the 7-position side chain to increase the water solubility. This modification resulted in a marked increase in the oral absorption.

KY-109 itself has no antibacterial activity. During the process of absorption from the gastrointestinal tract, however, it is de-esterified to become KY-087, an active compound, in which the 7-position side chain is the same as that of cefamandole[2].

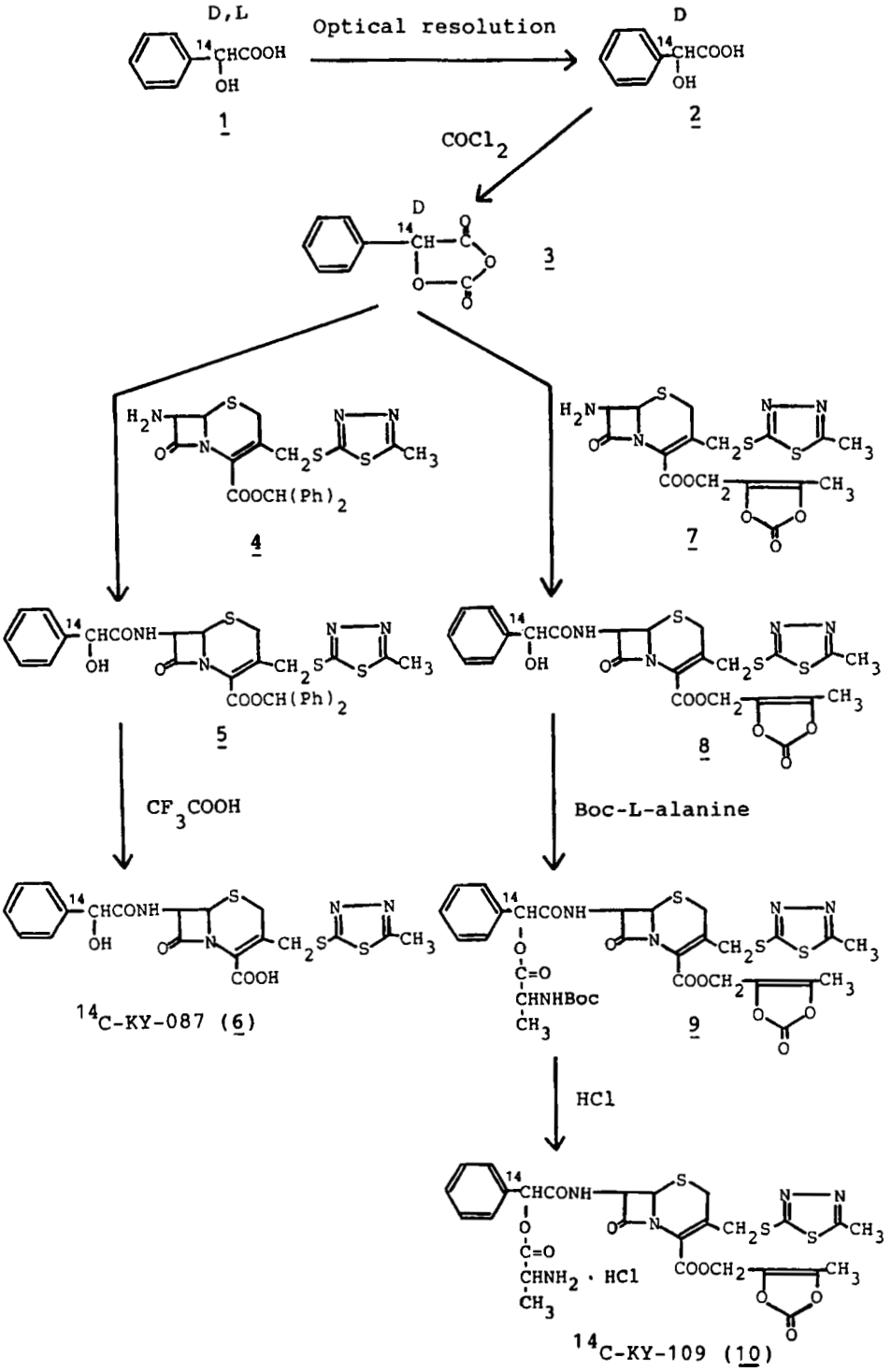
In order to conduct absorption and metabolism studies in animals, the radioactive drugs were required. Radioactive cefamandole similar in structure to KY-087 has been synthesized by Minato et al.[3] using mandelic-β-^{14}C acid. In this report, we describe the synthesis of ^{14}C-labelled KY-087 and KY-109 from mandelic-β-^{14}C acid, as in the case of cefamandole.

SYNTHESIS

^{14}C-KY-087 (6) was synthesized by the route shown in Scheme. DL-(±)-Mandelic-β-^{14}C acid (1), the starting material, was conveniently resolved by morphine in alcholic solution to give D-(-)-mandelic-β-^{14}C acid[3](2). The carbonate (3) of D-(-)-mandelic-β-^{14}C acid obtained by treatment with phosgene was condensed with diphenylmethyl 7-amino-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylate[4] (4) to yield diphenylmethyl 7-(D-[β-^{14}C]mandelamido)-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)-thio]methyl]-3-cephem-4-carboxylate (5). The diphenylmethyl group of 5 was cleaved by treatment with trifluoroacetic acid and anisole in dichloromethane to afford ^{14}C-KY-087 (6).

The synthesis of ^{14}C-KY-109 (10) is shown in Scheme. (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 7-amino-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]-methyl]-3-cephem-4-carboxylate[1] (7) was converted to ^{14}C-KY-106 (8) by

SCHEME


D,L
Optical resolution
D
1
2
COCl2
3
4
7
5
8
CF3COOH
Boc-L-alanine
14C-KY-087 (6)
9
HCl
14C-KY-109 (10)

treatment with 3. The reaction of 8 with Boc-L-alanine in the presence of DCC in dichloromethane gave an intermediate (9) having a protected group, which was cleaved by HCl-dioxane to afford ^{14}C-KY-109 (10).

EXPERIMENTAL

DL-(±)-Mandelic-β-^{14}C acid was purchased from Amersham international plc. The radiochemical purity was determined by TLC and HPLC. The radioactivity was determined using a Packard "Tri-Carb" 460 CD Liquid Scintillation Spectrometer.

Optical Resolution[3] of DL-(±)-mandelic-β-^{14}C acid

Morphine D-(-)-Mandelate-β-^{14}C : A solution of DL-(±)mandelic-β-^{14}C acid (1) (179 mg, 27 mCi) and unlabelled D-(-)-mandelic acid (268 mg) in ethanol (5 ml) was added to a solution of morphine (896 mg) in ethanol (35 ml). The mixture was left for two days at room temperature after addition of a nucleus of unlabelled morphine D-(-)-mandelate. The precipitated crystals were collected by filtration to give 850 mg (11.9 mCi) of morphine D-(-)-mandelate-β-^{14}C as coloress needles.

D-(-)-Mandelic-β-^{14}C acid (2) : To a solution of morphine D-(-)-mandelate-β-^{14}C (2.89 g, 40.2 mCi), prepared as described above from 1 (604 mg, 91 mCi), in chilled water (50 ml), 2N HCl (50 ml) was added, and the aqueous solution was extracted with ether (60 ml × 4). The extract was dried (Na_2SO_4) and evaporated to give 812 mg (32.6 mCi) of 2 (81% yield) with a specific activity of 6.1 mCi mmol^{-1}.

Diphenylmethyl 7-(D-[β-^{14}C]mandelamido)-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)-thio]methyl]-3-cephem-4-carboxylate (5)

Compound 2 (441 mg, 17.7 mCi) was dissolved in 37% $COCl_2$-tetrahydrofuran solution (5 ml). The solution was allowed to stand for 16 hr at room temperature and finally warmed to 50°C for 30 min. The solvent was evaporated under reduced pressure to leave a residue, which was washed with hexane to give a carbonate 3 (452 mg, 15.5 mCi).

The carbonate 3 was dissolved in anhydrous CH_2Cl_2 (7 ml) and the solution

was added dropwise to a solution of diphenylmethyl 7-amino-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylate (4) (1.62 g) in anhydrous CH_2Cl_2 (20 ml) with stirring in an ice bath. The reaction mixture was stirred for 2 hr at a temperature below 5°C and then for 1 hr at room temperature. To the mixture, unlabelled 3 (396 mg) was added with stirring at room temperature. After 2 hr, the solvent was evaporated under reduced pressure to leave a residue, which was dissolved in AcOEt (100 ml). The organic solution was washed with 2N HCl, 5% $NaHCO_3$ and brine, and dried (Na_2SO_4). The solvent was concentrated to about 15 ml, which was poured into iso-Pr_2O (150 ml) with stirring. The precipitate formed was collected by filtration to give 1.80 g (12.3 mCi) of 5.

7-(D-[β-^{14}C]Mandelamido)-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylic acid (^{14}C-KY-087, 7)

To a solution of 5 (1.77 g, 12.4 mCi) in anisole (8 ml) was added dropwise trifluoroacetic acid (6 ml) with stirring at 5°C, and the mixture was stirred at room temperature for an additional 30 min. To the reaction mixture, iso-Pr_2O (200 ml) was added, and the resulting precipitate was collected by filtration. The recrystallization was repeated twice from THF-H_2O (2:1) to give 350 mg (2.91 mCi) of 6 (23% yield) with a specific activity of 3.98 mCi $mmol^{-1}$ and a radiochemical purity greater than 97% as measured by TLC (SiO_2 : HCOOH/AcOEt/MeOH, 0.1:2:1). The overall radiochemical yield was 16.2% based on 2.

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 7-(D-[β-^{14}C]mandelamido)-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylate(^{14}C-KY-106, 8)

A solution of 3 (820 mg, 28.1 mCi), prepared as described above from 2 (812 mg, 32.6 mCi), in anhydrous CH_2Cl_2 (10 ml) was added to a solution of 7 (2.63 g) in anhydrous CH_2Cl_2 (30 ml) with stirring in an ice bath. After stirring at the same temperature for 1 hr and at room temperature for a further 2 hr, unlabelled 3 (717 mg) was added to the reaction mixture with stirring. After 2 hr, the solvent was evaporated under reduced pressure to leave a residue, which was dissolved in AcOEt (300 ml). The organic

solution was washed with 2N HCl, 5% $NaHCO_3$ and brine, dried and concentrated to about 30 ml, which was poured into iso-Pr_2O (300 ml). The precipitate thus formed was collected by filtration to give 3.21 g (26.6mCi) of 9 (95.0% yield) with a specific activity of 4.9 mCi $mmol^{-1}$.

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 7-(D-O-(L-alanyl)[β-^{14}C]mandelamido)-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylate hydrochloride(^{14}C-KY-109, 10)

A solution of DCC (1.20 g) in anhydrous CH_2Cl_2 (5.4 ml) was added to a solution of 8 (2.92 g, 24.1 mCi) and Boc-L-alanine (1.03 g) in anhydrous CH_2Cl_2 (50 ml) with stirring at 0 °C. To the mixture, 4-dimethylaminopyridine (60mg) was added at 0 °C. After stirring at the same temperature for 30 min, the resulting precipitate was removed by filtration. To the filtrate, CH_2Cl_2 (80 ml) was added, and the organic solution was washed with 5% citric acid and brine, dried (Na_2SO_4) and evaporated under reduced pressure. The residue (a crude intermediate 9) was dissolved in HCOOH (14 ml), and 2N HCl-dioxane (14 ml) was added to the reaction mixture. The resulting precipitate was dissolved in methanol (2.7 ml), and acetone (5.4 ml) was added with stirring. The resulting precipitate was recrystallized twice from methanol-acetone to give 2.17 g (14.45 mCi) of 10 (60% yield) as a white crystalline powder with a specific activity of 4.66 mCi $mmol^{-1}$ and a radiochemical purity greater than 97% as measured by TLC (SiO_2:HCOOH/CH_3CN/acetone/EtOH, 0.1:1:1:0.1). The overall radiochemical yield was 49.6% from 2.

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