SYNTHESIS OF 7-D-MANDEL-β-^{14}C-AMINO-3-{[(1-METHYL-1H-TETRAZOL-5-YL)-THIO]METHYL}-3-CEPHEM-4-CARBOXYLIC ACID (CEFAMANDOLE-^{14}C)

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Cefamandole, 7-D-mandelamino-3-{[(1-methyl-1H-tetrazol-5-yl)-thio]methyl}-3-cephem-4-carboxylic acid (I), is a new semisynthetic, parenteral cephalosporin antibiotic with a broad antibacterial spectrum, synthesized by the Lilly Research Laboratories.[1] It is highly active[2] against many gram-positive cocci and gram-negative bacilli, and especially active against many strains resistant to the other cephalosporins, such as the Enterobacter and indole-positive Proteus species.

In order to conduct absorption and metabolism studies with cefamandole (I), the radioactive drug was required. Kau[3] of the Lilly Laboratory has synthesized radioactive cefamandole nafate, 7-D-mandel-α-^{14}C-amino-{[(1-methyl-1H-tetrazol-5-yl)-thio]methyl}-3-cephem-4-carboxylic acid formate ester sodium salt (II) from D-(-)-mandelic-α-^{14}C acid for disposition studies in laboratory animals.

However, based on the facility of obtaining the ^{14}C-labelled starting material, we synthesized D-(-)-mandelic-β-^{14}C acid (III) by optical resolution of (±)-mandelic-β-^{14}C acid obtained from the ^{14}C-carboxylation of bromobenzene, followed by the reactions given in the Scheme.

(±)-Mandelic-β-^{14}C acid was conveniently resolved[4] by morphine in alcoholic solution to give D-(-)-mandelic-β-^{14}C acid (III) in 56.8% radiochemical yield. The carbonate (IV) of D-(-)-mandelic-β-^{14}C acid (III) obtained by treatment with phosgene was condensed with diphenylmethyl 7-amino-3-{[(1-methyl-1H-tetrazol-5-yl)-thio]methyl}-3-cephem-4-carboxylate (V), affording

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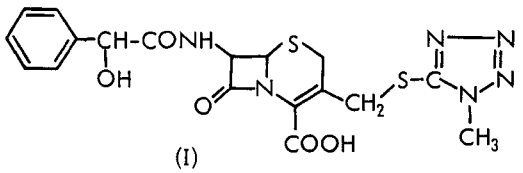
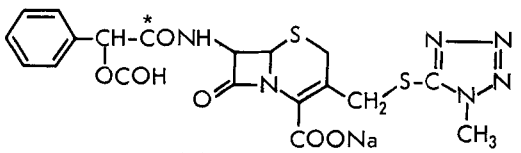


(I)



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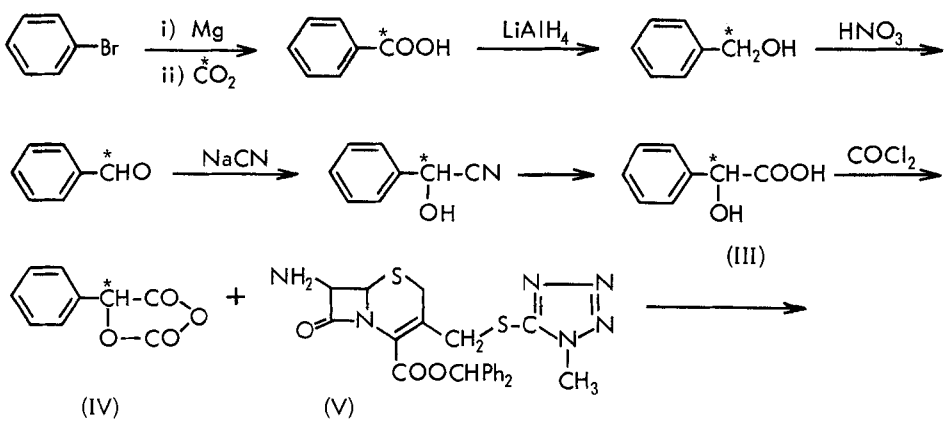
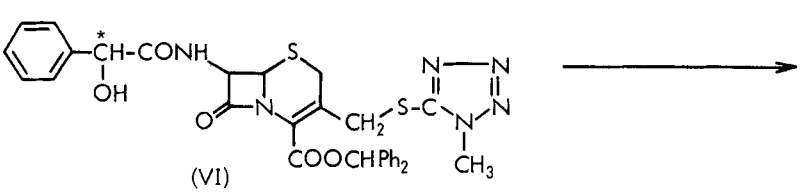
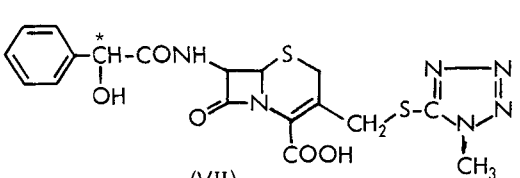


(III)

(IV) (V)



(VI)



(VII)

Scheme

diphenylmethyl 7-D-mandel-β-^{14}C-amino-3-{[(1-methyl-1H-tetrazol-5-yl)-thio]-methyl}-3-cephem-4-carboxylate (VI) in 84.3% radiochemical yield based on III. The diphenylmethyl group was removed from VI by treatment with trifluoroacetic acid and anisole in methylene dichloride with ice-cooling to obtain ^{14}C-labelled cefamandole, 7-D-mandel-β-^{14}C-amino-3-{[(1-methyl-1H-tetrazol-5-yl)-thio]methyl}-3-cephem-4-carboxylic acid (VII) in 94.4% radiochemical yield. The overall radiochemical yield was 11.27% based on barium carbonate-^{14}C.

EXPERIMENTAL

Radioactivity determination was carried out with a Packard "Tri-Carb" Liquid Scintillation Spectrometer 3380.

(±)-Mandelic-β-^{14}C Acid---Grignard reagent was prepared from 550 mg (3.5 mmol) of bromobenzene and 80 mg (3.3 mmol) of magnesium in 35 ml of anhydrous ether in a vacuum manifold Grignard apparatus under nitrogen atmosphere. Carbon dioxide-^{14}C derived from 86 mg (25 mCi, 0.435 mmol) of barium carbonate-^{14}C and 407 mg (2.065 mmol) of carrier barium carbonate and 60% perchloric acid solution (10 ml) were induced into the Grignard reagent in a liquid nitrogen bath and the mixture was stirred for 20 min. at -20°. Unreacted carbon dioxide was collected by cooling with liquid nitrogen and again induced into the reaction mixture. To acidify this mixture, it was placed in an ice bath and 2N H_2SO_4 was added dropwise. Next, the mixture was extracted with ether (20 ml x 3), then with 2N Na_2CO_3 (20 ml x 3). The aqueous layer was acidified with 2N H_2SO_4 with cooling and extracted with ether (20 ml x 3). The ether extract was washed with water, dried (Na_2SO_4), and slowly evaporated at 50-55° (bath temperature) for 30 min., leaving ^{14}C-labelled benzoic acid (310 mg).

A solution of ^{14}C-labelled benzoic acid (310 mg) in anhydrous ether (3 ml) was added dropwise to a solution of lithium aluminium hydride (160 mg) in anhydrous ether (16 ml) with stirring at 10° and stirred for 3 hr. at room temperature. To this mixture, ether (15 ml) and water (1 ml) were added dropwise with stirring in an ice bath and the mixture was stirred for 1.5 hr. The precipitates were collected by filtration, dissolved in 2N H_2SO_4 (15 ml),

and extracted with ether. The filtrate and the ether extract were combined, washed with water, dried (Na_2SO_4), and evaporated to leave an oily ^{14}C-labelled benzyl alcohol, which was oxidized without purification.

Chilled HNO_3 (d = 1.38, 2.5 ml) was added to ^{14}C-labelled benzyl alcohol with stirring and the yellow solution obtained was stirred in an ice bath. When the solution became green, ether (5 ml) was immediately added with ice-cooling. The mixture was poured into ice water (10 ml) and extracted with ether (15 ml x 2). The extract was washed with water, dried (Na_2SO_4), and evaporated slowly, leaving crude ^{14}C-labelled benzaldehyde.

Crude ^{14}C-labelled benzaldehyde was added to a solution of 450 mg (4.3 mmol) of sodium bisulfite in water (2 ml) and shaken vigorously in a stoppered bottle for 20 min., during which the sodium bisulfite adduct was separated from the solution. Water (10 ml) was added to this mixture to dissolve the adduct, followed by ether extraction (5 ml x 3). To the aqueous layer, fine powdered NaCN (900 mg, 18.4 mmol) was added with stirring, which was continued for 10 min. in an ice bath. Ether (5 ml) was added to the reaction mixture, which was stirred for 20 min. at room temperature. The aqueous layer was extracted with ether (10 ml x 2). The combined ether extract was washed with water, dried (Na_2SO_4), and evaporated to leave crude ^{14}C-labelled mandelonitrile (254 mg).

Crude ^{14}C-labelled mandelonitrile was dissolved in conc. HCl (1 ml) and allowed to stand for 48 hr. at room temperature. Next, it was diluted with water (5 ml) and extracted with ether (10 ml x 4). The extract was dried (Na_2SO_4) and evaporated to leave a crystalline residue (257 mg). The residue was recrystallized from benzene to give (±)-mandelic-β-^{14}C acid as colorless needles, m.p. 115-120° (184 mg, 12.5 mCi), in 50% radiochemical yield based on barium carbonate-^{14}C.

Optical Resolution[4] of (±)-Mandelic-β-^{14}C Acid---A solution of 753 mg (4.95 mmol, 50 mCi) of (±)-mandelic-β-^{14}C acid in ethanol (10 ml) was added to a solution of 1.5 g (4.95 mmol) of morphine monohydrate in ethanol (75 ml). A nucleus of morphine D-(-)-mandelate was added to this mixture, which then was allowed to stand at room temperature for two days while the alkaloidal salts separated from the solution as colorless crystals. Recrystallisation

from ethanol gave morphine D-(-)-mandelate-β-^{14}C as colorless needles (647.6 mg), m.p. 204-207°. Mother liquids were combined and concentrated to leave 30 ml of the ethanol solution. To this, a solution of 300 mg (1.97 mmol) of D-(-)-mandelic acid and 600 mg (1.97 mmol) of morphine monohydrate in ethanol (30 ml) was added. The mixture was left for 24 hr. at room temperature after addition of a nucleus of morphine D-(-)-mandelate. Morphine D-(-)-mandelate-β-^{14}C was obtained as colorless crystals (930.2 mg), m.p. 204-207°, which showed no melting point depression on admixture with an authentic sample of morphine D-(-)-mandelate.

Concentrated HCl (5 ml) was added to a solution of 1.577 g of morphine D-(-)-mandelate-β-^{14}C in ice water (25 ml). Water (30 ml) was added to the solution to dissolve separated morphine hydrochloride. This was extracted with ether (40 ml x 3), and the extract was washed with NaCl solution, dried (Na_2SO_4), and evaporated to leave a crystalline residue (533 mg). The residue was recrystallized from benzene (7 ml) and ether (3 ml) to give D-(-)-mandelic-β-^{14}C acid (III) as colorless needles (456 mg), m.p. 133-134°, $[\alpha]_D^{20}$ -145° (c, 2.7 in H_2O), 14.2 mCi (specific activity: 4.73 mCi/mmol), in 56.8% radiochemical yield based on D-(-)-mandelic-β-^{14}C acid.

Diphenylmethyl 7-D-Mandel-β-^{14}C-amino-3-{[(1-methyl-1H-tetrazol-5-yl)-thio]methyl}-3-cephem-4-carboxylate (VI)---Phosgene was passed through a solution of 456 mg (3 mmol, 14.2 mCi) of D-(-)-mandelic-β-^{14}C acid (III) in anhydrous tetrahydrofuran (15 ml) for 20 min. at 20°. This was left for 18 hr. in a stoppered bottle at room temperature, and then heated under reflux for 30 min. at 75-80° (bath temperature). After cooling, the solvent and excess phosgene were evaporated *in vacuo*. Anhydrous benzene (5 ml) was added to the residue and then evaporated *in vacuo*. This operation was repeated twice, leaving a carbonate (IV) as a crystalline residue. The carbonate (IV) was dissolved in anhydrous methylene dichloride (15 ml), and the solution was added dropwise to a solution of 1.45 g (2.94 mmol) of diphenylmethyl 7-amino-3-{[(1-methyl-1H-tetrazol-5-yl)-thio]methyl}-3-cephem-4-carboxylate (V) in anhydrous methylene dichloride (15 ml) with stirring in an ice bath and stirred for 2 hr. at the same temperature and for 1 hr. at room

temperature. Ethyl acetate (70 ml), ether (20 ml) and water (100 ml) were added to the reaction solution, and the mixture was shaken to extract the product. The aqueous layer was extracted with ethyl acetate (100 ml). The organic layers were combined and washed with water (30 ml x 2), dried (Na_2SO_4), and evaporated at below 45° in vacuo to leave a crystalline residue. The residue was dissolved in methylene dichloride (30 ml) and allowed to stand at room temperature after addition of a nucleus of cold VI. The separated crystals were recrystallized from methylene dichloride-ether to give diphenylmethyl 7-D-mandel-β-^{14}C-amino-3-{[(1-methyl-1H-tetrazol-5-yl)-thio]methyl}-3-cephem-4-carboxylate (VI) as colorless prisms (1.341 g), m.p. 205-207°, which showed no melting point depression on admixture with an authentic sample of cold VI. The mother liquid was evaporated to leave a residue, which was separated to give VI by preparative t.l.c. (KGF plate, 750 μm; solvent system, CH_2Cl_2:MeOH = 5:1). VI was recrystallized from methylene dichloride-ether to give colorless prisms (244 mg), m.p. 205-207°. The combined crystals (1.585 g) had 11.9 mCi total activity (radiochemical yield: 84.3%).

7-D-Mandel-β-^{14}C-amino-3-{[(1-methyl-1H-tetrazole-5-yl)-thio]methyl}-3-cephem-4-carboxylic Acid (Cefamandole-^{14}C) (VII)---Anisole (6 ml) was added to a solution of 1.585 g (2.52 mmol, 11.9 mCi) of VI in anhydrous methylene dichloride (65 ml). To this solution, trifluoroacetic acid (4.9 ml) was added dropwise with stirring for 5 min. in an ice bath, and the mixture was stirred for 2.5 hr. at the same temperature. The solvent and trifluoroacetic acid were evaporated at below 30° in vacuo. The residue was dissolved in ethyl acetate (100 ml) and extracted with 3% $NaHCO_3$ (30 ml x 3) with ice-cooling, and the extract then was washed with ethyl acetate (30 ml x 2). Ethyl acetate (100 ml) was added to the $NaHCO_3$ solution and the mixture was adjusted to pH 2.0 by addition of 2N H_2SO_4 with stirring in an ice bath, and the aqueous layer was extracted with ethyl acetate. The ethyl acetate extracts were combined, washed with water, dried (Na_2SO_4), and evaporated at below 40° in vacuo to leave 5 ml of the ethyl acetate solution. Ether (20 ml) was added to this residual solution, which was allowed to stand at room temperature for crystal

separation. The crystals were filtered and washed with ether to give 7-D-mandel-β-^{14}C-amino-3-{[(1-methyl-1H-tetrazol-5-yl)-thio]methyl}-3-cephem-4-carboxylic acid (cefamandole-^{14}C) (VII) as a pale yellow crystalline powder (1.105 g), 11.27 mCi (4.73 mCi/mmol, 10.2 μCi/mg), in 94.4% radiochemical yield and in 11.27% overall radiochemical yield based on $BaCO_3$-^{14}C. This compound was confirmed to be pure by t.l.c.-autoradiogram and t.l.c.-radioactinogram [X-ray film, silica gel KGF plate (Merck), solvent system: EtOAc-HOAc-H_2O (15:1:1)].

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