SYNTHESIS OF ^{14}C-LABELLED 7α-METHOXYCEPHALOSPORIN DERIVATIVE (CS-1170)

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SUMMARY

Sodium 7β-Cyanomethylthioacetamido-7α-^{14}C-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate (CS-1170), a new cephamycin derivative was prepared for metabolic studies. The key reaction involves the methoxylation of the diphenylmethyl 7-(3,4-di-tert-butyl-4-oxo-2,5-cyclohexadien-1-ylen)-methylimino-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate (I) with $^{14}CH_3OH$ to diphenylmethyl 7β-(3,5-di-tert-butyl-4-hydroxybenzylideneamino)-7α-^{14}C-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate (II). Cleavage of the Schiff base, acylation followed by hydrolysis produced the desired radioactive drug. The overall radiochemical yield was 14%, at a specific activity of 12.7 uCi/mg.

Key Words: Carbon-14, 7α-^{14}C-Methoxycephalosporin, Methoxylation, Antibacterial agent, CS-1170

INTRODUCTION

Sodium 7β-cyanomethylthioacetamido-7α-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate (CS-1170)[1] synthesized in our laboratories is a new semisynthetic cephamycin derivative with broad antibacterial activity. As a matter of course, studies on the metabolic fate of this agent in animals have required the preparation of the radioactive agent. There are three

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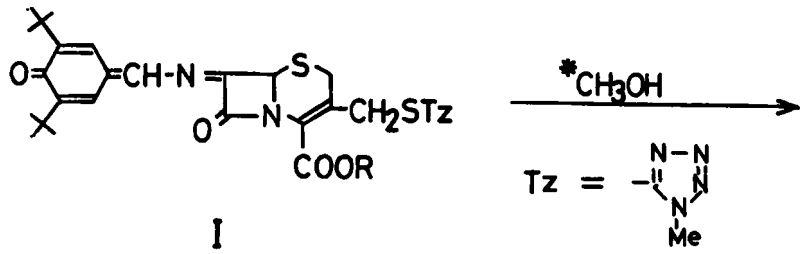
possible labelling moieties, i.e. the 7β-acyl, 7α-methoxy and 3-tetrazolylthio groups, which can be used to prepare a radioactive sample from 7-aminocephalosporanic acid. Previously we reported[2] the preparation of a sample labelled 7β-cyanomethylthioacetyl group with ^{35}S. Now we wish to report a preparation of CS-1170 labelled 7α-methoxy group with ^{14}C.

RESULTS AND DISCUSSION

In the previously reported procedure[1] for synthesis of unlabelled CS-1170 a large excess of methanol was used. For a radioactive synthesis, this procedure was considered impractical because of very low utility of methanol, and so an attempt was made to improve reaction conditions.

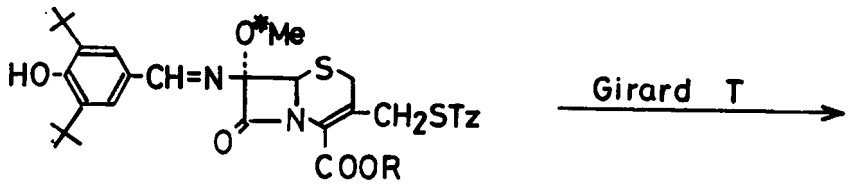
In the cold experiments, the reaction of the quinoidal compound (I) with an equimolar amount of methanol in benzene or dichloroethane gave the desired 7α-methoxy compound (II) in a poor yield. However, in the presence of catalytic amounts of BF_3-etherate the same reaction yielded II in a fairly good yield. In the hot experiments, II was obtained in 49% yield on the basis of $^{14}CH_3OH$ after purification with silica gel column chromatography. The resulting ^{14}C-labelled Schiff base (II)(44 mCi/mM) was diluted with pure unlabelled Schiff base to the specific activity 12.5 mCi/mM and treated with Girard T reagent to afford 7-amino compound (III), which was acylated with cyanomethylthioacetyl chloride to give the corresponding 7β-acylaminocephalosporin (IV). Subsequently the diphenylmethyl group was removed with trifluoroacetic acid to yield a free carboxylic acid, which was purified as the dicyclohexylamine salt (V). V was diluted with the same amount of unlabelled salt and converted into the sodium salt (CS-1170) by treatment with sodium 2-ethylhexanoate.

This procedure furnished the ^{14}C-labelled drug in overall radiochemical yield of 14% from ^{14}C-methanol. The specific activity was 12.7 uCi/mg (6.25 mCi/mM) and the radiochemical purity was 96%.

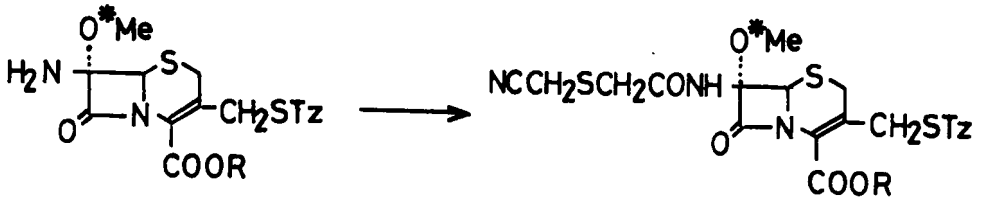


I

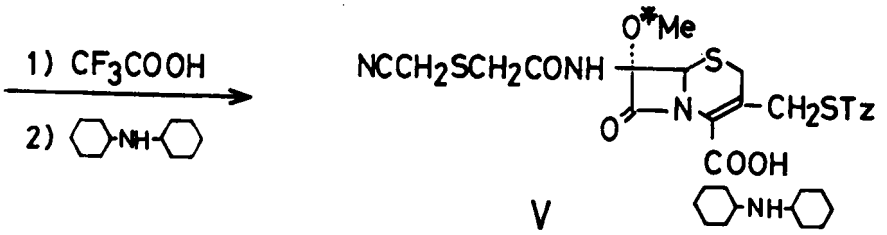
R = CH(C6H5)2



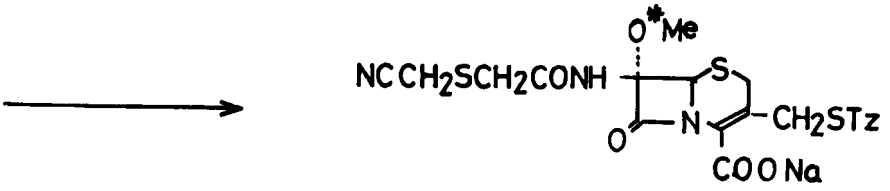
II



III IV



V



CS-1170

EXPERIMENTAL

Diphenylmethyl 7β-(3,5-di-tert-butyl-4-hydroxybenzylideneamino)-7α-^{14}C-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate (II) ——— To a solution of 1.6 g (2.25 mmole) of diphenylmethyl 7- (3,4-di-tert-butyl-4-oxo-2,5-cyclohexadien-1-ylen)-methylimino-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate[1] (I) in 8 ml of dichloroethane was added a solution of 73 mg (2.15 mmole, 100 mCi ; New England Nuclear, Boston, Mass.) of methanol-^{14}C in 8 ml of dichloroethane. To the mixture was added 1.6 ml of 0.1 M BF_3 anhydrous ether solution. The flask was stoppered and the resulting orange red solution was allowed to stand for 20 hr in a refrigerator. After addition of 1.6 ml of 0.1 M N,N-diethylaniline anhydrous ether solution at room temperature, the reaction mixture was concentrated under reduced pressure (aspirator) at 30-35°. The resulting residue was purified by silica gel (50 g) column chromatography with benzene-ethyl acetate (8:1). The fraction of II was collected and evaporated in vacuo. The residue was crystallized by addition of MeOH. The crystalline product was collected by filtration, washed with MeOH and dried in vacuo to give II (825 mg, 49% yield based on MeOH-^{14}C).

Diphenylmethyl 7β-Amino-7α-^{14}C-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate (III) ——— To 825 mg of the above 7-methoxy Schiff base (II) was added 2.075 g of unlabelled 7-methoxy Schiff base, 1.8 g of Girard T reagent and 30 ml of MeOH-THF-H_2O (10:1.3:0.1) mixture. Stirring at room temperature for 15 min afforded a pale yellow clear solution. Then the solution was allowed to stand for 2 hr in a refrigerator to produce colorless needles, which were collected, washed with MeOH and dried in vacuo to give III (1.5 g, 73% yield).

Diphenylmethyl 7β-Cyanomethylthioacetamido-7α-^{14}C-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate (IV) ——

——— To an ice-chilled solution of 1.5 g of III in 25 ml of dichloroethane was added dropwise 600 mg of N,N-diethylaniline followed by a solution of 600 mg of freshly distilled cyanomethylthioacetyl chloride[1] in 2 ml of dichloroethane. The mixture was stirred for 30 min under cooling, then washed with H_2O, 3% aqueous $KHSO_4$ solution, 10% aqueous NaCl solution and H_2O, successively. The dried ($MgSO_4$) organic layer was concentrated to dryness in vacuo to give 2 g of IV as a yellowish amorphous powder, which was employed in the next step without purification.

7β-Cyanomethylthioacetamido-7α-^{14}C-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic acid Dicyclohexylamine Salt (V) ——— To an ice-chilled solution of 2 g of IV in 15 ml of dichloroethane and 2 ml of anisole was added 3 ml of trifluoroacetic acid with stirring. After 30 min of stirring, the mixture was concentrated in vacuo at 35° to about 5 ml. The resulting residue was dissolved in 30 ml of AcOEt and extracted once with 35 ml of 10% aqueous K_2HPO_4 solution and once again with 10 ml of the same solution. The combined aqueous extract was washed two times with AcOEt, then covered with 40 ml of AcOEt and adjusted to pH 2 with 10% HCl with stirring. The organic layer was separated and the aqueous layer was extracted three times with AcOEt. The combined organic layers were washed with 10% aqueous NaCl solution, dried ($MgSO_4$) and evaporated in vacuo to give 1.5 g of crude product as a yellowish viscous oil. This crude product was dissolved in 8 ml of EtOH and 4 ml of CH_2Cl_2 and a solution of 550 mg of dicyclohexylamine in 3 ml of CH_2Cl_2 was added. The mixture was concentrated in vacuo to about 6 ml. To the residual solution was added 5 ml of EtOH to precipitate crystals. The mixture was allowed to stand for 3 hr in a refrigerator and the crystals were collected, washed with EtOH and dried to give 1.05 g of V. For further purification, this amine salt was dissolved in 24 ml of acetone-EtOH-CH_2Cl_2 (1:1:1) and the resulting solution was concentrated to about 5 ml in vacuo and then diluted

with 5 ml of EtOH, and the mixture was allowed to stand overnight in a refrigerator. The crystals were collected and washed with EtOH to yield V (816 mg, 43.3% yield from III) as colorless needles.

Sodium 7β-Cyanomethylthioacetamido-7α-^{14}C-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate (CS-1170) ——— 800 mg each of V and unlabelled V were dissolved in 35 ml of CH_2Cl_2 and 3.5 ml of iso-PrOH. To the solution was added a mixture of 1.7 ml of 2 M sodium 2-ethylhexanoate AcOEt solution and 3 ml of iso-PrOH at room temperature. After stirring for 5 min, 50 ml of cyclohexane was added. The mixture was allowed to stand for 15 min and the resulting precipitate was collected by filtration, washed with 40 ml of cyclohexane - dichloromethane (3:2) and dried in vacuo to give CS-1170 (1.07 g, 90% yield) as a colorless powder which showed analytical properties comparable to those of authentic unlabelled CS-1170[1]. The specific activity was 6.25 mCi/mmole or 12.7 uCi/mg and the radiochemical purity was 96.7%.

REFERENCES

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2. E. Nakayama and H. Nakao, Ann. Sankyo Res. Lab., 29, 147 (1977).