Synthesis of 7-[α-(2-aminothiazol-4-yl)-α-(z)-methoximinoacetamido]-3-(1-[^{14}C]methylpyrrolidinio)-methyl-3-cephem-4-carboxylate Sulfate

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SUMMARY

The synthesis of the title compound (6) is described. The reaction of sodium [^{14}C] formate with acetyl chloride produced the mixed anhydride. Treatment with pyrrolidine gave the N-formyl compound in a 4 to 1 ratio with the N-acetyl compound.[1] Separation and reduction with lithium aluminum hydride gave N-[^{14}C]methylpyrrolidine[2]. Reaction with 7-[α-(2-triphenylmethylaminothiazol-4-yl)-α-(z)-methoximinoacetamido]-1-oxo-3-iodomethyl-3-cephem-4-carboxylate diphenylmethyl ester introduced the label into the cephalosporin. Treatment with acetyl chloride converted the sulfoxide to the sulfide. Formic acid at 39°C removed the protecting groups and treatment with 4N sulfuric acid produced the title compound as a sulfate salt.

Key Words:

7-[α-(2-aminothiazol-4-yl)-α-(z)-methoximinoacetamido]-3-(1-[^{14}C]-methylpyrrolidinio)methyl-3-cephem-4-carboxylate sulfate, N-methyl-pyrrolidine, enterobacteriaceae

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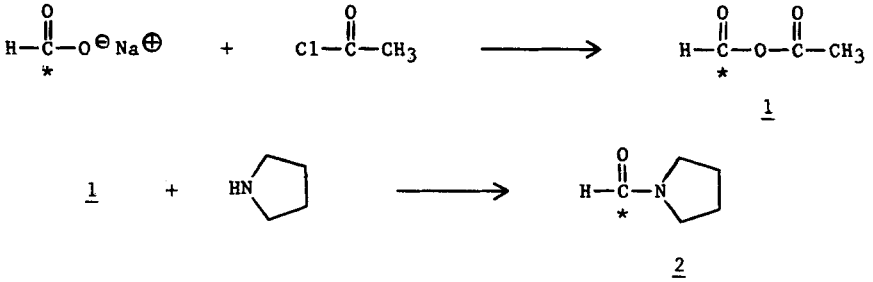
INTRODUCTION

Substitution with N-methylpyrrolidine of the 3-side chain of an aminothiazole cephalosporin afforded a quarternary compound showing high antipseudomonal activity. This compound is a parenteral cephalosporin with broad spectrum activity both *in vivo* and *in vitro*. When compared with ceftazidine, moxalactam and cefoperazone *in vitro*, against gram-positive organisms, the compound has greater activity. Among 84 strains of *Enterobacteriaceae* considered resistant, the cross-resistance was remarkable low. 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.

MATERIALS

The sodium [^{14}C] formate was purchased from Amersham Corporation. All chemicals used in the synthesis were purchased commercially and used without any purification. All other solvents were either distilled or analytical reagent quality. Thin layer chromatography plates used were Analtech silica gel GF scored 10 x 20 cm and high pressure liquid chromatography was carried out on Waters Associates instrumentation. Radioactivity was measured by a Beckman LS 9000 liquid scintillation counter. Nuclear magnetic resonance spectra were measured on a Bruker 360. Weighings were carried out on a Sartorius 200 balance and a Mettler Microanalytical M5AS balance.

SYNTHETIC PATHWAY



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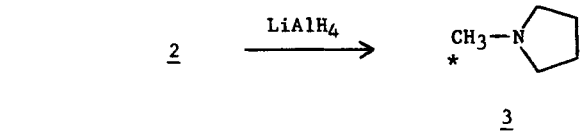
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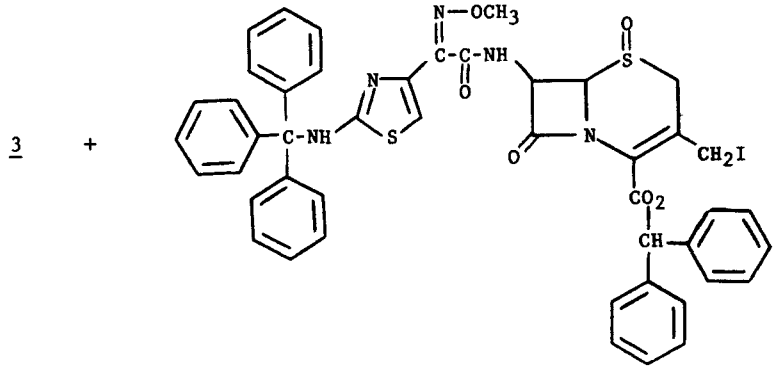
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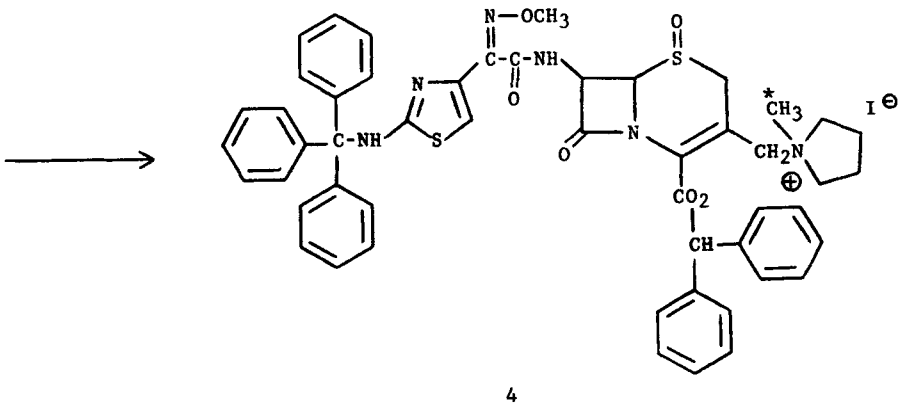
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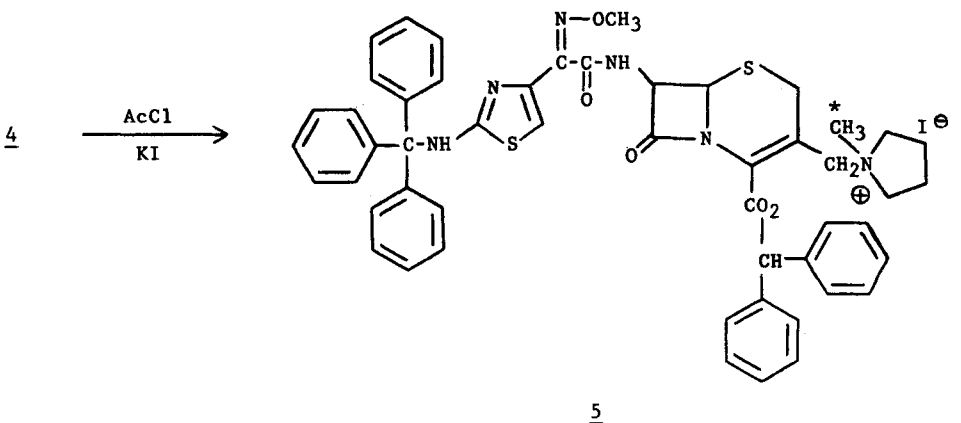
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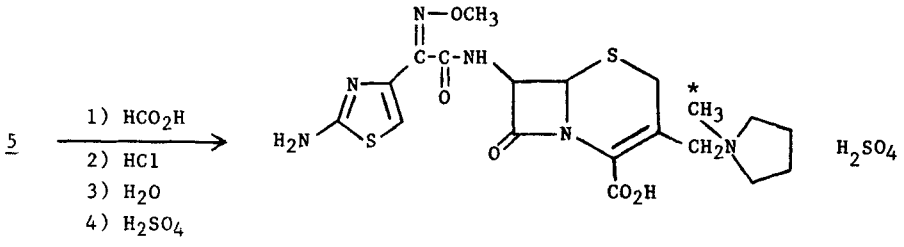

6

* = position of label

EXPERIMENTAL

N-[^{14}C]Formylpyrrolidine (2)

Into a 10 ml round-bottom flask was placed dry ether (0.5 ml) followed by acetyl chloride (0.6 ml). To this mixture was then added sodium [^{14}C] formate (100 mCi, 136 mg) followed by non-radiolabelled sodium formate (544 mg). The reaction was stirred at room temperature, in a nitrogen atmosphere, for 5 hrs. Dry ether (0.5 ml) was then added and the entire mixture added dropwise, over a period of 10 min. to a solution of pyrrolidine (1.3 ml, 2 eq) in dry ether (5 ml) in an ice bath. After the addition was complete, the mixture was stirred in the cold for 15 min. and then at room temperature for 45 min. The reaction was then added to a column of silica gel (15 g) in tetrahydrofuran and the desired product isolated (589 mg, 59% yield).

Thin Layer Chromatography:

Eluent - tetrahydrofuran, Plates - Analtech silica gel, Visualization - iodine vapors, Compound - Rf = 0.51.

N-[^{14}C]Methylpyrrolidine (3)

Into a 25 ml three-neck flask was placed dry ether (5 ml). To this was added lithium aluminum hydride (451 mg, 2 eq.) followed by the dropwise addition of a solution of N-[^{14}C]formylpyrrolidine in dry ether (5 ml). After the addition was complete, the reaction was heated under reflux

for 1 hr. To the refluxing reaction was carefully added dry ether (5 ml) followed by the dropwise addition of a saturated sodium sulfate solution (1.4 ml). After the addition was complete, the reaction mixture was refluxed for an additional 1.5 hr. It was then centrifuged and the mother liquors pipetted away from the solid. This solution was used directly in the next sequence.

7-[α-(2-Triphenylmethylaminothiazol-4-yl)-α-(z)-methoximinoacetamido]-1--oxo-3-(1-[^{14}C]methylpyrrolidinio)methyl-3-cephem-4-carboxylate diphenylmethyl ester (4)

To a 100 ml flask was added dry tetrahydrofuran (50 ml), followed by the addition of 7-[α-(2-triphenylmethylaminothiazole-4-yl)-α-(z)methoximinoacetamido]-1-oxo-3-iodomethyl-3-cephem-4-carboxylate diphenylmethyl ester (5.07 g). The solution was cooled in an ice bath under a nitrogen atmosphere. The mother liquors containing N-[^{14}C]methylpyrrolidine were added and the mixture stirred in the cold for 1 hr. Heptane (600 ml) was added and the reaction stirred at room temperature for 15 min. The resulting solid was removed by filtration and dried (4.82 g).

High Pressure Liquid Chromatography:

This was carried out on Waters Associates instrumentation having the following parameters: Eluent: -Eluent(A)-40% methanol-water, 0.005 M octanesulfonic acid sodium salt, pH adjusted to 3.0 with glacial acetic acid. Eluent(B)-90% methanol water, 0.005 M octanesulfonic acid sodium salt, pH adjusted to 3.0 with glacial acetic acid. The eluent was a gradient going from 0% B - 100% A to 100% B - 0% A over a 20 min. period. Flow Rate: 2 ml/min. Detector - ultraviolet at 254 nm Temperature: 22.5°C Column - Waters Associates C-18 Retention Time - 6.98 min.

7-[α-(2-Triphenylmethylaminothiazol-4-yl)-α-(z)-methoximinoacetamido]-3-(1-[^{14}C] methylpyrrolidinio)methyl-3-cephem-4-carboxylate diphenylmethyl ester (5)

Into a 125 ml flask was added a solution of compound (4)(4.82 g) in dry acetone (65 ml). To this was added 4A molecular sieves and the solution allowed to stand at room temperature for 1.5 hrs. The acetone solution was then pipetted away from the 4A molecular sieves into a 100 ml flask. To this was added a nitrogen atmosphere and cooled in an ice bath. The following was added, dimethyl acetamide (1.7 ml), potassium iodide (3.87 g, 5 eq.) and acetyl chloride (0.83 ml, 2.5 eq.) The mixture was stirred in the cold for 2 hrs. and then poured slowly into 0.1 M sodium metabisulfite solution (600 ml) with stirring. The resulting solid was removed by filtration, washed with water (10 ml) and dried under high vacuum for 16 hrs (4.07 g).

High Pressure Liquid Chromatography was carried out on Waters Associates instrumentation having the following parameters: Eluent - Eluent (A) - 40% methanol - water, 0.005 M octanesulfonic acid sodium salt, pH adjusted to 3.0 with glacial acetic acid. Eluent (B) - 90% methanol - water, 0.005 M octanesulfonic acid soldium salt, pH adjusted to 3.0 with glacial acetic acid. The eluent was a gradient going from 0% B-100% A to 100% B - 0% A over a 20 min. period. Flow rate - 2 ml/min. Detector - ultraviolet at 254 nm. Temperature - 22.5°C Column - Waters Associates C-18 Retention time - 8.22 min.

7-[α-(2-Aminothiazol-4-yl)-α-(z)-methoximinoacetamido]-3-(1-[^{14}C] methylpyrrolidinio) methyl-3-cephem-4-carboxylate sulfate (6).

Into a 125 ml flask was placed formic acid (16.2 ml). This was immersed into a preheated oil bath at 39°C. To this was then added with stirring compound (5) (4.07 g.) and the mixture stirred at 39°C for 0.5 hr. It

was then cooled to room temperature and concentrated hydrochloric acid (0.41 ml) added. This mixture was stirred at room temperature for 0.5 hr. Water (0.38 ml) was then added and stirred at room temperature for 0.75 hr. The reaction was then filtered through a glass filter and the filtrate concentrated under reduced pressure to an oil. Solidification occurred upon trituration with water (35 ml). The mixture was adjusted to pH 4 with 5% sodium bicarbonate solution. The insoluble material was removed by filtration and the filtrate placed on a C-18 column made up C-18 (40 g Waters Associates packing) in water. The column was first eluted with water (200 ml) and then with 15% methanol-water (450 ml). To this eluent was added concentrated sulfuric acid (0.3 ml) ;and concentrated under reduced pressure to a volume of 10 ml. It was seeded with non-radiolabelled compound and stirred at 4°C for 16 hr. The resulting solid was removed by filtration and dried (375 mg). Radiochemical purity was 97.5% as determined by high pressure liquid chromatography and the specific activity was 21.7 μCi/mg.

High Pressure Liquid Chromatography was carried out an Waters Associates instrumentation having the following parameters: Eluent - 6% acetonitrile, 0.015 M pentane sulfonic acid sodium salt, pH adjusted to 4 with glacial acetic acid. Flow rate - 2 ml/min. Detector - ultraviolet at 254 nm. Temperature - 22.5°C Column - Waters Associates C-18. Retention time - 11.0 min.

RESULTS AND DISCUSSION

The reaction of sodium [^{14}C] formate with acetyl chloride in dry ether produced the mixed anhydride. Treatment with pyrrolidine yielded a 4 to 1 mixture of N-formylpyrrolidine to N-acetylpyrrolidine which was separated using column chromatography of silica gel in tetrahydrofuran. A second chromatography using silica gel in acetone produced an analytical sample of the N-formyl compound. Reduction of the N-formyl

compound to the N-methyl compound went smoothly using lithium aluminum hydride in dry diethyl ether. A major problem was the volatility of the N-[^{14}C]methylpyrrolidine. This reaction had to be carried out carefully. Separation of the aluminum salts from the reaction by filtration caused a 60% loss of product. The compound was isolated out of the reaction mixture with only a 10% loss by centrifugation of the mixture and pipetting off the mother liquors into the next reaction. The reaction of the mother liquors, containing N-[^{14}C]methylpyrrolidine, with 7-[α-(2-triphenylmethylaminothiazol-4-yl)-α-(z)methoximinoacetamido]-1-oxo-3-iodomethyl- 3-cephem-4-carboxylate diphenylmethyl ester in tetrahydrofuran produced an 88% yield of a mixture containing 40% of desired product. Treatment with acetyl chloride and potassium iodide in acetone removed the sulfoxide. Removal of the protecting groups was carried out by the use of formic acid at 39°C for 0.5 hrs. Passing through a C-18 column and then acidifying with sulfuric acid produced crystalline product as the sulfate salt. The overall chemical yield, starting from labelled formate, was 10%. All experimental conditions were optimized using non-radioactive materials.

REFERENCES

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