SYNTHESIS OF CARBON-14 LABELLED ANTIMICROBIAL AGENTS.
II. SYNTHESIS OF APALCILLIN-^{14}C SODIUM

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SUMMARY

Sodium 6-[(R)-2-(4-hydroxy-1,5-naphthyridine-4-^{14}C-3-carboxy-^{14}C-amido)-2-phenylacetamido]penicillanate (apalcillin-^{14}C sodium)(1) was synthesized for use in metabolic studies. Reaction of ethyl malonate-1-^{14}C (2) with ethyl orthoformate in the presence of zinc chloride gave ethyl ethoxymethylenemalonate-1-^{14}C (3), which was condensed with 3-aminopyridine to give the aminopyridylacrylate-^{14}C (4). Cyclization of 4 in boiling Dowtherm A followed by hydrolysis of the resulting ester gave 4-hydroxy-1,5-naphthyridine-4-^{14}C-3-carboxylic-^{14}C acid (5). Condensation of 5 with 6-[(R)-2-amino-2-phenylacetamido]penicillanic acid by the activated ester method yielded apalcillin-^{14}C triethylammonium (7), which in turn was treated with sodium 2-ethylhexanoate, giving apalcillin-^{14}C sodium (1) in the overall yield of 17% based on 2.

Key Words: Carbon-14, Apalcillin Sodium, Semisynthetic Penicillin

INTRODUCTION

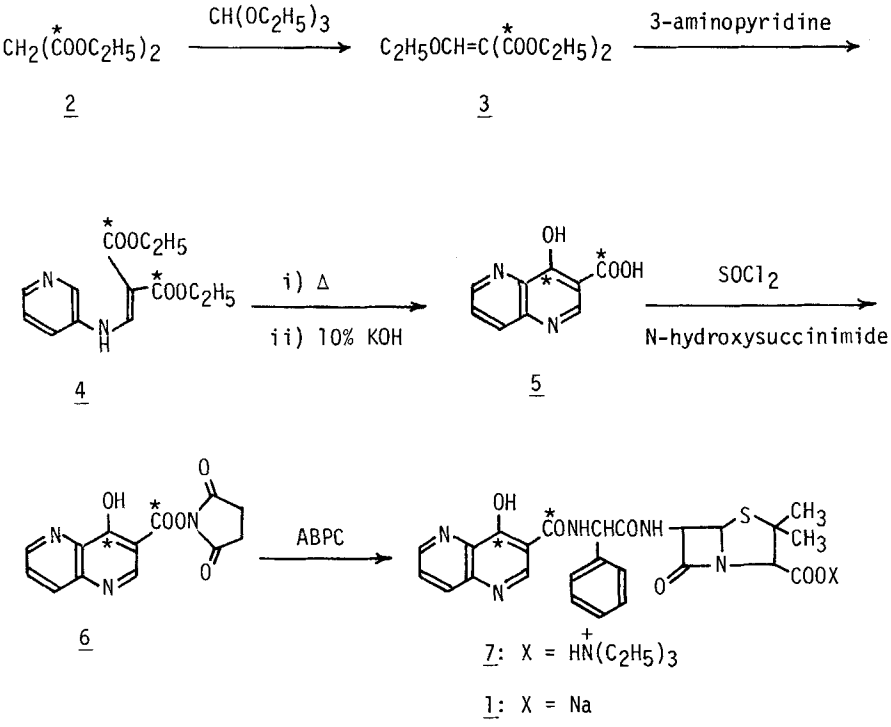
Sodium 6-[(R)-2-(4-hydroxy-1,5-naphthyridine-3-carboxyamido)-2-phenylacetamido]penicillanate (apalcillin sodium)(1) is a new semisynthetic penicillin[1,2]. This agent has been found to display excellent antimicrobial activities against gram-positive cocci and gram-negative bacilli including *Pseudomonas aeruginosa* [3,4]. These findings led to studies on the absorption and metabolism of this agent in animals. In this report, we describe the synthesis of apalcillin-^{14}C sodium labelled at both C-4 and carbonyl carbons of the 1,5-naphthyridine-carboxylic acid moiety for use in these studies.

0362-4803/81/101415-07$01.00

Received October 8, 1980

DISCUSSION

The scheme for the synthesis of apalcillin-^{14}C sodium (1) is illustrated in Figure 1. In the preceding paper[5], we reported that 3-(3,4-methylenedioxyanilino)acrylate-2-^{14}C was prepared in a good yield, without use of ethyl ethoxymethylenemalonate-2-^{14}C (EMME), by direct reaction of ethyl malonate-2-^{14}C and ethyl orthoformate with 3,4-methylenedioxyaniline. Similar reactions with 3-aminopyridine, however, gave the corresponding acrylate (4) in poor yields. The findings led to the use of Adams' method[6] which comprised a reaction of EMME with 3-aminopyridine (Fig. 1).



ABPC = 6-[(R)-2-amino-2-phenylacetamido]penicillanic acid

* ^{14}C

Fig. 1. Scheme for the synthesis of apalcillin-^{14}C sodium (1)

We reported previously that EMME-^{14}C (3) was prepared in 50% yield by reacting ethyl malonate-^{14}C with a small excess of ethyl orthoformate in the presence of anhydrous zinc chloride as catalyst[5]. After considerable re-investigations of this reaction, an improved reaction condition was found to give 3 with a higher radiochemical purity and in a better radiochemical yield. Ethyl malonate-1-^{14}C (2) was allowed to react in acetic anhydride with a large excess (4 equivalents) of ethyl orthoformate and a catalytic amount of anhydrous zinc chloride by heating the mixture gradually from 100° to 150° for 14 hr, the reaction being carried out for a longer period at higher temperature. EMME-1-^{14}C (3) thus obtained showed the radiochemical purity of 94%, and the radiochemical yield from 2 was 91%. The product was used for the next reaction without any purification.

To obtain 4-hydroxy-1,5-naphthyridine-3-carboxylic acid (5) from EMME in a large-scale preparation, Adams, *et al.*[6] carried out the series of reactions (Fig. 1) without any isolation of the intermediates. In this isotopic preparation, however, isolation of the acrylate-1-^{14}C (4) gave a better result. Condensation of 3 with 3-aminopyridine at 140-150° followed by column chromatography of the resulting product on silica gel afforded the acrylate-1-^{14}C (4) in 91% yield. Cyclization of 4 was effected by heating a dilute solution of 4 in Dowtherm A, giving the ethyl ester of 4-hydroxy-1,5-naphthyridine-4-^{14}C-3-carboxylic-^{14}C acid (5). High dilution, high temperature, and a brief reaction period appeared to be required for satisfactory results in this reaction. The ethyl ester was immediately hydrolyzed by heating with 10% potassium hydroxide solution to yield a crude product of 4-hydroxy-1,5-naphthyridine-4-^{14}C-3-carboxylic-^{14}C acid (5). Purification of the crude product was effected by repeating the re-precipitation method using alkari and acid; giving 5 with a radiochemical purity of 99% and in 50% yield from the acrylate-1-^{14}C (4).

Apalcillin is known to be stable only as the salt form, and in the form of the free acid, the compound readily decomposes mainly at the β-lactam moiety[1,2]. This chemical property made it difficult to purify the final product by column chromatography, preparative TLC, and recrystallization since these method caused

further decomposition. A good purity of apalcillin-^{14}C sodium was achieved by starting with the pure acid-^{14}C (5) and selecting the last sequence of reactions which could be carried out with milder conditions and give better yields.

Among the known methods for a condensation of the acid (5) with 6-[(R)-2-amino-2-phenylacetamido]penicillanic acid (ABPC)[1,2], the activated ester method was selected since the activated N-hydroxysuccinimide-ester (6) was relatively stable to be isolated as crystals. Thus, the acid-^{14}C (5) was allowed to react with thionyl chloride and N-hydroxysuccinimide below 30°, giving the activated ester-^{14}C (6) in 82% yield. Condensation of 6 with ABPC in dimethylsulfoxide containing triethylamine at room temperature afforded the triethylammonium salt of apalcillin-^{14}C (7) in 63% yield. The salt exchange was accomplished by treatment of 7 with sodium 2-ethylhexanoate in a mixed solvent of dimethylsulfoxide and methanol to give apalcillin-^{14}C sodium (1) in 80% yield. The radiochemical and chemical purities of the final product was 99% as determined by radio-TLC and high pressure liquid chromatography. The overall yield of 1 was 17% based on ethyl malonate-1-^{14}C.

EXPERIMENTAL

Ethyl Ethoxymethylenemalonate-1-^{14}C (3) -- A stirred mixture of ethyl malonate-1-^{14}C (2)(74.1 mCi, 2.25 g, 14.1 mmol)[5], ethyl orthoformate (4.20 g, 28 mmol), anhydrous zinc chloride (0.1 g) and acetic anhydride (5 ml) was heated as follows: at 100-110° for 2 hr, at 110-120° for 2.5 hr, and at 130-140° for 4.5 hr. After cooling, ethyl orthoformate (4.20 g, 28 mmol) and acetic anhydride (5 ml) were added to the mixture, and the mixture was heated again at 140-150° for 4.5 hr; during which ethyl acetate produced was removed by distillation. The mixture was poured into ice-water (15 ml) and extracted with ether. The extract was washed with water, dried over sodium sulfate and evaporated at atmospheric pressure to give ethyl ethoxymethylenemalonate-1-^{14}C (3)(72.0 mCi); the radiochemical purity 94% (containing 5% ethyl malonate-1-^{14}C) on radiogaschromatography (column: 3% DC-550 on Chromosorb, 2 m x 3 mm I.D.; column temperature: 200°; carrier gas: He 27 ml/min; retention times: ethyl malonate 1.0 min, ethyl ethoxy-

methylenemalonate 6.8 min). The product was used for the next reaction without any purification.

Ethyl 2-Carbethoxy-^{14}C-3-(3-aminopyridyl)acrylate-1-^{14}C (4) -- A mixture of ethyl ethoxymethylenemalonate-1-^{14}C (3)(72.0 mCi, 2.76 g, 12.8 mmol) and 3-aminopyridine (11.0 g, 117 mmol) was stirred at 140-150° for 1.5 hr. After cooling, the mixture was dissolved in ether (8 ml) and the solution was chromatographed on silica gel with ether. Evaporation of the main fraction under reduced pressure gave ethyl 2-carbethoxy-^{14}C-3-(3-aminopyridyl)acrylate-1-^{14}C (4)(61.3 mCi, 3.08 g); mp and mixed mp 64-66°; the radiochemical purity 99% on radio-TLC (silica gel, chloroform/methanol=9/1, R_f-value=0.63); IR νmax (nujol): 1680 cm^{-1} (C=O); NMR (δ, $CDCl_3$): 1.32 and 1.39 (each 3H, t, J=7.5 Hz, $-CH_2\underline{CH_3}$), 4.24 and 4.31 (each 2H, q, J=7.5 Hz, $-\underline{CH_2}CH_3$), 7.16-8.52 (5H, m, pyridine and C-3 H).

4-Hydroxy-1,5-naphthyridine-4-^{14}C-3-carboxylic-^{14}C Acid (5) -- A solution of ethyl 2-carbethoxy-^{14}C-3-(3-aminopyridyl)acrylate-1-^{14}C (4)(61.3 mCi, 3.08 g, 11.7 mmol) in Dowtherm A (12 ml) was added dropwise to boiling Dowtherm A (120 ml, 250-255°) with stirring within 5 min. The mixture was stirred at the same temperature for 6 min, allowed to cool rapidly, and added to 10% potassium hydroxide solution (45 ml). The mixture was refluxed for 1 hr, allowed to cool, and the aqueous solution was separated. The solution was acidified to pH 2.0 with concentrated hydrochloric acid, and the crystals produced were collected by filtration. The crude product was dissolved in 5% potassium hydroxide solution and treated with charcoal. After removal of the charcoal, the filtrate was acidified (pH 4.0) to give a crystalline product which was collected by filtration. The same procedure for purification was repeated two more times to give 4-hydroxy-1,5-naphthyridine-4-^{14}C-3-carboxylic-^{14}C acid (5)(30.7 mCi, 1.12 g); mp >300°; the radiochemical purity 99% on radio-TLC (silica gel, ether/butanol/acetone/water=1/1/1/1, R_f-value=0.15); IR νmax (nujol): 1740 cm^{-1}(C=O).

N-(4-Hydroxy-1,5-naphthyridine-4-^{14}C-3-carbonyloxy-^{14}C)succinimide (6) -- To a

stirred mixture of 4-hydroxy-1,5-naphthyridine-4-^{14}C-3-carboxylic-^{14}C acid (5) (27.9 mCi, 1.01 g, 5.3 mmol), N-hydroxysuccinimide (0.77 g, 6.7 mmol), pyridine (0.26 g), and dimethylformamide (6.90 g) was added dropwise thionyl chloride (0.76 g, 7.4 mmol) at 0-5°, and the mixture stirred at 20-30° for 4 hr. The crystals produced were collected by filtration, washed successively with dimethylformamide, water and acetone, and dried under reduced pressure to give N-(4-hydroxy-1,5-naphthyridine-4-^{14}C-3-carbonyloxy-^{14}C)succinimide (6)(23.0 mCi, 1.26 g); mp 301-303° (decomp); IR νmax (nujol): 1760 and 1740 cm^{-1} (C=O).

Triethylammonium 6-[(R)-2-(4-hydroxy-1,5-naphthyridine-4-^{14}C-3-carboxy-^{14}C-amido)-2-phenylacetamido]penicillanate (Apalcillin-^{14}C Triethylammonium)(7) -- A mixture of N-(4-hydroxy-1,5-naphthyridine-4-^{14}C-3-carbonyloxy-^{14}C)succinimide (6)(22.8 mCi, 1.25 g, 4.4 mmol), 6-[(R)-2-amino-2-phenylacetamido]penicillanic acid ($3H_2O$, 1.85 g, 4.8 mmol) and dimethylsulfoxide (5.56 g) was stirred at room temperature for 10 min. After addition of triethylamine (0.93 g, 9.2 mmol), the mixture was stirred at room temperature for 1 hr. Acetone was added to the mixture; and the precipitate was collected by filtration, washed with acetone, and dried under reduced pressure to give apalcillin-^{14}C triethylammonium (7) (14.3 mCi, 1.65 g); mp 210° (decomp); IR νmax (nujol): 1770 and 1660 cm^{-1} (C=O).

Apalcillin-^{14}C Sodium (1) -- Apalcillin-^{14}C triethylammonium (7)(14.3 mCi, 1.65 g, 2.7 mmol) was added portionwise, at room temperature, to a stirred mixture of sodium 2-ethylhexanoate (0.45 g, 2.7 mmol), dimethylsulfoxide (2.50 g) and methanol (1.50 g), and the mixture stirred at the same temperature for 10 min. After removal of insoluble materials, acetone (40 ml) was added to the filtrate. The crystals precipitated were collected, washed with acetone, and dried under reduced pressure to give apalcillin-^{14}C sodium (1)(11.5 mCi, 1.15 g, 5.25 mCi/mmol); mp 236-238°; the radiochemical and chemical purities 99% on both radio-TLC (silica gel, butanol/acetone/ethanol/water=1/1/1/1, R_f-value=0.41) and high pressure liquid chromatography [column: Zipax SAX (1 m x 2.1 mm I.D., Du Pont Instruments Inc., Wilmington, Del.); column temperature: 40°; pressure: 100

kg/cm^{-1}; mobile phase: 0.01 M citric acid-0.03 M sodium nitrate aqueous solution (pH 4.0); flow rate: 0.8 ml/min; retention time: 20.1 min]; IR νmax (nujol): 1650 and 1770 cm^{-1} (C=O). The product was identical in every respect with the unlabelled authentic sample.

ACKNOWLEDGEMENT

The authors wish to thank Dr. H. Tobiki and Mr. N. Tanno for helpful discussions and giving authentic samples.

REFERENCES

1. Tobiki H., Yamada H., Nakatsuka I., Shimago K., Eda Y., Noguchi H., Komatsu T. and Nakagome T. - Yakugaku Zasshi (Tokyo) 100: 38 (1980)
2. Tobiki H., Yamada H., Miyazaki T., Tanno N., Suzuki H., Shimago K., Okamura K. and Ueda S. - Yakugaku Zasshi (Tokyo) 100: 49 (1980)
3. Noguchi H., Eda Y., Tobiki H., Nakagome T. and Komatsu T. - Antimicrob. Agents Chemother. 9: 262 (1976)
4. Noguchi H., Kubo M., Kurashige S. and Mitsuhashi S. - Antimicrob. Agents Chemother. 13: 745 (1978)
5. Yoshitake A., Makari Y. and Endo M. - J. Labelled Compd. 10: 589 (1974)
6. Adams J.T., Bradsher C.K., Breslow D.S., Amore S.T. and Hauser C.R. - J. Amer. Chem. Soc. 68: 1317 (1946)