SYNTHESIS OF POTASSIUM [^{14}C]DICLOXACILLIN

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SUMMARY

A synthesis of potassium [^{14}C]dicloxacillin, labelled in the 3-position of the isoxazole ring, is described. The compound, having the specific activity 21 mCi/mmole, was obtained in 30% overall yield from ethyl [3-^{14}C]acetoacetate.

Key Words: Potassium [^{14}C]Dicloxacillin, [3-^{14}C]Isoxazole

INTRODUCTION

Certain studies in our laboratories on the microbial agent dicloxacillin [(5-(2,6-dichlorophenyl)-3-methyl-4-isoxazolylpenicillin)] required the [^{14}C]labelled compound. A [^{14}C]synthesis of this penicillin has not been published, which prompted us to report our preparation of the labelled compound.

DISCUSSION

Dicloxacillin was first prepared (1) as one of a series of substituted phenylisoxazolylpenicillins by condensation of (2,6-dichlorophenyl)-3-methyl-4-isoxazolylcarboxylic acid chloride with 6-aminopenicillanic acid (2). As shown below, the same synthetic scheme was used in the preparation of the labelled compound. The label was incorporated into the isoxazole nucleus by condensation of the sodium salt of ethyl [3-^{14}C]acetoacetate with the hydroxyamoyl chloride 1b to give the isoxazole-4-carboxylic acid ester 2a; hydrolysis of 2a in aqueous ethanolic potassium hydroxide produced the corresponding carboxylic acid 2b. The isoxazole-4-carboxylic acid 2b was converted to the acid chloride 2c which, in turn, was condensed with 6-aminopenicillanic acid at pH 7.2 to give dicloxacillin. The potassium salt 4 was formed by exchange with potassium 2-ethylhexonate.

Dicloxacillin was found to be stable only as the potassium or sodium salt: in the form of the free acid, the compound readily decomposes, as indicated

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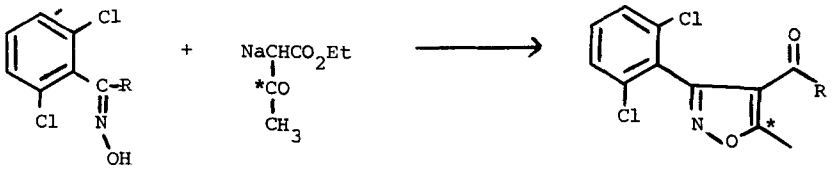
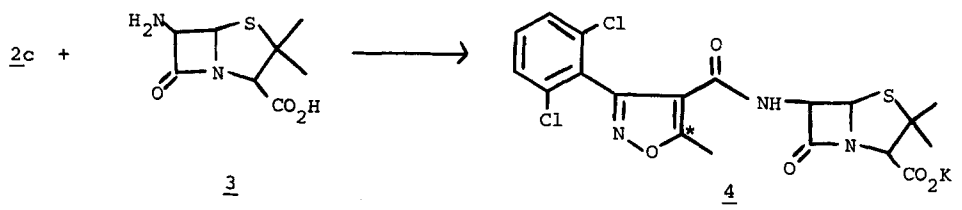


1 a R = H
b R = Cl

2 a R = OCH_2CH_3
b R = OH
c R = Cl



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* Position of the [^{14}C] label

by the disappearance of the β-lactam carbonyl absorption at 1770 cm^{-1} in the infrared spectrum. Therefore, the potassium salt was prepared as soon as possible after working up the condensation reaction. Further purification of the final product by recrystallization, preparative thin-layer chromatography (TLC) on silica gel or column chromatography was unsuccessful since all of these methods caused further decomposition.

A radiochemical purity of 97% of potassium [^{14}C]dicloxacillin was achieved by carrying out the last sequence of reactions starting with pure, i.e. chromatographed and recrystallized isoxazole-4-carboxylic acid 2b, and carrying out the final work-up in the cold, the potassium salt being formed as quickly as possible. It was found that, in methanol, even the potassium salt decomposed rapidly. However, the compound was stable in aqueous solution and was thus stored in sealed vials.

[^{14}C]Dicloxacillin (specific activities, 21 mCi/mmole and 4.3 mCi/mmole) was obtained in 30% overall yield from ethyl [3-^{14}C]acetoacetate, with radiochemical purity of 97 and 98% respectively as determined by TLC-autoradiography in three different solvent systems.

EXPERIMENTAL

Pilot experiments were carried out with unlabelled compounds, and suitable i.r. and n.m.r. spectra were obtained for the intermediates. The reactions with labelled compounds were monitored by TLC using the previously prepared intermediates as reference. Radioactivity measurements were carried out on a Packard Tri-Carb 3375 liquid scintillation spectrometer.

Ethyl [3-^{14}C]acetoacetate, 5 mCi, specific activity 24.7 mCi/mmole, was purchased from New England Nuclear, Boston.

2,5-Dichlorobenzohydroxamoyl chloride, 1b.

2,6-Dichlorobenzaldoxime 1a (2.0 g, 10.5 mmole) was dissolved in 800 ml chloroform and cooled to -5°. Chlorine gas was bubbled through the solution for 15 min and the solution allowed to stand at -5° for 15 hr. The solvent was evaporated and the residue chromatographed on silica gel (100 g, benzene elution). The purified hydroxamoyl chloride 1b (1.9 g, 80% yield) was kept in the dark at -20°; under these conditions the compound was stable for 2-3 weeks. I.r. ($CHCl_3$): 3540, 3300, 3380 cm^{-1}; n.m.r. ($CDCl_3$): δ 7.2 (3H, m, aromatic), 10.3 (1H, s, OH).

5-(2,6-Dichlorophenyl)-3-methyl-[3-^{14}C]-4-isoxazolylcarboxylic acid, 2b.

Ethyl [3-^{14}C]acetoacetate (29.8 mg, 0.229 mmole), dissolved in 1.0 ml anhydrous N,N-dimethylformamide (DMF), was transfered to a stirred slurry of sodium hydride (11.6 mg of a 57% oil dispersion, 0.275 mmole) in 1 ml DMF. This reagent was then carefully added, at 0°, to a solution of the hydroxamoyl chloride 1b (103 mg, 0.458 mmole) in 3 ml DMF, stirred for 1.5 hr at 0° and for 16 hr at room temperature. The solution was diluted with water and extracted with ether. Ether extracts were washed with water, 5% aq. sodium hydroxide, water, saturated saline solution, dried over anhydrous magnesium sulfate ($MgSO_4$) and concentrated to give a mixture (113 mg) of the isoxazolyl ethyl ester 2a, and excess hydroxamoyl chloride which could not be separated by chromatography. The ester was therefore directly hydrolyzed to the acid as follows: the mixture was dissolved in 3 ml ethanol; 1.38 g of potassium hydroxide in 3 ml of water was then added and the mixture was refluxed for 1 hr. After cooling, the solution was diluted with water and extracted with ether to remove all neutral material. The ether extract was washed with water and the aqueous layers were combined, acidified with 10% hydro-

chloric acid and again extracted with ether. The ether extract was washed with water, saturated saline solution, dried ($MgSO_4$) and concentrated to give a semi-crystalline residue (45 mg). This was further purified as follows: the residue was methylated with diazomethane then chromatographed on 7 g silica gel (benzene elution). The fractions containing the pure methyl ester were combined, concentrated and hydrolyzed again with potassium hydroxide in aqueous ethanol, as described above. The crude acid 2b (36.9 mg), obtained as white crystalline solid, was recrystallized twice from benzene-ethyl acetate to give the pure carboxylic acid 2b (18.6 mg) in 30% yield, homogeneous by TLC (benzene-acetone 8:2); i.r. ($CHCl_3$): 3000, 1700, 1610, 1565 cm^{-1}. The mother liquors containing 15.1 mg of less pure acid were concentrated, diluted with 20 mg of pure unlabelled carboxylic acid 2b and crystallized twice from hexane-ethyl acetate to give 19.5 mg of the pure 2b (with lower specific activity). The two batches were used separately in the subsequent reactions.

5-(2,6-Dichlorophenyl)-3-methyl-[3-^{14}C]-4-isoxazolylcarboxylic acid chloride, 2c.

The carobxylic acid 2b (18.6 mg, 0.0684 mmole) was refluxed in 3 ml thionyl chloride for 1 hr. The excess thionyl chloride was evaporated to give the acid chloride as a white solid: i.r. ($CHCl_3$), 1760 cm^{-1}.

Potassium 5-(2,6-dichlorphenyl)-3-methyl-[3-^{14}C]-4-isoxazolylpenicillin (potassium dicloxacillin), 4.

6-Aminopenicillanic acid 3 (20 mg), dissolved in 10 ml water at pH 7.2 (adjusted with 0.2 N NaOH), was added at 0° to the acid chloride 2c dissolved in 3 ml acetone. After stirring for 1 hr, the cold solution was extracted with ice-cold ether. Precaution was taken to keep all fractions at 0°. The combined ether extracts were washed with ice-water, saturated saline solution, dried ($MgSO_4$) and concentrated. The residue (39.7 mg) was dissolved in 1.5 ml anhydrous ether in a Craig tube and treated with a slight molar excess of potassium 2-ethylhexanoate. The potassium salt 4 precipitated immediately. The tube was centrifuged, the ether was removed with a pipet and the solid washed twice with anhydrous ether. The solvent was drawn off and the residue (30.5 mg, 88% yield) dried in vacuo at room temperature (specific activity 21 mCi/mole).

The second batch of 2b (19.5 mg, 0.0118 mmole), with the lower specific activity, was reacted with 6-aminopenicillanic acid (20 mg) as described above to give 36.8 mg (85% yield) of compound 4 (specific activity 4.3 mCi/mmole).

The purity of the final product was determined on 0.25 mm F-254 silica-gel plates (E.M. Laboratories, Elmsford, N.Y.) in three different solvent systems: (A) acetone-benzene-acetic acid, 50:45:5; (B) chloroform-acetic acid, 9:1, and (C) benzene-isopropanol-acetic acid, 70:25:5. The radioactive zones on the plates, located by autoradiography were scraped and counted in Aquasol (3). Although two-dimensional chromatography indicated that dicloxacillin decomposes on silica gel, TLC was satisfactory to determine the purity of the compound, provided that it was spotted from an aqueous solution and developed immediately. The 0.25 mm Cellulose F plates (E.M. Laboratories) were unsatisfactory because of extensive streaking.

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REFERENCES

1. Hanson J.C., Long A.A.W., Nayler J.H.C. and Stove E.R. - J. Chem. Soc., 5976, (1965)
2. Doyle F.P., Hanson J.C., Long A.A.W., Nayler J.H.C. and Stove E.R. - J. Chem. Soc., 5838, (1963)
3. Shaw W.A., Harton W.R. and Bennett A. - Anal. Biochem. 43: 119 (1971)