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SYNTHESIS OF D-[1-^{14}C]PENICILLAMINE HYDROCHLORIDE

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SUMMARY

Methods are described for the synthesis of DL-[1-^{14}C]penicillamine and D-[1-^{14}C]penicillamine hydrochloride from K^{14}CN. The method consists of additon of H^{14}CN to 2,2,5,5-tetramethyl-3-thiazoline followed by hydrolysis of the resulting thiazolidine to DL-penacillamine. Resolution was achieved through the salt of N-formylisopropylidene-DL-penicillamine with (-)-norephedrine. The specific activity of D-penicillamine was 21.7 mCi/mmole and the overall radiochemical yield from K^{14}CN was 3.3%.

Key Words: DL-[1-^{14}C]Penicillamine, D-[1-^{14}C]Penicillamine hydrochloride, Optical Resolution

INTRODUCTION

Penicillamine is the most characteristic degradation product of penicillin type antibiotics (1,2). D-Penicillamine, which itself possesses no antibiotic activity, was first used to treat Wilson's disease by acting as a chelating agent to remove heavy metal in physiological fluid (3,4). It is also an important medicine for the treatment of defect schizophrenia, schleroderma, cystinuria, agressive hepatitis (5), multiple sclerosis (6) and polyarthritis (7). It is only D-penicillamine which has therapeutic importance because the L-isomer is toxic (8). For investigations related to various aspects of D-penicillamine metabolism, D-penicillamine labeled with carbon-14 was required.

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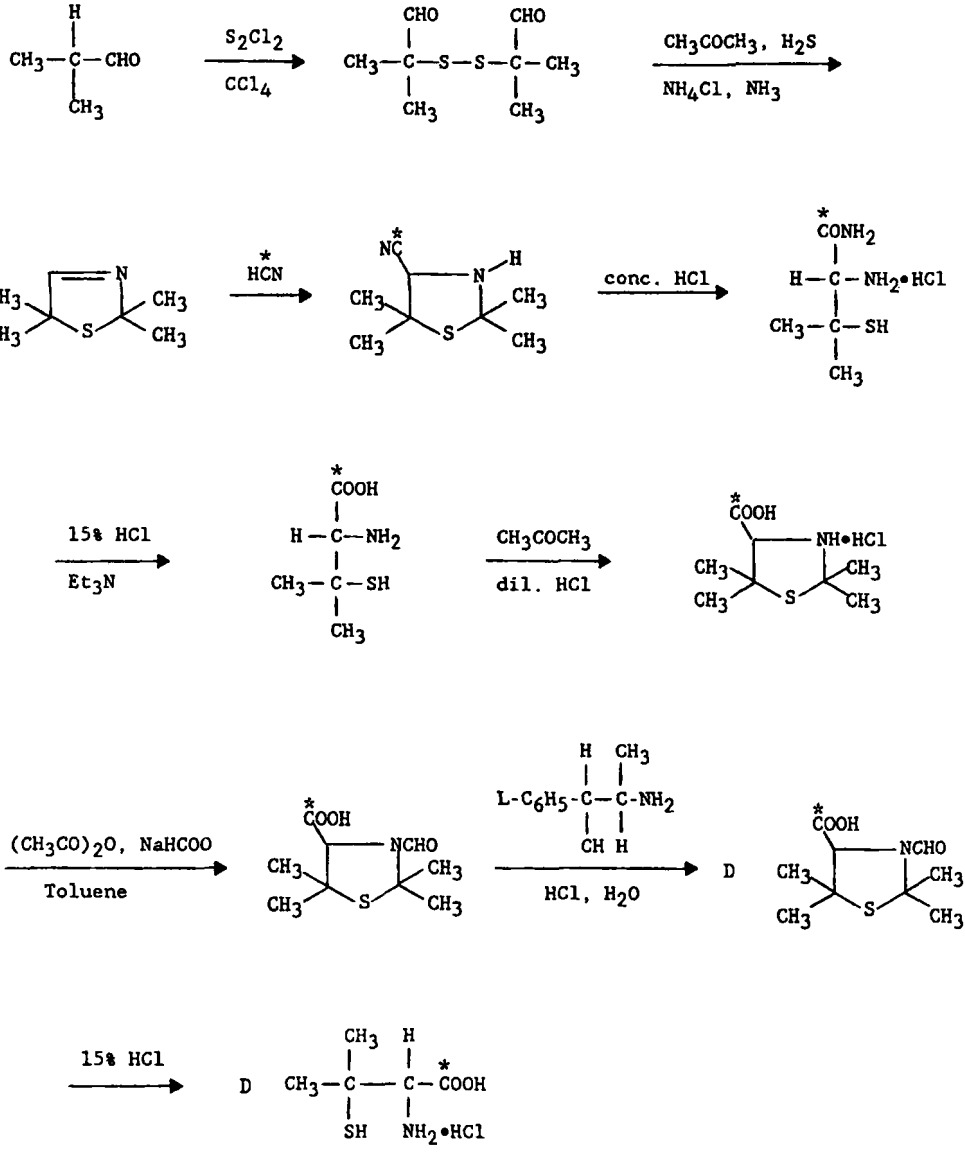
RESULTS AND DISCUSSION

Synthesis of unlabelled DL-penicillamine and D-penicillamine via the racemate has been described extensively in the literature (9). Of the various known methods, one involving addition of HCN across the double bond of 2,2,5,5-tetramethyl-3-thiazoline to provide the isopropylidene protected nitrile analog of penicillamine is the most direct and practical for labeling with carbon-14. Use of readily available hydrogen [^{14}C]cyanide leads to penicillamine labeled in the carbonyl carbon.

The synthesis of D-[1-^{14}C]penicillamine hydrochloride was accomplished starting with isobutyraldehyde, as shown in the Scheme 1. Thus, isobutyraldehyde in carbon tetrachloride was reacted with sulfur monochloride using a sweep of argon for mixing and to remove the hydrogen chloride to give 2,2'-dithiodiisobutyraldehyde (10). Simultaneous treatment of a mixture of 2,2'-dithiodiisobutyraldehyde and acetone with hydrogen sulfide gas and ammonia gas in the presence of triethylamine and ammonium chloride gave 2,2,5,5-tetramethyl-3-thiazoline (11). The published procedure for the latter step (kilogram scale) was modified to work smoothly on a multi-gram scale. The product was purified by column chromatography. Hydrogen [^{14}C]cyanide (prepared by dropping 85% phosphoric acid onto solid K^{14}CN) added smoothly across the azomethine double bond to give 2,2,5,5tetramethylthiazolidine-4-[^{14}C]carbonitrile (12) which was hydrolyzed to DL-[1-^{14}C]penicillamine hydrochloride without isolating the intermediate amide (12). The racemic free base was precipitated in high purity by adjusting a solution of the hydrochloride salt in ethanol to pH 6.5. Resolution of the racemic acid was carried out according to the patented procedure (13, 14). The reaction sequence entails protecting the reactive sulfhydryl group as the thiazolidine derivative and then the resulting ring amino group as the formamide. The latter step employs acetic-formic anhydride formed in situ from sodium formate and acetic anhydride. In this reaction the best yield was obtained with stoichiometrically equivalent amounts of formate and the amine. It is important to avoid a large excess of formate which gives a different product altogether. The resulting protected DL-penicillamine readily formed the diastereomeric salt between N-formylisopropylidene-D-penicillamine and (-)-norephedrine which precip-

Scheme I



itated from ethyl acetate in good yield (14). Surprisingly, we were unable to obtain any solid salt using (-)-pseudonorephedrine even though the literature indicates that this resolving agent is superior (15). The salt was decomposed with dilute HCl at room temperature and pure D-pennacillamine hydrochloride was obtained from the resulting free acid by heating in 15% HCl at 90 °C.

EXPERIMENTAL

Potassium [^{14}C]cyanide was purchased from Amersham International (London) and (-)-norepedrine was obtained from Aldrich Chemical Company. Melting points were determined on a Thomas Hoover capillary apparatus and are uncorrected. Thin-layer chromatography was carried out on silica gel 60A plates (Whatman LK6F) and radiochemical purity was determined on Bioscan Inc. Bid System 100. Radioactivity was measured with Beckman LS3801 liquid scintillation counter.

2,2'-Dithiodiisobutyraldehyde

Sulfur monochloride (2.7 g, 20 mmol) was added slowly to a solution of isobutyraldehyde (2.88 g, 3.64 mmol) in 4 mL of carbon tetrachloride at 40-50 °C. The resulting mixture was stirred at 40 °C for 3.5 h and then at 35 °C for an additional 23 h. A current of argon was passed through the mixture throughout the reaction period in order to remove the hydrogen chloride. The solution was then distilled under vacuum to give 3.48 g (84%) of the dialdehyde as a pale yellow oil, estimated to be about 95% pure by TLC.

2,2,5,5-Tetramethyl-3-thiazoline

Hydrogen sulfide was passed slowly into a stirred mixture of 2,2'-dithiodiisobutyraldehyde (3.47 g, 16.85 mmol), triethylamine (6.82 g, 67.4 mmol) and acetone (5.87 g, 101 mmol) at 0 °C. Then ammonium chloride (1.81 g, 33.7 mmol) and acetone (5.87 g, 101.1 mmol) was added and cooling was discontinued. Hydrogen sulfide and ammonia were simultaneously passed through the reaction mixture at the rate about 30 mL/min each. When the reaction was comlete the reaction mxture was diluted with 10 mL of water, and the product was extracted with ether. Drying ($MgSO_4$) and removal of solvent afforded 12 mL of crude product. A 5-mL portion was purified by column chromatography (silica gel, CH_2Cl_2, 30:1 CH_2Cl_2/acetone) to give 1.1 g (54%) of the title compound as a white solid, m.p. 50-53° [lit. (11) 49-53°].

2,2,5,5-Tetramethylthiazolidine-4-[^{14}C]carbonitrile

Hydrogen [^{14}C]cyanide (produced by adding 4 mL of 85% phosphoric acid to $K^{14}CN$, 204 mg, 80 mCi, 3.14 mmol) was added by vaccuum transfer to a solution of 2,2,5,5-tetramethyl-3-thiazoline (431 mg, 2.99 mmol) in 2 mL of methanol. The resulting mixture was stirred at room temperature for 3 h and then solvent was

evaporated to provide the crude nitrile (approxiamtely 70 mCi, 88%) which was used immediately in next step.

DL-[1-^{14}C]Penicillamine

The crude nitrile from above was mixed with 2 mL of conc. HCl and heated in a 50 °C bath for 6 h. Then another 2 mL of water was added the reaction was continued for another 15 h at 105 °C. Solvent was evaporated *in vacuo* and the residue was taken up in 2.5 mL of 95% ethanol and filtered. The filtrate was adjusted to pH 6 (pH paper) with triethylamine to give the product free base which was collected by filtration. Yield: 170 mg (25 mCi, 36%). Purity was estimated at ≥ 98% (TLC).

2,2,5,5-Tetramethylthiazolidine-4-[^{14}C]carboxylic acid

A mixture of DL-penicillamine (carboxyl-^{14}C] (136 mg, 20 mCi, 0.92 mmol), concentrated hydrochloric acid (0.175 mL, 2.1 mmol) and 4 mL of acetone was stirred at 80-90 °C for 1 h. Solvent was evaporated *in vacuo*, providing 181 mg of off-white solid (92%). This material (about 85% pure by TLC) was used in the next step without further purification.

3-Formyl-2,2,5,5-tetramethylthiazolidine-4-[^{14}C]carboxylic acid

To the suspension of 2,2,5,5-tetramethylthiazolidine-4-[^{14}C]carboxylic acid hydrochloride (191 mg, 18.5 mCi, 0.85 mmol) in 2 mL of toluene was added sodium formate (62 mg, 0.94 mmol) and acetic anhydride (191 mg, 1.87 mmol). The resulting mixture was stirred at 45-50 °C for 67 h then 1 mL of water was addeded and stirring was continued at room temperature for another hour. The product was extracted into chloroform and washed with water. Removal of solvent afforded 163 mg of light brown solid. Recrystallization from benzene/hexane yielded 112 mg (112 mCi, 61%) of off-white solid, estimated to be 95% pure by TLC.

N-Formyl-isopropylidene-D-[1-^{14}C]penicillamine

A mixture of 3-formyl-2,2,5,5-tetramethylthiazolidine-4-[^{14}C]carboxylic acid (112 mg, 11.2 mCi, 0.52 mmol), (-)-norephidrine (80.6 mg, 0.54 mmol) and 0.9 mL of ethyl acetate was stirred at 90 °C for 0.5 h. The suspension was cooled to room temperature and the resulting salt was collected by filtration and washed with small amount of ethyl acetate. Yield: 73.1 mg white solid (4.1 mCi, 37%), m.p. 184-186 °C [lit. (15) 200-204 °C, $(\alpha)_D^{20}$ + 33°]. This salt was treated with

water (0.267 mL) and conc. HCl (0.284 mL, 3.4 mmol) for 1 h at room temperature. The suspended product was collected and washed with a small amount of water. Yield: 29.3 mg (25%) of N-formyl-isopropylidene-D-[1-^{14}C]penicillamine as a white solid (25%), m.p. 180-181°C [lit. (15) 183-184 °C, $[\alpha]_D$ = + 53°].

D-[1-^{14}C]Penicillamine hydrochloride

A mixture of N-Formyl-isopropylidene-D-[1-^{14}C]penicillamine (29.3 mg, 2.9 mCi, 0.134 mmol) and 15% HCl (0.5 mL) was stirred at 90 °C for 4 h. Upon removal of solvent and drying in vacuo 22.9 mg (2.69 mCi, 93%) of the title compound having a radiochemical purity ≥ 98% by TLC (16).

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16. After trying many TLC systems on this compound, the following two systems are best, although some decomposition still occurrs during development. A. silica gel, butanol:acetic acid:water (25:4:10), R_f = 0.45; B. silica gel, chloroform: methanol:acetic acid (5:1:1), R_f = 0.25.