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PREPARATION OF [THIENYL-3-^{14}C]TEMOCILLIN

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SUMMARY

[thienyl-3-^{14}C]Temocillin has been prepared from [1-^{14}C]chloroacetic acid via 3-[3-^{14}C]thienylmalonic acid.

Key words: [thienyl-3-^{14}C]temocillin, [1-^{14}C]chloroacetic acid, 3-[3-^{14}C]thienylmalonic acid, chemical synthesis.

INTRODUCTION

In order to undertake metabolic studies on the novel penicillin antibiotic, temocillin (1), there was a requirement for a radiolabelled form of this compound, bearing an unambiguous carbon-14 label in the side-chain. Furthermore, we wished to incorporate the carbon-14 label into the thiophene ring of the side-chain because of the

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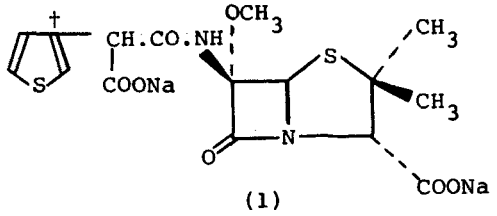
anticipated metabolic stability of this moiety. We therefore prepared 3-[3-^{14}C]thienylmalonic acid (2) as the key precursor for the synthesis of disodium 6β-(2-carboxyl-2-[3'-^{14}C]thien-3'-ylacetamido)-6α-methoxy-penicillanate, [thienyl-3-^{14}C]temocillin (1).



(1)

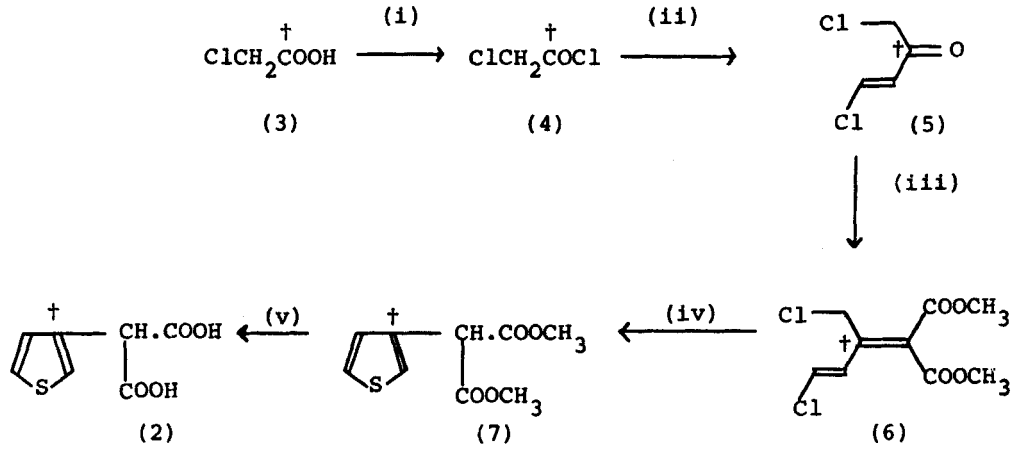
† denotes ^{14}C label

DISCUSSION

The overall strategy was to develop a synthetic route to 3-[3-^{14}C]thienylmalonic acid (2), which could then be used for the preparation of [thienyl-3-^{14}C]temocillin (1). 3-[3-^{14}C]-Thienylmalonic acid (2) was prepared from chloro[1-^{14}C]acetic acid (3), by the route shown in Scheme 1, in an overall 13% radiochemical yield. The synthesis followed a published route[2], and involved Lewis acid catalysed addition of chloro[1-^{14}C]acetyl chloride (4) to acetylene[3], Knoevenagel condensation of the resulting ketone (5) with dimethyl malonate by the procedure of Lehnert[4], cyclisation using sodium sulphide to form the thiophene ring and finally, base catalysed hydrolysis to give 3-[3-^{14}C]thienylmalonic acid (2) of greater than 97% radiochemical purity.

For the synthesis of temocillin (1), we envisaged coupling a mono-protected 3-thienylmalonic acid derivative, via the acid chloride, with a 6β-amino-6α-methoxypenicillanate ester. The latter was obtained from 6β-aminopenicillanic acid by a

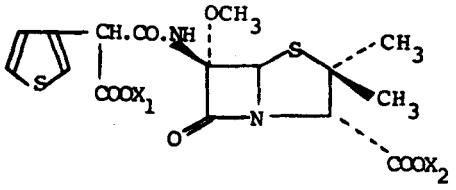
SCHEME 1



† denotes ^{14}C label

Reagents : (i) $SOCl_2$; (ii) $HC{\equiv}CH/AlCl_3$; (iii) a. $CH_2(COOCH_3)_2$; b. $TiCl_4$/THF; c. pyridine; (iv) $Na_2S.9H_2O$/THF; (v) dil. NaOH.

published procedure[5]. Accordingly, the di-protected temocillin derivatives (8a, b) were prepared. Difficulties were



(8)

(8a) : $X_1 = X_2 = C_6H_5.CH_2$ -

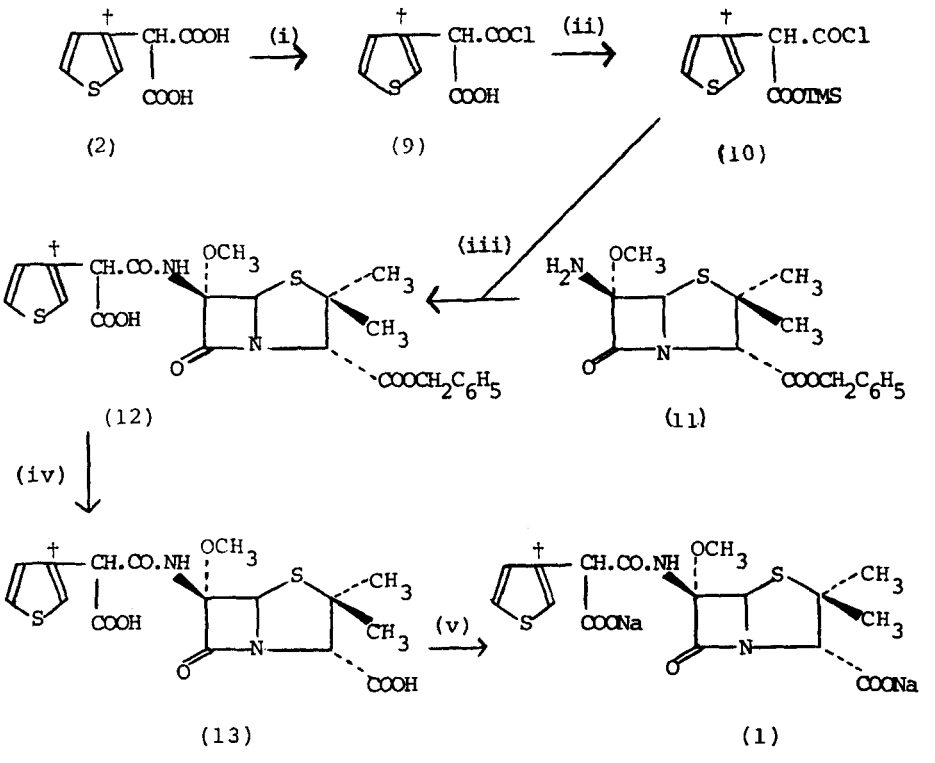
(8b) : $X_1 = X_2 =$ p-$O_2N.C_6H_4.CH_2$ -

(8c) : $X_1 = C_6H_5$ -; $X_2 =$ p-$O_2N.C_6H_4.CH_2$ -

experienced, however, in deprotecting these intermediates by hydrogenolysis, especially the cleavage of the side-chain protecting group (X_1). Some success was achieved by using a

phenyl protecting group (removed by di-sodium tetraborate) for the side-chain and a p-nitrobenzyl protecting group (removed by hydrogenolysis) for the ring carboxyl group (8c), but the overall yields were not satisfactory. The route finally adopted

SCHEME 2



† denotes ^{14}C label

[thienyl-3-^{14}C]temocillin

Reagents :

(i) $SOCl_2$;

(ii) chlorotrimethylsilane (TMSCl)/hexamethyldisilazane;

(iii) pyridine/THF;

(iv) Subtilisin A/pH 7.5 phosphate buffer;

(v) $NaHCO_3$.

for the preparation of [thienyl-3-^{14}C]temocillin (1) is shown in Scheme 2. 3-[3-^{14}C]Thienylmalonic acid (2) was converted to the monoacid chloride (9) and the remaining free carboxyl group was protected as the trimethylsilyl ester (10).

This ester (10) was coupled with benzyl 6β-amino-6α-methoxy-penicillanate (11)[5], and the trimethylsilyl group removed during aqueous work-up to give the monoprotected benzyl [thienyl-3-^{14}C]-temocillin monoacid (12). Removal of the benzyl protecting group was effected by enzymatic hydrolysis using Subtilisin A. Subtilisin A had been found to be the most effective of a large number of enzymes screened for this reaction[6]. Purification of the [thienyl-3-^{14}C]temocillin diacid (13) was achieved by selective solvent extraction. The diacid (13) did not significantly partition into chloroform from aqueous solution at pH 2.0, whereas diethyl ether extracted the diacid (13) but not the corresponding penicilloic acids. The overall chemical and radiochemical yields of [thienyl-3-^{14}C]temocillin (1) from 3-[3-^{14}C]thienylmalonic acid (2) were 13%[7] and the radiochemical purity was 89%.

EXPERIMENTAL

General

Chloro[1-^{14}C]acetic acid (3) was supplied by Physics and Radioisotope Services, Petrochemicals Division, Imperial Chemical Industries PLC, P.O. Box 1, Billingham, Cleveland TS23 1LB, England. Subtilisin A was obtained from Novo Enzyme Products Ltd., Windsor, Berkshire, England.

Analytical hplc was carried out using Waters μ-BONDAPAK C_{18} (10 μm)stationary phase (stainless steel column: 3.9 mm i.d. x 300 mm), Rheodyne 7125 Sample Injector, Waters M6000A Chromatography Pump (flow rate: 2 $ml.min^{-1}$) and a Cecil

Instruments Variable Wavelength UV Monitor Model CE 2012. The chemical purity was determined by comparison of the UV peak height of the product with respect to reference material using an internal standard. The radiochemical purity was determined by fractionation of the eluant from analytical hplc. The radioactivity in each fraction of eluant was determined by liquid scintillation counting. The radiochemical purity was calculated by comparing the radioactivity collected in the eluant fraction corresponding to the required product to the total radioactivity collected from the complete chromatographic run. Radioactivity was measured using a Packard Tri-Carb 2660 Liquid Scintillation System and Packard Scintillator ES 299, or a Packard Tri-Carb 460 Scintillation Counter and Omnifluor solution (8 $g.l^{-1}$ Omnifluor, 70% toluene, 30% 2-ethoxyethanol).

Thin layer chromatography (tlc) was carried out on precoated Merck silica gel 60 F_{254} plates. Column chromatography was carried out on Merck silica gel H, type 60.

All intermediates and products were characterised during preliminary non-isotopically labelled syntheses using standard spectroscopic and chromatographic methods.

The diisopropyl ether used had been freed from isopropyl alcohol by washing four times with an equal volume of water and drying over calcium chloride.

The evaporation of solvent from organic solutions was carried out on a rotary evaporator under reduced pressure.

1. Preparation of 3-[3-^{14}C]thienylmalonic acid (2)

1,4-Dichloro-3-[2-^{14}C]buten-2-one(5)

Chloro[1-^{14}C]acetic acid (3) (100 mCi, 58 $mCi.mmol^{-1}$, 166 mg, 1.72 mmol), supplied as a solution in diethyl ether, was diluted with unlabelled chloroacetic acid (1.34 g, 14.2 mmol). The diethyl ether was removed by evaporation at low temperature,

thionyl chloride (1.15 ml, 15.8 mmol) was added and the mixture was heated under reflux with stirring for 1 h. The reaction mixture was cooled, dissolved in dry dichloromethane, and added in portions over a period of 20 min to a mechanically stirred mixture of anhydrous aluminium chloride (2.26 g, 16.9 mmol) in dry dichloromethane (15 ml) through which a rapid stream of acetylene was passing. The reaction was allowed to proceed at room temperature for 4.5 h, with occasional additions of dry dichloromethane to keep the mixture at a constant volume. At this time, tlc (ethyl acetate:petroleum ether 60-80°C, 1:9 v/v) showed the reaction to be almost complete. The flow of acetylene was stopped and the reaction mixture diluted with dichloromethane (25 ml), cooled to 0°C and stirred, whilst water (25 ml) was added over a period of 5 min. The mixture was diluted with chloroform (50 ml) and water (50 ml), shaken and separated. The aqueous phase was further extracted with chloroform. The combined organic extracts were washed with water, sodium bicarbonate solution and brine prior to drying over magnesium sulphate. The magnesium sulphate was separated by filtration and the filtrate evaporated to give the crude 1,4-dichloro-3-[2-^{14}C]buten-2-one (5) (1.94 g, 14.0 mmol, 88%).

Methyl 2-methoxycarbonyl-5-chloro-3-chloromethyl-2,4-[3-^{14}C]-pentadienoate (6)

Titanium tetrachloride (3.5 ml) in dry carbon tetrachloride (8.0 ml) was added dropwise to dry tetrahydrofuran (80 ml) and stirred vigorously at 0°C for 10 min. The crude 1,4-dichloro-3-[2-^{14}C]buten-2-one (5) (1.94 g, 14.0 mmol) and dimethyl malonate (1.58 ml, 1.83 g, 13.8 mmol) were combined in dry tetrahydrofuran (17.5 ml), added to the above mixture in one portion, and stirring was continued at 0°C for 10 min. A solution of pyridine (4.52 ml) in dry tetrahydrofuran (35 ml) was added dropwise to the reaction mixture at 0°C over a period

of 10 min, and stirring was continued for 5 h at room temperature. The stirred mixture was cooled to 0°C, water (75 ml) was added dropwise over 15 min and, after stirring for a further 15 min, the majority of the tetrahydrofuran was removed by evaporation at low temperature. The remaining aqueous phase was extracted with dichloromethane and the combined organic extracts were washed with water, saturated sodium bicarbonate solution and brine prior to drying over magnesium sulphate. The magnesium sulphate was separated by filtration and the filtrate evaporated to give the crude product (6) (3.34 g). The crude product (6) was purified by column chromatography (60 g silica gel, eluted with ethyl acetate: petroleum ether, 60-80°C, 1:4 v/v) to give the purified methyl 2-methoxycarbonyl-5-chloro-3-chloromethyl-2,4-[3-^{14}C]pentadienoate (6) (2.27 g, 8.97 mmol, 64%).

Dimethyl 3-[3-^{14}C]thienylmalonate (7)

The following procedure was carried out in duplicate. Methyl 2-methoxycarbonyl-5-chloro-3-chloromethyl-2,4-[3-^{14}C]-pentadienoate (6) (1.13 g, 4.48 mmol) was dissolved in dry tetrahydrofuran (45 ml). Powdered sodium sulphide nonahydrate (1.71 g, 7.12 mmol) was added, and the mixture was stirred at room temperature for 20 h. Water (56 ml) and diethyl ether (50 ml) were added and the mixture was shaken and separated. The aqueous phase was further extracted with diethyl ether (brine was added to aid separation). The combined organic extracts were washed with water and brine, dried over magnesium sulphate and filtered. The solvent was removed by evaporation and the product was partially purified by column chromatography (5 g silica gel, eluted with ethyl acetate:petroleum ether 60-80°C, 1:4 v/v). The yields for the two runs were 618 mg and 615 mg. The two products were combined and purified by column chromatography (60 g silica gel, eluted with ethyl acetate:

petroleum ether 60-80°C, 1:4 v/v) to give dimethyl 3-[3-^{14}C]-thienylmalonate (7) (806 mg, 3.77 mmol, 42%).

3-[3-^{14}C]Thienylmalonic acid (2)

Dimethyl 3-[3-^{14}C]thienylmalonate (7) (806 mg, 3.77 mmol) was suspended in sodium hydroxide solution (5 M, 3.1 ml) and stirred at room temperature for 1.5 h. The solution was diluted with water (5 ml) and washed with diethyl ether. Diethyl ether (10 ml) was added to the aqueous phase, and the stirred mixture was acidified at 0°C with 5 M hydrochloric acid to pH 1.2. The mixture was separated and the aqueous phase was re-extracted with diethyl ether. The combined organic phases were washed with water, dried over magnesium sulphate, filtered and the solvent was removed by evaporation at low temperature to give 3-[3-^{14}C]thienylmalonic acid (2) (534 mg, 2.87 mmol, 13.0 mCi, 76%, 13% overall radiochemical yield from chloro[1-^{14}C]acetic acid (3)). The radiochemical purity of the product was >97%, (hplc;eluant: 0.05 M acetate buffer, pH 4.0: acetonitrile/water (75/25 v/v) (55:45 v/v); detection: 254 nm UV).

2. Preparation of disodium 6β-(2-carboxy-2-[3'-^{14}C]thien-3'-ylacetamido)-6α-methoxypenicillanate, ([thienyl-3-^{14}C]temocillin (1)

Benzyl 6β-(2-carboxy-2-[3'-^{14}C]thien-3'-ylacetamido)-6α-methoxypenicillanate (12)

3-[3-^{14}C]Thienylmalonic acid (2) (624 mg, 3.36 mmol, 13.9 mCi) was suspended in diisopropyl ether (4.5 ml), and 1.12 ml of a solution of dimethylformamide (15 µl) in diisopropyl ether (10 ml) was added. This was followed by the addition of thionyl chloride (441 mg, 269 µl, 3.71 mmol). The stirred mixture was heated at 40-45°C for 75 min. The resultant solution was evaporated to dryness at room temperature and the final traces of volatile

material were removed under high vacuum. The residual oil was dissolved in diisopropyl ether (5.6 ml) and the solution was cooled to 0°C. A solution of trimethylsilylchloride in diisopropyl ether (1:1 v/v; 840 µl, containing 3.36 mmol trimethylsilylchloride) was added, followed by a solution of hexamethyldisilazane in diisopropyl ether (1:1 v/v, 706 µl, containing 1.68 mmol). The mixture was stirred for 1 hr at 0°C. Benzyl 6β-amino-6α-methoxypenicillanate (11)[5](1.27 g,3.79 mmol, prepared simultaneously) was dissolved in dry tetrahydrofuran (27 ml) and the solution was cooled to -20°C. Pyridine (1.12 ml, dried over potassium hydroxide pellets) was added, and the mixture from the acid chloride preparation was added dropwise to the stirred solution over a period of 5 min. The reaction mixture was stirred at -20°C for a further 30 min. Dilute hydrochloric acid (1 M, 22.5 ml) was added and the solution was shaken and separated. The organic layer was washed with dilute hydrochloric acid (1 M, 11 ml), ethyl acetate (22.5 ml) was added and the solution was washed with water (17 ml). The organic layer was dried over magnesium sulphate, filtered and the solvent removed by evaporation. The last traces of solvent were removed under high vacuum to give the crude product (12) (1.59 g) which was dissolved in ethyl acetate (17 ml) and washed with dilute hydrochloric acid (1 M, 3 x 17 ml). Ethyl acetate (22.5 ml) was added to the organic phase which was washed with water (1 x 17 ml) and extracted with sodium bicarbonate solution (40% saturated, 1 x 28 ml; 30% saturated, 1 x 22.5 ml; 5% saturated, 1 x 17 ml). The combined bicarbonate extracts were washed with diethyl ether (3 x 22.5 ml) and acidified to pH 2.0 with 2 M hydrochloric acid in the presence of dichloromethane (22.5 ml). The mixture was transferred to a separating funnel, washed in with a further portion of dichloromethane (10 ml), shaken and separated. The dichloromethane extract was washed with water (4 x 20 ml), dried over magnesium sulphate, filtered and the solvent was removed by evaporation. The

last traces of solvent were removed under high vacuum to give benzyl 6β-(2-carboxy-2-[3'-^{14}C]thien-3'-ylacetamido)-6α-methoxypenicillanate (12) (878 mg, 1.75 mmol, 52%).

Disodium 6β-(2-carboxy-2-[3'-^{14}C]thien-3'-ylacetamido)-6α-methoxypenicillanate, ([thienyl-3-^{14}C]temocillin) (1)

Benzyl 6β-(2-carboxy-2-[3'-^{14}C]thien-3'-ylacetamido)-6α-methoxypenicillanate (12) (878 mg, 1.75 mmol) was dissolved in a solution of phosphate buffer (0.1 M, pH 7.5, 58.5 ml) containing sodium bicarbonate (439 mg, 5.25 mmol), and Subtilisin A (33.6 Anson units/g, 132 mg) was added. The solution was shaken at 30°C for 17 h, diluted with water (57 ml), washed with chloroform (3 x 57 ml) and acidified with 1 M hydrochloric acid to pH 2.0 in the presence of chloroform (57 ml). The mixture was shaken and separated, and the aqueous layer was washed with chloroform (57 ml) and extracted with diethyl ether (1 x 114 ml, 2 x 57 ml). The combined diethyl ether extracts were dried over magnesium sulphate, filtered and the solvent was evaporated. The final traces of solvent were removed under high vacuum to give 6β-(2-carboxy-2-[3'-^{14}C]thien-3'-ylacetamido)-6α-methoxypenicillanic acid ([thienyl-3-^{14}C]temocillin diacid) (13) (213 mg, 0.51 mmol, 29%). The oil was dissolved in ethyl acetate (10 ml) and water (10 ml) was added. Sodium bicarbonate solution (0.1 M) was added dropwise to the vigorously stirred mixture until the pH of the aqueous layer was 6.0. The mixture was transferred to a separating funnel, shaken, separated and the aqueous layer was lyophilised. This material was transferred to a vial, ground to a powder and dried over phosphorus pentoxide under high vacuum to give disodium 6β-(2-carboxy-2-[3'-^{14}C]thien-3'-ylacetamido)-6α-methoxy-penicillanate, ([thienyl-3-^{14}C]temocillin) (1) (203 mg, 0.44 mmol, 25%, 1.82 mCi) as an off-white solid. The radiochemical purity of the product was 89.2% and the chemical purity was 79.1% (in terms

of pure free acid), as determined by analytical hplc (eluant: 0.1M phosphate buffer, pH 6.5: methanol, 9:1 v/v, detection: 254 nm UV, internal standard for chemical purity: 1-naphthoic acid). The overall radiochemical yield was 13%[7] from 3-[3-^{14}C]thienylmalonic acid (2).

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7. 3-[3-^{14}C]Thienylmalonic acid (2) (1.26 mCi, 9%) was recovered from the reaction mixture after the first step of the synthesis.