THE PREPARATION OF 2-(4-CHLOROPHENYL)-[2-^{14}C]THIAZOL-4-YLACETIC ACID [I.C.I. 54,450]

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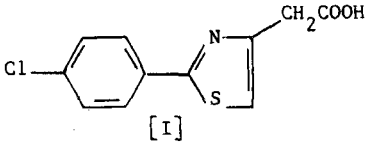
SUMMARY

The preparation of 2-(4-chlorophenyl)-[2-^{14}C]thiazol-4-ylacetic acid [I.C.I. 54,450] from potassium [^{14}C] cyanide in six stages is described. The overall radioachemical yield of product, at a specific activity of 12.56 μCi/mg, was 22.2%.

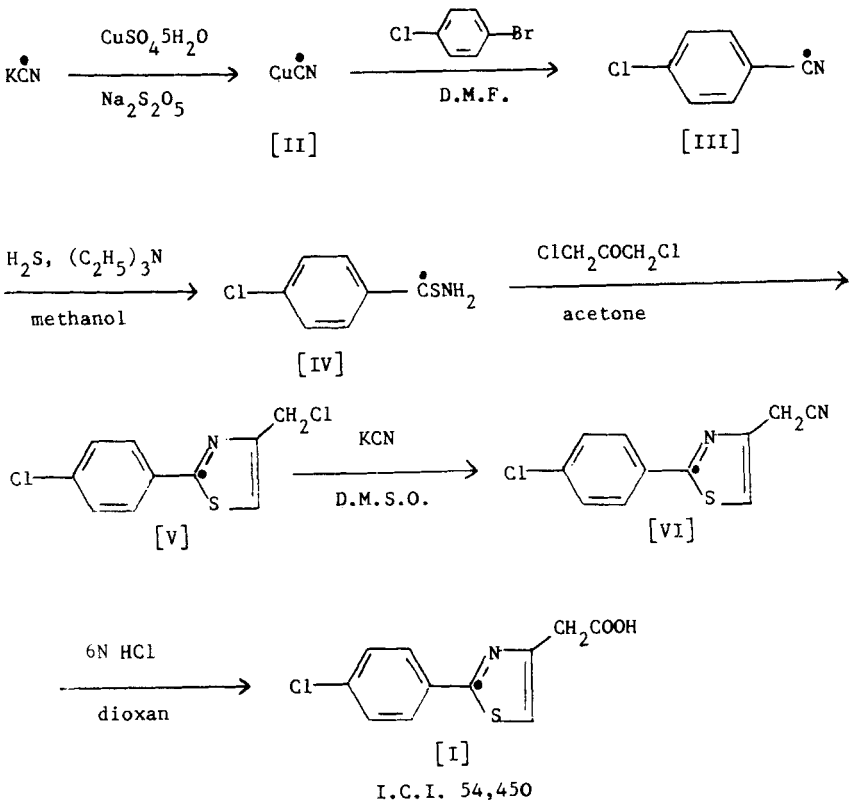
INTRODUCTION

In the course of investigations into the action of various compounds as possible drugs for the treatment of rheumatoid arthritis, it was necessary to prepare a [^{14}C]-labelled form of 2-(4-chlorophenyl)-thiazol-4-ylacetic acid [I] [I.C.I. 54,450] [1] for a study of its metabolism in various species. [2] The evaluation of I.C.I. 54,450 in man, [3] and its pharmacology [4], [5] in various animal species have also been reported.



[I] I.C.I. 54,450

The [^{14}C] material was prepared by the route indicated in the scheme below:-



I.C.I. 54,450

SCHEME

MATERIALS

Sulphur free toluene (May and Baker Ltd.) was used without further purification. The 2,5-diphenyloxazole (PPO) and 1,4-bis(4-methyl-5-phenyl oxazole) benzene (DMPOPOP) were purchased from Packard Instruments Ltd., Wembley; naphthalene (scintillation grade) was obtained from Thorn Electronics Ltd., Tolworth. The potassium [^{14}C] cyanide was purchased from the Radiochemical Centre, Amersham. Colloidal silica (Aerosil) was obtained from Buch, Beach, Segner Bayley Ltd. Dioxan was purified by the method of Vogel[6].

All samples were counted on a Packard Tri-Carb Liquid Scintillation Spectrometer Model 314; the sample containers were standard 20 ml glass screw-cap vials of low potassium content (Packard Instruments Ltd., Wembley). The photographic film used for autoradiography was 'Ilfex' X-ray film (Ilford Ltd., Essex, England). Merck Silica G.F. and Merck Silica H.R. were obtained from Andermanns Ltd. All solvents used were either redistilled or analytical reagent quality.

The solvent systems used for chromatography were as follows:-

(A)	light petroleum (b.p. 40 - 60) - ethyl acetate - chloroform	(90 : 5 : 5)
(B)	light petroleum (b.p. 40 - 60) - acetone	(80 : 20)
(C)	light petroleum (b.p. 40 - 60) - acetone	(95 : 5)
(D)	light petroleum (b.p. 40 - 60) - acetone	(90 : 10)
(E)	ethanol - ammonia (d 0.880) - water	(80 : 4 : 5)
(F)	n-butanol - acetic acid - water	(40 : 10 : 10).

EXPERIMENTAL

Cuprous [^{14}C] cyanide [II]

Copper sulphate (409.8 mg) was dissolved in distilled water (1.0 ml) and the solution stirred at 60°C. A solution of sodium metabisulphite (88.5 mg) in distilled water (1.0 ml) was added dropwise over 5 min immediately followed by a solution of potassium [^{14}C] cyanide (8.9 mg) with a specific activity of 45.2 mCi/mM and potassium cyanide (94.1 mg) in distilled water (1.5 ml). The reaction mixture was stirred for 20 min at 60°C. The product was filtered from the mother liquors, washed with hot water, methanol and ether and dried under reduced pressure at room temperature to give [II] (125.9 mg; 88.8%) as a buff coloured powder.

4-Chlorobenzo[^{14}C] nitrile [III]

4-Bromochlorobenzene (410 mg), cuprous [^{14}C] cyanide (125.9 mg), cuprous cyanide (26.8 mg), and dimethyl formamide (0.4 ml), were added to a 'Pyrex' glass tube, 0.5 mm internal diameter and 12 cm length. The tube was sealed and placed in an oil-bath maintained at 150 - 155°C for 7 hr. Higher yields were obtained at this stage if the tube was removed every 20 min and thoroughly shaken. On completion of the reaction and after allowing to cool to room temperature, the mixture was extracted with di-ethyl ether (10 X 4 ml). The combined extracts were washed with water (3 X 5 ml), dried over anhydrous sodium sulphate, and evaporated to dryness under reduced pressure at 20°C to give an off-white solid.

The product was examined by thin-layer chromatography (t.l.c.) using a Silica GF plate developed with solvent system (C), examined under UV 254 nm, and autoradiographed for 16 hr. Comparison of the UV and autoradiographic patterns showed that the product was mainly the required 4-chlorobenzo [^{14}C] nitrile plus two minor impurities of lower Rf values, one of which was labelled. No further purification was carried out at this stage.

4-Chlorobenz[^{14}C] thioamide [IV]

4-Chlorobenzo [^{14}C] nitrile (360 mg) and absolute alcohol (5 ml) were stirred until in solution. Triethylamine (0.4 ml) was added, the solution stirred at 70°C and dry hydrogen sulphide gas passed in for 2 hr. The reaction mixture was cooled, filtered and the residue washed with acetone (8 X 5 ml). The combined liquors were evaporated to dryness under reduced pressure to give a yellow solid.

The product was examined by t.l.c. using Silica GF plates developed with solvent systems (A) and (B). The plates were examined under UV 254 nm and autoradiographed for 16 hr. Comparison of the UV and autoradiographic

patterns showed that all the impurities were labelled.

A column (2.5 cm diameter) containing Merck Silica GF (40 g) was prepared and eluted with solvent system (B). The crude product was dissolved in the mobile phase (5 ml) and applied to the column; 120 X 2.0 ml fractions were collected, and 2 µl aliquots of alternate fractions were spotted on a Silica GF plate and developed with solvent system (B). The plate was run for 10 cm, dried, examined under UV 254 nm, and autoradiographed for 16 hr. The appropriate fractions containing one spot material with identical Rf to that of the pure reference compound were combined and evaporated to dryness under reduced pressure to give a yellow solid (199 mg; 44.3%). This material was examined by t.l.c. using a Silica GF plate developed with solvent system (B). An identical chromatographic pattern was shown both by examination under UV 254 nm and autoradiography.

4-Chloromethyl-2-(4-chlorophenyl)-[2-^{14}C] thiazole [V]

A solution of 4-chlorobenz [^{14}C] thioamide (199 mg) in acetone (40 ml) was added to a solution of 1,3-dichloro-2-propanone (250 mg) in acetone (10 ml). The acetone was slowly distilled off at atmospheric pressure. When all the acetone had been removed, a further solution of 1,3-dichloro-2-propanone (250 mg) in acetone (20 ml) was added and the acetone once again slowly distilled off. The residue was cooled to room temperature and dissolved in concentrated sulphuric acid solution (5 ml). The dark brown solution was stood at room temperature for 1 hr and then added slowly to a stirred ice/water mixture when a suspension was obtained which was stirred at room temperature for 1 hr. The product was filtered off, washed with water, and dried at 30°C under reduced pressure to give a grey powder (244 mg; 86.2%). The product was examined by t.l.c. on a Silica GF plate developed with solvent system (C). Examination of the plate under UV 254 nm followed by autoradiography for 16 hr showed that the product was mainly the required 4-chloromethyl-2-(4-chlorophenyl)-[2-^{14}C]-thiazole plus three minor impurities

which were labelled. No further purification was carried out at this stage.

2-(4-Chlorophenyl)-4-cyanomethyl-[2-^{14}C] thiazole [VI]

A solution of 4-chloromethyl-2-(4-chlorophenyl)-[2-^{14}C]-thiazole (244 mg) in dimethyl sulphoxide (6 ml) was added to a solution of potassium cyanide (23 mg) in dimethyl sulphoxide (6.5 ml) and the mixture stirred at room temperature for 1 hr. A further solution of potassium cyanide (55 mg) in dimethyl sulphoxide (10.5 ml) was then added and the mixture stirred at room temperature for a further 16 hr. The temperature was raised to 70 - 75°C and the mixture stirred at this temperature for 4 hr. The reaction mixture was cooled to 50°C and added slowly to distilled water (100 ml) stirring at 50 - 55°C. The suspension was stirred for 1 hr. The product was filtered off, washed with water, and dried at 30°C under reduced pressure to give a grey-white powder (159 mg; 67.8%). The product was examined by t.l.c. on a Silica GF plate developed with solvent system (D). The plate was examined under UV 254 nm and autoradiographed for 16 hr. The UV and autoradiographic pattterns showed that the product was the required 2-(4-chlorophenyl)-4-cyanomethyl-[2-^{14}C] thiazole.

2-(4-Chlorophenyl)-[2-^{14}C] thiazol-4-ylacetic acid [I.C.I. 54,450] [I]

A mixture of 2-(4-chlorophenyl)-4-cyanomethyl-[2-^{14}C] thiazole (159 mg), dioxan (1.7 ml), and 6N hydrochloric acid solution (6.7 ml) were stirred and refluxed for 3 hr. The mixture was cooled to room temperature and centrifuged to remove a little insoluble material. The residue was washed with 6N hydrochloric acid solution (3 X 2 ml) and the combined mother and wash liquors basified to pH 4 by the dropwise addition of 70° T.W. sodium hydroxide solution. The product was filtered off, washed with water (2 X 2 ml), and then dissolved in 1 : 1 ammonia (d 0.880) : water (9 ml). The solution was warmed with activated charcoal, filtered, and the residual charcoal washed with water (2 X 2 ml). The combined filtrates were acidified

with glacial acetic acid and the suspension produced was cooled at 5°C for 3 hr, filtered and washed with water (3 X 2 ml). The product was dried at 30 - 40°C under reduced pressure to give a white solid (132 mg). Examination of the solid by t.l.c. on a Silica GF plate developed with solvent system (E), followed by autoradiography for 16 hr, showed that the UV and autoradiographic patterns were identical. The one detectable impurity was labelled.

A column (2.5 cm diameter) containing Merck Silica HR (30 g) was prepared and eluted with solvent system (C). The crude product was dissolved in acetone (2 ml) and applied to the column; 50 X 5 ml fractions were collected, then the eluant was changed from solvent system (C) to methanol. A further 50 X 5 ml fractions were collected, and 5 µl aliquots of alternate fractions spotted onto a Silica GF plate and the plate developed with solvent system (E). The plate was run for 10 cm, dried, examined under UV 254 nm, and autoradiographed for 16 hr. The appropriate fractions containing one spot material with identical Rf to that of pure reference compound were combined and evaporated to dryness under reduced pressure. Traces of silica were removed by the centrifugation of absolute alcohol extracts. Removal of the solvent gave I.C.I. 54,450 as a white powder (106 mg) (Found: C, 52.0; H, 3.1; N, 5.4. $C_{11}H_8O_2NSCl$ requires C, 52.1; H, 3.2; N, 5.5), representing an overall chemical yield of 26.4%.

The product was examined by t.l.c. in solvent systems (E) and (F) on Silica GF plates and the plates autoradiographed for 16 hr. The autoradiographs were used to "map" the plates which were then segmented. Scintillation counting of the segmented plates in a dioxan-naphthalene-silica-butyl PBD (8%) phosphor indicated a minimum radiochemical purity of 99.7%. Mass spectrometry showed no detectable impurities. The specific activity was 12.6 µCi/mg (3.19 mCi/mM) which represented an overall radiochemical yield of 22.3%.

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