THE SYNTHESIS OF [^{3}H] AND [^{14}C] o-CHLOROBENZYLIDENEMALONONITRILE (CS)

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SUMMARY

Using similar procedures, the syntheses of [2-^{14}C] -malononitrile and malono[^{14}C]nitrile are described. Condensation with o-chlorobenzaldehyde affords [2-^{14}C]-o -chlorobenzylidenemalononitrile and o-chlorobenzylidenemalono [^{14}C]nitrile respectively. The syntheses of [^{3}H] o-chlorobenzaldehyde and o-chlorobenz[^{14}C]aldehyde, again by a common sequence of reactions, is described and with malononitrile gives [^{3}H]-o-chlorobenzylidenemalononitrile and [1-^{14}C]-o-chlorobenzylidenemalononitrile.

Key Words: Carbon-14, Tritium, Synthesis, o-Chlorobenzylidenemalononitrile.

INTRODUCTION

To facilitate biochemical studies of o-chlorobenzylidenemalononitrile (CS) 1, the synthesis of a radiochemically labelled form was required. More specifically, the catabolic nature of the *in vivo* metabolism of 1 (1) required that the molecule be extensively labelled so as to allow the fate of each portion to be ascertained. To this end, syntheses were devised which resulted in the incorporation of tritium into the aromatic ring and carbon-14 into each of the non-aromatic carbon atoms.

CS 1 is normally synthesised (2, Scheme 1) in high yield by condensation of o-chlorobenzaldehyde 2 with malononitrile 3. The radiochemical syntheses therefore divide naturally into the syntheses of the appropriately labelled precursors 2 and 3 which are reported in this paper.

For the synthesis of [^{14}C] - 1 labelled in the cyanide groups, the synthesis of malono-[^{14}C]-nitrile is required. The procedure used is

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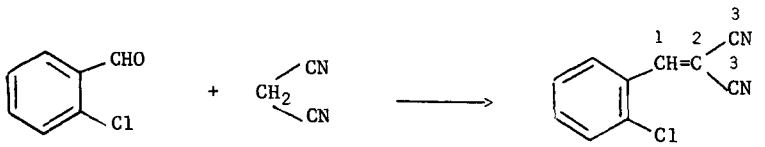
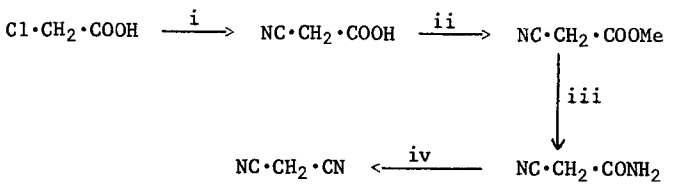


II III I

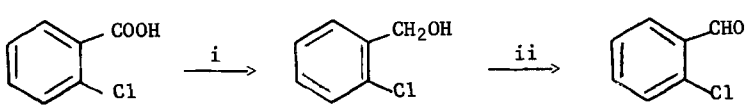
Scheme 1



III

Reagents: i, $NaCN-Na_2CO_3$; ii, CH_2N_2 ; iii, NH_3 ; iv, PCl_5-LiCl.

Scheme 2



IV V II

Reagents: i, $LiAlH_4$; ii, N_2O_4.

Scheme 3

illustrated in Scheme 2. Reaction i was effected using approximately equimolar quantities of sodium [^{14}C]-cyanide and chloroacetic acid. Some modifications to the literature procedure (3) were made (using non-labelled materials) before consistently good small scale yields were obtained. Reactions ii and iii consistently returned high yields of products. Reaction iv presented some difficulties and a variety of dehydrating agents and methods proved to be unsatisfactory. Ultimately, the modification of a procedure used previously (4) for the synthesis of labelled malononitrile gave adequate yields. This consisted essentially of heating an intimate mixture of silica (to assist uniform distribution of the reagents), cyanoacetamide, lithium chloride and phosphorus pentachloride in vacuo with a naked flame. Although this procedure was completely satisfactory with non-labelled materials, when applied to [^{14}C]-cyanoacetamide, unreacted material was obtained which had to be separated chromatographically from malononitrile and retreated with the dehydration mixture. Using the above procedures, o-chlorobenzylidenemalono[^{14}C]nitrile 1 was prepared in 22% overall yield from sodium[^{14}C]cyanide.

Similarly, using methyl[2-^{14}C]-cyanoacetate and reactions iii and iv (Scheme 2) to synthesise [^{14}C]-malononitrile, [2-^{14}C]-o-chlorobenzylidenemalononitrile 1 was prepared in 28% overall yield.

The synthesis of CS 1 with tritium in the aromatic ring and with carbon-14 at C-1 required the synthesis of the appropriately labelled o-chlorobenzaldehyde 2. For both procedures, o-chlorobenzoic acid was used as a common precursor.

Tritiation potentially represents a simple economical source of CS of high specific activity, i.e. several Ci/mmole. Attempts to tritiate o-chlorobenzaldehyde as the water soluble Girard T or bisulphite derivative by a heterogeneous catalytic exchange procedure were unsuccessful as a result of decomposition under the prevailing reaction conditions.

However, tritiation of sodium o-chlorobenzoate was readily achieved and converted to o-chlorobenzaldehyde and thus CS by the route outlined in Scheme 3.

With non-labelled materials, o-chlorobenzoic acid was reduced by lithium aluminium hydride to o-chlorobenzyl alcohol which was oxidised with dinitrogen tetroxide (5) to o-chlorobenzaldehyde and reacted with malononitrile to give CS in an overall yield of 51%. The direct reduction of acid 4 to aldehyde 2 using lithium aluminium hydride derivatives (6) proved to be unsatisfactory. Pfitzner-Moffatt oxidation (7) of alcohol 5 to 2 represents a less satisfactory alternative to the procedure adopted.

Using [G-^{3}H] sodium o-chlorobenzoate (tritiated in 1 g batches at the Radiochemical Centre, Amersham) with specific activities in the range 300 - 900 mCi/mmole, satisfactory yields of [^{3}H]-o-chlorobenzyl alcohol were isolated. However oxidation and condensation with malononitrile gave negligible yields of [^{3}H]-CS. Only by dilution of the crude [^{3}H]-o-chlorobenzoic acid with non-labelled material, adjusting the specific activity to ca. 100 mCi/mM, was it possible to obtain [^{3}H]-CS in acceptable quantities.

The synthesis of [1-^{14}C]-CS was achieved in similar manner using o-chlorobenzoic [^{14}C] acid. Reaction of the Grignard reagent from o-chloroiodobenzene with [^{14}C] carbon dioxide generated from barium [^{14}C] carbonate and sulphuric acid gave [^{14}C]-4 which was converted into o-chlorobenz[^{14}C]-aldehyde using the route outlined in Scheme 3. Again, it was apparent that the condensation of [^{14}C]-2 with malononitrile was less satisfactory than the reaction observed with non-labelled materials.

EXPERIMENTAL

General procedures are reported elsewhere (8). Tritiation of o-chlorobenzoic acid was performed at the Radiochemical Centre, Amersham using heterogeneous catalytic exchange process, TR1 (9).

o-Chlorobenzylidene malono[^{14}C]nitrile 1 (Scheme 2)

Reaction i. [3-^{14}C]-cyanoacetic acid.- Sodium [^{14}C]cyanide (32 mg, 25 mCi) was added to a mixture of chloroacetic acid (0.96 g), sodium carbonate (0.6 g) and sodium cyanide (0.47 g) in water. The mixture was warmed gently for 2 h and then boiled under reflux for 2 h. The mixture was concentrated, neutralised with dilute hydrochloric acid, concentrated and dried by evaporation with toluene. The residue was extracted repeatedly with ether. The extracts were filtered to give a solution of crude [3-^{14}C] -cyanoacetic acid.

Reaction ii. Methyl[3-^{14}C]cyanoacetate.- The ethereal solution of [3-^{14}C]cyanoacetic acid was treated with ethereal diazomethane until a pale yellow colour persisted. The solution was concentrated to yield essentially homogeneous methyl[3-^{14}C]cyanoacetate (0.94 g) (t.l.c., chloroform/methanol 9:1, R_f 0.7).

Reaction iii. [3-^{14}C]-cyanoacetamide.- A methanolic solution of methyl [3-^{14}C]cyanoacetate (0.94 g) was saturated with ammonia and stored at room temperature for 2 h. Concentration afforded the crude [3-^{14}C] -acetamide (0.79 g).

Reaction iv. Malono[^{14}C]nitrile.- [3-^{14}C]acetamide (0.79 g) was mixed thoroughly with silica (0.5 g), lithium chloride (0.05 g) and phosphorus pentoxide (1.0 g). The mixture was heated in vacuo (0.1 mm) and the volatile material collected in a trap at -70°. T.l.c. (chloroform/ methanol, 9:1) showed the product to be a mixture of malononitrile and unreacted cyanoacetamide which were separated by column chromatography. Unreacted cyanoacetamide was retreated with the dehydration mixture to afford malononitrile and unchanged acetamide (0.042 g). The malononitrile fractions were combined and treated with an excess of o-chlorobenzaldehyde. After 4 h, the solution was concentrated and the residue chromatographed (diisopropylether/petrol 7:3) to remove excess o-chlorobenzaldehyde (R_f 0.4). Recrystallisation from petrol (b.p. 60 - 80°) containing 5%

ethanol gave o-chlorobenzylidenemalono[^{14}C]nitrile (0.38 g, 22% from sodium [^{14}C]cyanide) with a specific activity of 2.53 mCi/mM.

[2-^{14}C]-o-Chlorobenzylidenemalononitrile 1.- [2-^{14}C]-3 was prepared from methyl[2-^{14}C]-cyanoacetate using reactions iii and iv of the procedure described above. Thus, methyl[2-^{14}C]-cyanoacetate (50.7 mg, 6 mCi) in methanol saturated with ammonia gave [2-^{14}C]-cyanoacetamide. Unlabelled cyanoacetamide (0.15 g) was added, the solution concentrated and the residue treated with dehydration mixture [silica (0.5 g), lithium chloride (0.04 g) and phosphorus pentoxide (1 g)] to afford [2-^{14}C]-malononitrile (80 mg). Treatment with o-chlorobenzaldehyde (0.17 g) in ethanol (5 ml) gave [2-^{14}C]-o-chlorobenzylidenemalononitrile (121 mg, 28% from cyanoacetamide) with a specific activity of 3.46 mCi/mM.

[^{3}H]-o-Chlorobenzylidenemalononitrile 1 (Scheme 3).

[^{3}H]-o-Chlorobenzoic acid 4.- Sodium [G-^{3}H]-o-chlorobenzoate was supplied as an aqueous solution (25 ml) and contained 4.65 Ci. A portion of this solution (8 ml) was acidified with dilute hydrochloric acid and extracted with ether. Unlabelled 4 (0.9 g) was added to give [^{3}H]-o-chlorobenzoic acid (1.0 g) with a specific activity of 120 mCi/mM.

[^{3}H]-o-Chlorobenzyl alcohol 5.- [^{3}H]-4 (1 g) in ether (20 ml) was added to a stirred suspension of lithium aluminium hydride (0.6 g) in ether (20 ml) and the mixture stirred at room temperature for 1 h. Conventional work up procedures gave [^{3}H]-o-chlorobenzylalcohol (0.58 g).

[^{3}H]-o-Chlorobenzaldehyde 2.- [^{3}H]-5 (0.58 g) was treated with dinitrogen tetroxide (1.5 ml) and the solution stored at room temperature for 18 h. Neutralisation of the solution with aqueous potassium carbonate and extraction with chloroform gave [^{3}H]-o-chlorobenzaldehyde as a brown oil.

[^{3}H]-o-Chlorobenzylidenemalononitrile 1.- [^{3}H]-2 was treated with a solution of malononitrile (0.24 g) in methanol (10 ml) for 3 h when t.l.c.

(petrol/ether, 9:1) showed the reaction to be essentially complete. The solution was concentrated and chromatographed (benzene) to give [^{3}H]-o-chlorobenzylidenemalononitrile (0.32 g, 26% overall from o-chlorobenzoic acid) m.p. 92 - 94° from petrol (b.p. 60 - 80°). The specific activity was 110 mCi/mM.

The remainder of the aqueous sodium [^{3}H]-o-chlorobenzoate solution (16 ml) was used diluted with unlabelled o-chlorobenzoic acid (0.9 g) to prepare [^{3}H]-1 (0.3 g, 22%) with a specific activity of 219 mCi/mM.

Using non-labelled materials, the overall yields for the conversion of 4 to 1 were in the order of 50%.

[1-^{14}C]-o-Chlorobenzylidenemalononitrile 1

For the preparation of [1-^{14}C]-1 from o-chlorobenzoic[^{14}C] acid, the procedures used are essentially those described for [^{3}H]-1. Using non-labelled materials, all reactions gave high yields and crude products that were essentially homogeneous (t.l.c., g.c. and spectroscopic analysis). CS 1, was consistently prepared in yields of 45 - 50%, overall from barium carbonate.

o-Chlorobenzoic[^{14}C] acid 4.- o-Chloroaniline (27 g) was diazotised in the usual way and treated with potassium iodide (36 g) in water (40 ml) to afford on steam distillation and subsequent distillation in vacuo, o-chloroiodobenzene (37 g, 74%) b.p. 58 - 60°/0.1 mm. A portion of this material (5.5 g) in ether (10 ml) was reacted with magnesium (0.6 g) stirred in ether (10 ml) to give a solution of o-chlorobenzene magnesium iodide. An aliquot (5 ml) was allowed to react at -78° with carbon dioxide generated from an intimate mixture of barium [^{14}C]carbonate (110 mg, 12.5 mCi) and unlabelled barium carbonate (690 mg) and conc. sulphuric acid (5 ml) using a simplified form of the procedure described by Dauben et al. (10). Conventional processing gave o-chlorobenzoic [^{14}C] acid (350 mg).

[1-^{14}C]-o-Chlorobenzylidenemalononitrile 1.- o-Chlorobenzoic[^{14}C] acid (350 mg) was treated with lithium aluminium hydride to yield [1-^{14}C]

-o-chlorobenzyl alcohol, which on oxidation with dinitrogen tetroxide gave o-chlorobenz[^{14}C]aldehyde. Reaction with excess malononitrile (500 mg) in methanol (5 ml) for 2 h, when t.l.c. (petrol/ether, 9:1) showed an absence of 2 (R_f 0.4), gave crude [1-^{14}C]-1. Column chromatography eluting with chloroform removed excess malononitrile. Further columns using chloroform/petrol, 1:1 as eluant and then benzene/petrol 1:1 gave pure [1-^{14}C] -o-chlorobenzylidenemalononitrile (100 mg, 13% overall from barium [^{14}C] carbonate) with a specific activity of 3.1 mCi/mM.

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