SYNTHESIS OF 2,4',5-TRICHLORO[^{14}C]BIPHENYL MERCAPTURIC ACIDS

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SUMMARY

The synthesis of 3- and 4-S-(N-acetyl)cysteinyl-2,4',5-trichloro[^{14}C]biphenyl is described. 4-Chloro[^{14}C]aniline was used as starting material. The products were obtained after an aryl coupling of the amine with 2,5-dichloronitrobenzene, a reduction of the nitrogroup and finally coupling of the diazotized trichlorobiphenyl amine with cuprous N-acetylcysteine mercaptide.

Key words: 2,4',5-trichlorobiphenyl, PCB, radiosynthesis, isotopes, mercapturic acids, metabolites

INTRODUCTION

Polychlorinated biphenyls (PCB) such as Aroclor 1016, 1242 and 1254 contain significant amounts of 2,4',5-trichlorobiphenyl (triCB) (1,2). TriCB is known to be metabolized by mice (3) and rats (4) to triCB methyl sulphones which accumulate in lung bronchial mucosa (4,5). With other xenobiotics, mercapturic acid pathway (MAP) metabolites are known to be precursors for methylthio-, methylsulphinyl- and methylsulphonyl-containing metabolites (6), and recently triCB has been shown to be metabolized in the rat to MAP metabolites that are excreted mainly in the bile (4,7). To prove that triCB mercapturic acids could be precursors of methyl sulphide and methyl sulphone metabolites of triCB, ^{14}C-labelled mercapturic acids of triCB were synthesized. The present paper describes the synthesis of two isomeric ^{14}C-labelled 2,4',5-triCB mercapturic acids.

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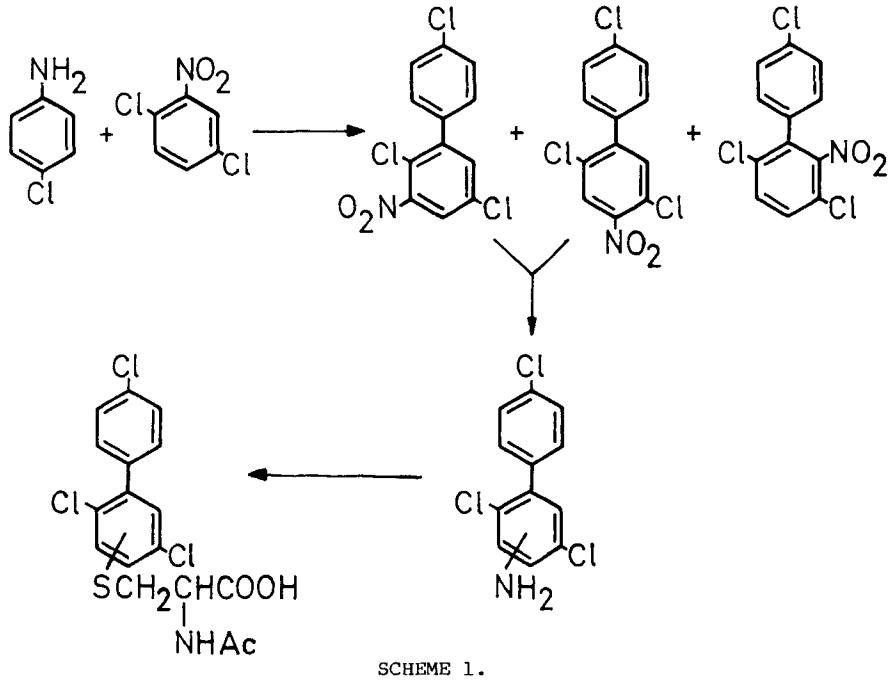

SCHEME 1.

RESULTS AND DISCUSSION

The synthesis of the labelled 2,4',5-trichlorobiphenyl mercapturic acids was performed as shown in Scheme 1. The initial reaction between the labelled 4-chloroaniline and the 2,5-dichloronitrobenzene was carried out with minor modifications of the Cadogan aryl coupling reaction (8). A mixture of three products was obtained from which 2-nitro-3,4',6-trichloro[^{14}C]biphenyl was isolated: however, 3- and 4-nitro-2,4',5-trichloro[^{14}C]biphenyl could not be separated from each other by straight phase TLC, LC or HPLC.

The nitro-group was reduced with stannous chloride in hydrochloric acid and ethanol (9). Although the yield of the mixture of 3- and 4-amino-triCB was less than 50%, no evidence was obtained that dechlorinated products had been formed. The two isomeric amino-triCBs were purified as a mixture by LC in a straight phase system.

Diazotation of aryl amines and coupling of diazo-compounds with cysteine or N-acetylcysteine is known to give poor yields of the corresponding derivatives(10,11). Several publications indicate the advantage of using cuprous mercaptides of cysteine or glutathione in synthesis of their aryl S-conjugates (11-14). Cuprous oxide was initially used by Hopkins (15) and Pirie (16) to precipitate amino acids containing a thiol group. Their results were later used in order to prepare cysteine and glutathione cuprous mercaptides for

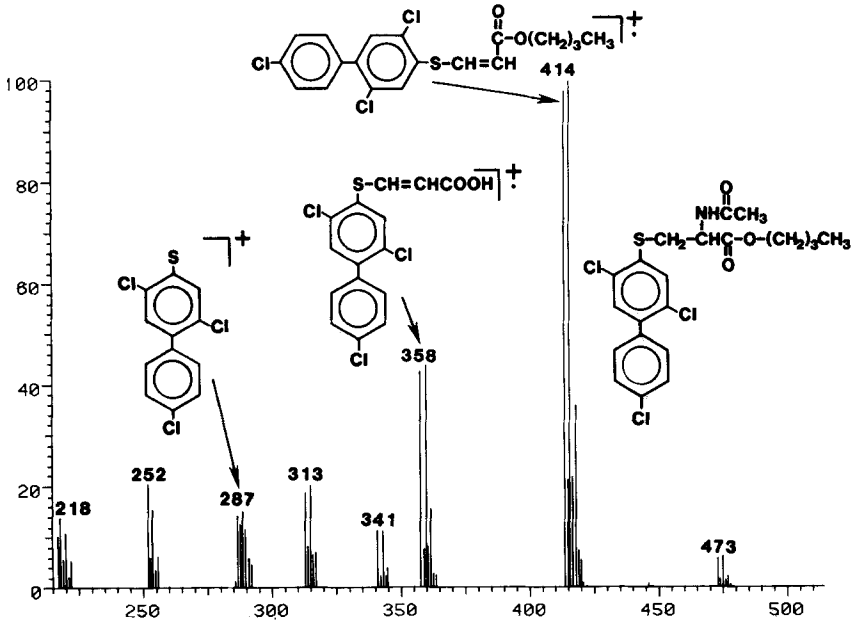

FIGURE 1. Mass spectrum of a mixture of the butyl esters of 3- and 4-S-(N-acetyl)cysteinyl-2,4',5-trichlorobiphenyl.

synthetic purposes. These mercaptides were shown to react with diazotized amines and give fair yields of e.g. cysteine conjugates of 4-bromobenzene (12) and benzene (13). The last step in the present synthesis was run based upon the coupling with cuprous mercaptides. The diazonium salt of 3- and 4-amino--2,4',5-trichloro[^{14}C]biphenyl was reacted with N-acetylcysteine cuprous mercaptide in water. Nickel sulphate was added to the mercaptide solution in order to facilitate a rapid decomposition of the initially formed aryldiazosulphide (17). The mercapturic acid products were isolated by extraction and purified by LC and HPLC. The two triCB mercapturic acid isomers did not separate on a reversed phase C_{18}-column. However, this shouldn't be a drawback in subsequent biological studies since evidence for the formation of the corresponding substituted MAP metabolites in the rat has been determined (4). The ratio of the 3- and 4-S-(N-acetyl)cysteinyl-2,4',5-trichlorobiphenyls was expected to be the same as the ratio of 3- and 4-amino-triCB (1:3.4).

The identity of the ^{14}C-triCB mercapturic acid mixture was established by comparison with the corresponding unlabelled material. Synthesis of unlabelled triCB mercapturic acids was carried out under exactly the same conditions as described in the present paper of the ^{14}C-labelled compounds. The mass spectrum of the butyl esters of 2,4',5-trichlorobiphenyl mercapturic acids is shown in Figure 1. The fragmentations shown are compatible with the assigned structures. The ions at m/z 341 and m/z 313 could arise from losses of C_4H_9O and $C_4H_9CO_2$ respectively from the m/z 414 ion. The ^{13}C NMR spectrum

was taken in DMSO-d_6 (the DMSO-septet at δ 39.5 was used as the reference) and was found to be compatible with a mixture of the mercapturate isomers. Absorptions at δ 51.6, 33.2 and 171.8 were assigned to methine, methylene and carboxyl carbons of the cysteine portion respectively. Absorptions at δ 22.3 and 169.4 were assigned to the methyl and carbonyl of the N-acetyl group. Absorptions at δ 135.6, 136.2 and 137.0 are likly due to the carbons in the 4-, 1- and 1'-positions of the biphenyl structure. Other major absorptions at δ 127.4, 128.3, 129.8, 130.5, 131.0 and 132.9 were not assigned. Off resonance experiments were limited by the amount of samples available: however, sufficient results were obtained to support the above assignments.

The radiochemical purity of the 2,4',5-trichloro[^{14}C]biphenyl mercapturic acids was determined as the activity recovered as the mercapturic acid, after injection to a HPLC radioactivity monitor (RAM) system. Even if not more than 94% of the activity was recovered no other peaks containing radioactivity could be determined in the chromatogram.

MATERIAL AND METHODS

4-Chloro[U-^{14}C]aniline (377 MBq, 178 MBq/mmol) was purchased from New England Nuclear (Boston, Mass.), 2,5-dichloronitrobenzene (Fluka AG), 3-methylbutyl nitrite (Aldrich), stannous chloride, sodium nitrite and cuprous oxide (Fischer Sci. Comp.), N-acetyl-L-cysteine (Sigma) and nickel sulphate (Pfaltz and Bauer). Liquid chromatography was performed on Silica gel 60 (< 63 um or 63-200 um) from E. Merck. Quantitative radioactivity measurements were performed by liquid scintillation. Mass spectra were run in a direct inlet mode at 70 eV using a Varian MAT CH-5DF mass spectrometer. The ion source temperature was 200°C. ^{13}C NMR was obtained on a Joel FX 90Q instrument.

Nitrotrichloro[^{14}C]biphenyls

4-Chloro[^{14}C]aniline (377 MBq, 178 MBq/mmol) was heated together with 2,5-dichloronitrobenzene (6.0 g, 31.2 mmol) to 60-65°C. Acetic acid (1.3 ml, 21.7 mmol) was first added to the solution and then 3-methylbutyl nitrite in portions of 150 ul, 140 ul, 130 ul and 100 ul at 0, 10, 20 and 90 min respectively. The temperature was rised to 120°C after 90 min and the reaction was finished after 3 h. Chloro[^{14}C]benzene from deamination of 4-chloro[^{14}C]-aniline and the excess of 2,5-dichloronitrobenzene was carefully destilled off under vacuum (0.25 mm Hg). The residue was dissolved in chloroform and transferred to a silica gel (63-200 um) column. The compounds of interest were eluted with chloroform. The polymeric residues on the column were eluted with

methanol and discarded. The three isomeric nitrotrichloro[^{14}C]biphenyls were eluted with hexane:ethylacetate (9:1) from a silica gel (<63 um, 40 g) column. 3-Nitro- and 4-nitro-2,4',5-trichloro[^{14}C]biphenyl (114 MBq) eluted together. 2-Nitro-3,4',6-trichloro[^{14}C]biphenyl was isolated from a fraction appearing after the main product. The identity of these compounds will be reported elswhere.

3- and 4-amino-2,4',5-trichloro[^{14}C]biphenyl

3- and 4-Nitro-2,4',5-trichloro[^{14}C]biphenyl (98 MBq, 0.55 mmol) was dissolved in abs. ethanol (16.4 ml). Conc. hydrochloric acid (5.8 ml) was added and the mixture obtained was kept at 50°C. Stannous chloride (451 mg, 2.4 mmol) was added. The reaction was run for 55 min. The reaction mixture was cooled in an ice bath and water (12 ml) was added. The products were extracted with methylene chloride after addition of sodium hydroxide to a slightly alkaline reaction. A dry chloroform solution (1.0 ml) of the reaction products was put on top of a slilica gel (<63 um, 15.0 g) column. The trichlorobiphenyl amines were eluted with chloroform. 3-amino- and 4-amino-2,4',5-trichloro[^{14}C]biphenyl (48.5 MBq, 178 MBq/mmol) were isolated in the same fraction. The ratio of 3-amino- and 4-amino-2,4',5-trichlorobiphenyl was 1:3.4 as determined by capillary gas chromatography.

3- and 4-S-(N-Acetyl)cysteinyl-2,4',5-trichloro[^{14}C]biphenyl

Cuprous oxide (79 mg, 0.55 mmol) was suspended in water (11.0 ml). N-Acetyl--L-cysteine (180.6 mg, 1.1 mmol) was added and the mixture was heated to 60°C and kept there for 2 h. The cuprous mercaptide obtained was cooled in an ice bath. A few crystals of nickel sulphate were added before the mercaptide was used in the reaction with the diazonium salt. 3- and 4-Amino-2,4',5-trichloro-[^{14}C]biphenyl (48.5 MBq, 0.27 mmol) was dissolved in conc. sulphuric acid (1.6 ml) and stirred at room temperature for 40 min. Water (6.6 ml) was added carefully in small portions to the cooled sulphuric acid solution. The diazotation was completed after the addition of sodium nitrite (0.30 M, 2.5 ml). A clear yellow solution was obtained. This diazonium salt solution was added in portions to the cold cuprous N-acetylcysteine mercaptide suspension under efficient stirring. The reaction was kept at <5°C over night.

The products were extracted with ether. A silica gel (63-200 um, 5.0 g) column was dry packed. The partly evaporated ether extract was put on top of the column and the products were eluted with ether. A first fraction of brownish coloured byproducts was obtained. The total radioactivity of this first fraction was 17.9 MBq. A second fraction was eluted with methanol. The activity of

the latter fraction, containing the trichlorobiphenyl mercapturic acids, was 25.2 MBq. The final purification of the mercapturic acids was carried out by HPLC. The separation was performed on a Bondapak TM/C18 column. Acetonitrile:water (the water contained 1% acetic acid) was pumped trough the column with a flow rate of 1.5 ml/min. An UV-detector was used for the preparative separation while a Packard Tricarb RAM was used for the determination of the radiochemical purity.

3- and 4-S-(N-Acetyl)cysteinyl-2,4',5-trichloro[^{14}C]biphenyl (16.1 MBq, 178 MBq/mmol) was obtained with a radiochemical purity of > 94%, determined as the radioactivity recovered from the fraction corresponding to the trichlorobiphenyl mercapturic acid from HPLC.

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