Synthesis of Radiolabelled Methylsulphonyl CBs with specific Retention in the Rat Liver

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Introduction

It is well known that polychlorinated biphenyls (PCB) are spread throughout the ecosystem and due to the lipophilicity and persistence several PCB congeners are strongly accumulated in the food webs. Methylsulphonyl-substituted chlorinated biphenyls ($MeSO_2$-CBs) is one of several types of metabolites from PCB (1,2), originating from CBs with chlorine atoms in 2,5- or 2,3,6-positions in at least one of the phenyl rings. Some of the $MeSO_2$-CBs are strongly retained in the liver (3) and lung (4), effects that might be due to binding to an uteroglobine like protein in the Clara cells of rat and mouse lung (5), binding to liver fatty acid binding protein (FABP) (6) and α-2u-globulin (α2u), a major urinary protein in mouse urine (7).

The aim of the present study was twofold (*i*) to identify $MeSO_2$-CBs with specific retention in rat liver and (*ii*) to develop a method for radiosynthesis of these $MeSO_2$-CBs with high specific activity.

Methylsulphonyl-CBs have been shown to be among the most persistent xenobiotics in free ranging mammals studied so far. In Baltic grey seals they were the third most abundant class of antropgenic substances, found in levels of 10-20 % of the total PCBs (8). In a comparative analytical study of grey seal, mink, otter and human liver samples, 3-$MeSO_2$-2,2',4,5,5',6-hexachlorobiphenyl (3-149) was the dominating PCB methyl sulphone congener in all species(9). The otter showed a highly selective retention for 3-$MeSO_2$-CBs in the liver whereas the grey seal only retained three 3-$MeSO_2$-CBs and the mink did not show any obvious specific retention of 3-$MeSO_2$-CBs.

Synthesis has been performed using the Cadogan coupling reaction through diazocoupling of a methylsulphonyl-substituted chlorinated aniline with a chlorinated benzene (10), coupling between a chlorinated aniline and chlorinated methylthiobenzene (11) or by nucleophilic

aromatic substitution of a chlorine atom in a polychlorobiphenyl by methane thiolate (12). More recently coupling of an aryl iodide and an aryl trimethylstannane with palladium as a catalyst has been described (13).
Two methods have been published for preparation of [^{14}C]-labelled $MeSO_2$-CBs. In the first one a [^{14}C]-pentachlorobiphenyl is reacted with sodium methanethiolate, resulting in substitution of a chlorine atom by the methyl sulphide group (12). In the other a [^{14}C]-chloroaniline is reacted with dichloromethylthiobenzene in a Cadogan coupling reaction (14).

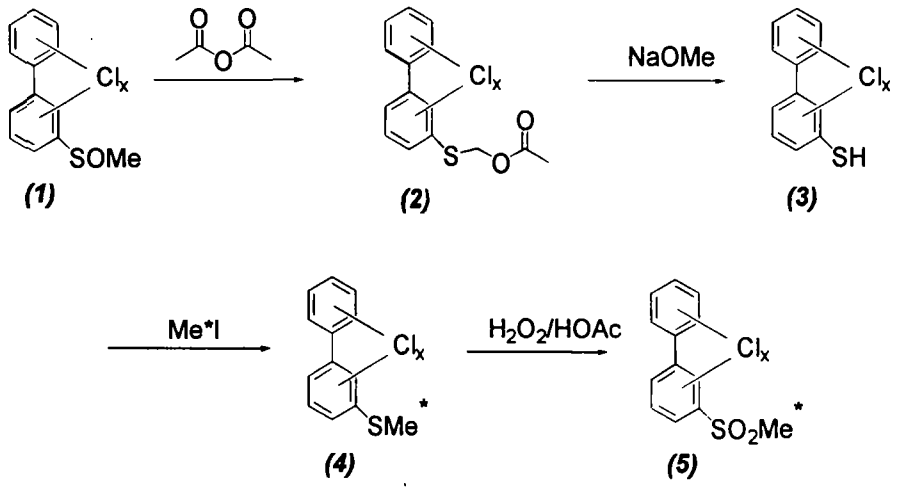
Material and methods:
Chemicals: All individual $MeSO_2$-CBs used as standards in the analysis were synthesised according to Haraguchi *et al.* (10). All solvents were of analytical grade, [^{14}C]-methyl iodide was purchased from Amersham (Sweden) and thin layer chromatography was performed on silica gel plates (Kiselgel 60 F_{254}, Merck) or RP-18 plates (RP-18 $F_{254}S$, Merck).

Instruments: Analysis of the liver samples was performed on a Varian GC 3700 fitted with a fused silica capillary column DB 5 (30m x 0.25 mm i.d., 0.25 µm film thickness). Gas chromatography-mass spectrometry (GC-MS) was performed on a Finnigan ITS40 instrument, connected to a Varian 3400 GC with a split-splitless injector kept at 270 °C and used in the splitless mode. The GC was equipped with a fused silica DB-5 capillary column (30 m x 0.25 mm i.d., 0.25µm film thickness) and helium was used as the carrier gas. The ITS40 was operated with an EI energy of 70 eV and an ion source temperature of 220 °C.
Radioactivity measurements were performed on a Wallac 1409 Emulsifier scintillator 299 (Wallac Oy, Finland.) and OptiScint HiSafe as scintillation cocktail.

Rat experiment: In the present experiment, rats were given a single, oral dose of a commercial PCB product (Clophen A50) dissolved in corn oil (25 mg/kg b.w.). After one, two, four and eight weeks the rats were sacrificed. Livers were taken out and analysed for their content of $MeSO_2$-CBs (15).

Synthesis: The first step in the synthesis of ^{14}C-methylsulphonyl-PCBs was a Cadogan coupling reaction between a chlorothioanisol and a chloroaniline (10) see Scheme 1. The metylthio-PCB was then selectively oxidised to the corresponding PCB methyl sulphoxide ***(1)*** with hydrogen peroxide in acetic acid. The sulphoxide was reacted with acetic acid anhydride in the "Pummerer reaction" (16,17) for 1.5 weeks. The isolated product ***(2)*** was treated with sodium methoxide in methanol to give the thiol ***(3)***. The thiol was methylated with either ^{3}H- or ^{14}C methyl iodide in an ion pair methylation (18) to give the PCB methyl sulphide ***(4)*** that was oxidised to the methylsulphonyl-PCB ***(5)*** with hydrogene peroxide in acetic acid (12).

So far we have synthesised 4-[^{3}H]-$MeSO_2$-2,2',4',5,5'-pentachlorobiphenyl (4-101), 3-[^{14}C]-$MeSO_2$-2,2',4',5,5'-pentachlorobiphenyl (3-101), 4-[^{14}C]-$MeSO_2$-2,2',4',5,5'-pentachlorobiphenyl (4-101),3-[^{14}C]-$MeSO_2$-2,2',4',5,5',6-hexachlorobiphenyl (3-149) and 3-[^{14}C]-2,2',4',5-tetrachlorobiphenyl (3-49). The binding of this [^{14}C]-3-149 to α2u and FABP has been studied and the results are presented at this meeting (19).



Scheme 1

Results and discussion

Looking at the total amount of $MeSO_2$-CBs formed in rats, dosed with Chlophen A50, it seems to reach a maximum after two weeks. The relative amount of 3-$MeSO_2$-2,2',4',5,5'-pentachlorobiphenyl (3-101) and 3-$MeSO_2$-2,2',3',4',5-pentachlorobiphenyl (3-87) were significantly increased in the rat livers.

To be able to study the observed specific tissue retention of $MeSO_2$-CBs in liver a method to prepare radiolabelled $MeSO_2$-CBs of high specific activity was developed and the ^{14}C was introduced in the last few steps of the reaction. The aim was to be able to use radiolabelled methyl iodide since high specific activity can be obtained for this chemical and it is also available at a reasonable price.

In a previously published method for synthesis of radiolabelled $MeSO_2$-CBs, the starting material has been a [^{14}C]-chloroaniline (14) or a [^{14}C]-pentachlorobiphenyl (12). Radiolabelled benzenes and anilines are not available with high specific activity. Our attempt was to demethylate a methylthio-CB to the corresponding thiol and to methylate the thiol with [^{14}C]- or [^{3}H]-methyl iodide. After several attempts to demethylate the methylthio-CB the Pummerer reaction (a reaction between a sulphoxide with at least one α-hydrogen and acetic acid anhydride) was found to work quite well for the production of an aryl thiol.

The original method had to be modified for the small-scale synthesis requested in the present preparation of radiolabelled substances. The amount of acetic acid anhydride was decreased one order of magnitude compared to the original volume used (16). The Pummerer reaction was performed in a sealed ampoule under inert atmosphere. The reaction time was changed to 1.5 weeks instead of 20 h. The yields were in the range of 90-100%.

Initial difficulties to hydrolyse the product *(2)* were overcome by using a stronger base, sodium methoxide in methanol (2 M) instead of sodiumhydroxide in methanol (0.12 M).

By this route for synthesis of radiolabelled methylsulphonyl-CBs we have reduced the number of reaction steps with radioactivity in the reaction material to only two steps. Since booth these steps give high yields, it is possible to obtain a good quantity of radiolabelled material.

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