RADIOLABELLED PCBs AND PCB METHYL SULPHONES - IMPROVED METHODS FOR SYNTHESIS AND TISSUE LOCALIZATION

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ABSTRACT

Synthesis of the coplanar PCB congeners, 3,3',4,4'-tetrachloro-[^{14}C]biphenyl and 3,3',4,4',5-pentachloro-[^{14}C]biphenyl (I-126) and five ^{14}C-labelled PCB methyl sulphones are descibed. A method for the preparation and isolation of I-126 in preparative scale is presented.

Data on tissue localizations of some PCB methyl sulphones are given. Also data on the binding of a PCB methyl sulphone to α_{2u}-globulin in rat kidney and urine and major-urinary-protein in mouse are presented.

KEYWORDS

PCB, PCB methyl sulphones, synthsis, radiolabelled, tissue distribution

INTRODUCTION

The global environmental burden of polychlorinated biphenyls (PCBs) is still of major concern due to their high levels in species at the top of the food-webs. It is well documented that PCB may induce reproduction failure in mink (1) and is probably also a cause of the decrease in seal and otter populations in Scandinavia (2). Even though enzyme induction properties and tissue localizations (3,4) are known for many of the PCB congeners present in technical mixtures, at least in mice, further studies are needed to explain mechanisms behind reproduction toxicity, and teratogenicity. Such studies also involve investigations on metabolism of PCB congeners and on the behavior of their metabolites - both hydroxylated PCB congeners and PCB methyl sulphones. In the present paper improved synthesis of some unlabelled and radiolabelled PCB congeners and five PCB methyl sulphone compounds are reported.

A large number of ^{14}C-labelled PCB congeners has been studied by autoradiography over the last fifteen years (4). The distribution of co-planar PCBs and PCBs with one chlorine atom in an *ortho*-position are very different. Certain PCBs, are known to form lipophilic metabolites, PCB methyl

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sulphones, that are retained in lung, kidney and in uterine fluid of pregnant animals. In lung PCB methyl sulphones of certain structures are non-covalenty but strongly bound to a uteroglobin-like protein (5) in mice and in kidney to $\alpha_{2\mu}$-globulin in rat and major-urinary-protein in mice (6). Furthermore, two of the synthesized PCB methyl sulphones have been reported as TCDD antagonists (7). It is thus of interest to investigate the fate of PCB methyl sulphones both with regard to tissue localization and toxicity.

EXPERIMENTAL

Synthesis

3,3',4,4'-Tetrachloro-(^{14}C)biphenyl and 3,3',4,4',5-pentachloro-(^{14}C)biphenyl were obtained from 3,4-dichloroiodo-(^{14}C)benzene and 3,4,5-trichloroiodobenzene in an Ullman reaction (8). The 3,4-dichloroiodo-(^{14}C)benzene was prepared via diazotation of 3,4-dichloro-(^{14}C)benzene and subsequent reaction with iodide (8). The Ullman reaction was performed in an ampoule containing copper bronze (100 mg) and 3,4-dichloroiodo-(^{14}C)benzene (790 µCi, 0.065 mmol). The ampoule was heated in a vertical steel furnace to 220 °C for 1.5 h. The ampoule was cooled and then opened. A small volume of chloroform was used to transfer the material in the ampoule to a Pyrex tube with screw cap. The tube was centrifuged and the solvent was transferred via a glass wool filter to a separate flask. The three PCB congeners formed were separated on silica gel TLC plates impregnated with tetrabutylammonium hydrogen phosphate (9) and finally the two labelled PCB congeners were further purified on RP_{18}-TLC plates. 3,3',4,4'-Tetrachloro-(^{14}C)biphenyl (67 µCi, 24.4 mCi/mmol) and 3,3',4,4',5-pentachloro-(^{14}C)biphenyl (202 µCi, 12.2 mCi/mmol) were isolated.

3,3',4,4',5-Pentachlorobiphenyl was prepared in 0.5 g scale from 3,4,5-trichloroaniline and 1,2-dichlorobenzene according to Sundström (10). The two PCB congeners form in the reaction were isolated as a mixture from a silica gel column. The mixture was dissolved in hexane:toluene (95:5, 300 ml) and separated on a charcoal column (40 g) prepared as described elsewhere (11). The solvent and an additional 300 ml of the same solvent was let through the column whereafter the solvent was changed to hexane:toluene (1:3, 500 ml). 2',3,3',4,5-Pentachlorobiphenyl (95% of total yield) was isolated via this procedure. The coplanar 3,3',4,4',5-pentachlorobiphenyl was isolated after a modified Soxhlet extraction of the charcoal column (11).

3-Methylsulphonyl-2,3',4,4'-tetrachloro-(^{14}C)biphenyl and 4-methylsulphonyl-3,3',4',5-tetrachloro-(^{14}C)biphenyl were prepared from 3,4-dichloro-(^{14}C)aniline (1.0 mCi, 12.2 mCi/mmol) and 2,6-dichloromethylthiobenzene (1 g) by addition of isopentylnitrite. Two isomeric methylthio-tetrachlorobiphenyls were isolated after separation on RP_{18}-TLC with methanol as mobile phase. The pure products were oxidized to the corresponding sulphones. 3-$MeSO_2$-2,3',4,4'-tetrachlorobiphenyl (192 µCi, 12.2 mCi/mmol) and 4-$MeSO_2$-3,3',4',5-tetrachlorobiphenyl (35 µCi, 12.2 mCi/mmol) were isolated on silica gel TLC with hexane:ethyl acetate as mobile phase.

2-Methylsulphonyl-3,3',4,4'-, 3-methylsulphonyl-3',4,4',5- and 4-methylsulphonyl-2,3,3',4'-tetrachloro-(^{14}C)biphenyl were prepared from 3,4-dichloro-(^{14}C)aniline (2.0 mCi, 12.2 mCi/mmol) and 2,3-

dichloromethylthiobenzene and isolated as described above. 2-Methylthio-3,3',4,4'-tetrachloro(14C)biphenyl (51 µCi, 12.2 mCi/mmol), 3-methylthio-3',4,4',5-tetrachloro-(14C)biphenyl (93 µCi) and 4-methylthio-2,3,3',4'-tetrachloro-(14C)biphenyl (806 µCi) were obtained. Aliquots of these PCB methyl sulphides were oxidized to the corresponding sulphones.

Autoradiography

The distribution of PCB methyl sulphones, injected i.v. were investigated in mice, female C57BL (20 g). The mice were kept at +22 °C with a 12/12 h light/dark cycle and given free access to pellet diet. The mice were killed, embedded in aqueous carboxymethyl cellulose, frozen and subjected to autoradiography at different levels of resolution.

Characterization of PCB methyl sulphone binding proteins in kidneys of mice and rat

4,4'-Bis((3H)-methylsulphonyl)-2,2',5,5'-tetrachlorobiphenyl ($MeSO_2$)$_2$-TCB (spec. act.: 15.6 Ci/mmol) was given i.p. to Sprague-Dawley male rats (250-300 g) and Swiss ICR male mice (30-35 g). The animals were killed 48 h after dosing. The purification of a protein - ($MeSO_2$)$_2$-TCB complex was performed as described elsewhere (6).

RESULTS AND DISCUSSION

The modified Ullman reaction was successfully adopted for preparation of the coplanar and most toxic PCB congeners known, labelled with carbon-14. Thus, approximately 20% yield was obtained of 3,3',4,4',5-tetrachloro-(14C)biphenyl in a pure form. It is possible to separate all the three most toxic PCB congeners,that are formed in the reaction, on tetrabutylammonium hydrogen phosphate impregnated silica gel TLC plates. The separating power of the charcoal column described for the purification of larger amounts of unlabelled 3,3',4,4',5-pentachlorobiphenyl is a prerequisite for biological/toxicological evaluation of this PCB congener. Because of unfavourable yield of I-126 in the Cadogan diarylcoupling reaction between 3,4-dichloroaniline and 1,2,3-trichlorobenzene I-126 should instead be prepared from 3,4,5-trichloroaniline and 1,2-dichlorobenzene.

It is shown that labelled PCB methyl sulphones are readily available through Cadogan reactions with only small amounts of the appropriate polychloromethylthiobenzene as a solvent. All PCB methyl sulphides synthesized were compared to the appropriate unlabelled reference compounds.

All 14C-labelled 4-$MeSO_2$-PCBs that have been studied by autoradiography have shown a strong uptake in the tracheobronchial mucosa. Among these ($MeSO_2$)$_2$-TCB is selectively accumulated in non-ciliated bronchiolar (Clara) cells and proximal tubular kidney cells in mice and rats (5). In the Clara cells the sulphone binds non-covalently to a uteroglobin-like protein as shown by Lund et al (5). In kidney, ($MeSO_2$)$_2$-TCB has been shown to be associated with α_{2u}-globulin, according to SDS-polyacryl amide gel electrophoresis, immunoblot and immunodiffusion analysis. The same α_{2u}-globulin complex was also isolated from rat urine. In mice, a ($MeSO_2$)$_2$-TCB-protein complex was characterized as mouse major-urinary-protein (MUP) but the complex was not detected in the kidney.

The observed nephrotoxicity of compounds such as 1,4-dichlorobenzene in male rats appears to be caused by reabsorption of the filtered xenobiotic complex. It is tempting to belive that a similar mechnism also occur in male rats dosed with $(MeSO_2)_2$-TCB. Studies on other PCB methyl sulphones need to be performed in order to further investigate the binding of these compounds to α_{2u}-globulin and to determine their nephrotoxicity.

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