Gas chromatographic enantiomer separations of chiral PCB methyl sulfons and identification of selectively retained enantiomers in human liver

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Introduction

Approximately 10 years after the discovery of PCBs in the environment [1], Jensen and Jansson reported on the identification of PCB methyl sulfones ($MeSO_2$-PCBs) in Baltic Grey seal blubber [2]. Twenty years later, $MeSO_2$-PCBs and $MeSO_2$-DDE have been detected in fish [3], birds [4,5] and mammals [6,7] including humans [8,9]. More recently some of the $MeSO_2$-PCBs in biota have also been observed to be selectively and strongly retained in liver tissue of mammals including man [6,9].

However, the mechanism for this selectivity is still unknown, although reversible protein binding plays a major role for their retention in the liver. The most important sulfones bound in mammalian liver are 3-$MeSO_2$-2,5,6,2',3',4'-hexachlorobiphenyl (3-132) (for abbreviations see Fig.1), 3-$MeSO_2$-2,5,6,2',4',5'-hexachlorobiphenyl (3-149) and 4-$MeSO_2$-2,5,6,2',4',5'-hexachlorobiphenyl (4-149) [6,9,10].

The $MeSO_2$-PCBs formed are persistent and only slightly less hydrophobic than their parent compounds which make them long lasting contaminants of the biosphere. From a toxicological viewpoint several of the 3-$MeSO_2$-PCBs have been shown by Kato and coworkers to induce strongly P-450 cytochrome enzymes such as P450 2B1, 2B2, 3A2 and 2C6 [11,12].

The present study is focused on the separation by gas chromatography (GC) of $MeSO_2$-PCBs into their enantiomers. The GC method was then applied to the analysis of $MeSO_2$-PCBs in human liver samples as a consult of the common knowledge that PCB methylsulfones with three or more chlorine atoms in the *ortho*-positions will give rise to atropisomers as they do for PCB congeners [13,14].

Materials and Methods

Chemicals and Instruments

All chemicals used were of analytical grade. The methods for synthesis of the $MeSO_2$-PCBs used are given in Figure 1 and $MeSO_2$-DDE was synthesized as described elsewhere [14]. Bis(4-chlorophenyl) sulfone (BCPS) was purchased from Merck (Darmstadt, Germany).
$MeSO_2$-PCB were quantified by GC-ECD as described in [15].
Full scan analyses of standards and a human liver sample were carried out using a VG 70-250 SE mass spectrometer (VG Analytical Ltd., Manchester, UK), coupled to a gas chromatograph HP 5890 (Hewlett-Packard, Palo Alto, USA). Gas chromatographic separation was performed on a BPX-5 (type SE 54) fused silica capillary column (50 m x 0.32 mm i. d., 0.25 µm film thickness, carrier gas He) using 1 min splitless injection at 280°C injector temperature and a temperature program of 60°C, 3 min isothermal, 10°/min to 220°C, 3°/min to 300°C. Mass spectra (70 eV, EI^+) were recorded at a resolution of 1000 (ion source temperature 200°C, trap current 500 µA) in the mass range of m/z 35-500.
For enantioselective gas chromatography/mass spectrometry the same instrumentation was used. The GC was fitted with a 10 m capillary column (0.25 mm i. d.) coated with a 1:1 (w:w) mixture of OV 1701 and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-hexyldimethylsilyl)-β-cyclodextrin (temperature program: 70°C, 3 min isothermal, 30°/min to 185°C, 30 min isothermal, 30°/min to 195°C, with helium as carrier gas). The column was connected to an on-column injector by means of a retention gap (1 m, 0.53 mm i.d.).
For further details see [15].

Samples

Six human livers were obtained from the „Institut für Rechtsmedizin" of the University of Hamburg who performed an autopsy of seven dead persons, who had passed away due to heart failure or accidents.
Liver samples (5 - 10 g) were thawed and then homogenized with threefold anhydrous sodium sulfate using an analytical mill. The samples were extracted in a soxhlet apparatus for 8 h with *n*-hexane (130 mL). The volumes of the extracts were determined and aliquots of 10 mL were removed. The solvent in the aliquots was evaporated and the lipid content determined gravimetrically. The remaining extracts were concentrated to 1.5 mL and purified by GPC with cyclohexane/ethyl acetate (1:1) as the eluent. The $MeSO_2$-PCB fraction was collected and the volume was reduced to 1 mL. These solutions were transfered to partially deactivated (5% water) aluminum oxide (5 g) open columns. Two fractions were taken from the column, first a *n*-hexane (20 mL) fraction followed by a dichloromethane (30 mL) fraction, where the $MeSO_2$-PCBs appeared. The solution was finally purified on open silica gel (3 g) columns mixed with KOH (1 M, 1.0 mL) according to [16]. Then, the sulfones were eluted with *n*-hexane/dichloromethane (1:1, 40 mL), isooctane (50 µL) was added to each sample and prior to GC-ECD and GC-MS analysis the volume was reduced to 50 µL.

Results and Discussion

Eight atropisomeric $MeSO_2$-PCBs previously found in environmental samples [6,9,10] were separated into their enantiomers by enantioselective GC. The chemical structures of the $MeSO_2$-PCBs and gas chromatograms are shown in Fig. 1. In the present work, the separation of the

latter PCB-metabolites was achieved on a derivatized cyclodextrine column (heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-hexyldimethylsilyl)-β-cyclodextrin). The $MeSO_2$-PCBs exert strong interactions with the chiral cyclodextrine phase and due to the rather low maximum temperature for the column (195°C), the retention times were long (from 50 min, 3-91, to 130 min, 4-174) despite the short length of the column (10 m). The peak width at half height was 30 s for the $MeSO_2$-pentaCBs and increased to up to 110 s for the $MeSO_2$-heptaCBs. Baseline separation was observed for most enantiomers and constitutional isomers. Only the $MeSO_2$-PCBs 3-91b/4-91b (Fig. 1a) were not baseline separated.

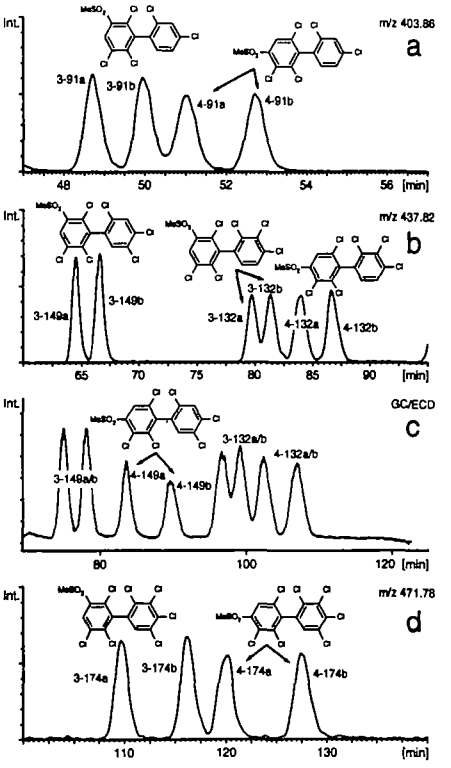

Figure 1
Chiral GC-MS ion chromatograms of seven $MeSO_2$-PCBs: 3-91, 4-91; 3-149; 3-132, 4-132; 3-174 and 4-174 resolved into their enantiomers (Fig. 1a, 1b and 1d) and a GC-ECD chromatogram with the enantiomers of 3-149, 4-149, 3-132 and 4-132 (Fig. 1c). The structural formulae and abbreviations of the sulfones are given. Methods for synthesis are described in [17,18].

Analysis of liver sample extracts

Two $MeSO_2$-PCBs (3-149 and 3-132) were detected in all liver samples by ECD, using an achiral column (NE 54), where for one sample GC/MS full scan analysis was applied. The mass spectra of 3-$MeSO_2$-PCBs 3-149 and 3-132 contain residual ions from matrix constituents, but clearly show the characteristic signals of hexachloro-methylsulfonyl-PCBs. The absence of interfering signals in the gas chromatographic peaks of the analytes was confirmed using the expected isotopic ratio of the two most intense signals from the molecular ion clusters of the target compounds. The assignment of enantiomer peaks by the retention time of the standards was also confirmed by the standard addition method.

Selective liver retention of the 3-132 and 3-149 $MeSO_2$-PCBs is observed, which is in agreement with previously reported findings for humans [9]. Weistrand and Noren report 3-132 and 3-149

levels that are somewhat higher than the levels determined in this study, where concentrations ranged between 300 to 3900 pg/g lipids for 3-132 and 10 to 700 pg/g lipids for 3-149. Still though, the concentrations are in about the same range. Further, the ratios 3-132/3-149 in the present study are similar to the mean ratio (10:1) reported there.
In all five liver samples exclusively the second eluting enantiomer of the two $MeSO_2$-PCBs 3-132 and 3-149 was found.
BCPS and 3-$MeSO_2$-DDE were detected in all liver samples in not negligable amounts, too.

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Literature

1 Jensen, S., *New Scientist* **1966**, *32*, 612
2 Jensen, S.; Jansson, B., *Ambio* **1976**, *5*, 257-260
3 Bergman, Å.; Haraguchi, K.; Kuroki, H.; Masuda, Y.; Olsson, M.; *Organohalogen Compounds* **1992**, *8*, 311-312
4 De Voogt, P.; van Raat, P.; Rozemeijer, M., *Organohalogen Compounds* **1996**, *28*, 517-521
5 Klasson-Wehler, E.; Bergman, Å.; Athanasiadou, M.; Ludwig, J. P.; Auman, H. J.; Kannan, K.; van den Berg, M.; Murk, A. J.; Feyk, L. A.; Giesy, J. P., *Environm. Toxicol. Chem.* **1998**, in press
6 Bergman, Å.; Norstrom, R. J.; Haraguchi, K.; Kuroki, H.; Béland, P., Environm. Toxicol. Chem. **1994**, *13*, 121-128
7 Letcher, R. J.; Norstrom, R. J.; Bergman, Å., *Sci. Tot. Environm.* **1995**, *160/161*, 409-420
8 Haraguchi, K.; Kuroki, H.; Masuda, Y., *Chemosphere* **1986**, *15*, 2027-2030
9 Weistrand, C.; Norén, K., *Environm. Health Perspec.* **1997**, *105*, 644-649
10 Bergman, Å.; Haraguchi, K.; Athanasiadou, M.; Larsson, C., *Organohalogen Compounds* **1993**, *14*, 199-201
11 Kato, Y.; Haraguchi, K.; Kawashima, M.; Yamada, S.; Isogai, M.; Masuda, Y.; Kimura, R., Chem. Biol. Interact. **1995**, *95*, 269-278
12 Kato, Y.; Haraguchi, K.; Tomiyasu, K.; Saito, H.; Isogai, M.; Masuda, Y.; Kimura, R., *Environm. Toxicol. Pharmacol.* **1997**, *3*, 137-144
13 Hardt, I.H.; Wolf, C.; Gehrke, B.; Hochmuth, D. H.; Pfaffenberger, B.; Hühnerfuss, H.; König, W. A., J. High Res. Chrom. **1994**, *17*, 859-864
14 Bergman, Å.; Wachtmeister, C. A., *Acta Chem. Scand. B.* **1977**, *31*, 90-91
15 Ellerichmann, T., Bergman, Å., Franke, S., Hühnerfuss, H., Jakobsson, E., König, W.A., Larsson, C., *Fresenius Envir. Bull.* **1998**, *7*, 244-257
16 Letcher R.J., Norstrom R.J., *Analytical Chemistry* **1995**, *67*, 4155
17 Haraguchi, K.; Kuroki, H.; Masuda, Y., *J. Agric. Food Chem.* **1987**, *35*, 178
18 Bergman, Å.; Wachtmeister, C. A., *Chemosphere* **1978**, *7*, 949-956