Isolation and structure elucidation of a further persistent octachloro congener of toxaphene from grey seal blubber

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Abstract

A persistent octachlorobornane was isolated from an extract of 1.7 kg grey seal blubber by liquid chromatography on 50 g silica gel followed by reversed phase HPLC on a C18 column. With GC-MS and ^{1}H-NMR-investigations we established the structure as 2-endo,3-exo,5-endo,6-exo, 8,8,9,10-octachlorobornane (B8-1412). Furthermore, it was shown that B8-1412 is a major compound of technical toxaphene (CTT) in adipose tissue of penguin and seal.

Introduction

Toxaphene is one of the world's most applied organochlorine pesticides[1)]. Technical mixtures of toxaphene contain several hundred compounds but only a few CTTs are bioaccumulated in species on a high trophic level[2)]. The goal of this study was the isolation of a so far unknown, persistent octachlorobornane which was detected together with two major CTTs B8-1413 (Parlar #26) and B9-1679 (Parlar #50) in seal species [3)4)]. The knowledge of the structure of these CTTs is important for understanding the factors leading the persistence.

Experimental Methods

Sample materials and matrix separation. Blubber of a grey seal (*Halichoerus grypus*) was taken from an adult male animal from the Baltic Sea. Adipose tissue of an Adelie penguin (*Pycoscelis adelis*) and blubber of a Weddell seal (*Leptonychotes weddellii*) were from animals from the Antarctic. Matrix was separated from 5 g or 2 g tissue as described in detail before [5)].
Solvents, chemicals, and reference standards. Silica gel, anhydrous sodium sulfate, and acetonitrile were obtained from E. Merck (Darmstadt, Germany) and n-hexane was from Promochem (Wesel, Germany). Standard solutions of B8-1413 (Parlar #26), B9-1679 (Parlar #50), B8-2229 (Parlar #44), B8-1414 (Parlar #40), B8-1945 (Parlar #41), and B9-1025 (Parlar #62) were from Dr. Ehrenstorfer (Augsburg, Germany). B7-515 (Parlar #32) was from Promochem (Wesel, Germany). B7-1453 was recently isolated in our lab [6)].
Liquid chromatography (LC). A 2.3 cm i.d. x 60 cm length chromatography tube with a frit was filled with 50 g silica gel (activated at 130°C for 18 hours) slurred in n-hexane. It was topped with a layer of 2 cm anhydrous sodium sulphate. The column was eluted with n-hexane.

Reversed phase high performance liquid chromatography (RP-HPLC). We used a LC-18 DB column (25 cm x 4.6 mm i.d., 5 µm) from Supelco (Deisenhofen, Germany) together with a HPLC Pump 64 (Knauer, Berlin, Germany) and a UV Detector 116 (Gilson, USA) operated at 254 nm. The eluent was acetonitrile/water 86:14 (v/v) at a flow rate of 0.9 mL/min. 95 µL were injected into a 100 µL sample loop.

Gas chromatography with electron capture detector (GC-ECD). Sample extracts, LC- and HPLC-fractions were analysed on an HP 5890 II gas chromatograph (Hewlett-Packard, Germany) equipped with two capillary columns (CP-Sil 2, CP-Sil 8/C18 20%; both Chrompack, Middelburg, The Netherlands) and two ^{63}Ni electron capture detectors. Nitrogen was used as carrier and make-up gas [7]. Enantiomer separations were performed on 35% heptakis (6-*O*-*t*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin diluted in OV 1701 (β-TBDM). The β-TBDM fused silica column (20 m x 0.25 mm i.d., 0.15 µm film thickness) was obtained from M. D. Müller, Eidgenössische Forschungsanstalt Wädenswil, Switzerland.

Isolation of B8-1412. The matrix of 1.7 kg grey seal blubber was separated as described before[8]. The resulting organochlorine extract in n-hexane was evaporated to approx. 2 mL and topped on the silica gel column. After elution of 250 mL, fractions of 25 mL were collected and analyzed by GC-ECD. B8-1412 containing fractions were combined, evaporated and dissolved in approx. 8 mL acetonitrile/water 86:14 (v/v) at 80°C. After blowing down with nitrogen to 4 mL this sample was further fractionated by RP-HPLC. Since CTTs gave no signal in the UV-detector, droplets were collected after a significant signal in the detector which was caused by a UV-active compound in the sample. Fractionating was started 80 droplets after a prominent UV-signal at 10.2 min. Seven fractions of 10 droplets were sampled and after extraction with n-hexane analyzed by GC-ECD. The fractions from 100 to 130 droplets contained the main amount of B8-1412 (approx. 7 µg) and allowed GC/ECNI-MS, GC/EI-MS, and ^{1}H-NMR-measurements (in $CDCl_3$).

Results and Discussion

Isolation of B8-1412. LC on silica gel allowed quantitative separation of PCBs (and other aromatic organochlorines) present at one to two orders of magnitude higher concentrations. PCBs were quantitatively eluted in front of CTTs and a lot of other organochlorines (up to 350 mL). Furthermore, the CTTs were also distributed. In agreement with recent findings [9], several important CTTs eluted on silica gel in the following order:

B8-1413 (Parlar #26) < B7-1453 < **B8-1412** < B9-1679 (Parlar #50) ≈ B8-2229 (Parlar #44) < B8-1414 (Parlar #40) < B8-1945 (Parlar #41) < B9-1025 (Parlar #62) < B7-515 (Parlar #32).

Note that the first eluted, more nonpolar CTTs are particulary persistent. B8-1412 was detected in the fractions from 500 mL to 700 mL. These fractions contained the CTTs in the first line above while the CTTs in the second line were already separated on silica. On RP-HPLC, the remaining CTTs eluted in the following order:

B7-1453 < **B8-1412** < B8-2229 (Parlar #44) < B9-1679 (Parlar #50) < B8-1413 (Parlar #26).

As expected, the elution order on a C18 column was different from silica gel. Particular B8-1413 (Parlar #26) and B9-1679 (Parlar #50) eluted later on C18 and could easily be removed from B8-1412. B7-1453 and B8-2229 (Parlar #44) were only partly separated. However, B8-1412 was the dominating compound in this fraction. The combination of LC with silica gel (separation of

nonpolar organochlorines like PCBs and more polar CTTs) and RP-HPLC with a C18 column (separation of CTT congeners) seems to be very efficient for the isolation of CTTs such as B8-1412. Figure 1 shows a part of the GC-ECD chromatogram of the ^{1}H-NMR-solution. It contained also some unknown compounds in low concentrations which, however, did not hinder structure elucidation.

The structure of the isolated compound was elucidated by GC/EI-MS and ^{1}H-NMR-measurements as: 2-endo,3-exo,5-endo,6-exo,8,8,9,10-octachlorobornane (B8-1412). The following lines give the ^{1}H-NMR data (1 = number of the carbon atom the proton is attached to; 2 = chemical shift [ppm]; 3 = signal multiplicity; 4 = coupling constants [Hz]):

H8[1]: 6.840[2] d[3] (1.85[4]); H2-exo: 5.094 dd (4.93, 0.64); H9b: 4.895 d (13.06); H6-endo: 4.746 d (5.57); H3-endo: 4.735 d (4.93); H5-exo: 4.691 dd (5.57, 4.12); H9a: 4.295 dd (13.06, 1.85); H10a: 4.013 d (13.03); H10b: dd (13.03, 0.64); H4: 3.268 m (4.12).

The detailed description of the structure elucidation was recently presented[7]. Figure 2 shows the structure-model of B8-1412.

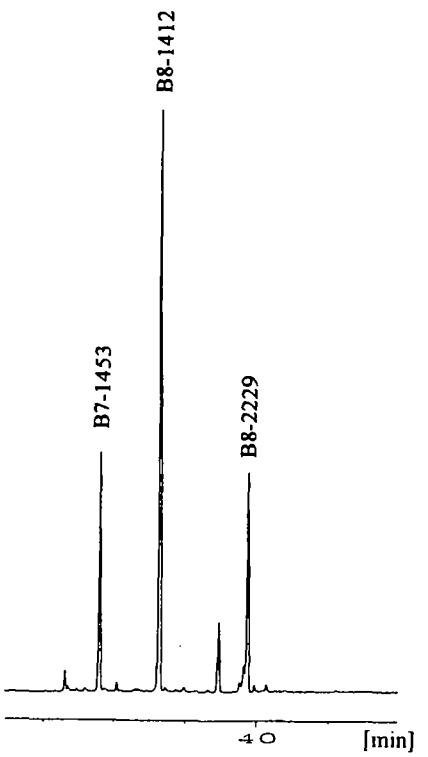

Figure 1: GC-ECD chromatogram (part) of the ^{1}H-NMR-solution on a CP-Sil 2 column

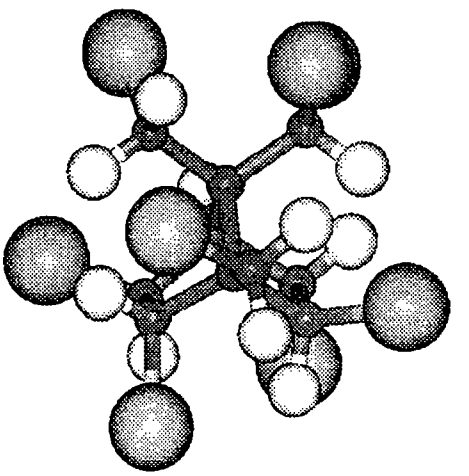

Figure 2: Structure-model of B8-1412

In accordance with the most chlorobornanes, B8-1412 is chiral. Several CTT enantiomers were separated on tert.-butyldimethylsilylated ß-cyclodextrin (ß-BSCD)[10]. So far, ß-BSCD was the only chiral stationary phase successfully applied to enantiomer separation of CTTs [10].

However, enantiomer separation of B8-1412 was poor on ß-BSCD[7]. On the other hand, we found improved enantiomer separation of B8-1412 on 35 % β-TBDM diluted in OV 1701 (Figure 3). On this phase we separated several chiral organochlorines, but enantiomer separation of B8-1413 (Parlar #26) and B9-1679 (Parlar #50) failed on ß-TBDM[11]. Furthermore, we found that ß-TBDM also separated the enantiomers of B7-1453, B8-1945 (Parlar #41), B8-1414 (Parlar #40), and B9-1025 (Parlar #62)[12]. Therefore, ß-TBDM seems to be a reasonable addition to ß-BSCD with respect to enantiomer separation of CTTs.

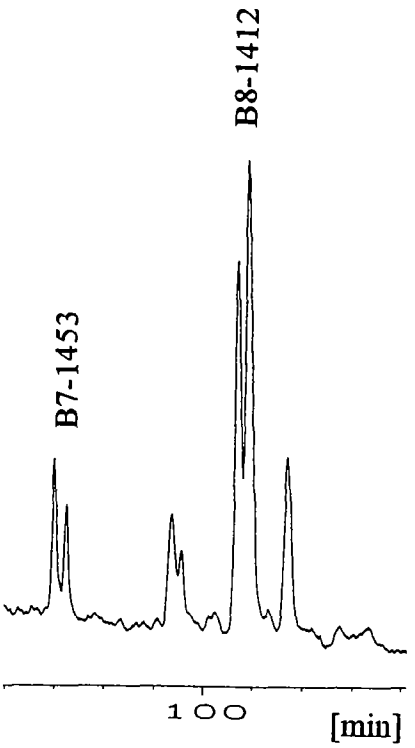

Figure 3: GC-ECD chromatogram (part) of the enantiomer separation of B8-1412 and B7-1453 in grey seal blubber on β-TBDM
GC oven program: 120°C (2 min), 15°C/min to 140°C (150 min), 20°C/min to 220°C (10 min)

Detection of B8-1412 in environmental samples by GC-ECD. Prior to GC-ECD quantitation, PCBs were separated on 8.0 g activated silica gel eluted with 48 mL n-hexane. After that, CTTs were eluted with 50 mL n-hexane/toluene (65:35, v/v)[9]. Figure 4 shows GC-ECD chromatograms of the CTT containing (n-hexane/toluene) fraction of adipose tissue of an Adelie penguin (*Pycoscelis adelis*), blubber of Baltic grey seal (*Halichoerus grypus*) and Antarctic Weddell seal (*Leptonychotes weddellii*). Important CTTs are labeled. Although B8-1412 was not quantified due to the lack of a calibrated standard solution, these examples clearly demonstrate that B8-1412 is a major persistent CTTs in environmental samples. E. g. in the Adelie penguin sample, the ECD signal of B8-1412 reached the height of B8-1413 (Parlar #26) (Figure 4a). Thus, a commercially available B8-1412 standard is important for the congener specific determination of CTTs.

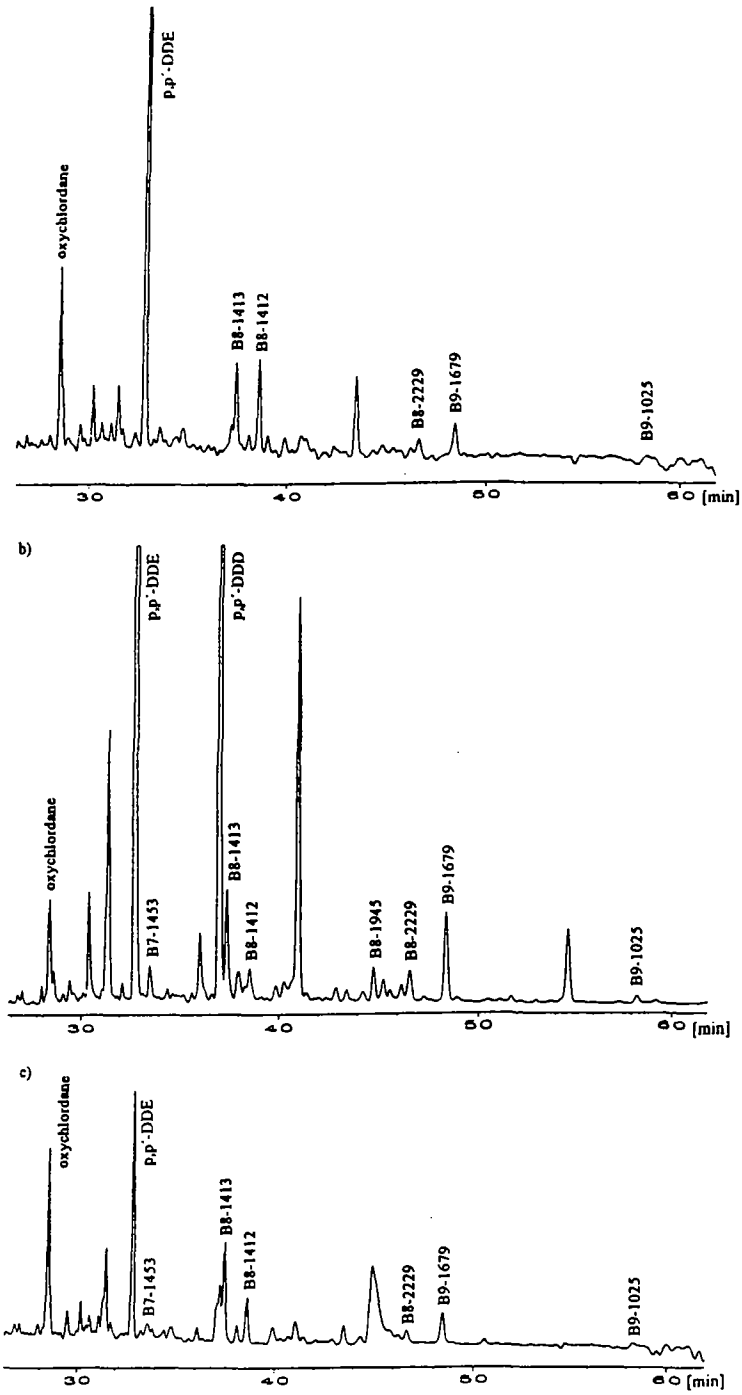

Figure 4: GC-ECD chromatograms of the aliphatic fractions of adipose tissue of Adelie penguin (a), Baltic grey seal (b), and Antarctic Weddell seal (c)

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