ELSEVIER

Journal of Chromatography A, 973 (2002) 123–133

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Chromatographic enrichment and enantiomer separation of axially chiral polybrominated biphenyls in a technical mixture

Urs Berger[a,*], Walter Vetter[b], Arntraut Götsch[b], Roland Kallenborn[a]

[a]*Norwegian Institute for Air Research (NILU), The Polar Environmental Centre, Hjalmar Johansens gt. 14, NO-9296 Tromsø, Norway*
[b]*Department of Food Chemistry, Friedrich-Schiller-University Jena, Dornburger Str. 25, DE-07743 Jena, Germany*

Received 29 April 2002; received in revised form 23 July 2002; accepted 24 July 2002

Abstract

The separation properties of different chromatographic methods regarding the enantioselective separation of axially chiral (atropisomeric) polybrominated biphenyls (PBB) were studied. For this purpose, the technical hexabromobiphenyl product Firemaster BP-6® was characterised by gas-chromatography coupled to electron capture detection (GC/ECD) and electron-capture negative ion mass spectrometry (GC/ECNI-MS) as well as by liquid chromatographic fractionating on active carbon and celite. Twelve individual PBBs including potential atropisomeric PBBs were isolated from Firemaster BP-6 by reversed-phase high-performance liquid chromatography (HPLC) on three serially coupled octadecylsilane columns. Six of the 12 isolated PBBs (three tri-*ortho* and di-*ortho* substituted PBBs, respectively) were separated into atropisomers on a HPLC column containing permethylated β-cyclodextrin on silica. Moreover, the temperature dependency of the enantiomer separations is discussed. Gas chromatographic enantiomer separation of PBBs is a very demanding task due to high elution temperatures. However, the atropisomers of one tri-*ortho* substituted PBB congener (PBB 149) could be resolved on a column coated with randomly modified heptakis(6-*O*-*tert*.-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin in OV 1701.

Keywords: Enantiomer separation; Atropisomerism; Firemaster BP-6; Polybrominated biphenyls; Cyclodextrins

1. Introduction

Technical products of polybrominated biphenyls (trade names Firemaster BP-6® or Firemaster FF-1®, Bromkal 80® and Flammex-B®) have been extensively used as flame-retardants in textile and electronic industries and as additives in plastics [1]. The structural similarity of polybrominated biphenyls (PBBs) to polychlorinated biphenyls (PCBs) brought PBBs already in the 1970s into the focus of environmental chemistry research [2,3]. The toxicological threat of this group of compounds was confirmed in 1973 when human food was accidentally contaminated with PBBs in Michigan [4–6]. In the late 1970s, the US industry voluntarily discontinued the production of PBBs. Despite a continuous reduction of the world-wide annual production that started already with the mentioned restrictions in the USA, the presence of PBBs in the environment was confirmed in a wide range of samples [7–9]. Recently, the European Union (EU) Commission has

*Corresponding author. Tel.: +47-7775-0385; fax: +47-7775-0376.

E-mail address: urs.berger@nilu.no (U. Berger).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)01112-3

concluded, ''research undertaken in the area of electrical and electronic equipment indicates that adequate fire safety of such equipment could be achieved without using PBB and polybrominated diphenyl ether (PBDE)''. Thus, other flame-retardants in electrical and electronic equipment should replace PBBs and PBDEs in the EU by January 2008 [10].

Likewise their chlorinated analogues, PBBs exist in a theoretical variety of 209 congeners [1]. Under environmental conditions, many PBB congeners containing two to four bromine *ortho*-substituents cannot fully rotate about the interannular phenyl–phenyl bond due to steric hindrance. Rotation hindered PBB congeners, which additionally possess asymmetric substitution patterns on both ring systems lack a symmetry plane and are axially chiral. This special form of chirality is called atropisomerism (Fig. 1) [11]. Seventy-eight of the 209 congeners have no symmetry element but only those with an energy barrier that is high enough to prevent full rotation form stable atropisomers. Nineteen axially chiral PCBs proved to be stable (high rotation barrier) under environmental conditions [12,13]. The presence of seven atropisomeric PCBs has already been confirmed in samples from the marine and terrestrial environment as well as in human milk extracts [14–18]. However, due to the more bulky bromine substituent (covalent radii for Cl=99 pm and Br=114 pm), it could be assumed that more than 19 atropisomeric PBBs exist at physiological temperatures.

The purpose of this work was to evaluate different chromatographic tools for the enantioselective separation of atropisomeric PBB congeners. Since such PBB congeners are not commercially available, the components of a technical product (Firemaster BP-6) had to be identified and relevant PBBs isolated. The high molecular masses of PBBs (pentabromo 548.7 Da, hexabromo 627.6 Da, heptabromo 706.5 Da, octabromo 785.4 Da) require high elution temperatures (typically $>200\,^{\circ}C$) in gas chromatography (GC). These temperatures are close to the maximum operational temperatures for chiral stationary GC phases. Furthermore, enantiomerization of an axially chiral PBB congener is likely to occur at elevated temperatures, though the same PBB congener may form stable atropisomers under physiological conditions. Therefore, both enantioselective GC and high-performance liquid chromatography (HPLC) were employed in this study on enantiomeric resolution of PBB atropisomers.

2. Experimental

2.1. Chemicals

Technical hexabromobiphenyl (Firemaster BP-6, 10 mg, Michigan Chemicals) was purchased from Promochem (LGC Promochem AB, Borås, Sweden). The single standards 2,2′,4,5,5′-pentabromobiphenyl (PBB 101, 5 mg) and 2,2′,4,4′,5,5′-hexabromobiphenyl (PBB 153, 5 mg) were obtained from Dr Ehrenstorfer GmbH (Augsburg, Germany). Envicarb (catalogue no. 57210-U) and celite 545-AW

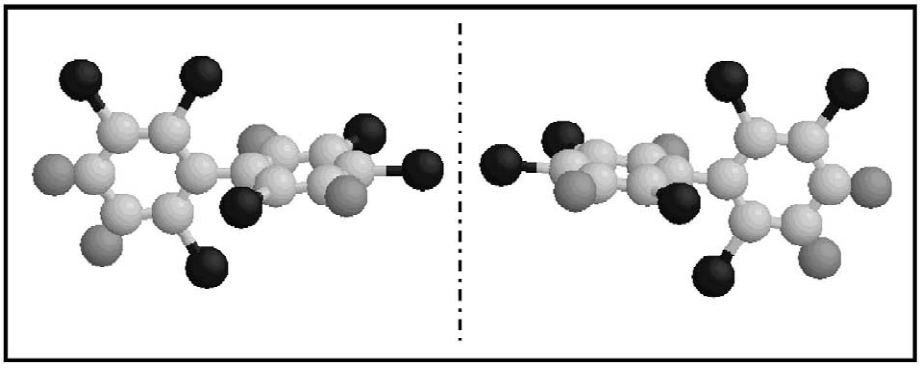

Fig. 1. Structure of the atropisomers of the axially chiral 2,2′,3,4′,5′,6-hexabromobiphenyl (PBB 149). Carbon is shown in light grey, hydrogen in dark grey and bromine in black.

(catalogue no. 2-0199) were purchased from Supelco (Deisenheim, Germany). Acetonitrile (HPLC gradient grade) was obtained from Baker (Mallinckrodt Baker B.V., Deventer, The Netherlands) and *n*-hexane (Uvasol®, for spectroscopy) and toluene (SupraSolv®, for organic trace analysis) from Merck (Darmstadt, Germany). Demineralised water was purified with a Milli-Q system (Millipore, Molsheim, France). Helium carrier gas (quality 6.0, Hydrogas, Porsgrunn, Norway or, alternatively, quality 5.0, Institute of Physics, University of Jena, Germany), methane reactant gas (quality 5.5, Hydrogas, Porsgrunn, Norway) and nitrogen make-up gas (quality 5.0, Linde, Leuna, Germany) were used for gas chromatographic analyses.

2.2. Fractionating of technical PBB mixture

A mixture of 2 g envicarb and 2 g celite 545-AW was slurry packed into a 1.0-cm I.D. glass column. The column was rinsed with 50 ml of *n*-hexane. The sample (27 μg Firemaster BP-6 in 1 ml *n*-hexane) was placed onto the column and eluted with *n*-hexane. Fractions of 4 ml (elution volume 8–40 ml) and 20 ml (elution volume 40–100 ml) were collected and analysed by GC/ECD and/or GC/ECNI-MS (see below).

2.3. High-performance liquid chromatography (HPLC)

2.3.1. HPLC systems

Three different HPLC systems (systems A, B and C) were employed. System A consisted of a series 200 binary gradient HPLC pump (Perkin-Elmer, Überlingen, Germany), a manual six-port injection valve equipped with a 20-μl loop (Rheodyne, Rohnert Park, CA, USA), an SPD-7A UV detector (Shimadzu, Jena, Germany), and a HP 3395 integrator (Agilent, Waldbronn, Germany). System B was built up from a GT-104 degasser unit, an LC-10AT VP quaternary gradient pump (both Shimadzu), a manual six-port injection valve equipped with a 20-μl loop (Rheodyne), a CTO-10AS VP column oven, an SPD-6A UV detector (both Shimadzu) and a HP 3395 integrator (Agilent). System C consisted of a GT-104 degasser, an LC-10AT quaternary gradient pump, an SIL-10A auto-injector equipped with a 50-μl loop, and an SPD-M10A VP photodiode array UV detector (all Shimadzu).

2.3.2. Semi-preparative HPLC

Semi-preparative separation of technical hexabromobiphenyl was carried out at room temperature (~25 °C) on system A or, alternatively, on system C, employing an isocratic flow of 1.2 ml/min acetonitrile/water (94:6; v/v). Three reversed-phase HPLC columns coupled in series were used. The first and the middle column were Prodigy ODS(3) (octadecylsilane, 15.5% carbon load, endcapped, 250 mm column length, 4.6 mm I.D., 5-μm particles, 100 Å pore size, Phenomenex, Torrance, CA, USA) and the third column was a Luna C_{18}(2) (octadecylsilane, 17.5% carbon load, 250 mm column length, 4.6 mm I.D., 5-μm particles, 100 Å pore size, Phenomenex). Approximately 50 μg technical hexabromobiphenyl in 20 μl acetonitrile was injected per chromatographic run and fractions of eluting PBB congeners (0.5–2 ml, depending on signal width) were collected manually during the detection of the compounds; 50–100 μl of each fraction were evaporated to dryness under a flow of nitrogen and the residues re-dissolved in 100 μl of *n*-hexane for GC analysis.

2.3.3. Enantioselective HPLC

Enantioselective separation of PBB congeners isolated by semi-preparative HPLC was performed on system A or, alternatively, on system B using the following HPLC columns: (i) α-PMCD: Nucleodex [α]-PM (hexakis(2,3,6-tri-*O*-methyl)-α-cyclodextrin on silica, 200 mm column length, 4 mm I.D., Macherey-Nagel, Düren, Germany). (ii) β-PMCD: Nucleodex [β]-PM (heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin on silica, 200 mm column length, 4 mm I.D., Macherey-Nagel). (iii) γ-PMCD: Nucleodex [γ]-PM (octakis(2,3,6-tri-*O*-methyl)-γ-cyclodextrin on silica, 200 mm column length, 4 mm I.D., Macherey-Nagel). Enantioselective separation on all columns was tested first at room temperature with an isocratic flow of 0.3 ml/min acetonitrile/water (75:25; v/v). The conditions for the β-PMCD column on system B were then optimised to 5 °C and an isocratic flow of 0.5 ml/min acetonitrile/water (60:40; v/v). Fractions from enantiomer separations (first and last eluting enantiomer as well as a fraction

in between) were collected manually. Approximately 20 drops of each fraction were evaporated to dryness and the residues re-dissolved in 100 μl of *n*-hexane for GC analysis.

2.4. Gas chromatography (GC)

2.4.1. GC–mass spectrometry

Technical hexabromobiphenyl was analysed on a Mega II 8065 gas chromatograph (Fisons, Milan, Italy) coupled to an MD800 low-resolution quadrupole mass spectrometer (Finnigan, San Jose, CA, USA) operated in the electron-capture negative ion mode (GC/ECNI-MS). Samples (2 μl, solvent *n*-hexane) were injected on-column with an AS800 auto-injection system (Fisons) onto a DB5MS capillary column (phenyl arylene polymer, 15 m column length, 0.25 mm I.D., 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). Separations were performed using helium carrier gas at a constant column head pressure of 80 kPa, applying the following temperature program: 110 °C (hold time 2 min), then at 12 °C/min to 180 °C (hold time 5 min) and at 5 °C/min to the final temperature of 280 °C (hold time 10 min). The interface temperature was held at 250 °C and the ECNI ion source temperature at 160 °C. The mass spectrometer was operated in full scan mode (*m/z* 75–800) for signal identification or in single ion monitoring mode (SIM) for more sensitive detection, using the 10 characteristic ions *m/z* 78.9, 80.9 (bromide), 547.6, 549.6 (from $[M]^-$ of pentabromobiphenyls), 625.5, 627.5 (from $[M]^-$ of hexabromobiphenyls), 705.4, 707.4 (from $[M]^-$ of heptabromobiphenyls), 783.3, and 785.3 (from $[M]^-$ of octabromobiphenyls).

2.4.2. GC-electron-capture detection

A HP 5890 series II gas chromatograph (Agilent, Waldbronn, Germany) equipped with two parallel capillary columns (CP-Sil 2 and 80% CP-Sil 8/20% octadecylmethyl polysiloxane, respectively, both 50 m column length, 0.25 mm I.D., 0.25 μm film thickness, Chrompack, Middelburg, The Netherlands) and two ^{63}Ni-electron-capture detectors (ECD) were used for analysis of technical hexabromobiphenyl and fractions from HPLC separations. Samples (1 μl, solvent *n*-hexane) were injected splitless (splitless time 1.5 min) at an injector temperature of 250 °C employing a HP 7673 auto-injection system (Agilent). The helium carrier gas (1.3 ml/min) was split onto the two columns using a T-piece from Gerstel (Mülheim/Ruhr, Germany). The following temperature program was applied: 60 °C (hold time 1.5 min), then at 40 °C/min to 150 °C (hold time 5 min), at 2 °C/min to 230 °C and at 5 °C/min to the final temperature of 270 °C (hold time 45 min). The detectors were operated at 300 °C with nitrogen as make-up gas.

2.4.3. Enantioselective GC

Gas chromatographic enantiomer separation of PBB congeners was studied using GC/ECD and GC/MS systems previously described in detail [19,20]. The helium carrier gas was kept at a constant flow of 1.3 ml/min if not explicitly mentioned. Temperature programs were developed in the following way. Standards were eluted within a heating ramp of 1.5 °C/min starting from 150 °C. After determination of the elution temperature, isothermal analyses were carried out with plateaus at 10, 20 and 30 °C below the elution temperature determined in the temperature programmed run. In the case of a partial resolution, the flow-rate was also set to 2 ml/min. The following chiral capillary columns were tested: (i) CB-β-PMCD: Chirasil-Dex (10% heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin chemically bonded to CP-Sil 5, 25 m column length, 0.25 mm I.D., 0.25 μm film thickness, Varian-Chrompack, The Netherlands). (ii) β-BSCD: (25% heptakis(2,3,6-tri-*O-tert.*-butyldimethylsilyl)-β-cyclodextrin in 85% dimethyl/15% diphenyl polysiloxane, 30 m column length, 0.25 mm I.D., 0.2 μm film thickness, BGB Analytik, Adliswil, Switzerland). (iii) β-TBDM: (35% randomly modified heptakis(6-*O-tert.*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin in OV 1701 (85% methyl/7% phenyl/7% cyanopropyl/1% vinyl polysiloxane), 20 m column length, 0.25 mm I.D., 0.15 μm film thickness, BGB Analytik).

3. Results and discussion

3.1. Initial experiments for characterisation of Firemaster BP-6 and for identification of potential atropisomeric PBBs

Though the composition of the technical hexa-

bromobiphenyl mixture Firemaster BP-6 was already studied in the 1970s, there is still a need to further characterize this product with respect to the identification of potential atropisomeric PBB congeners, since this topic has not been considered in previous work [21–23]. Initial characterization started with GC/ECD and GC/ECNI-MS experiments conducted both in full scan and single ion monitoring (SIM) mode (Fig. 2A). The following criteria were used for PBB congener identification:

- The identity of major PBB congeners in Firemaster BP-6 was derived from literature data [21–23]. Ratios of signal areas in GC/ECD and GC/ECNI-MS single ion monitoring (m/z 79 and 81) were compared with each other as well as with reports on the quantitative composition of Firemaster BP-6 [21–23].
- Two individual PBB standards (PBB 101 and PBB 153) were available for verification.
- The elution order of PBB congeners on different GC columns was supposed to be similar to the corresponding PCB congeners on the same columns.
- GC/ECNI-MS in the full scan mode resulted in mass spectra, which mainly consisted of both bromide ions (m/z 79 and 81) and molecular ions ($[M]^-$) [24]. The degree of bromination for each congener was derived from the isotope cluster of $[M]^-$.

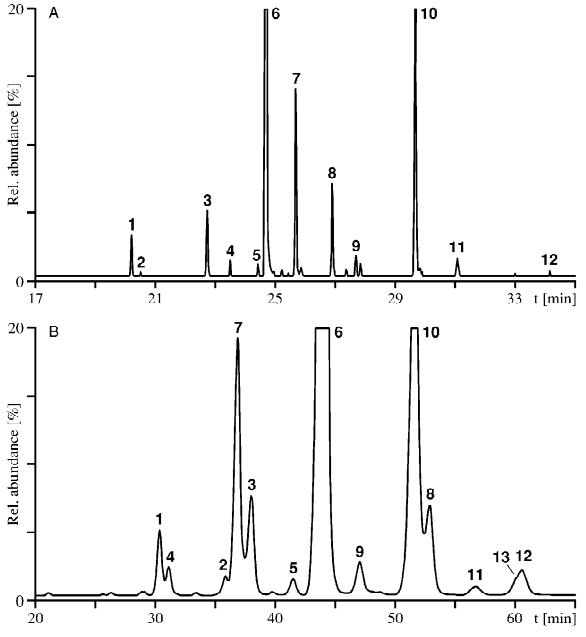

Fig. 2. (A) Extracted mass chromatogram (m/z 79+81) from a GC/ECNI-MS full scan analysis of 54 ng Firemaster BP-6. (B) HPLC/UV (220 nm) chromatogram of approximately 50 μg Firemaster BP-6. The relative abundances of signals No. 6 (100% in A and B) and 10 (22% in A, 25% in B) exceed the range shown. For signal identification, see Table 1.

Twelve compounds in the technical Firemaster product (Fig. 2A) were studied in detail (Table 1). Apart from peaks No. 2 (PBB 99) and No. 11 (PBB 170), the identification of PBBs can be considered as certain based on the criteria listed above. Interestingly, the bromide ion was only low abundant in the GC/ECNI mass spectrum of PBB 194 (the only octabromobiphenyl congener investigated) whose mass spectrum was mainly restricted to the molecular ion. Owing to that, the relative amount of PBB 194 in the mixture is underestimated by GC/ECNI-MS using m/z 79 and 81 compared to penta- to heptabromo congeners.

To further verify the peak assignment in Firemaster BP-6, as well as for identification of potential atropisomeric PBBs, liquid chromatographic fractionating on envicarb and celite (see Experimental) was carried out. Active carbon is often used as stationary phase in chromatography for separation of planar non-*ortho* and mono-*ortho* substituted biphenyls from the poly-*ortho* fraction [25,26]. Recently, this method has also been used for enrichment of PCB atropisomers [27]. The present fractionating on envicarb/celite allowed quantitative separation of mono-*ortho* from poly-*ortho* substituted PBB congeners in Firemaster BP-6 (Fig. 3). However, no separation of di-*ortho* from tri-*ortho* substituted PBBs was obtained. While this investigation additionally confirmed the assignment of structures to the PBB congeners (Table 1), it also indicated that distortion from planarity was already remarkable for di-*ortho* substituted PBBs and, thus, some of them might exist as stable atropisomers at physiological temperatures (<50 °C). In view of the high temperatures mandatory for elution of PBBs from GC columns (see Introduction), enantioselective HPLC was the first choice for initial experiments on PBB atropisomer separation. Since enantioselective HPLC phases do not provide the resolution and selectivity necessary for identification of PBB atropisomers in the technical product, an initial frac-

Table 1
Identification and characterisation of PBB congeners in technical hexabromobiphenyl Firemaster BP-6 by GC and HPLC. Signal numbers refer to Fig. 2

Signal no.	Bromine no.	Tentative structure IUPAC number; positions of Br atoms	Rt (min)				λ_{max} (nm) HPLC/DAD	Area (%) GC/ ECNI-MS[a]	Area (%) HPLC/DAD
			GC DB5MS	GC CP-Sil 2	GC CP-Sil 8	HPLC $3\times C_{18}$			
1	5	PBB 101 2,2′,4,5,5′	20.2	54.9	52.8	30.5	213	1.8	1.8
2	5	PBB 99 2,2′,4,4′,5	20.5	55.5	53.5	35.9	216	0.1	0.5
3	5	PBB 118 2,3′,4,4′,5	22.7	58.9	57.4	38.1	218	3.0	3.4
4	6	PBB 149 2,2′,3,4′,5′,6	23.5	60.2	57.7	31.2	212	0.6	0.9
5	6	PBB 132 2,2′,3,3′,4,6′	24.4	62.1	60.3	41.6	213	0.5	0.7
6	6	PBB 153 2,2′,4,4′,5,5′	24.7	62.5	60.8	44.1	215	60.2	59.6
7	6	PBB 138 2,2′,3,4,4′,5′	25.7	64.7	62.3	36.9	214	8.8	8.8
8	6	PBB 167 2,3′,4,4′,5,5′	26.9	68.0	66.7	53.0	224	4.3	3.3
9	7	PBB 174 2,2′,3,3′,4,5,6′	27.7	70.4	67.5	47.2	219	1.1	1.5
10	7	PBB 180 2,2′,3,4,4′,5,5′	29.6	76.9	74.8	51.7	221	16.0	16.7
11	7	PBB 170 2,2′,3,3′,4,4′,5	31.1	n.a.[b]	n.a.[b]	56.8	222	1.2	0.5
12	8	PBB 194 2,2′,3,3′,4,4′,5,5′	34.1	105.3	103.7	60.7	221	0.1	~1.1[c]
13	n.a.[b]		n.a.[b]	88.5	88.2	59.9	[c]	n.a.[b]	~0.4[c]

[a] Determined by single ion monitoring of m/z 79 and 81.
[b] Not analysed.
[c] Co-elution of compound Nos. 12 and 13.

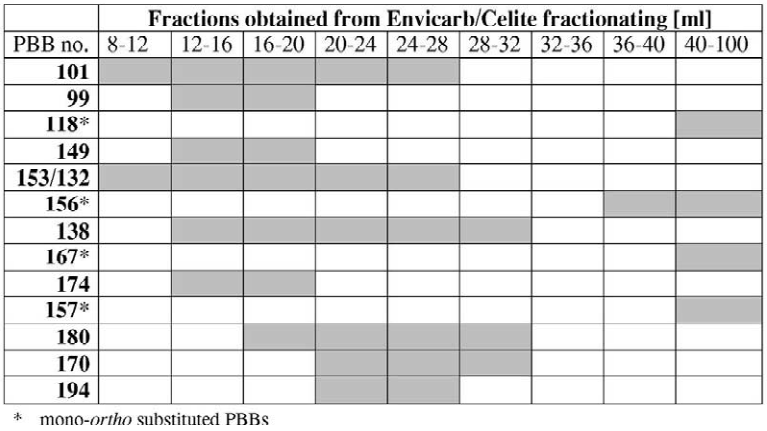

Fig. 3. Distribution of PBB congeners in fractions from chromatography on envicarb/celite and elution with *n*-hexane*.

tionating of Firemaster BP-6 was carried out using reversed-phase HPLC.

3.2. Isolation of PBB congeners by high-performance liquid chromatography

Three C_{18} reversed-phase HPLC columns were coupled in series (see Experimental) in order to obtain a sufficient separation of the most important congeners in the technical PBB mixture (Fig. 2B). HPLC retention times, UV absorption maxima on the range 190–500 nm and area percentages are given in Table 1. The good correspondence between area percentages found with HPLC/photodiode array detection (DAD) and GC/ECNI-MS (Table 1) was a further confirmation of the correct compound identification.

To obtain enough material of isolated congeners for enantioselective HPLC experiments, technical hexabromobiphenyl (~50 μg) was injected twice and fractions of PBB congeners No. 1 to 12 (Table 1) collected separately. Corresponding fractions of the two separations were pooled and fractionated once again for further purification. With this procedure, all congeners apart from PBB 99, PBB 149, and PBB 194 were obtained with purities >95% (GC/ECD confirmation). Remaining co-eluting PBB congeners could be separated on the enantioselective HPLC columns (see below).

3.3. Enantioselective separation of atropisomeric PBBs by HPLC

The first liquid chromatographic separation of two axially chiral PCBs into their atropisomers was carried out by Mannschreck et al. [28] on triacetylcellulose as chiral selector. Haglund [29] enantioselectively separated 14 of the 19 atropisomeric PCBs on two serially connected HPLC columns containing heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin on silica (β-PMCD) as chiral stationary phase. A similar β-PMCD column as well as α-PMCD and γ-PMCD columns (see Experimental) were therefore employed in this work. In a first series of tests, the enantioselective separation performance of the three columns with respect to the atropisomeric tri-*ortho* substituted PBBs 132, 149, 174 and the di-*ortho* substituted PBB 180 was evaluated. The α-PMCD column partially separated PBB 174 atropisomers, but none of the other PBB congeners. In contrast, the γ-PMCD partially resolved the enantiomers of PBB 149 and 180 but not PBB 132 and 174. However, the best results for all investigated PBBs were obtained with the β-PMCD column. This chiral phase partially resolved the atropisomers of PBB 149, 174, 180 and baseline separated the atropisomers of PBB 132. Therefore, an optimisation of column temperature, mobile phase flow-rate and composition (acetonitrile and water at different proportions) was performed with the β-PMCD column. The optimised parameters

are given in the Experimental part. In most cases, a decrease in the column temperature led to an increase in resolution of PBB atropisomers. The lowest possible temperature accepted by the column oven of HPLC system B (5 °C) was therefore chosen for further experiments. Fig. 4 shows the enantioselective separation of six axially chiral PBB congeners with the corresponding chiral resolution values R_s upon optimisation of the HPLC parameters. Since the HPLC/UV system did not provide information on the identity of the PBBs, the successful separation was confirmed by collecting fractions of the first and the second eluting atropisomer as well as a fraction in between (middle fraction). Analysis of these fractions by non-chiral GC/ECD showed peaks at the same retention time for the two atropisomers and no peak or a considerably lower peak at the expected retention time for the middle fraction. PBB congeners 101 and 153 were also investigated, but no enantiomer separation was achieved. As can be seen from Fig. 4 and Table 1, PBB congeners with three bromine substituents adjacent to the interannular phenyl-phenyl bond (tri-*ortho* substituted PBBs; PBB congeners 132, 149, and 174, Fig. 4A–C) and di-*ortho* 2,2′-substituted PBB congeners with at least one bromine in an adjacent *meta*-position (substitution type 2,2′,3; PBB congeners 138, 180, and 194, Fig. 4D–F) were at least partially separated into atropisomers at 5 °C as well as at room temperature

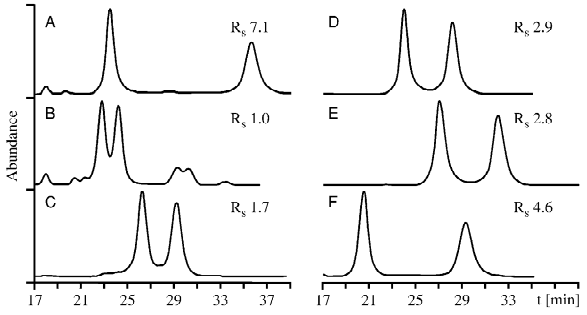

Fig. 4. Enantioselective HPLC separation of axially chiral PBB congeners on a β-PMCD column at 5 °C. For separation details see the Experimental section. (A) PBB 132; (B) PBB 149; (C) PBB 174; (D) PBB 138; (E) PBB 180 (A–E, flow 0.5 ml/min); (F) PBB 194 (F, flow 1 ml/min). Chiral resolutions R_s are calculated as follows: $R_s = 1.18\Delta t/\Sigma w_{1/2}$; Δt: retention time difference and $\Sigma w_{1/2}$: sum of the signal widths at half heights of the two atropisomers.

(~25 °C, not shown). Di-*ortho* 2,2′-substituted PBBs without bromine at a 3-position were not separated into enantiomers (PBB 101 and 153). The effect of substituents in adjacent *meta*-positions (3 and/or 3′) to increase the enantiomerization barrier was previously predicted [12] and observed [29] for PCBs and was referred to as ''buttressing effect''. However, only di-*ortho* 2,2′-substituted PCBs with chlorine atoms in both 3- and 3′-positions could be separated at 0 °C [29]. The larger covalent radius of bromine compared to chlorine (see Introduction) seems to be responsible for the higher stability of PBB atropisomers with only one buttressing substituent.

The stability of atropisomers can also be related to the twisting of the two phenyl rings. The enantiomerization barrier increases with the number of *ortho* substituents, and so also does the torsional angle between the two phenyl rings. This distortion from planarity gradually decreases the intramolecular π conjugation of the two phenyl rings which is paralleled by a blue shift of the UV absorption maximum (λ_{max} found at lower wavelengths, Table 1). Within an isomer group of PBBs (i.e. PBBs with the same degree of bromination), tri-*ortho* substituted PBBs showed the lowest λ_{max} followed by 2,2′,3-type PBBs (one or two buttressing Br), 2,2′-type (no buttressing Br) and finally mono-*ortho* substituted PBBs (Table 1). These findings are in agreement with literature data for di- to hexabromobiphenyls [30]. Thus, λ_{max} is an indicator for whether a PBB congener forms stable atropisomers or not.

The influence of temperature on the enantioselective HPLC separation has already been mentioned, and this parameter was studied in detail. As stated above, the atropisomers of PBB 153 could not be separated at 5 °C; however, this di-*ortho* 2,2′-substituted PBB without substituents in adjacent *meta*-position displayed a very broad signal on the β-PMCD HPLC column (~10 min at the baseline) but only one peak maximum. At 50 °C, elution of PBB 153 took 4 min. This difference can only to a minor extent be explained by the shorter retention time at 50 °C (20.3 min compared to 25.0 min at 5 °C), indicating that there was a much slower conversion of one atropisomer into the other and vice versa at low temperatures. Enantiomer resolution of PBB 174 and 180, which were almost baseline separated into atropisomers at 5 °C (Fig. 4C,E), was also studied at

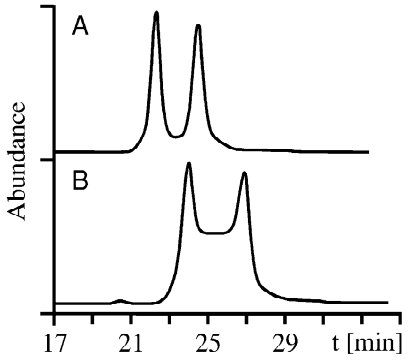

Fig. 5. Enantioselective HPLC separation of PBB 174 (A) and 180 (B) on a β-PMCD column at 50 °C. For separation details, see the Experimental section.

50 °C (Fig. 5). The tri-*ortho* substituted PBB 174 still showed no enantiomerization within the time of the chromatographic run (Fig. 5A). In contrast, due to sharper peaks at lower retention times, the two atropisomers were even better resolved at the higher temperature (R_s 2.0 instead of 1.7 at 5 °C, Fig. 4C). On the other hand, the di-*ortho* substituted PBB 180 was partially enantiomerized during chromatography at 50 °C, which is visible in the formation of a plateau between the atropisomers (Fig. 5B). The same behaviour was also observed for PBB 138.

Finally, the elution orders of the enantiomers of PBB 174 on α- and β-PMCD and of PBB 180 on β- and γ-PMCD were compared. For this purpose, racemic PBB 174 and 180 were spiked with the first eluting atropisomer, respectively, from the β-PMCD column. Injection of enantiomer enriched PBB 174 confirmed that the atropisomers eluted in the same order from α- and β-PMCD. An identical elution order was found for the atropisomers of PBB 180 on β- and γ-PMCD as well.

3.4. Enantioselective separation of atropisomeric PBBs by GC

Several chiral stationary phases have been successfully applied to resolve atropisomers of PCBs by GC [19,31–36]. Three corresponding chiral phases were available for this study (see Experimental) and were chosen due to the structural similarity of PCBs and PBBs. The HPLC studies (see before) indicated that di-*ortho* substituted PBBs cannot be separated at elevated temperatures mandatory for the application of gas chromatography, hence our screening was restricted to the tri-*ortho* substituted congeners PBB 149, 132, and 174. However, the results were poor for these hexa- and heptabromo congeners having molecular masses above 600 Da. This mass range requires very high elution temperatures on typical columns (25–30 m length, 0.25–0.32 mm I.D.) used for enantioselective separation of nonpolar organohalogen compounds [11,37,38]. The future goal, i.e. determination of atropisomeric PBBs in complicated environmental matrices, requires a good separation of isomers and other co-extracted compounds besides the enantioselective resolution. Therefore, shorter columns were not tested, although these would have allowed lower elution temperatures and higher selectivity.

None of the PBB congeners tested was at least partly resolved on CB-β-PMCD or β-BSCD (see Experimental). However, in contrast to PBB 132 and 174, a partial resolution of PBB 149 atropisomers was obtained on the β-TBDM column (Fig. 6). Successful gas chromatographic resolution of the PBB 149 enantiomers (molecular mass 627.6 Da) was verified by injection of single PBB 149 enantiomers isolated by enantioselective HPLC (see above). This also allowed to determine that the elution order of PBB 149 atropisomers on the β-TBDM GC column was the same as on the β-PMCD HPLC column. It is worth noting that the employed β-TBDM column was made of an impure cyclodextrin phase containing five differently modified cyclodextrin compounds in similar quantities [19]. This type

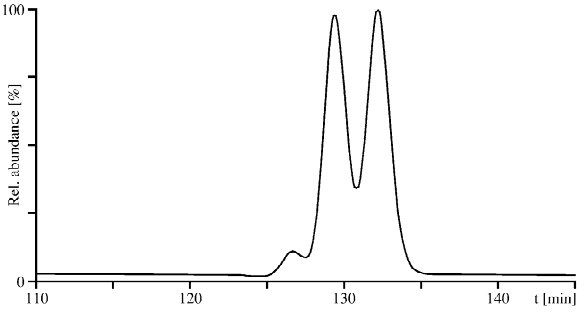

Fig. 6. Enantioselective GC separation of PBB 149 (isothermal elution at 190 °C) on randomly modified heptakis(6-*O*-*tert*.-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin (β-TBDM).

as well as the pure β-TBDM have been applied to the separation of PCB atropisomers [19,33,36]. Unfortunately, a column coated with the pure β-TBDM product as suggested by Hardt et al. [33] for the resolution of PCB atropisomers was not available in this work. This column could be a good choice for further attempts to resolve axially chiral PBBs by GC.

4. Conclusions

Enantioselective chromatography (GC and HPLC) allowed to separate the atropisomers of six PBB congeners. It was shown that di-*ortho* 2,2′-substituted PBBs with at least one additional substituent in adjacent *meta*-position and tri-*ortho* substituted PBBs can be separated into enantiomers by HPLC. Such PBBs most likely exist as stable atropisomers at conditions found in humans and wildlife. Thus, enantioselective processes may play a role for these compounds in nature. However, environmental samples contain PBBs at ultra-trace levels in a complex matrix together with other, significantly higher concentrated halogenated compounds. PBBs usually account only for 1–10% of the concentrations of polybrominated diphenyl ethers, which in turn are much less relevant than PCBs and other major contaminants. Thus, HPLC/UV is not sensitive enough for studies of enantioselective processes of PBBs in the environment. However, enantioselective HPLC will be the method of choice for preparative isolation of pure PBB enantiomers which would allow toxicological and ecotoxicological testing against the racemic mixture.

Requirements for enantioselective studies of chiral organochlorines in environmental samples are met with enantioselective GC [11,37,38]. The partial atropisomer separation of PBB 149 in this work demonstrates that it is in principle possible to separate the enantiomers of axially chiral PBB congeners by gas chromatography. Based on comparison with PCBs, PBB 149 may be one of the chiral PBB congeners of environmental relevance. Therefore, there is a chance for studying the environmental relevance of enantioselective processes for this PBB congener in biota. More work is necessary to receive a clear picture on atropisomerism of PBBs in wildlife. The results obtained in this study are a first and promising step in this direction.

Acknowledgements

The authors are grateful to the German Academic Exchange Service (DAAD) and the Scientific Council of Norway for supporting the bilateral project 313-PPP-N1-01. In this project, research grants were provided to A.G. for GC/MS studies, liquid chromatographic separation of Firemaster BP-6 as well as initial enantioselective GC studies in the laboratory of the Norwegian partner and to U.B. for isomer and enantiomer selective HPLC studies in the laboratory of the German partner. W.V. acknowledges a travel grant in this project for an initial working meeting of the four participants at NILU. Furthermore, we would like to thank Michael Oehme (University of Basel, Switzerland) for providing the enantioselective HPLC columns and Eldbjørg S. Heimstad (NILU, Norway) for providing Fig. 1.

References

[1] J. de Boer, K. de Boer, J.P. Boon, Polybrominated biphenyls and diphenylethers, in: J. Paasivirta (Ed.), The Handbook of Environmental Chemistry, Volume 3, Part K, Springer, Berlin, 2000, pp. 61–95.
[2] A. Norström, K. Andersson, C. Rappe, Chemosphere 4 (1976) 255.
[3] G. Sundström, O. Hutzinger, S. Safe, V. Zitko, Sci. Total Environ. 6 (1976) 15.
[4] S.M. Getty, D.E. Rickert, A.L. Trapp, CRC Crit. Rev. Environ. Control (1977) 309.
[5] K. Kay, Environ. Res. 13 (1977) 74.
[6] Anonymous, Toxicological profile for brominated biphenyls. US Department for Human Health Services (1995) 276.
[7] Anonymous, BFR 2001, Proceedings of the Second International Workshop on Brominated Flame Retardants, May 14–16, 2001, The Swedish Environmental Protection Agency.
[8] M. Alaee, L. Luross, D.B. Segeant, D.C.G. Muir, K. Solomon, D.M. Whittle, Organohalogen Comp. 40 (1999) 347.
[9] B. Jansson, R. Andersson, L. Asplund, K. Litzen, K. Nylund, U. Sellström, U. Ubemo, C. Wahlberg, U. Widequist, T. Odsjø, M. Olsson, Environ. Toxicol. Chem. 12 (1993) 1163.
[10] Anonymous, Off. J. Eur. Commun. (2001), April 18, 2001, C113E/134.

[11] R. Kallenborn, H. Hühnerfuss, Chiral Environmental Pollutants, Springer, Berlin, 2001.
[12] K.L.E. Kaiser, Environ. Pollut. 7 (1974) 93.
[13] H.-J. Lehmler, L.W. Robertson, Atropisomers of PCBs, in: Robertson and Hansen (Eds.), PCBs—Recent Advances in the Environmental Toxicology and Health Effects, University of Kentucky Press, 2001, Chapter 8, pp. 61–65.
[14] W. Vetter, U. Klobes, K. Hummert, B. Luckas, J. High Resolut. Chromatogr. 20 (1997) 85.
[15] H. Hühnerfuss, B. Pfaffenberger, B. Gehrcke, L. Karbe, W.A. König, O. Landgraff, Mar. Pollut. Bull. 30 (1995) 332.
[16] A. Glausch, G.J. Nicholson, M. Fluck, V. Schurig, J. High Resolut. Chromatogr. 17 (1994) 347.
[17] A. Glausch, J. Hahn, V. Schurig, Chemosphere 30 (1995) 2079.
[18] S. Reich, B. Jimenez, L. Marsili, L.M. Hernandez, V. Schurig, M.J. Gonzalez, Organohalogen Comp. 35 (1998) 335.
[19] W. Vetter, U. Klobes, B. Luckas, G. Hottinger, J. Chromatogr. A 846 (1999) 375.
[20] W. Vetter, B. Krock, U. Klobes, B. Luckas, J. Agric. Food Chem. 45 (1997) 4866.
[21] L.W. Robertson, S.H. Safe, A. Parkinson, E. Pellizzari, C. Pochini, M.D. Mullin, J. Agric. Food Chem. 32 (1984) 1107.
[22] D.L. Orti, R.H. Hill Jr., D.G. Patterson, L.L. Needham, R.D. Kimbrough, C.C. Alley, H.-C.J. Lee, Arch. Environ. Contam. Toxicol. 12 (1983) 603.
[23] S.D. Aust, G.A. Dannan, S.D. Sleight, P.J. Fraker, R.K. Ringer, D. Polin, Toxicology of polybrominated biphenyls, in: M.A.Q. Khan, R.H. Stanton (Eds.), Toxicology of Halogenated Hydrocarbons: Health and Ecological Effects, Pergamon, Oxford, 1981, pp. 73–96.
[24] H.-R. Buser, Anal. Chem. 58 (1986) 2913.
[25] C.S. Creaser, F. Krokos, J.R. Startin, Chemosphere 25 (1992) 1981.
[26] E. Storr-Hansen, T. Cederberg, Chemosphere 24 (1992) 1181.
[27] M. Schwinge, W. Vetter, B. Luckas, Organohalogen Comp. 40 (1999) 405.
[28] A. Mannschreck, N. Pustet, L.W. Robertson, F. Oesch, M. Püttmann, Liebigs Ann. Chem. (1985) 2101.
[29] P. Haglund, J. Chromatogr. A 724 (1996) 219.
[30] J.J. de Kok, A. de Kok, U.A.Th. Brinkman, R.M. Kok, J. Chromatogr. 142 (1977) 367.
[31] V. Schurig, A. Glausch, Naturwissenschaften 80 (1993) 468.
[32] W.A. König, B. Gehrcke, T. Runge, C. Wolf, J. High Resolut. Chromatogr. 16 (1993) 376.
[33] I.H. Hardt, C. Wolf, B. Gehrcke, D.H. Hochmuth, B. Pfaffenberger, H. Hühnerfuss, W.A. König, J. High Resolut. Chromatogr. 17 (1994) 859.
[34] P. Haglund, K. Wiberg, J. High Resolut. Chromatogr. 19 (1996) 373.
[35] W. Vetter, U. Klobes, B. Luckas, G. Hottinger, J. Chromatogr. A 769 (1997) 247.
[36] C. Wong, A.W. Garrison, J. Chromatogr. A 866 (1999) 213.
[37] W. Vetter, V. Schurig, J. Chromatogr. A 774 (1997) 143.
[38] W. Vetter, Food Rev. Int. 71 (2001) 113.