

Enantiomer separation of selected atropisomeric polychlorinated biphenyls including PCB 144 on *tert*-butyldimethylsilylated β -cyclodextrin

Walter Vetter^{a,*}, Ulrike Klobes^a, Bernd Luckas^a, Georg Hottinger^b

^aFriedrich-Schiller-Universität Jena, Institut für Ernährung und Umwelt, Dornburger Strasse 25, D-07743 Jena, Germany

^bBGB Analytik KG, Lettenstrasse 97, CH-8134 Adliswil, Switzerland

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Abstract

The enantiomer separation of eight atropisomeric polychlorinated biphenyls (PCBs) was studied by application of GC–MS and a chiral stationary phase (CSP). The modified cyclodextrin consisted of 25% *tert*-butyldimethylsilylated β -cyclodextrin (β -BSCD) in 15% diphenyl, 85% dimethylpolysiloxane. The enantiomers of seven atropisomeric PCBs were at least partly separated. Five of the separated enantiomers of PCB atropisomers had a 2,3,4,6-substitution pattern on one phenyl ring. Emphasis was put on the atropisomer separation of PCB 144 (2,3,4,6,2',5'-hexachlorobiphenyl) which was not reported before on other CSPs. Several enantiomers of PCB atropisomers not separated on permethylated β -cyclodextrin were separated on β -BSCD. The efficiency of the PCB atropisomer separation on β -BSCD is complement to permethylated β -cyclodextrin.

Keywords: Enantiomer separation; Chiral stationary phases, GC; Polychlorinated biphenyls

1. Introduction

The enantiomer separation of chiral organochlorine compounds and the determination of enantiomeric ratios (ERs) in biological samples is a new and growing research topic in analytical chemistry. Most of the chlorinated hydrocarbons only consist of C, H and Cl, and chirality is often caused rather by a plane or axis of symmetry than by an asymmetric carbon. Bulky substituents in *ortho* positions of biphenyl cause steric hindrance of rotation about the phenyl–phenyl axis. Requirement for chirality of polychlorinated biphenyls (PCBs) is a nonsymmetric substitu-

tion at both phenyl rings and restricted rotation about the phenyl–phenyl axis. Eighty-seven PCBs meet the first requirement [1]. At room temperature, the second requirement is fulfilled by PCBs with rotational energy barriers of at least 83 kJ/mol [1]. PCB atropisomers with three or four substituents in *ortho* positions can be enantiomer separated at room temperature [1]. Among di-*ortho* substituted rings only those with a 2,3,6- and a 2,3,4,6-pattern and among the mono-*ortho* substituted rings eight nonsymmetric patterns fulfill the requirements mentioned above. Combination of these ring patterns leads to 19 stable atropisomeric PCBs as predicted by Kaiser [1].

In 1993, GC enantiomer separation of nine of the

*Corresponding author.

19 atropisomeric PCBs was achieved on CSPs with peralkylated cyclodextrins [2,3]. Later the enantiomer separation of further six atropisomeric PCBs was reported [4,5].

The lacking atropisomers PCB 144, PCB 171, PCB 196 and PCB 197 resisted any GC enantiomer separation [4]. These four PCB congeners have been detected in technical Aroclor PCB mixtures [6]. Stability of the atropisomers PCB 171, PCB 196 and PCB 197 at physiological temperatures has been proven by application of chiral HPLC [7]. According to those results, the enantiomer separation of PCB 144 was lacking, thus not completely confirming Kaiser's pioneering account [1].

The GC enantiomer separation of PCB 144 and further atropisomeric PCBs was the goal of this study. For this purpose a CSP consisting of 25% *tert*-butyldimethylsilylated β -cyclodextrin (β -BSCD) diluted in 15% diphenyl, 85% dimethylpolysiloxane (PS086) was applied. On β -BSCD, we recently reported the enantiomer separation of several toxaphene and chlordane compounds [8,9]. β -BSCD phases were introduced by Blum and Aichholz and showed high selectivity for the enantiomer separation of a wide range of chiral compounds [10].

2. Experimental

GC-MS measurements were performed on an HP 5989B MS Engine (Hewlett-Packard, Waldbronn, Germany) using electron ionization (EI). In the selected ion monitoring (SIM) mode the two most abundant isotopic peaks of the molecular ion were recorded for each degree of chlorination. A 1- μ l aliquot was splitless injected at 240°C injector temperature. Helium was used as carrier gas. The GC was operated in the constant flow mode at 1.36 ml/min. The transfer line was set at 250°C, the ion source at 200°C and the quadrupole at 100°C.

Enantiomers of PCB atropisomers were separated on a fused-silica capillary column coated with 25% β -BSCD diluted in PS086 obtained from BGB Analytik (Adliswil, Switzerland). The column was 30 m \times 0.25 mm I.D., film thickness 0.20 μ m. The GC oven programs are given in the respective Figures.

GC-EI-MS full scan chromatograms and mass spectra were recorded on an HP 5971 mass-selective detector in combination with an HP-5 (30 m \times 0.25 mm I.D., 0.2 μ m film thickness) fused-silica capillary column (Hewlett-Packard). The GC oven program started at 80°C (4 min), followed by heating rates at 10°C/min to 160°C (20 min), 2°C/min to 235°C, and 25°C/min to 270°C (2 min).

3. Results and discussion

3.1. Problems in the enantiomer separation of PCB 144

Our first efforts to separate PCB 144 atropisomers started with a remarkable problem. We were not able to record peaks in the SIM chromatograms by GC-MS. The full scan GC-EI-MS of the standard solution showed a pentachloro pattern starting at *m/z* 324 and, therefore, the compound in the ampoule was not the hexachlorobiphenyl PCB 144. Unfortunately, the original ampoule has not been saved. Although we exclude an error in our laboratory, we refrain from mentioning the manufacturer. Wrongly labeled ampoules have been observed in the past [11] but to our knowledge the quality of standard solutions has improved during the last years. Consequently, we ordered PCB 144 from other manufacturers, i.e., Promochem and Dr. Ehrenstorfer. Since Dr. Ehrenstorfer was the first company to deliver the solution, we have used this product to study the enantiomer separation of the compound. The full scan mass spectrum was composed of the expected hexachloro pattern starting at *m/z* 358. To obtain further proof of the structure of the hexachlorobiphenyl we applied a method recently published [12,13]. Addition of time increments of PCBs allowed to predict the retention times (t_R) of PCBs [13]. At this method on the basis of Sissons and Welti [14], the rings of the biphenyl are regarded as independent. The t_R of a PCB (here: 2,3,4,6,2',5'-hexachlorobiphenyl, PCB 144) is estimated after measuring t_R of the 2,4,5-substituted PCBs with the respective ring patterns of the PCB in question (here: 2,4,5,2',5'-pentachlorobiphenyl, PCB 101 and 2,3,4,6,2',4',5'-heptachlorobiphenyl, PCB 183) and PCB 153 (2,4,5,2',4',5'-hexachlorobiphenyl). Divi-

Table 1
Estimation of t_R of PCB 144 by adding the time increments determined after separation of selected PCBs on a 30 m HP-5 column

IUPAC No.	Ring A	Ring B	t_R	Time increment (A/B)	MoA (%) ^a
PCB 153	2,4,5	2,4,5	37.10	18.55/18.55	
PCB 101	2,4,5	2,5	30.26	18.55/11.71	
PCB 183	2,3,4,6	2,4,5	42.12	22.57/18.55	
PCB 144	2,3,4,6	2,5	34.44	22.57/11.71 ($\Sigma=34.28$)	99.5

^a Measure of agreement (MoA) is the ratio of estimated and measured retention time in %.

sion of t_R of PCB 153 by a factor of two leads to the time increment of the 2,4,5-pattern. Subtraction of the time increment of the 2,4,5-pattern from t_R of PCB 101 and PCB 183 results in the time increment of the 2,5- and the 2,3,4,6-substitution pattern, respectively. Finally, combination of these time increments leads to the estimated t_R of PCB 144. Table 1 shows a good agreement of the predicted and the measured value. Note that t_R values have been measured in a time program (see Section 2).

The final proof of the right structure assignment was, however, the enantiomer separation of PCB 144 (see Section 3.2). At the moment it is not clear if also other researchers were served with a wrongly labeled PCB 144. Anyhow, on heptakis(2,3,6-tri-O-methyl)- β -cyclodextrin bonded to polysiloxane (Chirasil-Dex) the enantiomer separation of 2,3,4,6-substituted atropisomeric PCBs was problematic [5]. Therefore, enantiomer separation of PCB 144 is unlikely on permethylated β -cyclodextrin (Chirasil-Dex). We tested the enantiomer separation of PCB 144 on β -BSCD (see Fig. 1). Although no baseline

separation was achieved, the chirality of PCB 144 is proven. On the other hand, t_R of PCB 144 was comparably high and the peaks of the enantiomers were relatively broad. This problem may be overcome with a shorter column allowing enantiomer separation at lower temperatures. However, the present β -BSCD column shows excellent enantiomer separation of several compounds of technical toxaphene (CTTs), chlordanes, *o,p*-DDT and *o,p*-DDD, and is also used for the enantioselective determination of organochlorines in biological samples. A noninterfered determination of ERs in samples requires longer columns compared to the enantiomer separation of standard solutions. Therefore, we did not shorten the β -BSCD column.

3.2. Enantiomer separation of atropisomeric PCBs on β -BSCD

Eight of the 19 atropisomeric PCBs were available for this study. Table 2 lists results of the enantiomer separation of atropisomeric PCBs on β -BSCD and

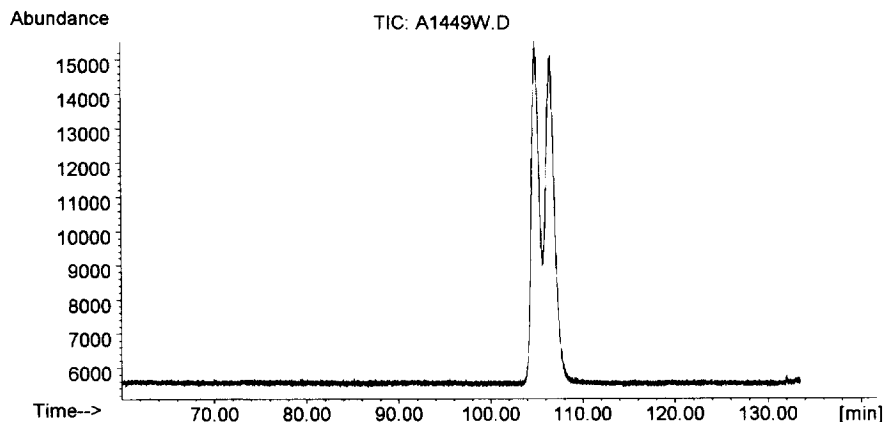


Fig. 1. Atropisomer separation of PCB 144 on β -BSCD. Temperature program: 120°C (2 min), 15°C/min to 170°C (100 min).

Table 2

GC enantiomer separation of the 19 stable atropisomeric PCBs on β -BSCD and literature data on other CSPs

IUPAC ^a No. (x/y) ^b	Ring A	Ring B'	β -BSCD (this study)	β -PMCD [2,5]	Heptakis(2,3-di-O-methyl-6-O-thexyldimethylsilyl)- β -cyclodextrin [4]
PCB 45 (4/3)	2,3,6	2	–	–	+
PCB 84 (5/3)	2,3,6	2,3	+	+	+
PCB 88 (5/3)	2,3,4,6	2	–	–	–
PCB 91 (5/3)	2,3,6	2,4	–	+	+
PCB 95 (5/3)	2,3,6	2,5	–	+	+
PCB 131 (6/3)	2,3,4,6	2,3	–	–	+
PCB 132 (6/3)	2,3,4	2,3,6	+	+	–
PCB 135 (6/3)	2,3,5	2,3,6	–	+	–
PCB 136 (6/4)	2,3,6	2,3,6	–	+	+
PCB 139 (6/3)	2,3,4,6	2,4	–	–	–
PCB 144 (6/3)	2,3,4,6	2,5	+	–	–
PCB 149 (6/3)	2,3,6	2,4,5	+	+	+
PCB 171 (7/3)	2,3,4,6	2,3,4	+	–	–
PCB 174 (7/3)	2,3,4,5	2,3,6	+	+	+
PCB 175 (7/3)	2,3,4,6	2,3,5	–	–	+
PCB 176 (7/4)	2,3,4,6	2,3,6	–	+	+
PCB 183 (7/3)	2,3,4,6	2,4,5	+	–	+
PCB 196 (8/3)	2,3,4,5	2,3,4,6	–	–	–
PCB 197 (8/4)	2,3,4,6	2,3,4,6	–	–	–

^a IUPAC number according to Ballschmiter and Zell [15].^b x=degree of chlorination; y=number of ortho substituents [16].

literature data obtained on permethylated β -cyclodextrin (β -PMCD) and on heptakis(2,3-di-O-methyl-6-O-thexyldimethylsilyl)- β -cyclodextrin. The latter two CSPs enantiomer separated nine and ten PCB atropisomers. Together with a CSP consisting of octakis(2,6-di-O-methyl-3-O-n-pentyl)- γ -cyclodextrin which separated PCB 88 and PCB 139 atropisomers [4], enantiomer separation of 15 of the 19 atropisomeric PCBs was reported by application of chiral GC.

Since enantiomerization was excluded, coelutions of the enantiomers of PCB 144, PCB 171, PCB 196 and PCB 197 were either caused of the high GC temperatures (the higher the temperature the longer a compounds remains in the gas phase and does not interact with stationary phase) or a too low host-guest interaction of the selectand with the CSPs applied so far (as a matter of geometry).

Results obtained by Haglund are speaking for the first point [7]. Haglund separated the enantiomers of several stable atropisomeric PCBs using chiral HPLC with a CSP consisting of permethylated β -cyclodextrin at 0°C–30°C. Interestingly, several di-ortho

PCBs like PCB 40 and PCB 170 were also enantiomer separated by chiral HPLC on β -PMCD [7]. Furthermore, PCB 171 which coeluted on chiral GC on β -PMCD (see Table 2) was partly enantiomer separated by chiral HPLC on β -PMCD [5,7].

Studies of the host-guest interactions of PCB atropisomers with CSPs are rare. Haglund and Wiberg reported problems in enantiomer separation of PCBs with a 2,3,4,6-substituted ring on chiral GC on β -PMCD [5]. Only one of the ten atropisomeric 2,3,4,6-substituted PCBs, i.e., PCB 176, was enantiomer separated on chiral GC on β -PMCD, which is the most applied CSP for separation of PCB atropisomers. König and coworkers separated the enantiomers of four 2,3,4,6-substituted PCBs by chiral GC on heptakis(2,3-di-O-methyl-6-O-thexyldimethylsilyl)- β -cyclodextrin (see Table 2) and PCB 88 and PCB 139 on octakis(2,6-di-O-methyl-3-O-n-pentyl)- γ -cyclodextrin [3,4]. However, the remaining four atropisomeric PCBs had also at least one 2,3,4,6-substituted ring.

Fig. 2 shows the enantiomer separation of PCB 132 together with five 2,3,4,6-substituted at-

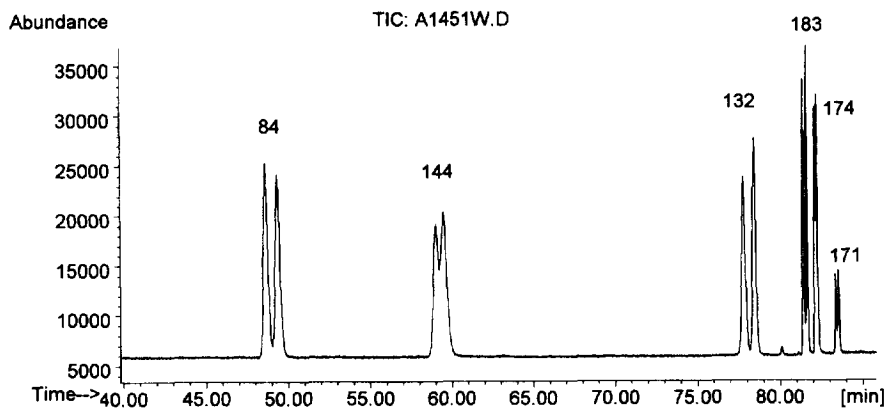


Fig. 2. Atropisomer separation of PCB 84, PCB 144, PCB 132, PCB 183, PCB 174 and PCB 171 on β -BSCD. Temperature program: 120°C (2 min), 15°C/min to 170°C (30 min), 20°C/min to 180°C (40 min), 10°C/min to 240°C (34 min).

ropisomeric PCBs by chiral GC on β -BSCD. The (partial) enantiomer separation clearly demonstrates the chirality of all compounds. The enantiomer separation of the compounds could be improved by lower elution temperatures (e.g., see Fig. 1, PCB 144) but this was not the goal of the present investigation. Note that the enantiomers of PCB 171 and PCB 144 were not separated by application of chiral GC with other CSPs (see Table 2). See also that the final temperature of 240°C was already reached with the elution of PCB 174. No enantiomerization by means of plateau formation between the PCB atropisomers was observed [17]. This clearly confirms the stability of PCB atropisomers under usual GC conditions which is in agreement with findings of Schurig et al. [17]. Therefore,

studies of the enantioselective accumulation as demonstrated in several works [18–25] can be carried out by GC with CSPs.

The elution order of PCB atropisomers in Fig. 2 is typical for medium polar (e.g., CP-Sil 19) and polar (e.g., CP-Sil 88) phases. For example, the high t_R of PCB 132 compared to PCB 144 (see Fig. 2) and PCB 149 reflect the comparably high polarity of CSPs. This is unavoidable owing to the polarity of the modified cyclodextrins [26].

The atropisomer separation of PCB 95 failed and PCB 149 atropisomers were only partly separated (see Fig. 3). These two atropisomeric PCBs, easily separated on other CSPs, have no 2,3,4,6-pattern on one of the rings. In other words, the separation principle on chiral GC on β -PMCD (good enantio-

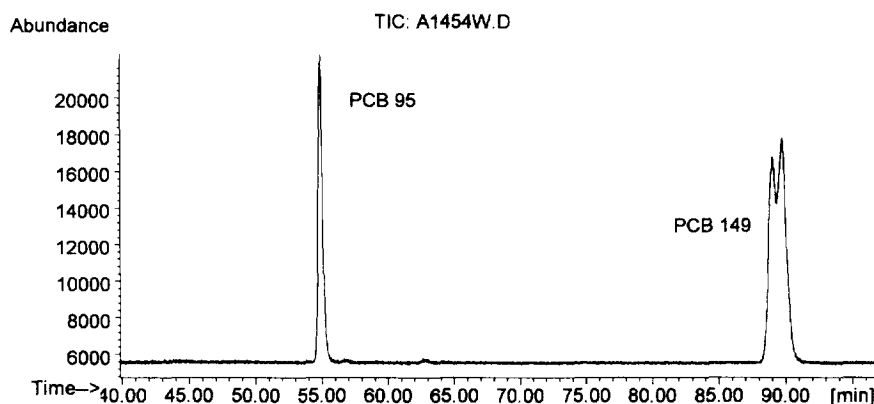


Fig. 3. Atropisomer separation of PCB 95 and PCB 149 on β -BSCD. Temperature program: 120°C (2 min), 15°C/min to 170°C (100 min).

mer separation of atropisomeric PCBs with 2,3,6-patterns and bad enantiomer separation of those with 2,3,4,6-patterns) and chiral GC on β -BSCD (good enantiomer separation of atropisomeric PCBs with 2,3,4,6-patterns and bad enantiomer separation of those with 2,3,6-patterns) seems to be inverse. Consequently, both CSPs complement one another with respect to the atropisomer separation of PCBs. An explanation might be significantly differing torus bodies of β -BSCD and β -PMCD. König et al. used modified cyclodextrins with either a bulky substituent in 3- or 6-position. Interestingly, the 6-O-thexyldimethylsilyl substituted CSP separated the enantiomers of some highly chlorinated 2,3,4,6-substituted atropisomers (see Table 2) while a 3-O-*n*-pentylated CSP enantiomer separated two early eluted PCB atropisomers. The key for the excellent atropisomer separations of 2,3,4,6-substituted PCBs on β -BSCD might be the modification of all terminal hydroxyl groups of the cyclodextrin with bulky substituents. According to that, the enantiomers of 2,3,4,6-substituted PCB 196 and PCB 197 which were not available as standard solutions may also be separated on β -BSCD.

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