Establishing the direction of light rotation of enantioenriched chiral organochlorines by HPLC with a chiral detector

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Abstract

Direction of light rotation of ß-pentachlorocyclohexene-1 (ß-PCCH), perdeuterated α-HCH (α-PDHCH), perdeuterated ß-PCCH, and the persistent compound of technical toxaphene 2-exo,3-endo,5-exo,9,9,10,10-heptachlorobornane (B7-1453) was determined in a chiral HPLC detector. Elution orders of enantiomers on four chiral gas chromatographic stationary phases are presented.

1. Introduction

During the last years, gas chromatographic enantiomer separation of chiral organochlorines has become an important topic in environmental chemistry[1)]. A prerequisite to the evaluation of enantiomeric ratios (ERs) of chiral organochlorines in environmental samples is the knowledge of the rotation of light of the enantiomers. This problem has been solved by chiroptical investigations of enantiopure or enantioenriched α-HCH[2)], chlordane-related compounds[2-3)], and atropisomeric PCBs[4)]. We have now established the direction of light rotation of a further four components including an enantioenriched compound of technical toxaphene (CTT).

2. Experimental Methods

Preparation of enantioenriched standards. Organochlorines (α-HCH, α-PDHCH, PCB 174, o,p´-DDT) were treated with (-)-brucine (2,3-dimethoxystrychnidin-10-one) as described by Cristol for α-HCH[5)]. 40 mg of the organochlorines (except 1 mg in the case of PCB 174) and 200 mg (-)-brucine (Merck) were added to 10 mL dioxane. In the case of α-HCH and α-PDHCH, the resulting metabolites ß-PCCH and the corresponding perdeuterated ß-PCCH (ß-PDPCCH) were separated (50 g silica gel, eluted with n-hexane) from the enantioenriched parent compounds. B7-1453 was isolated from the technical product Melipax[6)], and recently some of us showed that the heptachlorobornane was present in the technical mixture in non-racemic composition[7)]. Reaction of µg-amounts of B7-1453 with 40 mg (-)-brucine was carried out in dioxane.
High performance liquid chromatography with a chiral detector. The HPLC-system consisted of a PU-980 pump, a UV-975 (worked at 210 nm) and an OR990 chiral detector (all Jasco, Japan). The HPLC column (ET 200/8/4 NUCLEOSIL 100-5) was from Macherey-Nagel (Düren, Germany). The eluent was n-hexane at a flow of 0.7 mL or 0.5 mL.

Gas chromatography with electron capture detection. Nonchiral separations were carried out on an HP 5890 gas chromatograph equipped with two columns (CP-Sil 2 and CP-Sil 8/C18) and two ECDs in parallel. Enantiomer separations were performed on an HP 5890 equipped with one ECD. The chiral stationary phase consisted of 25% tert.-butyldimethylsilylated ß-cyclodextrin in PS086 (BGB Analytik Adliswil, Switzerland). All parameters were recently described[8-9] except the carrier gas which was changed in meantime from nitrogen to helium.

Results and Discussion

Reaction of organochlorines with (-)-brucine. Unfortunately, only α-HCH and α-PDHCH were enantioselectively degraded while o,p´-DDT and PCB 174 remained in racemic composition and the B7-1453 isolate from Melipax still showed the non-racemic ER 1.26[7]. Therefore, our first HPLC experiments were carried out with enantioenriched α-HCH. It was known that degradation with (-)-brucine leads to enantioenriched (-)-α-HCH[5] and this was confirmed by a significant negative signal in the chiral detector (definition: positive signal (dextrorotation) means higher than the baseline, a negative signal (levorotation) means lower than the baseline). Due to the small ER of α-HCH after treatment with (-)-brucine, the chiral detector required between 10 and 100 µg of the enantioenriched compounds to obtain a significant signal. The eluate at the retention time of the positive signal in the detector was collected. Diluted with n-hexane, this fraction was analyzed by GC/ECD. The chromatogram only contained α-HCH and therefore, levorotation of α-HCH after the (-)-brucine treatment was confirmed[5]. With the same technique we also investigated further enantioenriched solutions of chiral compounds with so far unknown direction of light rotation of enantiomers. First we established levorotation of the enantioenriched α-PDHCH obtained after treatment with (-)-brucine. The ß-PCCH isolate, however, caused a positive signal in the chiral detector and turned the light to the right (dextrorotation). Therefore, reaction of α-HCH with (-)-brucine led to enantioenriched (-)-α-HCH and enantioenriched (+)-ß-PCCH. ß-PDPCCH also caused dextrorotation and this is a further proof for the thesis that α-HCH and isotope labeled α-PDHCH will behave in the same way regarding enantioselectivity[9].
Recently we isolated B7-1453 from the technical product Melipax[6]. B7-1453 was abundant in fish[12] and blubber of seals[13] confirming persistence of that heptachloro CTT in biota. Buser and Müller showed that toxaphene mixtures might contain enantioenriched compounds[14]. Recently, we found this was at least true in the case of B7-1453 isolated from the technical mixture Melipax by enantiomer separation on ß-BSCD[7]. With GC/ECNI-MS and GC/EI-MS was found an ER of 1.26 ± 0.03[7]. The chiral detector required large amounts of substance (see above) and the difference in the levels of the B7-1453 enantiomers was relatively small for chiroptical experiments. Therefore, we concentrated the complete B7-1453 isolate of Melipax to approx. 50µL and injected 20 µL into the HPLC system. Two significant positive signals were obtained in the chiral detector (see Figure 1), The fractions which showed a signal in the chiral detector were collected and analyzed by GC/ECD. Figure 2 shows the GC/ECD chromatogram of the HPLC-cut of the major positive peak in the chiral detector which was identified as B7-1453. The fraction was concentrated and injected again into the HPLC system. This time we only recorded only the one positive signal in the chiral detector. Although the abundance of the positive signal in the chiral detector was low it is now established that the isolate from Melipax contained enantioenriched (+)-B7-1453. It is, to our knowledge, for the first time that the elution order of a CTT was established.
We have also injected a solution of 4.6 mg Melipax (see Figure 3). The chromatogram of the chiral detector showed several positive and negative signals which confirmed chiral components in the mixture. Note that Buser and Müller only found signals in one direction when they analyzed

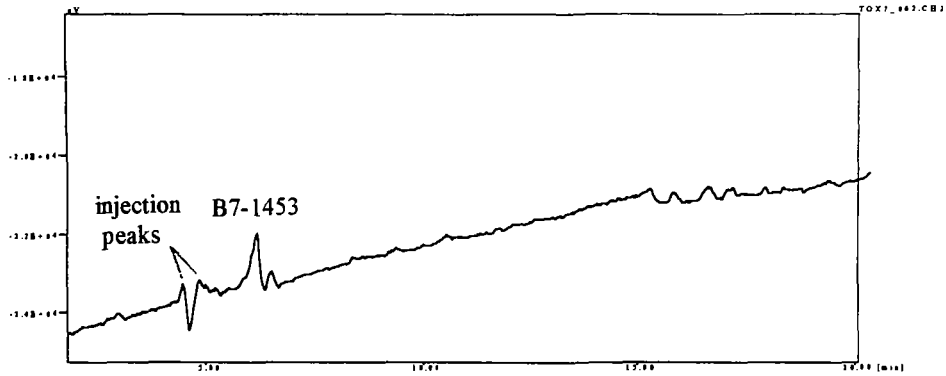

Figure 1: **HPLC chromatogram of enantioenriched (+)-B7-1453 isolated from Melipax with chiral detector (positive signals are caused by dextrorotation)**

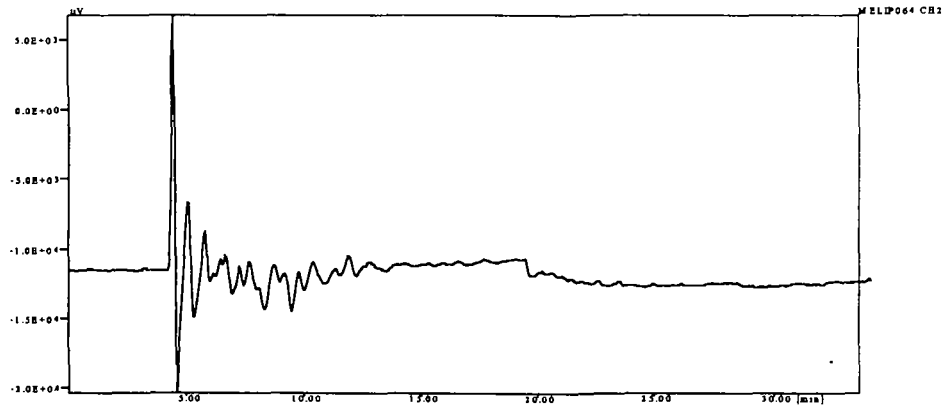

Figure 2: **HPLC chromatogram of technical mixture Melipax with chiral detector**

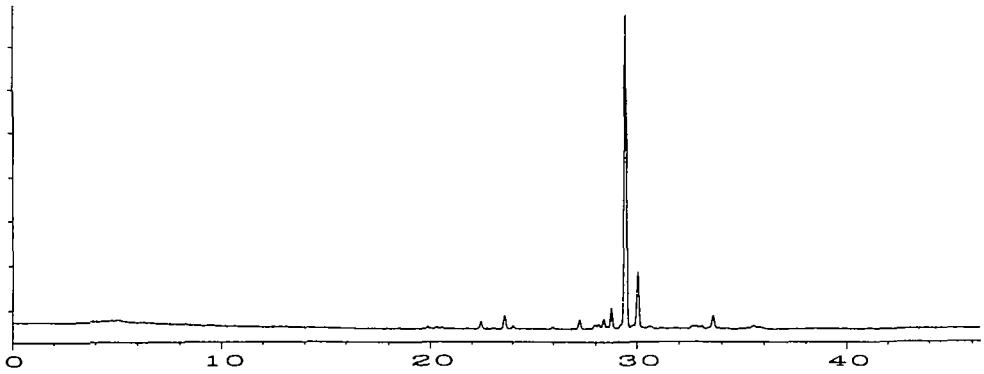

Figure 3: **GC/ECD chromatogram of the HPLC-cut of B7-1453 on CP-Sil 2**

technical toxaphene products[14]. For Melipax we found no clear trend. On the other hand, µg-amounts of further enantioenriched CTT congeners were not available. Anyhow, the ER of B7-1453 isolate from Melipax can now be determined in ratios of (+)/(-)-B7-1453.

Elution orders of the organochlorines on chiral gaschromatographic phases. Gas chromatographic enantiomer separation of chiral organochlorines on modified cyclodextrins has recently been reviewed[1]. It was shown that no CSP separated all enantiomers of chiral organochlorines. Several CSPs have been tested in our laboratory. Table 1 summarizes the elution order of the compounds under investigation on four chiral stationary phases.

Table 1: Elution order of chiral organochlorines on permethylated ß-cyclodextrin (ß-PMCD), permethylated γ-cyclodextrin (γ-PMCD), heptakis(6-O-tert.-butyldimethylsilyl-2,3-di-O-methyl)-ß-cyclodextrin (ß-TBDM), and tert.-butyldimethylsilylated ß-cyclodextrin (ß-BSCD)

	α-HCH/α-PDHCH	ß-PCCH/ß-PDPCCH	B7-1453
ß-PMCD	(+) < (-)*	(+) < (-)	not separated
γ-PMCD	(-) < (+)*	(-) < (+)	not separated
ß-TBDM	(+) < (-)	(+) < (-)	not separated
ß-BSCD	depends on synthesis procedure **		(+) < (-)

* elution order of α-HCH was already established in ref. (10) and (11)

** eluted order was inverse on ß-BSCD phases made by different manufacturers[8]: ß-BSCD is a mixture of several reaction products which elute the α-HCH enantiomers in inverse order. The composition varies with slight variations in the synthesis procedure.[8]

References.

(1) Vetter, W.; Schurig, V. *J. Chromatogr. A*, **1997**, in press
(2) König, W.A.; Hardt, I. D.; Gehrcke, B., Hochmuth, D. H.; Hühnerfuss, H.; Pfaffenberger, B.; Rimkus, G. *Angew. Chem.* **1994**, *106*, 2175-2177
(3) Müller, M. D.; Buser, H.-R. *Anal. Chem.* **1994**, *66*, 2155-2162
(4) Haglund, P. *J. High Resol. Chromatogr.* **1996**, *19*, 373-376
(5) Cristol, S. J. *J. Amer. Chem. Soc.* **1949**, *71*, 1894
(6) Krock, B.; Vetter, W.; Luckas, B.; Scherer, G. *Chemosphere* **1996**, *33*, 1005-1019
(7) Vetter, W.; Krock, B.; Luckas, B. *J. Agric. Food Chem.*, **1997**, submitted
(8) Vetter, W.; Klobes, U.; Luckas, B.; Hottinger, G. *Chromatographia*, **1997**, 45, 255-262
(9) Vetter, W.; Klobes, U.; Hummert, K.; Luckas, B.; Hottinger, G. *J. High Resol. Chromatogr.*, **1997**, 20, 85-93
(10) Mössner, S; Ballschmiter, K. Fresenius J. Anal. Chem., 1994, 348, 583-589
(11) Falconer, R.L., Bidleman, T.F., Gregor, D.J., Semkin, R., Reixeira, C. *Environ. Sci. Technol.* **1995**, 29, 1297-1302
(12) Krock, B.; Vetter, W.; Luckas, B. *Chemosphere* **1997**, in press
(13) Vetter, W.; Klobes, U.; Krock, B.; Luckas, B. *Chromatographia*, **1997**, 44, 65-73
(14) Buser, H.-R.; Müller, M. D. *J. Agric. Food Chem.*, **1994**,*42*, 393-400