PRODUCTION OF TOXAPHENE ENANTIOMERS BY ENANTIOSELECTIVE HPLC

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Introduction

Enantioselective studies of chiral organochlorines (chloropesticides and atropisomeric PCBs) attract currently growing interest in environmental chemistry[1,2]. In the past, different estrogenic activity of o,p´-DDT enantiomers and different insecticidal activity of enantiomers of chlordane-related compounds have been reported[3,4]. Application of enantioselective gas chromatography (eGC), i. e. the use of chiral stationary phases (CSP) based on modified cyclodextrins, allows to determine these compounds enantioselectively at trace levels in environmental samples. Suitable CSPs for the enantioseparation of the most of the chiral organochlorines are available. However, an objective interpretation of enantioselective data requires enantioenriched (or enantiopure) standards since reversals of the GC elution order of organochlorine enantiomers have been reported[5]. Such standards can be produced with the help of enantioselective HPLC (eHPLC). In the case of compounds of technical toxaphene (CTTs), this has not been achieved before. Here, we report on the enantioseparation of many environmentally relevant CTTs by using eHPLC.

Materials and Methods

Enantioselective high performance liquid chromatography (eHPLC). The HPLC system consisted of an LC-6a pump (Shimazu, Jena, Germany) and a LiChroCart 250-4 (ChiraDex, Katalogue no. 51333) HPLC column (Merck, Darmstadt, Germany). Different mobile phases composed of methanol with 0-35% water (v:v) were used at flow rates of 1.0 mL. An OR-1590 chiral detector (Jasco, Gera, Germany) was used for selected experiments.

GC/ECD parameters. All fractions were analyzed on a Hewlett-Packard 5890 gas chromatograph equipped with an HP 7673 auto sampler and a t-piece at the end of the injector that divided the GC flow onto two achiral columns (CP-Sil 2 and CP-Sil 8/C18) and two ECDs[6]. This system was used to determine the fractions with the respective target compound.

GC/ECNI-MS parameters. Enantioseparations were performed with a Hewlett-Packard 5890 series II/5989B GC/MS system in the ECNI-MS-SIM mode[7]. Two chiral stationary phases were used: (i) 25% randomly *tert.*-butyldimethylsilylated ß-cyclodextrin (ß-BSCD) diluted in PS086 (BGB Analytik, Adliswil, Switzerland). Column parameters were: 30 m length, 0.25 μm internal diameter, and 0.20 μm film thickness[5]. (ii) 35% impure 6-O-*tert.*-butyldimethylsilyl-2,3-di-O-methyl)-ß-cyclodextrin (ß-TBDM) diluted in OV-1701[8,9]. Column parameters were: 20 m length, 0.25 mm internal diameter, and 0.15 μm film thickness[8,9].

Toxaphene standards and analytical procedure. Melipax (technical toxaphene) was applied to sewage sludge and kept for 4 weeks under anaerobic conditions in our lab. This procedure yielded a significantly simpler CTT pattern in comparison with technical toxaphene The samples were extracted with n-hexane followed by adsorption chromatography on 60 g silica. Liquid

chromatographic fractionating yielded several fractions which contained persistent CTTs as the dominating ones. These fractions were fractionated by reversed phase HPLC (mobile phase: acetonitrile-water 86:14 (v:v). B9-1679 (P-50) was identical with an isolate from seal blubber that has been used earlier for NMR structure elucidation[10]. Solutions containing the most important CTTs were injected into the eHPLC system. 30 fractions of 1 min were collected and extracted twice with 0.5 mL n-hexane (except for pure methanol which was directly gas chromatographed). Aliquots were diluted and analyzed on the dual GC/ECD system. The fractions which contained the target compounds were then analyzed by enantioselective GC/ECNI-MS.

Results and Discussion

Table 1 lists the CTTs tested which comprise the most abundant CTTs in technical products (B7-515, B8-806), biota (B8-1413, B9-1679), and sediment (B6-923, B7-1001).

Table 1: HPLC enantioseparation (ChiraDex) of CTTs and elution order on enantioselective GC (ß-BSCD for all CTTs except B8-1412 that was analyzed on ß-TBDM)

CTT	Substitution pattern	% Methanol/Fraction: ER*	
B6-923 (-)	2-*exo*,3-*endo*,6-*exo*,8,9,10	75/10 min: E1	75/17 min: 0.1
B7-515 (P-32)	2,2,5-*endo*,6-*exo*,8,9,10	75/16 min: E2	100/>20 min: 2
B7-1000 (-)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10	75/9 min: E1	100/>30 min:E2
B7-1001 (-)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10	75/3 min: 0.2	75/6 min: 2.5
B7-1453 (-)	2-*exo*,3-*endo*,5-*exo*,9,9,10,10	75/4 min: E1	75/11 min: 0.05
B8-806 (P-42)	2,2,5-*endo*,6-*exo*,8,8,9,10	75/10 min:**	75/20 min: **
B8-1412 (-)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10	65/9 min: E1	65/14 min: 0.3
B8-1413 (P-26)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10	75/5 min: E1	75/9 min: 0.1
B8-1414 (P-40)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10,10	75/6 min: E2	75/ ?
B8-1945 (P-41)	2-*exo*,3-*endo*,5-*exo*,8,9,9,10,10	75/2 min: 2	75/ ?
B8-2229 (P-44)	2-*exo*,5,5,8,9,9,10,10	100/6 min: 3	100/9 min: 0.3
B9-1679 (P-50)	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10	75/4 min: E2	75/7 min: 4

* elution order of the isolated enantiomers was determined on ß-BSCD (eGC) except B8-1412 which was enantioseparated on ß-TBDM (eGC).
E1 (i. e. ER > 20) or E2 (i. e. ER < 0.05) means pure enantiomer 1 or 2.
Numbers (instead of E1 and E2) are enantiomeric ratio; in these cases only enantioenriched (and not enantiopure) standards were obtained.

** not enantioresolved by eGC. GC/ECD analysis provided two maxima which confirms the successful eHPLC enantiomer separation. Note that the anaerobically degraded sewage sludge only contained one of the two isomers of B8-806/9 (P-42) as recently described for sediment samples[11].

These and further tested CTTs were enantioseparated without exception. However, peak tailing was observed and, after a peak maximum, the respective enantiomer was usually found in several fractions. Therefore, fractions in which the second eluting enantiomer dominated usually also contained low amounts of the first eluted enantiomer (see **Table 1** and **Figure 1**). In this case, pure second eluted enantiomers was obtained by re-injection of the mixed fraction (data not shown).

Enantiomers of CTTs were usually separated with methanol-water (75/25, v:v). In selected cases, however, the second eluted enantiomer was only eluted with pure methanol (see e. g. B7-1000) whereas both B8-2229 enantiomers were enantioseparated with pure methanol. The latter example was surprising. B8-2229 (P-44) and B8-1412 were neither separated with normal phase nor with reversed phase HPLC[6]. We found that B8-1412 already eluted with 65% methanol while B8-2229 (P-44) was not eluted from ChiraDex using these conditions. Furthermore, the enantiomers of both CTTs were separated on by eHPLC (see **Table 1**).

Using methanol-water (75:25, v:v) the retention times were sometimes very short. E. g., the first eluting enantiomers of B8-1945 (P-41) and B9-1679 (P-50) left the HPLC column already after 2 and 3 min, i. e. shortly after the solvent peak. With other words, these first eluting enantiomers were not significantly retarded on the CSP while the second eluting enantiomers were. This illustrates the high enantioselectivity of ChiraDex for CTTs.

The enantiomer elution order on ChiraDex (eHPLC) was often different to that on the ß-BSCD phase (eGC). B6-923, B7-1000, B7-1453, B8-1412, B8-1413 (P-26), B8-1945 (P-41) and B8-2229 (P-44) eluted in the same order whereas B7-515 (P-32), B7-1001, B8-1414 (P-40) and B9-1679 (P-50) left both CSPs reversed order (see **Figure 1**). B8-1412 enantiomers eluted in the same order from the ß-TBDM (eGC) and the ChiraDex (eHPLC) column.

The amounts of pure enantiomers produced so far ranged from >100 ng to > 50 μg. This study clearly confirms that eHPLC is suitable to produce pure enantiomers in order to (i) determine the optical rotation of CTT enantiomers and (ii) to gain sufficient amounts for toxicological investigations. A semi-preparative HPLC column would simplify this by injection and enantioseparation of multiple amounts of racemic CTTs. Research in this field is on-going.

Our attempts to determine the optical rotation of pure enantiomers with the help of a chiral detector failed mainly since the enantiomers were not found in discrete sharp peaks but distributed over a wide range. Therefore, this CSP is not suitable for solving this problem. Establishing of the optical rotation of the enantiomers has to be performed by injection of pure enantiomers into a system consisting of a non-chiral stationary phase and a chiral detector[12].

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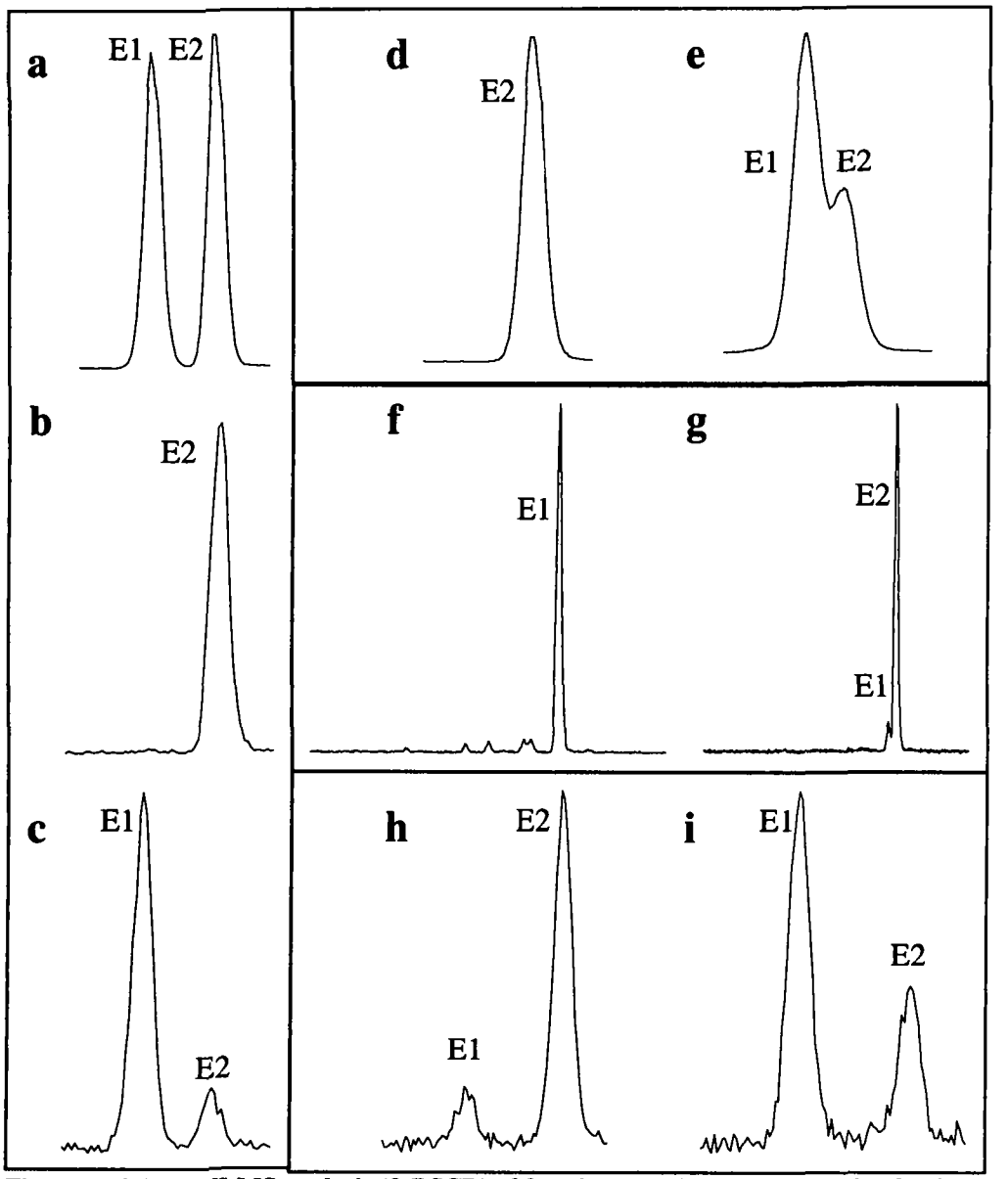

Figure 1: GC/ECNI-MS analysis (ß-BSCD) of fractions obtained with enantioselective HPLC (Chromatograms are corresponding with the fractions mentioned in Table 1).

(a-c) B9-1679 (P-50): (a) original solution; (b) pure enantiomer-2; (c) enriched enantiomer-1.
(d-e) B7-515 (P-32): (d) pure enantiomer-2; (e) enriched enantiomer-1.
(f-g) B8-1413 (P-26): (f) pure enantiomer-1; (g) enriched enantiomer-2.
(h-i) B7-1000: (h) enriched enantiomer-2; (i) enriched enantiomer-1.