STRUCTURE ELUCIDATION OF 2-*endo*,3-*exo*,5-*endo*,6-exo,8,8,10-HEPTACHLOROBORNANE, AN ABUNDANT TOXAPHENE COMPONENT IN TOP PREDATORS AND SEDIMENTS

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Introduction

Toxaphene was the most heavily used organochlorine pesticides in the U. S.[1]. The global production was estimated at 1.3 million tons[2]. Despite its ban in many countries over a decade ago, elevated concentrations of compounds of technical toxaphene (CTTs) are still found in the environment. Presently, CTTs are a major organochlorine contaminant of aquatic life worldwide[3]. However, only a subset of the several hundred CTTs in the original mixture are detectable in environmental samples[4]. In a previous study on Antarctic seals, the GC/ECNI-MS-SIM abundance of a heptachloro component designated as "7-1" was >10% of B8-1413 (P-26), a known, persistent CTT[5]. The purpose of this study was to elucidate the structure of 7-1.

Materials and Methods

Sample origin and reference standards. 7-1 was isolated from toxaphene-contaminated estuarine sediment[6]. A solution of the technical product Camphechlor (10 ng/μL) was obtained from Promochem (Wesel, Germany). Standard solutions of CTTs were obtained from Dr. Ehrenstorfer (Augsburg, Germany), Promochem, or isolated in our lab[7,8]. Both systematic AV-codes[9] and Parlar numbers[10] are used to name individual CTTs.

Isolation of 7-1 from sediment. CTTs including 7-1 were extracted from 1 kg sediment by shaking with hexane/acetone (1:1, v:v) overnight. The organic layer was partitioned into n-hexane and then treated with H_2SO_4 and acid-activated copper. The sediment extract (~1 mg toxaphene) was fractionated on 60 g silica, eluting with n-hexane. Fraction 325-350 mL containing the bulk of 7-1 was evaporated to dryness and re-dissolved in 500 μL acetonitrile. This extract was injected into a RP-HPLC system[7]. The HPLC fraction eluting between 12.5-13.0 min contained >90% 7-1.

Sample clean-up. Matrix interferences in biological samples were removed from the organochlorine fraction by digestion with acids, liquid-liquid extraction with n-hexane, repeated treatment with sulfuric acid, and adsorption chromatography on silica[11].

Gas chromatography/electron capture negative-ionization mass spectrometry (GC/ECNI-MS). Measurements were performed with a HP 5890 Series II plus/5989B GC/MS system using previously published parameters[12]. A 63 m x 0.25 mm i. d. column coated with 0.25 μm of CP-Sil 2 (Chrompack, The Netherlands) or a 30 m x 0.25 mm i. d. column coated with 0.2 μm of 25% randomly *tert.*-butyldimethylsilylated ß-cyclodextrin diluted in PS086 (BGB Analytik, Switzerland) was used. In the SIM mode we monitored m/z 343, 345, 378, and 380 for 7-1.

^{1}H-NMR measurements. The ^{1}H-NMR measurements of 7-1 were performed on a Bruker DRX

500 spectrometer. All chemical shifts were referenced to the solvent peak ($CDCl_3$) and recalculated with respect to TMS ($\delta(^1H)$ = 7.260 ppm for $CDCl_3$). Two dimensional shift correlated and phase-sensitive, time proportional phase incrementation spectra (COSY and NOESY, respectively) were recorded with a mixing time of 220 ms. Acquisition times for COSY and NOESY were 20.5 and 70.5 h, respectively. ^{1}H-NMR-data for 7-1 were as follows: H8 ($CHCl_2$), 6.68 ppm, s; H2-*exo*, 5.12 ppm, dd, 5.5 Hz and ~1.2 Hz; H3-*endo*, 4.66 ppm, m, 5.5 Hz; H6-*endo*, 4.56 ppm, ? Hz, ? Hz; H5-*exo*, 4.56 ppm, m, (to 2.68 ppm); H10a (CH_2Cl), 4.19 ppm, dd, 12.8 Hz, ? Hz; H10b (CH_2Cl), 3.57 ppm, d, 12.8 Hz; H4, 2.68 ppm, m, ~1.2 Hz (to 4.56); H9 (CH_3), 1.89 ppm, s.

Results and Discussion

Isolation and NMR structure elucidation. Based on ECD response factors for several early eluting CTTs, we estimated the isolated mass of 7-1 to be ~20 μg. Although chemical shifts of two protons were identical and some coupling constants could not be resolved (see above), the structure of 7-1 was unequivocally determined to be 2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10-heptachlorobornane (B7-1000) based on a comparison of the ^{1}H-NMR data with that for B8-1413 (P-26). Except for the additional proton H10 on B7-1000 (the signal of B8-1413 (P-26) at 6.42 ppm is distributed in 3.57 and 4.19 ppm), Δppm of all other protons matched that of B8-1413 (deviations of 0.01 to 0.17 ppm). B8-1412 also showed similar chemical shifts as B7-1000 for the two protons on C10[7]. Further confirmation of the structural assignment was derived from molecular modeling as reported elsewhere[13].

GC/ECNI-MS. The full scan mass spectrum of B7-1000 was dominated by the molecular ion starting at m/z 376 (**Figure 1**). Formation of the molecular ion is unusual for heptachlorobornanes, which usually show highest abundance for the $[M-Cl]^-$ fragment ion[14]. The $[M-Cl]^-$ fragment ion at m/z 341 was overlapped by the $[M-HCl]^-$ fragment ion which accounted for ~40% of the former (i.e. $[M-Cl]^-$) fragment ion. A large relative contribution of the $[M-HCl]^-$ fragment ion was also found for B8-1413 (P-26), providing further evidence as to the similarity of the two CTTs.

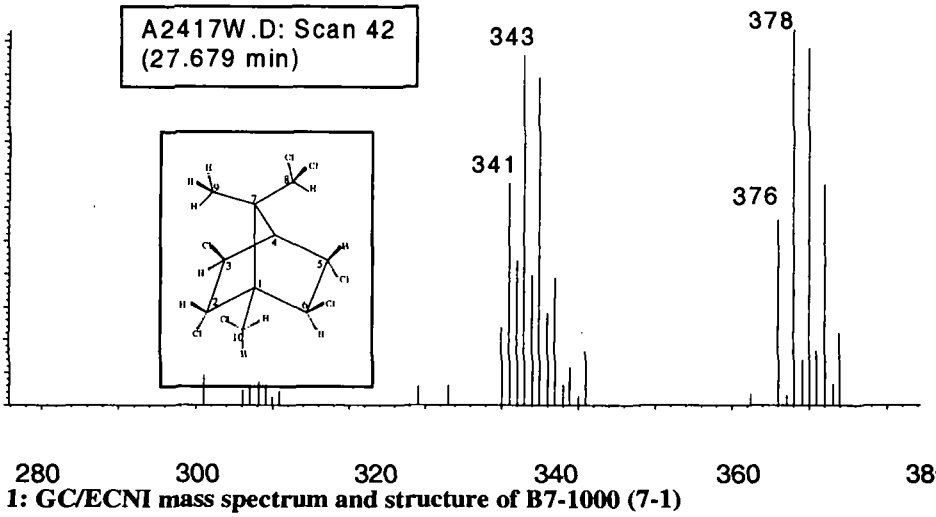

Figure 1: GC/ECNI mass spectrum and structure of B7-1000 (7-1)

Significance of B7-1000 in environmental samples. GC/ECNI-MS in the SIM mode was used to identify B7-1000 in technical toxaphene and environmental samples (**Figure 2**). B7-1000 is the first eluting heptachlorobornane in toxaphene on non-polar GC phases (e.g. DB-5). GC/MS analysis of technical toxaphene is therefore a suitable method for the identification of B7-1000. Because a quantitative solution was not available and ECNI-MS response factors can vary significantly for chlorobornanes, we cannot provide concentrations for B7-1000. Preliminary results suggest, however, that the detector response ratios of B7-1000 and B8-1413 (P-26) using ECD and ECNI-MS are similar. Under this assumption, B7-1000 accounted for ~10 % of the ECNI abundance of B8-1413 (P-26) in most of our samples (**Table 1**).

Table 1: ECNI-MS signal intensity of B7-1000 relative to B8-1413 (P-26)

Origin, Species	Sample size	% of B8-1413 (P-26) mean (range)
Iceland, harbor porpoise (*Phocoena phocoena*)	n = 4	9.2 (8.3 – 10.7)
Spitsbergen, harp seal (*Phoca sibirica*)	n = 9	10.0 (pool sample)
Antarctic, Weddell seals (*Leptonychotes Weddelli*)	n = 8	16.2 (11.6-25.6)

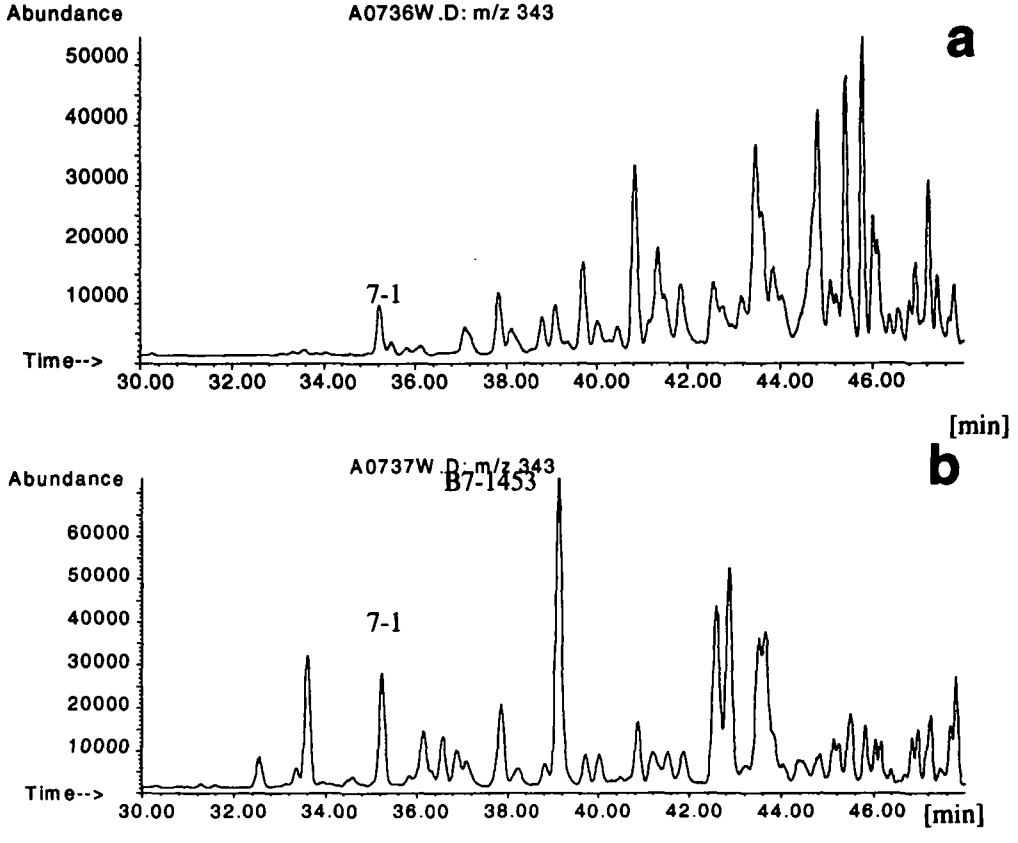

Figure 2: GC/ECNI-MS detection of B7-1000 in (a) technical toxaphene and (b) blubber of harbor porpoise (*Phocoena phocoena*). Unlabelled peaks in (b) are not chlorobornanes.

We also detected B7-1000 in human milk, adipose tissue of a polar bear, and fish samples (salmon fillet and cod liver). It is thus surprising that B7-1000 has not been reported more frequently in the literature. Monitoring a time window for m/z 343 that is too narrow is one possibility. Using non-selective methods such as GC/ECD, a pre-separation of PCBs should be performed before quantification of CTTs. Many authors have reported that some CTTs (particularly B8-1413 (P-26)) might smear into the PCB fraction[15,16]. Note that B7-1000 elutes earlier from silica than B8-1413 (P-26), and thus will elute in the PCB fraction generated using standard chromatographic clean-up protocols.

Conclusion. The structure elucidation of previously unidentified chlorobornanes like B7-1000 enhances our understanding of the environmental fate of toxaphene. Many environmentally relevant CTTs are currently available as reference standards, enabling the analyst to quantify CTTs on a congener-specific basis. It should be emphasized, however, that none of the commercially available heptachlorobornanes are regularly detected in higher organisms. Thus, the commercial availability of B7-1000 would be an important contribution to the analysis of toxaphene.

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