1,2,5,6,9,10-Hexabromocyclododecanes - a class of compounds with a complex stereochemistry

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Introduction

Hexabromocyclododecanes (HBCD) are the third most used brominated flame retardant. Concentrations are increasing for individual environmental compartments. Today, HBCD have entered the aquatic environment and are found in various aquatic organisms (1-4). We determined HBCD concentrations of 25-210 ng g^{-1} (lipid weight) in whitefish from six Swiss lakes (5). Enrichment of certain HBCD isomers were observed for these samples. The patterns differed from the one of a low-melting technical HBCD mixture as discussed herein.

Considering the increasing global use of HBCD, their ubiquitous occurrence even in remote areas and their increasing concentrations in biota, very limited information is available about environmental levels or toxic effects of individual HBCD stereoisomers. The stereochemistry of 1,2,5,6,9,10-HBCD is complex. Six stereocenters are formed upon complete bromination of 1,5,9-cyclododecatrienes (CDT) for which four isomers are known. In principle, 16 stereoisomers - 4 meso forms and 6 pairs of enantiomers have to be expected. However, environmental levels of individual HBCD enantiomers and analytical methods suitable for resolution and detection of different HBCD enantiomers have not been reported yet.

Herein, we report an analytical procedure relaying on chiral reverse-phase chromatography to differentiate individual HBCD stereoisomers. With the improved methodology, we identified 8 stereoisomers, all isolated from a low-melting, technical HBCD mixture. Six stereoisomers were found to be pairs of enantiomers and two additional HBCD stereoisomers were tentatively assigned as meso-forms.

Materials and Methods

The investigated stereoisomers were isolated from a low-melting, technical grade HBCD mixture (Saytex, mp = 168-184 °C) by liquid chromatography on silica with mixtures of *n*-hexane and dichloromethane. The recovered material was repeatedly crystallized to obtain material in a quality suitable for x-ray diffraction crystallography. Three crystalline samples of HBCD diastereomers were obtained with a purity > 98%, according to LC-MS analysis.

Resolution of enantiomers was achieved with a chiral permethylated-β-cyclodextrin reverse phase column (200 mm x 4 mm, Nucleodex 5, Macherey-Nagel) at a flow rate of 1 ml/min with a methanol/water gradient (85% methanol for 1 min, 85-98% in 15 min, 98% for 9 min).
Mass spectrometric analysis was performed on a triple stage quadrupole mass spectrometer (TSQ 7000, Thermo Finnigan) in the negative ion atmospheric pressure chemical ionization mode, recording the three most prominent anions at m/z 638.6, 640.6 and 642.6. Optical rotation was determined on a polarimeter (Polar Monitor, Büchi) calibrated with methanolic solutions of enantiomerically pure (+) and (-) carvon. Intensities for the crystal structure analysis were measured on a Bruker Nonius Kappa CCD with Mo-Kα radiation (λ=0.71073 Å) at room temperature. The structures were solved with SIR97 and refined with maXus and SHELXL97.

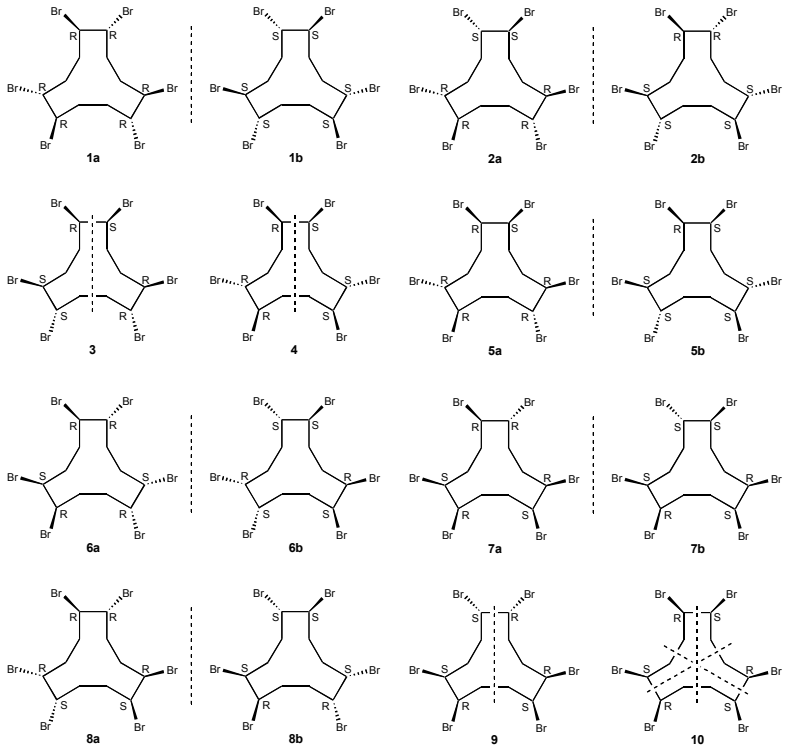

Figure 1 Scheme of all 16 1,2,5,6,9,10-HBCD stereoisomers. Dashed lines indicate mirror planes.

Results and Discussion

Stereochemistry: Six stereocenters at positions 1,2,5,6,9 and 10 are formed upon perbromination of 1,5,9-cyclododecatrienes. Figure 1 represents a scheme of all 1,2,5,6,9,10-HBCD stereoisomers theoretically possible. Overall, 16 stereoisomers - 6 diastereomeric pairs of enantiomers (**1a/b**, **2a/b**, **5a/b, 6a/b**, **7a/b**, **8a/b**) as well as 4 meso forms (**3**, **4**, **9**, **10**) - can be deduced.

Chromatographic separation: At most 10 diastereomers, but 16 stereoisomers can ideally be separated by achiral- and chiral-phase chromatography, respectively. Figure 2 displays chiral phase chromatograms of three pairs of enantiomers and two meso forms, named herein as α-, β-, γ-, δ- and ε-HBCD, which were all isolated from a low-melting, technical grade HBCD. So far, no diastereomerically pure sample of ε-HBCD could be obtained.

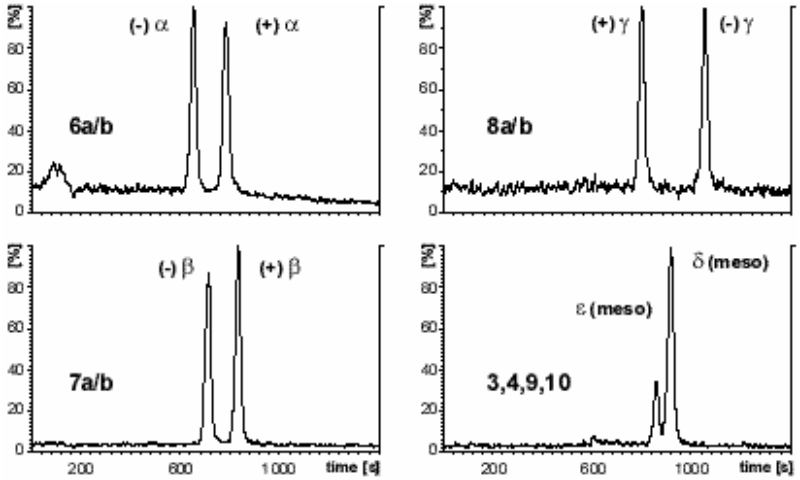

Figure 2 Chiral phase chromatograms of 8 stereoisomers found in low-melting, technical grade HBCD.

Measurements of optical rotation revealed that, indeed, three pairs of enantiomers were present with (-) α- and (-) β-HBCD eluting before the corresponding enantiomers, but the (+) γ-HBCD eluting ahead of the (-) form. No optical rotation was detected for δ- and ε-HBCD.

The proportion of the 5 diastereomers present in the technical product was estimated. We calculated relative amounts of α-, β-, γ-, δ- and ε-HBCD of 11.8, 5.8, 81.6, 0.5 and 0.3%, respectively, indicating that γ-HBCD is by far the most abundant diastereomer. Comparably low proportions of α- and β-HBCD and only trace levels of δ- and ε-HBCD were found.

Spectroscopic properties: Figure 3 displays the crystal structure of the most prominent HBCD diastereomer found at elevated levels in whitefish of Swiss lakes (5). The crystal structure data of two additional pairs of enantiomers is not shown but hopefully available soon (6). Based on our spectroscopic data, the α-, β- and γ-HBCD diastereomers are assigned to structures **6a/b**, **7a/b** and **8a/b**, respectively (Fig. 1). The (+/-) α-HBCD (**6a/b)** crystallized in a polar spacegroup $P2_1$ with an enatiomeric pair in the asymmetric unit. This stereoisomer shows the same conformation as the structure of cyclododecane (7). The (+/-) β-HBCD (**7a/b)** crystallized in the monoclinic space group $P2_1$/n. The (+/-) γ-HBCD (**8a/b)** crystallized in the centric, orthorhombic space group *P*bca.

Figure 3 Stereoview of one of the α-HBCD (**6a/b**) enantiomers with 50% vibration ellipsoids.

Conclusions

From these findings we conclude that the stereochemistry of HBCD is far more complex than discussed in the literature so far. From the 16 possible stereoisomers, 8 isomers - three diastereomeric pairs of enantiomers present as racemic mixtures, as well as two meso forms - were isolated from a low-melting, technical-grade HBCD mixture. X-ray crystal structures of the three pairs of enantiomers **6a/b** (α), **7a/b** (β) and **8a/b** (γ) were obtained. In addition, the chromatographic properties of the 8 stereoisomers on a chiral column as well as the optical rotation of enantiomerically pure stereoisomers were determined.

Nevertheless, further work will be necessary to determine the absolute configuration of all stereoisomers present in technical HBCD mixtures. So far they can only be distinguished based on optical rotation and chromatographic retention time on a chiral column. Two formerly unknown HBCD stereoisomers, assigned as δ- and ε-HBCD, were isolated from the technical product, as well, and tentatively assigned as meso forms. We hope that the presented work contributes to a better understanding of the complex stereochemistry of HBCD and induces environmental and toxicological research on individual HBCD stereoisomers.

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