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On the Enantiomerization Barrier of Atropisomeric 2,2',3,3',4,6'-Hexachlorobiphenyl (PCB 132)

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Abstract: The rotational barrier of chiral, tri-ortho-substituted 2,2',3,3',4,6'-hexachlorobiphenyl (PCB 132) was determined. After semipreparative HPLC separation of PCB 132 on polysiloxane-anchored permethyl-β-cyclodextrin (Chirasil-Dex), one single enantiomer was subjected to an enantiomerization kinetic and the enantiomeric ratio was determined by chiral gas chromatography. Furthermore, on-column inversion of PCB 132 was investigated in the presence of Chirasil-Dex via stopped-flow gas chromatography.

Anthropogenic polychlorinated biphenyls (PCBs) accumulate in the environment as persistent pollutants. 78 out of 209 congeners of PCBs display axial chirality in their nonplanar conformations. It has been predicted that 19 PCBs, at least nine present in commercial PCB formulations, exist as stable atropisomers at ambient temperatures due to restricted rotation about the C-C bond with estimated enantiomerization barriers $\Delta G^{\#}$ between 105 - 240 kJ/mol[1].

Chiral atropisomeric PCBs were resolved by liquid chromatography on triacetylcellulose[2] and by high-resolution capillary gas chromatography on cyclodextrin-based stationary phases[3, 4, 5]. Using a multidimensional approach[6], it has been demonstrated recently that the enantiomers of the Title compound 2,2',3,3',4,6'-hexachlorobiphenyl (PCB 132) are found in unequal amounts (enantiomeric ratio: 0.45-0.85) in human milk specimens[7], another approach showed, that one enantiomer of PCB 149 was slightly enriched in mussels[8, 9]. These remarkable findings raise the question whether the enantiomeric bias observed is due to *in vitro* or *in vivo* enantioselective degradation or is caused by deracemization in the presence of chiral biogenic matrices due to a low enantiomerization barrier. Moreover, it has been inferred that the height of the rotational barrier in PCBs is directly related to toxicity, *i.e.*, the low barrier of planar congeners lacking chloro substituents in ortho positions gives rise to toxicity while the high barrier of nonplanar congeners with four chloro substituents in ortho positions leads to low or no toxicity[10]. Thus, the precise knowledge of the rotational barriers $\Delta G^{\#}$ of PCBs and bromo analogs is of interest. For atropisomeric PCBs the rotational barrier can experimentally be determined via enantiomerization kinetics.

A barrier of 155.57 kJ/mol[10] and 157 kJ/mol[11] for 2,2',3,3'6,6'-hexachlorobiphenyl (PCB 136) and of as little as ≈ 116 kJ/mol[11] for PCB 95 and the Title compound (PCB 132) has been calculated by a molecular orbital method employing the AM1 formalism. The latter value can not be reconciled with the absence of interconversion profiles[12, 13, 14] during the gas chromatographic enantiomer separation of PCB 132 on Chirasil-Dex[15] in 55 min at 180°C indicating a barrier of at least $\Delta G^{\#} \geq 160$ kJ/mol[6]. A similar discrepancy between the low calculated barrier of PCB 95 ($\Delta G^{\#} = 114.1$ kJ/mol) and the absence of enantiomerization in the GC experiment has been explained[11] by the increase of the rotational barrier due to polar enantioselective interactions with Chirasil-Dex. Here we report on the chromatographic determination of the enantiomerization barrier of PCB 132 by on- and off-column methods. The enantiomers of PCB 132 were semipreparatively separated by HPLC on Nucleosil coated with immobilized Chirasil-Dex[15]. The first eluted enantiomer was isolated (ee = 98 %) and subjected to an enantiomerization kinetic in n-heptadecane at 280 - 300°C. The time-dependent decrease of the enantiomeric ratio was determined on Chirasil-Dex[15] by analytical gas chromatography. The linear Arrhenius plots (for three separate determinations) yielded 280°C: $k = 7.2 \pm 0.3 \times 10^{-5}\ s^{-1}$, $\Delta G^{\#} = 182 \pm 1$ kJ/mol; 290°C: $k = 1.4 \pm 0.1 \times 10^{-4}\ s^{-1}$, $\Delta G^{\#} = 182 \pm 1$ kJ/mol; 300°C: $3.8 \pm 0.1 \times 10^{-4}\ s^{-1}$, $\Delta G^{\#} = 181 \pm 1$ kJ/mol. These values are appreciably higher than the calculated value and explain the absence of plateau formation in the gas chromatographic enantiomer separation on Chirasil-Dex at 180°C within 55 min. The enhanced rotational barrier of PCB 132 with only three ortho chlorine atoms may be the result of a "buttressing effect"[16] of the two meta chlorine atoms.

To probe the asserted influence of polar interactions on the rotational barrier of PCBs[11], we investigated the on-column inversion of the enantiomers of PCB 132 in the presence of Chirasil-Dex[15] via a stopped-flow method[17, 18]. As shown in the Figure the enantiomers (a) and (b) and the racemic mixture (c) of PCB 132 were separately gas-chromatographed on a column coated with Chirasil-Dex[15]. After a specific time, the mobile phase flow was stopped and the setup was quickly heated to 320°C for 3 min to affect enantiomerization of PCB 132 in the enantiomerically pure elution zone(s). Afterwards, the column was rapidly cooled back to the previous temperature and the original flow rate was reinforced leading to enantiomer separation. Peak integration revealed the approximate enantiomeric ratio 32/68 for enantiomer (a) and 72/28 for enantiomer (b). The same ratio is observed for the racemate (c) taking into account that the fraction of inverted molecules is not resolved (cf. Fig.).

The enantiomeric ratios caused by inversion at 320°C in 3 min corresponds to a rotational barrier of $\Delta G^{\#} \approx 183$ -186 kJ/mol within experimental error in agreement with the value obtained by the off-column technique in the apolar environment of n-heptadecane.

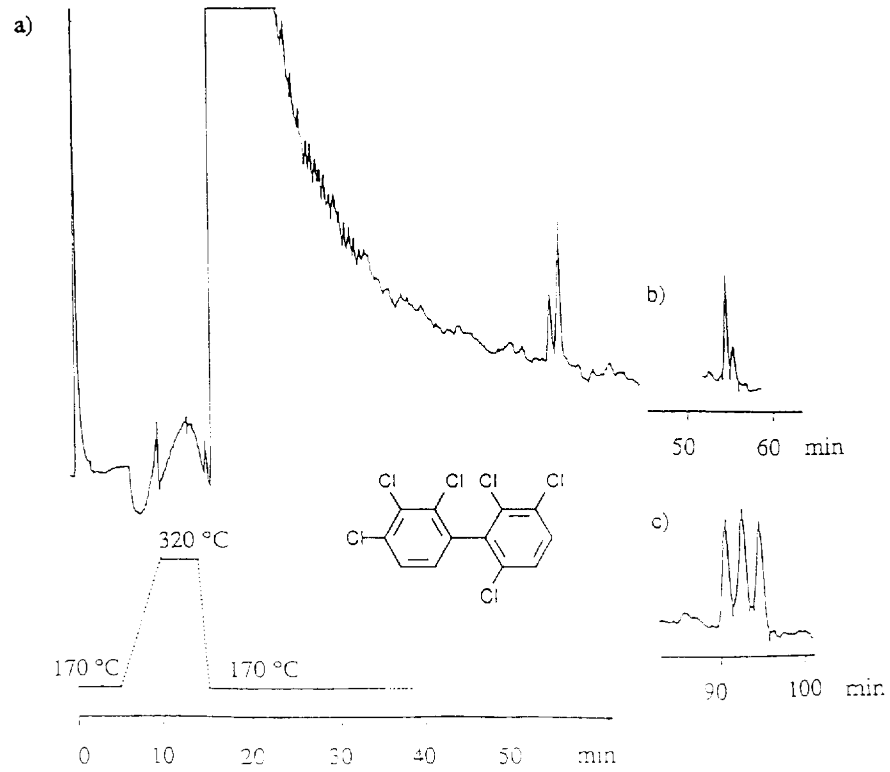

Fig. : On-column screening of the enantiomerization barrier by the stopped-flow technique during the gas chromatographic enantiomer separation of PCB 132. Fused-silica capillary column (25 m x 0.25 mm i.d.) coated with immobilized Chirasil-Dex[15], FID-detection. (a) Second eluted enantiomer, (b) first eluted enantiomer (elution order in HPLC and GC is identical): oven temperature: 170°C, after 5 min the carrier gas flow was stopped and the column was heated to 320°C, held for 3 min and after cooling to 170°C, gas chromatography was continued and the enantiomeric ratio recorded. (c) Racemic mixture; oven temperature: 160°C, after 20 min the carrier gas flow was stopped and the column was heated to 320°C, held for 3 min and after cooling to 160°C gas chromatography was continued and the enantiomeric ratio recorded. The noisy chromatographic baseline is due the heating process.

Thus, contrary to the recent suggestion[11] the polar chiral stationary phase does not exert an appreciable influence on the barrier of PCB 132[19]. The stopped-flow on-column method represents a fast and simple pre-screening method for the determination of enantiomerization barriers > 130 kJ/mol when dynamic chromatography ($\Delta G^{\neq} = 70 - 130$ kJ/mol)[10, 11] or NMR-spectroscopy ($\Delta G^{\neq} < 80$ kJ/mol) cannot be applied.

In conclusion, the low calculated rotational barrier of PCB 132 is not confirmed by on-column and off-column enantiomerization studies. An increase of the barrier due to polar interactions with Chirasil-Dex has not been detected. The data obtained strongly indicate that *in vivo* enantiomeric bias of PCB 132[7, 8, 9] is unlikely caused by a deracemization process.

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[19] However, as previously derived in dynamic chromatography[12, 13], a small difference in the rate constants between forward and backward enantiomerization is expected because the enantiomers reside in the environment of the chiral stationary phase and must possess a different energy to be separated.

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