On the Thermal and Environmental Stability of Atropisomeric PCBs

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Abstract

The internal barrier of rotation was determined for 12 atropisomeric PCBs by enantiomerization kinetics studies of enantiomerically pure PCB congeners. The average free energy of activation ($\Delta G^{\ddagger}$) values of the tri-*ortho* PCBs ranged between 42.2 and 44.2 kcal/mol. A buttressing effect of about 1.5 kcal/mol was observed for PCBs which have meta chlorines that obstruct the motion of the ortho chlorines in the transition state. The $\Delta G^{\ddagger}$ values of the tetra-*ortho* PCBs were about 59 kcal/mol.

The observed $\Delta G^{\ddagger}$ values are high enough to prevent enantiomization of the tri- and tetra-*ortho* PCB atropisomers at physiological temperatures. In fact, the atropisomers will also resist racemization at normal gas chromatography conditions, even if a standard split/ split-less injector is used.

Introduction

Of the 209 PCB congeners 78 display axial chirality, and it is suggested that 19 exist as stable atropisomers at ambient temperatures due to restricted rotation about the central C-C bond (1). This restriction of rotation is sustained by bulky substituents in the *ortho* positions of the phenyl rings. Oki predicted the existence of atropisomerism, where isomers can be isolated, for compounds which have a racemization half-life > 1000 s or 22.3 kcal/mol at 300 K (2). Only the 19 tri- or tetra-*ortho* chlorinated PCBs fulfil this criterion. Schurig et. al. reported a rotational energy barrier of 43 kcal/mol (3) for one of these atropisomeric PCBs, 2,2',3,3',4,6'-hexachlorobiphenyl (PCB#132 (4)).

However, several groups have analysed atropisomeric PCBs in various marine species without finding any large deviations in enantiomeric excess ratios (ERs) between the enantiomeric pairs (5,6). This can be interpreted in two ways: 1) no enantioselective processes are involved in the uptake, transport, metabolism or excretion of these PCBs, or 2) the enantiomers are not sufficiently stable and racemize in biota. Schurig's data suggests the first option.

The aim of this study has been to determine the rotational energy barriers for twelve atropisomeric PCBs including eight which are present in commercial PCB products at ≥ 1% by weight (7), to check if these compounds actually are stable enough to resist racemization at physiological temperatures. The results will also indicate whether or not special precautions are needed to prevent these compounds from racemizing during chemical analysis, e.g. using gas chromatography.

Experimental

Chemicals

The 12 atropisomeric PCB congeners, viz. 84, 131, 132, 135, 136, 144, 149, 174, 175, 176, 183, and 196, included in this study, were isolated in mg quantities by enantioselective high-performance liquid chromatography (HPLC) using either permethylated cyclodextrin (PMCD) or (+)- poly(diphenylpyridyl) methacrylate (OP(+)) phases. *n*-Docosane and *n*-hexatriacontane were of 99% purity obtained from by Aldrich-Chemie GmbH (Steinheim, Germany). Dichloromethane and n-hexane were of HPLC grade, supplied by Labscan Ltd. (Dublin, Ireland). Glass distilled toluene from Burdick and Jackson (Muskegon, MI, USA), and PA grade methanol was from Mallinckrodt Baker B.V. (Deventer, Holland). Water was purified with a Milli-Q apparatus (Millipore, Bedford, MA, USA).

Thermal enantiomerization experiments

The rotational energy barriers were determined by studying the enantiomerization kinetics at several temperature. In our experiments we used *n*-docosane ($C_{22}H_{46}$) as solvent (bp 369 °C) for the tri-*ortho* chlorinated PCBs. However, since significantly higher activation barriers are expected for tetra-*ortho* PCBs, *n*-hexatriacontane ($C_{36}H_{74}$, bp > 450 °C) was used for the PCBs 136 and 176. Enantiomerically pure, or highly enriched, PCBs were transferred to ampoules in toluene solutions, 250µl high boiling *n*-alkane was added, the toluene was evaporated, and the ampoules were argon flushed and flame sealed. One to four hundred ng were used of the PCBs which should be analysed by HPLC, and 10-40 ng was used then the analysis should be performed by GC. The enantiomerization kinetics was studied by heating the enantio-merically pure PCBs at 270 - 300°C for different length of time (32 - 128 min) in a GC oven. Due to the higher enantiomerization barrier for the PCBs 136 and 176 these were treated at 425°C for 21 hours.

After the heat treatment the samples were dissolved in 1 ml *n*-hexane and the *n*-docosane, or *n*-hexatriacontane, was removed by high-resolution gel permeation chromatography (HR-GPC). The separation was obtained using two serially connected HR-GPC columns (300 x 7.8 mm, 5 µm polystyrene-divinylbenzene particles, 50Å pore size, Polymer Laboratories, Church Stretton, UK) eluted with 50/50 (v/v) *n*-hexane/ dichloromethane, at a flow rate of 0.7 ml/min. The PCBs were recovered by collecting the eluent between 18 - 25 min. Following solvent exchange to iso-octane or methanol, and concentration to an appropriate volume, the samples were ready for analysis by GC and HPLC, respectively.

GC and HPLC analysis of enantiomerization products

The products of the enantiomerization experiments were analysed by either GC with an electron capture detector (ECD), or by HPLC with UV detection (λ=210 nm), c.f. Table 1.

For the GC analyses a 25 m x 0.25 mm fused silica column with covalently bound PMCD (CP Chirasil-Dex CB, Chrompack, The Netherlands), was used with hydrogen as the carrier gas. One microliter (ca. 1%) aliquots of the iso-octane solutions were split-less injected (220 °C). The GC oven temperature programme was optimised for each PCB atropisomer, and the best separation was generally obtained with a long period of isothermal operation at 145 °C to 160 °C.

The PMCD-HPLC analysis is described elsewhere (8), and the OP(+)-HPLC analyses were performed as follows: 10 µl methanol aliquots (ca. 10%) were injected onto the Chiralpak OP(+) column (250 x 4.6 mm, Daicel Chemical Industries, Japan), held at 0 °C, and eluted with 90/10 (v/v) methanol/water at 0.80 ml/min.

The relative peak areas were determined and used during the subsequent calculations. In some cases, the elution profiles were broad and difficult to integrate and a peak fitting procedure, using the PeakFit software (Jandel Scientific, Germany), was required to obtain reliable data.

Calculations

The rotational energy barriers can be determined by studying the temperature dependence of the racemization rate of pure PCB enantiomers. The kinetics can be expressed as a reversible first-order reaction:

$$\ln\left\{\frac{[A]_0}{[A]_0 - 2[A]}\right\} = 2kt \qquad (1)$$

there $[A]_0$, and [A] is the initial concentration, and the concentration at time t (s). The first-order rate constant k (s^{-1}) is obtained from a plot of $\ln\{[A]_0/([A]_0-2[A])\}$ vs. time. The slope of such a plot is 2k. If the transition-state theory is combined with the fundamental rate constant equation we can determine free energy of activation $\Delta G^{\ddagger}$, by this expression (9):

$$k = \frac{k_b T}{h}\exp\left(-\frac{\Delta G^{\ddagger}}{RT}\right) \qquad (2)$$

there k_b is Bolzmann's constant, T temperature in Kelvin, h is Planck's constant, R is the gas constant, and k is the first-order rate constant.

Results and Discussion

LC and GC Analysis

All atropisomeric PCBs were successfully separated by either enantioselective HPLC or GC. The OP(+) HPLC column resolved the enantiomers of the PCBs 144 and 183 ($\alpha > 1.2$, $R_s > 1.2$), and the PMCD column separated the enantiomers of the PCBs 131, 175, and 196 ($\alpha \approx 1.1$, $R_s \geq 1$). The remaining atropisomers, PCBs 84, 132, 135, 136, 149, and 176, were separation by GC on the Chirasil-Dex column ($\alpha \approx 1.0$, $R_s = 0.8$-1.8), see Table 1.

IUPAC	Analytical technique	Column	R_s	α	Enantiomer
84	Gas chromatography	Chirasil-Dex	1,12	1,02	(+)
132	Gas chromatography	Chirasil-Dex	1,81	1,01	(+)
135	Gas chromatography	Chirasil-Dex	0,97	1,01	(-)
136	Gas chromatography	Chirasil-Dex	1,16	1,02	(+)
149	Gas chromatography	Chirasil-Dex	1,65	1,03	E2
174	Gas chromatography	Chirasil-Dex	0,86	1,01	(-)
176	Gas chromatography	Chirasil-Dex	0,85	1,01	(+)
131	Liquid chromatography	Nucleodex β-PM	0,99	1,08	(+)
144	Liquid chromatography	Chiralpak OP(+)	1,27	1,20	E2
175	Liquid chromatography	Nucleodex β-PM	1,35	1,10	(+)
183	Liquid chromatography	Chiralpak OP(+)	1,46	1,23	E2
196	Liquid chromatography	Nucleodex β-PM	1,46	1,11	(+)

Table 1. PCBs separation technique and column, selectivity factors (α), resolution (Rs), and enantiomer (+/-), or if the optical rotation is unknown peak number (E2 = second eluting enantiomer).

Thermal racemerization experiment

The thermal racemization experiments performed on the tri-*ortho* chlorinated biphenyls in the temperature range 270 to 300 °C resulted in racemization half-lives between 7300 and 370 s, c.f. Figure 1 and Table 2. The reaction rate constants are 4.73×10^{-5} to 9.26×10^{-4} (s^{-1}), according to equation (1). The racemization half-life of the tetra-*ortho* PCBs 136 and 176, at 425 °C, was 56000 s (16 h) and 70000 s (19.6 h), respectively, with corresponding reaction rate constants of 6.14×10^{-6} and 4.91×10^{-6} (s^{-1}), c.f. Table 2. The regression constant (r^2) for the tri-*ortho* PCBS ranged between 0.82 and 1.00.

The free energy of activation ($\Delta G^{\ddagger}$) for the tri-*ortho* PCBs was estimated at each temperature according to Equation (2). The $\Delta G^{\ddagger}$ values for the tri-*ortho* PCBs were in the interval 42.1 to 44.4 kcal/mol with a variance (p=0.05) of 0.2 to 0.8 kcal/mol, and the $\Delta G^{\ddagger}$ values for the tetra-*ortho* PCBs were ~ 59 kcal/mol, see Table 2. Interestingly, our value for PCB#132 (44.2 kcal/mol) is in close agreement with the value reported by Schurig. (43.9 kcal/mol) (3). Further, in all cases the rotational energy is far above the energy barrier required for environmental stability, that have been estimated at 22 kcal/mol.

Some atropisomeric tri and tetra-*ortho* PCBs are expected to exhibit a buttressing effect, and some are not, e.g. PCB 84 and 144. By comparing the rotation energy barriers for compounds from these groups we could observe a buttressing effect for the PCBs. The average $\Delta G^{\ddagger}$ value of the first group (PCBs 84, 131, 132, 135, 174, 175, and 196) is 43.8 ± 0.27 kcal/mol (p=0.05), compared to 42.3 ± 0.12 kcal/mol for the second group (PCBs 144, 149, and 183). Thus, the buttressing effect account for about 1.5 kcal/mol in the first group. The difference between the groups is statistically significant at the p= 0.01 level. The significance of this effect can be illustrated by the racemization half-lives ($t_{1/2}$). PCBs which exhibit buttressing effect have average $t_{1/2}$ values of 5787 s (280 °C), 2933 s (290 °C), and 1804 s (300 °C), and the atropisomers which lack buttressing chlorines have $t_{1/2}$ values of 1492, 824, and 433 s (at the same temperatures).

IUPAC no	Temperature	Rate constant	r^2	$\Delta G^{\ddagger}$	$\Delta G^{\ddagger}$ average
84	280	6.24×10^{-5}	0.980	43.70	43.80±0.34
	290	1.24×10^{-4}	0.962	43.74	
	300	2.07×10^{-4}	1.000	43.96	
131	280	6.22×10^{-5}	0.980	43.71	43.82±0.26
	290	1.15×10^{-4}	0.995	43.83	
	300	2.15×10^{-4}	0.995	43.91	
132	280	4.73×10^{-5}	0.977	44.01	44.18±0.37
	290	7.70×10^{-5}	0.995	44.28	
	300	1.61×10^{-4}	1.000	44.24	
135	280	4.81×10^{-5}	0.984	43.99	44.15±0.56
	290	9.32×10^{-5}	-------	44.06	
	300	1.39×10^{-4}	0.910	44.41	
144	270	1.10×10^{-4}	0.998	42.28	42.43±0.19
	280	2.05×10^{-4}	0.949	42.40	
	290	3.66×10^{-4}	0.963	42.53	
	300	7.32×10^{-4}	--------	42.52	
149	270	1.20×10^{-4}	1.000	42.19	42.26±0.20
	280	2.28×10^{-4}	0.998	42.28	
	290	5.26×10^{-4}	0.993	42.13	
	300	7.69×10^{-4}	--------	42.46	
174	280	5.45×10^{-5}	0.999	43.85	43.85±0.83
	290	1.53×10^{-4}	0.949	43.51	
	300	1.71×10^{-4}	0.820	44.18	
175	280	7.55×10^{-5}	0.940	43.49	43.56±0.53
	290	1.70×10^{-4}	0.953	43.39	
	300	2.38×10^{-4}	0.970	43.80	
183	270	1.34×10^{-4}	0.997	42.07	42.21±0.28
	280	2.74×10^{-4}	0.991	42.08	
	290	4.00×10^{-4}	0.976	42.43	
	300	9.26×10^{-4}	-------	42.25	
196	280	8.88×10^{-5}	0.977	43.34	43.47±0.32
	290	1.58×10^{-4}	0.977	43.47	
	300	2.86×10^{-4}	0.993	43.59	
136	425	6.14×10^{-6}	--------	58.70	
176	425	4.91×10^{-6}	--------	59.01	

Table 2. Values of the first-order rate constant, free energy activation ($\Delta G^{\ddagger}$), and the regression coefficient (r^2) for each temperature and PCB.

Thermal degradation of PCBs

Tetra-*ortho* PCBs have a high rotational energy barrier and therefore experiments were conducted at 425 °C for an extended period of time, 21 h. As the reaction mixtures were analysed by GC it was realised that some thermal degradation products were formed during the experiment. By using GC/ mass spectrometry the presence of degradation products from both PCB 136 and 176 could be verified. In the reaction mixture of PCB#176 only hexachlorobiphenyls (PCBs 132, 136, 144, 145, 149, and 150) were detected, while both penta- (PCBs 84, 95, and 96) and tetrachlorobiphenyls (PCB#53 and 54) were formed from PCB#136, cf. Table 3. In both cases, the amount of degradation products approached the amount of parent PCB. Dechlorination and chlorine migration reactions were suggested by the structure of the pyrolysis products.

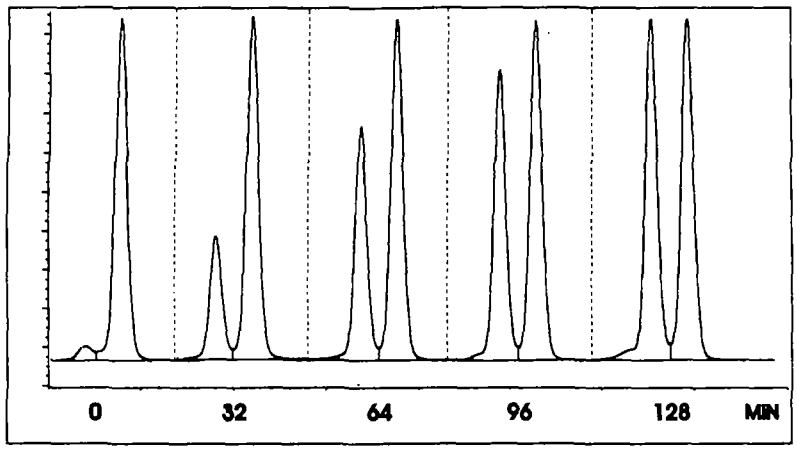

Figure 1. Gas chromatograms from the analysis of the reaction mixtures obtained by heating (+)-PCB#149 for different length of time.

IUPAC	% of total PCB	IUPAC	% of total PCB
136	64.8	*176*	58.9
96	22.4	150	10.6
95	6.6	136	8.9
84	3.7	132	5.3
54	1.5	149	4.5
53	1.1	164	4.2
		145	3.8
		168	2.2
		144	1.6

Table 3. Thermolysis mixture composition after treatment of PCB#136 (left column) and PCB#176 (right column) at 425°C for 21 h, in argon atmosphere.

Conclusions

The experimentally determined rates of racemization clearly indicates an environmental stability of the atropisomeric PCBs. The most labile isomer, PCB#183, has a free energy of activation value of 42.2 kcal/mol which translates to a half-life of 7×10^{18} s, or 2×10^{11} years, at 37 °C! Thus, unless the PCB atropisomers are affected by some enantioselective process in a living organism they will maintain their enantiomeric ratio for the foreseen future. In the tetra-ortho PCBs the resistance towards rotation about the central C-C bond is actually of the same magnitude as the bond strengths in the molecule, as illustrated by the partial decomposition of the PCBs 136 and 176 at 425 °C.

However, the rotation barrier of the tri-*ortho* PCBs is not high enough to totally resist conversion at in the full temperature interval used in GC analyses, i.e. 50- 320 °C. At 320 °C the half-life of PCB#183 is about three minutes. Thus, high temperatures for prolonged periods should be avoided. Under standard conditions for enantioselective gas chromatography, e.g. injector temperature 250 °C, detector temperature 300 °C, and a column length of 10 to 20 m, the conversion will be negligible. The residence time in the injector and detector areas are much lower than the half-lives at the temperatures in question. For highly chlorinated tri-*ortho* PCB atropisomers, e.g. PCB#196, the use of excessively long columns should be avoided since the elution temperature would approach the hazardous region. This is not a major problem since long columns should be avoided anyway in enantioselective GC as the capacity factor decrease, and thus the resolution deteriorate, at elevated elution temperatures.

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