MASS SPECTROMETRIC IONIZATION PATTERN OF 209 POLYCHLORINATED BIPHENYLS

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Abstract

Electron impact (EI) and electron capture negative ionization (ECNI) mass spectra of 209 polychlorinated biphenyls (PCBs) were obtained. With increasing level of chlorine substitution, PCB total ion responses decreased upon EI and dramatically increased upon ECNI. [M]+ and [M-nCl]+ are major ions for EI. For ECNI, [Cl]- is major ions for lighter congeners, while [M]- and [M-nCl]- for heavier ones, with large variation of relative abundance in the same homolog. Fragmentation patterns are different for congeners within the same homolog under both EI and ECNI. Ortho-substituted PCBs tend to have a higher [M-nCl]+ abundance upon EI and [Cl]- abundance upon ECNI than their meta and para counterparts. PCB ionization upon ECNI was strongly dependent of ion source temperature. Higher source temperature facilitated the fragmentation, causing lower molecular and higher [Cl]- abundances.

Introduction

Polychlorinated biphenyls (PCBs) have been intensively investigated for decades due to their ubiquitous biotic and abiotic presence and human health effects. Many interesting findings on their toxicity and molecular properties are related to the number and positions of chlorine substitution. Non-ortho or one-ortho-chlorinated PCBs can assume coplanar configuration [1], while ortho-rich congeners tend to have restrained rotations of the biphenyl rings around the central C-C σ-bond [2]. Among the 209 congeners, 78 congeners display axial chirality and 19 of them with three or four ortho chlorine substituents have been predicted to exist as astropisomers due to the rotation restriction imposed by the bulky chlorines [3]. The energy required for activation of such congeners into a planar transition state is higher than ortho-poor congeners [4,5]. Meanwhile, the dihedral angle between the two rings in ortho-rich congeners is relatively large due to steric congestion and electronic effects compared to the ortho-poor counterparts [6,7].

Although PCBs have been studied for decades, publications on the mechanisms and structural dependence of the mass spectrometric fragmentation are surprisingly scarce. It has been suggested that the number of chlorines, substitution positions and molecular symmetry influence PCB stability in EIMS ionization [8,9]. In this study, we examined PCB EI and ECNI mass spectrometric fragmentation patterns in terms of chlorine substitution pattern.

Materials and Methods

In this work, 209 PCB congeners in 9 mixtures were purchased from AccuStandard (New Haven, CT). The numbering and the nomenclature of the congeners are based on the system of the International Union of Pure and Applied Chemistry (IUPAC).

The data acquisition was conducted using Agilent Model 6890 gas chromatography (GC) coupled with 5973 mass spectrometer (MS). In each run, 60 µL PCB solutions (100 ng/mL) in n-hexane were injected into the programmable temperature vaporization (PTV) inlet operated in the solvent vent mode, which has been evaluated in our previous work and the results show a good repeatability [10]. A DB-XLB column (30 m × 0.25 mm ID, 0.25 µm film thickness; J&W Scientific) was used. Helium was the carrier gas at the constant pressure of 14.4 psi. The initial oven temperature was 100 °C, which lasted for 10 min, then increased to 130 °C at 10 °C/min and further to 255 °C at 2 °C /min. Afterwards, the temperature was increased to 285 °C at 10 °C /min. The final temperature was held for 5 min until the end of the run. All PCB spectra were scanned from m/z 34 to 800 in EI and ECNI modes. In the EI mode, the ion source and quadrupole temperatures were 230 °C and 150 °C, respectively. The electron energy was 70 eV. In the ECNI mode, the ion source and quadruple temperatures were 150 °C and 106 °C, respectively. Methane was used as the reagent gas at 40% of total mass flow rate. GC/MS interface temperature was 280 °C for both modes. In nature, chlorine, carbon and hydrogen exist each with two isotopes. So the molecular weights of PCB congeners are in ranges accordingly, which generate isotopic clusters for molecular and fragment ions upon EI and ECNI. The m/z ranges of molecular and dechlorinated fragment ions were selected based upon the full-scan mass spectra.

Results and Discussion

1. Fragmentation upon EI

In the EI mode, PCB total ion responses decreased slightly with increasing level of chlorine substitution. The dominant ions are the molecular ion [M]+ and fragment ions [M-nCl]+ (where n may range from 1 up to the homolog number or the number of chlorines in the PCB molecule). The sum of all [M-nCl]+ ions is denoted as Σ[M-nCl]+. The sum [M]+ and Σ[M-nCl]+ responses for all congeners are close, accounting for majority of the total ion responses (68-81%), although CB79 (3,3',4,5') has a little lower response portion of the total (62%). This indicates that C-Cl cleavage is the main fragmentation pathway upon electron impact.

The relative abundance of molecular and fragmental ions is similar among isomers in each homolog, which makes it difficult to identify individual isomers simply by retrieving and matching from those in a mass spectra library. In general, the abundance of [M]+ is the highest. For congeners with ≥2 Cl substituents, the abundance of [M-2Cl]+ is higher than that of [M-Cl]+. The ratios of [M-2Cl]/[M-Cl] are in the range of 1.3 to 16, indicating that 2 chlorines are more preferably eliminated to form [M-2Cl]+. The process could involve the direct loss of two chlorines from [M]+ and/or the loss of one chlorine from metastable ion [M-Cl]+, depending on the number and positions of chlorine substitutents of the congeners [11]. Ceccarini and Giannarelli [12] also reported abundant ions with even number of chlorine eliminated, i.e. [M]+, [M-2Cl]+ and [M-4Cl]+. Note that 70 eV was used in this study for EI. Different electron energy could produce fragments with different intensities, affecting the abundances of PCB fragments.

PCB molecular ion [M]+ is usually used for quantification in EI due to the relatively high abundance compared to [M-nCl]+ fragments. The PCB mass-based molecular ion response decreased slightly with increasing chlorine substituents, indicating lower sensitivity for higher chlorinated PCBs. However, the mole-transformed response, which better explains congener ionization tendency, does not show a trend with increasing homolog number (Figure 1a).

The abundance ratio Σ[M-nCl]+/[M]+ is an indicator of the relative EI stability of PCBs. A higher ratio indicates lower stability. Within the same homolog, Σ[M-nCl]+/[M]+ varied, in a wide range for some of the homologs (Figure 1c), suggesting that chlorine substitution position plays an important role. The general trend is the followings. (a) Ortho-chlorinated congeners have relatively high ratios of Σ[M-nCl]+/[M]+. Meta and para-chlorinated isomers tended to have lower ones. For instance, in mono-CBs, the ratios are in the order of ortho > meta > para. And the more ortho chlorines, the higher the ratio. The descending order of the Σ[M-nCl]+/[M]+ ratios for dichloro biphenyls is: 2,2’ > 2,6 > 2,3 > 2,3’ > 2,5 > 2,4’ > 2,4 > 3,5 > 3,4 > 3,3' > 3,4’ > 4,4’. In tri-PCBs, Σ[M-nCl]+/[M]+ ratio of 2,2’,3 is the highest and that of 3,3’,4 is the lowest. In each homolog from tetra to nona, congeners with chlorines at 2,2’3,3’ positions had the highest Σ[M-nCl]+/[M]+ ratios and those with meta and para chlorines had the lowest ratios. (b) The Cl spatial arrangement of the two rings also affects the abundance, which could explain Σ[M-nCl]+/[M]+ ratio variation of PCB isomers from tri to nona homologs. For example, in tetra to octa-PCBs, four-ortho-chlorinated congeners did not generate the highest Σ[M-nCl]+/[M]+ ratios. The torsional angle of two rings are highest (90º) for these congeners [7]. This may indicate that the conformation of the perpendicular rings somewhat stabilizes the chlorines. (c) Good symmetry with Cl spreading out on the two rings is associated with low Σ[M-nCl]+/[M]+ ratios. Chlorines crowded on the same ring are repulsive to each other, making the molecule relatively unstable, and causing high Σ[M-nCl]+/[M]+ ratios.

2. Fragmentation upon ECNI

In the ECNI mode, PCB total ion responses increased dramatically with increasing level of chlorine substitution. Due to the soft chemical ionization, the m/z clusters of PCBs are much “clearer” upon ECNI than upon harsh electron impact ionization. [M]-, [Cl]- and [M-nCl]- (usually n=1~3 for abundant [M-nCl]- ions) contribute to almost all the total ion responses. For mono to tri PCBs, [Cl]- is the dominant ions. The [Cl]- responses accounts for 51-98% (mean of 89% and median of 93%) of the total ion responses for di and tri-CBs except for CB36 (3,3',5), which has a minor [Cl]- response (11%) and major molecular ion response (70%) of the total. However, [Cl]- response is low, which explains the low response of total ions for these congeners. Relatively high [Cl]- response was observed in the majority of 3-5 Cl substituted congeners. With the increasing number of Cl substituents, [M]- and [M-nCl]- responses were getting stronger.

As shown in Figure 2a, [M]- response increased dramatically by four orders of magnitude from mono to deca PCBs. In the same homolog, the responses of isomers varied. It strongly depends on the arrangement of chlorines on the two rings. Congeners with more chlorines may also produce fragments of [M-nCl]- (n=1~3). With less Cl substituents, the [M-nCl]- response is smaller (n is smaller). Usually, the [M-nCl] fragments are not observed for most of congener with <4 Cl substituents. Unlike in EI that [M-1Cl]- < [M-2Cl]-, the descending abundance order in ECNI is [M]- > [M-1Cl]- > [M-2Cl]- > [M-3Cl]-.

In ECNI, the consistent dominance of halogen ions is not expected for the majority of PCBs as it is for PBDEs, although [Cl]- is predominant in the mass spectra of less chlorinated PCBs, accounting for majority of the total ion response. As Σ[M-nCl]+/[M]+ ratio in EI, isomeric [Cl]-/[M]- ratio in ECNI is also an indicator of chlorine stability at different positions since both involve C-Cl bond cleavage. In the same homolog, it appears that [Cl]-/[M]- ratio varied in a wide range (Figure 2c). Congeners with Cl at meta and para positions had relatively low [Cl]-/[M]- ratios. Low [Cl]-/[M]- ratio is also corresponding to symmetrical and balanced distribution of chlorines on the two rings. In each homolog, the isomers with the lowest [Cl]-/[M]- ratios are: 3 in mono; 3,4' in di; 3,3',5 in tri; 3,3',5,5' in tetra; 3,3',4,5,5' in penta; 3,3',4,4',5,5' in hexa; 2,3,3',4,5,5',6 (then 2,3,3',4,4',5,5') in hepta; 2,3,3',4,4',5,5',6 in octa; 2,2',3,3',4,4',5,5',6 in nona. In contrast, chlorines at ortho position, and crowdedly and unbalancedly distributed on the same ring tend to break, resulting in high [Cl]- response. For instance, the higest [Cl]-/[M]- ratios in some of the homologs are from: 2 in mono; 2,6 in di, 2,4,6 and 2,3',6 in tri, 2,2',3,6' in tetra, 2,2',4,6,6' in penta, 2,2',3,3',6,6' in hexa, 2,2',3,3',5,6,6' in hepta and 2,2',3,3',4,4',6,6', in octa. The trends of Σ[M-nCl]+/[M]+ and [Cl]-/[M]- ratios are similar, but not the same probably due to the different natures of proton affinity in EI and electron affinity in ECNI.

The ion source temperature affects the ECNI mass spectra [13]. As the ECNI ion source temperature increases, the probability of C-Cl bond cleavage increases, resulting in the production of more halogen anions from halogenated compounds [14]. In this study, we tested PCB congeners at the ion source temperatures of 150, 200 and 250 °C with 39 congeners from mixture#1. With the increasing ion source temperature, the total ion response increased for di- tri- and two-ortho-chlorinated-tetra-PCBs, and decreased for higher chlorinated congeners. The trend can be explained by that of their dominant ion. As presented above, molecular ion is generally dominant for heavier congeners and chlorine ion for lighter ones. Re-examination showed that [Cl]- dominated the total ion response of the above mentioned di- tri- and two-ortho-chlorinated-tetra- PCBs, while molecular ion dominated for others. High temperature facilitates molecular fragmentation, causing low [M]- response and high [Cl]- response due to the C-Cl bond cleavage. In the case of ECNI used for PCB quantification with molecular ion monitored (presumably for highly chlorinated ones due to the relatively high response), low ion source temperature setting is preferred.

Acknowledgements

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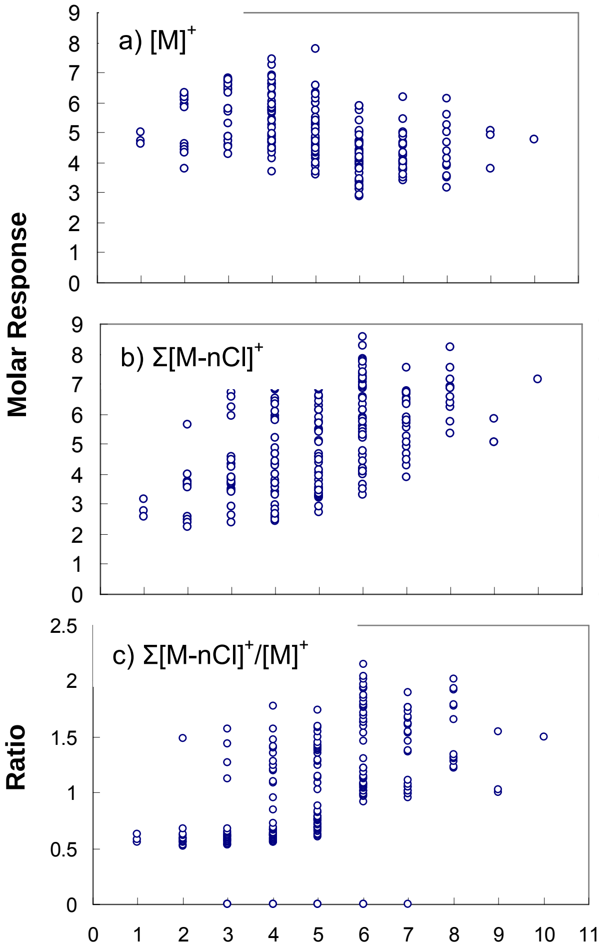

Figure 1. The molar responses of molecular ion [M]+ (a) and fragmental ions Σ[M-nCl]+ (b) for PCBs in EI mode; Ratio of Σ[M-nCl]+ and [M]+ responses (c).

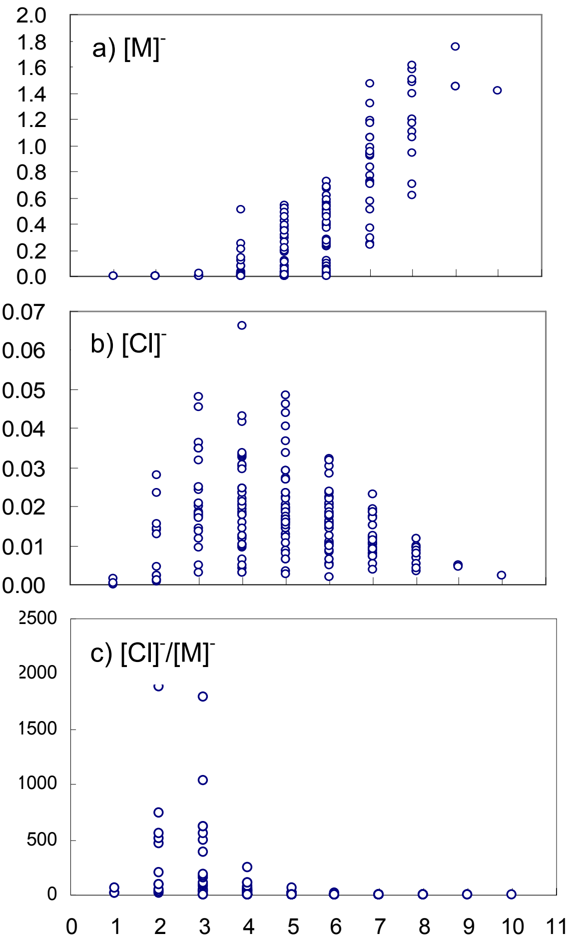

Figure 2. The molar responses of molecular ion [M]- (a) and fragmental ion [Cl]- (b) for PCBs in the ECNI mode; Ratio of [Cl]- and [M]- responses (c).