HEXABROMOCYCLODODECANE: ADDUCTS & IMPURITIES

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Introduction

The production of technical Hexabromocyclododecane (HBCD) for use as a flame retardant in expanded polystyrene foams and upholstery textiles has made it the third most globally produced brominated flame retardant (BFR).[1-4] The additive manner in which it is incorporated into products makes it susceptible for release into the environment while its seemingly limited thermal and chemical stability predisposes it to both abiotic and biotic degradation. A number of studies have been published that identify possible HBCD metabolites and degradents[5,6]. This, taken together with the fact that all of the HBCD isomers display different physiochemical properties[7,8], results in a variation in the diastereomer profiles of technical material and biological samples[9,10]. Indeed, the analysis of HBCD and related compounds will continue to be very important considering the ubiquitous nature of this BFR in the environment.

Since the structural isomers of HBCD can not be separated by high resolution gas chromatography (HRGC) due to thermal isomerization, the utilization of high pressure liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) has emerged as the preferred analytical method for the analysis of these compounds[11]. Although this method allows for an effective separation of the main isomers of the technical material, there are some analytical challenges associated with these compounds that can affect their detection limits in environmental samples, especially adduct formation[12]. It is well known that the HBCD isomers can form adducts with a number of anions that are present as trace impurities in solvents commonly used for LC-MS/MS analysis. This work details the identification of unique hexabromocyclododecane adducts, which we have designated as "HBCD sandwiches".

During the HBCD analysis of biological samples, it is also very important to be able to distinguish between the bioaccumulation of impurities present in the technical material versus metabolites of the HBCD diastereomers. It has been previously reported that the presence of 1,5,9-cyclododecatriene (CDT) impurities in the *ctt*-CDT utilized for HBCD production can lead to the formation of minor isomers, however alkoxylated HBCD species and compounds containing less than 6 bromine atoms have also been observed in technical material. Most of these impurities arise from incomplete bromination of the starting material and/or use of hydroxylic solvents during the production process. The presence of by-products, such as the minor HBCD isomers and pentabromocyclododecenes (PBCDenes), further complicate the analysis of HBCD. In order to aid in the identification of PBCDene diastereomers present in environmental samples, we have completed and now report the synthesis, purification, and characterization of a single PBCDene isomer.

Materials and methods

All "HBCD sandwich" experiments were conducted using a Quattro *micro* API mass spectrometer in full scan mode (40-1600 amu). Technical HBCD samples (Sigma Aldrich, USA) with a concentration of approximately 35µg/ml in methanol (1.5% toluene) containing 1 of 4 additives (HCl, HBr, KI, or formic acid) were infused directly into the mass spectrometer at a flow-rate of 10µl/min. The full scan spectra were collected using the following source conditions: capillary voltage = 3.00kV, cone voltage = 20.00V, source temperature = 110°C, desolvation temperature = 200°C, and desolvation gas flow-rate = 200L/hr.

The pentabromocyclododecene reported here was synthesized at Wellington Laboratories Inc. using proprietary methods. It was purified by preparative thin layer chromatography (TLC) and obtained as a thick oil. It was characterized by ^{1}H NMR and LCMS. Reaction of this oil with bromine afforded a single allylic bromination product which crystallized readily. Its structure was determined by x-ray crystallography.

Results and discussion:
Quantification accomplished by monitoring only the transitions of the HBCD molecular ion (e.g. m/z 640.5 → 79/81) may result in increased detection limits if adducts are being formed in the LCMS system. Many common LC grade solvents contain trace impurities that can result in the formation of multiple adducts during electrospray ionization. It was previously believed that the adducts were being formed in a ratio of 1:1 (HBCD:anion). However, we have observed the formation of adducts in the ratio of 2:1 (HBCD:anion). Data were collected using a mass range that is larger than what is typically used for these compounds (40-1600 amu), and significant amounts of adducts in the range of 1300-1450 amu were observed for a sample of HBCD in methanol. The subsequent addition of HCl, HBr, KI, and formic acid allowed us to confirm the identity of the observed adducts (see Figure 1). Small amounts of these novel adducts, termed HBCD sandwiches, were also observed when a sample containing a mixture of alpha, beta, and gamma HBCD was run on a C18 column. HBCD sandwiches were observed for all of the additives investigated in varying intensity. It appeared that certain sandwiches were formed preferentially over others, however this needs to be investigated further.

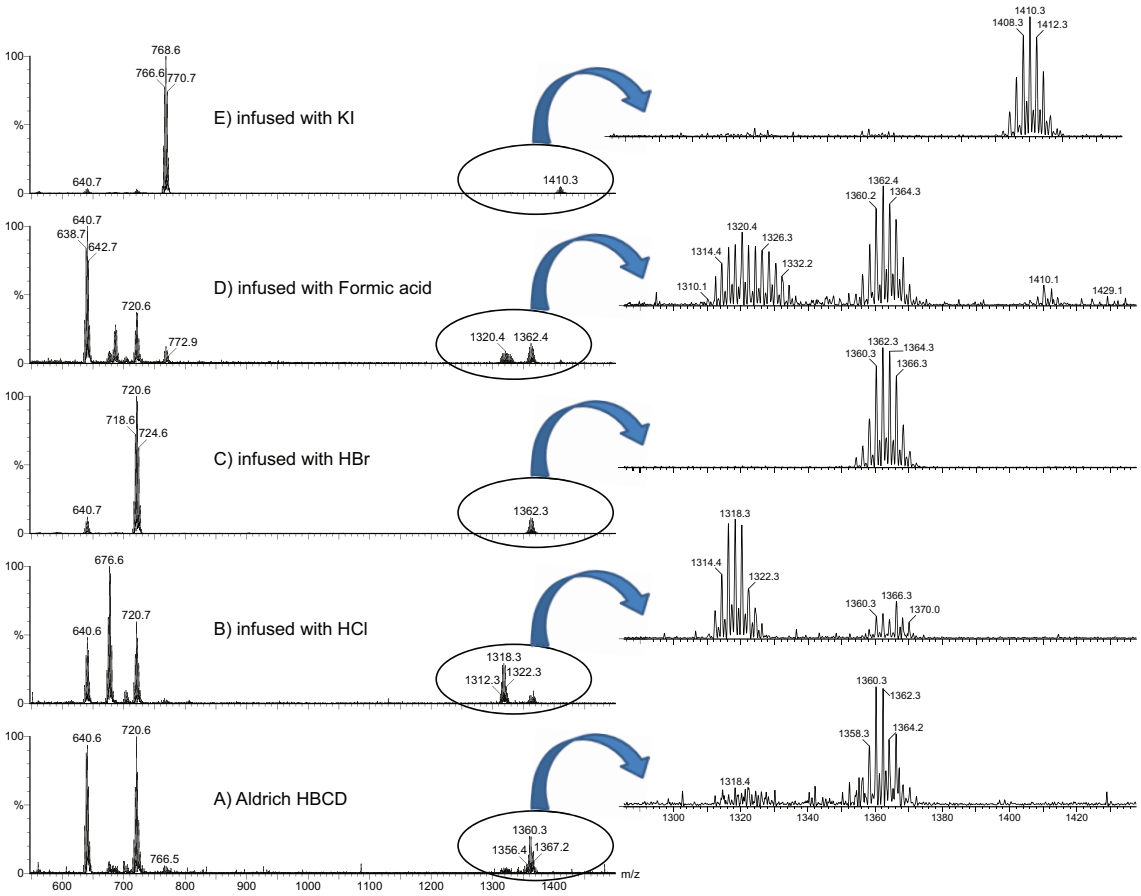

Figure 1: Spectra illustrating the presence of adducts and sandwiches for a sample of Aldrich HBCD A) without any additive, B) infused with HCl, C) infused with HBr, D) infused with formic acid, and E) infused with KI.

In order to identify debromination products and metabolites of hexabromocyclododecane in environmental and biological samples, it is advantageous to have fully characterized reference standards for retention time and spectral comparisons. Unfortunately due to the large number of potential degradation products of HBCD, such

standards are not yet available. Pentabromocyclododecene has been identified as one of the more prominent degradation products and for this reason its synthesis and characterization was completed. The synthesis of pentabromocyclododecene resulted in a major isomer and minor isomer. As previously stated, the structure of the major isomer was determined by allylic bromination followed by x-ray analysis of the resulting product (Figure 2). From this data, we identified the major pentabromocyclododecene product as *rac*-(1,5*R*,6*S*,9*S*,10*R*)-pentabromocyclododecene. The LCMS retention time of this isomer was very close to that of the gamma HBCD isomer on a C_{18} column. The relevance of this isomer in the technical product still needs to be investigated.

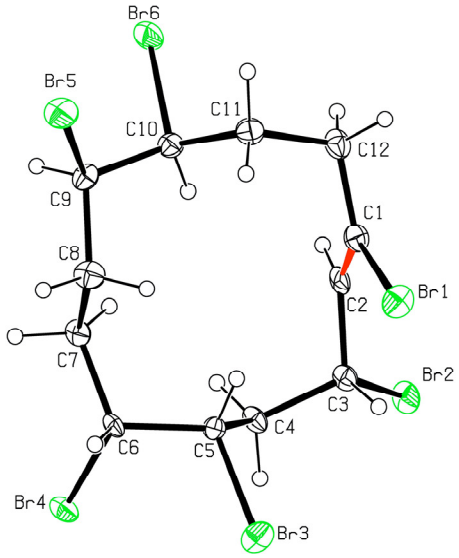

Figure 2: X-ray structure of the major bromination product of a single isomer of pentabromocyclododecene.

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