Characterization of 1,2,3,4-tetrabromocyclohexane isomers by GC-matrix isolation FTIR-MS

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Isomers of 1,2,3,4-tetrabromocyclohexane have been synthesized and characterized in an effort to identify methodologies capable of detecting and identifying nanogram levels of unknown individual degradation products and residues of brominated flame retardants (BFRs) in environmental and biological matrices. Analysis of the reaction mixture by GC-matrix isolation FTIR-MS indicates the presence of four epimeric products, all of which are identified on the basis of comparison of their vibrational (FTIR) spectra with simulated vibrational spectra derived by molecular modelling techniques. The results demonstrate that GC-matrix isolation FTIR-MS can be used to definitively identify individual BFRs and/or their degradation products present at trace levels in environmental or biological matrices, even in cases where individual analytes may not be isolatable in sufficient quantities for conventional analysis.

Introduction

Brominated flame retardants (BFRs) are used in a wide range of products to reduce ignition hazards. Under thermal stress, bromine atoms dissociate from the BFR and retard flame propagation by (i) limiting combustion access to oxygen *via* creation of heavy bromine gasses and (ii) interference with gas-phase $HO^{\bullet}$ and $H^{\bullet}$ radicals.[1] Although initially assumed to be environmentally benign due to their high MW and low solubility, these compounds and/or their degradation products are increasingly being detected in both environmental samples[2] and biological systems.[3]

A range of polybrominated organic materials have been, or are currently, used as BFRs, including: 1,2,3,4,5,6-hexabromocyclohexane (HBCH), hexabromobenzene (HBB), tetrabromobisphenol-A (TBBPA), 1,2,5,6,9,10-hexabromocyclododecane (HBCD), polybrominated biphenyls (PBB) and various polybrominated diphenyl ether (PBDE) congeners.[4] Many of these compounds are structurally analogous to known polychlorinated products, such as chlorinated pesticides (*e.g.* hexachlorocyclohexane [HCCH]) and polychlorinated biphenyls (PCBs), for which extensive studies of environmental behavior and biological activity have been reported. In many instances these investigations have found that the environmental and/or biological behavior of these (chlorinated) products is isomer and stereoisomer specific.[5] Mounting evidence suggests that the same is true of analogous BFR-derived products.[6]

Analytical methodologies, especially chromatographic methodologies, for accurate and specific identification of trace levels of individual chlorinated congeners and their biological metabolites and environmental degradation products have been developed, many of which are based on gas chromatography-mass spectrometry (GC-MS). However, due to the difficulty of differentiation of many isomeric compounds such as PCBs or epimeric HCCHs on the basis of MS alone, these methods rely heavily on the use of carefully defined and prescribed chromatographic conditions in combination with MS for assignment of individual analytes. Development of these methods has required painstaking effort and, in particular, requires access to well characterized and purified reference materials.

Assessment of the environmental and biological behavior of BFRs likewise requires analytical methodology capable of identification of trace levels of individual brominated/polybrominated organic materials. In the case of the parent BFRs, for which individual isomers and/or stereoisomers can be synthesized or readily isolated from bulk BFR by conventional means, this problem, while challenging, is not extraordinary. Unambiguous identification of trace metabolites and/or environmental degradation products, which may be difficult to isolate from source matrices in sufficient quantities for conventional bulk analysis and which are likely to consist of complex mixtures of isomeric/stereoisomeric products in-and-of themselves, poses a more complex analytical challenge.

One potential approach to this problem is application of GC-matrix isolation FTIR-MS (GC-mi FTIR-MS).[7] This technique allows investigators to simultaneously acquire both MS and highly resolved FTIR data for nanogram quantities of individual analytes. GC-mi FTIR-MS has been used successfully for differentiation of all 209 PCB congeners[8] and also for differentiation of epimeric hexachlorocyclohexanes.[9] As these polychlorinated compounds are structurally analogous to certain BFRs, it was decided to apply this technique to analysis of HBCH and a potential reductive debromination product, 1,2,3,4-tetrabromocyclohexane (TBCH) as a case study in order to demonstrate the utility of the technique for trace analysis and identification of polybrominated BFR-derived materials.

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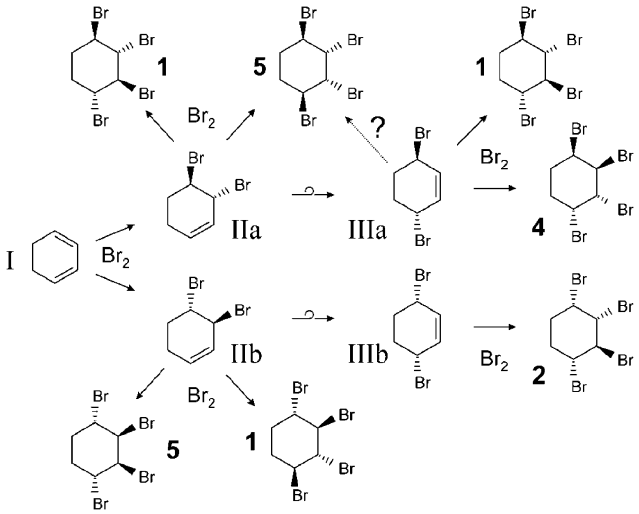
Results and discussion

1,2,3,4-Tetrabromocyclohexane (TBCH) is a one of the potential products of reductive debromination of HBCH. Synthesis of this product has been previously described by a number of workers,[10,11] and it is well established that bromination of 1,3-cyclohexadiene results in the formation of a number of isomeric TBCH species. The isomer distribution obtained from this reaction has been shown to be dependent on the reaction conditions used (primarily temperature). In principle, there are six potential bromine substitution geometries for TBCH, four of which may exist as discrete conformers, as illustrated in Fig. 1. However, due to the propensity of Br_2 to add *trans* across double bonds, two of the structures (nos. **3** and **6** in Fig. 1) may be excluded because all plausible mechanisms for formation of these structures require at least one *cis* bromination. All of the remaining four structures may be formed by mechanistically reasonable processes, as illustrated in Scheme 1.

Based on the possible reaction pathways, it is reasonable to expect that of the four plausible isomers, compounds **1** and **5** are likely to predominate due to the existence of multiple reaction routes to formation of these structures. Compounds **2** and **4**, each of which is produced by only one pathway, are expected to be less significant. Han *et al.*[10] have shown that the reaction proceeds stepwise *via* either of two 1,2-dibromo-3-ene intermediates (IIa and b) and that these can rearrange under the reaction conditions to either of two 1,4-dibromo-2-ene compounds (IIIa and b). Han *et al.*[10] also report observation of three tetrabromo products (**1**, **2** and **5**) from this reaction. However, GC-mi FTIR-MS analysis of the crude TBCH product (illustrated in Fig. 2) indicates the presence of a fourth discrete, albeit minor, product.[12]



Scheme 1 Reaction scheme for bromination of 1,3-cyclohexadiene *via* transient 1,2- and 1,4-dibromo intermediates, modified after Han *et al.*[10,12]

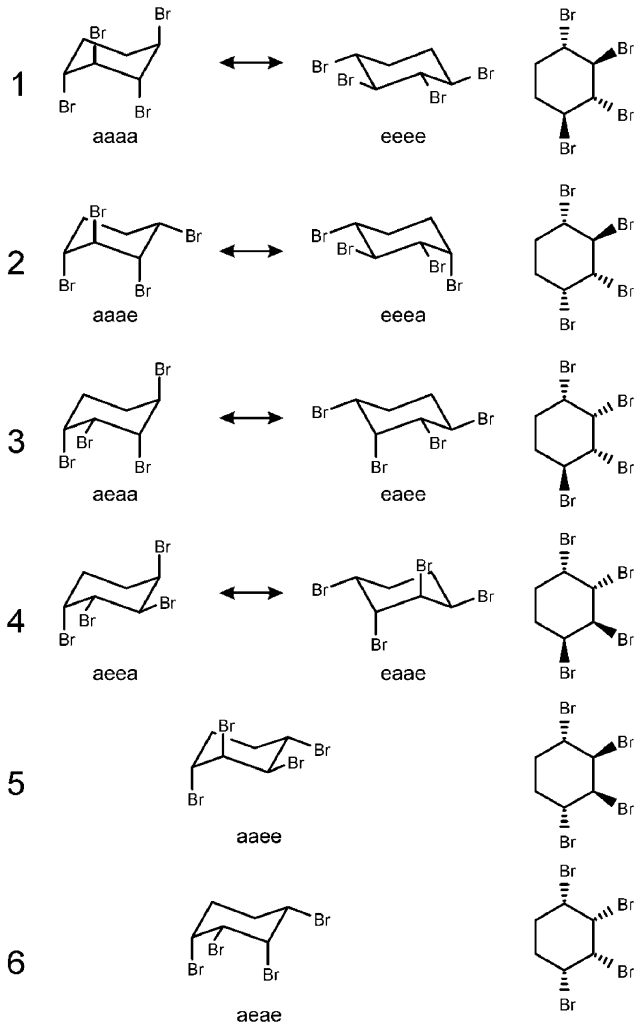


Fig. 1 All potential 1,2,3,4-tetrabromocyclohexane (chair) geometries without regard for mechanistic restrictions on Br addition.

Mass spectra of each of the four eluants observed in Fig. 2 are illustrated in Fig. 3. MS data establish that all four eluants are isomeric TBCH structures, but beyond this, due to the close similarity of the fragmentation patterns, these data alone are insufficient for unambiguous differentiation of the various isomeric possibilities (Fig. 1 and Scheme 1).

In contrast, matrix isolation FTIR data obtained from the same analysis, illustrated in Fig. 4, show marked differences. Previous GC-FTIR studies of halogenated aromatic compounds of environmental interest, especially PCBs, have also demonstrated the capacity of this approach to differentiate isomeric compounds that are not easily differentiated by

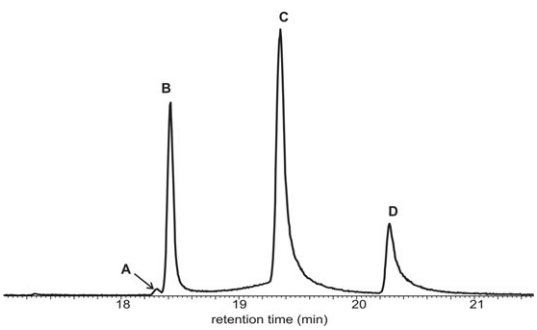


Fig. 2 Total ion chromatogram obtained from analysis of 1,2,3,4-tetrabromocyclohexane mixture.

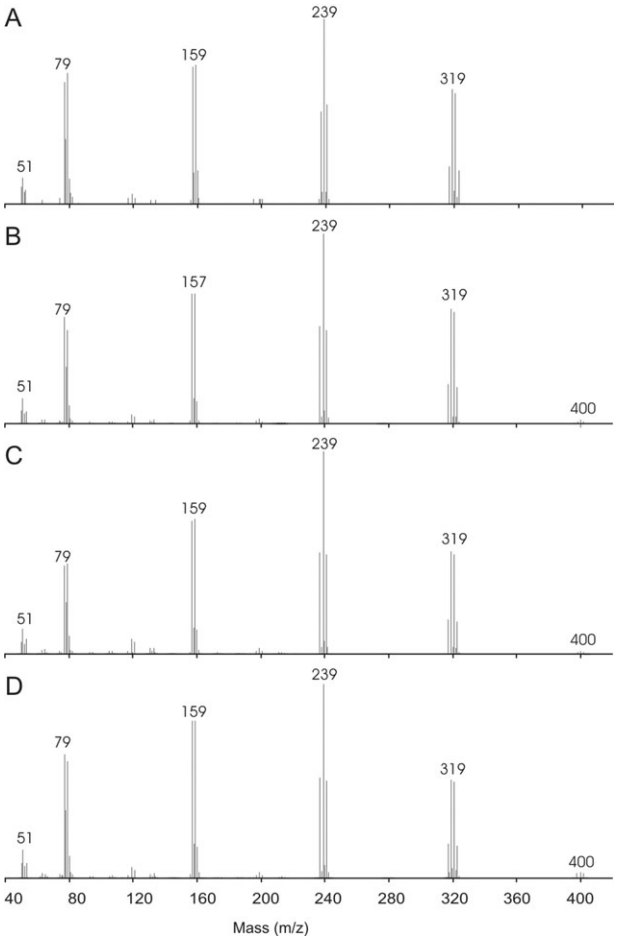


Fig. 3 Mass spectra of isomeric 1,2,3,4-tetrabromocyclohexanes, in elution order, top to bottom (from GC-MS data illustrated in Fig. 2).

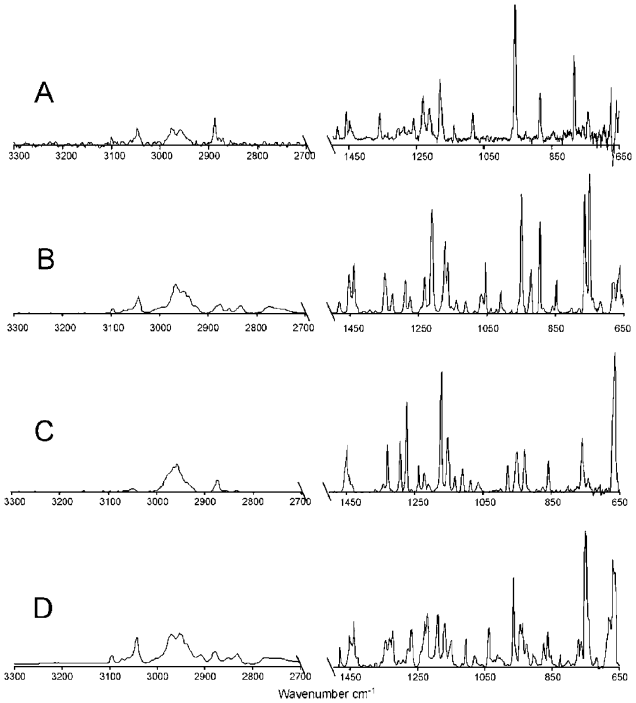


Fig. 4 Matrix-isolation FTIR spectra of 1,2,3,4- tetrabromocyclohexane isomers, in elution order from top to bottom. C–H stretching region (2700–3300 cm^{-1}) on left (vertical scale exaggerated for clarity) Right (650 to 1500 cm^{-1}) shows absorbance due to a variety of C–H (flexing and torsional modes), C–C and C–Br vibrational modes.

GC-MS alone.[13] Assignment of individual analytes in these studies relied on either (i) comparison of data for unknown analytes with FTIR data for relevant purified standards and analogues, or (ii) took advantage of the well known relationship between aromatic substitution patterns and the distributions of absorbances arising due to C_{Ar}–H out-of-plane bending vibrations.[13] In the present study, neither of these approaches is applicable. In this case the compounds of interest are aliphatic and differ only in the relative stereochemistry of Br substitution (*i.e.* positional isomerism is not relevant). The available literature on the vibrational spectra of polybrominated aliphatic compounds is sparse and insufficient experimental data are available on which to base credible definitive structural assignments and relevant purified analogues or reference standards are not available. However, in principle vibrational spectra obtained are *a prioi* sufficient for complete structural assignment. Therefore, in order to differentiate between the various possible isomers and thereby derive structural assignments from these data, vibrational spectra of all TBCH isomers and conformers illustrated in Fig. 1 were simulated using commercially available modeling/simulation software and the results compared with the experimental data illustrated in Fig. 4.

Molecular modeling of brominated, and especially polybrominated, compounds is complex and although force fields for these calculations are improving, simulations of complex vibrations involving motion of one or more bromine atoms and/or motion of both C and Br tend to give less than completely satisfactory results.[14] Vibrational modes involving only C and H are generally better simulated and tend to give results that better reflect experimental reality. In general, modeling results (for C–H vibrations) provide a good simulation of the intensity and relative frequency distributions of vibrational modes arising from specific individual structural elements (*e.g.* CH_2 or CH), although absolute absorbance frequencies predicted by computational approaches often differ from experimental values. That is, the relative frequencies and intensities of related vibrations of a single type of structural element (*e.g.* in-phase and out-of-phase, symmetric and asymmetric methylene stretching vibrations) appear to be well simulated, although modeling may incorrectly predict the absolute frequency of these vibrations (*i.e.* all may be offset from experimental values by an approximately constant factor). The relative frequencies of vibrations arising from distinct types of structural elements (*e.g.* CH_2 *vs.* CH) are not necessarily well modeled. Hence, vibrations of different functional groups may be offset from experimental values by unequal amounts. This ultimately derives from differences in the degree of error associated with the force constants used by the modeling software to estimate C–H bond strengths for methylene and methyne (CHBr) structures. It is therefore necessary to 'anchor' modeling data to experimental empirical reality in order to compare the distribution of absorbances arising due to different structural elements.

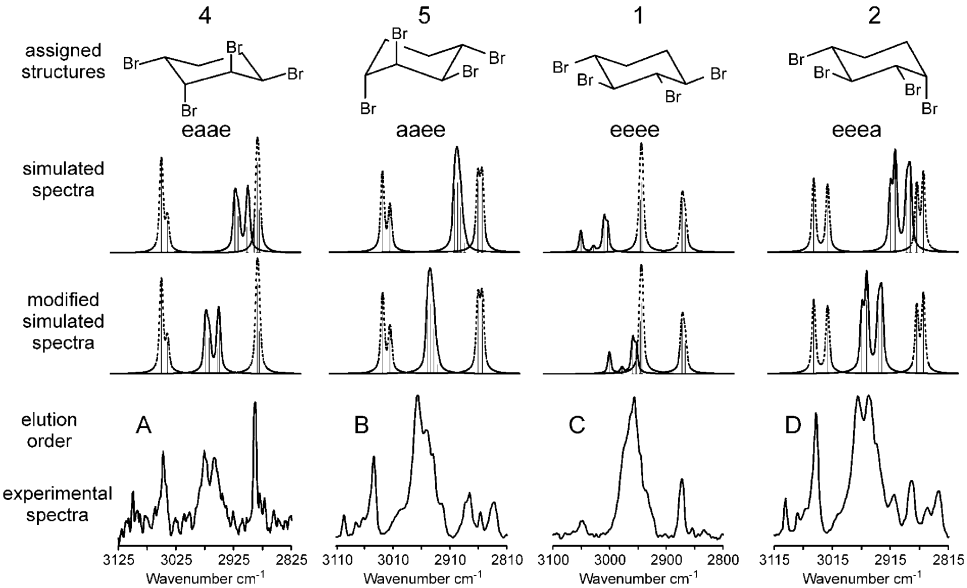


Fig. 5 Comparison of simulated and experimental vibrational spectra. Structural assignments, in elution order from are also given. From top down: assigned structure with numeric notation (bold number) from Fig. 1/Scheme 1; unmodified simulated spectrum of C–H stretching vibrations (CH_2 modes shown as dashed lines and CHBr modes as solid lines); modified simulated spectra (see text for additional description of basis for modification); detail of experimental spectra showing C–H bending region from matrix isolation FTIR.

In this system, C–H vibrations arise due to motion of only two basic C–H functional structures: CH_2 and CHBr. Methylene vibrations consist of four modes (in-phase and out-of-phase symmetric and asymmetric stretching) that closely approximate the vibrations of typical hydrocarbons such as the parent cyclohexane. Absorbances due to vibration of these structures are clearly discernible in the experimental spectra with the symmetric vibrations occurring at $\sim$2880 cm^{-1} (shifted slightly relative to pure hydrocarbon due to the electronegativity of Br). Vibrations due to CHBr structures (also 4 modes) are more complex and differ between isomeric structures (and conformers) due to differences in the distribution of axially oriented and equatorially oriented H. In the experimental data, these vibrational modes are centered $\sim$80 wavenumbers up-frequency from the absorbance due to CH_2 symmetric stretching vibrations. Using this observation to "anchor" the relative frequencies of the CH and CH_2 related absorbances derived from the modeling results facilitates useful comparison of the distribution of observed and predicted absorbances, as illustrated in Fig. 5. Although not perfect by any means, the modeling results give a satisfactory match to the observed experimental mi FTIR data and are certainly sufficient for confident assignment of the individual analytes.[15]

Experimental

1,2,3,4-Tetrabromocyclohexane (TBCH). Bromination of cyclohexa-1,3-diene was carried out at -70 °C according to the method described by Han *et al.*[10] A 100 ml two-necked round bottom flask, fitted with a 25 mL addition funnel and drying tube ($CaSO_4$) was charged with CH_2Cl_2 (10 mL, Fisher HPLC-GC/MS grade) and cooled by dry ice–ethanol. 85 μL Cyclohexa-1,3-diene (Acros Organics) and 67 μL pyridine (Fisher) were added *via* syringe and the mixture was gently stirred. Aluminium foil was utilized to shield the apparatus from unnecessary exposure to light. 0.30 ml bromine (Aldrich reagent grade) in 10 ml CH_2Cl_2 was then added dropwise over $\sim$1 h. This mixture was allowed to react and warm to room temperature overnight, after which the resultant solution was rotary evaporated to a pale yellow solid residue. This product was dissolved in a small quantity of CH_2Cl_2 and washed twice with dilute HCl. The organic phase was then dried over $CaSO_4$, filtered, and the resulting solution refrigerated in darkness until analysis.

GC-matrix isolation FTIR measurements were carried out using a ClearIR® GC-matrix isolation FTIR-MS. The system consists of a conventional Agilent 6890/5973 GC-MSD coupled with a custom built matrix isolation cell which houses a rotating, optically polished gold cylinder held at 9 K by a closed cycle helium refrigerator. GC carrier gas is doped with $\sim$2% Ar and column effluent is split $\sim$30% to the MSD, 70% to the matrix isolation cell. On contact with the matrix isolation cell, Ar and entrained eluants are captured as a helical solid argon matrix which is subsequently (post run) interrogated with an IR microscope coupled with a conventional FTIR (Nexus 670) spectrometer.[16]

Products were analyzed using a variety of chromatographic conditions to obtain optimal separations. Data illustrated were obtained using a 30 m HT-8 (0.25 mm id × 0.25 μm film thickness) programmed as follows: 40 °C (2 min), 3 °C min^{-1} to 250 °C, then 20 °C min^{-1} to 300 °C (4 min).

Theoretical simulations of vibrational spectra of TBCH were calculated using HyperChem (v 7.5, Hyper Cube, Inc.) using *ab initio* basis set STO-3G with 1 extra D orbital (exp 1) and 1e005 convergence limit, core Hamiltonian molecular orbitals with an Amber force field. Simulated spectra were generated by fitting calculated vibrational frequencies and

intensities with Gaussian–Lorentzian [0.70 Gaussian, 0.30 Lorentzian] curves to simulate typical matrix isolation IR absorbance line shapes and widths.

Conclusions

Overall, the data obtained in this investigation demonstrate that GC-mi FTIR-MS can be used, in conjunction with relatively low level molecular modeling techniques, to derive confident structural assignments for trace levels of unknown polybrominated aliphatic compounds without the necessity for isolation and purification of individual analytes. This technique is likely to have considerable utility for analysis of BFR-derived compounds in environmental and/or biological matrices where low total concentrations and limited overall sample size make isolation and purification of individual degradation products and metabolites impractical.

In the case of the TBCH isomers investigated, all four theoretically plausible TBCH isomers (not three as previously reported) are observed in the reaction produced by bromination of 1,3-cyclohexadiene and the distribution of products obtained is consistent with plausible mechanistic pathways for their formation. GC-MS data alone are clearly insufficient for differentiation or confident assignment of individual TBCH isomers. However, matrix isolation FTIR data obtained by GC-mi FTIR-MS clearly differentiates individual TBCH isomers and in conjunction with molecular modeling are *a priori* sufficient to derive unambiguous structural assignments of individual analytes.

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