GC/ECNI-MS/MS for the Structural Determination of Chlorinated Bicyclic Monoterpenes in Michigan Great Lakes Sport Fish

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Abstract:

The mass spectrometry technique of CID-MIKES is used in an attempt to differentiate between the parent carbon skeletal structures for the chlorinated bicyclic monoterpenes, specifically the chlorinated campha(e)nes (toxaphenes) and pina(e)nes in Michigan Great Lakes sport fish. If the pina(e)nes are found, some other source in addition to atmospheric translocation-deposition may be required for the Great Lakes system.

Introduction:

As part of the Michigan Great Lakes Sport Fish Consumption Advisory Program, various species of fish from around the state of Michigan are monitored on a yearly basis for a number of pesticides, and industrial and environmental contaminants. Of particular interest has been the class of pesticides related to the chlorinated bicyclic monoterpenes (PCBMTs)[1,2]. Until recently, the PCBMTs found in the Great Lakes ecosystem have been thought to be the result of the presence of the commercial pesticide known as toxaphene. Toxaphene was manufactured by the chlorination of camphane, camphene, and camphadiene resulting in a complex mixture of more than 200 chlorinated isomers/congeners (not including stereomers). Toxaphene is quite volatile and was used extensively throughout the south, southwestern, and western parts of the United States resulting in the popularly accepted atmospheric translocation - deposition mechanism for the contamination of the Great Lakes ecosystem.

We believe that these toxaphene-like contaminants found in Michigan Great Lakes sport fish may not be completely due to the presence of chlorinated camphanes (commercial toxaphene) but to a mixture of chlorinated bicyclic monoterpenes including the chlorinated pinenes, brought about as unwanted by-products from the chlorination of naturally occurring, plant derived product materials[3,4,5,6,7,8]. The natural product family of bicyclic monoterpenes include thujane (3.1.0), carane (4.1.0), fenchane (2.2.1), isocamphane (2.2.1) in addition to camphane (bornane, 2.2.1) and pinane (3.1.1)[9,10].

Experimental Method:

Fish tissues are prepared for instrumental analysis by extraction and clean-up procedures developed in-house [11]. Four fraction extracts are generated based on polarity of the analytes present. The PCBMTs are found in fraction III along with the technical chlordanes and chlorinated diphenyl ethers. Unless the fish sample is heavily contaminated with PCBs, no PCB components are found in this sample fraction. The PCBs are found in fractions I and II.

Gas chromatography/mass spectrometry with electron capture negative ionization (GC/ECNI-MS) is the method of choice for detecting these PCBMTs in biological tissue extracts [12, 13]. Two instrument systems have been used for this work; a VG Tritech TS-250E mass spectrometer with EBEE geometry coupled to an HP 5890 series A gas chromatograph and a Micromass (VG/Fisons) Prospec mass spectrometer with EBE geometry coupled to an HP 5890 series II GC. Both instruments were equipped with MIKES collision cells immediately before the last electrostatic sector.

The gas chromatograph is used with a DB-5, 60M x 0.25mm ID fused silica capillary column (J&W Scientific) in the splitless mode of injection with a split/splitless valve time of 45 seconds. Helium gas (Airco, grade 5.0) was used as the carrier at a delivery pressure of 50 psig and an injection backpressure of 20 psig. The GC oven temperature was programmed, starting at 70°C for two minutes followed by a two step temperature rate profile (25°C/min. to 150°C, then 5°C/min.) until a final temperature of 310°C is reached. The injection port temperature was maintained at 250°C while the GC/MS transfer line temperature was held at 250°C. The capillary column is directly inserted into the ion source zone.

The mass spectrometer is operated in the negative ion mode with the ion source parameters optimized to produce electron capture and dissociative electron capture anion species (ECNI-MS). The ionization potential is set for 40 eV with an emission current of 300 mA and a source temperature of 120°C. Methane gas (Airco, grade 4.0) is used as the reagent/moderator gas. The mass resolution was adjusted for 700 to 1,000 (depending on the instrument used) with a detector voltage of 300 to 350. The mass scan range is 500 to 200 Daltons (Da) at a scan time of 1.5 seconds. For CID-MIKES operational mode, mass resolution is set for 400 to 700 (again depending on the instrument used), the magnet is adjusted to pass the desired parent anion and the last electrostatic energy analyzer is scanned from the parent mass down to an energy level equivalent to mass 180 Da. Helium is used as the collision gas in the MIKES cell and adjusted to reduced the signal of a calibrant gas peak by 50 percent.

Results and Discussion:

Initial work was done to determine if any structural differences between individual components of toxaphene and strobane (a source of chlorinated pina(e)nes) could be discerned by mass spectrometry. Strobane, a pesticide closely related to toxaphene, was manufactured by the

chlorination of a mixture of monoterpenes that contain mainly pina(e)nes [14, 15]. Toxaphene and strobane contain many isomers that are isobaric and cannot be readily separated or distinguished from one another. With the exception of some subtle differences, the total ion current (TIC) chromatograms for both toxaphene and strobane standards are essentially indistinguishable from one another (figure 1). The mass spectra for individual TIC peaks are for the most part identical as well (figure 2). All PCBMTs undergo a dissociative electron capture ionization process yielding energetically stable (M-Cl) anions. As a result, no structural information concerning the identity of the parent molecule (campha(e)ne vs. pina(e)ne) can be extracted from the mass spectral data.

The structural differences between the parent molecule campha(e)ne, a (2.2.1)-bicyclo compound (with two fused cyclopentane rings) and pina(e)ne, a (3.1.1)-bicyclo compound (with cyclohexane and cyclobutane fused rings), is the placement of the bridgehead carbon atom. Thus, the internal energies of these two parent molecules should be different. Calculated molecular mechanic strain energies with geometries minimized yield 25.033 Kcal/mol for camphane and 51.381 Kcal/mol for pinane [16]. With the use of the mass spectrometry technique of collisionally-induced-dissociation mass analyzed ion kinetic energy spectrometry (CID-MIKES), it was hoped that these energy differences would become apparent through the daughter ion spectra allowing us to distinguish between the chlorinated campha(e)nes and pina(e)nes. For the parent anion (M-Cl) at 341 or 375 Da (hepta- or octachloro congeners), toxaphene CID-MIKES spectra exhibit daughter ions at 297 to 304, 263 to 269, 229 to 234 or 197 to 199 Da's apparently from the loss of the carbon group ($C_3H_xCl_y$) between the two bridgehead carbon atoms. However, at these collision energies (3,000 to 4,000 electron volts), not all strobane species (the chlorinated pina(e)nes) lose the carbon group between the bridgehead carbons (figure 3) but may undergo a direct decyclization to yield a chlorinated diisoprenyl species from which further fragmentation can occur (use of ECNI-CID-MIKES with charge inversion). It is interesting to note that the positive ion electron impact ionization mass spectra for toxaphene yields a highly abundant fragment ion at 159 Da for all components. This ion at 159 Da is apparently brought about by the loss of chlorine followed by the loss of the carbon group attached to the bridgehead carbons and then rearrangement to give the dichloro tropylium ion [17].

We have used this CID-MIKES experiment to investigate a fish extract (Ciscowet trout from the Lake Superior region) that appeared to have a high concentration of PCBMTs. The TIC chromatogram for this extract is shown in figure 4. The mass spectrum for the peak at 27:26 (figure 5) indicates the presence of an octachlorobicyclic monoterpene. The CID-MIKES spectrum shows a daughter ion peak at an approximate mass of 263 Da, suggesting that this species is a chlorinated campha(e)ne (possibly from toxaphene). Other chromatographic peaks at 25:25, 26:18, 28:17 and 28:47 also revealed similar daughter ion spectra. The mass spectrum for the TIC peak at 27:43 (figure 6) also indicates the presence of an octachlorobicyclic monoterpene but the CID-MIKES spectrum gives no indication for the loss of a bridgehead carbon group suggesting that this species may be the result of a chlorinated pina(e)ne or other monoterpene not related to the (2.2.1)-bicyclic configuration. Additional chromatographic peaks at 25:37, 25:46,

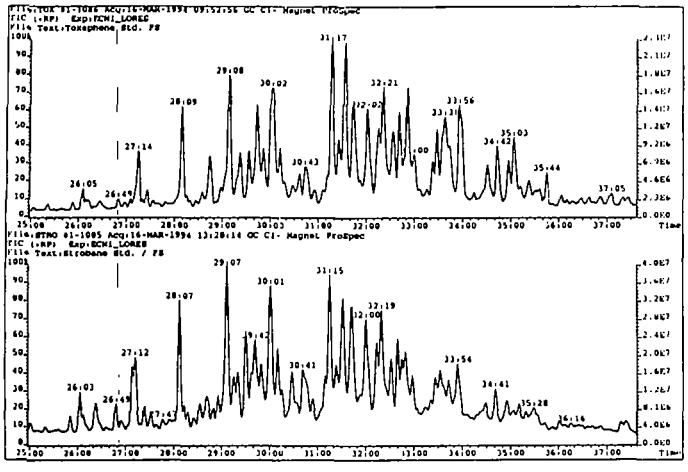

Figure 1) TIC chromatograms for toxaphene (top) and strobane (bottom).

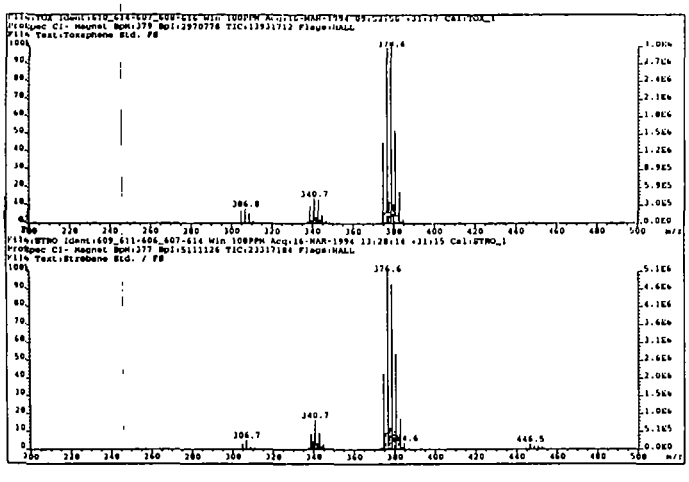

Figure 2) Mass spectra for octachloro species from toxaphene (top) and strobane (bottom).

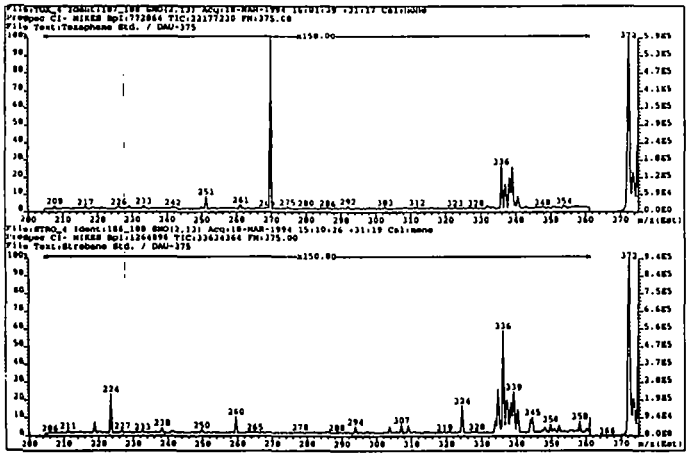

Figure 3) CID-MIKES spectra for octachloro species from toxaphene (top) and strobane (bottom).Parent anion set at 375 Da.

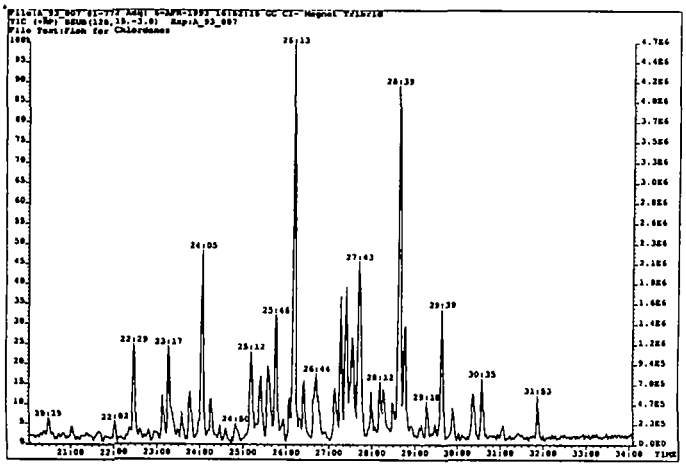

Figure 4) TIC chromatogram for Ciscowet trout fraction III extract.

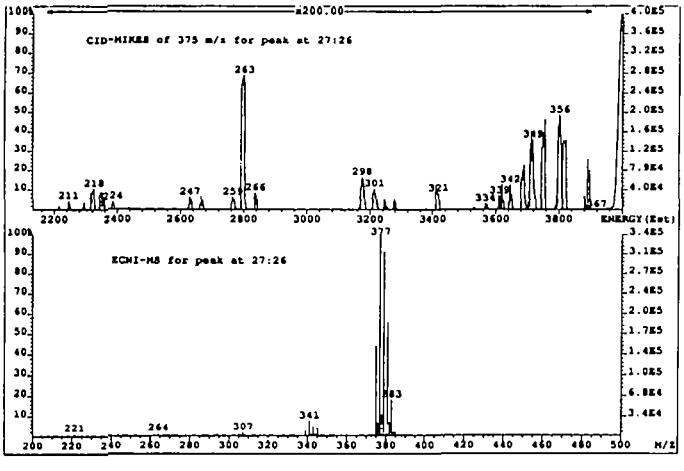

Figure 5) MIKES spectrum (top) and mass spectrum (bottom) for chromtographic peak at 27:26.

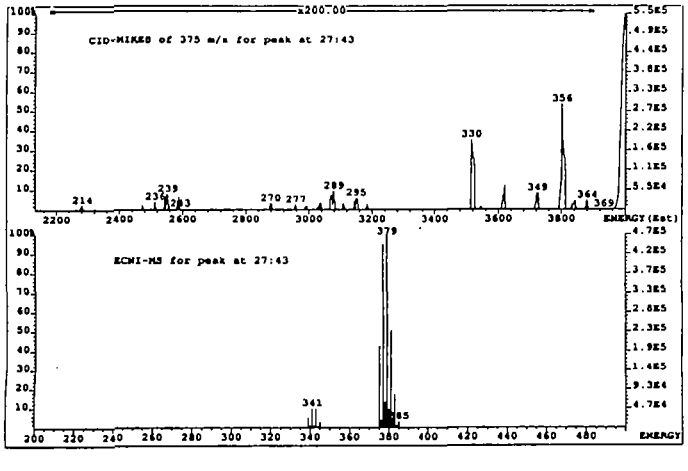

Figure 6) MIKES spectrum (top) and mass spectrum (bottom) for chromtographic peak at 27:43.

27:17 and 27:33 likewise yield CID-MIKES spectra that do not indicate the presence of daughter ions resulting from this loss.

The above results would suggest that not all of the PCBMTs found in this fish tissue may be the result of chlorinated campha(e)nes from toxaphene, but due to other chlorinated bicyclic monoterpenes from other sources. Further studies of more Michigan Great Lakes sport fish are presently underway.

Acknowledgment:

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