Characterization of Five Major Environmentally Significant Chlorobornane Congeners

By

Mark D. Loewen

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

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CHARACTERIZATION OF FIVE MAJOR ENVIRONMENTALLY SIGNIFICANT CHLOROBORNANE CONGENERS

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Abstract

Three environmentally significant chlorinated bornane (CHB) congeners were extracted from ringed seal blubber (Arviat, NWT) and identified by gas chromatography/mass spectrometry (HRGC/ECNIMS (CH_4), low resolution EIMS, and linked-field scanning). They are referred to as TS2 (2,2,3-exo,5-endo,6-exo,8,9,10-octachloroborane), TS3 (2-exo,3-endo,5-exo,6-endo,8,9,10,10-octachlorobornane) and TS4 (one or both of 2,2,5-endo,6-exo,8,8,9,10-octachlorobornane or 2,2,5-endo,6-exo,8,9,9,10-octachlorobornane). Two environmentally significant CHB congeners isolated from the surface sediment of a toxaphene-treated lake and from toxaphene standard were characterized. These congeners, Hp-Sed (2-exo,3-endo,5-exo,6-endo,8,9,10-heptachlorobornane) and Hx-Sed (2-exo,3-endo,6-exo,8,9,10-hexachlorobornane) were identified by mass spectrometry (HRGC/low resolution EIMS, and linked-field scanning) and ^1H NMR spectroscopy (500 MHz normal ^1H NMR, difference decoupling, NOESY and 2D COSY). Using a molecular modelling program the minimum energy onformations of the identified CHB congeners were estimated.

Acknowledgements

I would like to thank the Arctic Environment Strategy, Northern Contaminants Program, initiated by the Department of Indian Affairs and Northern Development, for their financial support for this project. I would also like to thank my academic supervisors Derek Muir and John Westmore, and my project supervisor Gary Stern. H. Parlar graciously supplied toxaphene standards which were necessary for characterizing chlorobornane congeners in seal blubber. Thanks also to Brenda Miskimmin and J. Curtis from the University of Alberta for collecting sediment samples from Chatwin Lake. Thanks to Terry Wolowiec from the Prairie Regional NMR Centre for assistance with NMR experiments and with interpretation of the spectra.

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Abbreviations:

HRGC	High Resolution Gas Chromatography
HPLC	High Pressure Liquid Chromatography
MS	Mass Spectrometry
EI	Electron Ionization
ECNI	Electron Capture Negative Ion
CNL	Constant Neutral Loss
CHB	Chlorinated Bornane
SIR	Selected Ion Response
2D COSY	2 Dimensional Correlation Spectroscopy
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy

Glossary:

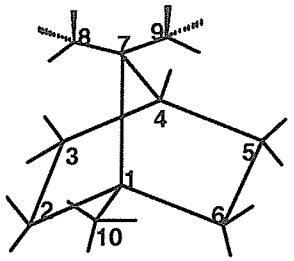
TS2, Parlar #39	2,2,3-exo,5-endo,6-exo,8,9,10-octachlorobornane
TS3, Parlar #40	2-exo,3-endo,5-exo,6-endo,8,9,10,10 octachlorobornane
TS4α, Toxicant A, Parlar #42	2,2,5-endo,6-exo,8,8,9,10 octachlorobornane
TS4β, Toxicant A, Parlar #42	2,2,5-endo,6-exo,8,9,9,10-octachlorobornane
Hx-Sed, hexachlorobornane III	2-exo,3-endo,6-exo,8,9,10-hexachlorobornane
Hp-Sed, TC1	2-exo,3-endo,5-exo,6-endo,8,9,10 heptachlorobornane

Introduction

Toxaphene, a complex mixture of chlorinated compounds, consisting primarily of chlorinated bornanes (CHBs), was widely used as a pesticide in the US, Canada and other parts of the world before its ban in the early 1980's due to its environmental persistence and toxicity[1]. When DDT was banned in the early 70's, toxaphene became the most widely used agricultural pesticide in the United States. Cumulative world use of toxaphene during the period of 1950-1993 was estimated to be 1.33 megatonnes[2]. Unfortunately, it is still in use in a number of developed nations[2]. Its primary use was as an insecticide for cotton and soybean crops in the southern United States[3]. It was also used briefly as a piscicide in the late 1950's, and 1960's to rid lakes of less desirable fishes before subsequently restocking them. In some cases, the restocking scheme was not successful due to the persistence of the toxaphene in water and sediment, and subsequent problems with viability of the stocked fish[4,5].

Technical toxaphene is thought to consist of more than 600 compounds mainly consisting of penta- to undecachlorobornanes and to a lesser extent, chlorinated camphenes and camphadienes[6]. Of these compounds only a fraction are known to be environmentally persistent. It is known that toxaphene samples extracted from environmental samples give, by HRGC, a profile that differs by matrix and from

the analytical standard itself[7-10]. While the basic structure of CHB congeners is known, the specific pattern of chlorination of most of the congeners is unknown.



Bornane skeleton

The characterization of environmentally ubiquitous CHB congeners is important in order to better understand their environmental fate and health hazards. To date, numerous experiments on the toxicity of technical toxaphene have been performed (Table 1&2) but only a few have studied the toxic effects of a single CHB congener[11-14]. Data regarding the toxicity of technical toxaphene are valuable; however it is important to assess the toxic effects of all those compounds that are environmentally persistent.

Of perhaps more realistic concern are the chronic effects of these compounds on aquatic wildlife (and the species in their food chain) and on humans. Toxaphene has been shown to give dramatic increases in hepatic tumor formation in rats exposed to low concentrations of toxaphene over an extended period of time[1]. Those aquatic species that have large deposits of fat (e.g. marine mammals, cod, burbot) will often have high tissue organochlorine contaminant burdens[15]. These

Table 1: Toxicity of Toxaphene[1]

Acute LD_{50} of toxaphene to several mammals (mg/kg)

Species	Route	LD_{50} (mg/kg)	Reference
Rat (males)	Oral	90	Gaines (1969)
Rat (males)	Dermal	1075	Gaines (1969)
Dog	i.v.	5-10	EPA (1976)
Man (estimated)	oral	60	FAO/WHO (1969)

Table 2: Toxicity of Toxaphene[1]

Acute LC_{50} of toxaphene to several aquatic organisms (µg/L)

Species	LC_{50} (µg/L)	Reference
Stonefly	1.3	Johnson and Finley (1980)
Daphnid	14.2	Johnson and Finley (1980)
Largemouth Bass	2.0	Johnson and Finley (1980)
Fathead minnow	18.0	Johnson and Finley (1980)
Pinfish	0.2	Johnson and Finley (1980)

hydrophobic contaminants move from the water phase and bind strongly to organic particles in the water column. These particles are ingested by particle feeders, and hence become contaminated. Certain CHB congeners bioaccumulate up the marine food chain (passed from prey to predator) if they are not metabolized, and eventually become stored mainly in the blubber of marine mammals[16]. Identification of CHB congeners in marine mammals is important because they are a major part of the diet of the arctic indigenous people.

Physical properties of only the technical mixture have been measured or estimated (Table 3). These data are inadequate because they are not specific for any one component. Now that that a few standards are available and their structures characterized[17], work on physical properties of individual CHB congeners is underway. With knowledge of the chlorine substitution patterns of environmentally persistent CHB compounds we can pursue research involving the determination of physical properties of these compounds. Environmental modeling is an important part of understanding the way a given compound behaves in nature. By testing the physical properties of a compound (e.g. water solubility, Henry's Law constant, octanol:water partition coefficient, toxicity) one can estimate its effect in the environment (e.g. if a compound has an appropriate

volatility to reach the arctic, will the compound solubilize in ringed seal tissue and if so is it toxic to the ringed seal or those that feed on it) . It is clear then that if CHB congeners differ in their physical properties, an adequate model will not be possible by assuming the technical mixture behaves like any given congener.

Table 3: Physical Properties of Toxaphene[1]

Vapour Pressure	$3x10^{-7}$mm Hg @ 293K	Atkins and Eggleton (1971)
	10^{-6} mm Hg @ 293 K	Korte et al. (1979)
	0.17-0.4 mm Hg @ 298 K	Brooks (1974)
Water Solubility	estimated 500 µg/L	Guyer et al. (1971)
	400 µg/L	Sandborn et al. (1976)
log $K_{octanol:water}$	estimated 6.44	Magnuson et al. (1979)
Average specific gravity	1.630 g/ml @ 298 K	
Henry's Law Constant	estimated $1.7x10^{-6}$ atm m^3/mol	Bidleman (1987)

Another reason to isolate and identify CHB congeners is to advance the sophistication of quantitative analysis of these compounds. To date, quantitation techniques involve a variety of detectors connected to HRGC columns. The environmental standards are quantified with respect to the technical standard which, as already mentioned, is a mixture of many compounds. In such cases we must make assumptions as to the response factors for each compound, as well as make an assumption that compounds do not co-elute. Needless to say, it is important to improve this technique as CHB compounds are one of the most widely prevalent class of environmentally persistent compounds[1].

CHB congeners present in seals

Chlorobornane compounds reach the arctic via atmospheric transport[18]. These semi-volatile compounds exist in the arctic in concentrations ranging from part per trillion levels in water to part per million levels in organisms near the top of the food chain[15]. The HRGC chromatograms of chlorobornane compounds extracted from burbot liver, narwhal and technical toxaphene by HR-ECNIMS-SIR are shown in Fig. 1[10]. Differences between the two chromatograms (toxaphene extracted from environmental matricies have fewer CHB congeners than the technical standard) are a result of destruction of most of the congeners in the toxaphene standard by environmental factors (e.g. photodegradation during

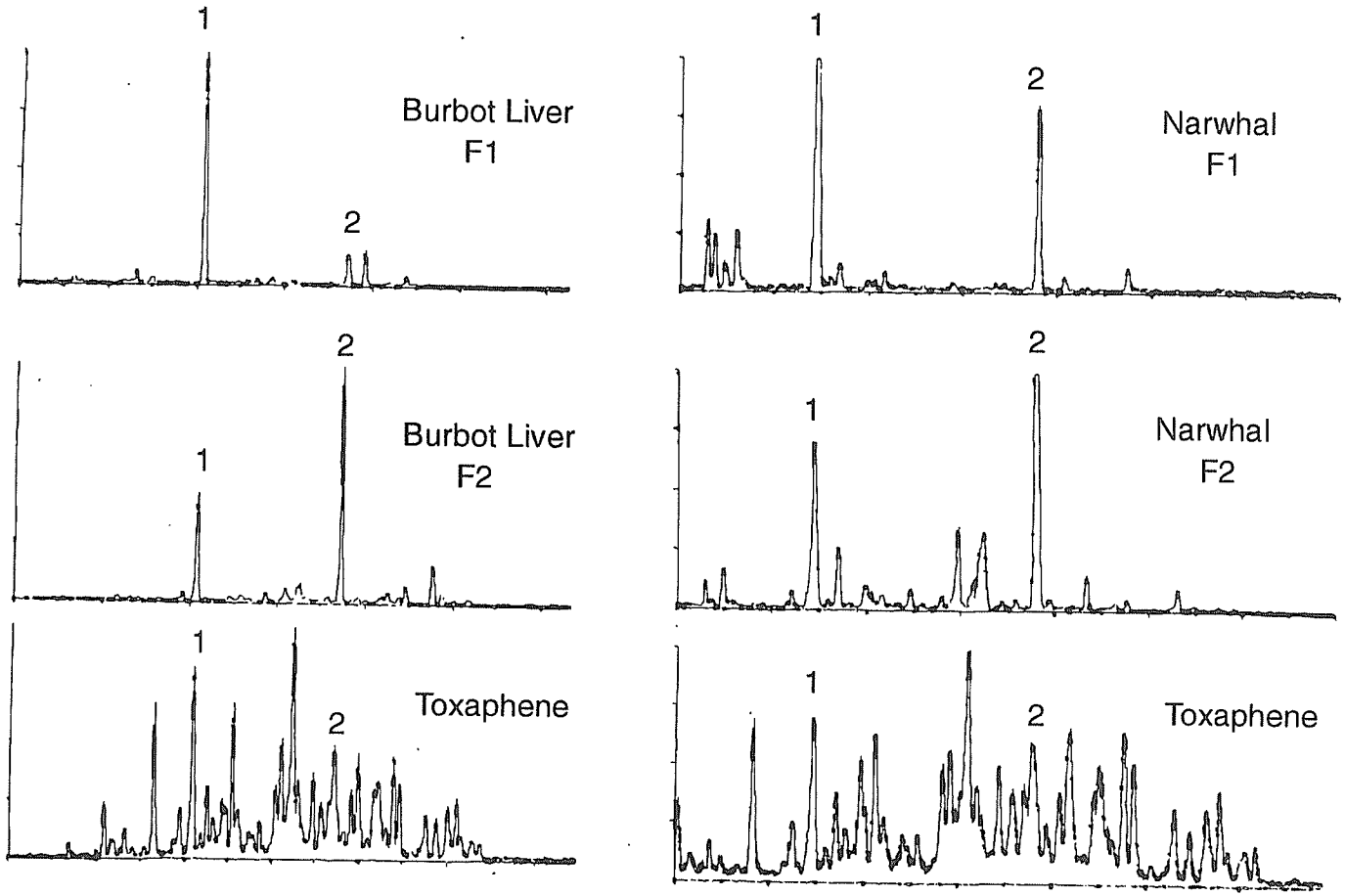


Figure 1: GC/ECNIMS chromatogram of Burbot Liver and Narwhal with toxaphene reference (F1, elution from florisil with100% hexane; F2, 15% DCM in hexane).

atmospheric transport, biological metabolism and differential uptake)[8,9].

CHB congeners present in surface sediment

Hexa- and heptachlorobornane (Hx-Sed and Hp-Sed, respectively) were detected in the sediment of Chatwin Lake, located in Alberta, Canada[19]. It was treated in the early 1960's by addition of toxaphene to the water column at low ug/L concentrations. Highest toxaphene concentrations (~500-1600 ng/g dry wt.) were found in sediments in slices dated to the early 1960's[19]. In these slices the chromatographic pattern resembled that of the toxaphene standard while, in more recent slices, the number of CHB peaks was greatly reduced, with the two most prominent peaks corresponding to Hx-Sed and Hp-Sed (Figure 2)[19]. Surface sediments of a similar but untreated basin of Peanut Lake in Alberta, Canada were also analyzed for toxaphene and were found to contain levels < 0.1 ng/g. This suggests that external sources of toxaphene (i.e. atomospheric deposition) to the treated lakes are negligible and that the observed chromatographic pattern in the surface sediment is solely the result of the introduction of the toxaphene during treatment[19]. The observation of hexa- and heptachlorobornane in the surface sediment of toxaphene treated lakes (i.e. top 10 cms) and not the rest of the technical toxaphene standard is likely a result of those two congeners having higher mobility in the sediment and diffuse to the uppermost layers in the

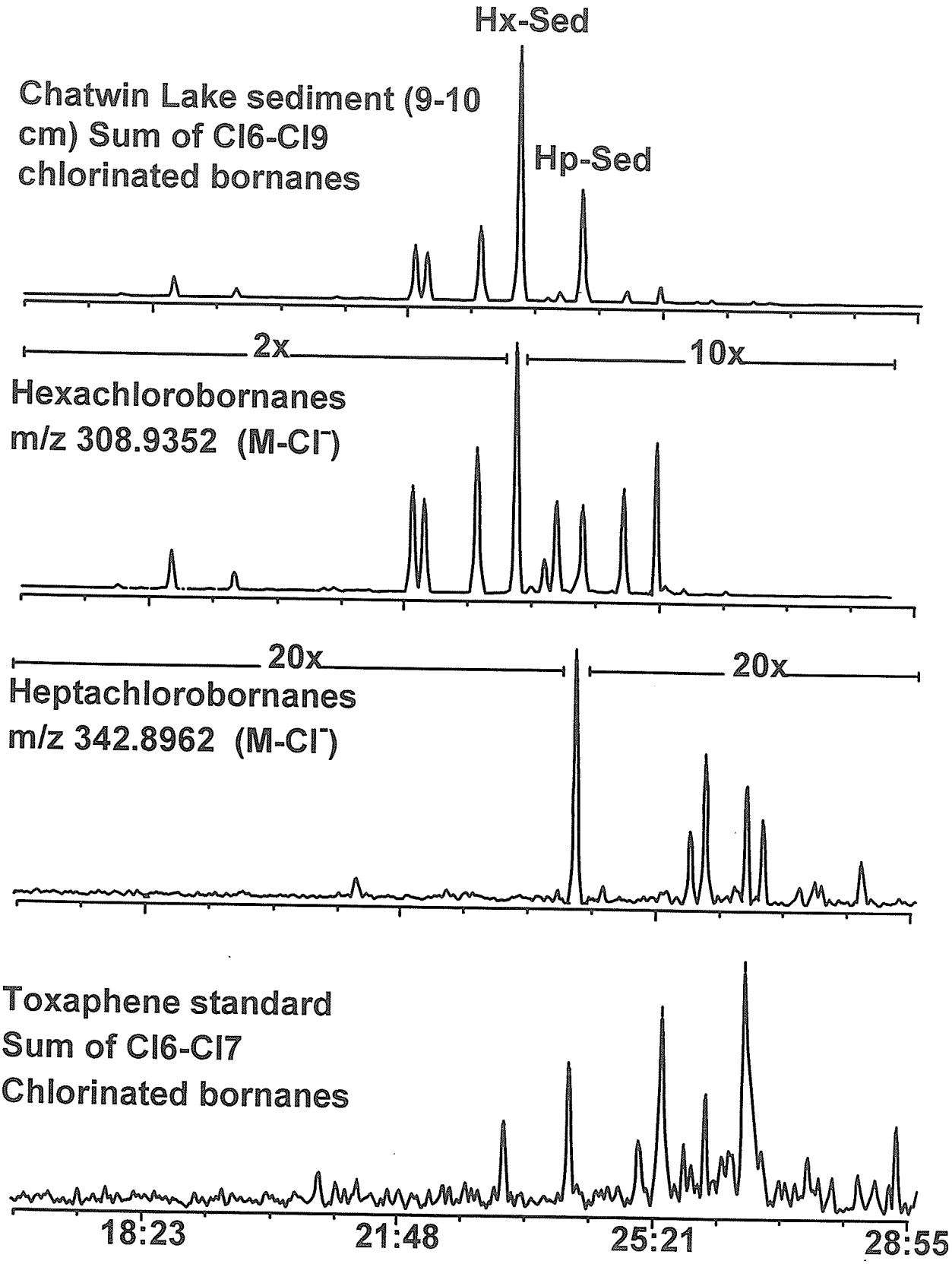


Figure 2: HRGC/SIR/ECNIMS chromatograms of toxaphene in surface layer sediment of Chatwin Lake, Alberta, Canada

sediment. The rate of diffusion of a compound in sediment is a function of a compound's sorption coefficient with sediment (related to water solubility, dissolved organic content in the water that makes up 90-95% of the surface sediment, and organic content of sediment) sediment porosity and bulk density[19]. Much of the toxaphene that was initially applied in the 1960's is tightly bound to its corresponding sediment layer. As that layer was buried by new sediment, more mobile congeners exhibiting greater diffusivity (normally those with lower levels of chlorination) moved away from the level of the toxaphene application horizon and hence predominate in the uppermost sediment layers[19].

At present, only one Cl_9 compound, T12 (2-exo,3-endo,5-exo,6-endo,8,8,9,10,10-nonachlorobornane), and one Cl_8 compound, T2 (2-exo,3-endo,5exo,6-endo,8,8,10,10-octachlorobornane), have been extracted and structurally identified in environmental matrices[20,21]. About 19 other CHB compounds have been identified in the technical toxaphene standard, a number of which have been found in environmental matrices as supported by HRGC retention times[17]. Characterization of compounds in this study will aid not only in the task of determining a general chlorine pattern that is resistant to environmental degradation but also in the further characterization of CHB congeners by mass spectrometry.

Experimental

Seal Sample Preparation

Extraction

Ten kilograms of ringed seal blubber (Arviat, NWT) were homogenized in hexane in successive 100g portions by using a polytron. The hexane/lipid suspension was allowed to settle. Then the hexane solution was decanted from the solid residue, and evaporated using a rotary evaporator, to remove most of the hexane. The lipid residue was added to a 55 cm length of hexane-rinsed 'layflat' polyethylene tubing (Cope Plastics, Fargo, ND, 5.1 cm wide and 51μm thick), and then the tubing was sealed. The tubing was placed in a dialysis chamber containing 3 litres of hexane. After dialysis for 24 h at room temperature, the hexane solution was siphoned off and its volume was reduced to approximately 5 mL with a rotary evaporator; the recovered hexane was saved for re-use. The dialysis extraction was repeated for another 24 h using the recycled hexane. To remove as much as possible of the co-dialysed lipid, extracts were combined, and redialysed, as above.

Cleanup

The dialysed sample was cleaned by chromatography on a Florisil column (40 g, 1.2% deactivated) by elution with hexane, followed by 15% dichloromethane (DCM) in hexane. This process removed a majority of the remaining lipid in the sample as well as some co-dialyzed organic compounds. The resulting eluate was evaporated and the residue was taken into approximately 200μL of acetone. This was fractionated on a Varian 5000 HPLC (Mississauga, ON) fitted with a Waters (Missisauga, ON) Nova-Pak Preparatory C_{18} HPLC column, employing an isocratic mobile phase of 70% acetonitrile and 30% water. To isolate individual compounds, fractions were collected into vials on an Isco (Lincoln, NE) Foxy 200 fraction collector. After successive 20 μL injections, the sample was extracted from the HPLC effluent with hexane. Each fraction was then taken to dryness and treated with 1:1 concentrated sulphuric acid: fuming nitric acid to remove remaining lipid and any contaminating aromatic and olefinic compounds. In experiments CHB congeners in sediment comparing CHB chromatic profiles with and without acid oxidation, it was found that this process did not degrade CHB compounds. After extraction with hexane, the samples were cleaned up with 8g of Florisil by eluting with hexane and 15% DCM in hexane. Samples were taken into 300μL of isooctane .

Sediment Sample Preparation

Extraction

Approximately 10 kg (wet wt.) of Chatwin Lake sediment was collected by B. Miskimmin and J. Curtis (Dept. of Biological Sciences, University of Alberta) with a modified Ekman dredge designed to penetrate the sediment surface to a depth of 10 cm; it was stored in three 4 L brown glass bottles. This corresponded to 735 g (dry wt.) of sediment after freeze drying. The freeze dried sediment was refluxed, 100 g at a time, in 800 mL DCM for 5 hours.

Cleanup

Extracts were treated with 1:1 fuming nitric:sulfuric acid to remove organic pigments and chlorinated aromatics, and with activated copper to remove sulfur compounds. They were further cleaned up by Florisil chromatography [8g, 1.2% deactivated]. The approximately 20 μg of Hx- and Hp-Sed isolated in this manner was more than sufficient for mass spectrometric studies. ^{1}H NMR studies, however, required milligram amounts, and therefore Hx- and Hp-Sed were isolated from the technical standard, by injection of successive 2mg amounts of technical toxaphene in acetone on HPLC employing a Waters Nova-Pak HR C18 preparative column, and an isocratic solvent system consisting of an acetonitrile-water (65:35, v/v) mixture (3.5 ml min^{-1}). The CHBs were extracted from the

HPLC effluent (diluted with HPLC grade water) with hexane. Hx- and Hp-Sed were further fractionated on Florisil [16g; 1.2% (v/w) water deactivated]. The sample was added to the top of the column in 1 mL of hexane and then eluted with 30 mL of hexane, which was discarded; then 1 mL aliquots of hexane-DCM (85:15) were collected. Each aliquot was taken up into 1 mL of hexane for GC analysis.

Instrumentation

Gas Chromatography

The sample extracts were injected in 1-2μL portions into a Hewlett Packard (Wilmington, DE) model 5890 Series gas chromatograph fitted with a 60m X 0.25mm i.d. DB-5ms fused silica column (Chromatographic Specialties, Brockville, ON) connected directly to the ion source of the mass spectrometer. Helium was used as the carrier gas. Samples were injected by splitless injection (2 min.) with the injector port at 260°C. The initial column temperature was 80°C; at 2 minutes the oven was ramped at 20°Cmin^{-1} to 200°C, then at 2°min^{-1} to 230°C then at 10°Cmin^{-1} to a final temperature of 300°C and held for 8 minutes. Electronic pressure programming was used to increase the pressure during the injection cycle and then to maintatin a constant flow of 1mL min^{-1} during the remainder of the run. All injections were made by a CTC A200SE autosampler

under data system control.

Mass Spectrometry

GC-EIMS, GC-ECNIMS, and linked-field scanning, were performed on a Kratos (Manchester, England) Concept high mass spectrometer (EBE geometry) controlled by a Mach 3X data system. EI mass spectra were scanned from 35-450 daltons at a scan rate of 1 sec per decade. The ion source was maintained at a temperature of 220°C, the trap current was 500μA, the ion acceleration voltage was 8 kV and the electron energy was adjusted for maximum sensitivity (~50 eV). Decompositions of selected ions in the first field-free region were identified by a series of linked field scans (B/E, B^2/E and CNL(daughter and parent)). Ion decompositions were enhanced by collisional activation by introducing argon into the collision cell at a pressure to give approximately ~50% attenuation of the m/z 231 ion of PFK. Selected ion ECNIMS was performed at a spectrometer resolution of M/ΔM ~14000. Methane was used as the moderating gas and PFK as the mass calibrant. Optimum sensitivity was obtained at a gas pressure of $\sim 2 \times 10^{-4}$ torr as measured by the source ion gauge. The electron energy was adjusted for maximum sensitivity (~180 eV), the accelerating voltage was 5.3 kV and the ion source temperature was 120°C. The following characteristic ions were monitored from the $[M-Cl]^-$ isotopic cluster of the hexa- to nonachlorobornane homolog

groups; Cl_6 308.9352, 310.9323; Cl_7 342.8962, 344.8962; Cl_8 376.8573, 378.8543; Cl_9 410.8183, 412.8154.

NMR Spectrometry

^1H NMR spectra of compounds dissolved in perdeuterobenzene were recorded at 500 Mhz on a Bruker (Billerica, MA) AMX500 spectrometer (Prairie Regional NMR Centre. Difference decoupling and NOE experiments were performed with a digital resolution of 0.18 Hz.

Structural Calculations

Relative stabilities of proposed structures and conformations were estimated with the Molecular Modeling Pro program (WindowChem Software Inc., Fairfield, CA).

Results and Discussion

After a review of the probable structures of chlorinated bornanes, the results and discussion section will continue with describing general mass spectral degradation patterns in chlorobornane compounds. This will be followed by a detailed analysis of the mass spectra of three major chlorobornane compounds in ringed seal blubber. Finally, the mass spectra and ^{1}H NMR data of two chlorobornane compounds found in lake sediment of a toxaphene treated lake will be discussed.

Synthesis of Toxaphene

The Hercules Chemical Company used the following method to synthesize toxaphene[1]:

1) Wash and chip pine tree stumps.
2) Extract resin with methyl iso-butyl ketone under heat and pressure and distill.
3) Isomerize α-pinene by heating over a catalyst of benzoyl peroxide to produce camphene, bornylene, and some α-terpineol.
4) Chlorinate camphene with liquid chlorine in CCl_4 using UV light as a catalyst until chlorine content reaches 70% by weight.

Enantioners

Pinene, the starting material in the synthesis process of CHBs is a chiral compound. Upon conversion to camphene the chiral character of the molecule remains. This leads to CHB compounds that exist in enantiomeric pairs classified as series-1 and series-2 CHB compounds (Figure 3)[22]. Corresponding atoms in a given CHB enantiomeric pair are magnetically identical. In other words they each experience an identical environment. Hence these compounds are not differentiable by mass spectrometry or NMR experiments. However, there is evidence that both enantiomeric pairs normally exist in technical toxaphene and in natural environmental samples as confirmed by chiral column HRGC[22,23]. Enantiomers are resolved on this type of column and measurements show that the pairs are present in near 1:1 ratios. It should be noted that, when a given CHB congener is identified in this thesis, it is not identified as a single compound and its assignment is ambiguous at the enantiomeric level. Nevertheless, the likelihood of the pairs both existing in our samples is considerable.

An exercise in understanding the chemical synthesis pathway of toxaphene is helpful since it provides insights as to the possible CHB congeners in the technical toxaphene standard. Before starting characterization of chlorobornane congeners, it is worthwhile to narrow the possibilities by discounting improbable congeners.

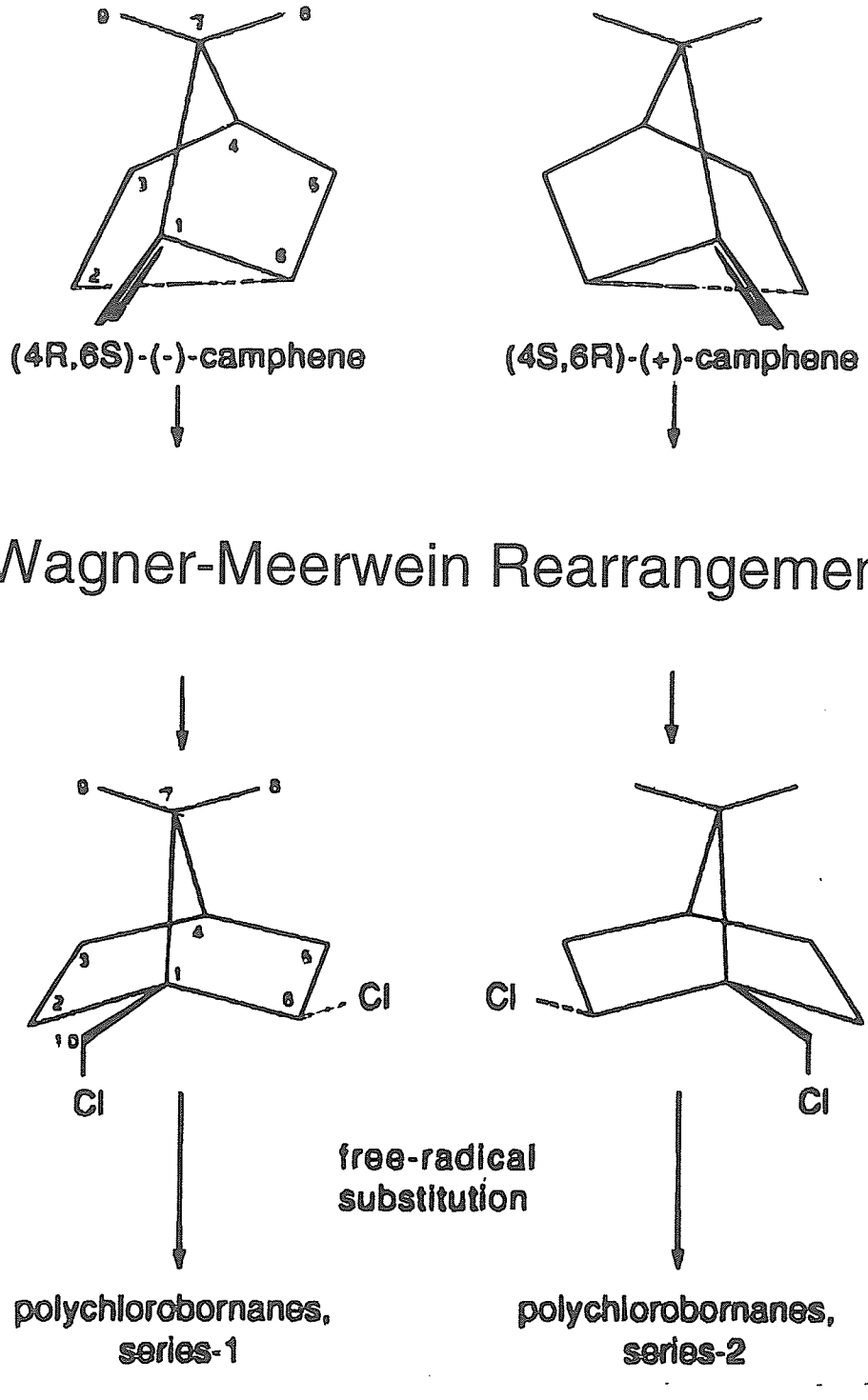


Figure 3: Synthesis of CHB congeners from enantiomeric camphene

Rules governing the formation of chlorobornanes from the starting material indicate the following: All CHBs must have a chlorine atom in the 2-exo position and at least one chlorine on C10, as 2-exo,10-dichloro- and 2-exo,10,10-trichlorobornane are initial intermediates in the production of toxaphene[24]. Due to the nature of the starting product and to steric factors, many of the theoretically possible chlorobornane congeners (calculated to be a maximum of 32768 congeners[25]) are not produced in the toxaphene synthesis process. For example, vicinal chlorine atoms on the ring cannot both be in the endo position[24]. The intermolecular distance between 2 exo chlorines is greater than that of 2 endo chlorines and is therefore more energetically favourable. When three chlorine atoms are observed on one side of the ring, the chlorine atoms on the same face are in the exo position[24]. Also energetically favourable is the chlorine substitution pattern where 2 chlorine atoms across the ring are anti to one another (e.g. 2-exo,6-endo). Analysis of a CHB model indicates that a chlorine substiturtion pattern where chlorine atoms are located both in the endo position across the ring, are somewhat crowded, and therefore this conformation is less energetically favourable. These types of steric factors govern the type of CHB that can be synthesized. What is most often observed in CHB congeners that are environmentally stable is a staggered pattern of chlorine atoms on the ring (2-

exo,3-endo,5-exo,6-endo). Another class of environmentally stable CHB (however less common than the staggered type) compounds includes those with two chlorine atoms on one ring carbon and an adjacent hydrogen-saturated ring carbon.

Rules governing the synthesis of the bridge portion of a given CHB congener are less well known. However, we can speculate from observations on previously identified CHB congeners that a maximum of two chlorines can be found on either C8 or C9[24]. When three chlorine atoms are found on the bridging group (C7-C9), two will be substituted on one carbon. To date, only those CHB congeners with 2-4 chlorine atoms on the bridging group have been identified. Similarly, only those compounds with one or two chlorine atoms on C10 have been identified. These rules reduce the number of possible CHB candidates.

Linked Scanning Experiments

Linked scanning experiments were performed to confirm certain ion fragmentation reactions. Normally, in a double focusing triple sector instrument such as the one used in this study, ions are produced in the source and accelerated to a certain kinetic energy (8 kV). The first electric sector filters out those ions that do not have this kinetic energy (ions with KE other than 8 kV collide with the sides of the electric sector). Linked scanning experiments work on the premise of producing

ions in the source, accelerating them to a given kinetic energy (in this case 8 kV), and then (optionally) colliding them with a collision gas (argon) in a collision cell located in the first field free region (between the source and the electric sector), to induce ion decomposition. Ions formed by unimolecular decomposition after acceleration from the source are called metastable ions; decomposition is alternatively induced by collision. Consider the ion reaction in which a parent ion of mass m_1 decomposes to a daughter ion A^+ of mass m_2 and a neutral fragment N:

$$\begin{array}{ccccc} P^+ & \text{------>} & A^+ & + & N \\ m_1 & & m_2 & & (m_1-m_2) \end{array}$$

After acceleration P^+ has a KE of 8 kV. Upon decomposition, A^+ and N, share this KE in proportion to their resulting masses. This process gives product ions with kinetic energies proportional to their mass and thus we can predict the kinetic energies of ions resulting from a given ion degradation reaction. By scanning the electric sector (E), which sorts ions according to their KE, ions can be selectively transmitted into the magnet (B). The magnet is concurrently scanned at a rate determined by the given experiment so that the ratios B/E (product ion scan) or B^2/E (precursor ion scan) are held constant for a given ion with a certain mass. Constant neutral loss scans ($B(E_o-E)^{1/2} E^{-1}$ held constant) monitor ions that lose a certain neutral fragment. This type of experiment gave conclusive evidence as to the ion fragmentation pathways that were useful for characterizing certain CHB

moieties.

Ion abundance

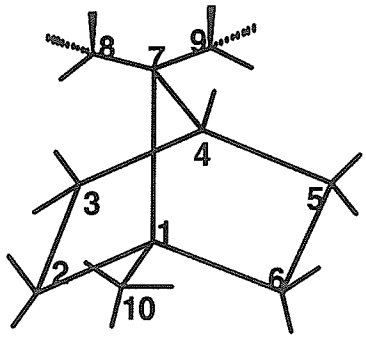
Of importance is the relative abundance of ions involved in each process. The degree to which each reaction takes place will give information regarding the chlorine substitution pattern. This is especially true of the abundance of $[M\text{-}HCl]^{+\cdot}$ and $[M\text{-}Cl]^{+}$. It has been shown that a high relative abundance of $[M\text{-}HCl]^{+\cdot}$ indicates that the ring chlorines are trans staggered, i.e. 2-exo,3-endo,5-exo,6-endo. This is likely due to the good leaving characteristics of HCl and the fact that there are 4 separate ways HCl can be lost, thus giving this process a higher probability. In the mass spectra of previously studied CHB congeners T2 and T12, competitive losses of $Cl^{\cdot}$ and HCl from the molecular ion were observed, while those of toxicants A and B (2,2,5-endo,6-exo,8,8,9,10/8,9,9,10-octachlorobornane and 2,2,5-endo,6-exo,8,9,10-heptachlorobornane, respectively) showed an enhanced elimination of a chlorine atom from the molecular ion[21,26]. It was suggested that the enhanced HCl elimination from the molecular ions in T2 and T12 with respect to that of toxicants A and B was to be expected from the greater acidity of the 5-endo and 6-exo hydrogens of T2 and T12 relative to the 5-exo and 5-endo hydrogens of toxicants A and B, due to the electron-withdrawing properties of the 5-exo and 6-endo chlorines. Another factor that increases the

likelihood of ·Cl loss is increased steric energy in toxicants A and B arising from crowding of chlorine atoms at the 2 position. This energy is released when one of the chlorines is lost as a radical. It appears that compounds exhibiting the staggered chlorine substitution pattern show the $[M\text{-}HCl]^{+\cdot}$ ion degrading to form a large relative abundance, even-mass, odd-electron ion by loss of C_2H_3Cl, $C_2H_2Cl_2$, or C_2HCl_3. Conversely, those compounds with 2 chlorine atoms on one ring carbon with an adjacent hydrogen- saturated ring carbon will have a small relative abundance, odd-electron, even-mass peak as a result of loss of C_2H_3Cl, $C_2H_2Cl_2$, or C_2HCl_3 from $[M\text{-}HCl]^{+\cdot}$. This is usually observed with a like abundance of the even-electron odd-mass ion cluster that is a result of loss of C_2H_3Cl, $C_2H_2Cl_2$, or C_2HCl_3 from $[M\text{-}Cl]^{+}$.

Mass Spectrometry of Chlorinated Bornanes

It has been found that three major ion degradation pathways in the positive ion spectra can be used to help identify a given CHB congener[21]. The first is the loss of C_2H_3Cl, $C_2H_2Cl_2$, or C_2HCl_3 after an initial loss of HCl or Cl_2 from the molecular ion. This process has been observed to occur in the reverse order as well. Both the forward and reverse reactions give rise to an even-mass odd-electron ion that is characteristic of the number of chlorine atoms located on C2-

C3 and C5-C6. The second pathway is elimination of a neutral fragment from $[M\text{-}HCl\text{-}Cl]^+$ or $[M\text{-}Cl_2\text{-}Cl]^+$ comprised of C7 and its two attached substituents. This yields information regarding the bridging group's (C7+C8+C9) chlorine content, while observations of decompositions involving the elimination of $^{\cdot}CH_2Cl$ or $^{\cdot}CHCl_2$ from $M^{+\cdot}$ and other characteristic ions give evidence as to the identity of the substituted chloromethyl groups on C7. The third decomposition, would lead to the presence of $CHCl_2^+$ ions at m/z 83, and provide information with regard to the chlorine substitution on C10. The abscence of this ion in the mass spectrum indicates that rather a monochloromethyl group is substituted on C10.



Bornane skeleton

Fragmentation Theory

A commonly observed reaction pathway in CHB congeners is a process that involves the loss of molecular $C_2H_2Cl_2$ from $[M\text{-}HCl]^{+\cdot}$. The molecular ion loses molecular HCl, comprised of one Cl and one H from one and the same face of the six-membered ring. Each atom contributes one electron to the HCl sigma bond,

while a double bond is formed between adjacent carbons of the ring. The [M-HCl]$^{+\cdot}$ radical cation undergoes a concerted electron movement to the opposite side of the ring so that molecular ClHC=CHCl is lost (retro Diels-Alder reaction, Figure 4) to produce the even-mass, odd-electron cyclopentadiene structure.

Alternatively, $C_2H_2Cl_2$ or C_2HCl_3 loss may precede, respectively, the elimination of Cl_2 or HCl from $M^{+\cdot}$. This suggests that an electron is lost from a chlorine atom on the six-membered ring forming $M^{+\cdot}$. The electrons from the C4-C5 bond form a π bond between C5 and C6 since the C4 site is best suited to stabilize the positive charge on the ion. The electrons in the C6-C1 bond are shared between the leaving neutral group ClHC=CHCl and the radical cation (Figure 5). The radical cation then loses molecular Cl_2. A decomposition involving the initial loss of C_2HCl_3 followed by loss of HCl occurs in the same fashion as previously described.

The next reaction deals with the loss of the chlorobornane bridging group (C7-C9). Followed by consecutive losses of HCl and $^{\cdot}$Cl from $M^{+\cdot}$, concerted electron shifts (Figure 6) allow the bridging group to leave as molecular $ClH_2CC{:}(CH_2Cl)$ with a lone pair of electrons on the C7 carbon. This results in a chloro-cyclohexadiene structure where the π electrons are likely delocalized around most of the ring

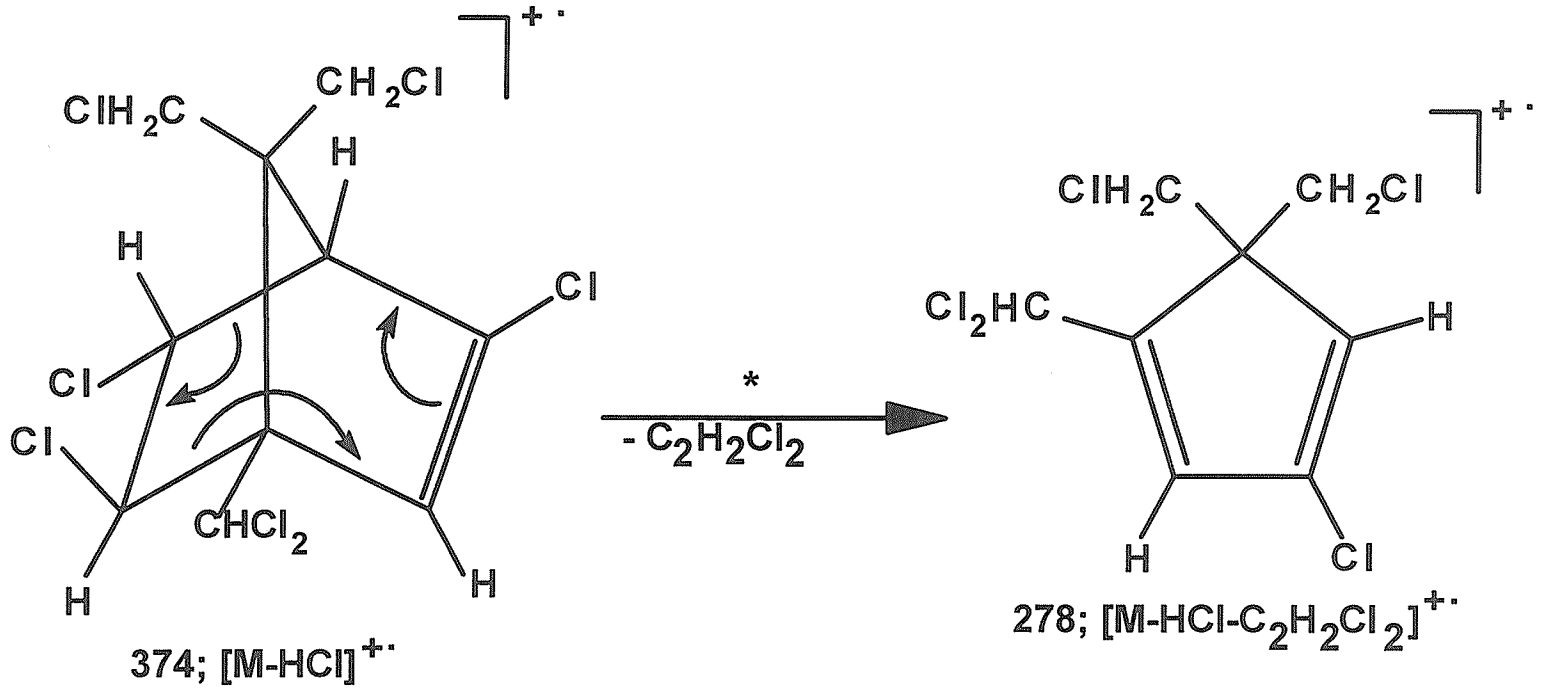


Figure 4: Proposed mechanism (retro-Diels-Alder reaction) for loss of the neutral fragment $C_2H_2Cl_2$ from $[M\text{-}HCl]^{+\cdot}$ (m/z 374) and formation of the characteristic odd-electron even mass ion $[M\text{-}HCl\text{-}C_2H_2Cl_2]^{+\cdot}$ (m/z 278) in the mass spectrum of TS3.

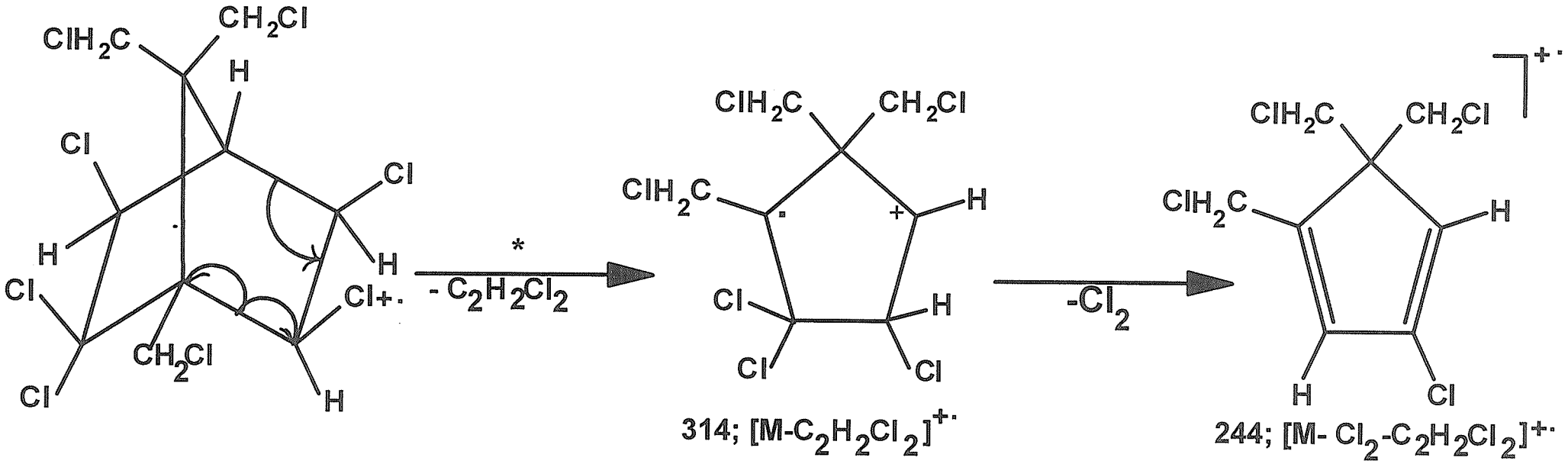
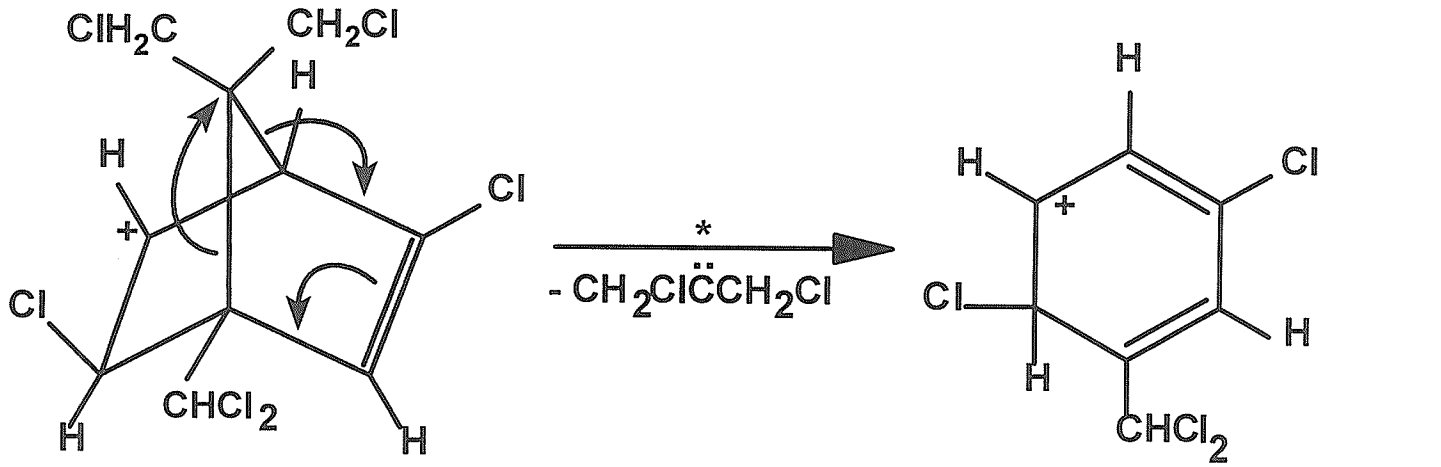


Figure 5: Proposed mechanism for sequential losses of $C_2H_2Cl_2$ and Cl_2 from $M^{+\cdot}$ in mass spectrum of TS2.



339; $[M\text{-}HCl\text{-}Cl]^+$

229; $[M\text{-}HCl\text{-}Cl\text{-}CH_2ClCCH_2Cl]^+$

Figure 6: Proposed mechanism for the elimination of the neutral species $CH_2ClC{:}(CH_2Cl)$ from $[M\text{-}HCl\text{-}Cl]^+$ (m/z 339) in the mass spectrum of TS3.

structure.

CHB congeners present in Arviat ringed seal blubber

TS2, 3, and 4 were determined to be octachlorobornanes by SIR/ECNIMS (Figure 7). This technique of mass spectrometry is extremely sensitive for toxaphene analysis, giving information on the level of chlorination of a given congener at the femtogram level when monitoring the $[M\text{-}Cl]^-$ ion. The $[M\text{-}Cl]^-$ ion is very stable, but the ECNI (soft ionization method) mass spectra lack much fragmentation. Although this stability aids in sensitivity for quantitative analysis, it does not provide much information about a congener's identity. For this purpose we use positive ion EIMS which is a harsher mode of ionization and thus induces greater fragmentation of CHBs. This consequently, provides greater insight into fragmentation patterns, and ultimately helps characterize a given CHB congener.

Upon extraction of organic compounds from ringed seal blubber using the dialysis method previously described, the extracts were further cleaned-up using fuming nitric and sulfuric acid to destroy any aromatic and olefinic compounds. Using a combination of florisil chromatography and reverse phase C18 HPLC, TS2, TS3 and TS4 were sufficiently separated from one another. This aided in obtaining superior EI and linked scanning spectra for each compound as a number of the

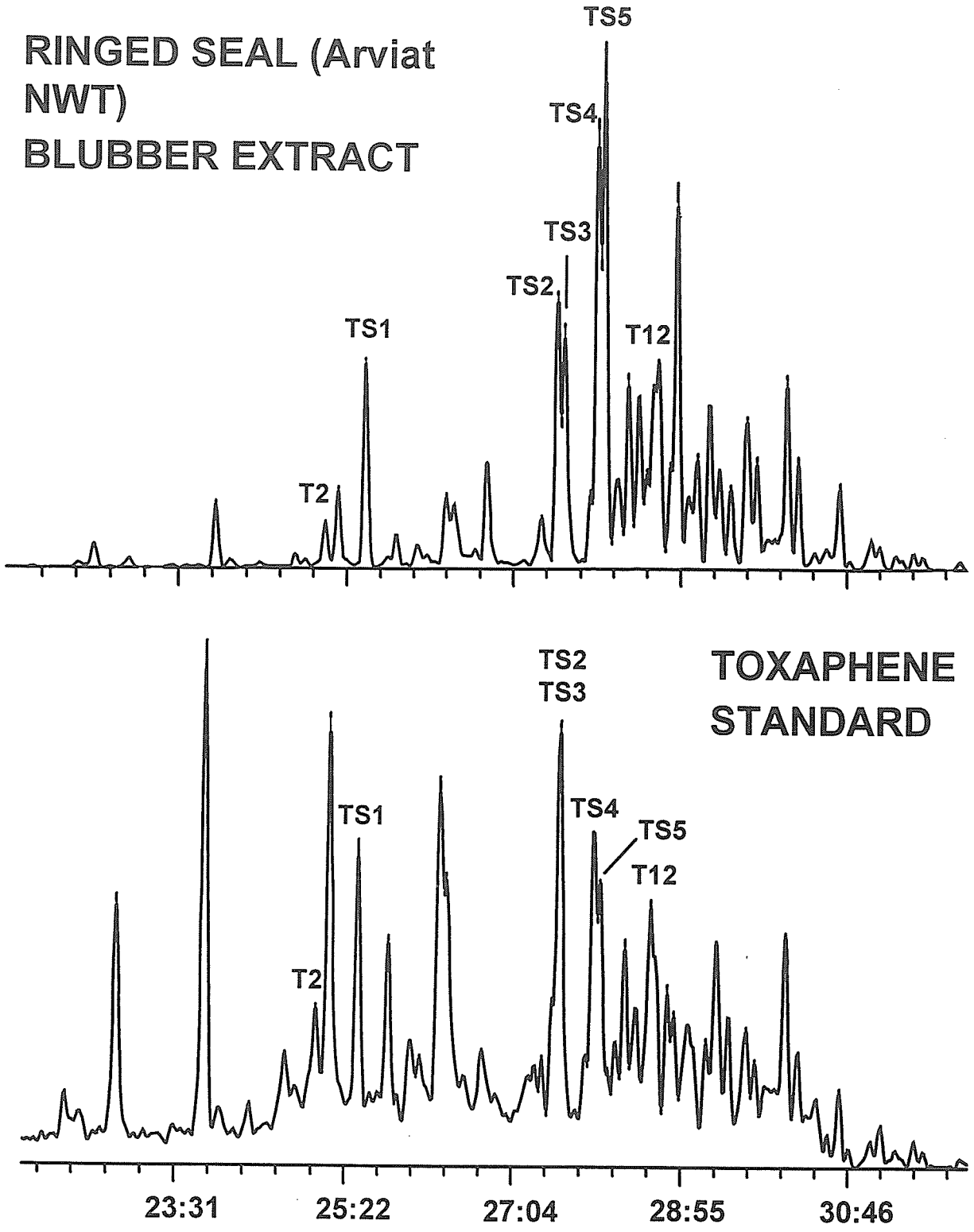


Figure 7: GC-ECNIMS selected ion chromatograms (sum of ions m/z 208.9352, 342.8962, 376.8573 and 412.8154) of ringed seal blubber extract (top) and technical toxaphene (bottom). RP = 14000, 60m x 0.25mm i.d. DB5-ms column (He carrier gas).

CHB congeners nearly co-elute. As a result of separating the seal extract into three separate fractions, TS2, TS3, and TS4 were sufficiently resolved from other compounds for GC/MS experiments, but were neither concentrated enough nor adequately pure for ^{1}H NMR studies.

Table 4: Relative abundances of important ions in mass spectra of TS2, 3 and 4

Ion	m/z	TS2 %RA	TS3 %RA	TS4 %RA
$[M-Cl]^+$	375	<1	4.4	1
$[M-HCl]^{+\cdot}$	374	<1	<1	-
$[M-CH_2Cl]^+$	361	1.4	1.1	-
$[M-Cl_2]^+$	340	<1	2.5	-
$[M-HCl-Cl]^+$	339	1.3	21.4	4.9
$[M-CHCl_2]$	327	<1	<1	1.1
$[M-CH_2Cl-HCl]^+$	325	2.1	19.9	1.4
$[M-Cl_2-Cl]^+$	305	1.5	-	-
$[M-HCl-Cl-CH_2Cl]^+$	303	1.5	52.8	10.5
$[M-CHCl_2-HCl]^+$	291	15.7	<1	9.5
$[M-HCl-C_2H_2Cl_2]^{+\cdot}$	278	<1	29.4	2.7
$[M-HCl-Cl-2HCl]^+$	267	3.1	43.4	20.3
$[M-Cl_2-C_2H_2Cl_2]^{+\cdot}$	244	46.6	-	-
$[M-HCl-C_2HCl_3]^{+\cdot}$	244		-	-
$[M-HCl-C_2H_2Cl_2-Cl]^+$	243	<1	56.7	13.2
$[M-HCl-C_3H_4Cl_2]^+$	229	1.5	35.9	4.4
$[M-HCl-C_2H_2Cl_2-Cl-HCl]^+$	207	-	32.8	10.5
$[M-HCl-Cl-C_3H_3Cl_3]^+$	195	-	-	19.6
$[M-Cl_2-Cl-C_3H_4Cl_2]^+$	195	15.7	-	-

Table 4 shows ion abundances that are important in assessing the favourability of certain ion decompositions. In this table, and in the discussion, m/z values of ions, and masses of neutral species, are based on ^{35}Cl. The following is an interpretation of the EIMS of three major CHB congeners in ringed seal blubber.

In the mass spectrum of TS2 (Figure 8), the even mass odd-electron ion at m/z 244 is the result of two processes, as verified by linked scanning experiments. Successive losses of C_2HCl_3 and HCl (Figure 9a), or $C_2H_2Cl_2$ and Cl_2 (Figure 9b), from $M^{+\cdot}$ indicate that there must be five chlorine atoms on the six-membered ring, two on one side and three on the other. The exact positioning of these ring chlorines, however, could not be exactly determined by mass spectrometry. It is possible that owing to steric factors, the side with 3 chlorines has 2 chlorines in an exo position. It is also significant that the abundance of the ion fragment cluster at m/z 244 is high most likely because there two major processes resulting in this radical cation. This indicates that the chlorine atoms on the opposite side (the one with 2 chlorine atoms) are on different carbon atoms and in staggered formation.

An ion giving rise to a peak at m/z 195 results from loss of $C_3H_4Cl_2$ from $[M\text{-}Cl_2\text{-}Cl]^+$ (m/z 305) (Figure 10) indicating there are two chlorines on the bridging group. The observed losses of $\cdot CH_2Cl$ from $M^{+\cdot}$ and $[M\text{-}Cl_2]^{+\cdot}$ and the absence of decompositions involving loss of $\cdot CHCl_2$ suggest that the bridging group consists

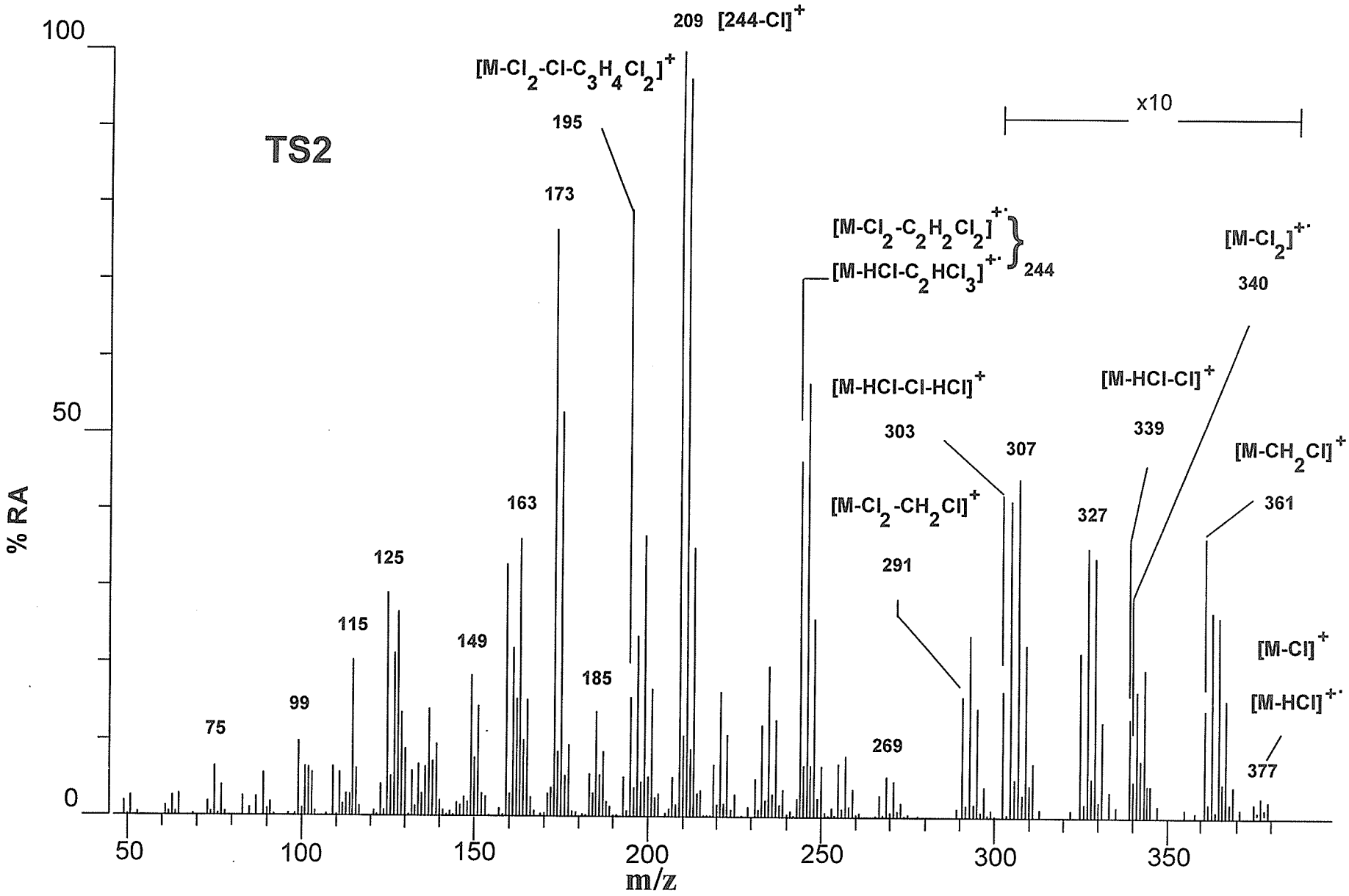


Figure 8: Full scan positive EI mass spectrum of TS2

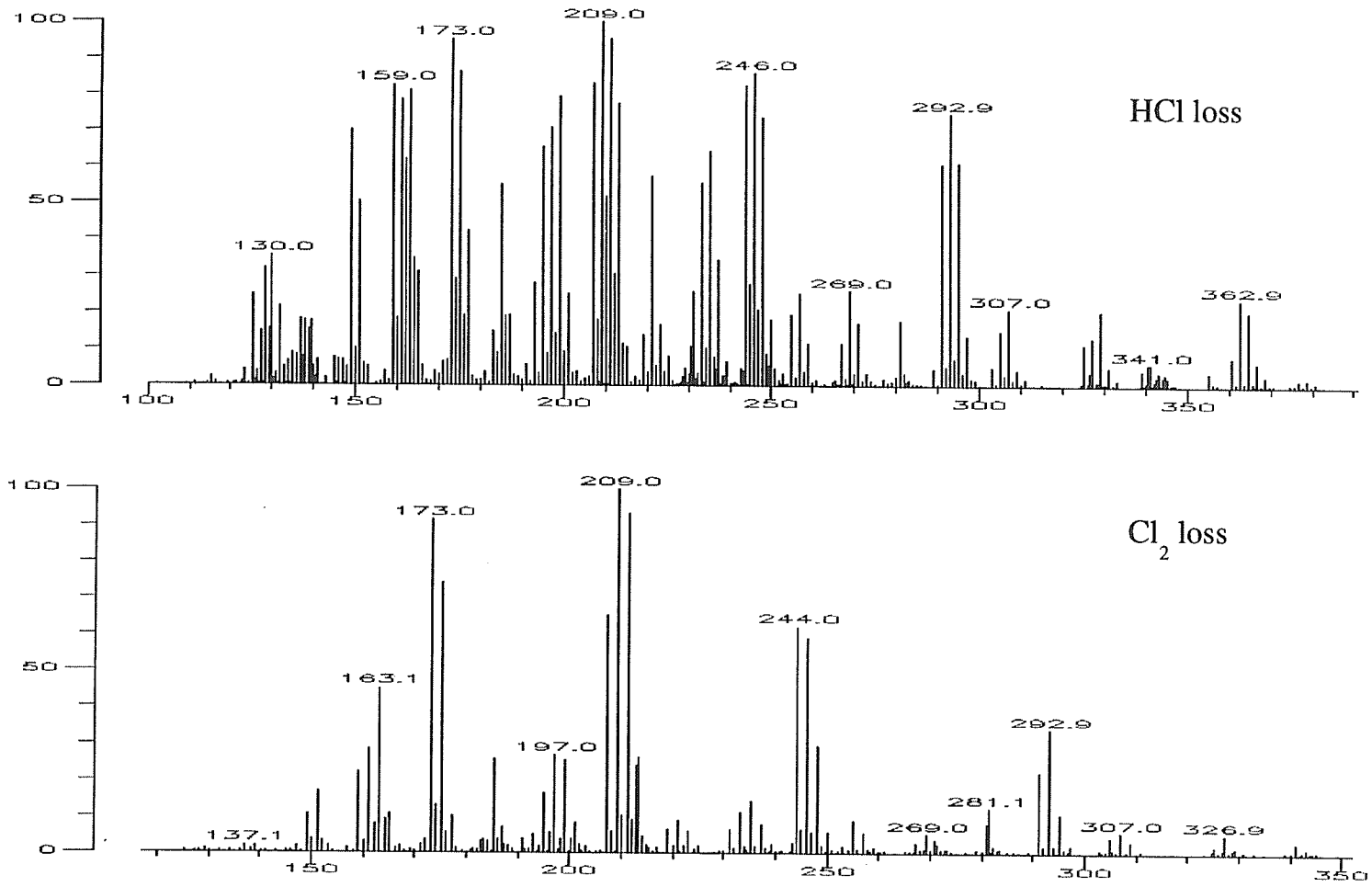


Figure 9a: TS2 CNL (daughter) of HCl. Ion at m/z 244 indicates loss of HCl from $[M\text{-}C_2H_3Cl]^{+\cdot}$.

Figure 9b: TS2 CNL (daughter) of Cl_2. Ion at m/z 244 indicates loss of Cl_2 from $[M\text{-}C_2H_2Cl_2]^{+\cdot}$.

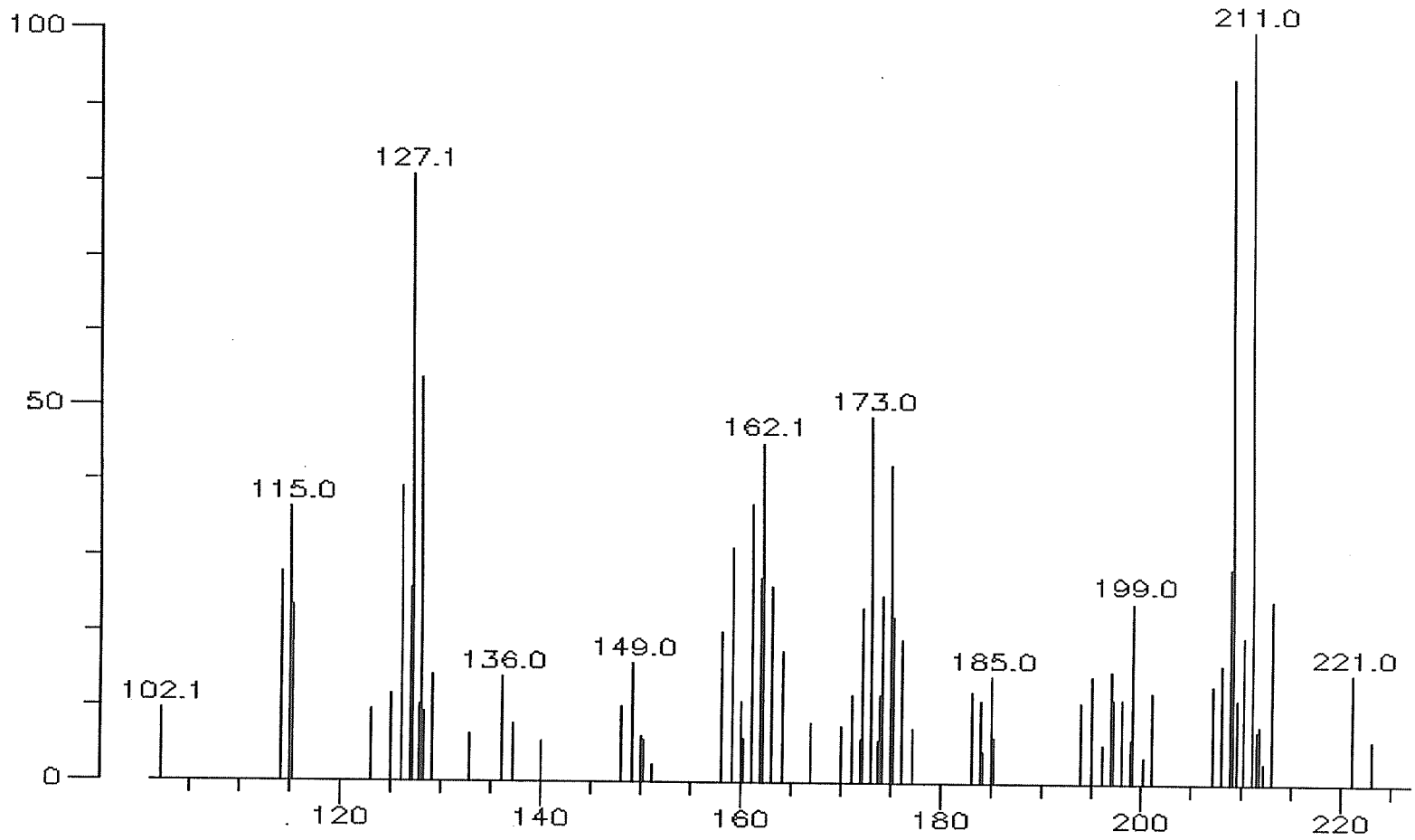


Figure 10: CNL (daughter) scan of TS2. Cluster of peaks at m/z 195 indicates loss of $C_3H_4Cl_2$ from $[M\text{-}Cl_2\text{-}Cl]^+$.

of two monochloromethyl substituents on C7. The one remaining chlorine atom must be positioned on C10.

In the mass spectrum of TS3 (Figure 11), a peak observed at m/z 278 is the result of successive losses of HCl and $C_2H_2Cl_2$ from $M^{+\cdot}$ (Figure 12). There is no other significant pathway resulting in an even-mass odd-electron ion, as verified by linked scanning experiments, and it can therefore be concluded that there must be two chlorine atoms on each of the C2-C3 and C5-C6 sides of the ring. The high abundance of $[M\text{-}HCl]^{+\cdot}$, relative to the spectrum of TS2, indicates that there are likely many pathways to the formation of this ion. This suggests that chlorine atoms are distributed one to each carbon atom in the ring and that adjacent chlorine atoms are anti to one another (e.g. 2-exo,3-endo). Since there must be chlorine in the 2-exo position we can tentatively propose the ring as 2-exo,3-endo,5-exo,6-endo substituted. Loss of the neutral fragment $C_3H_4Cl_2$ from $[M\text{-}HCl\text{-}Cl]^+$ indicates the presence of two chlorine atoms on the bridging group (Figure 13). Losses of $\cdot CH_2Cl$ from $M^{+\cdot}$ and from $[M\text{-}HCl]^{+\cdot}$, and the absence of losses involving $\cdot CHCl_2$, verify that the two chlorines in the bridging group are the result of two monochloro substituents on C7. The remaining two chlorine atoms must be positioned on C10. Support for a dichloromethyl group at C10 is shown by the presence of a peak corresponding to $CHCl_2^+$ at m/z 83.

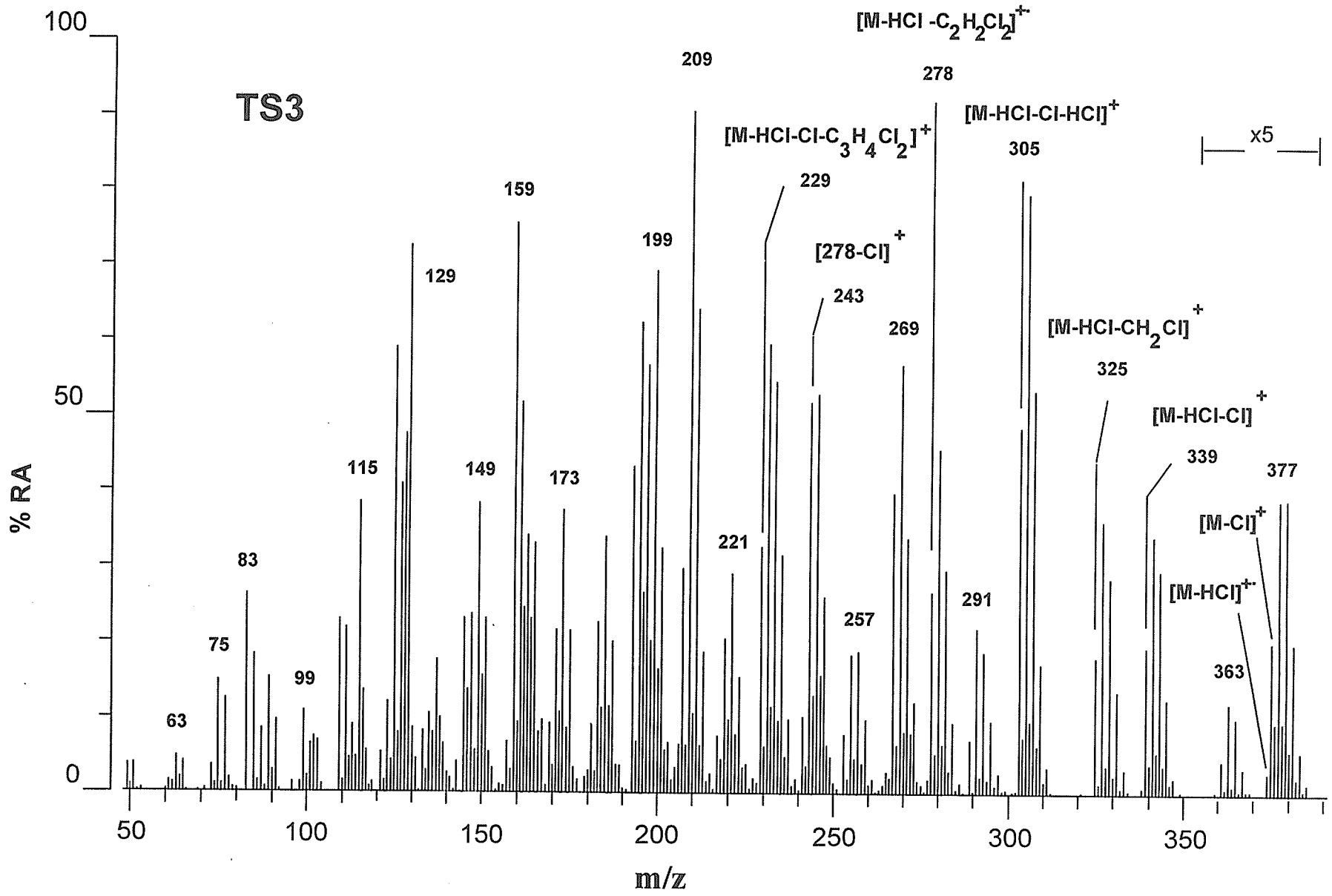


Figure 11: Full scan positive EI mass spectrum of TS3

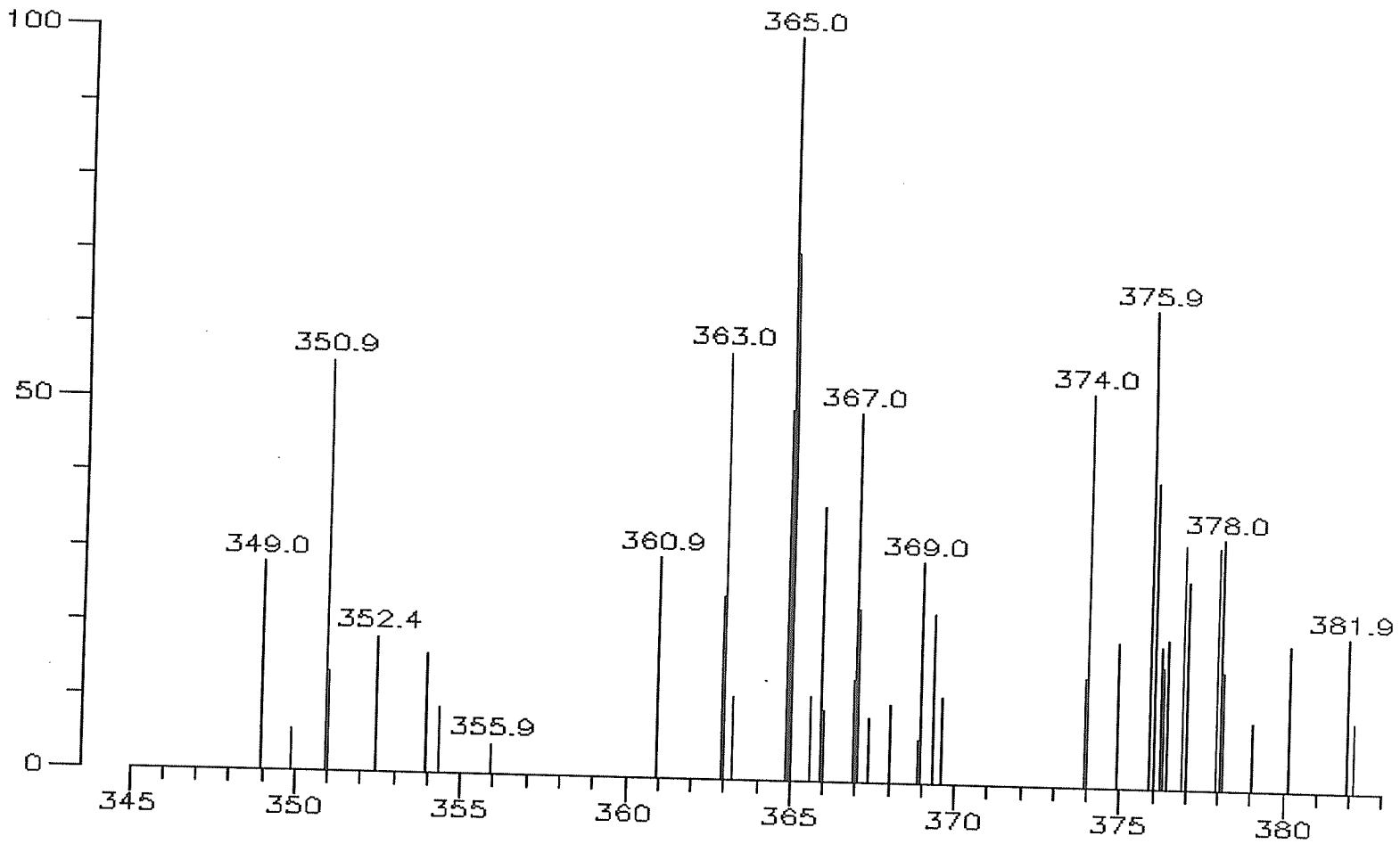


Figure 12: TS3 CNL (parent) scan for loss of $C_2H_2Cl_2$ (96 amu). The cluster of peaks starting at m/z 374 indicates the loss of $C_2H_2Cl_2$ from $[M\text{-}HCl]^{+\cdot}$.

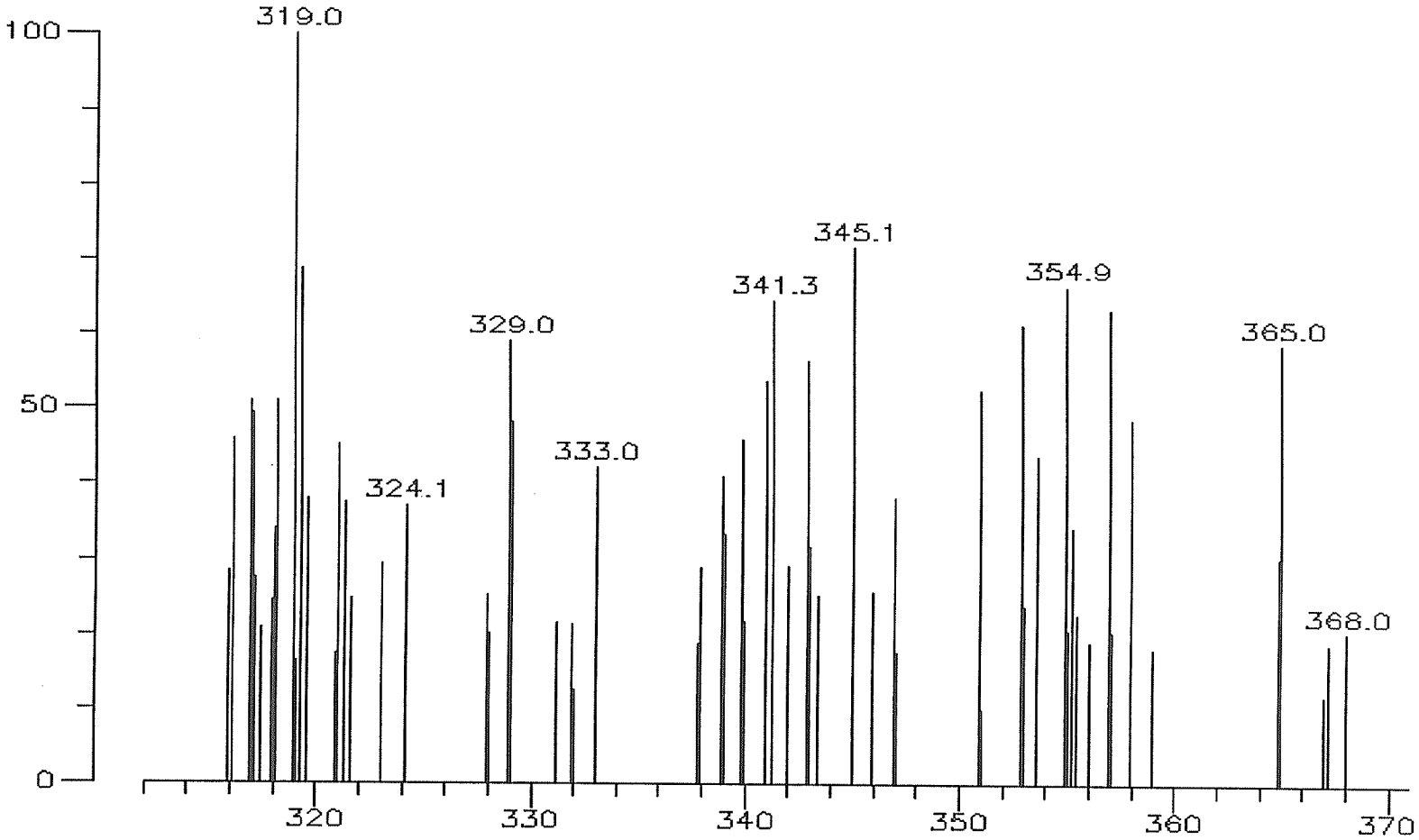


Figure 13: TS3 CNL (parent) scan for loss of 110 u indicates elimination of $C_2H_4Cl_2$ from $[M\text{-}HCl\text{-}Cl]^+$, m/z 339.

A significant feature of the mass spectrum of TS4 (Figure 14) is the very low relative abundance of the even-mass odd-electron ion at m/z 278 resulting from the loss of HCl from $[M\text{-}C_2H_2Cl_2]^{+\cdot}$(Figure 15). As with TS3, the loss of only $C_2H_2Cl_2$ indicates the presence of two chlorine atoms on both C2-C3 and C5-C6. Lack of a predominant even-mass odd-electron ion (e.g. $[M\text{-}C_2H_2Cl_2\text{-}HCl]^{+\cdot}$) would be consistent with a ring structure having a geminal dichloro-substitution and an adjacent unchlorinated carbon. The actual chlorine substitution pattern on the ring cannot be verified by mass spectrometry. Loss of $C_3H_3Cl_3$ is observed from $[M\text{-}HCl\text{-}Cl]^+$ (Figure 16). This confirms the presence of three chlorine atoms on the bridging group. Decompositions involving the losses of $^{\cdot}CH_2Cl$ and $^{\cdot}CHCl_2$ from M^+ and $[M\text{-}HCl]^{+\cdot}$ indicate the presence of a dichloromethyl and monochloromethyl substituent on C7. The remaining chlorine must be located on C10.

Recently, Parlar *et al.* were successful in isolating 19 CHBs in the technical mixture by a combination of uv radiation, column chromatography on silica gel, and preparative HPLC[17]. One gram samples were dissolved in 120 mL oxygen-free *n*-hexane and irradiated for 1 h with a high-pressure mercury lamp (HPK 125 W) while stirring. The solvent was removed and the photochemically modified toxaphene was applied to a silica gel column and separated by liquid

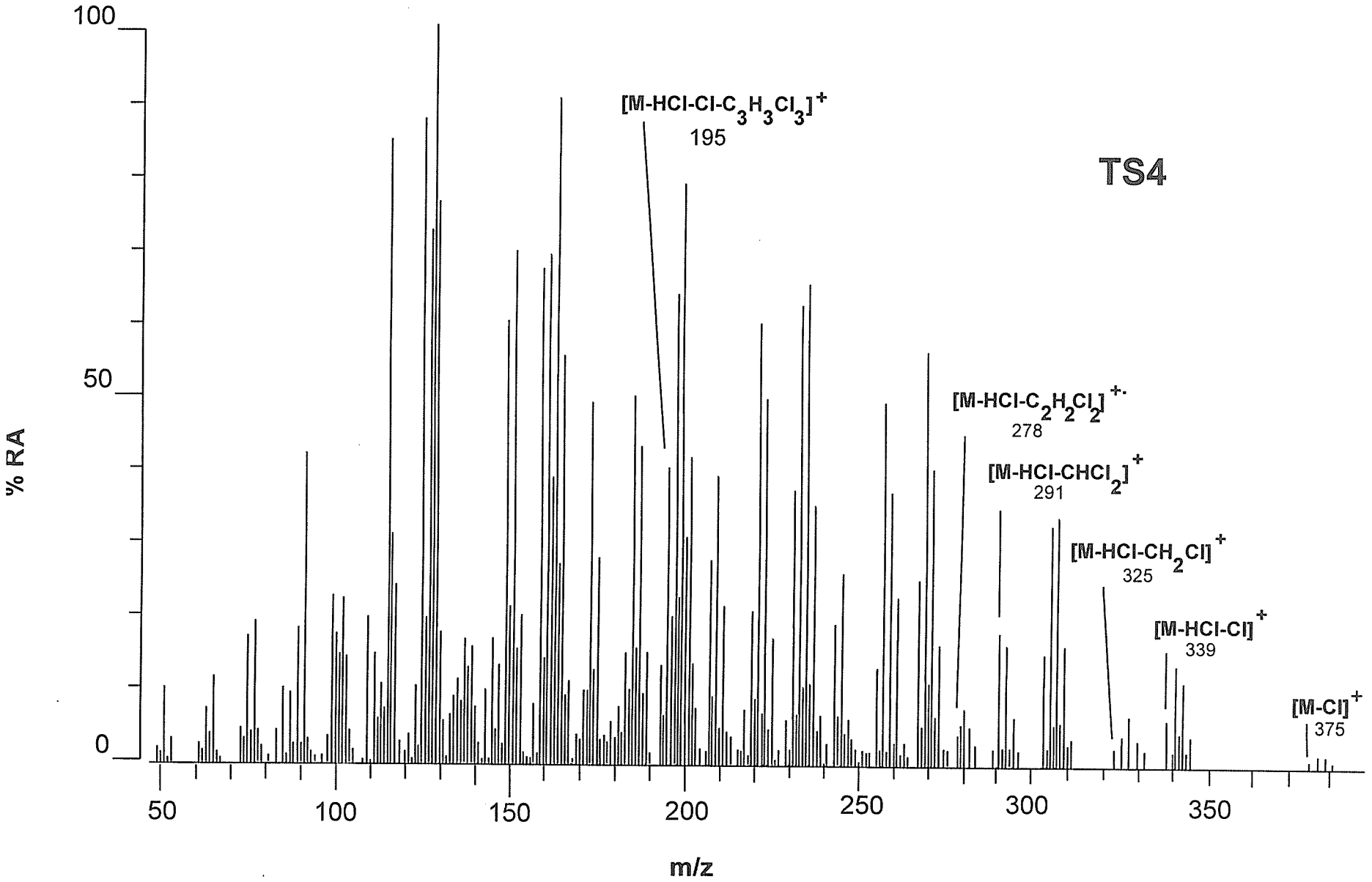


Figure 14: Full scan positive EI mass spectrum of TS4

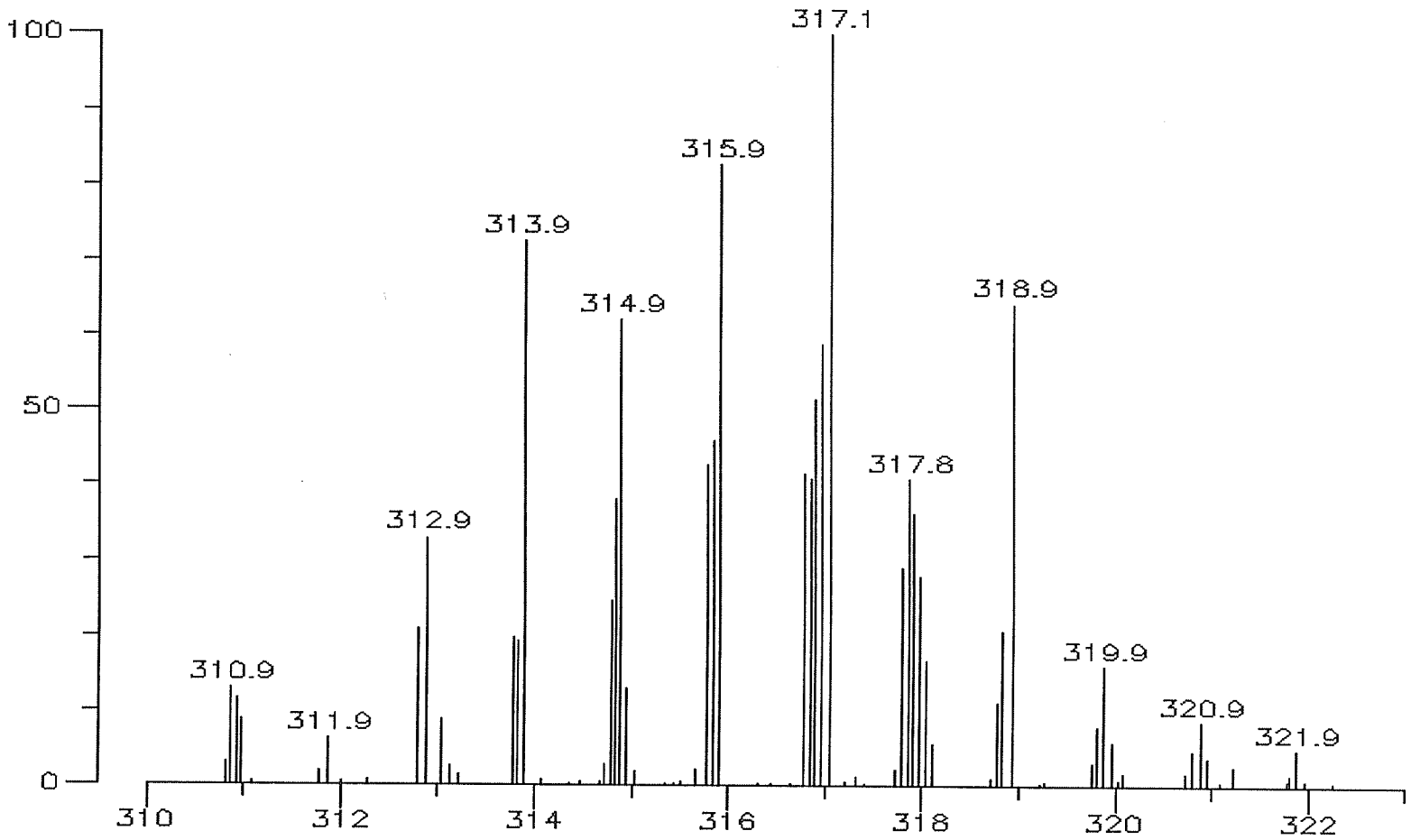


Figure 15: TS4 CNL (parent) scan for loss of 36 u. The cluster starting at m/z 314 indicates the loss of HCl from $[M\text{-}C_2H_2Cl_2]^{+\cdot}$.

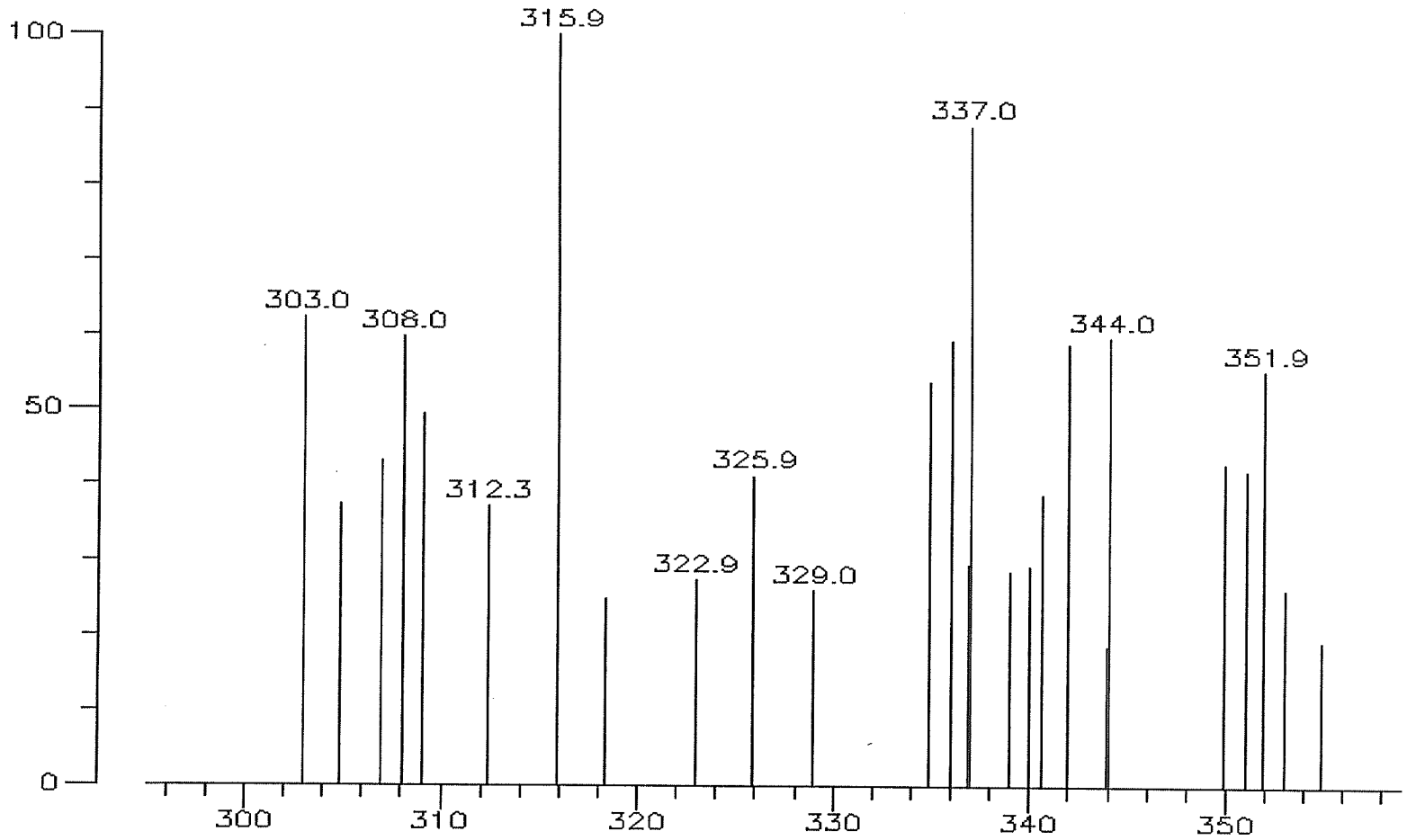


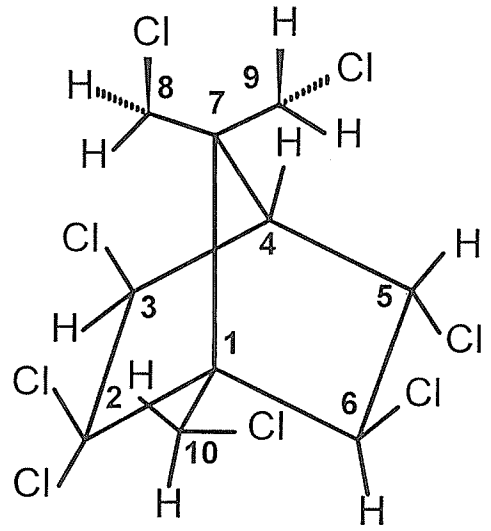
Figure 16: TS4 CNL (parent) scan for loss of 144 u. The cluster at m/z 339 indicates elimination of $C_3H_3Cl_3$ from $[M\text{-}HCl\text{-}Cl]^+$.

chromatography. Similar fractions were combined and further purified on a preparative C18 reverse phase column. Isolated compounds were identified by using a combination of mass spectrometry, ^{1}H and ^{13}C NMR.

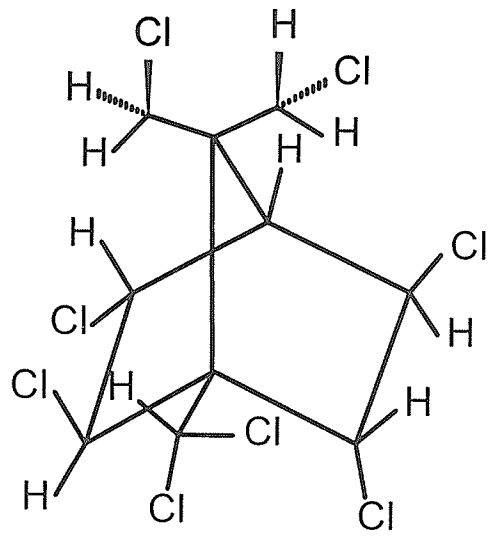
Parlar supplied a standard mixture of these CHB compounds for the purpose of comparison with TS2, TS3, and TS4. Based on their GC retention times, and by comparison with full scan positive EIMS spectra, TS2, 3 and 4 were found to correspond to Parlar #39, 2,2,3-exo,5-endo-6-exo,8,9,10-octachloroborane, Parlar #40, 2-exo,3-endo,5-exo,6-endo,8,9,10,10-octachlorobornane, and Parlar #42, which is a mixture of 2,2,5-endo,6-exo,8,8,9,10-octachlorobornane (TS4α) and 2,2,5-endo,6-exo,8,9,9,10-octachlorobornane (TS4β) respectively (Figure 17). The two compounds in Parlar #42 are identical except for the proximity of the substituted chloromethyl groups on C7 to the ring substituents. Since these two compounds would likely give the same mass spectrum we cannot determine whether TS4 is one of or a mixture of TS4α, and TS4β.

Spatial Conformation of CHB congeners in Seal Blubber

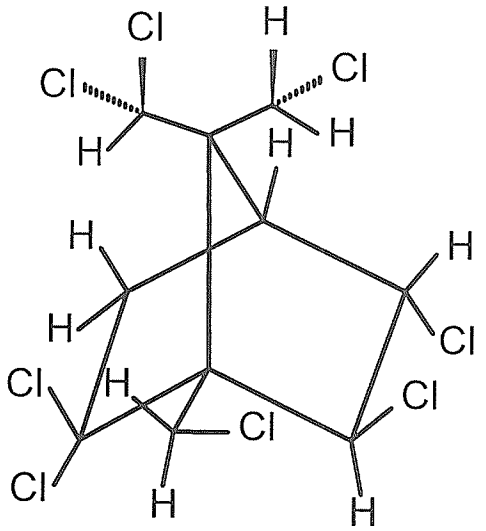
To simplify the discussion of the conformations of the C-8 and C-9 chloromethyl groups, we first define a vertical plane passing through C-7 and the mid-points of the C-2, C-3 and C-5, C-6 bonds. Because of the large sizes of the chlorine atoms,



2,2,3-*exo*,5-*endo*,6-*exo*,8,9,10-octachlorobornane (TS2, Parlar #39)



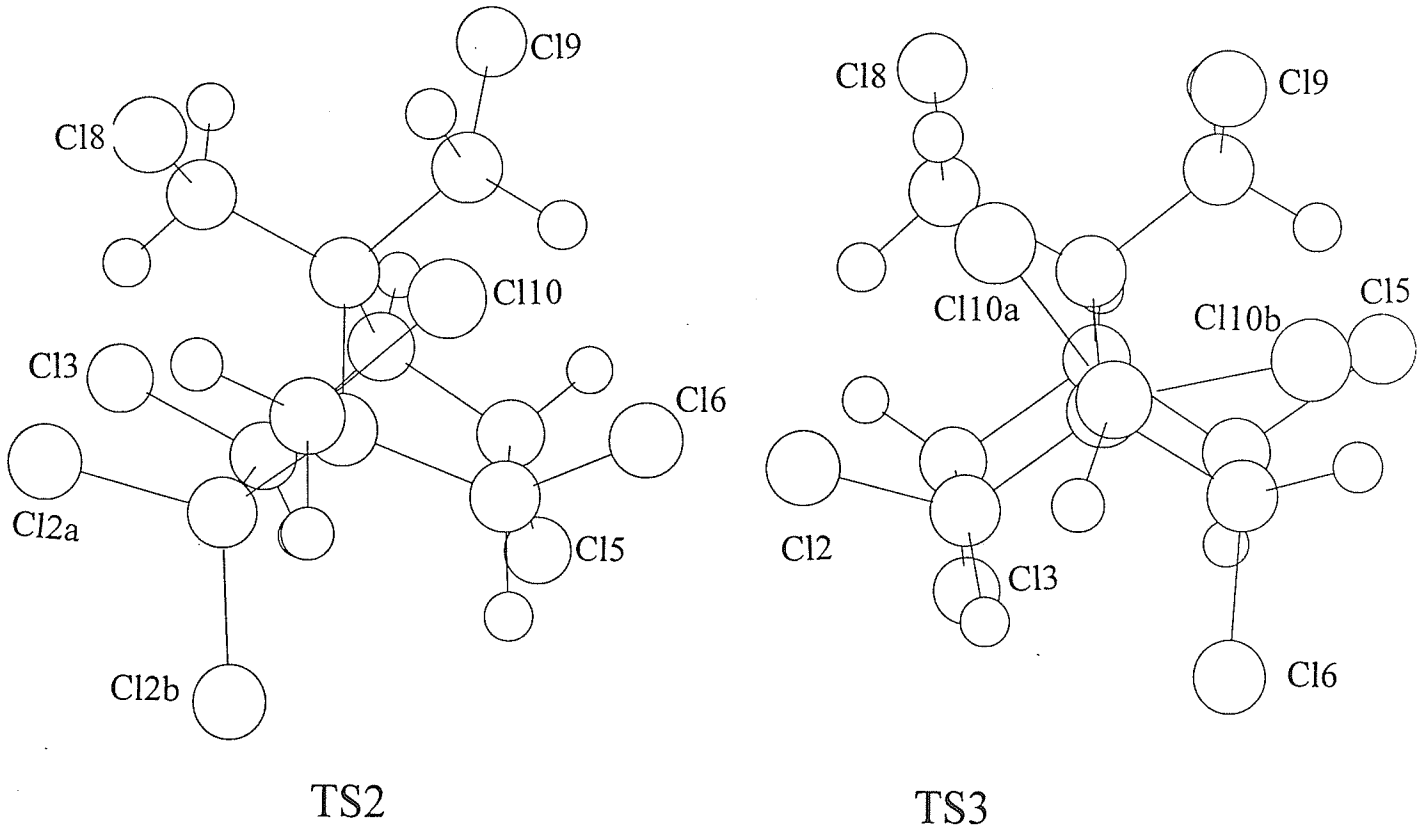
2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,9,10,10-octachlorobornane (TS3,Parlar #40)



2,2,5-*endo*,6-*exo*,8,8,9,10-octachlorobornane (TS4,toxicant A, Parlar #42)

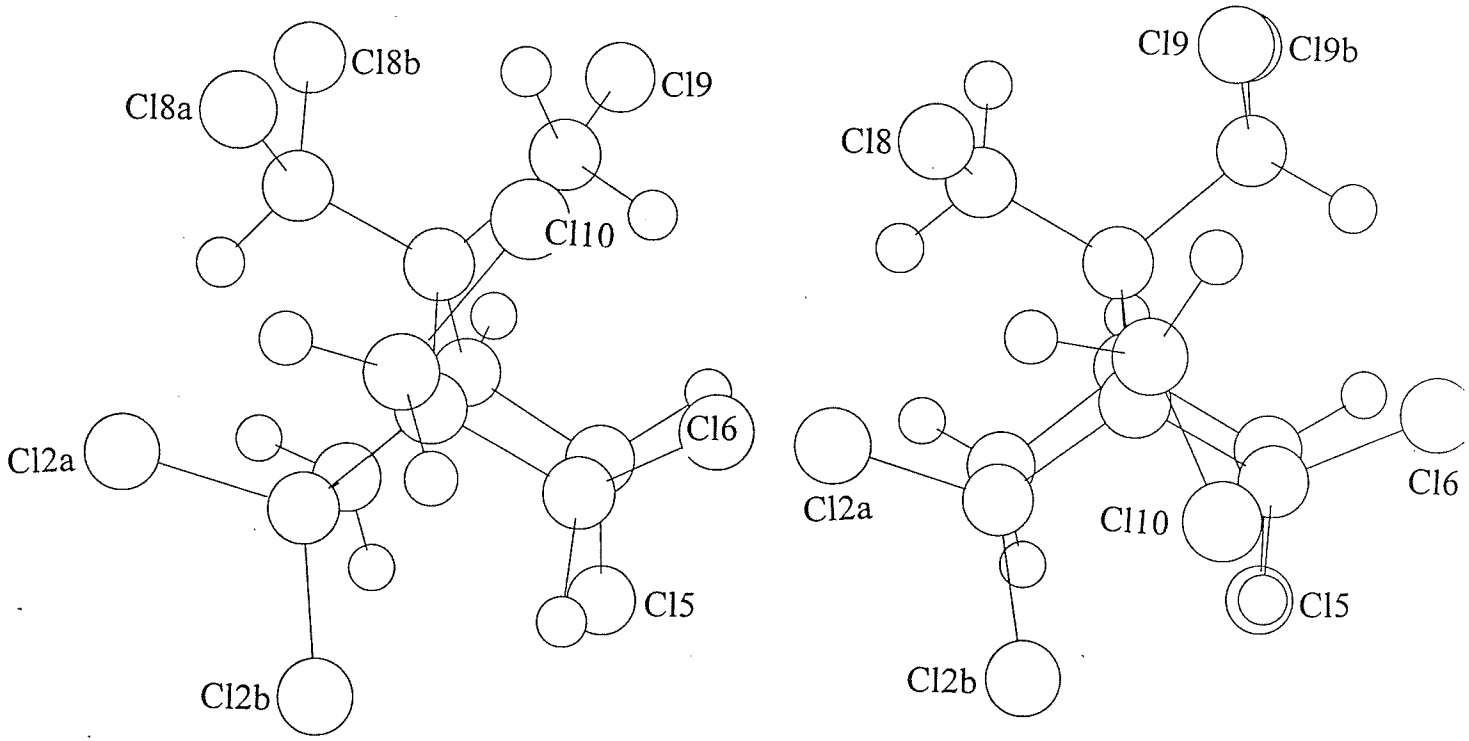
Figure 17: Postulated structures of three major chlorinated bornanes isolated from ringed seal blubber (Arviat, NWT).

for steric reasons chlorine atoms on C-8 and C-9 will tend to avoid exo chlorine atoms on the six-membered ring. Thus, complete rotations of the chloromethyl groups are prevented by the development of very large steric interferences between these chlorine atoms. In the most probable conformations, protons H-8a and H-9a will be located in, or close to, the defined vertical plane. An oscillating internal "rotation" (rather like the swinging motion of an oscillating water sprinkler) of each of these groups is the only motion possible, with time-averaged conformations approximating those depicted in Fig. 18a (TS2 & 3) and 18b (TS4α and β). Probable spatial conformations of TS2, TS3, and TS4 were examined using molecular mechanics calculations. The Molecular Modeling Pro program is based on the MOLY minimizer[27]. First the C10 chloromethyl group was rotated so that Cl-10 was equidistant from C-2 and C-6 in the cases of TS2 and TS4. For TS3 dichloromethyl group was rotated as to position the proton on C10 in the plane passing through C-1, C-7 and C-4. Then the structures were derived by alternately performing conformational analysis (rigid rotor model) by incrementally (5°) rotating the C-8 chloromethyl groups and then allowing C-9 to rotate through a complete 360° cycle to find the lowest energy configuration. Then C-10 was rotated through 360° to minimize its energy, followed by minimization of the total strain energy in the respective molecules. If we first



TS2 TS3

Figure 18a: Molecular mechanics models of lowest energy conformation of TS2 and TS3



TS4α TS4β

Figure 18b: Molecular mechanics models of lowest energy conformation of TS4α and TS4β.

consider rotation of the C-8 chloromethyl group, with the Cl atoms of the C-9 and C-10 groups moved to positions in which they do not interfere, the strain energy developed exceeds 100 kcal mol^{-1}, with respect to the minimum strain, as Cl-8 passes the exo Cl-2 and/or exo Cl-3. A similar large strain energy is developed as Cl-9 passes the exo positioned Cl-5 or Cl-6 for a rotating C-9 chloromethyl group. Of special note here are the two possible isomers of TS4 (TS4α with 2 chlorine atoms on C-8 and TS4β with two chlorine atoms on C-9). The added complexity of three chlorine atoms on the bridging groups yields structures less like the simpler TS2 and TS3 compounds. The strongest interaction governing the spatial position of the chlorine atoms on C-8 and C-9 is their interaction with adjacent exo chlorine atoms on the six-membered ring. The chlorine atoms also avoid the previously described vertical plane through the molecule. Therefore the dichloromethyl group (C-8 in TS4α and C-9 in TS4β) shows rotation so that chlorine atoms straddle the previously defined vertical plane. The monochloromethyl group is subjected to the same interactions with exo chlorine atoms and therefore is forced close to one of the chlorine atoms in the dichloromethyl group. This has an effect on the conformation of the bridging group as the congener has slightly increased steric energy when two chlorine atoms are eclipsed. In each molecule's lowest energy conformation, it follows that

H-8a and H-9a are not as close to the plane as in the other two compounds.

At the extremes of their swinging excursions, the chlorine atoms of the C-8 and C-9 groups do not significantly interfere with the motion of the other group so that positioning these atoms on the other sides of the vertical plane from those depicted in Figure 18a and 18b is, in principle, possible. However, as previously described, very large steric interferences between these chlorine atoms develop when they approach the vertical plane and these would prevent them from passing each other in any reasonable oscillations of the chloromethyl groups. Thus, the magnitude of the oscillations will be quite restricted.

For a rotating C-10 chloromethyl group the models and calculations show that the only significant steric interferences develop between Cl-10 and chlorines on the bridging group which are rotated towards C-10. These are very large (>100 kcal mol^{-1}); however, complete rotation of the C-10 chloromethyl group is permitted in less probable, but low energy conformations of the C-8 and C-9 chloromethyl groups so that the motion of the C-10 group could be either complete rotation or a swinging oscillation. In the case of TS2, Cl-10 is rotated away from Cl-8, which is rotated in front of the defined plane. It also tends to move away from the bulky chlorines at C-2. For TS3, in its lowest energy state, the two Cl-10 chlorine atoms are rotated away from Cl-9 which is in front of the plane. There are two maxima

of steric energy as one or the other chlorine atoms passes Cl-9. For TS4, Cl-10 is rotated away from Cl-8. TS4β experiences its lowest energy state when the chlorine on C-8 and a chlorine on C-9 are both rotated in front of the plane. Cl-10 is rotated away from both of these and slightly away from the bulky chlorine atoms at C-2. In general, however, there seems to be very little restriction in the rotation of the C-10 chloromethyl group.

Ringed seal blubber CHB congener toxicity

TS4, also known as Toxicant A, was first isolated and characterized long ago[26]. It is thought to be the most toxic of all the components in technical toxaphene with a relative toxicity to gold fish 25 times that of the technical standard[12]. This component has been found in fish (salmon, Baltic herring) but is normally absent in warm-blooded species that feed on fish (harp seal, penguin)[23]. This is the first instance where this compound has been found in such high quantities in marine mammals and indicates that the ringed seal differs markedly from other warm-blooded species in its ability to metabolize toxaphene. The presence of Toxicant A is of concern for ecosystem and human health reasons. Ringed seal is a large part of the diet of Polar bears. Inuit people in the Canadian North also eat ringed seal tissue, especially muscle, kidney and liver in their traditional diet. What biological effect Toxicant A has in the Canadian Arctic and other arctic regions is

unknown at this time. A study of the chlorination by-products of Toxicant B, revealed that the toxicity of TS2 (a chlorination by-product of Tox B) was about one half as toxic to goldfish and houseflies as is technical toxaphene[13].

CHB congeners present in surface sediment

Two major toxaphene components, which we call Hx-Sed and Hp-Sed, were isolated from surface sediment (Figure 2). After refluxing the freeze-dried lake sediment and evaporating the solvent, the residue was further cleaned-up using the acid oxidation method removing pigments aromatic and olefinic compounds. Using both florisil chromatography and reverse phase C18 HPLC chromatograpy, both compounds were succesfully isolated from other organic compounds.

Table 5 shows the ion abundances for important ions in the EI mass spectra, which are important in assessing the favourability of certain ion decompositions.

The following is an interpretation of the EIMS of these two major CHB congeners in surface sediment of toxaphene treated lakes:

The EI positive ion mass spectrum of Hp-Sed is shown in Figure 19. The groups of peaks starting and at m/z 340 and 341 in the mass spectrum of Hp-Sed

Table 5: Relative abundances of important ions in the mass spectra of Hx-Sed and Hp-Sed

	Hp-Sed		Hx-Sed	
Ion	m/z	%RA	m/z	%RA
$[M-Cl]^+$	341	4.0	307	29.4
$[M-HCl]^{+\cdot}$	340	2.2	306	2.2
$[M-CH_2Cl]^+$	327	3.0	293	33.3
$[M-HCl-Cl]^+$	305	23.4	271	42.0
$[M-HCl-CH_2Cl]^+$	291	13.0	257	33.0
$[M-Cl-C_2H_3Cl]^{+\cdot}$	279	-	245	19.4
$[M-HCl-C_2H_3Cl]^{+\cdot}$	278	-	244	10.2
$[M-HCl-Cl-HCl]^+$	269	44.2	235	100
$[M-CH_2Cl-2HCl]^+$	255	10.5	221	35.3
$[M-HCl-C_2H_2Cl_2]^{+\cdot}$	244	55.1	210	20.5
$[M-HCl-C_2H_3Cl-Cl]$	243	-	209	20.0
$[M-HCl-Cl-C_3H_4Cl_2]^+$	195	42.2	161	52.0

correspond to the ions $[M-HCl]^{+\cdot}$, and $[M-Cl]^+$ respectively. The neutral fragment $C_2H_2Cl_2$ is eliminated from the former ion and gives rise to the even-mass odd-electron ion $[M-HCl-C_2H_2Cl_2]^{+\cdot}$ (m/z 244) . This was verified by linked scanning (Figure 20). In the mass spectrum of Hp-Sed, loss of only $C_2H_2Cl_2$ suggests a total of four ring chlorines, two on each side of the six-membered ring. The competitive loss of HCl and $Cl^{\cdot}$ from the molecular ion is observed in the mass spectrum of Hp-Sed. This result suggests that Hp-Sed, like T2 and T12, has a 2-exo,3-endo,5-

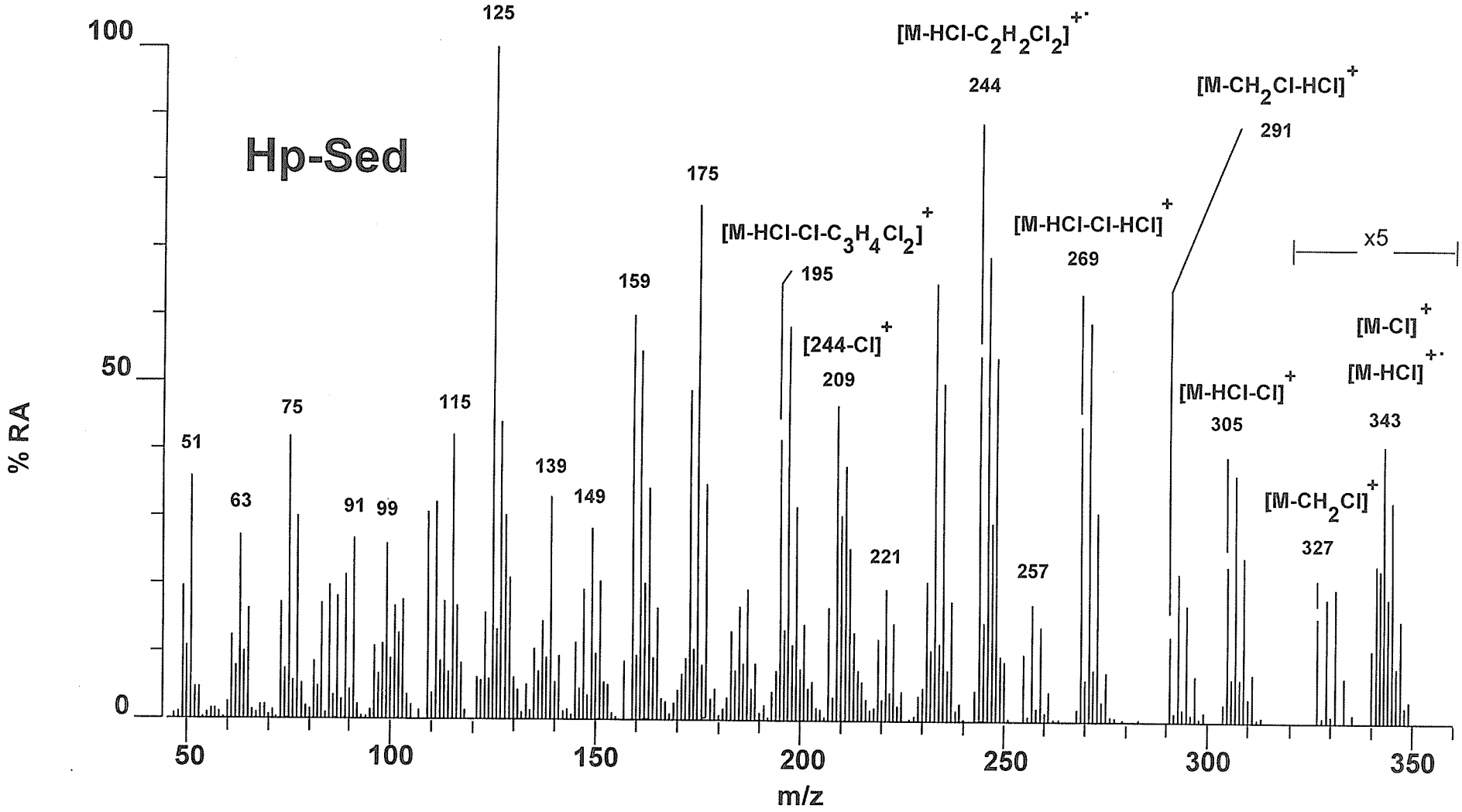


Figure 19: Positive EI mass spectrum of Hp-Sed

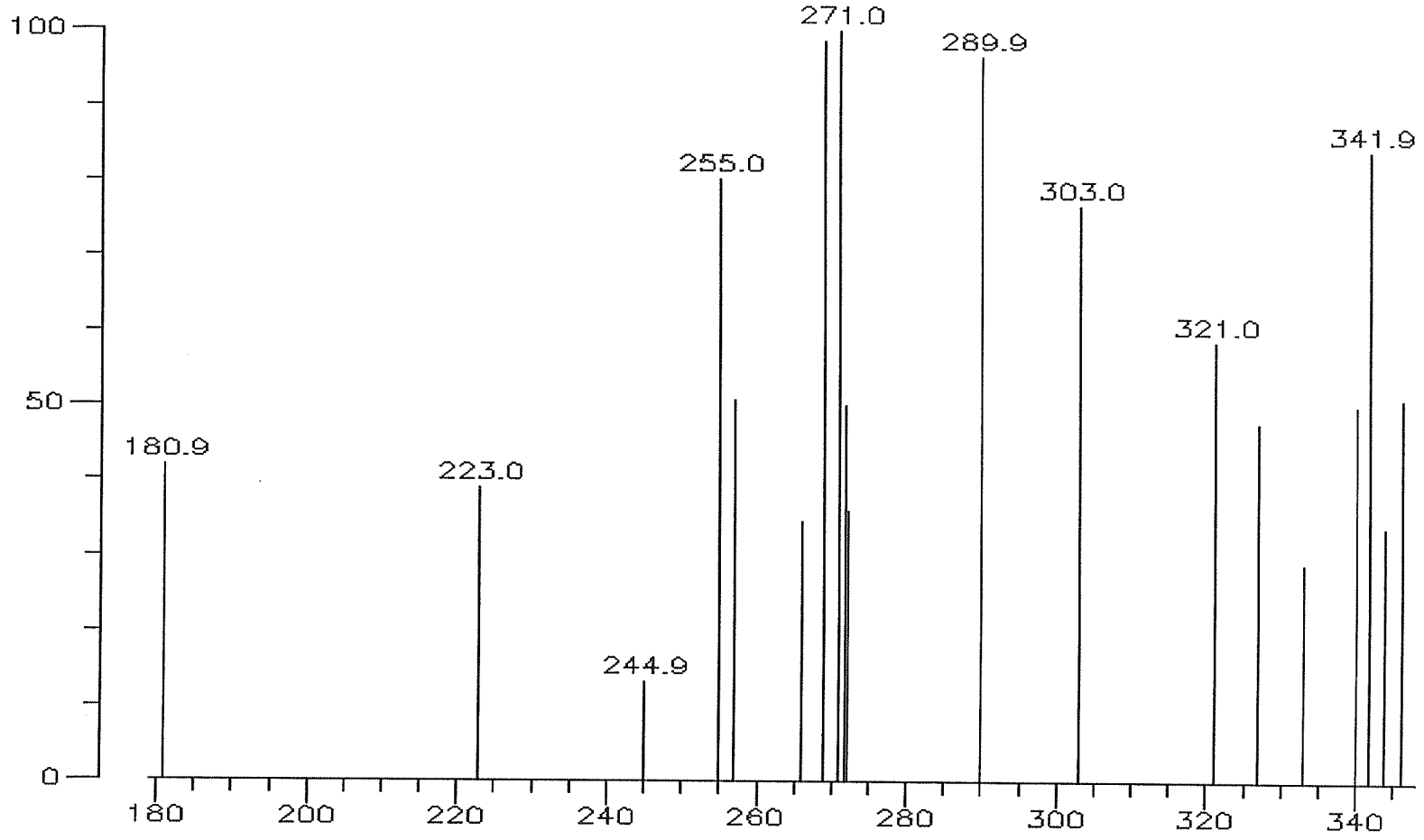


Figure 20: Hp-Sed CNL (parent) scan for loss of 96 u. The cluster of ions at m/z 340 indicates loss of $C_2H_2Cl_2$ from $[M\text{-}HCl]^{+\cdot}$.

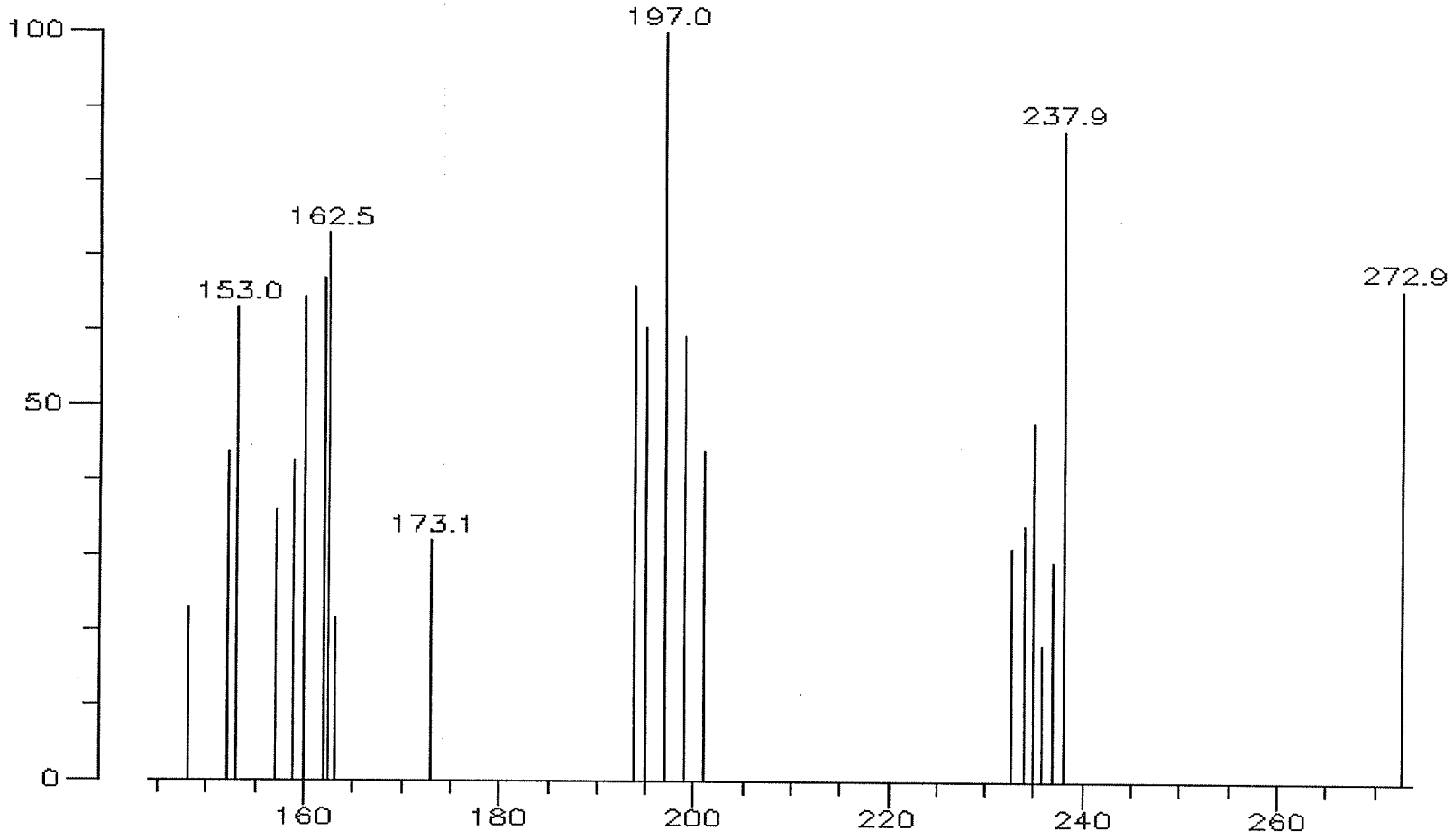


Figure 21: Hp-Sed CNL (daughter) scan for neutral loss of 110 u. The cluster at m/z 195 indicates the loss of $C_3H_4Cl_2$ from $[M\text{-}HCl\text{-}Cl]^+$.

exo,6-endo ring conformation.

In the CNL spectrum of Hp-Sed, a 110 Da neutral species is eliminated from $[M\text{-}HCl\text{-}Cl]^+$ (m/z 305) (Figure 21) to yield the m/z 195 ion. The observation of this decomposition, verified by linked field scanning, provides us with important structural information because the neutral species lost is comprised of the bridging carbon (C7) and its two substituents, i.e. it is $CH_2ClC{:}(CH_2Cl)$. Observed decompositions involving the loss of $^{\cdot}CH_2Cl$ in the mass spectrum, and the absence of corresponding decompositions involving the loss of $^{\cdot}CHCl_2$, further support the positioning of two monochloromethyl moieties on C7. The postulated structure for Hp-Sed is thus, 2-exo,3-endo,5-exo,6-endo,8,9,10-heptachlorobornane.

The EI positive ion mass spectrum of Hx-Sed is shown in Figure 22. The groups of peaks starting at m/z 306 and 307 correspond to the ions $[M\text{-}HCl]^{+\cdot}$ and $[M\text{-}Cl]^+$, respectively. The neutral fragment $C_2H_2Cl_2$ is eliminated from the former ion, and gives rise to the even-mass odd-electron ion $[M\text{-}HCl\text{-}C_2H_2Cl_2]^{+\cdot}$ (m/z 210, Figure 23). The losses of C_2H_3Cl from $[M\text{-}Cl]^+$ and $[M\text{-}HCl]^{+\cdot}$ are also observed and give rise to the $[M\text{-}Cl\text{-}C_2H_3Cl]^+$ ion (m/z 245) and to the even-mass odd-electron ion $[M\text{-}HCl\text{-}C_2H_3Cl]^{+\cdot}$ (m/z 244); both are verified by linked scanning (Figure 24). The loss of C_2H_3Cl verifies the existence of a carbon atom with no

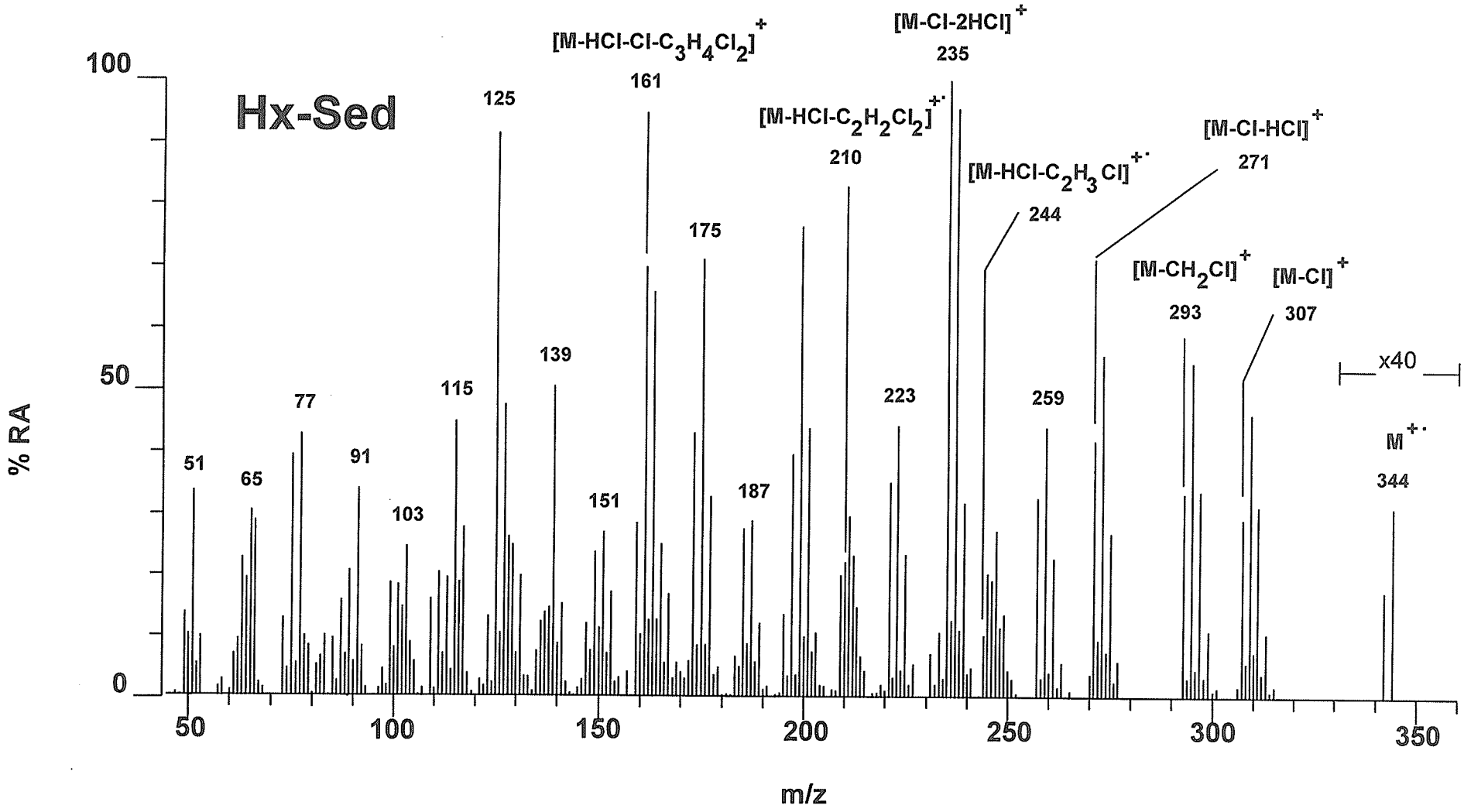


Figure 22: Positive EI spectrum of Hx-Sed

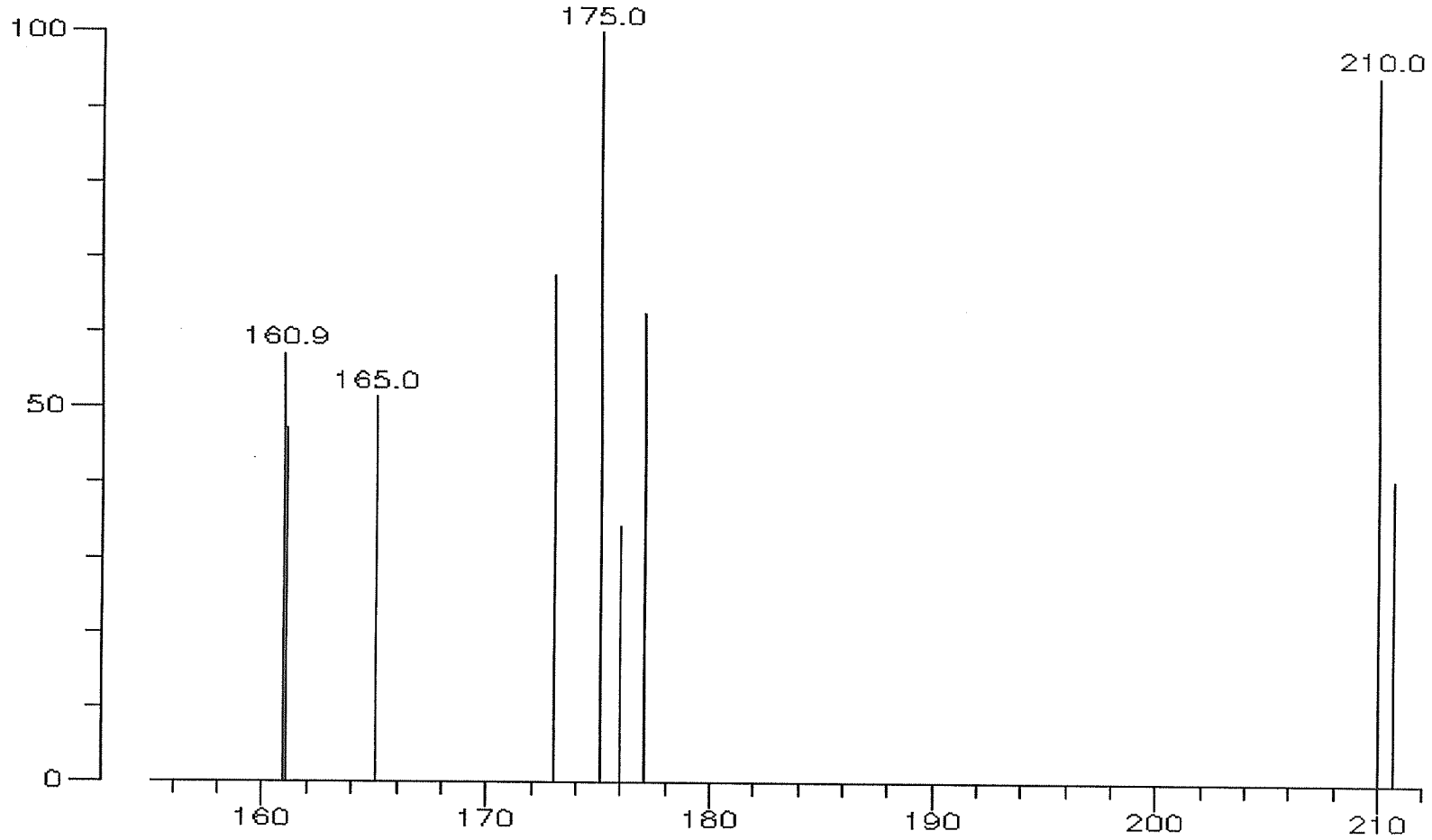


Figure 23: Hx-Sed CNL (daughter) scan for neutral loss 96 u. The cluster at m/z 210 indicates the loss of $C_2H_2Cl_2$ from $[M\text{-}HCl]^{+\cdot}$.

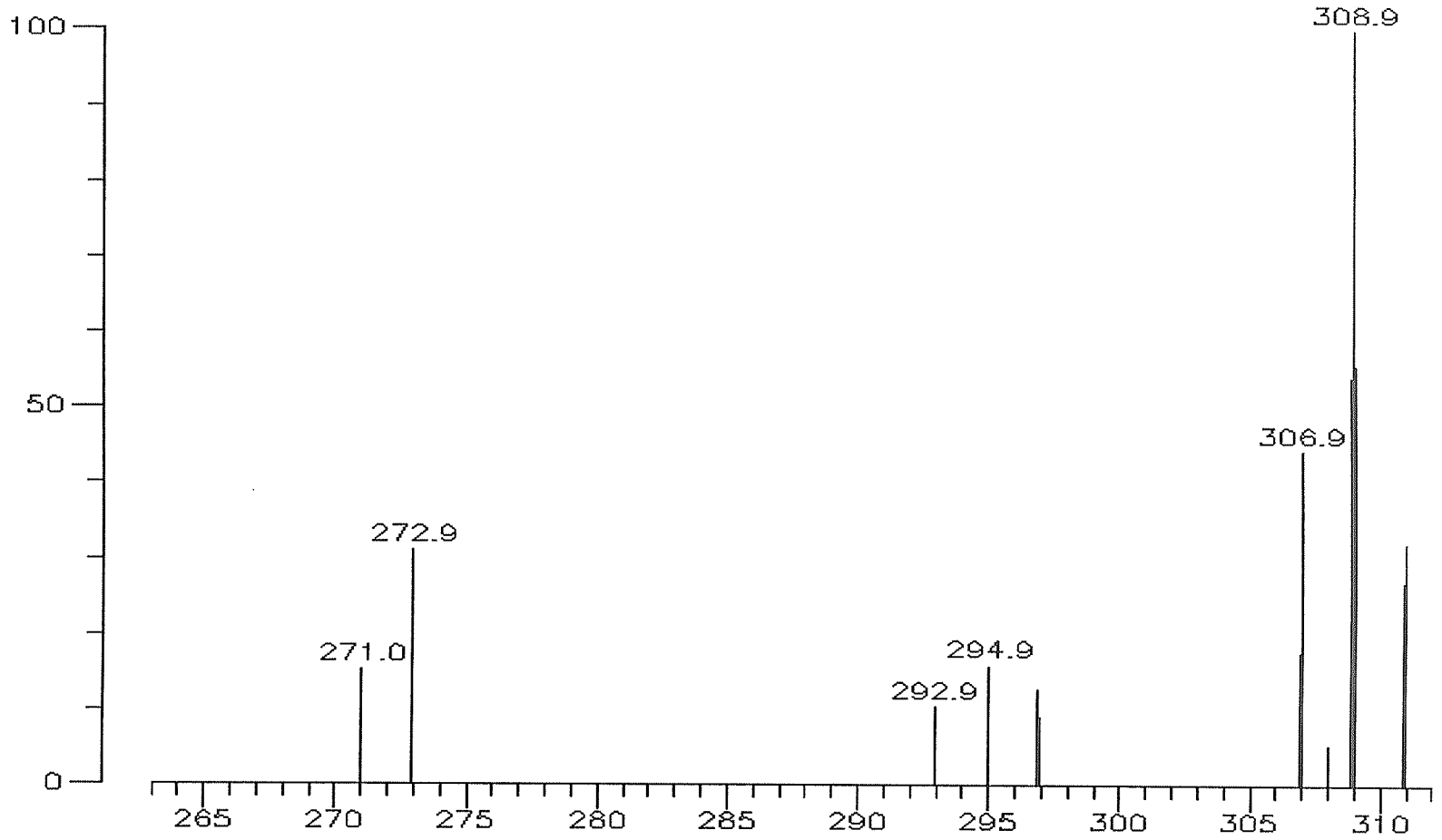


Figure 24: Hx-Sed CNL (parent) scan for loss of 62 u. The spectrum is dominated by by loss of C_2H_3Cl from $[M\text{-}Cl]^+$ shown by the cluster beginning at m/z 307. The small peak at m/z 308 is the M+2 peak in the $[M\text{-}HCl]^{+\cdot}$. This indicates that Hx-Sed loses C_2H_3Cl from $[M\text{-}HCl]^{+\cdot}$.

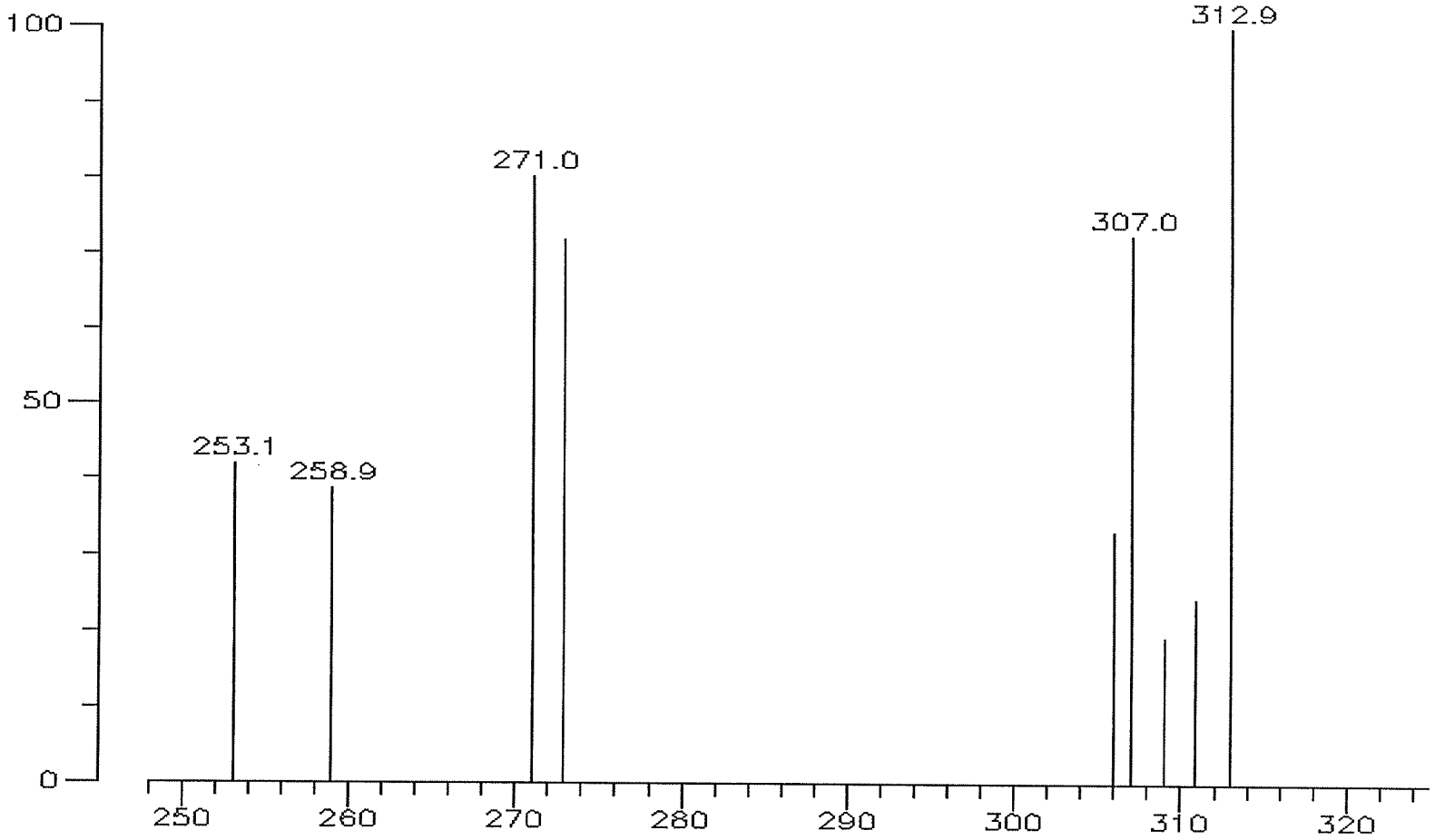


Figure 25: Hx-Sed CNL (parent) scan for loss of 110 u. the cluster at m/z 271 indicates the loss of $C_3H_4Cl_2$ from $[M\text{-}HCl\text{-}Cl]^+$.

chlorine atoms attached and is consistent with the observation of greater abundance of ·Cl loss from the molecular ion with respect to loss of HCl. Losses of both C_2H_3Cl and $C_2H_2Cl_2$ in the mass spectrum of Hx-Sed indicate that there are three chlorines on the six-membered ring, two on one side and one on the other. Positioning of the ring chlorines on Hx-Sed however, could not be confirmed by mass spectrometry.

A CNL spectrum of Hx-Sed also shows elimination of a 110 Da neutral species from $[M\text{-}HCl\text{-}Cl]^+$ (m/z 271, Figure 25) to yield the m/z 161 ion in the mass spectrum of Hx-Sed. The observation of these decompositions, verified by linked field scanning, provides us with important structural information because the neutral species lost is comprised of the bridging carbon (C7) and its two substituents, i.e., it is $CH_2ClC{:}(CH_2Cl)$. Observed decompositions involving the loss of $\cdot CH_2Cl$ in the mass spectra of both compounds and the absence of corresponding decompositions involving the loss of $\cdot CHCl_2$ further support the positioning of two monochloromethyl moieties on C7.

NMR Spectroscopy and Molecular Modelling of Hx and Hp-Sed

^{1}H NMR of sediment congeners

Sample amounts extracted from Chatwin lake sediment were not adequate for

NMR analysis. The two congeners, Hx-Sed and Hp-Sed, were confirmed to be the same compounds as two congeners found in the technical toxaphene standard as verified by EIMS and HRGC retention time. These two congeners were isolated and purified from the technical mixture using florisil chromatography and reverse phase C18 chromatography, in order to obtain a suitable sample concentration for ^{1}H NMR analysis (Appendix 1).

Hp-Sed ^{1}H NMR

The 500-MHz ^{1}H NMR spectrum of Hp-Sed was essentially first order (Figure 26). The chemical shifts and coupling constants are presented in Tables 6 and 7, respectively. Integration confirmed the presence of 11 protons in Hp-Sed and therefore 7 chlorines. The absence of geminal coupling in the spectrum of Hp-Sed normally 16-17 Hz for protons of a ring methylene group in a norbornane ring system[28] indicates that one proton (and therefore one chlorine) is attached to each of C-2, C-3, C-5 and C-6. In a norbornane ring system a proton is always bound to C-4. Because C-4 does not have a chlorine substituent, the chemical shift of H_4 in a ring system such as this, will normally be up field relative to those of the other ring protons, provided there is a chlorine atom attached to each of the other ring carbon atoms. The doublet occurring at 2.26 ppm is assigned to H_4. The triplet observed at 3.79 ppm is assigned to H_3. Spin decoupling (difference double

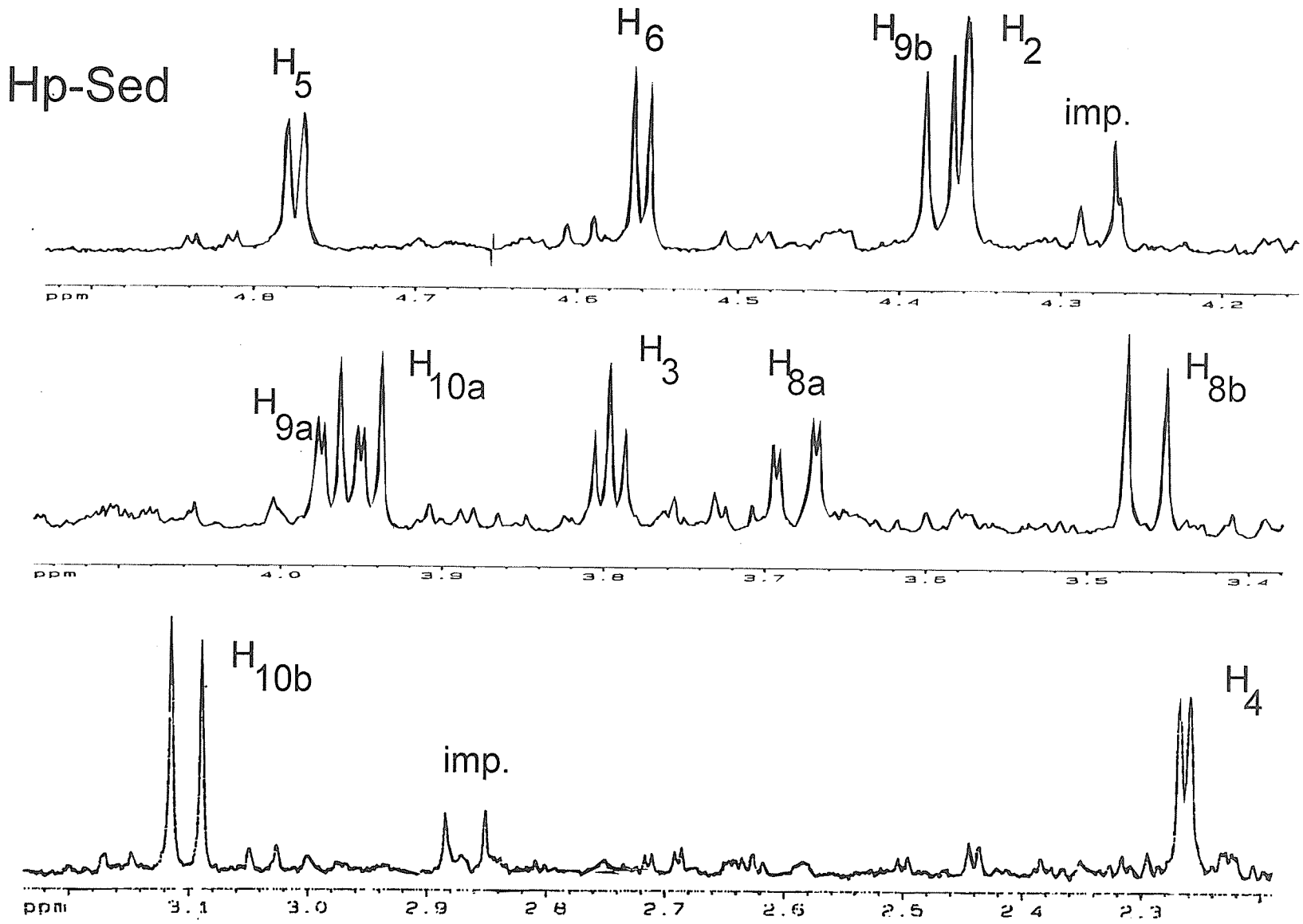


Figure 26: ^{1}H NMR spectrum of Hp-Sed

Table 6. ^{1}H NMR Chemical Shift Data (ppm) for Hx- and Hp-Sed[a].

	H_2	H_3	H_4	H_{5a}	H_{5b}	H_{6a}	H_{6b}	H_{8a}	H_{8b}	H_{9a}	H_{9b}	H_{10a}	H_{10b}
Hx-Sed	3.58	4.02	1.86	1.43	2.27	-	3.58	3.69[b]	3.63[b]	3.80[b]	3.76[b]	3.84[c]	3.61[c]
	(d)	(dt)	(t)	(ddt)	(dd)	-	(dd)	(Ad)	(B)	(Ad)	(B)	(d)	(d)
Hp-Sed	4.36	3.8	2.26	-	4.56	4.77	-	3.68	3.46	3.96	4.37	3.95	3.1
	(d)	(t)	(d)	-	(d)	(d)	-	(dd)	(d)	(dd)	(d)	(d)	(d)

[a]In C_6D_6 with C_6D_5H as the reference; doublet centers are reported; s=singlet, d=doublet, dd=doublet of doublets, t=triplet, dt=doublet of triplets, ddt=doublet of doublets of triplets.
[b]The assignments may be interchanged, 8 ↔ 9
[c]The assignments may be interchanged, a ↔ b

Table 7. ^{1}H NMR Coupling Constant Data (Hz) for Hx- and Hp-Sed[a].

	J_{23}	J_{34}	J_{35a}	J_{45a}	J_{5ab}	J_{5a6b}	J_{5b6a}	J_{5b6b}	J_{8ab}	J_{8a9a}	J_{9ab}	J_{10ab}
Hx-Sed	4.4	4.4	2.2	4.7	15.6	4.8	-	8.8	11.9	1.2	12.4	11.6
Hp-Sed	4.8	4.3	-	-	-	-	4.8	-	12.2	2.0	12.3	12.7

[a]In C_6D_6

resonance experiments[29]) of this proton reveals that it is coupled to H_4, and to the proton giving rise to the doublet at 4.36 ppm (H_2). This is confirmed by observance of H_2 and H_4 resonances collapsing to singlets from doublets (Figure 27). For the H_3 and H_4 protons to be coupled (J_{34} = 4.3 Hz) H_3 must be in the exo position. If H_3 were in an endo configuration, vicinal coupling with H_4 would be very small (<0.5 Hz) due to a dihedral angle near 90° [30]. The magnitude of these vicinal couplings (J_{32} = 4.3 Hz) requires that H_2 be in an endo configuration. The doublet signal observed for H_4 indicates coupling to only one proton (H_3). Therefore the proton on H_5 must be in the endo position since its coupling to H_4 is less than 1 Hz. The absence of long range coupling between H_3 and H_5 (planar W pathway) similarly indicates that H_5 must be in an endo configuration. A W pathway coupling constant of 1.8 Hz has been reported for the toxaphene congeners 2,2,5-endo,6-exo,8,9,10-heptachlorobornane[12,31], 2,2,5-endo,6-exo,8,8,9,10-octachlorobornane[13,31] and 2,2,5-endo,6-exo,8,9,9,10-octachlorobornane[13,31] where the corresponding H_3 and H_5 protons are both in the exo configuration. The two remaining ring proton resonances (H_5, 4.77 ppm and H_6, 4.56 ppm) are coupled to one another by a 4.8 Hz coupling (both are 4.8 Hz. doublets). The magnitude of this coupling (J_{56} = 4.8 Hz) requires that H_6 be in the exo configuration since H_5 has already been shown to be endo. Assignment in

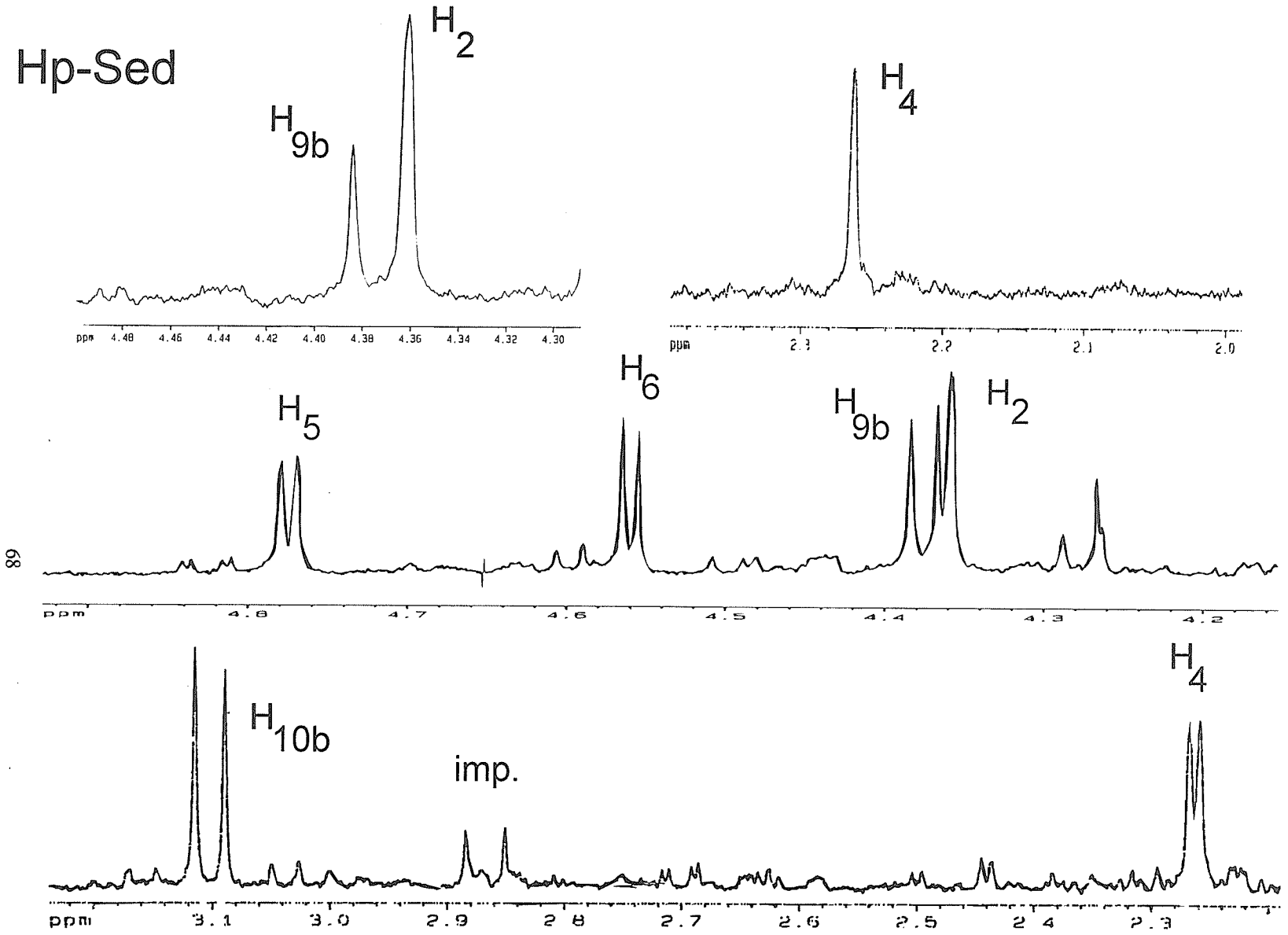


Figure 27: Spectrum obtained by decoupling proton 3 (top) compared to normal spectrum (middle and bottom)

this case is difficult because these two protons are coupled to each other and not simply coupled to any other proton. A way to assign these protons follows from the assumption that the exo proton resonance is upfield of the endo proton, as is normally observed[30]. Therefore H_6 and H_5 are assigned the resonances 4.56 and 4.77 ppm, respectively.

Yet to be accounted for are the six protons and three chlorines located on C-8, C-9 and C-10. Based on the magnitude of their coupling constants, the doublets observed at 4.37 (J = 12.3 Hz), 3.95 (J = 12.7 Hz), 3.46 (J = 12.2 Hz) and 3.10 ppm (J = 12.7 Hz) and the doublet of doublets at 3.68 ppm (J = 12.2, 2.0 Hz) and 3.96 ppm (J =12.3, 2.0 Hz) must arise from the geminal protons of three monochloromethyl groups. The long-range 2.0 Hz coupling between the protons giving rise to the resonances at 3.68 and 3.96 ppm (Figure 26) is ascribed to a W pathway (protons in the same plane) between them, indicating that these two protons are on different carbons (C8 and C9) attached to C7. Difference NOE (nuclear Overhauser effect) experiments[29] were used to determine which one of these two protons is located on C-8 or C-9. Irradiation of the H_3 resonance (3.80 ppm) results in the enhancement of the signal at 3.68 ppm (H_{8a}). Since this proton and the proton at 3.96 ppm are coupled by a 2 Hz coupling we know then that the proton giving rise to the resonance at 3.96 ppm (H_{9a}) is located C-9 and in the

same plane as H_{8a}. Spin decoupling by irradiation at 3.68 (H_{8a}) (Figure 28) results in the collapse of the doublet at 3.46 to a singlet (H_{8b}) and the 2 Hz coupling in proton H_{9a} (3.96 ppm) to disappear. Spin decoupling of the signal at 3.10 ppm (Figure 28) results in the collapse of the signal at 3.95 ppm. This process did not result in the collapse of any signals associated with 2 Hz (W) couplings and therefore cannot be on C8 or C9. The resonances at 3.10 and 3.95 ppm are therefore attributed to protons on C10. A difference NOE experiment involving the saturation of the H_2 resonance, resulted in the enhancement of the signal occurring at 3.95 ppm. This result dictates that the doublet at 3.95 is nearest to H_2 in space and is therefore attributed to H_{10a} and then the resonance at 3.10 ppm to H_{10b}. The final signal yet to be accounted for then (4.37 ppm) is a result of H_{9b}. Hp-Sed has therefore been identified as 2-exo,3-endo,5-exo,6-endo,8,9,10-heptachlorobornane (Figure 35).

Hp-Sed Spatial Conformation

As with conformational analysis of TS2, TS3, and TS4, we first simplify the discussion of the conformations of the C-8 and C-9 chloromethyl groups by defining a vertical plane passing through C-7 and the mid-points of the C-2, C-3 and C-5, C-6 bonds. Because of the large sizes of the chlorine atoms, Cl-8 and Cl-9 will, for steric reasons, avoid Cl-2 and Cl-5, respectively. Therefore, in the most

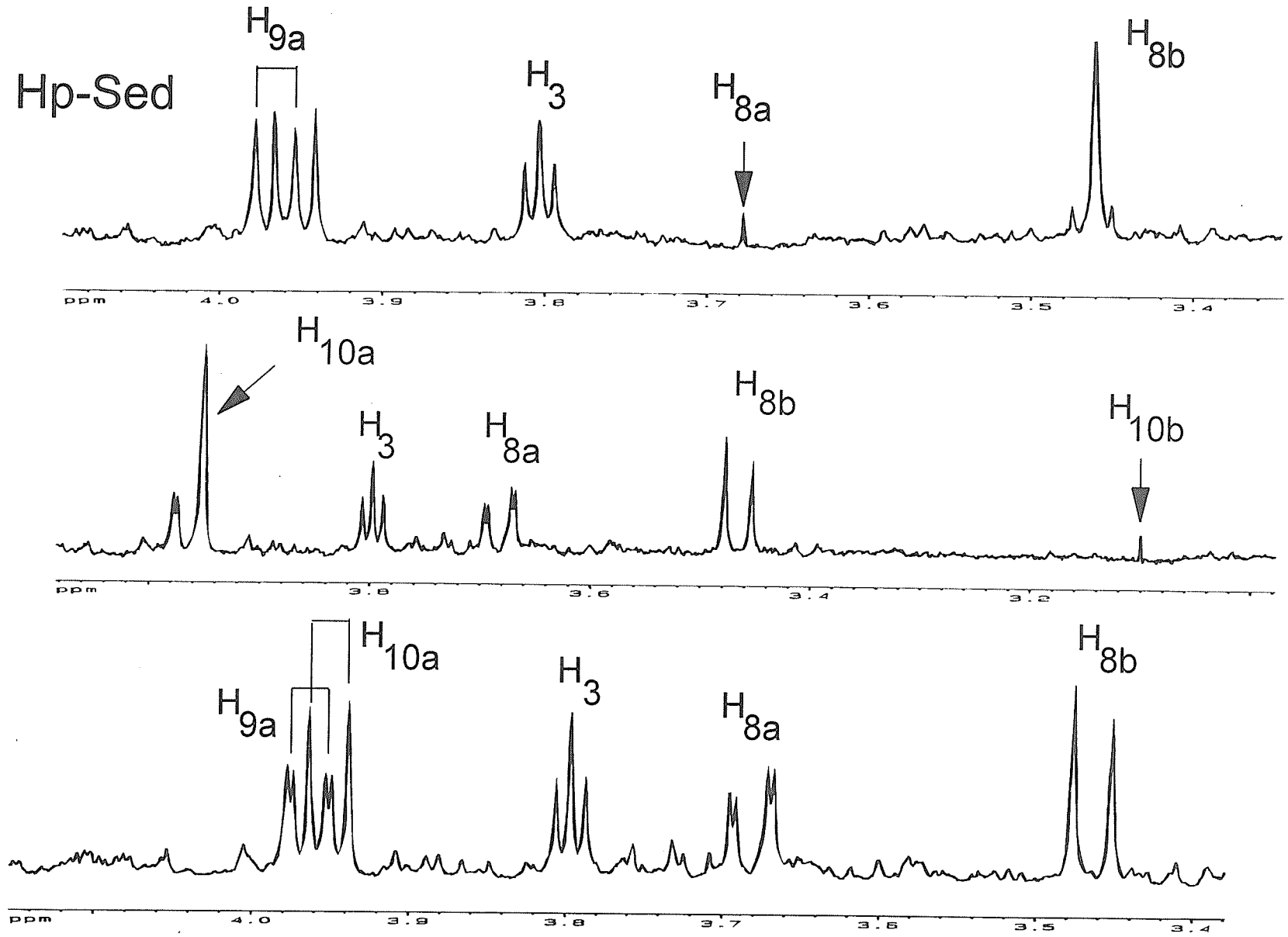


Figure 28: Spectra obtained by decoupling proton 8a (top) decoupling proton 10b (middle) and normal spectrum (bottom)

probable conformations, protons H-8a and H-9a will be located near the defined vertical plane, as shown in Fig. 37, while H-8b and Cl-9 will be located on the same side of the plane as C-2 and C-6, and Cl-8 and H-9b will be located on the other side of this plane. This reasoning is supported by the NOE experiments where the intensity of the H-8a signal only is significantly enhanced by irradiation of H-3 (proton H-8b is much further away from H-3). In the most probable conformations of the C-8 and C-9 groups, a W pathway, or near W pathway, for long range coupling exists between H-8a and H-9a, but not between H-8b and H-9b (the dihedral angle between the C-H bonds of the latter pair is ~120°), as observed experimentally. If, as expected, these conformations dominate, then the general form of the NMR signals will be independent of the rate, or form, of motion of the chloromethyl groups although, in fact, both molecular models, and molecular mechanics calculations (Molecular Modeling Pro), indicate that complete rotations of the chloromethyl groups are prevented by the development of very large steric interferences between Cl-8 and Cl-2, and between Cl-9 and Cl-5. An oscillating internal "rotation" (rather like the swinging motion of an oscillating water sprinkler) of each of these groups is the only motion possible, with time-averaged conformations approximating those depicted in Fig. 36.

Support for this interpretation of the NMR spectra is provided by molecular

mechanics calculations. The structures were derived by alternately performing conformational analysis (rigid rotor model) on the rotating chloromethyl groups and then minimizing the strain energy in the structures. If we first consider rotation of the C-8 chloromethyl group, with the Cl atoms of the C-9 and C-10 groups moved to positions in which they do not interfere, the strain energy developed exceeds 100 kcal mol^{-1}, with respect to the minimum strain, as Cl-8 passes Cl-2. A similar, large strain energy develops as Cl-9 passes Cl-5 for a rotating C-9 chloromethyl group. At the extremes of their swinging excursions, the chlorine atoms of the C-8 and C-9 groups do not significantly interfere with the motion of the other group so that positioning these atoms on the other sides of the vertical plane from those depicted in Fig. 36 is, as in TS2, TS3 and TS4, in principle, possible. However, very large steric interferences between these chlorine atoms develop when they approach the vertical plane and these would prevent them from passing each other in any reasonable oscillations of the chloromethyl groups. Thus, the magnitude of the oscillations will be quite restricted. In the minimum energy conformation the dihedral angle between the outside bonds and opposite inside bonds of the "W" are ~3.5° and ~2°, and between the outside bonds of the "W" is ~2.5°, i.e. the "W" is almost planar, as deduced above from the NMR results. Similar conclusions about the predicted

NMR spectrum can be drawn for the alternative conformations in which the C-8 and C-9 groups are rotated by 120° to place Cl-8 and Cl-9 on the respective other sides of the vertical plane. The NMR spectrum shows that only one of the possibilities occurs, but cannot distinguish between them (a single resonance pattern, and retention of long-range W coupling between one pair of protons, are observed). The force field calculations suggest that the structure shown in Fig. 36 is slightly more stable (~1 kcal mol^{-1}) than the alternative.

For a rotating C-10 chloromethyl group the models and calculations show that the only significant steric interferences develop between Cl-10 and Cl-9, in the most probable conformation of the C-9 chloromethyl group. These are very large (>100 kcal mol^{-1}); however, complete rotation of the C-10 chloromethyl group is permitted in less probable, but low energy conformations of the C-8 and C-9 chloromethyl groups, so that the motion of the C-10 group could be either complete rotation or a swinging oscillation. In any event, the most probable conformations of the C-10 group have Cl-10 far away from Cl-9. The difference in strain energy between the conformation shown in Fig. 36 and that in which Cl-10 and H-10b move to the positions occupied by H-10a and Cl-10, respectively, is <0.5 kcal mol^{-1}, with a barrier of ~10 kcal mol^{-1} between them. Thus, on average, H-10b is closer to H-2 than is H-10a; in this way, the NOE enhancement of the

resonance of one proton only on the C-10 group when H-2 is irradiated, can be explained.

Hx-Sed ^{1}H NMR

Hx-Sed gives rise to a more complicated ^{1}H-NMR spectrum than that of Hp-Sed due to the presence of an additional proton, and fewer chlorines that influence the proton chemical shifts (Figure 29). This gave rise to overlapping and second order resonances for some protons. Initially only 11 of the 12 protons were accounted for by integration. Due to the decrease in the number of chlorine atoms in this molecule it was thought that the remaining proton was relatively upfield of the other resonances. The chemical shifts and coupling constants are presented in Tables 6 and 7, respectively. Unfortunately contamination in the sample obscured the region upfield of 1.8 ppm. This contamination did not show up in GC/ECD analysis of the sample and is likely due to non-volatile column bleed from the florisil column. In an effort to find the missing resonance a 2D COSY spectrum was acquired (Figure 30). COSY uses a pulse sequence to reveal the coupling pattern in the molecule. The frequency of the first pulse is plotted along one axis and the spectrum acquired after a suitable delay. In the 2D plot, the normal ^{1}H spectrum lies along the diagonal while the couplings lie as topographical peaks at the crossing point of 2 resonances. This revealed the position of a proton at 1.43

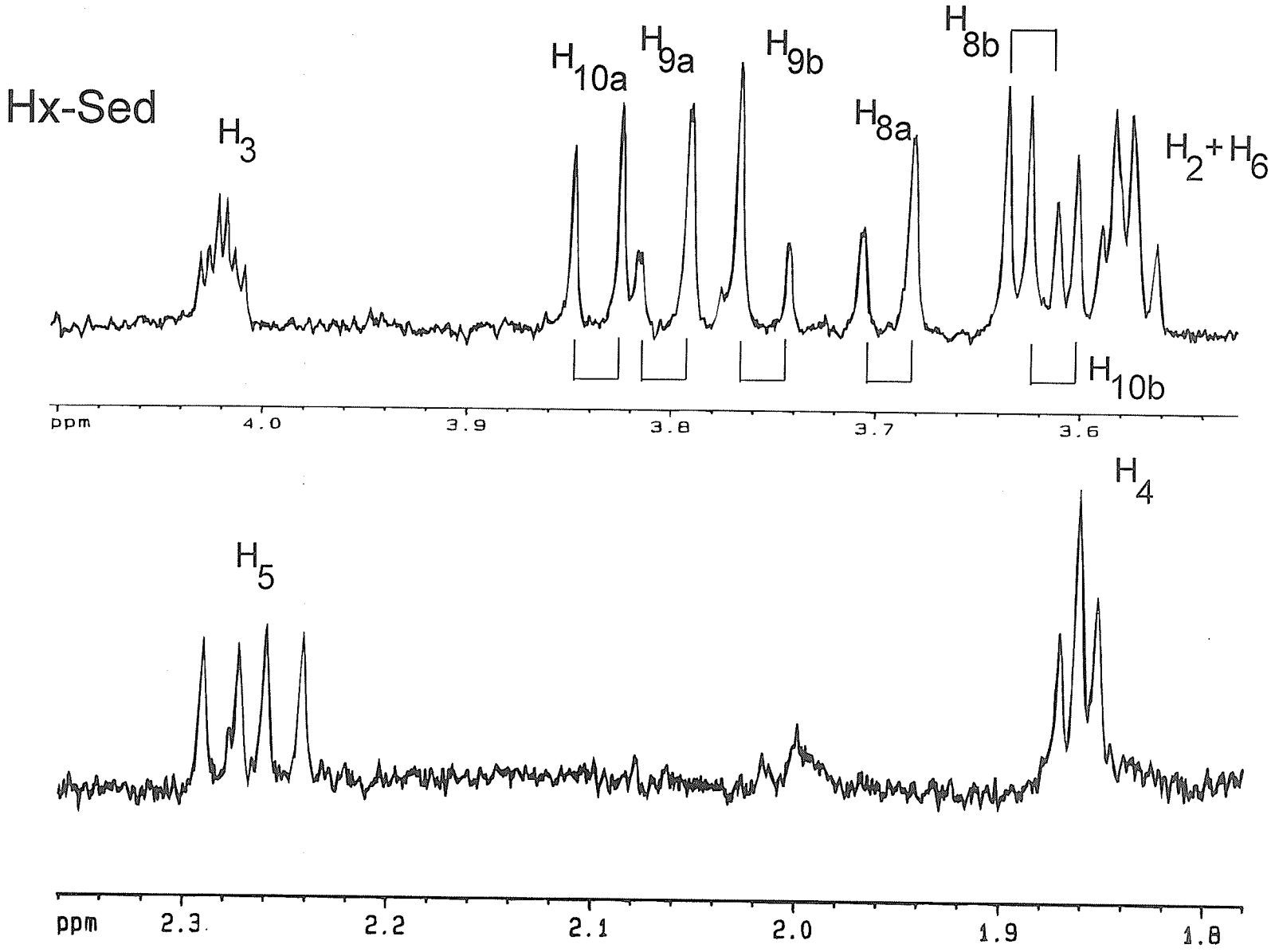


Figure 29: ^{1}H NMR Spectrum of Hx-Sed

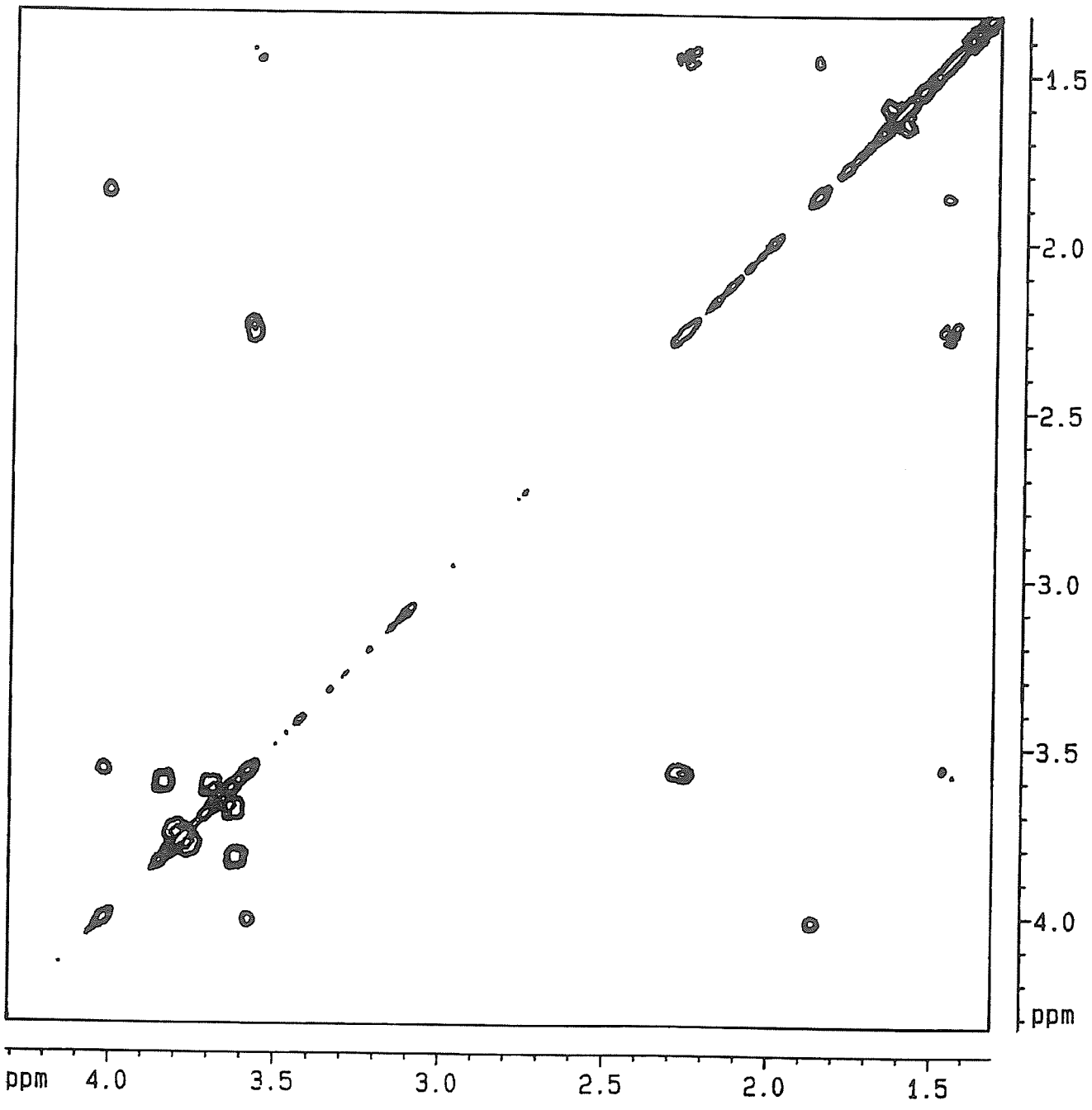


Figure 30 : 2 dimensional ^{1}H COSY NMR spectrum of Hx-Sed.

ppm. According to the COSY spectrum this proton was coupled to three other protons at 1.86, 2.26, and 3.58 ppm respectively. Due to the number of couplings this proton cannot be H_4. H_4 can theoretically be coupled to four other protons (two each on C-3 and C-5 respectively). Since the coupling between H_4 and adjacent endo protons is near zero, two is the maximum number of crosspeaks we could possibly observe. Since the coupling constants with the two possible exo protons are expected to be about the same we would observe a triplet (J=4.7 Hz), or a doublet (J=4.7 Hz) if only one adjacent exo proton were present. The high field triplet observed at 1.86 ppm is consistent with this argument and is therefore assigned to H_4. Spin decoupling of this proton reveals that it is coupled to H_3 (4.0 ppm, J = 4.7 Hz) which must be in an exo configuration (Figure 31). It must also be coupled to another proton by the same coupling (J= 4.7 Hz). This is attributed to H_{5a} (1.43 ppm, J = 4.7 Hz) which is not shown in the normal spectrum but is evident in the COSY spectrum. As noted above, if either H_3 or H_{5a} were in an endo configuration, vicinal coupling with H_4 would be very small (<0.5 Hz) due to a dihedral angle close to 90°. H_3 gives rise to a triplet of doublets attributed to vicinal coupling to both H_4 and to the proton giving rise to the doublet (J = 4.4Hz) at 3.58 ppm (H_2), and a four-bond W coupling to H_{5a} (J = 2.2 Hz). The H_2 doublet collapses upon irradiation of H_3 (Figure 32) and overlaps a signal arising from the

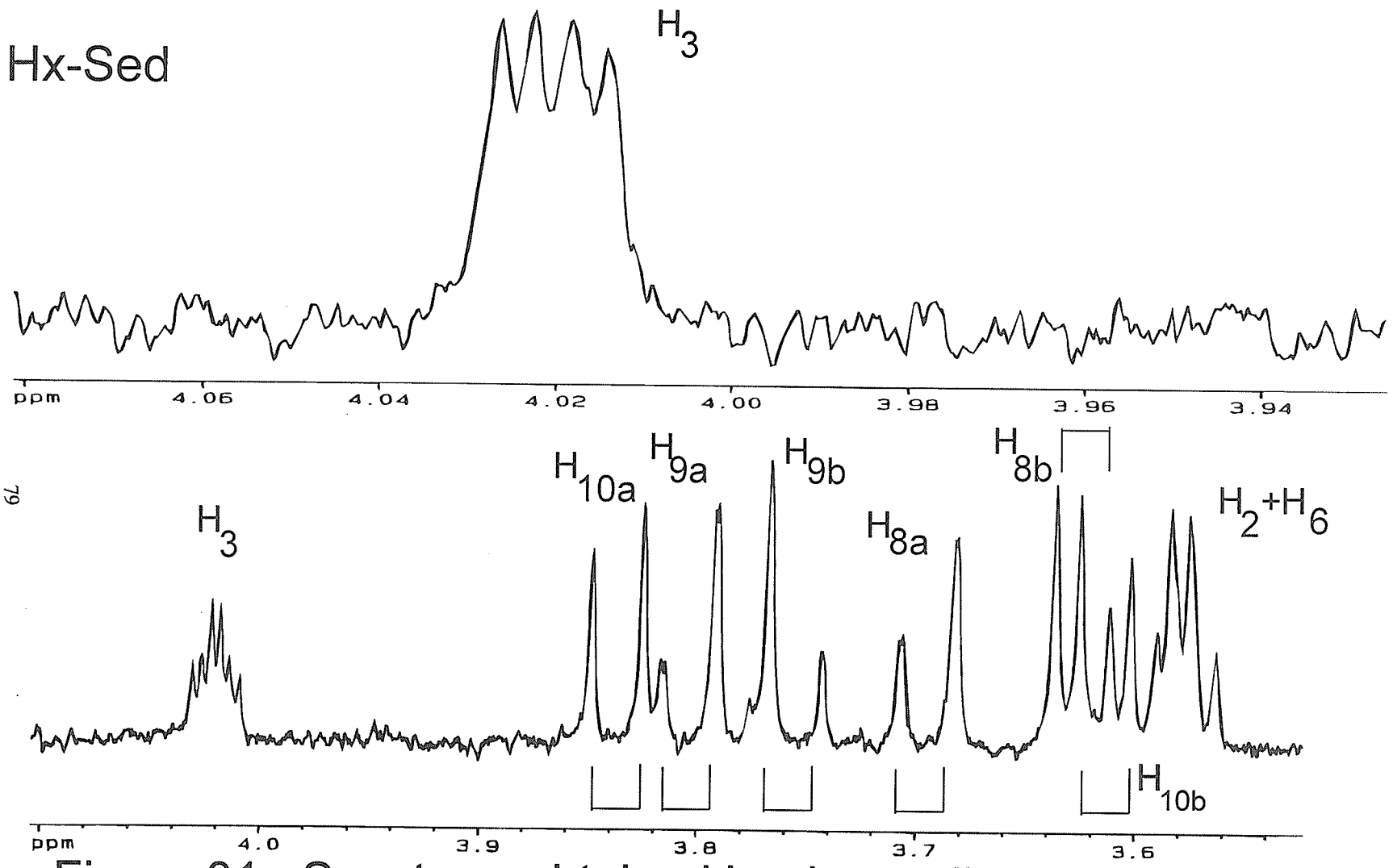


Figure 31: Spectrum obtained by decoupling proton 4 (top) compared to the normal spectrum (bottom)

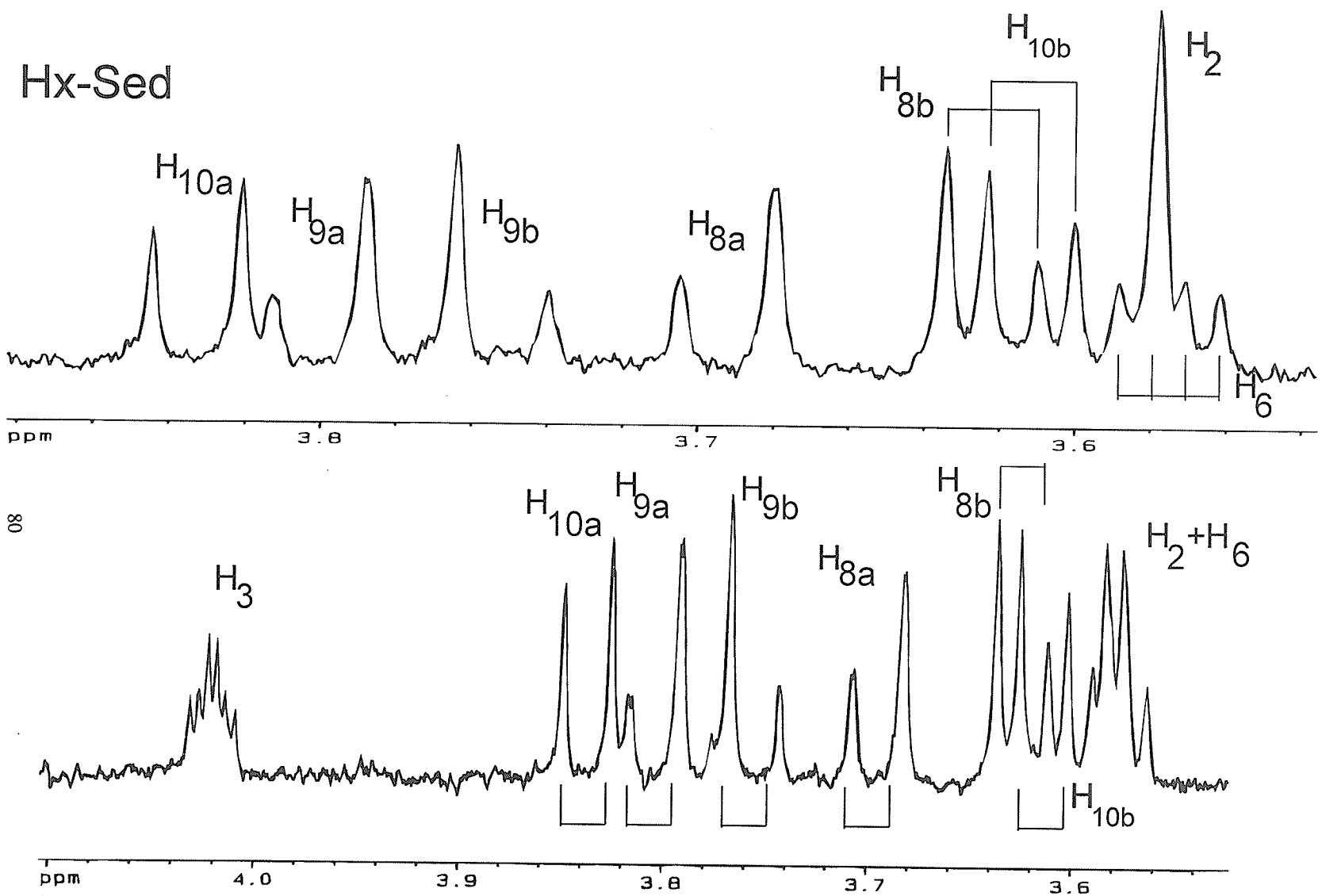


Figure 32: Spectrum obtained by decoupling of proton 3 (top) compared to normal spectrum (bottom)

presence of another proton at 3.58 ppm (doublet of doublets). This underlying signal (J_1=8.8Hz, J_2=4.8Hz) collapses to a 4.8 Hz doublet upon irradiation of the proton at 2.26 ppm (Figure 33). This signal (3.58 ppm, dd) is consistent with a proton that is vicinally coupled to two geminal protons and is also geminally positioned to a chlorine, as can be seen by its relatively low field resonance. The magnitude of the vicinal couplings show that this proton is eclipsed with the proton at 2.26 ppm, according to the Karplus relationship[29], which would predict J~8 Hz for vicinal eclipsed protons (e.g. 5-endo,6-endo). The proton at 2.26 ppm (J_1=8.8 Hz, J_2=15.6 Hz) is geminally coupled to another proton, as seen by its large coupling (15.6 Hz). Due to the relatively high field resonance of this proton we can assume that it is not geminal to a chlorine atom. This proton must be on the same carbon as the proton at 1.43 ppm. Since we know the proton at 1.43 ppm is in the 5-exo position the proton at 2.26 ppm must be in the 5-endo position. We have also shown that the proton at 2.26 ppm is in the same face as the proton at 3.58 ppm. That proton must be in the 6-endo position, leaving the chlorine in the 6-exo position.

As for Hp-Sed, two of each of the remaining six protons are located on C-8, C-9 and C-10. However, increased amounts of second order complexity ($\Delta\delta_{AB}\nu_o/J >$ 1.5) are observed for the geminal protons located on C-8 and C9 in Hx-Sed and, in

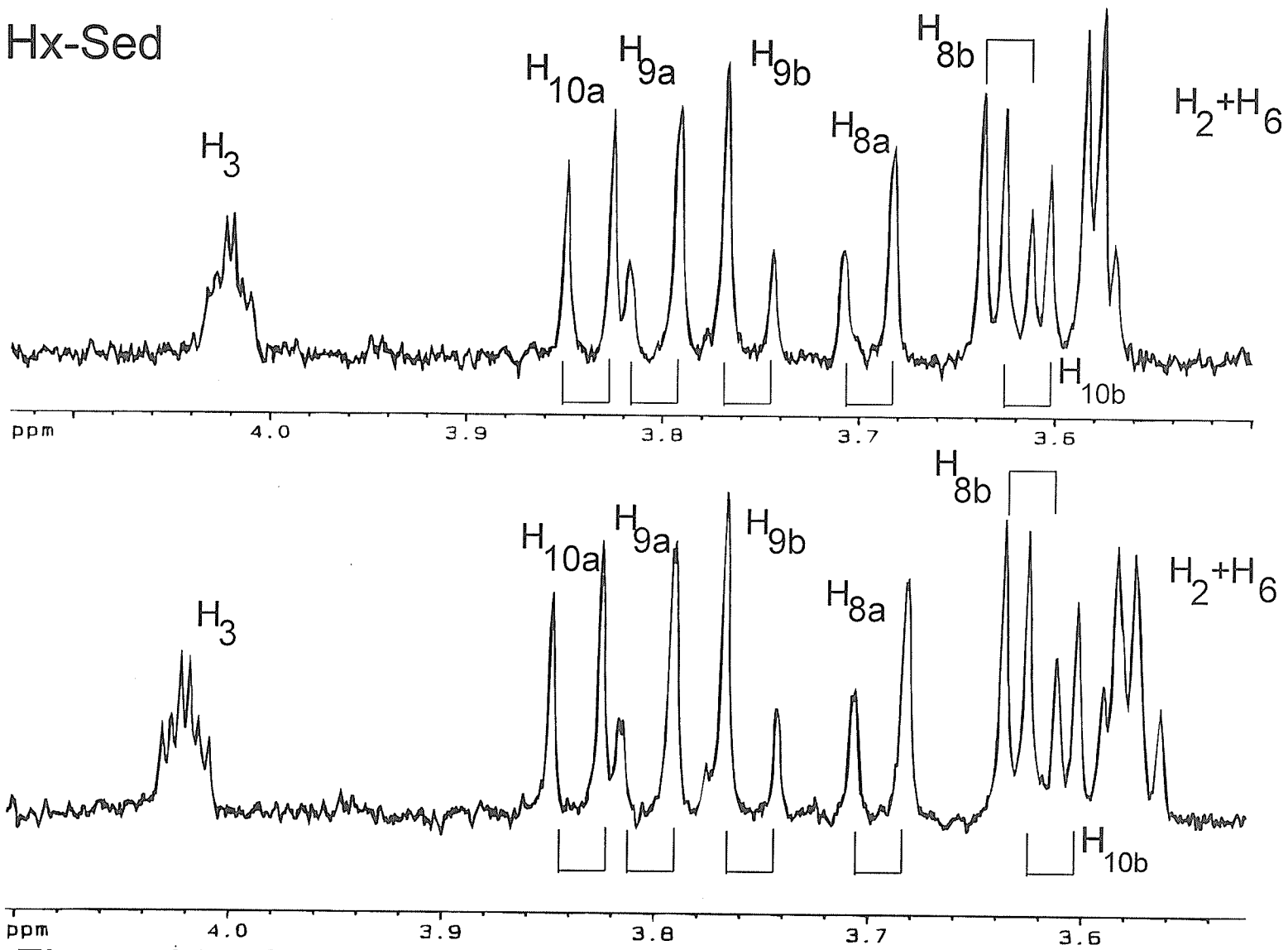


Figure 33: Spectrum obtained by decoupling proton 5 (top) compared to normal spectrum (bottom)

both cases, correspond to a two spin (AB) system. In this type of system, the coupling (J_{AB}) can be obtained directly from the spacing between a central peak and its adjacent satellite peak, but no specific positions correspond to the chemical shifts. Because the A peaks have unequal intensities, δ_A is defined by the weighted average position. The difference, $\Delta\delta_{AB}$ between the A and B chemical shifts were obtained from the following equation:

$$\Delta\delta_{AB}\nu_o = (4C^2\text{-}J^2)^{1/2}$$

where 2C is the spacing between alternate peaks and ν_o is 500 Mhz, the operating frequency of the spectrometer. The actual values of δ_A and δ_B were determined by adding and subtracting $\frac{1}{2}\Delta\delta_{AB}$ to the midpoint of the spectrum[27]. H_{8a} and H_{9a} give rise to the A parts of the AB patterns arising from the geminal protons on C-8 and C-9, and occur at 3.69 and 3.80 ppm, respectively. They were easily recognizable because each of the two peaks is split into a doublet (J = 1.2 Hz) due to the W-coupling which occurs between them (Figure 26). NOE experiments involving irradiation of the H_3 resonance (4.02 ppm) produced no conclusive results even after a 16 hour run time (6528 scans). Therefore the above assignment of the signals at 3.69 and 3.80 ppm to H_{8a} and H_{9a}, respectively, was based on the fact that the corresponding H_{8a} resonance in the ^{1}H-NMR of Hp-Sed occurred at a

higher field than that of H_{9a}; however this is quite speculative. Spin decoupling, by irradiation at 3.69 ppm (H_{8a}) results in the collapse of the doublet at 3.63 ppm (H_{8b}) [B part of the AB systems], to a singlet (figure 34). Spin decoupling, by irradiation at 3.84 ppm (H_{10a}), results in the collapse of the doublet at 3.61 ppm (H_{10b}) (in this case $\Delta\delta_{AB}\nu_o/J \gg 10$ and can therefore be classified as a first order spin system) (Figure 34). The final geminally coupled proton (3.76 ppm) is therefore attributed to H_{9b}. Hx-Sed has therefore been identified as 2-exo,3-endo,6-exo,8,9,10-hexachlorobornane (Figure 35).

Hx-Sed Spatial Conformation

By using molecular models, molecular mechanics calculations, and arguments similar to those for Hp-Sed, the most probable conformations of the C-8 and C-9 chloromethyl groups are as shown in Fig. 36. Because H-8a and H-9a are in, or close to, a vertical plane defined as for Hp-Sed, long range coupling between them is possible through a W pathway. Because the environments of the C-8 and C-9 chloromethyl groups, and the chemical shifts of their protons, are so similar, further ambiguities in their assignments must remain. The C-10,Cl-10 bond is at a dihedral angle of ~180° to the C-1,C-7 bond. This places H-10a and H-10b in similar, but not identical, environments so their chemical shifts are not very different.

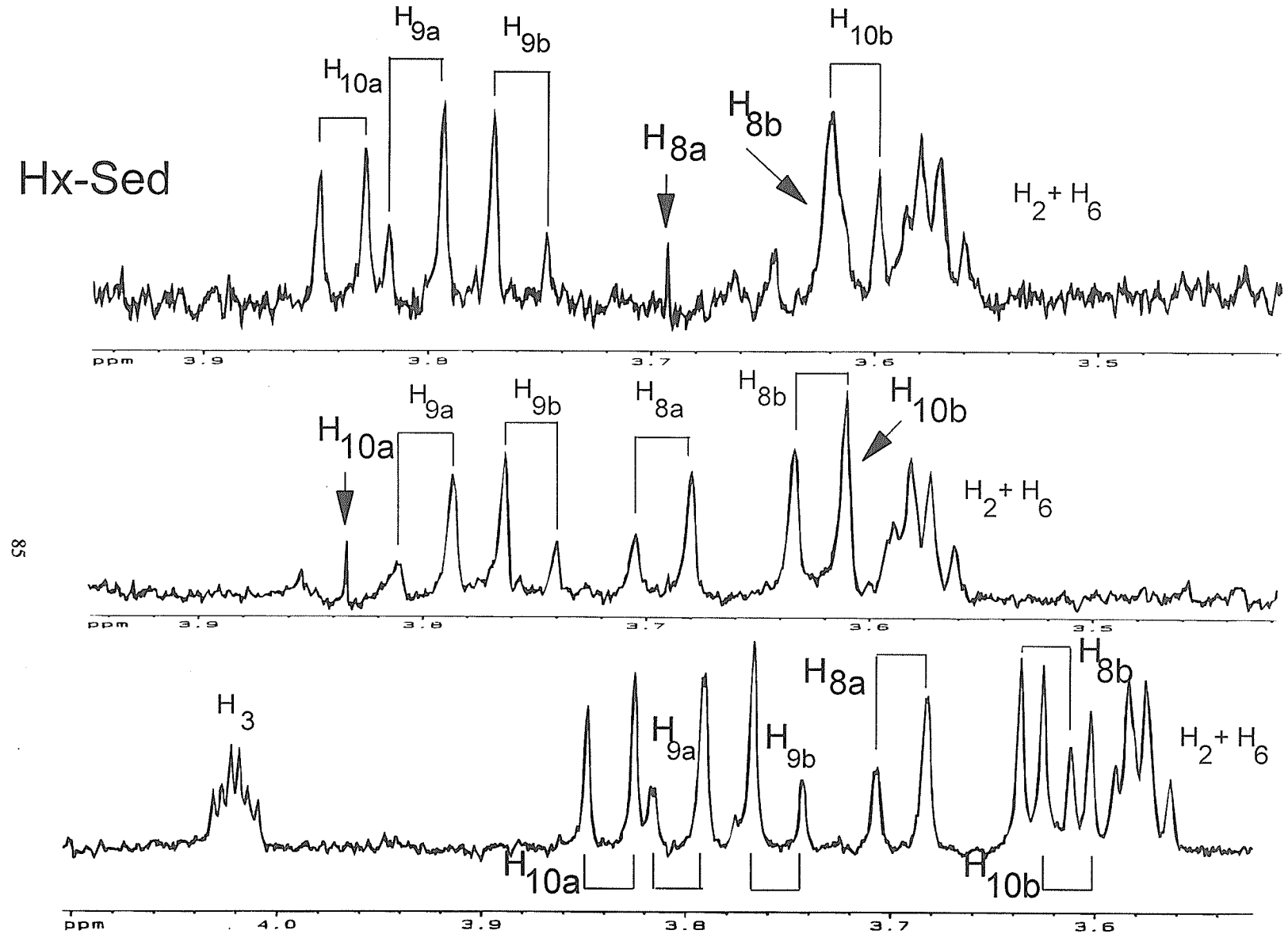
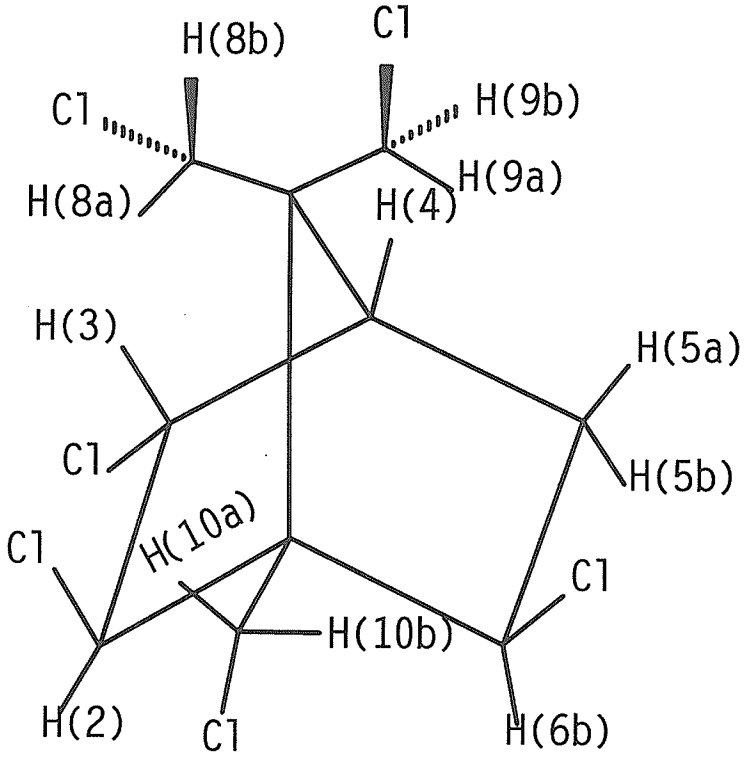
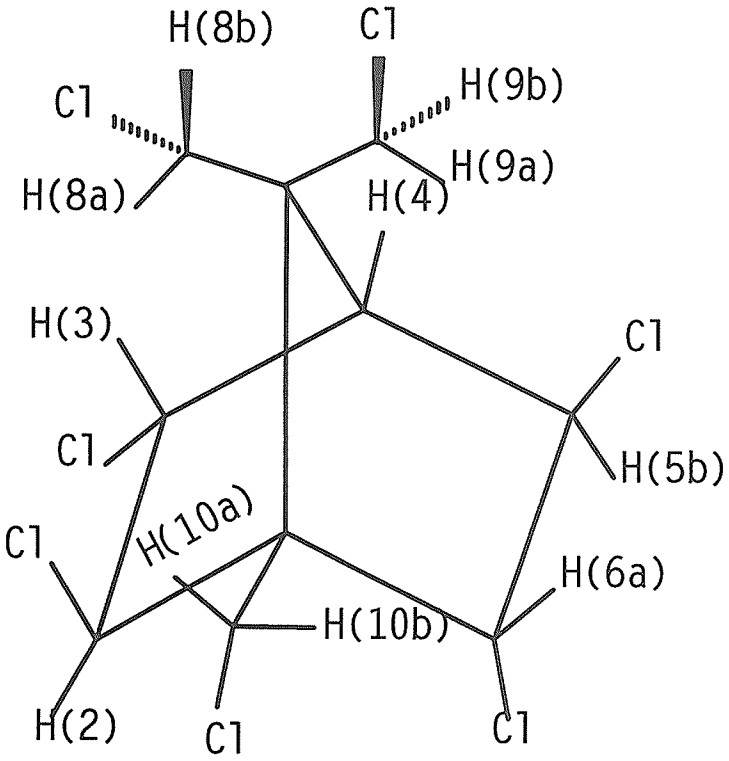


Figure 34: Spectra obtained by decoupling proton 8a (top) and proton 10a (middle) normal spectrum (bottom)



2-*exo*,3-*endo*,6-*exo*,8,9,10-hexachlorobornane (Hx-Sed)



2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,9,10-heptachlorobornane (Hp-Sed)

Figure 35: Structure of Hx-Sed and Hp-Sed

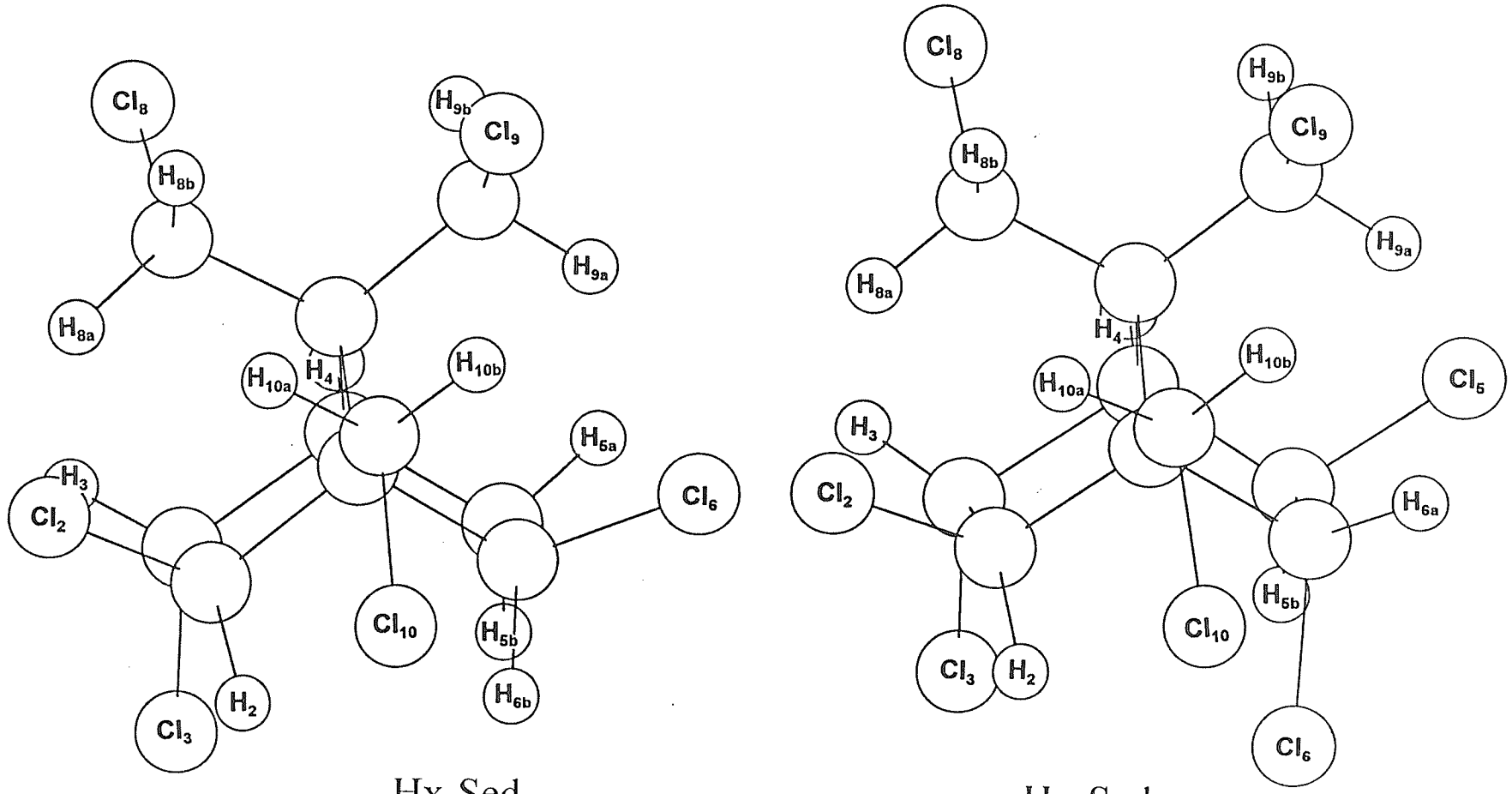


Figure 36: Molecular mechanics models of lowest energy conformations of Hx-Sed and Hp-Sed.

Sediment congener stability and toxicity

Toxicant B (2-exo,3-endo,6,6,8,9,10-heptachlorobornane), one of the most toxic components of the technical mixture, was shown to undergo reductive dechlorination under various chemical, photochemical and metabolic systems[11]. Using various spectroscopic techniques, two of its major metabolites were identified as 2-exo,3-endo,6-exo,8,9,10-hexachlorobornane (hexachlorobornane III/Hx-Sed) and its isomer, 2-exo,3-endo,6-endo,8,9,10-hexachlorobornane (hexachlorobornane II). Relative toxicities of these compounds and technical toxaphene are given in Table 8. Data from 2 separate studies (Turner 1975, and Saleh 1978) are compiled here and relative toxicities given. Since both studies compare each congener's toxicity relative to Toxicant B, it was used as a reference benchmark compound.

In a study by Buser *et. al.* [23], a heptachlorobornane referred to as TC1 was reportedly present in the technical material and all aquatic species studied (Baltic herring, salmon and seal, Arctic seal and Antarctic penguin). The positive EI mass spectrum reported for TC1, extracted from Antarctic penguin tissue, was virtually identical to that reported here for Hp-Sed. It is almost certain, therefore, that TC1and Hp-Sed correspond to the same compound. Using chiral high resolution gas chromatography, it was found that TC1 was present as a racemic mixture (ER

= 1.02 ± 0.02) in the technical product while, in penguin, a preference for the later eluting enantiomer (TC1-2) was observed (ER = 0.74 ± 0.03). That Hp-Sed (TC1) was found to be one of the major toxaphene components in penguin and seal is not surprising because, like T2 and T12, two CHB's known for their persistance in marine mammals[20,21], Hp-Sed has the same staggered 2-exo,3-endo,5-exo,6-endo ring configuration. CHB's possessing this structural feature should be less vulnerable to both metabolism by anaerobic reductive dechlorination and by oxidative processes.

Table 8: Relative toxicity of some toxaphene congeners

Compound	Toxcity relative to technical toxaphene	Reference
Toxaphene	1	
Toxicant A	25	Turner (1975)
Toxicant B	5	Turner (1975) Saleh (1978)
Hx-Sed (hexachlorobornane III)	2.5	Saleh (1978)
hexachlorobornane II	0.03	Saleh (1978)

Bioaccumulation of Hx- and Hp-Sed was observed in two-year-old rainbow trout

muscle from both Peanut and Chatwin Lakes[19]. This result suggested that the chemicals were being transferred either directly or indirectly from the sediment to the fish. The peak intensity of Hx-Sed in the ECNIMS selected ion chromatogram of Chatwin Lake near surface sediment was approximately twice that of Hp-Sed; however, in corresponding chromatograms of the trout muscle, the Hx- and Hp-Sed peaks were of nearly equal size[19]. While the absence of geminal ring chlorines on both congeners will reduce their susceptibility to reductive dechlorination, Hx-Sed, because of lack of chlorination on the C-5 ring carbon, should be more vulnerable than Hp-Sed to oxidative metabolism once up-take to fish has occurred. Further studies involving the analysis of older fish might reveal an even greater shift to higher Hp-Sed levels.

Conclusion

Three environmentally significant CHB congeners in ringed seal blubber were successfully characterized as 2,2,3-exo,5-endo-6-exo,8,9,10-octachloroborane (TS2), 2-exo,3-endo,5-exo,6-endo,8,9,10,10-octachlorobornane (TS3) and one or both of 2,2,5-endo,6-exo,8,8,9,10-octachlorobornane, and 2,2,5-endo,6-exo,8,9,9,10-octachlorobornane (TS4), respectively. Two environmentally significant CHB congeners in the surface sediment of toxaphene treated lakes were identified as 2-exo,3-endo,5-exo,6-endo-8,9,10-heptachlorobornane (Hp-Sed), and 2-exo,3-endo,6-exo-8,9,10-hexachlorobornane (Hx-Sed).

Of perhaps the greatest importance of this study is the recognition that at least one of the congeners present in seal blubber (TS4) is Toxicant A, a toxaphene compound previously shown to be toxic to insects and mammals. This is the first time this congener has been found in a warm-blooded species. This is an important discovery, and the first that shows elevated levels of such a particularly toxic congener in a species near the top of its food chain. It may be of concern to those that use ringed seal blubber in their diet and to those that are interested on toxic effects on the ringed seal species itself. Although the toxicity of TS2 is not very high it would wise to study the toxicity of other congeners in ringed seal blubber including TS3.

Similarly, a study of toxicity on Hp-Sed would be useful as it has not only found in the sediment of toxaphene treated lakes but also in antarctic penguin as well as fish in these toxaphene treated lakes. Hx-Sed, which was also identified in fish in toxaphene treated lakes, is also known to be relatively toxic compared to technical toxaphene.

It should be noted that most of the toxaphene congeners have not been quantified individually because of the difficulty purifying them for the purpose of making analytical standards. Now that more congeners are becoming commercially available (Parlar) and being purified and characterized in the technical standard, (Hx- and Hp-Sed, Loewen) it is possible to carry out further studies on each congener's biological effects and physical properties for modelling purposes. This study will aid significantly in the ease of quantification methods. As a result of knowing the relevent CHB compounds in a given matrix (e.g. sediment) and having access to individual CHB congeners (with know concentration and identity), simplified standard CHB mixtures for each matrix, may be created. Rather than employing technical toxaphene as a standard, (a mixture of several hundred compounds, perhaps some co-eluting, and not environmentally significant), use of these simpler matrix relavent CHB standards make quantification of CHB congeners more accurate.

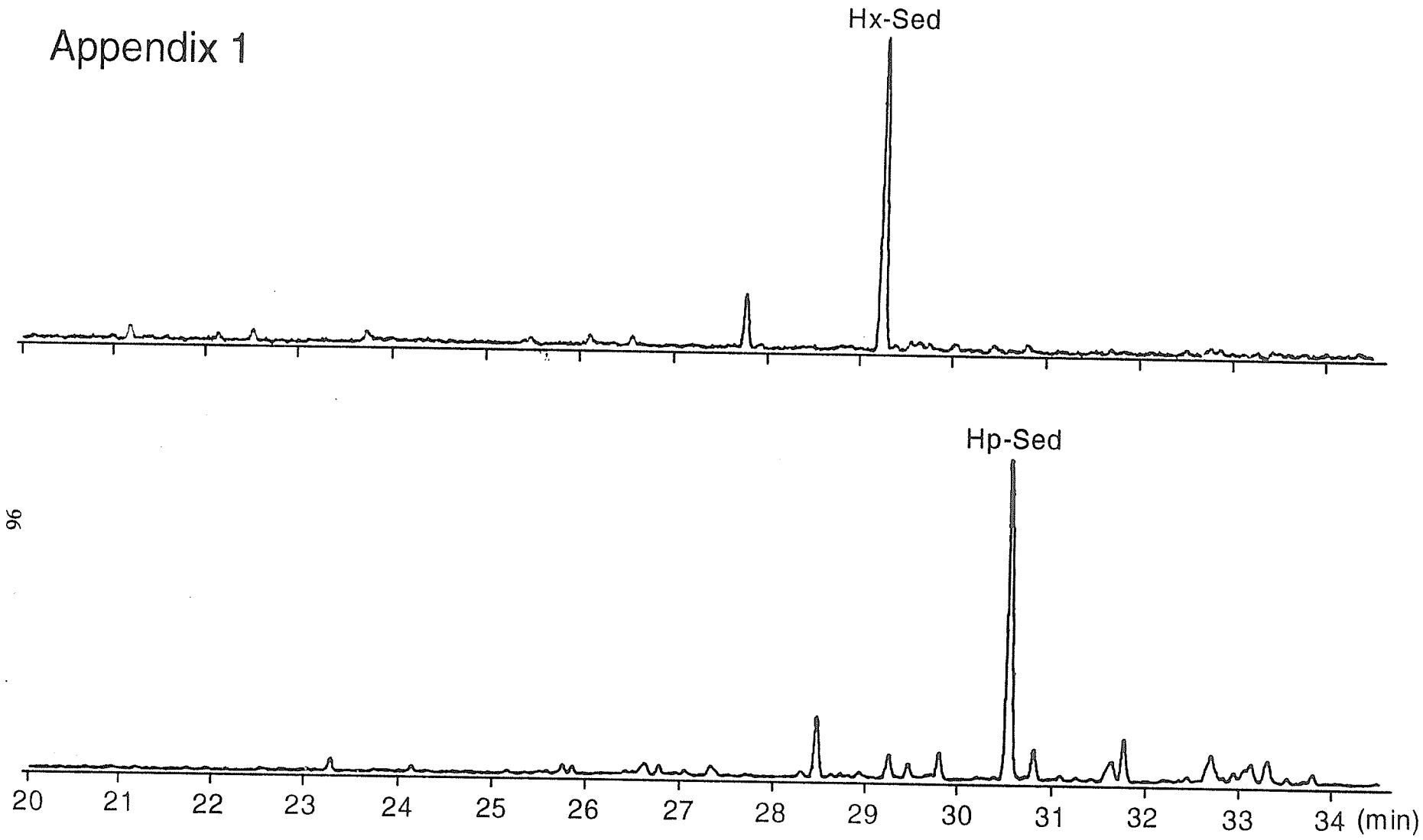
Finally the various mass spectral experiments give insight into general EI-MS fragmentation patterns of CHBs, making mass spectrometry a powerful tool for structural analysis of CHBs.

References

(1) M.A. Saleh, Reviews of Environmental Contamination and Toxicology. Springer-Verlag New York. 1991, 118, 1-83.
(2) E.C. Voldner, Y.F. Li. Chemosphere. 1993, 27, 2073-2078.
(3) T. Cairns, E.G. Siegmund, J.E. Foberg. Biomed. Mass Spectrom. 1981, 8, 569.
(4) G. Lee, R. Hughes, G. Veith. Water Air Soil Pollut. 1977, 8, 479.
(5) D. Webb. Entomol. Soc. 1980, 53, 731.
(6) J. Zhu, M.J. Mulvihill, R.J. Norstrom. J. Chromatog. 1994, 669, 103-117.
(7) M.A. Saleh. J. Agric. Food Chem. 1983, 31, 748.
(8) C.J. Musial, J.F. Uthe. Int. J. Environ. Anal. Chem. 1983, 14, 117.
(9) J.W. Gooch, F. Matsumura. Arch. Environ. Contam. Toxicol. 1987, 16, 349.
(10) T.F. Bidleman, M.D. Walla, D.C.G. Muir, G.A. Stern. Environ. Toxicol. Chem. 1993, 12, 701-709.
(11) P.S. Chandurkar, F. Matsumura, T. Ikeda. Chemosphere. 1978, 2, 123-130.
(12) M.A. Saleh, J.E. Casida. J. Agric. Food Chem. 1978, 26, 583-590.
(13) W.V. Turner, S. Khalifa, J.E. Casida. J. Agric. Food Chem. 1975, 23, 991.
(14) W.V. Turner, J.L. Engel, J.E. Casida. J. Agric. Food Chem. 1977, 25, 1394-1401.
(15) D.C.G. Muir, C.A. Ford, N.P. Grift, R.E.A. Stewart, T.F. Bidleman. Environ. Pollut. 1992, 75, 307.
(16) Hargraves
(17) D. Hainzl, J. Burhenne, H. Barlas, H. Parlar. Fresenius J. Anal. Chem. 1995, 351, 271-285.
(18) L.A. Barrie, D. Gregor, R. Lake, D. Muir, R. Shearer, B. Tracey, T. Bidleman, Sci. Tot. Environ. 1992, 122, 1-74.
(19) B.M. Miskimmin, D.C.G. Muir, D.W. Schindler, G.A. Stern, N.P. Grift. In press
(20) G.A. Stern, D.C.G. Muir, C.A. Ford, N.P. Grift, E. Dewailly, T.F. Bidleman, M.D. Walla, Environ. Sci. Technol. 1992, 26, 1838-1840.
(21) G.A. Stern, D.C.G. Muir, J.B. Westmore, W.D. Buchannon. Biological Mass Spectrom. 1993, 22, 19.
(22) H.R. Buser, M.D. Müller. J. Agric. Food Chem. 1994, 42, 393-400.
(23) H.R. Buser, M.D. Müller. Environ. Sci. Technol. 1994, 28, 119-128.
(24) D. Hainzl, J. Burhenne, H. Parlar. Chemosphere, 1994, 28, 245-252.
(25) W. Vetter. Chemosphere, 1993, 26, 1079-1084.
(26) R.G. Nash, E.A. Woolson. Science, 1967, 157, 924.

(27) T.M Dyott, A.J. Stuper, G.S. Zander. J. Chem. Inf. Comput. Sci. 1980, 20, 28-35.
(28) K.J. Palmer, R.Y. Wong, R.E. Lundin, S. Khalifa, J.E. Casida. J. Am. Chem. Soc. 1975, 97, 408-413.
(29) J.K.M. Sanders, J.D. Mersh. Prog. Nucl. Magn. Reson. Spectrosc. 1982, 15, 353-400.
(30) J.B. Lambert, H.F. Shurvell, D. Lightner, R.G. Cooks. Introduction to Organic Spectroscopy; Macmillan: New York, 1987.
(31) F. Matsumura, R.W. Howard, J.O.Nelson. Chemosphere. 1975, 5, 271-276.

Appendix 1



HRGC/ECD chromatogram of Hx-Sed and Hp-Sed