GC/MS ANALYSIS OF 5-*exo*,6-*exo*,8,10-TETRACHLORODIHYDRO-CAMPHENE AND A HEPTACHLORO ISOLATE FROM MELIPAX

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Introduction

Toxaphene, a non-systemic organochlorine pesticide, has been produced since the 1940s in many countries all over the world. Technical products comprise an unknown number of chlorinated trimethyl-bicyclo[2.2.1]heptanes and heptenes. The typical hydrocarbon backbone is bornane, but several other backbones like camphene, dihydrocamphene, and bornene have been reported in the literature for compounds of technical toxaphene (CTTs) [1][2][3]. The study and understanding of the chemical nature of toxaphene is limited by the fact that total synthesis of highly chlorinated single components has not been achieved, yet. In a recent publications plausible pathways were discussed for the formation of the bicyclic components mentioned above [4].
In this manuscript we discuss the GC/MS properties of two non-chlorobornane components. The first one, 5-*exo*,6-*exo*,8,10-tetrachlorodihydrocamphene was recently formed in a four-step synthesis [4]; the second one was isolated from the technical product Melipax.

Material and Methods

Synthesis of 5-*exo*,6-*exo*,8,10-tetrachlorodihydrocamphene [4]. The synthesis started with a Diels-Alder addition of cyclopentadiene and anhydrous methylmaleic acid. The double bond of the cyclo adduct was chlorinated with $MnCl_2$/acetic chloride, followed by reduction of the carboxylic groups with $LiAlH_4$ to the diol, subsequent esterification with p-tosyl chloride, and substitution of the tosylates by chloride using LiCl [4].
Isolation of a heptachloro component (Hp-ene) from the technical product Melipax. Hp-ene was isolated from Melipax by normal phase HPLC (ET 250/8/4 Nucleosil 100-7 column, Macherey-Nagel, Germany) followed by RP-HPLC (ET 250/8/4 Nucleosil 120-5 C_{18} column, Macherey-Nagel) using the parameters described for the isolation of B7-1453 (TOX7) [5]. After the HPLC procedures, B7-1453 and the hp-ene were the most abundant compounds in the extract. The final separation was obtained on 50 g silica and elution with n-hexane. B7-1453 eluted between 325-375 mL and hp-ene eluted between 250-325 mL and therefore prior to B7-1453.
Gas chromatographic and mass spectrometric parameters. Both components were analyzed with an HP 5890 series II GC coupled to an HP 5989B MS (Hewlett-Packard). GC/EI-MS was performed in the full scan mode (m/z 50-400) as recently described in detail [6]. GC/ECNI-MS was performed in the SIM mode. The ion source temperature was set at 150°C and the quadrupole temperature at 100°C. Methane and helium were used as CI reagent gas and carrier gas. A PAS 1 split/splitless injector (Gerstel, Germany) was used in the splitless mode (1.5 min) heated at 250°C. The GC capillary column consisted of 25% randomly *tert*.-butyldimethylsilylated ß-cyclodextrin (ß-BSCD) diluted in PS086. The column parameters were: 30 m length, 0.25 mm internal diameter, 0.20 µm film thickness. The ß-BSCD phase was from BGB Analytik (Adliswil, Switzerland). The GC oven program was as follows: 80°C (hold 4 min), 20°C/min to 180°C (hold 15 min), 20°C/min to 200°C (hold 25 min), and 20°C/min to 230°C (hold 15 min).

Results

Study of the GC/EI-MS of the unsaturated heptachloro component (hp-ene)

The most plausible backbones of unsaturated CTTs are camphene and bornene (see Figure 1). Note that there are some discrepancies with respect to the nomenclature of unsaturated CTTs. According to the definite rules of organic chemistry [7], the backbone numbering of camphene has the two methyl groups on C-2 and the methylene group on C-3. The present IUPAC rules do not distinguish between the carbons C-8 and C-9, but due to the lower second letter we assign C-8 to the *endo*-methyl group and C-9 to the *exo*-methyl group (see Figure 1, left). Note that the most of the chlorocamphenes reported in the literature do not apply the IUPAC numbering. Bornenes have the sp^2 hybrided carbons on C-2 and C-3, respectively (see Figure 1, right).

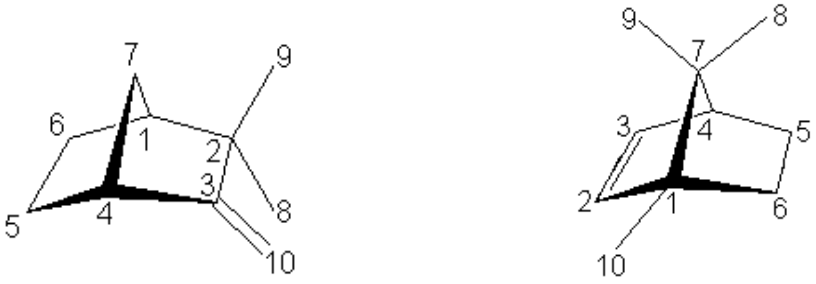

Figure 1: Hydrocarbon backbones of camphene (left) and bornene (right) with the carbon numbering according to IUPAC

Despite the different structures, the distinguishing of the two unsaturated backbones of chlorinated CTTs is not trivial by GC/MS. The EI-MS of hp-ene is shown in Figure 2. The dominating fragment ion at m/z 291 displays a pentachloro isotope pattern. m/z 291 is formed from the low abundant molecular ion at m/z 374 by elimination of 83 amu which corresponds to a $CHCl_2$ group. Subsequent elimination of HCl from 291 (5 Cl) leads to m/z 255 (4 Cl) and m/z 219 (3 Cl), respectively. Due to the lack of high mass fragments with entire backbone - the highly abundant fragment ion at m/z 291 has already a degraded backbone - structure elucidation of hp-ene by one dimensional mass spectrometry is difficult. An abundant signal at m/z 195 (3 Cl) may be formed via a retro-Diels-Alder reaction from m/z 291 (5 Cl) by elimination of a fragment containing $C_2H_2Cl_2$. This fragmentation is more plausible for chlorobornenes. Further minor abundant fragments between m/z 180 and 300 could not be interprested due to overlaps. E. g. m/z 183 is overlapped by m/z 185, both most likely daughter ions of m/z 219 and m/z 221, respectively.

High abundance was found for m/z 161, a sister ion of the tropylium cation m/z 159. There is some evidence that the ratio of m/z 159 to m/z 161 decreases with decreasing degree of chlorination. m/z 125 is the monochloro tropylium cation [8].

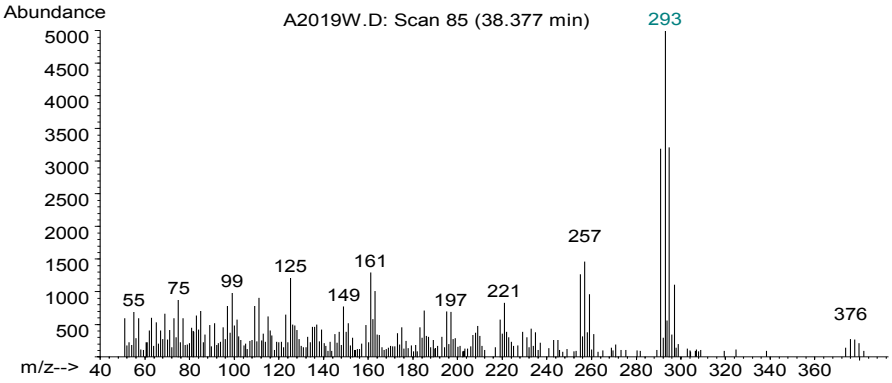

Figure 2: GC/EI-MS spectrum of hp-ene

Chlorocamphenes easily rearrange to chlorinated bornenes/pinenes and this may also happen in the ion source. Therefore, a definite conclusion if the typical bornane/bornene fragmentation may also be generated after intramolecular camphene-bornene rearrangement cannot be excluded. Thus, we have to leave the question open.

Study of the GC/EI-MS of tetrachlorodihydrocamphene (TDC)

The EI-MS of TDC is dominated by m/z 203 (2 Cl) or M-71 while the molecular ion (m/z 274) is very lowly abundant (Figure 3). Elimination of Cl and HCl, respectively, leads to m/z 238 and m/z 239 which overlap each other. Elimination of a CH_2Cl-group from C-8 or C-9 leads to m/z 225 (3 Cl). Further loss of HCl leads to m/z 189 (2 Cl). There are further fragment ions which were not elucidated. Buser and Müller claimed that m/z 100 is a typical fragment ion of chlorodihydrocamphenes [9]. However, this fragment ion was neither observed in the EI-MS of hept-ene nor in the EI-MS of TDC.

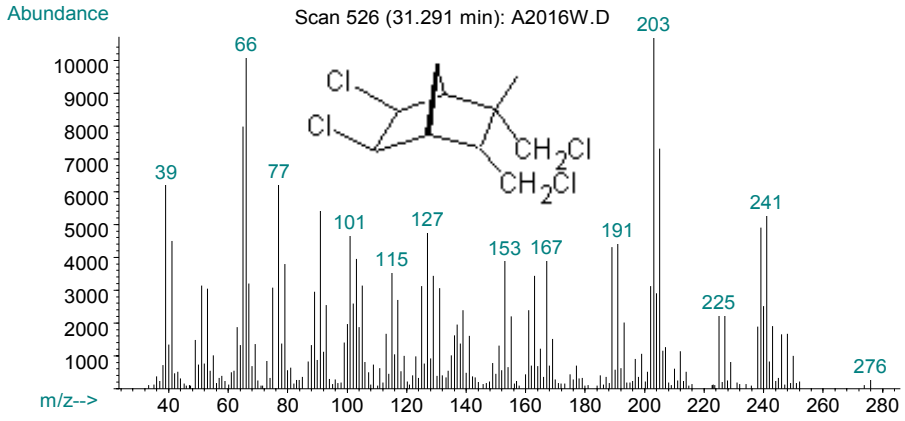

Figure 3: Structure and GC/EI-MS spectrum of TDC

Discussion

There are frequent reports on one-fold unsaturated components in toxaphene. Literature known chlorinated bornenes have been produced by reductive dehydrochlorination of chlorobornanes [10]. Chlorocamphenes reported by Hainzl et al. were isolated from photochemically modified toxaphene [11]. According to our knowledge, this is the first report on the isolation of an unsaturated compound from a technical toxaphene product (here: Melipax). So far, structure elucidation has not been achieved. The presence of chlorinated bornenes and camphenes has been discussed controversely. Krock et al. found plausible pathways for the formation of camphenes, while the formation of bornanes was only explainable by elimination of HCl for a chlorobornane [4]. It was concluded that such formation may not be reproducible and thus vary from synthesis product to another. Here, we add another interesting point to the recent knowledge: Fractions obtained during HPLC analysis of Melipax were colorless but after a storage of approx. 1 year they turned brown, even in the dark. Therefore, we conclude that toxaphene products (toxaphene standards) may include substances which are degraded with time. This also points towards low and varying amounts in toxaphene products. Recently, Parlar and coworkers have identified traces of bornenes in technical products [12]. These results clearly confirm our results.
At the moment it is not clear if non-chlorobornanes can be accumulated in the food web. Modelling of chlorobornenes contradict this possibility [13], but in samples of marine mammals from the Antarctic, an unsaturated component was detected and it was suggested to be a chlorocamphene [14]. The data of the group of Parlar and our results make this to be the most-likely case. Our data suggest that m/z 291 (and the respective values for homologs) should be added as EI-MS-SIM masses for the control of toxaphene and samples on unsaturated CTTs.
Note also that all chlorinated bornenes, camphenes, (and dihydrocamphenes) are chiral. Both components were enantioseparated on ß-BSCD.

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