HYDROXYLATED POLYCHLOROBORNANES – SYNTHESIS AND CHARACTERISATION OF NEW POTENTIAL TOXAPHENE METABOLITES

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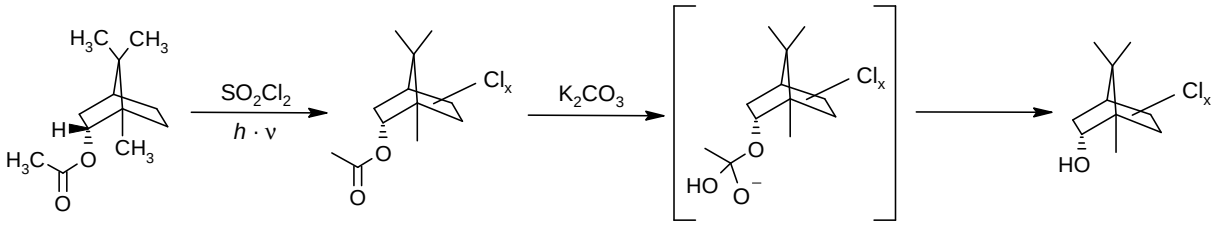
Introduction

The multicomponent mixture toxaphene belongs to the most intensively used organochlorine pesticides worldwide.[1,2] The total amount of toxaphene that has been applied is estimated to exceed one million tons.[3] Although its use was discontinued in most countries in the 1980s, toxaphene residues are still frequently detected in various environmental samples and biota due to long-range atmospheric transport and bioaccumulation along the food chain.[1] The congener patterns found in samples differ significantly from the pattern in the original technical mixture. In sediment samples, for instance, lower chlorinated compounds like B6-693 (HxSed) or B7-1001 (HpSed) dominate due to reductive dechlorination of higher chlorinated precursors in the anaerobic environment.[4] In biota of high trophic levels, residue patterns show a strong enrichment of selected congeners, usually bearing eight or nine chlorine atoms per molecule.[2] For some congeners like B8-2229 (Parlar 44) or B9-1025 (Parlar 62), nonracemic enantiomer ratios in biological samples are indicative of enzymatic biotransformation.[5] Furthermore, it is known that toxaphene acts as an inducer of hepatic mixed-function oxidases.[1,6] However, only very few studies explicitly address the existence of hydroxylated toxaphene metabolites.[7,8] Possibly, these compounds have simply been overlooked in the past due to a lack of methodological information concerning their analysis. Therefore, the aim of this study was to synthesize potential hydroxylated toxaphene metabolites, thus facilitating studies on the chromatographic and mass spectrometric behaviour of this poorly characterized compound class.

Materials and methods

Preparation of hydroxylated polychlorobornanes

A solution containing 1.00 g of L-bornyl acetate (Merck, Darmstadt/Germany) in 15 mL of sulfuryl chloride (Fluka, Buchs/Switzerland) was prepared in a quartz beaker and irradiated using a TQ150 medium pressure mercury vapor UV lamp (150 W, Heraeus Noblelight, Hanau/Germany). After two hours, the reaction mixture was added dropwise to ice-cold water to hydrolyse excessive sulfuryl chloride. The hydrolysate was then extracted twice with *n*-hexane/TBME (9:1, v/v). The combined organic layer was washed with sodium hydrogencarbonate solution and water until pH neutrality and then dried over anhydrous sodium sulfate.[9] Solvent removal using a rotary evaporator yielded 2.09 g of crude polychlorinated bornyl acetate.



Scheme 1: Synthesis pathway of polychlorobornanes with hydroxyl moiety at 2-*endo* position

Conversion to the corresponding 2-hydroxy polychlorobornanes (scheme 1) was performed by dissolution of the crude product in methanol (2.5 mg mL^{-1}) and subsequent saponification under mild conditions using 0.33 M

potassium carbonate solution in methanol/water (2:1, v/v) at room temperature. The hydrolysis was stopped after 60 min by the addition of hydrochloric acid. The target compounds were then extracted from the neutral hydrolysate with cyclohexane and subjected to a cleanup step on activated silica gel which is usually employed in analytical methods for the separation of PCBs from toxaphene.[10] As a modification, a third fraction consisting of pure ethyl acetate was introduced to elute compounds more polar than toxaphene from the column.

Gas chromatography/mass spectrometry (GC/MS)

All measurements were performed on a CP3800 GC coupled to a 1200 triple-quadrupole mass spectrometer (Varian, Darmstadt, Germany), equipped with a 30 m HP-5ms capillary column (0.25 mm i.d., 0.25 µm d_f). Helium 5.0 was used as carrier gas at a constant flow rate of 1.4 mL min^{-1}. Injector and transfer line temperatures were set at 230 °C and 280 °C, respectively. Sample introduction occurred by splitless injection (2 min splitless time). The oven temperature program started at 80 °C (1 min), was then ramped at 40 °C min^{-1} to 180 °C, followed by a 2 °C min^{-1} ramp to 270 °C (held for 4.5 min). The total run time was 45 min.

For GC/ECNI-MS and GC/EI-MS measurements, the ion source of the MS was maintained at 150 °C and 200 °C, respectively. In ECNI mode, methane 5.0 was used as buffer gas at a source pressure of approximately 8.5 Torr. The electron energy and emission current were set at 70 eV and 150 µA, respectively. All spectra were recorded in full scan mode, covering the range of *m*/*z* 30-500.

Results and discussion

With the synthesis pathway described above it was possible to obtain a mixture of bornane compounds that were both multiply chlorinated and equipped with a hydroxyl group. According to the proposed reaction mechanism (Scheme 1), the hydroxyl group should be located at the 2-*endo* position. It has previously been shown that toxaphene metabolites featuring a hydroxyl moiety are very likely to exist.[7,8,11] However, in order to successfully obtain these hydroxylated toxaphene derivatives, we found it mandatory to make use of a protecting group for the hydroxyl group during the photochlorination procedure. Experiments to directly chlorinate 2-*endo*-borneol were not successful as the reaction mixture turned dark almost instantly, even without turning on the UV lamp (data not shown).

The degree of chlorination achieved under the above photochlorination conditions ranged from four to six chlorine atoms per hydroxylated bornane molecule (Figure 1). Higher chlorinated homologs can probably be achieved using stronger conditions during chlorination (longer irradiation time, greater excess of sulfuryl chloride)[9].

After subjecting the reaction product to a modified PCB/toxaphene group separation and subsequent GC/ECNI-MS analysis, no peaks were found in the first *n*-hexane fraction as expected. The second fraction (*n*-hexane/ethyl acetate 9:1, v/v), which usually contains toxaphene[10], exhibited five peaks which could be assigned to our target compounds according to their GC/ECNI mass spectra. Polychlorinated bornanes or bornenes without hydroxyl moiety were not observed. By far the biggest part of the hydroxylated derivatives, however, was in the third fraction which was eluted with pure ethyl acetate. Hence, higher amounts of ethyl acetate or similarly polar solvents might be needed when hydroxylated toxaphene derivatives are to be determined in samples using conventional column cleanup steps.

The GC retention time window of tetra- to hexachlorinated 2-*endo*-hydroxybornanes ranged from about 10 to 22 min using the above GC method (Figure 1). For comparison, this range corresponds roughly with penta- to heptachlorinated bornanes when using the same GC conditions. Consequently, hydroxylated toxaphene metabolites seem to exhibit longer retention times than their non-hydroxylated homologs. Additionally, it can be concluded that hydroxylated toxaphene metabolites are very likely to be directly amenable to GC analysis as no derivatization step was required to obtain suitable chromatograms.

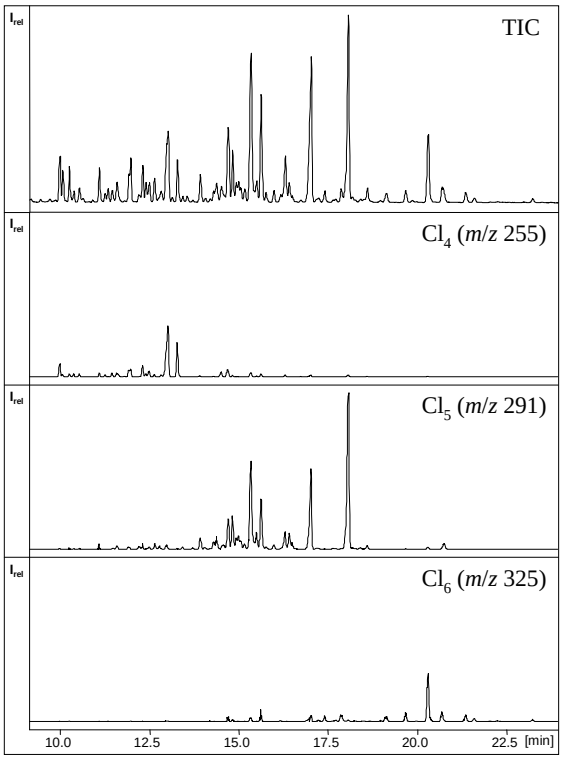


Figure 1: GC/ECNI-MS chromatogram and homolog distribution of the synthesized hydroxylated toxaphene derivatives

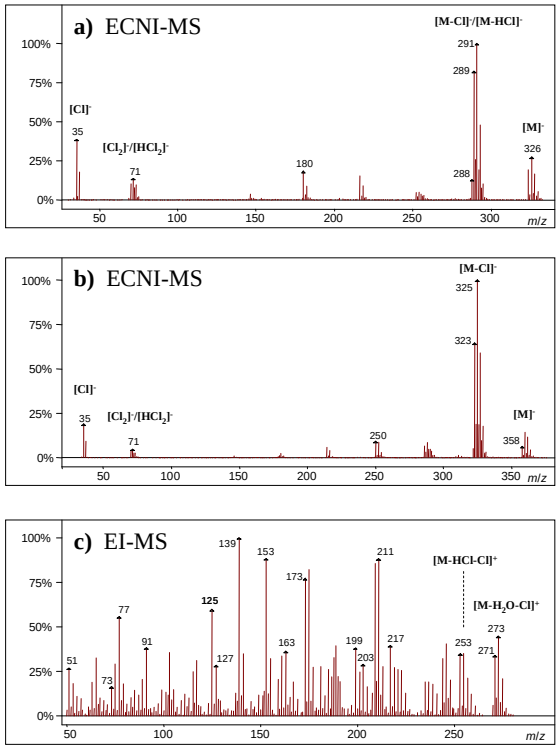


Figure 2: Mass spectra of selected penta- and hexachlorinated 2-hydroxybornanes. a) Penta, ECNI spectrum, b) Hexa, ECNI spectrum, c) Penta, EI spectrum

Examination of GC/ECNI-MS full scan mass spectra representative for the synthesized compounds revealed that, like polychlorobornanes, the hydroxylated derivatives form abundant $[M\text{-}Cl]^-$ fragment ion clusters (Figure 2a,b). In addition, the molecular ion cluster was always present to some extent in the GC/ECNI-MS spectra of the hydroxy derivatives. A further difference between the synthesized compounds and conventional toxaphene was the occurrence of practically the complete homolog series of possible fragment ion clusters caused by successive abstraction of Cl and/or HCl from the molecular ion. Interestingly, in GC/ECNI-MS no fragment ions attributable to the loss of the hydroxy group were observed.

It should be noted that OH-chlorobornanes and polychlorinated biphenyls are isobaric. For instance, the molecular ion of hexachloro isomers is both *m/z* 358 at low resolution. This may also hinder the identification of OH-CTTs in samples. However, PCBs and OH-CTTs can easily be separated by adsorption chromatography on silica (see Materials and methods). Alternatively, using GC/EI-HRMS with a resolution of >6500 provides sufficient power for the non-interfered determination of the most abundant isotopic peaks, i.e. *m/z* 359.84147 (100 %) and *m/z* 361.838520 (81 %) for hexachlorobiphenyls and *m/z* 359.89899 (100 %) and *m/z* 361.89604 (81 %) for hexachloro-OH-CTTs (Figure 3). $[M\text{-}Cl]^-$ fragment ions of OH-CTTs and PCBs overlap the same way.

Figure 2c shows the GC/EI-MS full scan mass spectrum of a pentachloro-2-*endo*-hydroxybornane. In general, EI spectra were very complex and thus hard to interpret when compared to ECNI mass spectra. One of the most obvious drawbacks was the complete absence not only of the molecular ion, but also of the $[M\text{-}Cl]^+$ or $[M\text{-}HCl]^+$ fragment ions which are normally formed by polychlorobornanes under EI conditions.[12] Instead, EI spectra of

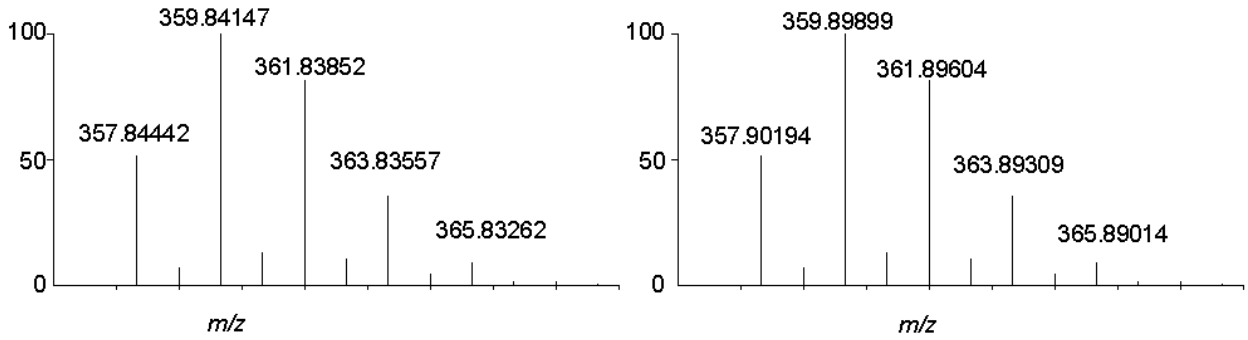


Figure 3: Isotope pattern of the molecular ion of hexachlorobiphenyl (left) and hexachloro-hydroxy-bornanes at high resolution

hydroxylated toxaphene derivatives often showed a fragment ion cluster corresponding to $[M\text{-}H_2O\text{-}Cl]^+$ as the fragment ion with the highest observed *m*/*z* value. Thus, it appears very difficult to identify hydroxylated toxaphene metabolites as such when only EI mass spectra are examined. Confusions with "conventional" toxaphene compounds might be possible. In this context, it is also interesting to note that it seemed to be a characteristic feature of both non-hydroxylated and hydroxylated toxaphene to form the monochlorotropylium ion $[C_7H_6Cl]^+$, which appears at *m*/*z* 125 (Figure 2c). This ion, together with its dichlorinated homolog (*m*/*z* 159), is often used for GC/EI-MS(MS) quantitation of toxaphene.[13]

The findings described here might help to establish a method for the determination of hydroxylated metabolites of toxaphene. Such a method could greatly improve our knowledge about the oxidative metabolism of toxaphene whereof only little is known even today. Furthermore, the synthesis described allows for the preparation of quantities sufficient for toxicological investigations.

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