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Hydroxylated polychlorobornanes – Synthesis and characterization of new potential toxaphene metabolites

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ABSTRACT

For decades, toxaphene had been used as a major chloropesticide. Degradation of the multicomponent mixture in the environment was mainly reported to be due to anaerobic dechlorination and hydrodechlorination. Little was known about oxidative transformation processes and the potential hydroxylated metabolites were not available as standard compounds. For this reason we synthesized hydroxylated polychlorobornanes by the UV-induced photochlorination of 2-*endo*-bornyl acetate with sulfuryl chloride followed by hydrolysis of the acetate moiety. The released polychlorinated 2-*endo*-hydroxybornanes were slightly higher chlorinated the longer the reaction was maintained. After 8 h, the main products were pentachlorinated hydroxybornanes followed by hexa- and heptachlorinated homologues. Traces of octachlorinated hydroxybornanes were also observed. The GC/ECNI-MS spectra of the products were characterized by the molecular ions and the $[M–Cl]^-$ fragment ions. The molecular ions of the polychlorinated hydroxybornanes are isobaric with those of polychlorinated biphenyls. E.g. hexachlorohydroxybornanes ($C_{10}H_{12}Cl_6O$) and hexachlorobiphenyls ($C_{12}H_4Cl_6$) show the molecular ion at *m/z* 358. Based on fractionation experiments on silica with the synthesis products it might be possible that OH-CTTs if present in samples will elute into a more polar fraction usually discarded or not collected. Both problems might explain why these compounds have not been more frequently described in the scientific literature.

1. Introduction

Toxaphene belongs to the most intensively used organochlorine pesticides worldwide (Saleh, 1991; Vetter and Oehme, 2000). The total amount of toxaphene that has been applied mainly to soils was estimated to exceed 1 million tons (Voldner and Li, 1993). Although its use was discontinued in most countries in the 1980s, toxaphene residues are still frequently detected in various environmental and food samples due to long-range atmospheric transport and bioaccumulation along the food chain (Saleh, 1991). As a consequence, toxaphene residues are still abundant in environmental samples some 30 years after the initial ban of the pesticide in the United States (Lamb IV et al., 2008; Pulster et al., 2009). Toxaphene is mainly composed of polychlorinated bornanes with 5–11 (average: 8) chlorine substituents. Minor constituents are chlorinated camphenes, dihydrocamphenes, and bornenes as well as a few compounds with lower or higher chlorine content (Saleh, 1991). While technical toxaphene is composed of ~1000 compounds (Korytár et al., 2003; Kapp and Vetter, 2009), the toxaphene residue pattern in environmental samples is much simpler (Vetter and Oehme, 2000). Only a few recalcitrant compounds are enriched while the bulk consists of readily degradable congeners which are metabolized. In sediment samples, for instance, lower chlorinated compounds like B6-693 (HxSed) or B7-1001 (HpSed) are dominant (Fingerling et al., 1996; Stern et al., 1996; Vetter and Maruya, 2000). These lower chlorinated congeners are formed by reductive dechlorination of higher chlorinated precursors as was shown in lab-experiments (Fingerling et al., 1996; Ruppe et al., 2003). However, Saleh (1991) concluded that toxaphene might also be degraded aerobically.

In biota of high trophic levels, toxaphene residue patterns are characterized by a strong enrichment of selected congeners, usually bearing eight or nine chlorine atoms per molecule (Vetter and Oehme, 2000). According to Ohsawa et al. (1975), toxaphene must contain compounds readily degradable while others are extremely persistent. The predominant recalcitrant congeners are B8-1413 (P-26) and B9-1679 (P-50) (Stern et al., 1992; Burhenne et al., 1993; Vetter et al., 1994). For some congeners like B8-2229 (P-44) or B9-1025 (P-62) non-racemic enantiomer ratios in biological samples are indicative of enzymatic biotransformation (Vetter and Luckas, 2000). Of special interest appear to be the compounds that can be degraded, because acidic compounds were reported to occur in this fraction (Ohsawa et al., 1975). First evidence for the existence of hydroxylated compounds of technical toxaphene (OH-CTTs) was produced by means of a shift in the GC profile after

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silylation (Chandurkar and Matsumura, 1979). Based on their findings, Saleh proposed five hydroxylated toxaphene metabolites (Saleh, 1991). In addition, polychlorinated hydroxybornanes have been reported as reaction intermediate products of the photoreaction of toxaphene (Parlar, 1988). However, the existence of hydroxylated toxaphene metabolites was explicitly verified only in very few studies (Boon et al., 1998; van Hezik et al., 2001). Possibly, OH-CTTs have simply been overlooked in the past due to a lack of methodological information concerning their analysis. In addition, the study of OH-CTTs was hampered by the lack of reference standards.

In this study an attempt was made to overcome these drawbacks in the analysis of OH-CTTs. For this purpose, we aimed at synthesizing potential hydroxylated toxaphene metabolites and to characterize their mass spectra. We also intended to define the likelihood and nature of interference of toxaphene metabolites by PCBs using commonly used instrumental techniques (EI- and ECNI-MS). We also tested an existing sample clean-up method on the chromatographic behaviour of this poorly characterized class of toxaphene metabolites.

2. Materials and methods

2.1. Chemicals and standards

L-bornyl acetate was from Merck (Darmstadt/Germany) and sulfuryl chloride (for synthesis) was from Fluka (Buchs/Switzerland). The solvents *n*-hexane (Unisolv, for organic trace analysis, Merck, Darmstadt, Germany), *t*-butylmethylether (TBME, technical grade, distilled prior to use), methanol (Rotisolv, HPLC gradient grade, >99.9%, Carl Roth, Karlsruhe, Germany), ethyl acetate (purity > 99.5%, Acros Organics, Geel, Belgium), and cyclohexane (purity > 99%, Acros Organics, Geel, Belgium) were used.

2.2. Synthesis of hydroxylated polychlorobornanes

L-bornyl acetate (1.0 g) was diluted in 15 mL sulfuryl chloride in a quartz beaker and irradiated by means of a TQ150 medium pressure mercury vapor UV lamp (150 W, Heraeus Noblelight, Hanau/Germany). After 2, 4, 6 or 8 h of reaction, the samples were dropped into ice-cold water to hydrolyse excessive sulfuryl chloride. The hydrolysate was extracted twice with *n*-hexane/TBME (9:1, v/v). The combined organic layer was washed with sodium hydrogen carbonate solution and water until pH neutrality and then dried over anhydrous sodium sulphate (Kapp et al., 2006). Solvent removal using a rotary evaporator yielded ~2 g of crude polychlorinated bornyl acetate.

Conversion to the corresponding 2-hydroxy polychlorobornanes (Fig. 1) was performed with the polychlorinated bornyl acetate sample obtained after 8 h reaction. An aliquot of the crude product (5 mg) was dissolved in methanol (2 mL) and subsequently saponified under mild conditions using 300 μL 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 100 μL 1 M hydrochloric acid. The target compounds were then extracted from the neutral hydrolysate with 3 mL cyclohexane.

2.3. Fractionation of synthesized OH-CTTs on activated silica gel

The cyclohexane fraction was subjected to a cleanup step on activated silica gel using a modification of the method of Klobes et al. (1998) originally developed for the separation of PCBs from toxaphene. Using activated silica, PCBs are eluted with 48 mL *n*-hexane into fraction 1 while toxaphene and other non-aromatic chlorinated pesticides and polybrominated compounds are collected in a second fraction with 50 mL *n*-hexane/ethyl acetate (9:1, v/v). In this study, the method was modified by the addition of a third fraction consisting of 50 mL pure ethyl acetate to elute more polar compounds compared to the conventional toxaphenes from the column.

2.4. 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-5 ms 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 isothermal). Then the temperature was ramped at 40 °C min^{-1} to 180 °C, and directly by 2 °C min^{-1} to 270 °C (held for 4.5 min). The total run time was 53 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 reagent 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.

3. Results and discussion

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). In order to successfully obtain hydroxylated toxaphene derivatives, we found it mandatory to make use of a protecting group for the hydroxyl group during the photochlorination procedure. For this purpose, our revised synthesis strategy followed the pathway suggested in Fig. 1 in order to produce a mixture of polychlorinated bornanes that were equipped with a hydroxyl group. According to the proposed reaction mechanism, the hydroxyl group should be located at the 2-*endo* position (Fig. 1). Chlorination of bornyl acetate with sulfuryl chloride produced a wide range of halogenated products (Fig. 2). With increasing reaction time, the average number of chlorine in the products slightly increased. This can be

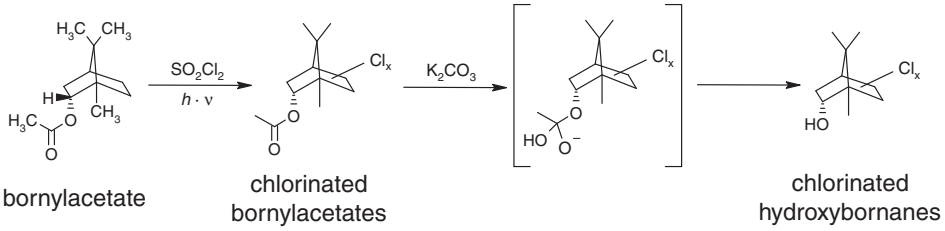

Fig. 1. Synthesis pathway of polychlorobornanes with hydroxyl moiety at 2-*endo* position.