A Simplified Approach to Studies of Toxic Toxaphene Components

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Toxaphene is a chlorinated hydrocarbon insecticide that is extensively used for control of cotton insect pests. It is produced by the chlorination of technical camphene to 67 to 69 percent chlorine by weight. The product is an extremely complex mixture of chlorinated terpenes. Little is known about the chemistry, toxicity or environmental fate of individual toxaphene components. Only recently has a toxic component of toxaphene been identified as 2,2,5-endo,6-exo,8,9,10-heptachlorobornane (CASIDA, et al., 1974). This group also isolated a $C_{10}H_{10}Cl_8$ component which was more toxic to mice and houseflies than the above component, however no structure was proposed for the latter compound. At least 175 polychlorinated 10-carbon components were claimed to be recognized in toxaphene by their methods and these were characterized as polychlorobornanes, polychlorobornenes, and polychlorotricyclenes with 6 to 10 chlorine atoms per compound.

Chlorination of bornyl chloride, isobornyl chloride and camphene hydrochloride to 66 to 67 percent chlorine yielded products more toxic to insects than toxaphene (WITEK, 1964). Chlorination products of fenchyl chloride and dipentene hydrochloride were almost non-toxic. WITEK (1973) stated that the chlorination product of exo-2,10-dichlorobornane was 1.7 times more toxic to certain insects than toxaphene. Since toxaphene is synthesized by the chlorination of camphene (BUNTIN, 1951) and exo-2,10-dichlorobornane is the major early chlorination product of camphene (JENNINGS AND HERSCHBACH, 1965; RICHEY, et al., 1965), this compound would appear a key intermediate in synthesis of toxic components of toxaphene. For the above reasons, we have investigated the chlorination of exo-2,10-dichlorobornane to form a "simplified toxaphene" which was used for the isolation of toxic components of toxaphene.

MATERIALS AND METHODS

Exo-2,10-dichlorobornane was synthesized as described by JENNINGS AND HERSCHBACH (1965). Camphene (Aldrich Chemical, Milwaukee, Wisc.) 13.6 g, was dissolved in 50 ml of carbon tetrachloride and 10.5 g of sodium bicarbonate was added. The reaction vessel, a 125 ml Erlenmeyer flask, was wrapped in aluminum foil and cooled in an ice bath. Chlorine gas (Matheson Gas Products) was bubbled into the solution at 40

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ml/min for 60 minutes. The course of the reaction was monitored by gas chromatography. The solvent was removed by rotary evaporation and products were vacuum distilled. Two fractions were collected, a mixture of monochloro compounds (bp 75-118°C/ 23 mm) and the dichloro compound which solidified in the condenser (bp 135°C/16 mm). This material was collected and recrystallized from ethanol. Infrared and NMR spectra agreed with reported literature values (JENNINGS AND HERSCHBACH, 1965). Chlorination of exo-2,10-dichlorobornane was similar to the chlorination of camphene except sodium bicarbonate was excluded and a UV lamp illuminated the reaction. The progress of the reaction was followed by gas chromatography.

Gas chromatographic (GC) analyses and separations were performed on a Varian model 1848 gas chromatograph equipped with a flame ionization detector (FI) and a ^{3}H electron capture detector (EC). Columns used in this work included SE-30, QF-1 and OV-1 liquid phases at 3% on Chromasorb G, 80/100 mesh and packed in 1.6 m x 3 mm I.D. stainless steel columns. Preparative GC separations were performed on a 2 m x 6 mm I.D. column packed with 10% QF-1 on GasChrom P, 60/80 mesh. Common operating conditions for the gas chromatograph were: injector temperature, 215-225°C; column temperature, 180 or 190°C; detector temperature, 200-210°C, N_2 carrier gas flow rate of 30-40 ml/min.

Mass spectra were taken on a Finnigan 1015 quadropole mass spectrometer. Both direct inlet and GC inlet systems were employed. Infrared (IR) spectra were obtained on KBr pellets of purified components using a Beckman IR33 spectrophotometer. Fourier Transform NMR spectra were obtained on a 90 MHz Bruker FTNMR spectrometer.

Three test organisms were used for bioassay of toxaphene fraction toxicity. They were: 1) mosquito larvae, Aedes aegypti; 2) freshwater blue-green algae, Anacystis nidulans (TX20); and 3) brine shrimp, Artemia salina. Bioassay procedures involved LC_{50} determinations for brine shrimp and mosquito larvae (NELSON, 1974), while toxicity to algae was measured as a decreased k value, a growth rate constant (BATTERTON et al., 1971).

RESULTS AND DISCUSSION

Products of chlorination reactions of purified exo-2,10-dichlorobornane were examined by gas chromatography. Gas chromatograms of four of the chlorination products are shown in Figure 1. The GC patterns show an increasing degree of chlorination as evidenced by GC peaks with longer retention times. The toxicities of these products are presented in Table 1. Interestingly, all the chlorination products, one through four, are only slightly toxic to algae. This is in contrast to toxaphene which strongly inhibits algal growth at the same concentration (1 ppm). One reason for this difference may be

that the precursor(s) for the components toxic to algae are eliminated when exo-2,10-dichlorobornane is separated from the other early chlorination products of camphene. The LC_{50} values for brine shrimp decrease with increasing degree of chlorination, however, the extent to which this generalization holds true was not pursued past chlorination product 4. Both products 3 and 4 are as toxic to brine shrimp as crude toxaphene. The LC_{50} values for mosquito larvae, however, never reached the levels of toxaphene. Again, other toxic components of toxaphene are probably excluded by this simplification method. With this bioassay organism there appears to be a degree of chlorination which is optimum for toxicity and it is roughly approximated by product 3.

Chlorination product 3 has approximately the same GC pattern as toxaphene. It also appears to be a simpler mixture than toxaphene as judged by GC and TLC comparisons. Toxaphene and product 3 were spotted on silica gel G thin layer plates (0.25 mm thickness) and developed 4 times with n-heptane. Separation of toxaphene components was poor, but product 3 gave fairly distinct spots. Silica gel adjacent to the spots was scraped from the plates, washed with diethyl ether, and the extracts were compared by gas chromatography. While gas chromatograms of

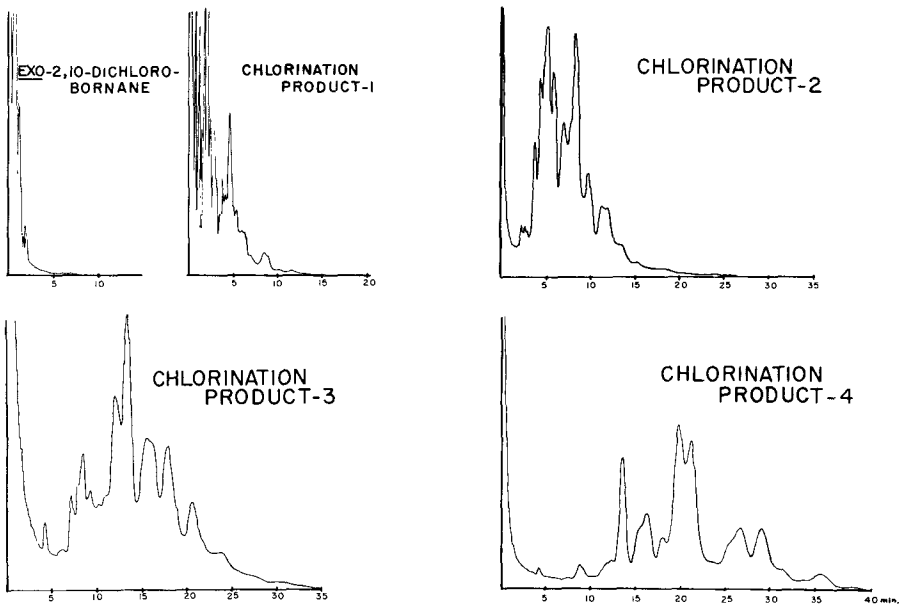

Fig. 1: Gas chromatograms of the chlorination products of exo-2,10-dichlorobornane. Conditions: 3% QF-1 on Chromasorb G (80/100) packed in 1.6 m x 3 mm stainless steel column. Injector, column and FI detector temperatures were 225°C, 190°C and 200°C, respectively. Nitrogen carrier gas flow rate was 25 ml/min.

TABLE 1

Toxicities to algae, brine shrimp and mosquito larvae of chlorination products of exo-2,10-dichlorobornane[a/]

	Algae, Anacystis nidulans 1.0 ppm		Brine shrimp Artemia salina	Mosquito larvae Aedes aegypti
	k[b/]	σ	LC_{50} (ppm)	LC_{50} (ppm)
exo-2,10-dichlorobornane	1.081	0.150	>4.0	>10.0
Chlorination Product 1	0.935	0.019	0.90	3.5
Chlorination Product 2	0.763	0.047	0.34	0.65
Chlorination Product 3	0.829	0.038	0.27	0.32
Chlorination Product 4	0.775	0.064	0.26	0.63
Toxaphene	0.327	0.090	0.24	0.105
Acetone control	0.960	0.072	--	--

a/ See Fig. 1 for the gas chromatograms of these products.

b/ Growth of the liquid cultures was followed colorimetrically after the method of KRATZ AND MYERS (1955), using a Bausch and Lomb Spectronic 20 Colorimeter. Linearity between O.D. and cell concentration was verified. The growth rate of algae is expressed by the equation: $kt = \log (N_t/N_o)$, where k = growth rate constant, t = 24 hr., N_t = cell number at time t, N_o = cell number at time 0. The growth values reported are the specific growth rate constant, k, in $\log_{10}$ units/day. When k = 0.301, the generation time is one day. The sensitivity of the algae to the insecticide compounds was reflected by growth rate depression (BATTERTON et al., 1971).

fractions separated by TLC were still complex for both toxaphene and chlorination product 3, the corresponding gas chromatograms for chlorination product 3 were consistently simpler than the fractions from toxaphene.

In order to scale up the separation effected by the TLC system, a Florisil column (2 x 28 cm) was employed to separate chlorination product 3 by eluting with hexane and hexane diethyl ether mixtures. Sixty-nine 15 ml hexane fractions and additional 200 ml fractions of hexane were collected followed by 200 ml of hexane:diethyl ether (9:1) and 250 ml of hexane:diethyl ether (1:1).

Florisil fractions 13, 14 and 15 have a major peak with a retention time equal to a toxic component isolated from toxaphene (NELSON, 1974). Preparative GC of these fractions on 10% QF-1 was used to isolate this peak, designated Peak 2 (Fig. 2). The purified peak was checked for purity and gave a single major peak on SE-30, OV-1, QF-1 columns. The LC_{50} of Peak 2 was 0.057 ppm for mosquito larvae, 0.32 ppm for brine shrimp and had a k value of 0.211 for algae growth inhibition. These compare favorably to toxicity values for the toxic component, isolated from toxaphene, which were 0.056, 0.32, and 0.243 for mosquito larvae, brine shrimp, and algae, respectively.

The infrared and mass spectra of Peak 2 were identical with the toxic compound isolated from toxaphene. Infrared absorptions were observed at cm^{-1} 2920 (w), 2850 (w), 1460 (s), 1450 (s), 1430 (s), 1305 (s), 880 (s), 850 (s), 840 (s), 805 (s), and 770 (s). The mass spectrum of this compound has a weak parent ion cluster (8 Cl) at 410 (Cl=35). Major fragments include: m/e 375 (7 Cl); 361 (7 Cl); 339 (6 Cl); 303 (5 Cl); 291 (5 Cl); 267 (4 Cl); 243 (3 Cl); 231 (3 Cl); 195 (3 Cl); 193 (3 Cl); 159 (2 Cl) and 83 (2 Cl). The major routes of fragmentation appear to be removal of chlorines, elimination of hydrogen and chlorine, and cleavage of chloromethyl and dichloromethyl moieties. The NMR spectrum of this compound is quite complex and exact proton assignments were not possible.

Although no exact structure can be assigned for this toxic compound, it is speculated on the basis of infrared and mass spectral data that this eight-chlorine compound is similar to the toxic seven-chlorine compound identified by CASIDA et al. (1974). One major difference is the presence of a dichloromethyl group (m/e=83, 2 Cl) and a trichloroisopropyl fragment (m/e= 145, 3 Cl) in the mass spectrum which indicates that methyl carbons 8 and 9 of the bornane structure contain 3 chlorines instead of 2 as in 2,2,5-endo,6-exo,8,9,10-heptachlorobornane (CASIDA et al., 1974). An exact structure for this compound is awaiting an x-ray crystal structure determination.

The use of a "simplified toxaphene" for the isolation of toxic components of toxaphene appears a valid and useful approach,

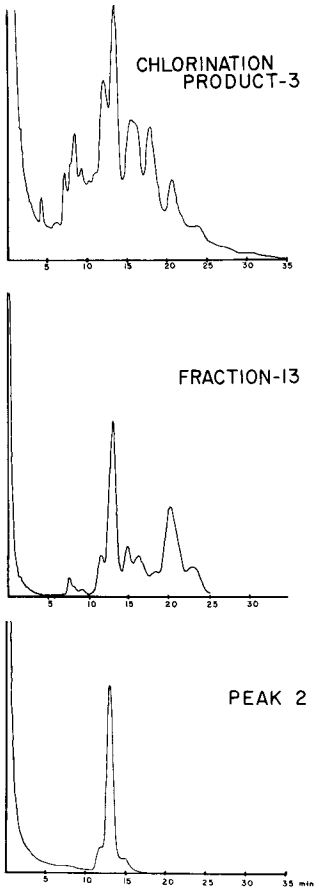

Fig. 2: Gas chromatograms showing the purification of a toxic compound from a simplified toxaphene mixture. (Fraction 13 was obtained from chlorination product 3 by Florisil column chromatography, as described in text. Peak 2 was purified from Fraction 13 by preparative GC on 10% QF-1 column 2 m x 6 mm.) GC conditions: same as Fig. 1.

since at least one of the toxic components isolated from a chlorination reaction product of exo-2,10-dichlorobornane was found to be identical to a toxic compound isolated from toxaphene (NELSON, 1974) as judged by its toxicity to three organisms, its chromatographic behavior and its IR and mass spectra. With suitable GC monitoring of reaction progress, it is possible to maximize the amount of any one chlorination product in the chlorination of exo-2,10-dichlorobornane, or for that matter, any other purified intermediate in chlorination of camphene to toxaphene. The result is a simplified toxaphene, from which the purification of toxaphene components for chemical identification and toxicological studies is considerably easier.

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