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ISOLATION AND GAS CHROMATOGRAPHIC CHARACTERIZATION OF SOME TOXAPHENE COMPONENTS

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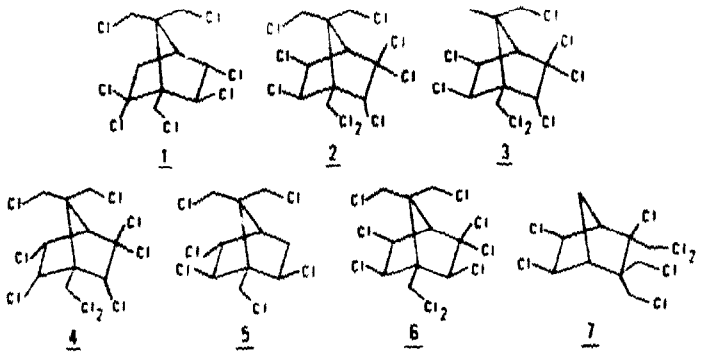
SUMMARY

Column chromatography of technical toxaphene on activated alumina reproducibly yields eight major fractions from which 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane and 2,5,6-*exo*, 8,8,9,10-heptachlorodihydrocamphene may be readily isolated by preparative gas–liquid chromatography. These and some other reported toxaphene components were characterized by gas–liquid chromatographic retentions relative to aldrin on packed columns of OV-101, OV-17, and QF-1, and on a capillary open-tubular column of OV-101. Structural assignments were revised for some components on the basis of retentions. The fractionation and isolation procedure may have general utility for separating toxaphene components, and the retention data form a convenient basis for component comparison.

INTRODUCTION

Recent interest in the metabolic and environmental fate of toxaphene insecticide has prompted efforts towards isolation and characterization of components of the toxaphene mixture. From technical toxaphene Casida *et al.*[1,2] isolated two components (A and B) of high mammalian toxicities by a sequence of steps involving liquid–liquid partition and adsorption column chromatography (CC), preparative gas–liquid chromatography (GLC), and eventual crystallization. Component B, $C_{10}H_{11}Cl_7$, was identified by X-ray crystallography as 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (**1**)[3] while component A, $C_{10}H_{11}Cl_8$, was not identified. Anagnostopoulos *et al.*[4] isolated three additional components (substances I, II, and III), each of greater topical toxicity to houseflies than technical toxaphene. Purification of I–III involved CC of recrystallized toxaphene, and preparative GLC of the resulting fractions. Their structures (**2**, **3**, and **4**, respectively) were assigned based on mass spectrometry (MS) and proton magnetic resonance (PMR) spectroscopy. Additional components were separated by Black[5] by a combination of CC, liquid–liquid partition, preparative GLC, and crystallization; two components (13 and nonpolar 19) were tentatively assigned structures **5** and **6**, respectively, while several others were not as completely characterized. Nelson and Matsumura[6] have recently isolated a component apparently quite similar, or identical, to component A[2] from a "simplified toxaphene" made by chlo-

rinating *exo*-2,10-dichlorobornane, a postulated intermediate in chlorination reactions involving camphene.



Most of the reported isolations were tailored to one or a few components of high toxicity, and vary considerably in the specific techniques employed. It is thus difficult to compare the relative merits of one reported procedure with another, or with new ones as they are devised. We report here details of a simple and straightforward isolation method and its application to isolation of component B (**1**) and a new component (**7**) of technical toxaphene. GLC retention data, obtained on three packed columns and one open-tubular (OT) column, are included for **1**, **7**, and a few other toxaphene components. We offer the isolation procedure as one which may have general utility in separations involving toxaphene, and the GLC tabulation as a basis for cataloguing known components and new ones as they become isolated.

EXPERIMENTAL

Materials

Technical toxaphene was provided by Hercules, Wilmington, Del., U.S.A. Merck (Darmstadt, G.F.R.) neutral alumina, 1077 aluminum oxide activity I, was activated at 240° for at least two days before use.

Column chromatography

One gram of technical toxaphene, dissolved in 10 ml of hexane, was introduced to a 27 × 2 cm I.D. column containing 67 g of alumina, topped with 3 cm of anhydrous sodium sulfate, and prewashed with hexane. The mixture was then eluted with 50-ml portions of each solvent, commencing with pure hexane, through various mixtures of hexane–benzene, to 20% methylene chloride in benzene and 100% methanol as final eluting solvent. The eluent was collected in 10-ml cuts and each cut analyzed by GLC. These were combined into eight fractions, which corresponded to the first elution of toxaphene with 20% benzene in hexane and thereafter the change in solvent constitution according to Table 1.

This chromatographic experiment was scaled to accept 10 g of toxaphene on 700 g of alumina, using a 115 × 3 cm I.D. column and 500 ml of each solvent mixture. Fractions III, IV, V, VII, and VIII were each collected as two subfractions (a and b) of approximately equal solvent volume.

Preparative GLC

Fractions III and VII, or more satisfactorily IIIa and VIIa, were subjected to preparative GLC using a 2.4 m × 4 mm O.D. column containing 10% OV-101 on 100–120 mesh Chromosorb W operated at 220° with a nitrogen carrier gas flow-rate of 82 ml/min. An F & M Model 402 gas chromatograph equipped with a 300:1 glass splitter and flame ionization detector was used; collection of the majority of the column effluent was effected through a 15 cm × 1 mm I.D. glass capillary. The back pressure created by these tubes resulted in a final split ratio, measured by flow, of 4.5:1. In one experiment 20 mg of the major component of fraction III was collected upon processing 150 mg of fraction III in 5-mg portions. In another experiment 23 mg of a major component of fraction VII was obtained by processing 120 mg of fraction VII in 8-mg portions. A column containing 10% DC-200 on 100–120 mesh Chromosorb Q operated at 220° with a nitrogen carrier gas flow-rate of 30 ml/min was used to check the purity of the collected fractions.

In a subsequent, somewhat more productive experiment, 77 mg of the major component of fraction IIIa was obtained by processing 720 mg of IIIa in 30-mg batches. This was accomplished by a change in column and collection procedures. A 1.2 m × 1.27 cm O.D. column containing 20% SE-30 on 40–60 mesh Chromosorb P was operated at 220° with a carrier flow-rate of 500 ml/min. The F & M Model 402 was equipped as before except that the collection tube sizes were 25 cm × 3 mm O.D. and these were cooled in a dry ice–acetone bath. The final split ratio, measured by flow, was 200:1.

Crystallization and physical characterization

The GLC-purified component of fraction IIIa crystallized as colorless orthorhombic prisms upon slow evaporation of hexane–chloroform (5:1). It had a m.p. (164–167°) and infrared (IR) spectrum which agreed closely with those reported for **1** (ref. 2). Unit cell dimensions, measured from a Weissenberg and oscillation photograph[7] of a crystal mounted along the c axis, were a = 8.53 ± 0.04 Å, b = 21.30 ± 0.05 Å, and c = 7.60 ± 0.04 Å, while those of **1** are reported as a = 8.603 ± 0.005 Å, b = 21.344 ± 0.009 Å, and c = 7.608 ± 0.005 Å.

The major component of fraction VIIa was crystallized by allowing a solution in hexane–diethyl ether (5:1) to slowly evaporate to near dryness. It had a m.p. of 131–132°, and MS molecular ion of 376 *m/e*, indicating an empirical formula of $C_{10}H_{11}Cl_7$.

Retention times by packed column GLC

An F & M Model 402 gas chromatograph with a flame ionization detector was used with the following 2.5 m × 3.2 mm O.D. columns: (1) 10% OV-101 on 100–120 mesh Gas-Chrom Q at 220°, 40 ml/min nitrogen; (2) 3% OV-17 on 100–120 mesh Gas-Chrom Q at 210°, 32 ml/min nitrogen; and (3) 10% QF-1 on 100–120 mesh acid-washed Chromosorb W at 200°, 35 ml/min nitrogen. Aldrin was an internal standard in all analyses.

Retention times by OT capillary column GLC

A Hewlett-Packard Model 5700 A gas chromatograph was employed with a Model 18713A ^{63}Ni electron capture detector (ECD). The chromatograph inlet was

fitted with a variable split injector splitter[8]; a split ratio of 46: 1 was used in this work. The column was 18 m × 0.25 mm I.D. glass coated with OV-101 containing 5% BTPPC and 5% Ionox according to Jennings *et al.*[9]. The detector inlet was modified to accept the capillary column by adapting common Swagelok fittings to the 6-mm fitting supplied with the instrument. The column was threaded through all these fittings, and through a 6 × 2 mm I.D. glass sleeve placed inside the 6-mm fitting, to just inside the detector cavity. Injector, column, and detector temperatures were 250°, 200°, and 350°, respectively. Nitrogen carrier and argon–methane detector makeup gas flow-rates were 0.7 and 60 ml/min, respectively.

RESULTS AND DISCUSSION

Alumina column chromatography gave a reproducible fractionation of technical toxaphene into eight major fractions. All of the fractions were mixtures and gave only a small discrimination on the basis of acute mammalian toxicity (Table I). Highest toxicity was observed in the major fractions (II and III); the trend was towards lower toxicity with the fractions eluted with more polar solvents, with the exception that fraction VII was more toxic than immediately preceding and following fractions. There was no indication of dehydrochlorination on the activated alumina employed in the chromatography.

Preparative GLC of fraction III gave **1**, purified by crystallization from hexane–chloroform (Fig. 1). This route to **1** is considerably more direct than that of a previously reported procedure[2]. The identity and purity of crystalline **1** was established by comparing its m.p., IR spectrum, PMR spectrum, and unit cell dimensions with those reported[2,3].

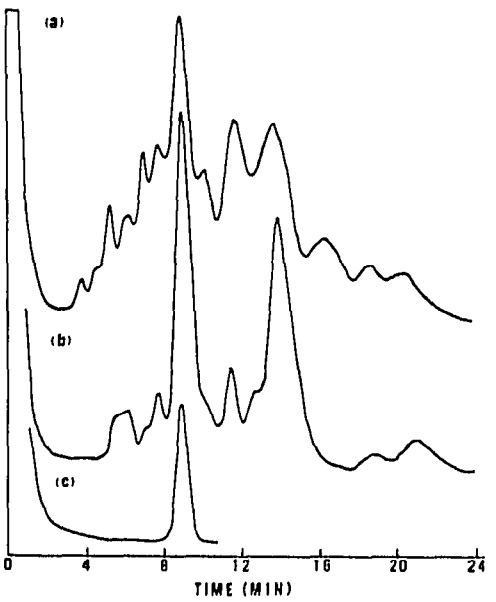
The major component of fraction VII was readily isolated by preparative GLC and crystallization from hexane–ether (Fig. 2). Its structure, determined by X-ray crystallography[10], is that of 2,5,6-*exo*,8,8,9,10-heptachlorodihydrocamphene, **7**. A striking feature of its mass spectrum was the appearance of an ion cluster at 305–311

TABLE I

COLUMN CHROMATOGRAPHY OF 1 g OF TECHNICAL TOXAPHENE ON 67 g OF ALUMINA

Fraction No.	*Solvent**	*Weight (mg)*	*LD_{50}** (mg/kg)*	*Toxicity relative to toxaphene*
(toxaphene)	—	—	32	1.0
I	30 (A)	21	—	—
II	40 (A)	381	29	0.9
III	50 (A)	339	23	0.7
IV	60 (A)	120	65	2.0
V	70, 80 (A)	80	95	3.0
VI	90, 100 (A)	31	79	2.5
VII	100 (A) 10 (B)	15	53	1.7
VIII	20 (B) 100 (C)	22	177	>5.5

* A = % Benzene in hexane; B = % methylene chloride in benzene; C = % methanol.
** Upon intraperitoneal injection in mice, in dimethyl sulfoxide solvent.



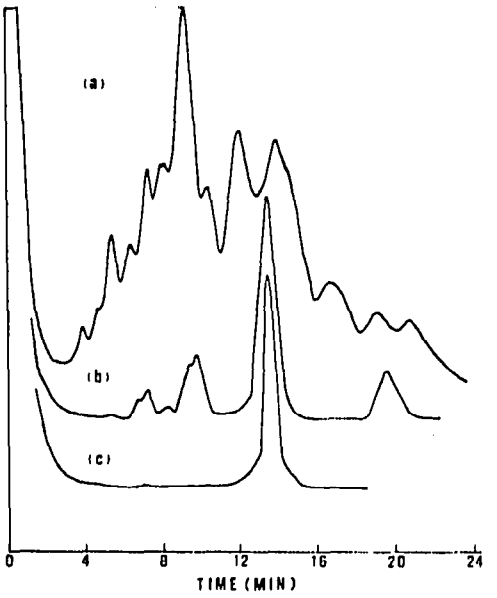


Fig. 1. Gas chromatograms of (a) toxaphene, (b) fraction IIIa and (c) isolated component **1** on 10% DC-200, 220°.

Fig. 2. Gas chromatograms of (a) toxaphene, (b) fraction VIIa, and (c) isolated component **7** on 10% DC-200, 220°.

m/e which was the most intense of those observed above 160 *m/e*. This corresponds to loss of Cl and HCl from the M^+ ion, with formation of a relatively stable fragment $C_{10}H_{10}Cl_5$. More complete information on the properties of **7** will be published elsewhere[10].

The GLC retentions of five toxaphene component preparations on packed columns of three common liquid phases are chromatographically displayed in Figs. 3 and 4, and summarized in Table II. The close similarities of **1** and component 13[5] on all three columns suggested identity; however, the reported m.p. of the latter (215–220°[5]) is considerably higher than that of **1** (166–167° reported[2], and 164–167° from our preparation). Furthermore, as noted by Black[5], the PMR spectra of the two components, while quite similar, exhibit differences not ascribable to conditions of purity. It does appear extremely unlikely from the retention data that component 13 contains one less Cl than **1**, as originally postulated; its structure, then, cannot be that of **5**. It is apparently a $C_{10}H_{11}Cl_7$ component, which is a close isomeric relative of **1**, of an as yet undetermined structure.

Substance I[4] and nonpolar component 19[5] also appeared identical from GLC retention data. Their identity has been further established by comparison of MS and PMR spectral data. Whether the correct structure of this component is represented by structures **2** or **6** was not determined in this study. Both represent plausible interpretations of the PMR spectral data[4,5], and final differentiation must await more definitive characterization.

Component **7** exhibited the most dramatic shift in retention from the least polar (OV-101) to the more polar (OV-17 and QF-1) columns. The greater polarity

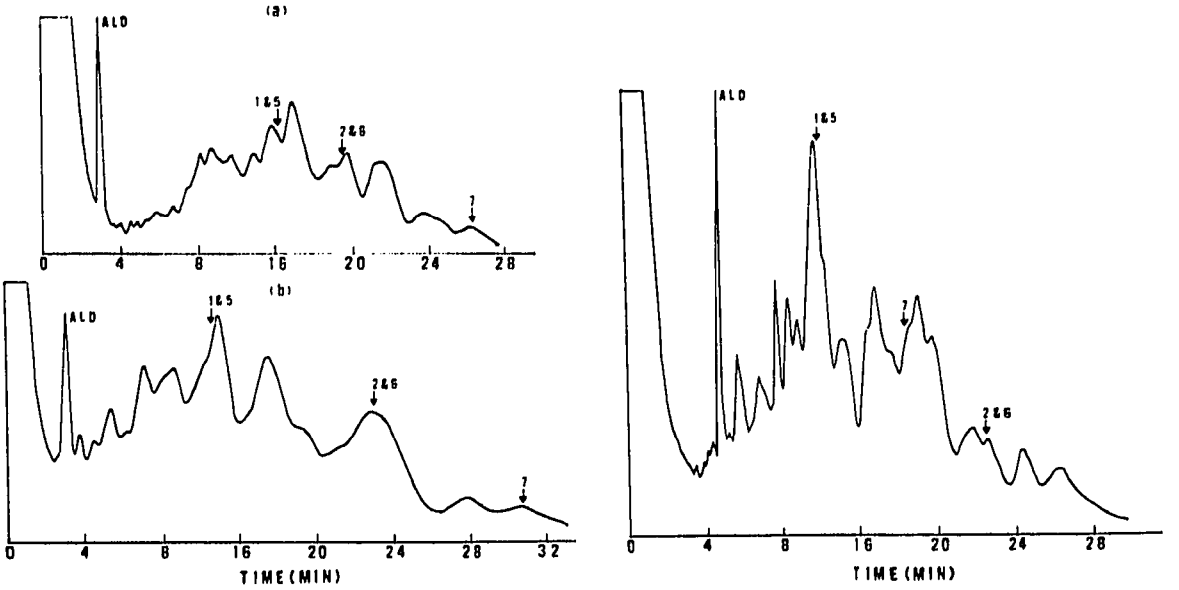


Fig. 3. Gas chromatograms of toxaphene with aldrin (ALD) as an internal standard on (a) 10% QF-1 at 200° and (b) 3% OV-17 at 210°.

Fig. 4. Gas chromatogram of toxaphene with aldrin (ALD) as an internal standard on 10% OV-101 at 220°.

of this component indicated by this retention shift, in keeping with its late elution from alumina, apparently reflects a greater polarity of chlorodihydrocamphenes than the related chlorobornanes. If this is indeed a proper generalization, further examination of the late-eluting alumina fractions may yield additional chlorodihydrocamphenes.

Technical toxaphene and the five components were examined by OT capillary GLC on OV-101, with a considerable gain in efficiency and resolution. The chromatograph was fitted with an inlet splitter[9] to prevent overloading, and the column interfaced with a commercial ^{63}Ni ECD to maximize sensitivity. For successful interfacing

TABLE II

GLC RETENTIONS RELATIVE TO ALDRIN (R_A) FOR SOME TOXAPHENE COMPONENTS

Component postulated structure	*R_A* on packed columns*			*Capillary column, OV-101*	
	OV-101	*OV-17*	*QF-1*	*R_A***	*R_A**
Component B[2], 1	2.15	3.50	4.29	2.04	2.33
Substance I[4], 2	4.04	6.24	5.46	3.83	4.60
Component 13[5], 5***	2.14	3.50	4.26	2.04	2.33
Nonpolar component 19[5], 6***	4.04	6.28	5.46	3.82	4.60
Heptachlorodihydrocamphene, 7	3.19	7.46	7.85	2.88	3.40

* Based on retention times adjusted for solvent (hexane) holdup.
** Based on unadjusted retention times.
*** Reported structure.

it was necessary to modify the detector inlet slightly to reduce dead volume, primarily by addition of a glass sleeve to the column coupling supplied with the instrument. The capillary column was threaded through the insert directly to the base of the detector inlet. With this configuration column effluent was swept directly into the detector cavity by argon–methane purge gas with little back diffusion or loss of resolution. Response of the system to aldrin, *ca.* 10% f.s.d. with 20 pg aldrin, and its efficiency, 28,000 theoretical plates for aldrin at 200°, were comparable to those of a similar system described by Franken and Vader[11].

Similarity of **1** and component 13[5], and identity of substance I[4] and nonpolar component 19[5] were again indicated by retentions obtained with the capillary system (Table II). In addition, it becomes quite apparent from examination of the capillary chromatogram (Fig. 5) that the major peaks of toxaphene are not represented by any of the standards examined. For example, **1** is only a shoulder on the largest peak B, and substance I represents a relatively small peak of long retention time. Major peaks A, B, and D elute largely in column fraction II as components of a still complex mixture, and C and E elute largely in column fractions IIIb and IV. Further fractionation of these fractions may be required to furnish simpler mixtures for isolation of these major components.

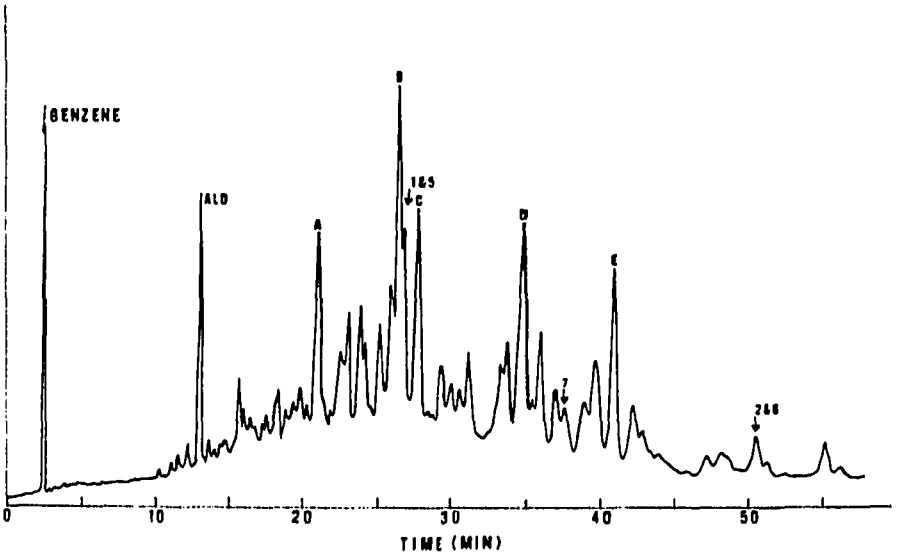


Fig. 5. Open tubular capillary gas chromatogram of toxaphene with aldrin (ALD) as an internal standard on OV-101 at 200°.

CONCLUSIONS

Alumina column chromatography may serve as the basis for a fractionation of technical toxaphene to facilitate isolation of a highly toxic component, 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (**1**). From a polar column fraction an additional component, $C_{10}H_{11}Cl_7$ (**7**), may be readily isolated in pure form. These and other reported components were characterized by GLC retentions on packed columns

containing stationary phases of different polarities. From resulting data it appears that a component previously formulated as $C_{10}H_{12}Cl_6$ (**5**)[5] is in fact a $C_{10}H_{11}Cl_7$ isomeric to **1**, and that two $C_{10}H_9Cl_9$ components for which isomeric structures had been proposed[4,5] were in fact identical.

The above assignments were supported by OT capillary GLC on OV-101. In addition, it became apparent that the major components of toxaphene are not represented by any of the five standards examined. The capillary system offers a means of fraction and component analysis which could greatly assist future efforts aimed at isolation and comparison of components of this complex mixture. In addition, the coupling of the capillary column with an ECD offers an approach to selective examination of toxaphene components in environmental residue samples.

From the work described here it is apparent that a systematic means of characterizing isolated toxaphene components is needed to prevent duplication. GLC retention data, referenced to a common standard, offer an approach to standardization.

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