crossing) are probably important factors contributing to the photolability of 1.

The photoproducts of 1 are less toxic on an acute basis to mice than 1 itself. This includes the individual esters resulting from isomerization in the acid moiety and racemization in the alcohol moiety and the overall mixture of photoproducts. Thus, as far as acute toxicity is concerned, the amount of 1 is of greater toxicological significance than that of any of its photoproducts.

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Toxaphene Components and Related Compounds: Preparation and Toxicity of Some Hepta-, Octa-, and Nonachlorobornanes, Hexa- and Heptachlorobornenes, and a Hexachlorobornadiene

Walter V. Turner, Judith L. Engel, and John E. Casida*

Five major toxaphene components [2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (B) and its 3-*exo*-chloro, 8-chloro, 9-chloro, and 10-chloro derivatives] collectively account for up to 23% of the GLC–electron-capture properties of chlorinated technical grade camphene (i.e., toxaphene insecticide) and up to 34% of those of chlorinated 2-*exo*,10-dichlorobornane. Chlorination of 2-*exo*,10-dichlorobornane provides a convenient source of B, which on further chlorination gives the indicated octachlorobornanes and the 5-*exo*-chloro derivative of B plus two nonachlorobornanes, one with the introduced chlorines at C-8 and C-10 and the other with these chlorines at the 3-exo position and at C-10. On dehydrochlorination B yields two hexachlorobornenes and the 3-*exo*-chloro derivative of B gives one heptachlorobornene and one hexachlorobornadiene. The toxicity to mice, houseflies, and goldfish of the octachlorobornanes formed by introducing chlorine substituents into B, relative to B itself, generally decreases in the order: 9-chloro > 8-chloro > no added chlorine (i.e., B) > 3-*exo*-chloro, 5-*exo*-chloro, or 10-chloro.

The amount of toxaphene used in the United States continues to be larger than that of any other insecticide. It is therefore essential to define the composition of toxaphene and the toxicological properties of its components.

Toxaphene is produced by chlorination of camphene to yield a very complex mixture with an overall composition approximating $C_{10}H_{10}Cl_8$. Two of its most toxic components are 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (compound B) and a mixture of the 8-chloro and 9-chloro derivatives of B (referred to here as 8-Cl-B and 9-Cl-B,

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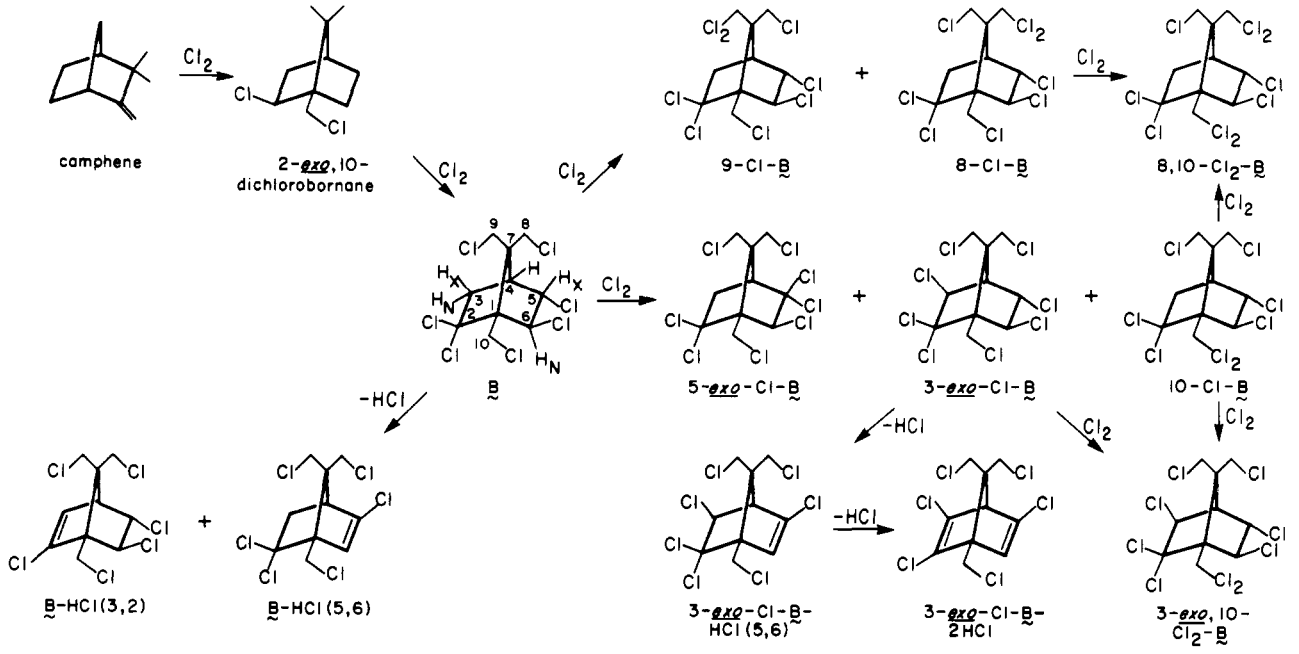

Figure 1. Conversion of camphene and 2-*exo*,10-dichlorobornane to 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane (B) and several octa- and nonachlorobornanes, hexa- and heptachlorobornenes, and a hexachlorobornadiene.

respectively) (Casida et al., 1974; Khalifa et al., 1974; Matsumura et al., 1975; Palmer et al., 1975; Seiber et al., 1975; Turner et al., 1975). A probable precursor for these toxicants is 2-*exo*,10-dichlorobornane, a major product at an early stage in chlorination of camphene (Jennings and Herschbach, 1965; Richey et al., 1965). This dichlorobornane has been used to prepare trichlorobornanes as models for identification of toxaphene components (Parlar et al., 1977) and to obtain a product from which the mixture of 8-Cl-B and 9-Cl-B can be isolated by thin-layer chromatography (TLC) and preparative gas–liquid chromatography (GLC) (Nelson and Matsumura, 1975).

This report gives a procedure for chlorination of 2-*exo*,10-dichlorobornane to prepare B on a gram scale and to obtain 3-*exo*-Cl-B. Additional octa- and nonachlorobornanes, hexa- and heptachlorobornenes, and a hexachlorobornadiene are obtained on chlorination or dehydrochlorination of B or dehydrochlorination of 3-*exo*-Cl-B as shown in Figure 1. These products are identified by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) and used in comparative toxicity studies to determine the relationship of chemical structure and biological activity.

METHODS

Chromatography. The composition of reaction mixtures and the purity of individual products were determined by open tubular column GLC (Saleh and Casida, 1977a), assuming the same response for each component with the electron-capture detector. TLC utilized 20 × 20 cm silica gel 60 F-254 chromatoplates of 0.25 mm layer thickness (EM Laboratories, Inc., Elmsford, N.Y.) for analysis and silica gel F-254 chromatoplates of 0.5 mm layer thickness (EM Laboratories) for product isolation on a 5–10-mg scale. The products on the chromatoplates were detected by spraying with diphenylamine (10% w/v) in acetone and irradiating with ultraviolet light. Column chromatography utilized silicic acid (AR-100 mesh, Mallinckrodt Inc., St. Louis, Mo.; column length, ~10 times the diameter) packed in technical grade hexane or hexane saturated with dimethylformamide (DMF) and developed with the same solvent under 7–9 lb of N_2 pressure to increase the elution rate. In most cases, bifluorenylidene (~10 mg) was added as a marker dye (Saleh and Casida, 1977a), since it elutes in almost the same position as B.

Spectroscopy and Elemental Analyses. NMR spectra (360 MHz) were obtained on a Bruker HX-360 spectrometer at the Stanford Magnetic Resonance Laboratory (Stanford University, Stanford, Calif.) by arrangement with W. Conover and as supported by NSF Grant GR 23633 and NIH Grant RR 00711. Spectra (90 MHz) were run on a Perkin-Elmer R-32 spectrometer. Chemical ionization (CI)–MS and GLC–CI–MS determinations utilized a Finnigan Model 1015D mass spectrometer as previously described (Holmstead et al., 1974) but with isobutane as the ionizing gas. Elemental analyses were carried out at the Department of Chemistry, University of California, Berkeley, Calif., on samples prepared by evaporating hexane solutions and drying at pump vacuum for 14 h.

Bioassays. Procedures for determining the 24-h LD_{50} values with male albino mice, adult female houseflies, and goldfish were as previously reported (Saleh and Casida, 1977a; Turner et al., 1975) except that the volume of water for the goldfish was 3 L rather than 4 L. In synergism studies, piperonyl butoxide (PB) was applied topically to the houseflies at 250 μg/g 30 min prior to the chlorinated hydrocarbon or administered intraperitoneally (ip) to the mice at 150 mg/kg 1 h prior to the test compound. The carrier solvent for PB was 1 μL of acetone for the houseflies and 50 μL of dimethyl sulfoxide for the mice.

EXPERIMENTAL PROCEDURES

B and 3-*exo*-Cl-B from Chlorination of 2-*exo*,10-Dichlorobornane. 2-*exo*,10-Dichlorobornane (52 g) (Richey et al., 1965) containing up to 25% 2-*exo*,10,10-trichlorobornane (Parlar et al., 1976, 1977) was dissolved in CCl_4 (1 L) which was then heated to boiling to drive out O_2 and cooled under N_2. Chlorine was bubbled into the solution at ~0 °C, the amount added being determined by weight gain. Stirring the solution under a sunlamp resulted in rapid HCl evolution (probably with some loss of Cl_2) and disappearance of the yellow color within a few minutes. GLC monitoring revealed that addition of 105.5 g of Cl_2 gave the optimal yield of B (12%) (Table I). Half

Table I. Products from Chlorination of Camphene, 2-*exo*,10-Dichlorobornane, and 2,2,5-*endo*,6-*exo*,8,9,10-Heptachlorobornane (B)

Compound or mixture analyzed and elemental composition of mixture, %	Hepta- and octachlorobornanes					
	B	3-*exo*-Cl-B	5-*exo*-Cl-B	8-Cl-B	9-Cl-B	10-Cl-B
	GLC t_R, min (peak no. designation)[a]					
Individual polychlorobornanes	63.8 (9)	79.4 (14)	95.8	82.2 (16)	82.2 (16)	90.7 (23)
	Composition, %[b]					
Chlorination of tech. grade camphene[c] C, 28.7; H, 2.4; Cl, 68.9 ($C_{10}H_{10.0}Cl_{8.1}$)	8	3	<1	5	3	4
Chlorination of 2-*exo*,10-dichlorobornane C, 30.90; H, 2.62; Cl, 66.68 ($C_{10}H_{10.1}Cl_{7.3}$)	12	2	<1	5		3
C, 27.43; H, 2.15; Cl, 70.27 ($C_{10}H_{9.3}Cl_{8.7}$)	6	4	<1	12	7	5
Chlorination of B C, 29.03; H, 2.67; Cl, 67.92 ($C_{10}H_{11.0}Cl_{7.9}$)	70	4	2	11	5	6
No elemental analysis	22[d]	12	1	27	13	13

[a] Procedure of Saleh and Casida (1977a). The identity of 3-*exo*-Cl-B with 14 and of 10-Cl-B with 23 is based on GLC cochromatography and the TLC-GLC method of Saleh and Casida. Additional t_R values (min) for four nonachlorobornanes are 104.0 for 3-*exo*,10-Cl_2-B, 107.6 for 8,10-Cl_2-B, and 102.7 and 104.4 for two unidentified compounds. [b] Based on GLC analysis. The yields of B and possibly some other components include other materials not adequately separated by GLC, particularly in the chlorination products of tech. grade camphene and 2-*exo*,10-dichlorobornane. The 8-Cl-B plus 9-Cl-B content is based on GLC and their ratio on NMR (Turner et al., 1975, for toxaphene; this study for the chlorination products of 2-*exo*,10-dichlorobornane and B). [c] Data for standard toxaphene from Saleh and Casida (1977a). [d] Additional components are 3-*exo*,10-Cl_2-B (2%), 8,10-Cl_2-B (5%), and two unidentified nonachlorobornanes (2 and 3%). These nonchlorobornanes also appear in small amounts on chlorination of B to 30% conversion but they are not detected (<1%) in toxaphene or in the chlorination products of 2-*exo*,10-dichlorobornane.

of the product mixture was combined with the marker dye and chromatographed on a silicic acid column (1 kg) with hexane. Details on this column chromatographic procedure are given by Saleh and Casida (1977b). Elution of the yellow dye and B began after 10.6 L of hexane had been eluted and was essentially complete after an additional 3.8 L of hexane. The crystals obtained on concentration of the fractions weighed 1.2 g (2.5% yield of B from 2-*exo*,10-dichlorobornane) after washing with hexane and 0.7 g after recrystallization from hot acetone (>98% pure B; mp 222 °C).

In a second preparation, pure 2-*exo*,10-dichlorobornane (20 g) was chlorinated as above, using 52 g of Cl_2, yielding 6% B, 4% 3-*exo*-Cl-B and 19% 8-Cl-B plus 9-Cl-B (Table I). Fractions from chromatography as above containing 65–71% B were processed in the usual manner to obtain 220 mg of B. Those containing 22–25% 3-*exo*-Cl-B were concentrated and on standing yielded crystals, which were washed with hexane and recrystallized from hot acetone to give 3-*exo*-Cl-B (220 mg, >98% pure, mp >240 °C). Fractions containing 44–55% of 8-Cl-B plus 9-Cl-B were combined (7.2 g) and rechromatographed on a silicic acid column (265 g) with DMF-saturated hexane as eluent to yield 8-Cl-B plus 9-Cl-B in ~70% purity.

Octa- and Nonachlorobornanes from Chlorination of B. A solution of B (1.63 g; 99% pure) in CCl_4 (100 mL) was heated to boiling, cooled, and stirred under N_2. To this solution was added Cl_2 (0.2 g) in CCl_4 previously boiled and stored under N_2. On stirring the solution under a sunlamp, the Cl_2 color disappeared within 3 min. Conversion of B was 30% to the products shown in Table I. Most of the unreacted B and a small portion of the 3-*exo*-Cl-B were removed as crystals on recrystallization of the chlorination product mixture from hexane and then acetone. Chromatography of the supernatant from recrystallization on a silicic acid column (265 g) with hexane separated the octachlorobornanes and two of the nonachlorobornanes as shown in Figure 2. The fractions richest

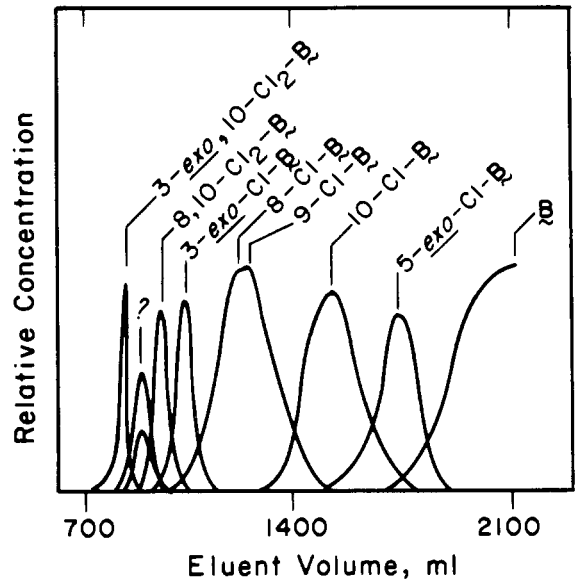

Figure 2. Chromatography of B and its chlorination products on a silicic acid column (265 g) developed with hexane. The elution position of two unidentified nonachlorobornanes is designated by ?.

in each component were combined. The 5-*exo*-Cl-B obtained (25 mg; 70% purity containing 9% B, 2% 8-Cl-B plus 9-Cl-B and 14% 10-Cl-B) was not further purifiable by TLC or recrystallization. Early fractions of the mixture of 8-Cl-B and 9-Cl-B were found to be >90% 8-Cl-B (NMR). Recrystallization of one fraction gave relatively pure 8-Cl-B (23 mg; <2% 9-Cl-B; 5% 3-*exo*-Cl-B). The last fractions of the 8-Cl-B and 9-Cl-B mixture gave an 8-Cl-B:9-Cl-B ratio of 1:1.5 but they were already rich in 10-Cl-B. The next eluting portion was used to obtain 10-Cl-B after recrystallization from hot hexane (32 mg; 98% purity). The small amount of 3-*exo*,10-Cl_2-B obtained

(12 mg) was >86% pure (containing no B, 8-Cl-B or 9-Cl-B) and was not further purified. 8,10-Cl_2-B from this preparation (22 mg) was combined with that from another preparation and subjected to recrystallization from hot hexane, further purification by preparative TLC with hexane and another crystallization (>75% purity containing no B, 8-Cl-B, or 9-Cl-B).

Hexa- and Heptachlorobornenes and a Hexachlorobornadiene from Dehydrochlorination of B and 3-*exo*-Cl-B. A solution containing B (143 mg; >90% purity) and KOH (~2 g) in ethanol (70 mL) was held at 25 °C for 28 h, yielding B (10%) and two dehydrochlorination products [90%; B–HCl(5,6) and B-HCl(3,2) in a 2.4:1 ratio]. The GLC t_R values (min) at 200 °C isothermal are 20.2 for B, 11.6 for B–HCl(3,2), and 10.5 for B–HCl(5,6). Their TLC R_f values with hexane for development are 0.08 for B, 0.12 for B–HCl(3,2), and 0.19 for B–HCl(5,6). Water was added to the residue after ethanol evaporation, and the hexane-soluble products were purified by TLC as above or by chromatography on a silicic acid column (36 g) with hexane. The column separation yielded B–HCl(5,6) (39 mg) eluting in fractions from 370–480 mL total volume and B–HCl(3,2) (31 mg) in the 620–780-mL region. Recrystallization from hot hexane gave B–HCl(3,2) in 95% purity, while recrystallization from a small volume of hexane gave B–HCl(5,6) in >98% purity. On dehydrochlorination of B by dissolving it in *n*-propylamine and holding for several days, the product mixture consisted of 10% B and 90% B–HCl(5,6) plus B–HCl(3,2) in a 6:1 ratio. Both of the dehydrochlorination products, when exposed to air and light, became discolored by a brown, hexane-insoluble material.

3-*exo*-Cl-B (51 mg) in ethanol (40 mL) containing KOH (250 mg) reacts rapidly (>99% in 1 h) to give 3-*exo*-Cl-B-HCl(5,6) (>97%) and 3-*exo*-Cl-B–2HCl (1%). The GLC t_R values at 200 °C isothermal are 25.1, 12.7, and 7.3 min, respectively, for 3-*exo*-Cl-B and its mono- and didehydrochlorination products. After preparative TLC with hexane (R_f 0.17 for 3-*exo*-Cl-B and 0.26 for both dehydrochlorination products), 3-*exo*-Cl-B–HCl(5,6) was recrystallized (>99% purity) from a 2:1 mixture of tetramethylsilane and hexane, the tetramethylsilane serving to reduce the solubility in hexane. On treatment with more concentrated KOH, 3-*exo*-Cl-B-HCl(5,6) eliminates another HCl to give 3-*exo*-Cl-B-2HCl. This hexachlorobornadiene was also obtained directly from 3-*exo*-Cl-B (10 mg) on treatment with KOH (~100 mg) in ethanol (3 mL) for 1 h (>99% conversion). Several such preparations were combined and subjected to preparative TLC to obtain 3-*exo*-Cl-B–2HCl [17 mg; oil; 95% with 5% 3-*exo*-Cl-B–HCl(5,6)]. A brown impurity, removable by preparative TLC, was formed on exposure of its CCl_4 solutions to light and air.

RESULTS AND DISCUSSION

Preparation of Polychlorobornanes and Polychlorobornenes. On photochlorination, camphene and 2-*exo*,10-dichlorobornane yield relatively large amounts of B and its 3-*exo*-Cl, 8-Cl, 9-Cl, and 10-Cl derivatives (Table I). This is the first report of 3-*exo*-Cl-B and 10-Cl-B as toxaphene components. Since all these octachlorobornanes, along with 5-*exo*-Cl-B, are also formed on photochlorination of B (Table I), it appears likely that B is their major precursor in both toxaphene and chlorinated 2-*exo*,10-dichlorobornane.

There is remarkable selectivity in chlorination of 2-*exo*,10-dichlorobornane to B, since this product is one of 943 isomers derivable by addition of five chlorine atoms to the dichlorobornane. Considerable site selectivity also exists in photochlorination of B, i.e., C-8 > C-9 = C-10 ≥ C-3 > C-5. The two endo hydrogens (H-3N and H-6N) and the bridgehead hydrogen (H-4) appear not to be substituted at all. The greater resistance of position 5-exo to substitution relative to position 3-exo may be due to the deactivating effect of the 5-endo chlorine or to steric protection of this site by chlorines at positions 5-endo, 6-exo, and 8, as observed in comparison of a space-filling model with the crystal structure of B (Palmer et al., 1975). The 3-endo position of B is considerably protected by the two endo chlorine atoms, and the 6-endo position is almost completely occluded by these chlorine atoms and the one on C-10. Studies on norbornane also indicate that the bridgehead hydrogen is very unreactive, presumably because the dihedral angle at the bridgehead does not lend itself to stabilization of a radical, and that the endo positions are substituted less readily than the exo positions, possibly because of steric effects on chlorine approaching the radical (Kooyman and Vegter, 1958; Poutsma, 1969; Walling and Mayahi, 1959).

Chlorination of 2-*exo*,10-dichlorobornane with GLC monitoring of the product composition provides a convenient source of B on a gram scale. At an optimal B content of 12%, about one-fifth of this amount can be isolated pure by a single column chromatography, followed by recrystallization. The easiest products to isolate are B and 3-*exo*-Cl-B because of their low solubility in hexane, acetone, and some other solvents relative to most toxaphene components. On chlorination of the dichlorobornane to a higher chlorine content, the amount of B is reduced to 6% and that of 3-*exo*-Cl-B, 8-Cl-B, and 9-Cl-B is multiplied by a factor of 2 to 4 (Table I). Chromatography of this product yields a mixture containing 70% 8-Cl-B and 9-Cl-B, but recrystallization does not provide further purification. Thus, chlorination of 2-*exo*,10-dichlorobornane provides convenient access to B and 3-*exo*-Cl-B but not to the other individual octachlorobornanes. Fortunately, direct chlorination of B yields 8-Cl-B and 9-Cl-B in a purity such that 8-Cl-B can be obtained by chromatography and recrystallization. In addition to the five identified octachlorobornanes and two identified nonachlorobornanes which are easily separated by column chromatography, two unidentified nonachlorobornanes are also formed, but they are not separated by this chromatographic technique (Table I, Figure 2).

Dehydrochlorination of B and 3-*exo*-Cl-B with KOH or propylamine yields polychlorobornenes. An alternative dehydrochlorination of either compound via the equivalent of a Wagner-Meerwein rearrangement might give cis and trans isomers of two 8-chlorocamphene derivatives. An analogy for this rearrangement is the dehydrochlorination of 2-*exo*,10-dichlorobornane with dimethylaniline (Jennings and Herschbach, 1965). However, the NMR spectra of the dehydrochlorination products of B and 3-*exo*-Cl-B are not appropriate for the 8-chlorocamphene derivatives which would result from such a rearrangement. The preponderance of HCl elimination from the 5,6 positions of B over the 3,2 positions is expected from the greater acidity of the 5-*exo*-hydrogen over the 3-*exo*-hydrogen, due to the electron-withdrawing properties of the 5-*endo*-chlorine. The 5-*exo*-hydrogen is more easily removed than the 6-*endo*-hydrogen. This is predictable from the almost exclusive exo-cis dehydrohalogenations observed in *trans*-2,3-dihalonorbornanes (LeBel et al., 1964). Lacking an exo hydrogen in position 2 or 3, 3-*exo*-Cl-B can form a 2,3-olefin only by some mechanism other than exo-cis dehydrochlorination, and less than 1% of such a product is obtained in the initial dehydrochlorination. With more

concentrated base, however, the monodehydrochlorination product from 3-*exo*-Cl-B undergoes further loss of HCl to form a hexachlorobornadiene.

Chromatographic Properties. Consistent chromatographic patterns related to the number of chlorine atoms are evident for B and its polychlorobornane derivatives on a silicic acid column (Figure 2) and on open tubular column GLC (Table I). With the silicic acid column, the sequence of elution is four nonachlorobornanes first, then five octachlorobornanes, and finally the heptachlorobornane (B). The GLC t_R values of these bornane derivatives decrease in the same sequence. In addition, the GLC t_R values decrease further in the sequence of two heptachlorobornenes, two hexachlorobornenes, and one hexachlorobornadiene. These patterns are probably restricted to compounds within a closely related series, since many exceptions are evident in the variety of components in toxaphene (Casida et al., 1975; Holmstead et al., 1974).

Identification of New Compounds. Mass spectrometry confirmed that the major products of chlorination of B are $C_{10}H_{10}Cl_8$ and $C_{10}H_9Cl_9$ compounds and that base treatment of B and 3-*exo*-Cl-B leads initially to $C_{10}H_{10}Cl_6$ and $C_{10}H_9Cl_7$ derivatives, respectively. In CI–MS with methane as the ionizing gas, toxaphene components generally give no M + 1 ions, but instead have their highest masses at M – Cl (Holmstead et al., 1974). In the present study using isobutane as the ionizing gas, the identified products from chlorination of B conform to this rule, as do B–HCl(5,6) and 3-*exo*-Cl-B–HCl(5,6). However, B–HCl(3,2) gives a weak molecular ion (*m/e* 340) and a weak M – 1 ion (*m/e* 339) as well as the base peak at *m/e* 305 (M – Cl). The fragmentation patterns of B–HCl(5,6) and B–HCl(3,2) are almost identical in other respects, with major fragment ions at *m/e* 305, 304, 269, 244, 209, 195, 173, 159, and 125. 3-*exo*-Cl-B–2HCl has a molecular ion at *m/e* 338 and an M + 1 ion at *m/e* 339, as well as prominent fragment ions at *m/e* 303, 302, 267, 266, 231, and 193.

NMR spectroscopy, employed previously to assign the structures of 8-Cl-B and 9-Cl-B (Matsumura et al., 1975; Turner et al., 1975), also proved useful in structural assignments for the new compounds in the present study (Table II). The structure of each compound, except the dehydrochlorination products of 3-*exo*-Cl-B, is readily evident from the number and coupling patterns of protons at low and high fields. However, some difficulty is encountered in resolving and assigning all the resonances, particularly the chloromethyl and chloromethylene protons. Determining the spectra in several different solvents and in some cases at 360 MHz allowed the chemical shifts of protons incompletely resolved in CCl_4 solution to be estimated.

These structural assignments proceeded from the assumption that the bornane skeleton of 2-*exo*,10-dichlorobornane and B does not undergo any rearrangement on chlorination. While rearrangement during photochlorination is reported for bicyclic compounds with considerably strained three- and four-membered rings, it is not expected in less strained systems (Poutsma, 1969) such as bornane derivatives. The resemblance of observed coupling constants in the new compounds to analogous coupling constants in B (Palmer et al., 1975) supports their proposed bornane skeletons. A departure from the coupling patterns of B, however, is the absence of some long-range coupling between protons on the bridge chloromethyl groups C-8 and C-9. In B, each of the chloromethyl group protons, in addition to geminal coupling, shows ~1.8 Hz four-bond coupling across the bridge to one other proton. The fact that none of these protons shows long-range coupling to two protons suggests the importance of the relative conformations of the chloromethyl groups in B (Palmer et al., 1975). It is thus not surprising that addition of chlorine to the molecules should alter these conformations and thus the long-range coupling. Addition of chlorine is less likely to affect angles of protons attached to the relatively rigid six-membered ring of bornane, and thus four-bond coupling of H-3X to H-5X, as observed in B, is expected in related compounds which contain both of these protons.

10-Cl-B has a singlet at δ 6.81, indicating the presence of one dichloromethyl group. Two of the three possible isomers with one dichloromethyl group are already identified (8-Cl-B and 9-Cl-B), so the dichloromethyl group in the third isomer must be C-10. The absence of long-range coupling to this dichloromethyl proton is in accord with this structure. The coupling constants of the ring protons are essentially the same as in B. Although H-5X is isochronous with chloromethyl group proton 9b, its distinctive coupling pattern can be observed in the 360 MHz spectrum, and it can be decoupled from H-3X, H-4, and H-6N. Several resonances of this compound are broad, probably reflecting long-range coupling: 6N and 8b are broadened doublets, while 8a (and probably the obscured 9b) appears as a broad singlet.

3-*exo*-Cl-B was examined in several solvents and solvent mixtures, but complete resolution of the resonances was not achieved in any individual spectrum; a benzene–$CHCl_3$ mixture (2:1) gave the best resolution. The C-3 methylene group is not present, since there is only a single proton resonating at high field (a doublet, H-4), compared with three in B. If the remaining H-3 were exo, H-4 would be an apparent triplet (overlapping doublet of doublets), rather than a simple doublet, since it would be coupled to both H-3X and H-5X; the absence of long-range coupling to H-5X also indicates that H-3X is lacking. Irradiation of H-5X collapsed the doublets for H-4 and H-6N to singlets. In this compound, only one proton on C-8 is coupled to one on C-9.

5-*exo*-Cl-B was the most difficult of these octachlorobornanes to identify, because neither the three protons at higher field nor the six chloromethyl group protons are fully resolved, even at 360 MHz. After identification of 3-*exo*-Cl-B, 8-Cl-B, 9-Cl-B, and 10-Cl-B, however, there remain just four positions (3-endo, 4, 5-exo, and 6-endo) to which a chlorine can be added to B. Only addition of chlorine to position 5-exo would yield a compound with three protons at higher field (H-3N, H-3X, and H-4) and an isolated singlet at δ 5.78 (H-6N). Support for the structure was obtained by computer simulation of the complex multiplets for the three upfield protons as they appear in two different solvents, CCl_4 and benzene, using the same set of coupling constants, but different chemical shifts. As expected, H-3X lacks the four-bond coupling to H-5X anticipated if the latter proton remained in the molecule.

8,10-Cl_2-B has resonances of two dichloromethyl group protons, one of which has 2 Hz coupling to one of the protons of the remaining chloromethyl group. This must be four-bond coupling, indicating that the chloromethyl group and coupled dichloromethyl group are on the bridge. Thus, 8,10-Cl_2B is related to 10-Cl-B in the same way in which 8-Cl-B or 9-Cl-B is related to B, so the assignment of the dichloromethyl group to C-8 or C-9 is similar to its earlier assignment in 8-Cl-B and 9-Cl-B. In the latter two compounds, relative to B, there is significant deshielding of the 3-exo or 5-exo proton to which the dichloromethyl

Table II. NMR Spectra of Hepta-, Octa-, and Nonachlorobornanes, Hexa- and Heptachlorobornenes, and a Hexachlorobornadiene

Compound	Protons										
	3N	3(X)	4	5X	6(N)	8a	8b	9a	9b	10a	10b
					Chemical Shifts[a]						
3-*exo*-Cl-B	5.20		2.73	4.67	5.24	4.10[b]	4.75[c]	4.16[b]	4.54[c]	3.76[b]	4.67[b]
5-*exo*-Cl-B	3.58	3.22	3.15		5.78	4.06[d]	4.26[d]	4.26[d]	4.31[d]	4.43[d]	4.51[d]
10-Cl-B	3.36	3.05	2.65	4.66	5.32	4.15[e]	4.28[e]	4.44[e]	4.66[e]	6.81	
3-*exo*,10-Cl_2-B	5.22		2.77	4.73	5.24	3.99[e]	4.73[c,e]	4.45[e]	4.57[e]	6.88	
8,10-Cl_2-B	3.54	~3.10	~3.15	5.04	5.52	6.94		4.54	5.15	6.70	
B-HCl(3,2)		6.32	3.28	4.59	4.00	3.97[c,e]	4.30[e]	3.79[e]	4.29[c,e]	4.08	4.31
B-HCl(5,6)	2.62	3.00	2.93		6.05	4.14[c,e]	4.24[e]	4.03[e]	4.16[c,e]	3.98	4.51
3-*exo*-Cl-B-HCl(5,6)	4.57		3.14		6.15	4.02[c,e]	4.35[e]	4.72[c,e]	4.10[e]	3.96	4.58
3-*exo*-Cl-B-2HCl[f]			3.08		5.79	3.57[e]	3.76[c,e]	3.65[c,e]	3.83[e]	3.46	3.46
					Coupling Constants[g]						
3-*exo*-Cl-B	s		4.6	4.5, 4.5	4.5	12.5	13, 2.5	12	12, 2.5	13	12
5-*exo*-Cl-B	15.5	15.5, 4.5	4.5		s	~12	~12	~12	~12	~12	~12
10-Cl-B	16.5	16.5, 5,2	5,5	5, 5, 2	5[h]	i	~12[h]	13	*j*	s[h]	
3-*exo*,10-Cl_2-B	s		5	5, 5	5	~13[h]	~13, 2[h]	13	~13[h]	s[h]	
8,10-Cl_2-B	17	17, 4.5, 2	4.5, 4.5	4.5, 4.5, 2	4.5	2		14	14, 2	s	
B-HCl(3,2)		4	4,4	4,4	3.5	12,2	12.5	12.5	12.5, 1.5	12.5	11.8
B-HCl(5,6)	15	15, 3	3[h]		s[h]	11.8, 2.5	11.5	12.5	12.5, 2.5	12	12
3-*exo*-Cl-B-HCl(5,6)	s		1.2[h]		1.2	11.5, 2.5	12[h]	13, 2.5	12[h]	12.5	12
3-*exo*-Cl-B-2HCl			1.7		1.7	*k*	*c,k*	*c,k*	*k*	*l*	*l*

[a] Ppm downfield from tetramethylsilane in CCl_4, except for 3-*exo*-Cl-B-2HCl which was run in C_6D_6. [b] These assignments are arbitrary. [c] These appear as distinct doublets (geminal and four-bond coupling) and are therefore assigned to C-8 or C-9. [d] These are not assigned because impurities interfered with measurement of relative line intensities. The downfield pair, however, is coupled to each other. [e] It could not be ascertained which set of protons is on C-8 and which on C-9. [f] The data tabulated are in C_6D_6. In CCl_4, H-4 appears at δ 3.33 and H-6 at δ 6.53. The other resonances, except for a doublet at δ 4.28 (J = 13 Hz), are incompletely resolved at δ 3.85–4.2. [g] Except for H-3N, H-3X, and H-4 for 5-*exo*-Cl-B, for which coupling constants were obtained by computer simulation, these numbers represent observed line separations, not calculated or averaged coupling constants; s = singlet. [h] Broad. [i] Singlet but broad enough to include 12–13 Hz coupling. [j] Obscured by H-5X. [k] The resonances at δ 3.57 and 3.76 are coupled to each other (J ~ 13 Hz) as are those at δ 3.65 and 3.83. The 360 MHz spectrum was not expanded to allow accurate measurement of coupling constants. [l] Presumably if these were not isochronous they would show ~13 Hz coupling to each other.

Table III. Biological Activity of 2,2,5-*endo*,6-*exo*,8,9,10-Heptachlorobornane (B) and Related Octa- and Nonachlorobornanes, Hexa- and Heptachlorobornenes, and a Hexachlorobornadiene

Compound	Purity, %	LD_{50}, 24 h						
		Mouse ip, mg/kg			Housefly topical, μg/g			Goldfish, ppb
		−PB	+PB	−PB/+PB	−PB	+PB	−PB/+PB	
Comparison Standard								
Toxaphene		47	42	1.1	18.0	9.5	1.9	20
Heptachlorobornane								
B	>98	75	9.5	7.9	11.5	2.4	4.8	2.9
Octachlorobornanes								
3-*exo*-Cl-B	>98	>100	>100		18.5	3.2	5.8	43
5-*exo*-Cl-B	70	~24	~28	~0.9	26	7.5	3.5	13
8-Cl-B	>93	3.3	3.1	1.1	5.5	2.2	2.5	1.1
8-Cl-B (57%) + 9-Cl-B (43%)	>92	2.5	1.9	1.3	3.1	1.9	1.6	0.55
10-Cl-B	98	>100	48	>2.1	80	34	2.4	36
Nonachlorobornanes								
3-*exo*,10-Cl_2-B	>86				95	65	1.5	>100
8,10-Cl_2-B	>75				60	22	2.7	44
Hexachlorobornenes								
B-HCl(3,2)	95				36	11	3.3	27
B-HCl(5,6)	>98	~65	~50	~1.3	225	85	2.6	>100
Heptachlorobornene								
3-*exo*-Cl-B-HCl(5,6)	>99	>100	>100		105	35	3.0	>100
Hexachlorobornadiene								
3-*exo*-Cl-B-2HCl	95				105	29	3.6	>100

group is syn, without significant change in the chemical shift of the exo proton to which it is anti (Matsumura et al., 1975; Turner et al., 1975). In benzene solution, where this effect is seen most dramatically, protons 3X and 5X of 8,10-Cl_2-B are shifted downfield 0.05 and ≥0.58 ppm, respectively, relative to their chemical shifts in 10-Cl-B; thus, the dichloromethyl group must be C-8, i.e., syn to the 5-exo proton. In 8,10-Cl_2-B, H-4 resonates 0.75 ppm downfield of its chemical shift in 10-Cl-B, and this effect also has an analogy in the chemical shift of H-4 of 8-Cl-B and 9-Cl-B relative to B.

3-*exo*,10-Cl_2-B has an NMR spectrum similar to that of 3-*exo*-Cl-B in that both have a doublet for H-4 as the single proton at high field, indicating that chlorine has been added to position 3X. The broadness of the dichloromethyl proton might suggest that it is on C-8 or C-9 with small four-bond coupling, but this is not likely, since H-4 is not significantly shifted downfield relative to H-4 of 3-*exo*-Cl-B as noted above in compounds with C-8 and C-9 dichloromethyl groups. As in 10-Cl-B, other resonances (H-8a and H-9b) are broad. The resonances of H-5X, although isochronous with those of chloromethyl proton 8b at δ 4.73, are apparent in the 360 MHz spectrum; absence of coupling of H-5X to a 3-exo proton is confirmed by an INDOR experiment involving monitoring the resonances of H-4 or H-6N.

The dehydrochlorination products of B have either one proton [B–HCl(3,2)] or three protons [B–HCl(5,6)] at high field. Thus the methylene group at C-3 of B is preserved in B–HCl(5,6) but not in B–HCl(3,2). The vinyl proton of B–HCl(5,6) appears as a singlet. If it were at C-5, it would be expected to show 3 to 4 Hz coupling to H-4, as H-3 does in B–HCl(3,2). The broadness of H-4 and H-6 suggests small allylic coupling in B–HCl(5,6). In comparison with B, protons 3N in B–HCl(5,6) and 6N in B–HCl(3,2) are shifted considerably to higher field. This effect is probably due to decreased deshielding by chlorine atoms on C-2 and C-5 in B, which are lost or repositioned on formation of the double bond; this chemical shift change is not expected to arise from mere introduction of the double bond into the bornane system (Jackman and Sternhell, 1969).

3-*exo*-Cl-B–HCl(5,6) has a vinyl proton, indicating that the double bond is in position 5,6, rather than 2,3. Although this vinyl proton is coupled to H-4, the observed coupling of 1.2 Hz (confirmed by a decoupling experiment) is more likely to be allylic $J_{4,6}$ than vicinal $J_{4,5}$ (see above). The broadness of the resonances for H-4 and H-6 in the analogous B–HCl(5,6) is attributed to small allylic coupling. The remaining endo proton (H-3N), like those of the dehydrochlorination products of B, resonates at higher field than in its saturated precursor.

3-*exo*-Cl-B–2HCl also has a single vinyl proton coupled to the bridgehead proton, and here the coupling constant is increased to 1.7 Hz. The protons of the three chloromethyl groups fall in a range of δ <0.4. Although a 360 MHz spectrum in C_6D_6 separated the protons on C-8 and C-9, those on C-10 were isochronous and thus appeared as a singlet.

Relationship of Chemical Structure and Biological Activity. Toxaphene is moderately toxic to mice and houseflies and highly toxic to goldfish (Table III). The synergist PB increases its housefly toxicity but not its mouse toxicity. Compound B (>98% purity) is less toxic to mice than toxaphene, but with PB-treated mice it is more toxic than toxaphene, as a result of the 7.9-fold synergism. The previous finding that B is more toxic than toxaphene to mice (Khalifa et al., 1974) suggests the presence of a minor impurity (<10%) of high toxicity in the sample used in the earlier studies. This impurity is not removed by column chromatography or preparative GLC, but is minimized on recrystallization. With houseflies, in the presence or absence of PB, and with goldfish, B is 1.6 to 7 times as toxic as toxaphene. Addition of a chlorine atom at the 3-exo or 5-exo position generally reduces the toxicity of B to each species. A large toxicity increase results in introducing the 8-chloro substituent into B, except with houseflies in the presence of PB. The mixture of 8-Cl-B and 9-Cl-B is 1.2 to 2.0 times as toxic as 8-Cl-B alone, so 9-Cl-B is as much as four times as toxic as 8-Cl-B. Introduction of chlorine at C-10 greatly reduces the toxicity of B and its 3-*exo*-chloro- and 8-chloro derivatives. The toxicity of B and 3-*exo*-Cl-B is reduced by dehydrochlorination, particularly when the olefin is formed at the 5,6 position. Three of the samples assayed (5-*exo*-Cl-B, 3-*exo*,10-Cl_2-B, and 8,10-Cl_2-B) were of only

moderate purity (70–>86%). It is not known to what extent the reported potency values for these compounds are due to the assigned structures as opposed to impurities. However, it is clear that each of these compounds is of low toxicity relative to B, 8-Cl-B, and 9-Cl-B.

In general, the potency of compounds formed on introducing one chlorine substituent into B decreases in the order: 9-chloro > 8-chloro > none > 3-*exo*-chloro or 5-*exo*-chloro or 10-chloro. It appears that PB-sensitive mechanisms detoxify B more readily than its 8-chloro- and 9-chloro derivatives.

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α- and β-Alkyl Substituted Cinnamates as Pyrethrum Synergists

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Series of α- and β-alkyl substituted cinnamates have been investigated as pyrethrum synergists against the red flour beetle, *Tribolium castaneum* Herbst. All the methylenedioxy cinnamates showed synergism, the factors of synergism ranging between 1.71–6.43 and 1.72–4.07 for the α- and β-substituted esters, respectively. The 1:5 insecticide:synergist level invariably showed better activity over 1:1 level. The increased length of the alkyl substitutent at these positions did not correspondingly increase the synergistic activity of these esters, maximum activity being attained with a C-2 substituent. On the contrary, lipophilicity of such molecules increased directly with the increased number of carbon atoms of the alkyl substituent, indicating thereby that a C-2 substituent provides enough lipophilicity for such molecules to exhibit maximum synergism. The esters without the methylenedioxy group, mostly, showed antagonism.

Importance of methylenedioxyphenyl compounds as synergists for oxidatively metabolized insecticides is well known. Moore and Hewlett (1958) investigated the effect of different side chains on the synergistic properties of such compounds. Schroeder et al. (1948) and Carson and Eddy (1949) reported that the methylenedioxy cinnamates also possessed synergistic properties toward pyrethrum. However, information on the effect of alkyl substitution on this property of cinnamates is, in general, lacking and the same is reported here.

MATERIALS AND METHODS

n-Alkanoic acids from C_3 to C_{10}, methyl iodide, *n*-alkyl bromides from C_2 to C_4, and piperonal were procured and used without further purification. Benzaldehyde was washed with sodium bicarbonate solution followed by water, dried, and distilled. Anhydrides of *n*-alkanoic acids from C_3 to C_8 were prepared from the corresponding acid chlorides and their potassium or sodium salts by the general procedure detailed by Vogel (1948). 3,4-Methylenedioxyphenyl alkyl ketones were made by Jones oxidation (Bowden et al., 1946) of the corresponding carbinols obtained by condensing piperonal with the corresponding *n*-alkylmagnesium bromides. Structures of all new compounds agreed with their spectral and analytical data.

Infrared spectra were recorded on a Perkin-Elmer Model 437 spectrometer either as neat film or solution in CCl_4. Nuclear magnetic resonance spectra were recorded on a Varian A-60 spectrometer using Me_4Si as internal standard.

Synthesis of Test Chemicals. *α-Alkylcinnamic Esters.* The α-alkylcinnamic acids were prepared by the

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