Photolysis of *cis*-Chlordane: Identification of Two Isomers of *cis*-Photochlordane

Muhammad Feroz, Jacques Kagan, Swaminathan Ramesh, and M. A. Q. Khan*

The insecticide *cis*-chlordane was photolyzed under UV light. The major photoreaction product, *cis*-photochlordane, was isolated and resolved into two isomeric forms. One of these products was a previously described (1,3,4,7,8,9,10,10-octachlorotetracyclo[5.2.1.$0^{2,6}$.$0^{4,8}$]decane), and the other was a new compound. The new photoproduct was analyzed by GC–MS, IR, and NMR spectrometry and tentatively assigned the chemical formula 1,3,4,7,8,9,10,10-octachlorotetracyclo[5.2.1.$0^{2,6}$.$0^{4,9}$]decane. The approximate ratio of the former to the latter was 3:1.

Reported studies on photoisomerization of *cis*-chlordane (Figure 1, 1) (Benson et al., 1971; Knox et al., 1973; Lahaniatis et al., 1976; Onuska and Comba, 1975; Parlar and Korte, 1977) suggest the formation of a half bird cage configuration arising from intramolecular rearrangement which involves migration of a proton from the cyclopentane ring to the dichloroethylene moiety of the molecule followed by a cross-linkage of carbon atoms. However, such a cross-linkage is possible between any of the three carbons (C_1; C_2; C_3) of the cyclopentane ring and any of the two carbons (C_5; C_6) of the dichloroethylene bond in *cis*-chlordane. Six isomeric structures are therefore possible (Figure 1, 2–7) in photoisomerization of the compound. Two of these, 2 (Figure 1) (Knox et al., 1973) and 4 (Figure 1) (Lahaniatis et al., 1976), have already been characterized. This report concerns another photoisomer of *cis*-chlordane, the existence of which was realized in metabolic studies with *cis*-photochlordane (Feroz and Khan, 1980).

MATERIALS AND METHODS

Chemicals. Pure unlabeled *cis*-chlordane (Chemical Abstracts nomenclature: 1-*exo*,2-*exo*,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane) was provided by the Velsicol Chemical Corp., Chicago, IL. ^{14}C-Labeled *cis*-chlordane (hexachloronorbornene moiety uniformly labeled, sp act. 10.9 mCi/mmol), was purchased from New England Nuclear Corp., Boston, MA, and was reported to be 97%+ pure. The impurities (~1%) in the ^{14}C-labeled compound were removed by thin-layer chromatography (TLC) and autoradiography. Gas–liquid chromatography (GLC) of the purified material showed only one peak.

The reference standard of *cis*-photochlordane (PC) was also provided by Velsicol Chemical Co. The compound was reported to be 80% pure. Prior to use, the impurities in the standard were removed by TLC.

Photolysis of *cis*-Chlordane. The photoreaction of the ^{14}C-labeled or unlabeled compound was carried out as described elsewhere (Podowski et al., 1979). Briefly, 0.1 M solution of unlabeled *cis*-chlordane with equimolar benzophenone in acetone (5 mL) contained in 16 × 100 mm Kimax tubes was exposed to UV light. The tubes were closed with screw caps having Teflon liners. ^{14}C-Labeled *cis*-chlordane was photolyzed similarly except that concentration of the compound was lower than that of unlabeled compound. The source of UV light was 30- or 96-W germicidal lamps (Westinghouse, Blacklight F15T8, or General Electric, G24T8) mounted in a thin layer plate viewing assembly. The distance of the tubes from the source was 10 cm.

The reaction was monitored with time by employing TLC and autoradiography in the case of ^{14}C-labeled compound and TLC and GLC in the case of the unlabeled compound. Under the conditions, 50% photoisomerization occurred around 288 h although longer exposure periods were used in some experiments to procure higher yields. The photoisomer was isolated at the termination of the exposure.

Thin-Layer Chromatography. Unlabeled PC in the reaction mixture was isolated and purified by TLC using 0.5-mm silica gel G Prekotes (Applied Science Laboratories, State College, PA). After being concentrated under nitrogen, the reaction mixture (~25–30 mg equiv of *cis*-chlordane) was streaked on the plates which were then developed with pure heptane. A single development in the solvent did not give satisfactory separation of PC from the parent compound. Duplicate development (occasionally triplicate) was therefore routinely employed. Initially, chlorine-containing compounds in the mixture (along with Cis-chlordane and PC reference standards) were visualized by spraying one edge (keeping most of the plate covered with aluminum foil) of the plate with acetonic silver nitrate (Mitchell, 1958) and exposing the edge to intense UV irradiation from germicidal lamps. Later, however, a more convenient method was found. Developed chromatoplates were placed against fluorescent light and major products (PC and *cis*-chlordane) could be observed as opaque bands on a translucent background. The areas were marked, scraped, and eluted with diethyl ether or chloroform. The TLC of the PC was repeated 5 more times to achieve near complete purity.

[^{14}C]PC was isolated by a similar procedure except that it involved TLC on 0.25-mm silica gel G-F254 (Brinkmann Instruments, Des Plaines, IL) followed by autoradiography (Kodak No-Screen X-ray Films, NS-5T; G. W. Brady, Skokie, IL) for location of the labeled products. Final purification of [^{14}C]PC was carried out on 0.5-mm silica gel G Prekotes (Applied Science Laboratories) as described above for unlabeled PC.

Gas–Liquid Chromatography. GLC was carried out on a Series 7300 Packard gas chromatographs (Packard Instruments, Downers Grove, IL) equipped with ^{3}H or ^{63}Ni electron capture detectors with the following conditions. (1) 3% SE-30 coated on 80–100-mesh Gas-Chrom Q packed in a 107 × 0.2 cm (i.d.) glass column; operating temperatures were inlet 215 °C, column 190 °C, and detector 215 °C; the Nitrogen flow rate was 40 mL/min. (2) 3% QF-1 coated on 80–100-mesh Chromosorb W-HP packed in a 152 × 0.2 cm (i.d.) glass column; operating temperatures were as in (1); the nitrogen flow rate was 30 mL/min. (3) 6% SE-52 on 60–80-mesh Chromosorb

Department of Biological Sciences (M.F. and M.A.Q.K.) and Department of Chemistry (J.K. and S.R.), University of Illinois at Chicago Circle, Chicago, Illinois 60680.

0021-8561/81/1429-0272$01.25/0

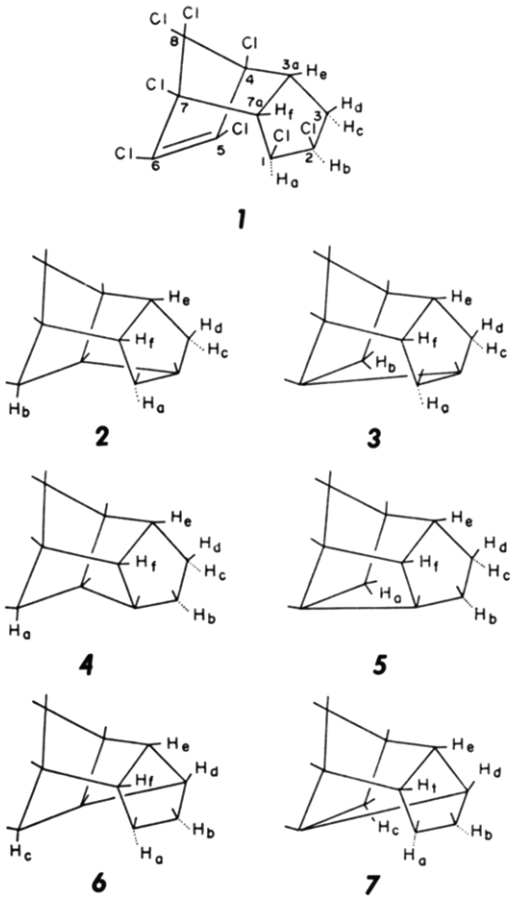

Figure 1. Structural formulas of *cis*-chlordane (1) and some of its possible photoisomers. H's with small letter subscripts show protons and arabic numerals indicate carbon atoms. Chlorine atoms in the photoisomers are not shown for clarity.

W-AW, HMDS packed in a 183 × 0.2 cm (i.d.) glass column; operating temperatures were inlet 230 °C, column 205 °C, and detector 230 °C; the nitrogen flow rate was 55 mL/min. (4) 3% OV-101 on 80–100-mesh Chromosorb W-HP packed in a 183 × 0.2 cm glass column; operating temperatures were as in (3); the nitrogen flow rate was 35 mL/min.

Quantitation. [^{14}C]PC and other ^{14}C-labeled photolysis products of *cis*-[^{14}C]chlordane were quantitated by liquid scintillation counting employing a Model 3390 Packard Tricarb scintillation spectrometer (Packard Instruments). Insta-Gel (Packard Instruments) was used as the scintillation cocktail. The radiometric technique has been described elsewhere (Feroz and Khan, 1979). Unlabeled PC was analyzed by GLC.

Mass Spectrometry. GC–MS analysis was carried out with a Varian MAT 112S mass spectrometer equipped with Varian MAT 166 data system. The mass spectrometer was interfaced with a Varian Aerograph Series 1400 gas chromatograph. A glass column (183 × 0.2 cm) packed with 3% OV-101 on 800–100-mesh Chromosorb W-HP was used in the gas chromatograph. Inlet, column, and interface temperatures were respectively 250, 190, and 235 °C. Helium served as a carrier gas. The mass spectrometer was operated at 70 eV.

Infrared Spectrophotometry. Infrared spectra were recorded on a Perkin-Elmer Model 257 spectrophotometer. KBr micropellets were prepared in an 8-mm Minidie (Beckman Instruments, Lincoln Park, IL), and a beam attenuator was used in recording the spectra.

NMR Spectrometry. Proton magnetic resonance (^{1}H NMR) spectra were recorded on a Varian spectrometer at 60 MHz or on a Bruker WP-80 FT spectrometer (Bruker Instruments, MA) operated at 80 MHz. The compounds were dissolved in $CDCl_3$ (10%) solutions, and tetramethylsilane (Me_4Si) was used as an internal standard. Decoupling studies were conducted on the Bruker spectrometer.

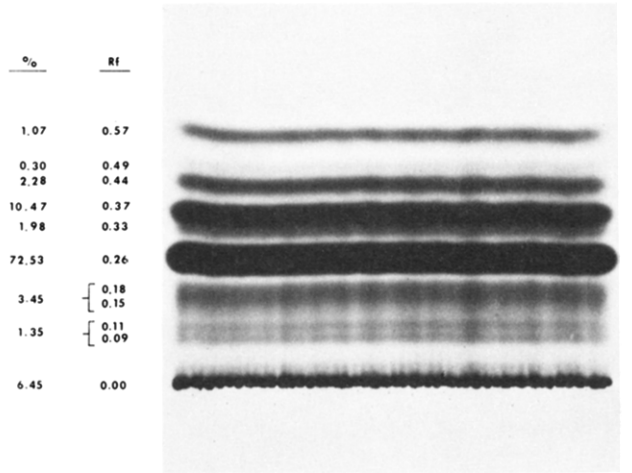

Figure 2. Autoradiogram of photolyzed *cis*-[^{14}C]chlordane (silica gel G-F254, duplicate development with pure heptane). The compounds at R_f 0.26 and 0.37 were respectively *cis*-photochlordane and *cis*-chlordane. The column on the left side indicates percentage recovery of different compounds. About 0.12% of the radioactivity was present in the residual gel on the plate.

Table I. Retention Times of *cis*-Chlordane and *cis*-Photochlordane on Various Gas Chromatographic Columns

column[a]	retention time, min	
	cis-chlordane	PC[b]
QF-1	1.38	2.26
SE-30	4.13	6.59
SE-52	8.26	13.58
OV-101	4.72	7.56

[a] Operating conditions are given in the text. [b] *cis*-Photochlordane.

RESULTS AND DISCUSSION

Photolysis of *cis*-Chlordane and Purification of the Major Product. An autoradiogram of photolyzed *cis*-[^{14}C]chlordane is shown in Figure 2. At least 11 compounds were recognizable in the reaction mixture, of which PC (R_f 0.26) was the major product (72.53%) and the parent compound, *cis*-[^{14}C]chlordane (R_f 0.37), comprised 10.47%. These identities were based on comparative TLC and GLC analyses of the isolated compounds using authentic reference standards.

The band constituting PC, ^{14}C labeled or unlabeled, was scraped from the chromatoplates, eluted with diethyl ether or chloroform, and tested for purity by GLC. The process was repeated until the product eluted as a single peak (and therefore appeared homogeneous) in GLC analysis. Retention times on four different GLC columns are listed in Table I. The compound appeared as a single peak on all these columns.

Separation of Isomeric Forms of PC. Notwithstanding the homogeneity in GLC analysis, metabolic behavior of the compound in rats suggested its complex nature with two isomeric forms (Feroz and Khan, 1980). Several TLC solvent systems of varying polarities (Table II) were employed to separate the suspected PC components which, as Table II shows, had identical R_f values in all except two solvent systems. Hexane–ethyl acetate (9:1)

Table II. Thin-Layer Chromatographic R_f Values of the Two Components in *cis*-Photochlordane[a]

solvent system	R_f values PC I	PC II
n-octane (pure)[b]	0.20	0.20
n-heptane (pure)[b]	0.26	0.26
diethyl ether (pure)	0.61	0.61
chloroform (pure)	0.62	0.62
ethyl acetate (pure)	0.61	0.61
methyl ethyl ketone (pure)	0.62	0.62
cyclohexane-chloroform (9:1)	0.48	0.48
cyclohexane-chloroform (4:1)	0.32	0.32
hexane-methylene chloride (4:1)	0.30	0.30
hexane-ethyl acetate (9:1)	0.40	0.44
hexane-ethyl acetate (3:1)	0.49	0.49
hexane-ethyl acetate (1:1)	0.63	0.63
hexane-methyl ethyl ketone (4:1)	0.43	0.43
hexane-benzene-ethyl acetate (18:1:3)	0.45	0.47
benzene-ethyl acetate (3:1)	0.66	0.66
benzene-ethyl acetate (1:1)	0.66	0.66
chloroform-acetone (9:1)	0.64	0.64
chloroform-methanol (7:3)	0.69	0.69
propanol-ethyl acetate-water (3:2:1)	0.73	0.73

[a] Silica gel G-F254 plates. [b] Duplicate development.

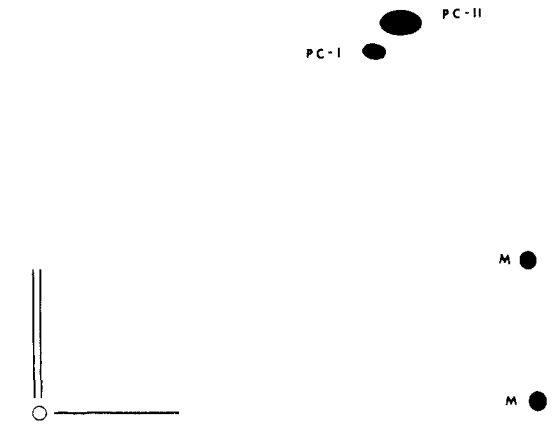

Figure 3. Autoradiogram of *cis*-[^{14}C]photochlordane showing separation of two isomers, PC I and PC II. The TLC plate (silica gel G-F254) was developed two dimensionally with duplicate development on each side using hexane–ethyl acetate (9:1). The hollow circle at the left indicates the origin and the lines show dimensions. The spots on the right side were radioactive markers, applied after development, to locate the spots.

and hexane–benzene–ethyl acetate (18:1:3) indicated duplicity in the compound but the difference in their R_f values was very small (Table II). Satisfactory separation of the compounds was affected by two-dimensional duplicate development in hexane–ethyl acetate (9:1), and an autoradiogram of the separated PC components is presented in Figure 3. The compound with lower R_f value was designated as PC I and that with higher R_f value as PC II. Radiometry of the two spots gave a 1:3 ratio of PC I to PC II. The two isolated components were indistinguishable from each other in GLC analyses. From the chloroform solution, the purified compound PC I yielded an oily liquid very slowly crystallizing to pale flaky crystals, and PC II gave white crystals.

Mass Spectrometry. Mass spectra of PC I, PC II, and their mixture are shown in Figure 4. The molecular ion (m/e 406) was not detected in any of the compounds. The fragment with highest mass appeared at m/e 371 which was due to loss of a chlorine from the molecular ion ($M^+ - Cl$). The remainder of the spectra in all cases were in conformity with previous studies on similar molecules (Benson et al., 1971; Knox et al., 1973; Onuska and Comba, 1975), involving further losses of Cl, HCl, or their combination and retro Diels–Alder collapse of molecular skeleton. The fragmentation patterns of PC I (Figure 4b) and PC II (Figure 4c) were essentially similar. The most obvious difference in the spectra was that whereas PC II exhibited a base peak at m/e 375, the base peak in PC I

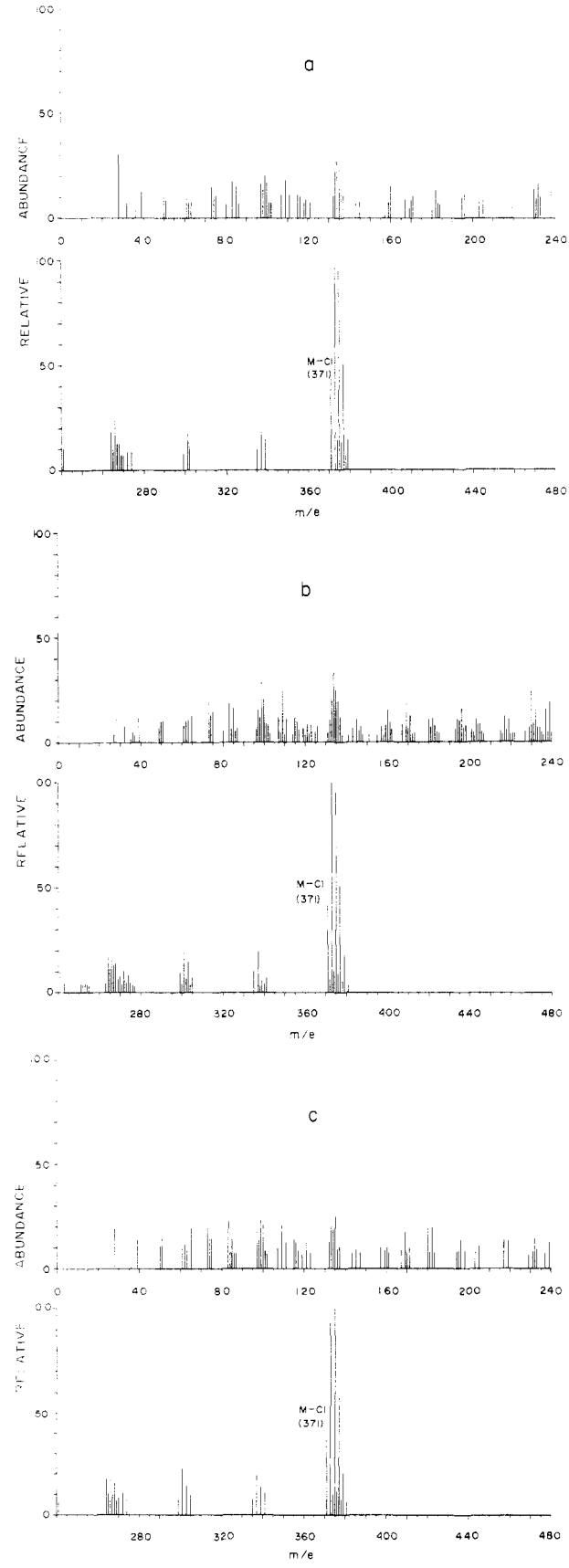

Figure 4. Mass spectra of PC I (b), PC II (c), and their mixture (a), the original isolate from photolysis of *cis*-chlordane.

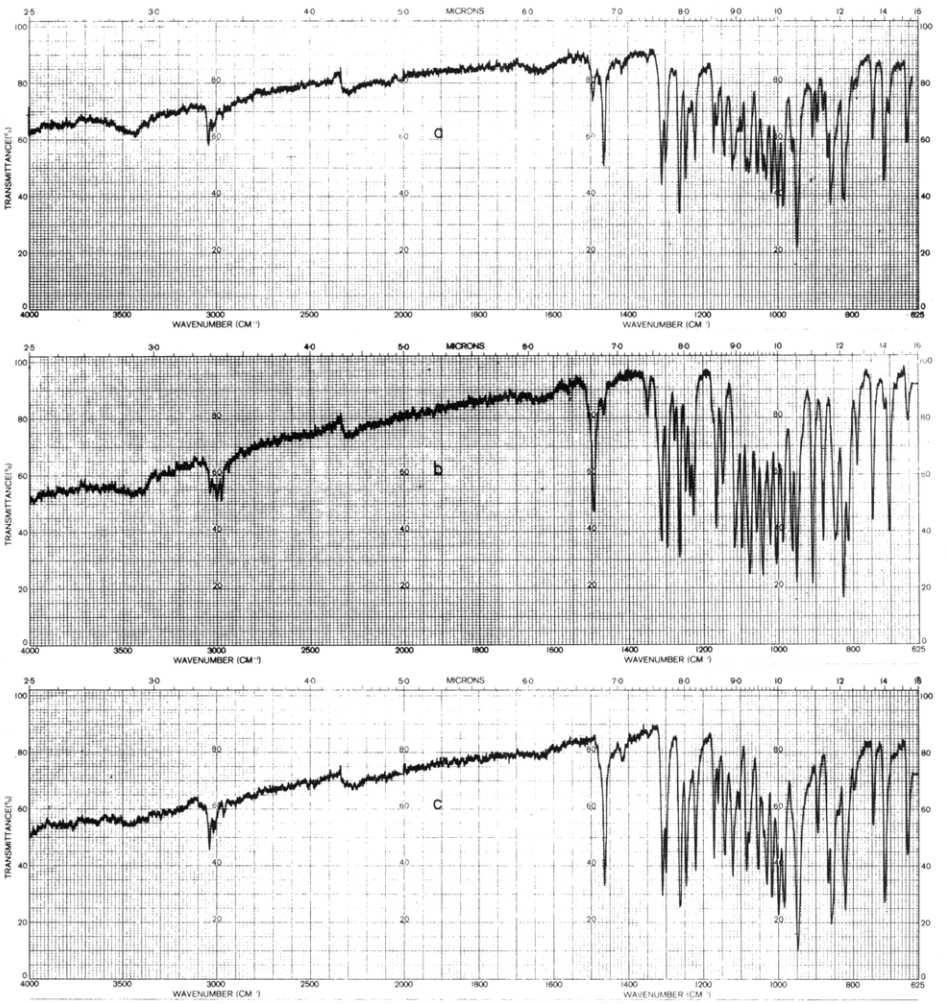

Figure 5. Infrared spectra of PC I (b), PC II (c), and their mixture (a).

was present at m/e 373. The similarity of mass spectra in conjunction with identical retention times on GLC columns and closeness of behavior on thin-layer plates (Table II) showed that PC I and PC II were isomeric molecules.

Infrared Spectrophotometry. Infrared scans of PC I, PC II, and the original PC isolate (mixture) are presented in Figure 5. In al spectra, absorption at 1597 cm^{-1} characteristic of the dichloroethylene bond of *cis*-chlordane (and other related molecules) was absent, indicating molecular rearrangement consequent upon opening of the double bond. Besides significant differences in the fingerprint region (625–1300 cm^{-1}), PC I (Figure 5b) showed strong absorption at 1492 cm^{-1} while PC II (Figure 5c) exhibited a strong band at 1457 cm^{-1}. The ratio of the peak heights at 1492 and 1457 cm^{-1} in the mixture (Figure 5a) was 1:3, the same as the ratio estimated radiometrically (see above).

NMR Spectrometry. Figure 6 shows ^1H NMR spectra of the PC mixture, PC I, and PC II. Whereas spectra of PC I (Figure 6b) and PC II (Figure 6c) are distinct, the spectrum of the PC mixture (Figure 6a) is a mere superimposition of the two.

Two features of the spectra are noteworthy. First, the ratios of the heights of the downfield signals at δ 4.96–4.86 and of those at δ 4.44–4.66 in Figure 6a are almost the same as the ratios of PC I to PC II observed in radiometric and infrared analyses. Second, the ^1H NMR spectra of PC I (Figure 6b) and PC II (Figure 6c) showed the presence of six protons in each molecule, indicating their isomeric nature and rearrangement products of *cis*-chlordane.

Leaving aside any other structural rearrangements in the molecule, possible photoisomeric forms with cross-links between the dichloroethylene bridge and cyclopentane ring are shown in Figure 1. The spectrum of PC II conforms to the previously published data for PC shown to have a bridge between C_2 and C_5 (Figure 1, **2**; Knox et al., 1973).

Rationalization of the ^1H NMR spectrum of PC I together with the published information on photoisomerization of *cis*-chlordane rules out structures **6** and **7** (Figure 1). First, the higher field part of the spectrum (Figure 6b) showed a geminal coupling of 16.9 Hz, indicating the presence of a methylene group in the molecule. Second, deshielding due to α-chlorines of only two protons (instead of the three expected in structures **6** or **7**) was observed.

The structures **4** and **5** (Figure 1) are also ruled out. Nuclear magnetic resonance analysis of the former has been reported, and the compound does not conform to the data (Lahaniatis et al., 1976). Furthermore, with both the structures H_b should be strongly coupled to the adjacent methylene group which was not the case.

Although not entirely precluded, the structures **4**–**7** are less favorable products than the structures **2** or **3** because of the stereochemistry of the molecule. Study of molecular models of *cis*-chlordane shows that the carbon C_2 is closer to the dichloroethylene bridge than the carbon C_1 or C_3. Consequently, in cases where such cross-links [for example, structure **4** from *cis*-chlordane (Lahaniatis et al., 1976) and photoisomers of *trans*-chlordane and *trans*-nonachlor which have an *endo*-chlorine at C_2 (Knox et al., 1973)] are formed, yields of the products are lower than we observed in this study. Thus based on the balance of evidence and

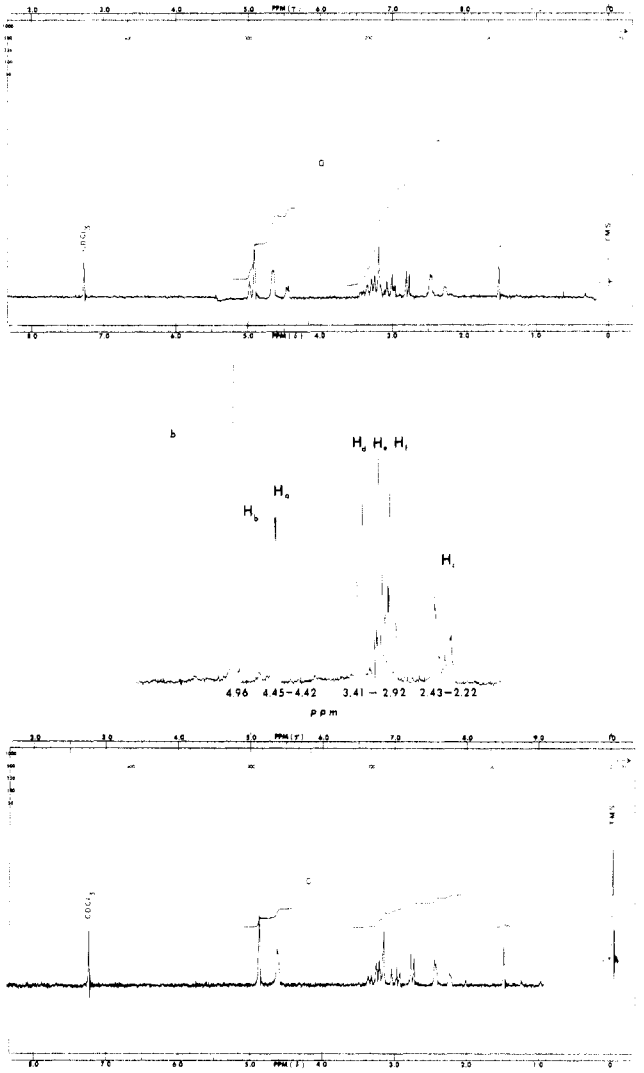

Figure 6. NMR spectra of the mixture (a), PC I (b), and PC II (c). The spectra of the mixture and PC II were recorded at 60 MHz and that of PC I was recorded at 80 MHz.

^{1}H NMR analysis (Figure 6b), PC I appears to have structure **3** (Figure 1).

Table III is a list of ^{1}H NMR resonances of PC I. The assignments were based on the analysis of decoupled spectra. Irradiation at δ 4.96 did not affect any part of the spectrum. The singlet occurring at δ 4.96 can thus be assigned to the strongly deshielded proton H_b migrating during photolysis to the dichloroethylene bridge. The other downfield signal (δ 4.45–4.42) can be assigned to H_a which is deshielded by the neighboring chlorine on the C_1 and is coupled with one of the methylene protons (δ 2.43–2.22, more likely H_c because of its endo position). Irradiation at δ 2.30 eliminated the ω coupling between H_a and H_c, sharpened the signal at δ 4.44, and changed the quartet to a doublet. Conversely, irradiation at δ 4.44 changed the broad doublet at δ 2.43–2.22 to a quartet, showing geminal coupling with a J value of 16.9 Hz. H_d, the methylene counterpart of H_c, along with H_e and H_f, resonates in the multiplet at δ 3.41–2.92. This part of the spectrum was not amenable to firstorder analysis.

Table III. NMR Analysis of Protons in PC I

protons	chemical shift, ppm
H_b (singlet)	4.96
H_a (quartet)	4.45, 4.44, 4.43, 4.42
H_d, H_e, H_f (multiplet)	3.41, 3.33, 3.17, 3.13, 3.05, 3.02, 2.99, 2.99, 2.92
H_c (doublet)[a]	2.43–2.22

[a] Broad doublet, due to one of the methylene protons, H_c or H_d. The signals appear to be more likely due to H_c because of ω coupling with H_a.

Systematic Nomenclature (IUPAC). *cis*-Chlordane: 1,3,4,7,8,9,10,10-octachlorotricyclo[5.2.1.$0^{2,6}$]dec-8-ene; PC I: 1,3,4,7,8,9,10,10-octachlorotetracyclo[5.2.1.$0^{2,6}$.$0^{4,9}$]decane; PC II: 1,3,4,7,8,9,10,10-octachlorotetracyclo-[5.2.1.$0^{2,6}$.$0^{4,8}$]decane.

ACKNOWLEDGMENT

Diem-phuong Ngo provided technical assistance in this work.

LITERATURE CITED

Benson, W. R.; Lombardo, P.; Egry, I. J.; Ross, R. D., Jr.; Barron, R. P.; Mastbrook, D. W.; Hanson, E. A. *J. Agric. Food Chem.* **1971**, *19*, 857.
Feroz, M.; Khan, M. A. Q. *J. Agric. Food Chem.* **1979**, *27*, 108.
Feroz, M.; Khan, M. A. Q. *J. Agric. Food Chem.* **1980**, *28*, 740.
Knox, J. R.; Khalifa, S.; Ivie, G. W.; Casida, J. E. *Tetrahedron* **1973**, *29*, 3869.
Lahaniatis, E. S.; Parlar, H.; Gab, S.; Korte, F. *Synthesis* **1976**, *47*, 1.
Mitchell, L. C. *J. Assoc. Off. Anal. Chem.* **1958**, *41*, 781.
Onuska, F. I.; Comba, M. E. *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 6.
Parlar, H.; Korte, F. *Chemosphere* **1977**, *6*, 665.
Podowski, A. A.; Banerjee, B. C.; Feroz, M.; Dudek, M. A.; Willey, R. L.; Khan, M. A. Q. *Arch. Environ. Contam. Toxicol.* **1979**, *8*, 509.

Received for review February 25, 1980. Accepted October 24, 1980. Financial support for this study was provided by U.S. Public Health Service Grant No. ES 01479 from the National Institute of Environmental Health Sciences.