Confirmation of Hexachlorobenzene by Chemical Reaction

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Introduction

Several techniques have been used for the identification of pesticide residues including thin-layer chromatography, mass spectrometry, multiple column gas chromatography and chemical reactions. KOEMAN et al. (1969) used mass spectrometry for the confirmation of hexachlorobenzene (HCB) in fish, muscle and birds and GILBERTSON and REYNOLDS (1972) used multiple column gas chromatography for the confirmation of the same compound in the eggs of common terns. NEWTON and GREENE (1972) employed thin-layer chromatography with silver nitrate - UV visualization to confirm the presence of HCB. Additional confirmation was accomplished by removal of the developed and UV-exposed spot from the plate, extraction of the alumina with hexane and injection of the concentrated hexane solution in one of the two columns that were used for HCB analysis. As pesticide laboratories are not always equipped with a mass spectrometer and the use of a multiple column technique or thin-layer chromatography are not completely satisfactory for confirmation evidence, an attempt has been made in the present work to find a chemical reaction which could be applied to the confirmation of HCB. Experiments showed that monoethoxypentachlorobenzene was formed when HCB was treated with sodium ethoxide and that this derivative could be used to identify HCB in the presence of HCH.

Experimental

Reduction. - Hexachlorobenzene [1 mg; Pechinex-Progil (Pepro), Lyon, France] was dissolved in hot, absolute ethanol (10 ml). Freshly prepared sodium ethoxide [10 ml; 5 g Na/100 ml ethanol], and palladium on carbon catalyst (5%) (1 g) were then added

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followed by aqueous ethanol [1 ml; $EtOH/H_2O$ = 10/1]. After the initial reaction had subsided, the reaction mixture was heated under reflux for one hour; it was then cooled, water (25 ml) was added followed by sufficient hydrochloric acid to give an acid reaction. The mixture was extracted with three, 20-ml portions of hexane and the extract was dried over sodium sulfate. The sodium sulfate was removed by filtration and the volume of the hexane solution was adjusted to 100 ml. Fig. 1 shows a typical chromatogram that was obtained with an appropriate dilution of the extract using an Aerograph Model 600, operated under the following conditions: 5' x 1/8" Pyrex column packed with Chromosorb W-HMDS, 80-100 mesh; liquid phase - QF-1, 5%; oven temperature 130°C. Peaks A and B had the same retention times as 1, 2, 3, 5-tetrachlorobenzene and pentachlorobenzene, respectively. The use of more catalyst or longer heating times did not increase markedly the conversion of HCB to pentachlorobenzene.

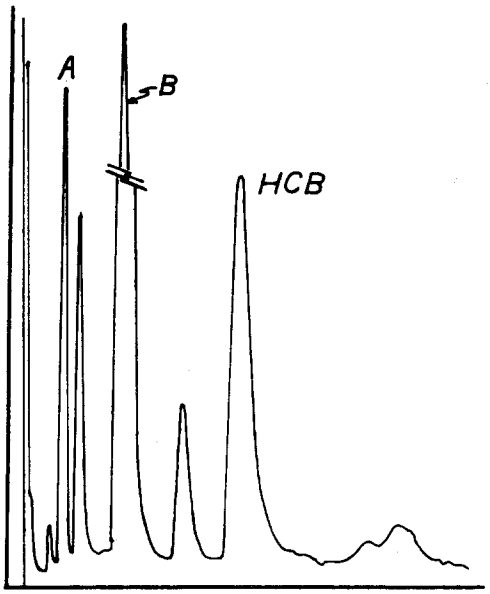

Fig. 1. Chromatogram obtained with reduced HCB. Column temperature 130°C. Peaks A and B had same retention times as 1, 2, 3, 5-tetrachlorobenzene and pentachlorobenzene, respectively.

Treatment with sodium ethoxide. - The above experiment was repeated but without the addition of the catalyst. Gas chromatography of the hexane extract showed the presence of unreacted

HCB, pentachlorobenzene (a contaminant in the HCB used in the experiments) and a third substance which had a longer retention time than did HCB. Another experiment was performed using the following conditions: HCB (0.5 g) dissolved in hot absolute ethanol (100 ml) plus sodium ethoxide (50 ml; 5 g Na/100 ml ethanol); the mixture was heated under reflux. Samples were taken after 1 hour, 3 hours and 8 hours and were analyzed by gas chromatography. The remaining reaction mixture was added to water (300 ml) and sufficient hydrochloric acid was added to give an acid reaction. The mixture was extracted with hexane, the extract was dried over sodium sulfate and then evaporated to dryness. The resultant product was dissolved in hot absolute ethanol (75 ml), treated with activated carbon and sufficient water (60 ml) was added to the hot ethanolic solution to give a slightly cloudy solution. The mixture was then cooled in an ice bath. A white crystalline product (0.25 g) melting at 87-88°C was obtained. Mass spectrometry gave the following results: m/e 292 ($C_6{}^{35}Cl_5OC_2H_5$); m/e 264 (292-28 corresponding to C_6Cl_5OH by loss of C_2H_4 in parent). BILTZ and GIESE (1904) prepared monoethoxypentachlorobenzene (MEPCB) by reaction of pentachlorophenol with a mixture of ethyl iodide and sodium ethylate. They reported a melting point of 89-90°C. Fig.2 shows gas chromatograms that were obtained with the samples taken at reaction times of 1, 3 and 8 hours and with the recrystallized product. Peak A had the same retention time as pentachlorobenzene. Peak B was not identified but it might represent diethoxytetrachlorobenzene.

Conversion of HCB to MEPCB on a microscale. - A hexane solution (0.2 ml) containing 0.1 mg of HCB/ml was added to a mixture of hexane (20 ml) and sodium ethoxide (20 ml; 5 g Na/100). The resultant solution was heated under reflux and samples (1 ml) were taken after 0, 1, 2, 4, 6 and 10 hours. Fig.3 shows gas chromatograms that were obtained with these samples. It will be noted that the HCB was converted to MEPCB at a lower rate than under the conditions of the previous experiment (Fig.2). Peak A increased in size during the reaction. The addition of pentachlorobenzene to the hexane extracts obtained from each sample increased the height of peak A.

Identification of HCB in the presence of isomers of HCH. - A hexane solution (1 ml) containing 1 µg of HCB and 1 µg of lindane (γ-HCH) was analyzed by gas chromatography (Fig.4a). One milliliter of sodium ethoxide (5 g Na/100 ethanol) was added to the hexane solution and 1 ml of the resultant mixture (A) was added to 10 ml of water. Sufficient hydrochloric acid was added to give an acid reaction and the mixture was extracted with hexane. The hexane extract was dried over sodium sulfate then analyzed by gas chrom-

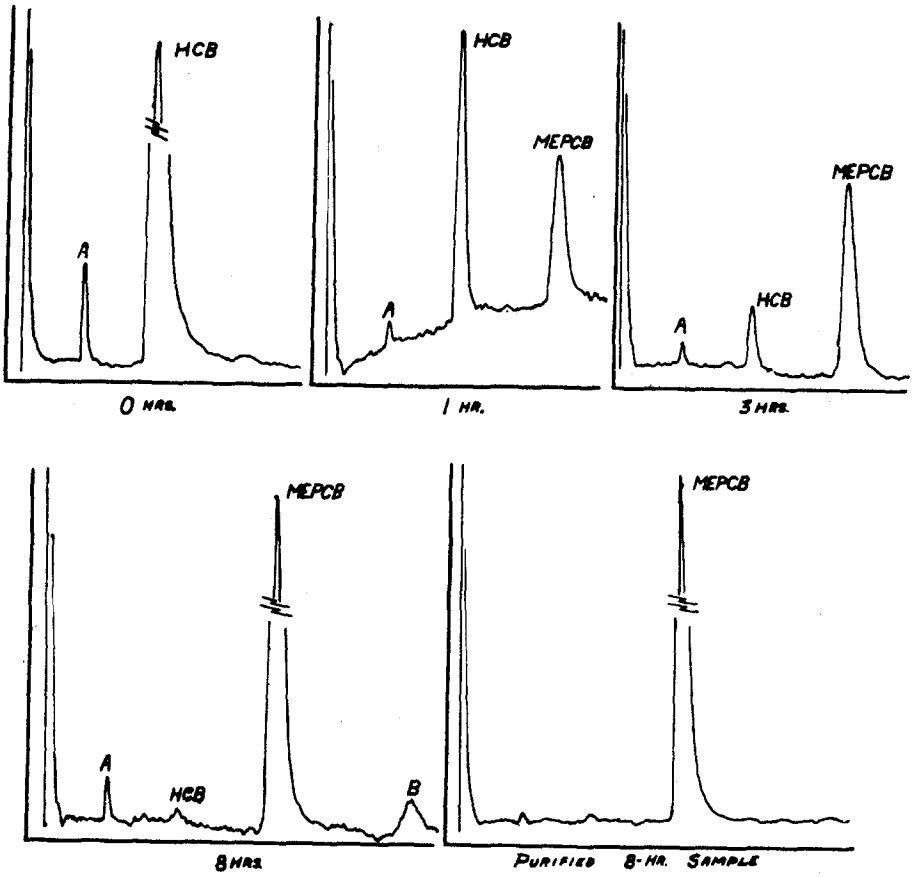

Fig. 2. Chromatograms showing the conversion of HCB to MEPCB. Column temperature, 135°C. Peak A had the same retention time as pentachlorobenzene. Peak B was not identified.

atography. Fig. 4b shows that the lindane had been destroyed and that only HCB gave a peak on the chromatogram. The remaining 1 ml of the mixture (A) was refluxed 1 hour. Analysis of the product [Fig. 4c] shows the presence of unreacted HCB, MEPCB and an unidentified peak X. Additional experiments showed that β-HCH was not completely destroyed simply by the addition of sodium ethoxide to the mixture of HCB and β-HCH but required a heating period of at least one hour. α-HCH required a heating period of at least 4 hours to convert it into substances with lower retention times than HCB and hence into substances that did not interfere with the appearance of the MEPCB peak which served to identify HCB.

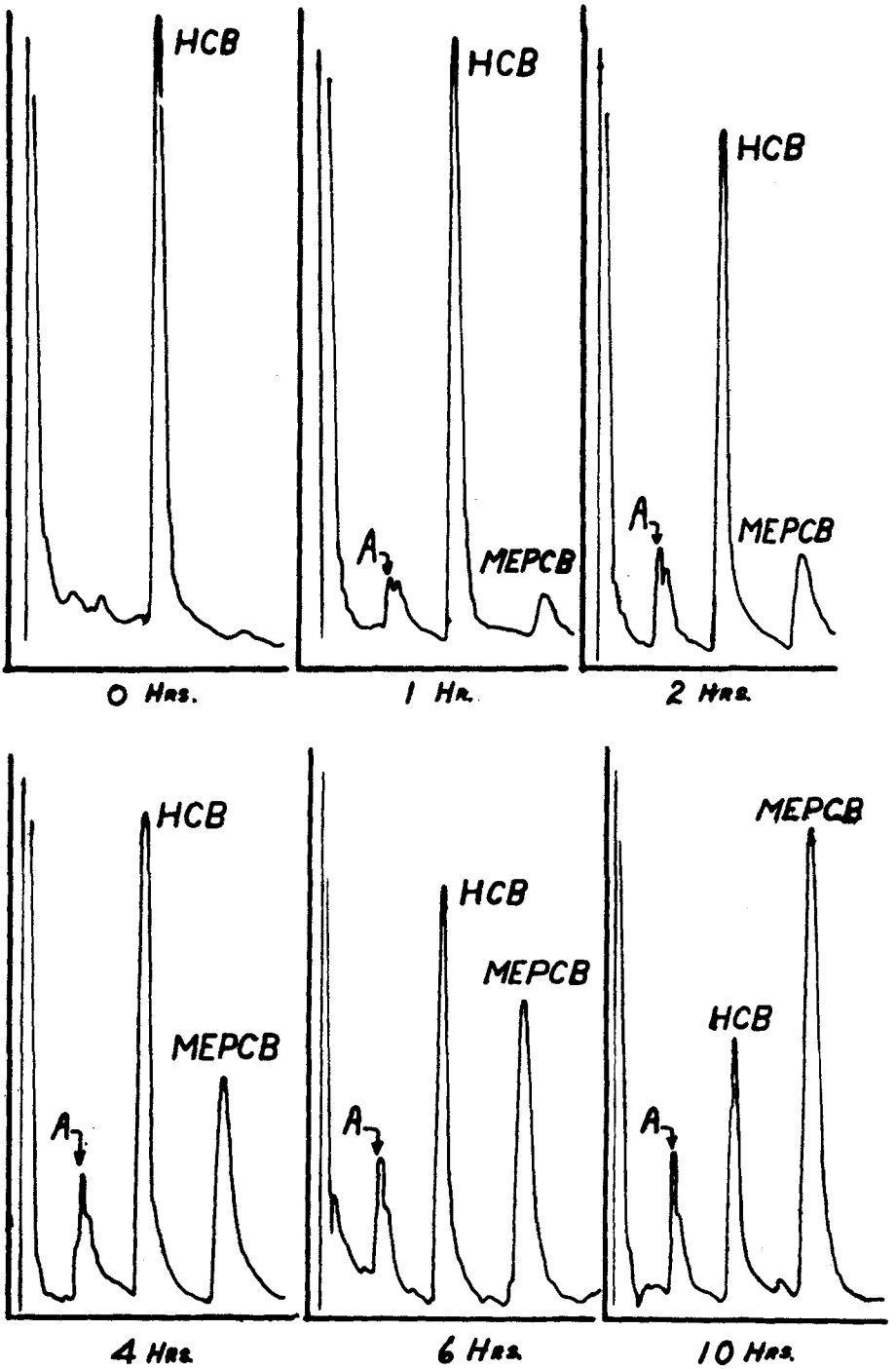

Fig. 3. Chromatograms showing the conversion of HCB to MEPCB. Column temperature 150°C. Peak A had the same retention time as pentachlorobenzene.

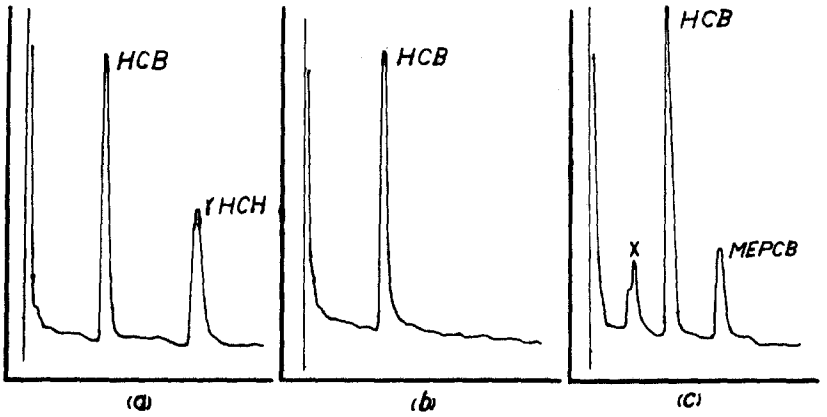

Fig. 4. Chromatogram illustrating the identification of HCB in the presence of HCH. Column temperature 150°C. (a) Mixture of HCB and α-HCH. (b) Mixture after addition of sodium ethoxide. (c) Mixture after addition of sodium ethoxide and heating under reflux for 1 hour. Peak x was not identified.

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