Differentiation of Polychlorinated Biphenyls from DDT by Carbon-Skeleton Chromatography

Application of carbon-skeleton chromatography to the qualitative differentiation between polychlorinated biphenyls (PCB's) and DDT is demonstrated. PCB's and biphenyl yield identical carbon-skeleton chromatograms that are strikingly different from that of DDT. Comparisons of the relative retention times of products yielded by the PCB's with known compounds, plus partial identification by their uv spectra, suggested that the products formed at 300° C catalyst temperature were cyclohexylbenzene and biphenyl, and at 260° C were cyclohexylbenzene and a small amount of bicyclohexyl.

Polychlorinated biphenyls (PCB's) are stable compounds extensively used in the manufacture of rubber goods, plastics, fireproofing preparations, paints and lacquers, resins, and many other products. Those commercially marketed by Monsanto Co. under the trade name "Aroclors" are complex mixtures of chlorinated biphenyls and terphenyls with various chlorine contents. Because of their similarities in structure and properties to the DDT pesticide group, the PCB's, if present, are carried through the usual pesticide extraction and screening procedures, and since they possess electron capturing properties, they will interfere with the gas chromatographic analysis of the organochlorine compounds using either the electron capture or the microcoulometric detector.

In the analysis of biological samples for pesticides, especially those of fish and sea birds, a large number of unknown but chlorine-containing compounds other than the ordinary pesticides have been detected using both electron capture and microcoulometric detectors. Mass spectra of some of these compounds obtained with a combined gas chromatograph-mass spectrometer have shown that most of the unknown compounds were PCB's (Widmark, 1967).

Articles have recently appeared reporting on current progress in the determination of PCB's (Risebrough *et al.*, 1969) and their interference with pesticide residue analysis (Reynolds, 1969). On gas chromatographic columns most frequently used for pesticide residue analysis, peaks for the PCB's usually interfere with those for *p,p'*-TDE, *p,p'*-DDT, and *o,p'*-DDT, and as a result, many of the DDT values reported in recent literature may be erroneous.

There exists a need for a simple and reliable means for removing the doubts that arise in many instances where either DDT or its degradation products are indicated by gas chromatographic responses, yet there may be reason to doubt its presence. A description of the technique known as carbon-skeleton chromatography and its analytical applications has been included in a review on reaction gas chromatography (Beroza and Coad, 1966). In this process three vapor-phase reactions of hydrogen may occur: hydrogenation, dehydrogenation, and hydrogenolysis. Thus, the PCB's should yield carbon-skeleton chromatograms identical to that obtained for biphenyl. The products should be cyclohexylbenzene and/or bicyclohexyl. In an earlier work we presented typical carbon-skeleton patterns for DDT and its analogs, indicating the products to be largely ethylbenzene and/or ethylcyclohexane (Asai *et al.*, 1967). The patterns for the two types of compounds should be sufficiently different to allow clear differentiation.

We have investigated the carbon-skeleton chromatography of *p,p'*-DDT and nine Aroclors. The dissimilarity in responses is striking and offers a simple and reliable means for detecting the presence of PCB's in suspect samples.

EXPERIMENTAL

Apparatus. An N.I.L.-Beroza carbon-skeleton determinator (National Instrument Laboratories, Inc., Rockville, Md.), attached to the injection port of an Aerograph Model A-600-B gas chromatograph with flame ionization detection, was employed for obtaining the carbon-skeleton chromatograms. For the electron capture gas chromatograms, the same gas chromatograph equipped with a tritium detector was used.

Catalyst. An NaCl-neutral palladium catalyst, 1% by weight as the metal, on DMCS-treated Gas Chrom Q, 80/100 mesh, was used to charge the carbon-skeleton determinator. Preparation of this catalyst has been described in an earlier report (Asai *et al.*, 1967). The temperature of the catalyst bed was maintained at either 260° or 300° C.

Columns. For the chromatograms obtained with the electron capture detector, a 2 ft × 1/8 in. stainless steel column packed with 10% DC-200 on DMCS-treated Gas Chrom Q, 80/100 mesh, was used. The column temperature was maintained at 180° C and the nitrogen flow rate was maintained at 85 ml/min. Except where noted, the carbon-skeleton chromatograms were obtained using a 3 ft × 1/8 in. stainless steel column packed with 5% DC-200 on DMCS-treated Gas Chrom Q, 80/100 mesh. The column temperature was held at 105° C, and the hydrogen flow rate was held at 20 ml/min. The carbon-skeleton chromatograms of bicyclohexyl, cyclohexylbenzene, biphenyl, and Aroclor 1260 were obtained on a 3 ft × 1/8 in. stainless steel column packed with 80/100 mesh Carbowax 400 on Porasil S. The column temperature was 178° C, and the hydrogen flow rate was 20 ml/min.

Materials. Aroclor samples were obtained from Monsanto Co., St. Louis, Mo. The first two digits of the identification number represent the type of material: 12 = chlorinated biphenyls; 44 = 60:40 blend of chlorinated biphenyls and terphenyls; and 54 = chlorinated terphenyls. The last two digits indicate the approximate weight percent of chlorine in the product. One-microliter aliquots of the samples dissolved in *n*-hexane were injected into the gas chromatograph or carbon-skeleton determinator for obtaining the chromatograms.

RESULTS AND DISCUSSION

Carbon-skeleton chromatograms for the Aroclors were obtained using catalyst temperatures of 260° and 300° C. Eight Aroclors which are complex mixtures of PCB's, with chlorine contents ranging between 21 and 68%, and the ninth,

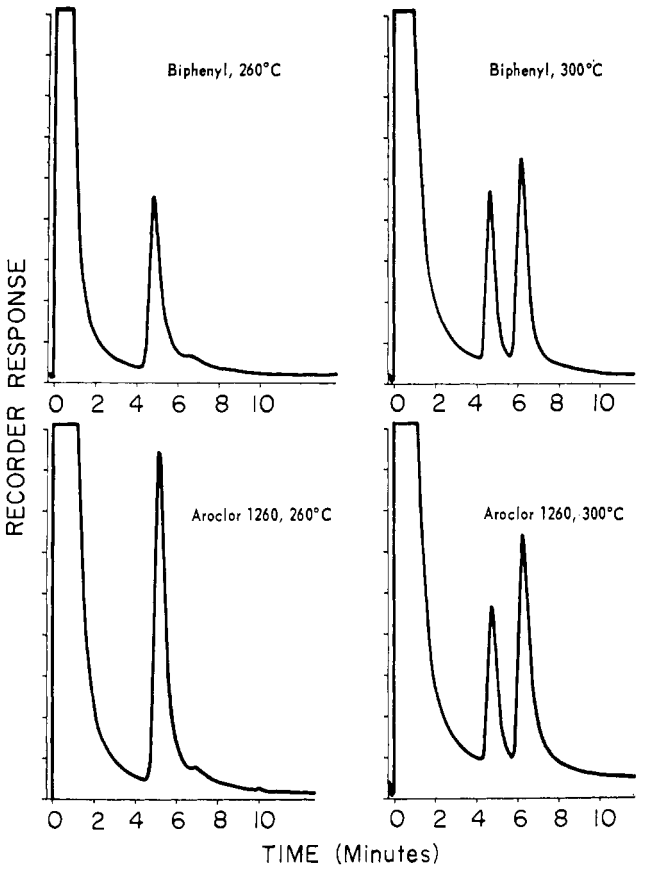

Figure 1. Carbon-skeleton chromatograms of biphenyl and Aroclor 1260 at two catalyst temperatures

a 60:40 blend of chlorinated biphenyls and terphenyls containing 65% chlorine, gave carbon-skeleton patterns identical to that from biphenyl. Typical chromatograms obtained at the two temperatures are reproduced in Figure 1 for biphenyl and Aroclor 1260. Two peaks are observed at the higher catalyst temperature due to incomplete hydrogenation of the biphenyl.

The carbon-skeleton chromatograms of Aroclor 1260, bicyclohexyl, cyclohexylbenzene, and biphenyl were obtained at 260° and 300° C catalyst temperatures using a Carbowax 400 chromatographic column which separates the latter three compounds eluted from the column in the following order: bicyclohexyl; cyclohexylbenzene; and biphenyl. No detector response was obtained from 50 μg of Aroclor 1260.

At 260° C catalyst temperature 1 μg of cyclohexylbenzene, 1 μg of biphenyl, and 5 μg of Aroclor 1260 each gave chromatograms qualitatively similar to Figure 1 (Biphenyl, 260° C) but containing a minor peak preceding the major peak. One microgram of bicyclohexyl gave a chromatogram in which the positions of the major and minor peaks were reversed. At 300° C catalyst temperature 1 μg of cyclohexylbenzene, 1 μg of biphenyl, and 5 μg of Aroclor 1260 each gave two-peak chromatograms qualitatively similar to Figure 1 (Biphenyl, 300° C). One microgram of bicyclohexyl gave a chromatogram with a reduced two-peak pattern preceded by a large single peak. At 300° C catalyst temperature, the materials representing each peak from 20 μg injections of Aroclor 1260 were collected with the detector flame extinguished. The material collected from the first peak to elute had an unidentifiable uv spectrum which had a maximum in cyclohexane solution at 218 nm similar to Aroclor 1260. The presence of cyclohexylbenzene would have been obscured due to its relatively low extinction coefficient. The material collected from the second peak to elute had uv maxima in cyclohexane solution at 246 and 211 nm which corresponded to those of biphenyl. The data suggest that at 300° C catalyst temperature, Aroclor 1260 gives a mixture of cyclohexylbenzene and biphenyl. Since hydrogenation is favored at lower temperatures (Beroza and Sarmiento, 1964), Aroclor 1260 is converted to cyclohexylbenzene and a small amount of bicyclohexyl at 260° C catalyst temperature.

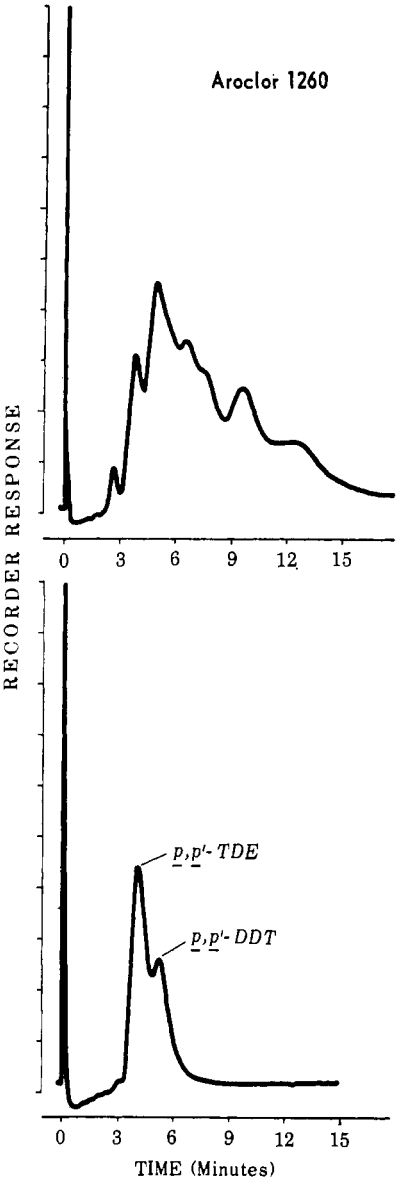

Figure 2. Gas chromatograms of Aroclor 1260 and a mixture of *p,p'*-TDE and *p,p'*-DDT using the electron capture detector

Gas chromatograms for Aroclor 1260, and a mixture of *p,p'*-TDE and *p,p'*-DDT obtained with the electron capture detector are shown in Figure 2 to illustrate how the PCB's can interfere with the analysis of the DDT pesticide group. Carbon-skeleton chromatograms obtained at 260° and 300° C for the same samples, at much higher concentrations, are reproduced in Figure 3. It is evident that the carbon-skeleton chromatographic technique can be an extremely useful means for ascertaining the presence or absence of PCB's in samples

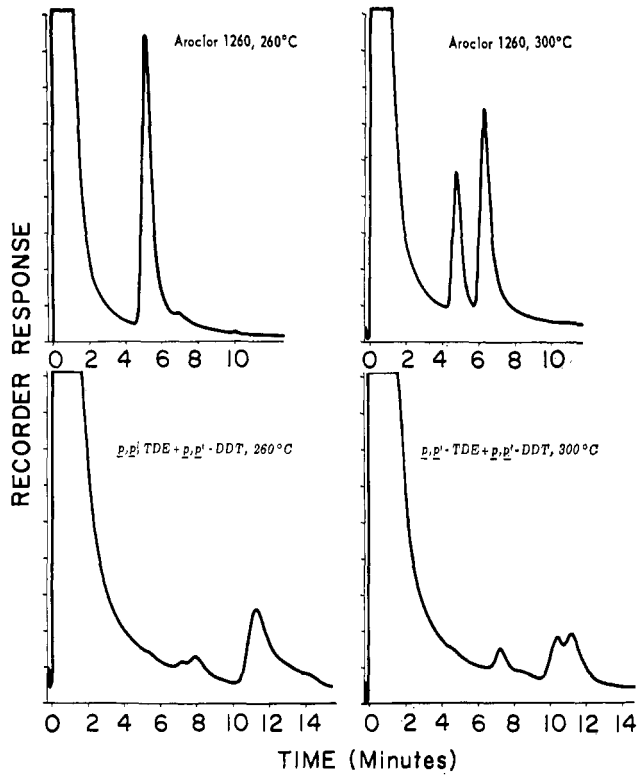

Figure 3. Carbon-skeleton chromatograms at two catalyst temperatures of Aroclor 1260, and a mixture of *p,p'*-TDE and *p,p'*-DDT

which, from gas chromatographic responses, show the presence of the DDT pesticide group.

To be useful for the qualitative identification of PCB's in metabolism studies and some surveillance situations, carbon-skeleton chromatography should be able to detect quantities of a microgram or less. The response of this technique for a particular Aroclor will depend upon its chlorine content; the greater its chlorine content, the larger will be the amount required for detection. For 1 μg of Aroclor 1260 with the electrometer attenuator at 8×, a response of 13% of full-scale deflection was obtained. For 1 μg quantities of the Aroclors with less chlorine content than Aroclor 1260, responses should increase as the chlorine content decreases.

The carbon-skeleton chromatographic technique was applied to the identification of Aroclor residues in seven rat tissue samples. Each was derived from 0.5 g of tissue after feeding the rat approximately 2 mg of Aroclor per day for 15 days. Only the sample of the rat fed Aroclor 5442 gave no response; the others yielded carbon-skeleton patterns identical with the parent material and with biphenyl.

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