PCB - Articles

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Identities of Polychlorinated Biphenyl (PCB) Isomers in Aroclors

by

Ronald G. Webb

and

Ann C. McCall

Southeast Water Laboratory
Environmental Protection Agency
Athens, Georgia

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ABSTRACT

Thirty PCB isomers in Aroclor 1221, 1242, and 1254 were separated and identified by matching both their GC retention times and infrared spectra with known compounds prepared by the Gomberg or Ullmann reactions. The retention times of the PCBs are presented relative to p,p'-DDE.

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Identities of Polychlorinated Biphenyl (PCB) Isomers in Aroclors

Environmental scientists are finding PCBs everywhere (1-4). In pollution studies here they have been observed by gas chromatography-mass spectrometry in sediments from a bay in Florida, in river water from Ohio (5) and in sewage outfalls from Wisconsin.

PCBs are made in this country by the Monsanto Company and sold under their registered trademark, Aroclor. Their manufacture is apparently by the direct chlorination of biphenyl with iron turnings as catalyst (6). The dark product is separated from the catalyst and refined by distillation. The Aroclors, complex mixtures of chlorinated biphenyl isomers, are identified by the percent by weight of chlorine in the total mixture; for example, Aroclor 1254 contains 54% by weight organically bound chlorine.

The toxicity, metabolic fate, and environmental chemistry of PCBs are the subject of much current study. Such studies would be simpler if the identities of the individual isomers were known so that they could be tested one at a time. Peakall and Lincer (1) point out "that we still relatively unsophisticated in our PCB quantitation methodology and will continue to estimate only relative amounts of PCBs in field samples until we synthesize the individual PCB components commonly found in the ecosystem and are able to speak in terms of these individual peaks as we do for most pesticide residues." This paper reports the results of research

to solve these problems.

Experimental

Analytical gas chromatography was performed with a Perkin-Elmer 990 instrument* equipped with a standard 1/8" glass-lined injector maintained at 300°. Detection was by flame-ionization. The column was 100' x 0.02" SCOT SE-30 operated as in Figure 1.

Several instruments were used for preparative GC: Nester/Faust Prepkromatic 850, Aerograph A-700 and PE-990. Because PCBs corroded the thermal conductivity detector elements, later work was done with a FID. Aroclor 1242 (0.6 ml/injection) was chromatographed in the Prepkromatic 850 instrument through 5' of 3/4" bi-wall column packed with 25% DC-200 on 80/90 mesh Anakrom ABS. A run required 80 min. at 245° and carrier gas flow of 150 ml/min.; injector 300° and collector 275°. An electrostatic precipitator on the six collection tubes greatly reduced losses due to aerosol formation. Aroclors 1221 and 1254 were separated similarly. A more rapid technique for isolation of smaller quantities involved chromatographing the Aroclor, or a fraction from earlier work, through a 1/8" x 10' stainless steel column of 5% OV-101 in an analytical GC equipped with a heated effluent splitter. Less-than-milligram fractions were collected in glass capillaries attached to the splitter exit by teflon tubing. The sample was washed onto KBr and a micro (1.5 mm dia.) pellet made and the IR spectrum taken with a 4X beam condenser in a Perkin-Elmer 457 instrument. The PCB was then dissolved out of

*All brand names are for description only and do not imply endorsement by EPA.

the pellet and its purity checked by capillary GC. This technique was also used to separate and obtain IR spectra of the synthetic PCBs.

Mass spectra of the Aroclors were taken with a Hitachi Perkin-Elmer RMU-7 mass spectrometer interfaced with a Perkin-Elmer model 900 gas chromatograph. The GC column was 50' x 0.02" SCOT SE-30. The spectra do not give any structural information other than the total number of chlorines in the molecule. In general our data agreed with that of Stalling and Huckins (7).

The Aroclors were a gift of the Monsanto Company. No lot numbers were given. All chlorobenzenes and anilines were commercial materials except 2,3,5-trichloroaniline which was prepared by published methods (8,9).

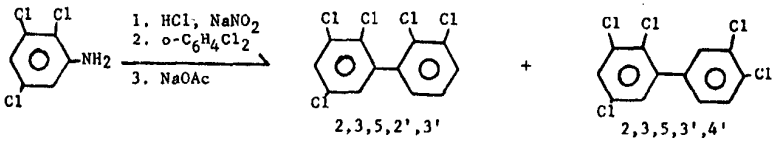
The Ullmann reaction (10) is illustrated by the preparation of 2,4,5,2',4',5'-hexachlorobiphenyl. 2,4,5-Trichloroiodobenzene, prepared from the aniline by the method of Van de Lande (11), was mixed with an equal weight of copper (electrolytic dust, Fisher Scientific Co.) which had been activated by the method of Kleidener and Adams (12). The mixture was heated at gentle reflux, about 250°, for 18 hours. The reaction was monitored by GC. After extraction from the copper with chloroform the product was collected by preparative GC and recrystallized from methanol, mp 100-102°.

A modification of Weingarten's (13) procedure was used for the Gomberg reaction as illustrated by the preparation of 2,4,5,2',5'-pentachlorobiphenyl. 2,4,5-Trichloroaniline (19.6g) was heated briefly with 30 ml conc. HCl and then cooled to 0-5° with an ice bath.

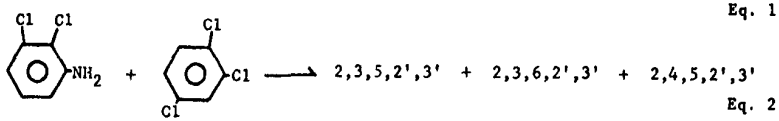
Sodium nitrite (15g in 10ml water) was added dropwise with stirring to the paste at such a rate that the temperature did not rise above 5°. After stirring 10 minutes, any solid remaining was removed by filtration. A cold solution of 80g p-dichlorobenzene in 100 ml of CCl_4 was added to the aqueous diazonium salt solution followed immediately by 50g sodium acetate trihydrate in 100 ml of water. The ice bath was removed and the mixture was stirred for at least 8 hours. After the organic layer was separated and washed several times with water, the solvent and excess dichlorobenzene were removed by evaporation and sublimation, or by steam distillation. The dark oily residue was extracted with 500 ml of hexane, filtered, dried over Na_2SO_4 and evaporated under water pump vacuum to a dark oil. The oil was taken up in a minimum volume of hexane and equal portions placed on two 2 x 30cm columns of neutral alumina. Four 100 ml fractions were collected from each column and evaporated to give light pink oils which crystallized on standing. GC showed the third and fourth fractions considerably contaminated with longer-retention-time impurities. The first and second fractions from both columns were combined, the solvent evaporated, and the residue recrystallized from methanol, 3.8g, mp 74-76°.

Results and Discussion

PCB isomers of known structure were prepared by the Ullmann and Gomberg reactions. The Gomberg reaction (13,14) was more useful and is illustrated by Equation 1.



Eq. 1



Eq. 2

The identities of the products in the first reaction were established by running a parallel reaction as shown in equation 2. The 2,3,5,2',3'-isomer is common to both reactions. Its physical identity was shown by the identical GC retention time of one compound from each reaction. As a separate check the common material was sometimes isolated from different reactions and the infrared spectra shown to be identical. Although isomer identification could sometimes be predicted based on product ratios and order of elution from the GC, the identity of every synthetic PCB in this report was confirmed by a parallel reaction.

To positively identify an Aroclor isomer a GC test and an IR test were applied. The synthetic PCB, when added to the Aroclor and gas chromatographed under the conditions shown in Figure 1, had to enrich the appropriate peak. The infrared spectrum of the synthetic isomer and the isolated Aroclor isomer had to match. When an isolated Aroclor fraction contained only one PCB isomer, the infrared spectrum perfectly matched that of the synthetic material. However, some of the isolated Aroclor fractions were mixtures of isomers. In several of these cases

the IR spectrum of this mixture could be reproduced by carefully mixing the correct synthetic materials. An example of this technique is shown in Figure 2. In those cases where an isomer was only a small portion of the fraction isolated and therefore contributed only a small amount to the infrared absorption, the judgment of its presence or absence was sometimes difficult. Figure 3 illustrates this situation. The bottom spectrum is of the Aroclor 1254 fraction containing a major peak at the relative retantion time 48 and a minor peak at 49.5 (Cf. Figure 1). The spectrum of 2,5,2',5'-tetrachlorobiphenyl above it almost matches the Aroclor fraction except for three minor peaks at 840, 770, and 590 cm^{-1}. The top spectrum of 2,4,2',5'-tetrachlorobiphenyl does not resemble the overall shape of the Aroclor spectrum. However, 2,4,2',5' does not have any peaks that are not found in the Aroclor fraction, although in different proportions, and the three peaks unexplained by the major isomer are explained by this isomer.

The compounds shown to be present in the Aroclors by these methods are shown in Table 1. The compounds that failed either the GC or IR tests and therefore are absent from the Aroclors are listed in Table 2.

The fraction containing isomers of retention times 29-29.5 from 1242 consisted of a solid and a liquid phase. The isolated solid had a retention time of 29.5 and was 4,4'-dichlorobiphenyl. The liquid phase, as already shown in Figure 2, was 2,5,2'-(ret. 29) and 2,4,2'-(ret. 29.2). Although 4,4'- was isolated from 1242 it does not appear as a separate peak at 29.5 in the 1242 chromatogram (Figure 1)

presumably because it is present in such low concentrations.

There is a marked difference in the identities of the peaks in the 70-71 region between Aroclors 1248 and 1254. Two peaks are found in 1248 at 70 and 71. Neither of these are observed in 1254 but a separate peak appears at 70.5. The two compounds in 1242 and 1248 are principally 2,5,3',4'- and 2,4,3',4'-tetrachlorobiphenyls. Infrared indicated that some other materials are also present in this same fraction of 1242 and MS showed a weak signal for a pentachloro isomer. The IR of the 70.5 peak from 1254 matched 2,3,6,2',5'-pentachlorobiphenyl. Some strong IR bands from 2,3,6,2',5' are completely missing in the spectrum from 1242 (70 and 71) confirming the absence of this material from 1242.

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Table 1. Summary of Aroclor Retention Times and Identities

Retention time[a]	Synthetic Chloro-biphenyl	Aroclor				
		1221	1232	1242	1248	1254
10	Biphenyl	X[b] C[c]	X	X	X	
13	2	X C	X	X	X	
16.9	3	X				
17	4	X C	X			
17.7				X		
18.5	2,2'	X C	X	X C	X	
21.3		X	X	X		
21.6		X	X	X		
23.5	2,3'	X C	X	X	X	
24	2,4'	X C	X	X C	X	
26	2,6,2'		X	X D[d]	X	
29	2,5,2'		X	X C	X	
29.2	2,4,2'	X	X	X C	X	
29.5	4,4'	X C	X	C		
34	2,3,2'		X	X C[e]	X	
38	2,4,3'		X	X C	X	
38.5	2,4,4'		X	X C	X	
38.7	2,5,4'		X	X C	X	
41.5				X	X	
42.5	3,4,2'			X	X	
43				X	X	
44				X	X	
44.5					X	
48	2,5,2',5'			X C	X	X C
49.5	2,4,2',5'			X C	X	X C
50.5	2,4,2',4'			X D	X	
51.5				X	X	
55	2,3,2',5'			X	X	X C
57				X	X	
59	2,3,6,2',6'			X	X	X D
60				X	X	
64				X	X	
69				X	X	
69.5					X	X
70	2,5,3',4'			X C[e]	X	
70.5	2,3,6,2',5'					X C
71	2,4,3',4'			X C[e]	X	
72				X	X	
76	2,3,6,2',4'					X C

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Table 1 (continued)

Retention time[a]	Synthetic Chloro-biphenyl	Aroclor				
		1221	1232	1242	1248	1254
82					X	
83	2,3,6,2',3'				X	X C
84					X	
85	2,4,5,2',5'				X	X C
87	2,4,5,2',4'				X	X C
96						X
99	2,4,5,2',3'				X	X C
101					X	X
104					X	X
107					X	X
115						X
117					X	X
119						X
125	2,4,5,2',3',6'					X C
126	2,4,5,3',4'				X	X C
135						X
147						X
148	2,4,5,2',4',5'					X C
149						X
152						X
165						X
178						X

[a]Relative to p,p'-DDE=100 at 190° on 100' X 0.02" SCOT SE-30 column.

[b]X indicates a GC peak in this Aroclor at this retention time.

[c]C means that the synthetic compound in the second column was confirmed by GC and IR in this Aroclor. Because of the labor involved, most compounds were only confirmed in one Aroclor. For example, biphenyl is probably present in 1232-1248 as well as 1221.

[d]Only GC data available.

[e]Something else also present.

Table 2. PCB Isomers Found to be Absent from Aroclors 1221-1254

Compound	Relative retention time[a]
2,3,4'	44
3,4,4'	59.5
2,5,3'	36
3,5,3'	46
2,6,3'	32
2,3,2',4'	57
2,3,3',5'	68
2,3,2',6'	47
2,3,5,2',3'	93
2,3,5,2',4'	85
2,3,5,2',5'	83
2,3,5,2',6'	68
2,3,5,3',4'	121
2,4,5,2',6'	70.5
2,4,5,3',5'	104
2,4,6,2',4'	65
2,4,6,2',4',6'	78
2,3,5,2',3',5'	138
2,3,5,2',4',5'	142
2,3,5,2',3',6'	116

[a]As in Table 1.

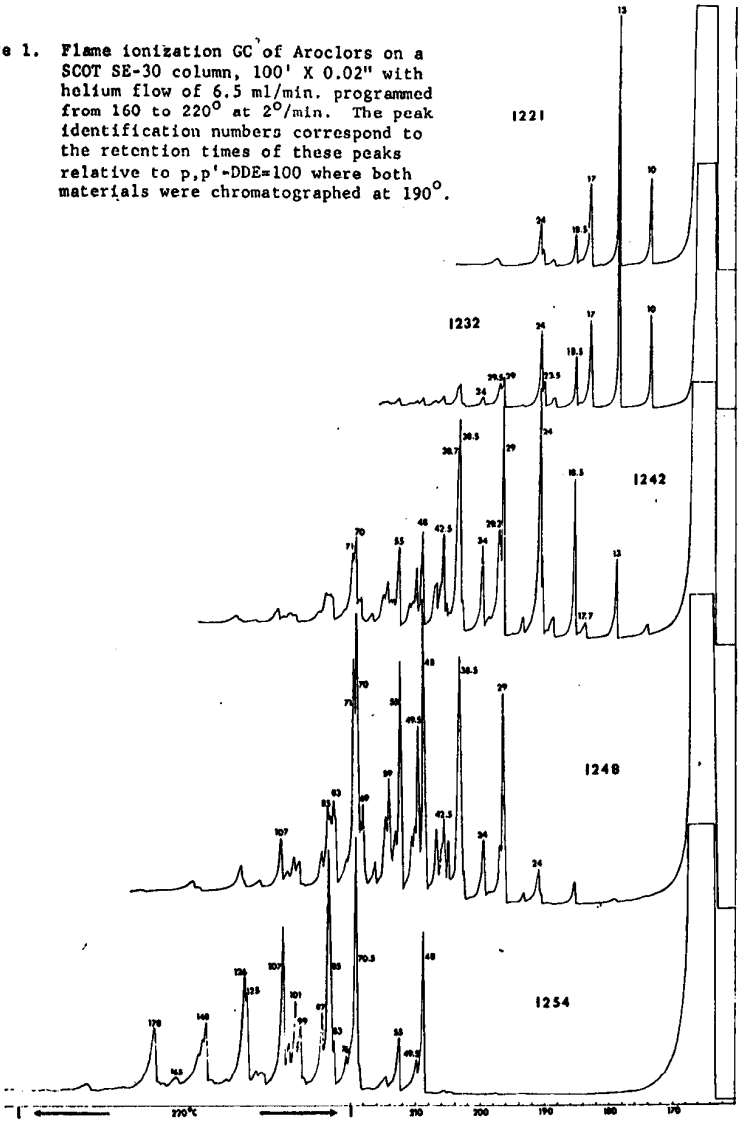

Figure 1. Flame ionization GC of Aroclors on a SCOT SE-30 column, 100' X 0.02" with helium flow of 6.5 ml/min. programmed from 160 to 220° at 2°/min. The peak identification numbers correspond to the retention times of these peaks relative to p,p'-DDE=100 where both materials were chromatographed at 190°.

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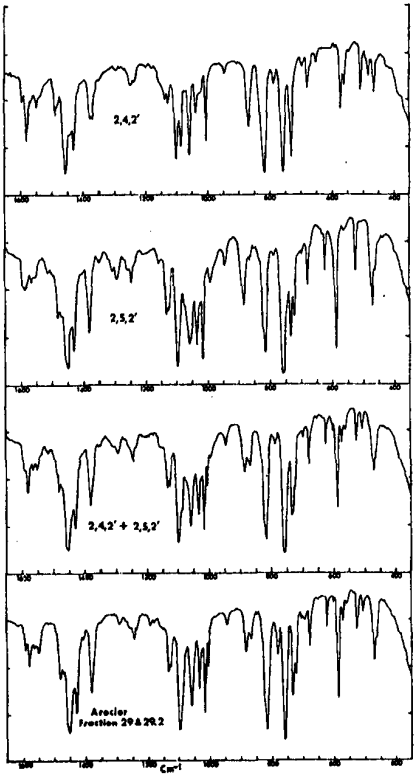

Figure 2. Comparison of IR spectrum of an Aroclor fraction containing at least two isomers with the spectrum of a mixture of known isomers.

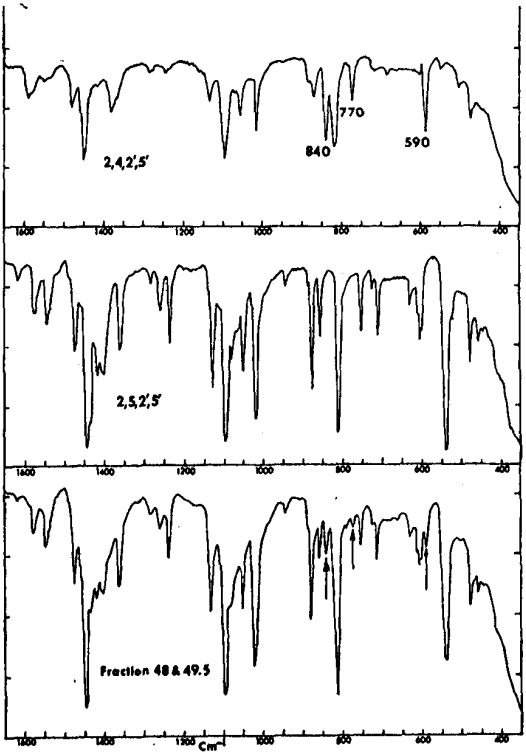

Figure 3. Infrared spectra of synthesized PCB isomers and of an Aroclor fraction containing a major and a minor isomer. The bottom spectrum is of an Aroclor 1254 fraction containing peaks 48 and 49.5 (Cf. Figure 1). The middle spectrum is of synthetic 2,5,2',5'-tetrachlorobiphenyl, the major isomer. The top spectrum is of the minor isomer, 2,4,2',5'-tetrachlorobiphenyl.

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