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QUANTITATIVE AND QUALITATIVE ANALYSIS OF POLYCHLORINATED BIPHENYLS BY GAS-LIQUID CHROMATOGRAPHY AND FLAME IONIZATION DETECTION

I. ONE TO THREE CHLORINE ATOMS

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SUMMARY

Retention indices on six liquid phases are given for the mono-, di- and trichlorobiphenyls, and the additivity of half-retention index values in predicting retention indices is confirmed for these compounds.

A method is given for predicting the relative molar response of the hydrogen flame ionization detector to polychlorinated biphenyls for which no pure standard is available. Solute-solvent interactions between these "non-polar" compounds and a variety of liquid phases are discussed.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are generally assayed in extracts of biological materials by gas-liquid chromatography (GLC). Analytical conditions are usually those employed for chlorinated pesticides, involving such liquid phases as SE-30 (or OV-1, OV-101)[1-5], DC-200/QF-1[6], or XE-60[7]. Apiezon L has been reported to give greater resolution of the many components in the Arochlor series of commercial PCB mixtures than a large number of other liquid phases tested[8]. Even with Apiezon L, however, long capillary or SCOT columns are required for resolution of the large number of similar isomers occurring in the Arochlors[8].

Quantitative analysis for PCBs is complicated by the number of peaks appearing on chromatograms and by the variation in detector response to each component. This variation may be 3000-fold using an electron capture detector[5]. Although a correlation between total electron capture peak area and amount of a particular mixture of PCBs can be made[6], any variation in the relative composition of the mixture will cause an unpredictable change in the total peak area response. This is particularly troublesome in analyzing PCBs extracted from biological materials, since the PCB mixtures obtained often do not exactly match any of the commercial PCB mixtures.

STALLING[9] has recommended programmed-temperature gas chromatography on

SCOT columns using electrolytic conductivity or microcoulometric detectors for reliable quantitative analysis of PCB mixtures. In conjunction with mass spectrometry both to confirm the identification of a given peak as being due to a PCB, and to determine the number of chlorine atoms per molecule (necessary for quantitation by microcoulometry), this recommended procedure should indeed cope adequately with the major problems in PCB analysis where large numbers of PCB isomers are involved.

Increasing interest in environmental aspects of PCBs and development of specific methods for the synthesis of many individual isomers[2,10] may be expected to result in an increasing amount of research involving less complex mixtures of PCBs than those discussed above. Preparative fractionation of a small number of very similar isomers will become increasingly necessary to those involved in synthetic work. Accordingly, we have studied the GLC properties of the PCBs on a variety of liquid phases using packed columns. Results for the PCBs having up to three chlorine atoms per molecule are presented in detail here, with some information on a few higher analogues.

MATERIALS AND METHODS

GLC liquid phases and solid supports were from Applied Science Laboratories, Inc., State College, Pa., and from Supelco, Inc., Bellefonte, Pa. Sources for the PCBs including those synthesized here have been described elsewhere[11].

ROHRSCHNEIDER constants, where not available in the literature, were determined as described originally[12] except that the column loadings were not 20% liquid phase. The constants so determined are therefore "apparent" and apply strictly only to the specified loading on the specified solid support. (For published ROHRSCHNEIDER constants see ref. 13)

Varian Aerograph Model 1200 and Hewlett-Packard Model 5750 gas chromatographs, both equipped with hydrogen flame ionization detectors and 1-mV recorders were used for these studies. Injection port and detector temperatures were 260° and 300°, respectively. Hydrogen and air flows were 30 and 320 ml/min, respectively. Samples were injected in less than 5 μl of *n*-heptane or methylene chloride.

The columns used in this study were 0.318 cm (O.D.) stainless steel, packed by vibration while drawing an aspirator vacuum at the outlet end. Ends were plugged with silylated glass wool. Column packings were as follows: (1) OV-101, 10% on 80–100 mesh High Performance Chromosorb W, column length 3 m; (2) Versilube F-50, 5% on 80–100 mesh High Performance Chromosorb W, column length 2.75 m; (3) Apiezon L, 6% on 100–120 mesh AW Chromosorb W, column length 2 m; (4) OV-17, 10% on 80–100 mesh Supelcoport, column length 3 m; (5) OV-225, 10% on 80–100 mesh Supelcoport, column length 2.5 m; (6) cyclohexanedimethanol succinate (CHDMS), 3% on 100–120 mesh Gas-Chrom Q, column length 4 m. Some samples were run on two additional columns: a 1½ m × 0.2 cm (I.D.) column packed with 4% paraffin wax on 80–100 mesh High Performance Chromosorb W; and a 3 m × 0.2 cm (I.D.) column packed with 5% purified Apiezon L on 100–120 mesh Gas-Chrom Q.

For the latter, 1 g of Apiezon L was passed through a column of 50 g of Florisil in *n*-heptane. 0.75 g of colorless grease was eluted with 300 ml of *n*-heptane, after which 0.23 g of orange wax was eluted with 250 ml of 5% methyl acetate in *n*-heptane. The white grease showed only aliphatic hydrocarbon absorption in the infrared and had the same thermal stability as a GLC liquid phase as Apiezon L. This white material is here referred to as purified Apiezon L.

Retention indices (*R.I.*) were determined graphically[12] at the column temperatures specified below by injecting mixtures of PCBs and *n*-alkanes. Indices were calculated for those PCB's for which no standards were available by making use of the additivity of $\frac{1}{2}$ (*R.I.*) values for the two substituted phenyl groups. This additivity has been shown to hold with Apiezon L[8], and our results show that it holds with the other liquid phases tested here as well.

Quantitation was in terms of peak area measured with a Disc integrator. Molar responses relative to biphenyl (set equal to 100) were determined, and the data presented here are averages of at least six determinations for each compound. Group response factors were determined from the relative molar responses to biphenyl, the monochlorobiphenyls, 2,3-, 2,4'-, 2,6- and 3,4-dichlorobiphenyls, and 2,4,4'-trichlorobiphenyl. Relative molar responses were predicted for the other PCB standards available and compared with their experimentally observed response values. After finding good agreement between predicted and observed responses to the standards, responses to the other compounds of concern to this paper were calculated.

RESULTS AND DISCUSSION

A comparison of calculated and observed relative molar responses for eighteen PCBs is given in Table I, and it is noteworthy that the relative molar flame responses do not differ by more than 60% for any of the PCBs tested.

TABLE I

RELATIVE MOLAR FLAME RESPONSES[a] OF SOME REFERENCE PCBs

PCB[b]	*Calculated*[c]	*Observed*[d]
2,2'	98 ± 2	96
2,4	81 ± 1.5	83
3,3'	88 ± 2	91
3,4'	83 ± 2	82
4,4'	79 ± 2	79
2,5,2'	87 ± 3	88
3,5,2'	79 ± 3	77
3,4,2'	83.5 ± 3	83
3,4,3'	81.5 ± 2.5	81
2,6,2',6'	90 ± 4	87
2,5,2',5'	84 ± 2.5	86
2,4,2',4'	78 ± 3	84
2,3,5,6	70 ± 3	69
2,3,4,5	80 ± 4	84
3,5,3',5'	76 ± 3.5	82
3,4,3',4'	85 ± 4.5	84
2,4,5,2',5'	79.5 ± 4.5	83
2,4,5,2',4',5'	86.5 ± 3	88

[a] Molar flame response relative to biphenyl = 100.
[b] The biphenyl positions substituted with chlorine are given.
[c] Errors are confidence limits for the calculations described in text.
[d] Averages of 6–15 determinations; all ± 1 (S.D.).

TABLE II

RETENTION INDICES ON DIFFERENT LIQUID PHASES AND RELATIVE MOLAR RESPONSES FOR PCBs

Compound[a]	*Retention index values*						*Relative molar response*[b,c]
	OV-101 200°	*F-50 200°*	*Apiezon L 205°*	*OV-17 200°*	*OV-225 190°*	*CHDMS 200°*	
Bicyclohexyl	1360	1364	1424	1448	1495	1517	121
Biphenyl	1396	1408	1490	1656	1831	2001	100
2	1509	1519	1578	1792	1969	2120	97
3	1575	1593	1678	1854	2060	2247	89
4	1584	1601	1688	1875	2080	2267	84
2,6	1619	1628	1679	1913	2105	2246	88
2,2′	1621	1629	1670	1918	2123	2264	96
2,5	1658	1674	1748	1942	2136	2319	86
2,4	1667	1679	1757	1941	2140	2318	83
2,3′	1688	1696	1763	1990	2195	2376	96
2,4′	1695	1707	1782	2012	2203	2382	91
2,3	1696	1703	1772	1995	2215	2370	87
3,5	1721	1738	1842	1982	2208	2424	78
3,3′	1756	1775	1870	2047	2292	2512	91
3,4	1763	1779	1881	2058	2315	2516	83
3,4′	1764	1780	1880	2073	2318	2520	82
4,4′	1774	1789	1895	2093	2342	2557	79
2,6,2′	1735	1737	1765	2049	2243	2381	89
2,5,2′	1772	1784	1831	2080	2275	2446	88
2,4,2′	1779	1788	1848	2080	2284	2457	82
2,6,3′	1799	1811	1866	2112	2334	2511	87
2,6,4′	1808	1819	1878	2132	2400	2536	82
2,3,2′	1809	1815	1864	2132	2355	2496	90
2,4,5	1830	1831	1925	2106	2312	2514	80
3,5,2′	1833	1840	1932	2118	2357	2551	77
2,5,3′	1837	1858	1932	2142	2366	2570	85
2,5,4′	1848	1866	1944	2163	2389	2592	80
2,4,3′	1849	1862	1949	2141	2375	2574	80
2,4,4′	1855	1870	1962	2162	2398	2597	76
2,3,3′	1875	1889	1963	2193	2445	2622	87
2,3,4′	1885	1897	1971	2214	2468	2643	82
3,4,2′	1890	1889	1975	2217	2458	2652	83
3,5,3′	1900	1914	2029	2180	2438	2676	77
3,5,4′	1910	1922	2041	2239	2461	2697	72
3,4,3′	1958	1963	2073	2279	2550	2772	81
3,4,4′	1967	1971	2085	2303	2570	2792	77
2,6.2′,6′	1840	1848	1859	2163	2380	2526	87
2,5,2′,5′	1930	1935	1995	2232	2442	2642	86
2,4,2′,4′	1938	1941	2027	2230	2464	2650	84
2,3,5,6	1955	1959	2020	2221	2452	2612	69
2,3,2′,3′	1998	2005	2055	2335	2599	2745	89
2,3,4,5	2039	2043	2127	2330	2570	2750	84
3,5,3′,5′	2045	2052	2192	2310	2587	2848	82
3,4,3′,4′	2161	2156	2282	2553	2802	3055	84
2,4,5,2′,5′	2095	2096	2175	2375	2618	2830	83
2,4,5,2′,4′,5′	2262	2250	2356	2558	2800	3025	88

[a] Only the positions substituted with chlorine are indicated for the PCBs.

[b] Relative to biphenyl = 100.

[c] Observed for reference compounds listed in the text, calculated for the others.

The calculated responses were derived as follows: The biphenyl skeleton was arbitrarily assigned a response value of 100. Substitution of chlorine into positions 2, 2′, 6, or 6′ required that 3 response units be subtracted for each such substitution, and 6 additional units be subtracted for each such substitution *after the first* (a "twist" correction). This "twist" factor has also been observed in the electron capture response of these compounds[14]. Each chlorine at positions 3, 3′, 5, or 5′ required that 11 units be subtracted, and 16 units were subtracted for each substitution at 4 or 4′. If both phenyl rings were substituted, 10 units were added for each chlorine in the least substituted ring ("transanular" correction). Substitution of chlorine adjacent to another chlorine raised the response by 1.5 units for each 2,3- (or 5,6-), and 9.5 units for each 3,4- (or 4,5-) substitution ("vicinal" corrections). Confidence limits for these calculations as given in Table I increase as the number of terms increases, but do not exceed 5 units (approx. 5%) for up to six chlorine atoms per molecule. Lower confidence limits can be attained by using the observed response of more highly substituted PCBs in the calculations. The observed responses for the reference compounds in Table I have an average deviation from the calculated responses of 2.2, *i.e.* 2.6% of the average response.

The six compounds listed above (biphenyl, 2,3-, 2,6-, 2,4′-, and 3,4-dichlorobiphenyls, and 2,4,4′-trichlorobiphenyl), the PCBs listed in Table I, and the three monochlorobiphenyls comprise the reference compounds used throughout this study, twenty-seven in all. Information presented in Tables II, IV and VI for PCBs other than these reference compounds has been calculated as indicated above and in what follows.

Table II lists retention indices and relative molar responses for all of the PCBs having up to three chlorines per molecule as well as for those PCB reference compounds available to us having more than three chlorines per molecule.

PCBs having both rings substituted could have their retention indices calculated by summing ½ (*R.I.*) values[8]. The observed retention indices of the seventeen reference compounds substituted in both rings, examined on all six liquid phases (102 terms), had an average deviation of ± 3.7 retention index units from their corresponding calculated retention indices. Since this is in excellent agreement with the sum of the average errors of estimate inherent in our graphical estimation of two ½ (*RI*) values (± 2 units each), it is apparent that additivity of ½ (*R.I.*) values is generally valid for at least the six liquid phases tested and probably for all liquid phases applicable to PCB analysis. In experiments not reported here in detail we found the retention indices for several of the PCBs to vary logarithmically with the column temperature. Additivity of ½ (*R.I.*) values would thus require a very reliable control of the column temperature throughout the duration of an analysis. The data in Table II will permit calculation of retention indices for several tetra-, penta-, hexa-, hepta-, and octachlorobiphenyls.

Table III lists boiling points for a few of the substituted biphenyls. When these values are applied to a plot of boiling point against chain length × 100 for *n*-alkanes, theoretical retention indices that should apply in the absence of any interaction with a GLC packing are obtained.

Comparison of Table III, which also lists observed retention indices on paraffin wax and purified Apiezon L, with Table II reveals that the theoretical retention indices are most closely matched on Versilube F-50 (a trichlorophenyl-substituted

TABLE III

BOILING POINTS, AND CALCULATED AND OBSERVED RETENTION INDICES (*R.I.*) FOR PCBs

Compound[a]	*Boiling point (°C)*	*R.I. value*		
		Calculated[b]	*Observed on column packing*	
			Paraffin wax (125°)	*Purified Apiezon L (205°)*
Bicyclohexyl	238	1242	1354	1410
Biphenyl	256	1417	1394	1452
2	274	1521	1479	1543
3	285	1587	1569	1640
4	291	1625	1580	1650
4,4'	317	1800	1766	1848

[a] The chlorine-substituted biphenyl positions are given.
[b] Calculated from boiling points as described in text.

silicone). Due to dispersion forces, even *n*-alkanes would not have theoretical retention indices if they could be gas chromatographed in reference to compounds lacking even dispersive interaction with a liquid phase. Thus the apparent correspondence between retention indices observed on F-50 and calculated for PCBs from their boiling points is coincidentally due to having selected 200° as the operating temperature. Since the retention indices calculated from PCB boiling points are higher than those observed on OV-101 at 200°, it appears that dispersion forces are less for PCBs than for *n*-alkanes at this temperature. This is particularly clear in the case of biphenyl itself which is unlikely to interact with OV-101 via hydrogen bonding, dipole orientation, or electron repulsion, and for 4,4'-dichlorobiphenyl which similarly lacks any permanent dipole. Thus dipole induction (σ or π interaction) and, in some cases, orientation forces on F-50 coincidentally balance out the low dispersion forces (relative to *n*-alkanes) at 200°.

We have attempted to use an approach based on Δ*R.I.* values (*i.e.*, retention index on one liquid phase minus retention index on another) to examine the nature of the interactions of the PCBs with the various liquid phases used in this study. The six liquid phases in Table II give fifteen permutations of Δ*R.I.* values producing positive numbers. We have selected six of these as giving the most useful information, as summarized in Table IV.

ROHRSCHNEIDER constants[12] (Table V) suggest that Δ*R.I.* values for F-50 minus OV-101 would mainly reflect orientation forces involving hydrogen bonding (sample as donor) and induction forces (sample as σ or π acceptor). Δ*R.I.* for Apiezon L minus F-50 should reflect primarily the ability of the PCB to serve as a σ or π donor in inductive interactions, which in this case would also reflect the degrees of electron delocalization in the PCBs. Δ*R.I.* for OV-17 minus Apiezon L should give information concerning interactions of permanent dipoles as well as charge transfer phenomena, while Δ*R.I.* for CHDMS minus OV-225 should emphasize both types (directions) of dipole induction as symbolized by X and U ROHRSCHNEIDER constants. Δ*R.I.* values for CHDMS minus OV-101 and OV-225 minus OV-101, respectively, should, in effect, summarize all of the polar interactions.

Induced dipole interactions occur on all "selective" liquid phases. While

TABLE IV

SELECTED $\Delta R.I.$ VALUES FOR PCBs

Compound[a]	*Liquid phase*					
	F-50 minus OV-101	*OV-225 minus OV-101*	*CHDMS minus OV-101*	*Ap. L minus F-50*	*OV-17 minus Ap. L*	*CHDMS minus OV-225*
Bicyclohexyl	4	135	157	60	25	22
Biphenyl	12	435	605	82	166	170
2	10	460	611	59	214	151
3	18	485	672	85	176	187
4	17	496	683	87	187	187
2,6	9	486	626	51	234	143
2,2′	8	502	643	41	248	141
2,5	16	478	661	74	194	183
2,4	12	473	651	78	184	178
2,3′	8	507	688	67	225	181
2,4′	12	508	687	75	230	179
2,3	7	519	674	69	223	155
3,5	17	487	703	104	140	216
3,3′	19	536	756	95	172	220
3,4	16	552	753	102	177	201
3,4′	17	555	757	100	192	202
4,4′	15	568	783	106	198	215
2,6,2′	2	508	646	28	274	131
2,5,2′	12	503	674	47	249	170
2,4,2′	9	505	678	60	232	177
2,6,3′	12	535	712	55	236	170
2,6,4′	11	592	728	59	254	128
2,3,2′	6	546	687	49	244	166
2,4,5	1	482	682	94	181	202
3,5,2′	7	524	718	82	186	194
2,5,3′	21	539	733	74	210	179
2,5,4′	18	541	744	78	219	204
2,4,3′	13	526	725	87	192	204
2,4,4′	15	543	742	92	200	207
2,3,3′	14	570	747	74	230	177
2,3,4′	12	583	758	74	243	175
3,4,2′	− 1	568	762	86	242	194
3,5,3′	14	538	776	115	151	238
3,5,4′	12	551	787	119	198	236
3,4,3′	5	592	816	110	206	222
3,4,4′	4	603	825	114	218	222
2,6,2′,6′	8	540	686	11	214	146
2,5,2′,5′	5	512	712	60	237	180
2,4,2′,4′	3	526	712	86	203	190
2,3,5,6	4	497	657	61	201	160
2,3,2′,3′	7	601	747	50	280	146
2,3,4,5	4	531	711	84	203	180
3,5,3′,5′	7	542	803	60	118	261
3,4,3′,4′	− 5	641	894	126	271	253
2,4,5,2′,5′	1	523	735	79	200	212
2,4,5,2′,4′,5′	−12	538	763	106	200	225

[a] Only the positions substituted with chlorine are indicated for the PCBs.

TABLE V

ROHRSCHNEIDER CONSTANTS USED IN THIS STUDY

Liquid phase	*X*	*Y*	*Z*	*U*	*S*
OV-101	0.16	0.56	0.50	0.85	0.48
Versilube F-50	0.13	0.98	0.51	1.11	0.43
Apiezon L	0.32	0.39	0.25	0.48	0.55
Purified Apiezon L	0.29	0.28	0.17	0.44	0.30
OV-17	1.30	1.66	1.79	2.83	2.47
OV-225	2.17	3.20	3.33	5.16	3.69
CHDMS	2.50	4.34	3.54	5.43	4.05
Arochlor 1254	1.27	1.59	1.83	2.19	2.04

bicyclohexyl shows some slight dipole induction (low polarizability) in that its retention index increases by 157 units between OV-101 and CHDMS, biphenyl shows high polarizability as its retention index increases 605 units between these liquid phases. All of the PCBs show *ΔR.I.* values (CHDMS minus OV-101) higher than 605, and since several of them do not have permanent molecular dipole moments, it is probable that interactions between the liquid phases and individual group dipoles are of significance. Thus whereas the *ΔR.I.* (CHDMS minus OV-225) for 3,3′-dichlorobiphenyl is higher than that for 4,4′-dichlorobiphenyl, the opposite order is seen in *ΔR.I.* for (CHDMS minus OV-101). *ΔR.I.* (CHDMS minus OV-225) is identical for the 3- and 4-monochlorobiphenyls, confirming that this parameter is relatively independent of the absolute value of the permanent molecular dipole moment. *ΔR.I.* (CHDMS minus OV-101) increases with both the dipole moment and polarizability of the monochlorobiphenyl, so the observed *ΔR.I.* values for the 3,3′- and 4,4′-isomers clearly suggest an interaction based on individual group moments.

ΔR.I. values indicate that there is greater selective interaction between the 3- and 4-monochlorobiphenyls and the various liquid phases than between the liquid phases and biphenyl itself. However, *ΔR.I.* values for F-50 minus OV-101, Apiezon L minus F-50, and CHDMS minus OV-225, respectively, are higher for biphenyl than for 2-chlorobiphenyl. Under these conditions we are measuring primarily induction forces (for OV-17 minus Apiezon L, where as discussed above dipole moment is emphasized, *ΔR.I.* for 2-chlorobiphenyl is higher than that for biphenyl), indicating either that the polarizability of the 2-chlorobiphenyl or its ability to serve as a π-donor is less than that of biphenyl. The latter might be due to the tendency of substitution in the 2-position to twist the two rings out of coplanarity. This phenomenon is particularly conspicuous in terms of *ΔR.I.* for Apiezon L minus F-50. Here we primarily see the interaction between chlorinated biphenyls as π-donors and Apiezon L as a π-acceptor; the ROHRSCHNEIDER X constant for Apiezon L is higher than that for F-50 while Y, Z, U and S constants are all lower. As we go through the series 2-, 2,2′-, 2,6,2′-, 2,6,2′,6′-PCB, *ΔR.I.* for Apiezon L minus F-50 drops rapidly from 59 through 41 and 28 to 11, indicating that overall electron delocalization is decreasing rapidly.

ΔR.I. for CHDMS minus OV-101 increases continually with increasing substitution in 2- and 6-positions. Although the permanent dipole moment does not increase between 2,6,2′- and 2,6,2′,6′-PCB, as reflected in the observation that *ΔR.I.* (OV-17 minus Apiezon L) increases from 2 < 2,2′ < 2,6,2′ and drops 2,6,2′ > 2,6,2′,6′, it appears that dipole *induction* does increase throughout. This further suggests that (1) in terms of *ΔR.I.* for CHDMS minus OV-101, inductive forces outweigh orientation forces in significance, and (2) for the 2-chlorobiphenyl/biphenyl comparison, reduced net polarizability of the chlorinated biphenyl is a less satisfactory explanation of the *ΔR.I.* behavior than is reduced π-donor capability resulting from the ring "twist".

The monochlorobiphenyls show another peculiarity in their *ΔR.I.* values for OV-17 minus Apiezon L. Here the sequence of *ΔR.I.* values is biphenyl < 3-chlorobiphenyl < 4-chlorobiphenyl < 2-chlorobiphenyl. One possible explanation for this observation, assuming that *ΔR.I.* for OV-17 minus Apiezon L reflects dipole moments, is related to the fact that electron delocalization tends to reduce effective dipole moments. The reduced electron delocalization resulting from the "twist" in the 2-chlorobiphenyl would tend to minimize the resonance reduction of its dipole moment.

A second factor may enter into the *ΔR.I.* values for CHDMS minus OV-225. Here the order is 2-Cl < biphenyl < 3-Cl = 4-Cl. Since these values are all positive, there presumably is greater interaction between the electrophilic chlorine atoms and the oxygen functions of CHDMS than between the chlorines and the cyano groups of OV-225. However, the OV-225 cyano groups are extended from the polymeric backbone of the molecule by propyl side chains, while the CHDMS ester groups are buried in the polymeric matrix. Steric interference by the adjacent phenyl group may impede interaction of the 2-chlorobiphenyl chlorine with CHDMS while less steric interference would be expected against OV-225. Thus the similarity in *order* of *ΔR.I.* values between CHDMS minus OV-225 and Apiezon L minus F-50 for biphenyl and the monochlorobiphenyls, respectively, is postulated to be due to two entirely different causes.

TABLE VI

ELUTION ORDER AND *ΔR.I.* VALUES OF HOMOANNULAR (H) *vs.* TRANSANNULAR (T) PAIRS

Pair[a]	*Elution order*	*ΔR.I. value*	
		Liquid phase minus OV-101	
		F-50, Apiezon L	*OV-17, OV-225, CHDMS*
2,6–2,2′	H < T[b]	T < H	H < T
2,5–2,3′	H < T	T < H	H < T
2,4–2,4′	H < T	T ≤ H	H < T
3,5–3,3′	H < T	H < T	H < T
2,3–2,3′	T < H[c]	H < T[b]	H < T[d]
3,4–3,4′	T ≃ H	H ≤ T	H ≤ T

[a] Only the chlorine-substituted positions are indicated for the PCBs.
[b] Except Apiezon L.
[c] Except CHDMS.
[d] Except OV-225.

Table VI summarizes some observations on the elution order of various dichlorobiphenyl pairs. The prevalent elution order is apparently homoannular < transannular in spite of the presumably higher dipole moment of the homoannular species. *Vicinal* substitution would possibly be expected to reduce the effective dipole moment (considered as the absolute sum of individual group moments) compared to the transannular species by shifting the C–Cl bonds out of the plane of the rings. Instead we find that *vicinal* substitution increases the retention of the homoannular species such that the elution time becomes equal to or greater than that of the transannular species. The exceptions in Table VI provide some information. On Apiezon L 2,6-dichlorobiphenyl elutes after 2,2′-dichlorobiphenyl suggesting that π-interactions are somewhat greater in the case of the homoannular species. *Vicinal* substitution has less retarding effect on OV-17 and CHDMS (which emphasize dipolar interactions) than on the other columns.

The $\Delta R.I.$ values for any liquid phase minus OV-101 are also summarized for the dichlorobiphenyl pairs in Table VI. It can be seen that $\Delta R.I.$ values for the homoannular species on OV-17, OV-225, and CHDMS are always less than or equal to those of the transannular species. On F-50 and Apiezon L the values are lower for transannular than for homoannular species in some cases, reversed for others. These observations suggest that the transannular species take part more strongly than their corresponding homoannular isomer in dipole-induced dipole interactions not involving their role as π-donors in charge transfer interactions, but tend to support the view that the homoannular species enter into charge transfer, π-interactions more strongly than do their transannular analogues. *Vicinal* substitution then appears to have a greater effect on decreasing the π-interaction than on decreasing the polarizability overall.

Polarizability effects are emphasized in $\Delta R.I.$ for OV-225 minus OV-101 (see Table IV). Here it can be seen by subtracting $\Delta R.I.$ for biphenyl from that of the chlorinated compounds that the $\Delta R.I.$ increments produced by adding chlorines may be an approximately additive function for transannular substitutions (3-Cl = 50, 3,3′-Cl_2 = 101; 4-Cl = 61, 4,4′-Cl_2 = 133; 3,5-Cl_2 = 52, 3,5,3′,5′-Cl_4 = 107), or non-additive for homoannular substitutions (2-Cl = 25, 4-Cl = 61, 2,4-Cl_2 = 38; 3-Cl = 50, 3,5-Cl_2 = 52). This apparently reflects interaction between the chlorine atoms in the same ring, possibly through the ring itself since vicinal substitution, forcing the C–Cl bond out of the plane of the ring, restores additivity (3-Cl = 50, 4-Cl = 61, 3,4′-Cl_2 = 120, 3,4-Cl_2 = 117).

Three conspicuous entries in Table IV are the $\Delta R.I.$ values (F-50 minus OV-101) for 3,4,2′-trichloro-, 3,4,3′,4′-tetrachloro-, and 2,4,5,2′,4′,5′-hexachloro-PCBs, which are negative. It is not clear whether this relates to mutual repulsion between the trichlorophenyl group in F-50 and 3,4-substituted rings in the biphenyls or simply to increased dispersion forces for these PCBs.

A comprehensive treatment of the interaction forces accessible to study through consideration of $\Delta R.I.$ values as related to PCB structures is beyond the scope of this paper. We have attempted through qualitative discussion to give a few examples of the utility of this approach which, we believe, may lend itself to quantitative treatment as further data become available. The liquid phases chosen for these experiments were not selected for maximum yield of information on solute–solvent interactions, but rather to provide maximum resolution of similar PCB isomers and permit quantitative analysis. The discussion above makes it seem probable that liquid phases for GLC could be selected such that the effects on retention indices of the PCBs (and other classes of compounds) of dispersive interactions, hydrogen bonding, dipole–dipole orientation forces, charge transfer interactions, and dipole-induced dipole (induction) forces could be effectively "isolated" for quantitative study. We see reason to hope that this approach may eventually overcome the limitations in the present use of ROHRSCHNEIDER constants to characterize liquid phases, such that a quantitative utility may be achieved.

REFERENCES

1 F. J. BIROS, A. C. WALKER AND A. MEDBERY, *Bull. Environ. Contam. Toxicol.*, 5 (1970) 317.
2 O. HUTZINGER, S. SAFE AND V. ZITKO, *Bull. Environ. Contam. Toxicol.*, 6 (1971) 209.
3 M. L. PORTER AND J. A. BURKE, *J. Ass. Off. Agric. Chem.*, 54 (1971) 1426.
4 A. C. TAS AND R. H. DEVOS, *Environ. Sci. Technol.*, 5 (1971) 1216.
5 V. ZITKO, O. HUTZINGER AND S. SAFE, *Bull. Environ. Contam. Toxicol.*, 6 (1971) 160.
6 J. W. ROTE AND P. G. MURPHY, *Bull. Environ. Contam. Toxicol.*, 6 (1971) 377.
7 D. T. WILLIAMS AND B. J. BLANCHFIELD, *J. Ass. Off. Agric. Chem.*, 54 (1971) 1429.
8 D. SISSONS AND D. WELTI. *J. Chromatogr.*, 60 (1971) 15.
9 D. L. STALLING, *Workshop on Pesticide Residue Analysis, 2nd International Congress of Pesticide Chemistry, Tel Aviv, Israel, February 22–26, 1971.*
10 B. MELVAS, *PCB Conference, Nat. Swed. Environ. Prot. Board, Stockholm, September 29, 1970.*
11 P. W. ALBRO AND L. FISHBEIN, *Bull. Environ. Contam. Toxicol.*, in press.
12 L. ROHRSCHNEIDER, *J. Chromatogr.*, 22 (1966) 6.
13 SUPELCO, INC., *Catalog 1971*, (1971) 3.
14 N. L. GREGORY, *J. Chem. Soc.*, (1968) 295.