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RELATIONSHIP BETWEEN STRUCTURE AND RETENTION OF POLY-HALOGENATED AROMATICS IN TWO ADSORPTION CHROMATOGRAPHIC SYSTEMS*

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SUMMARY

High-performance liquid chromatography of a large number of halogenated benzenes, biphenyls and naphthalenes has been carried out in two adsorption systems, *viz.*, silica gel-*n*-hexane and aluminium oxide-*n*-hexane. The dependence of retention on the type, number and position of the halogen atoms present in the aromatic nucleus is discussed and a number of rules are presented.

INTRODUCTION

It has been shown that, in addition to gas chromatography, high-performance liquid chromatography (HPLC) also has great potential for the analysis of halogenated aromatics. A considerable amount of data on the retention behaviour of polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs) and polychlorinated naphthalenes (PCNs) has been published¹⁻⁴ for the system silica gel-dry *n*-hexane. The HPLC of PCBs using, *e.g.*, pyrocarbon-modified silica gel^{5,6} or a polystyrene-type cation-exchange resin⁷ as the stationary phase and partly aqueous solvent mixtures as the eluent has also been considered; gel-permeation chromatography has been applied⁸ to the analysis of PCBs present in a polymer matrix.

In this paper, for the system silica gel-*n*-hexane, previously published tentative rules concerning the dependence of retention on the number and position of the halogen atoms in the biphenyl and naphthalene nuclei are summarized. In addition, results for a large number of halogenated benzenes are discussed.

Secondly, data obtained for the same classes of compounds using the adsorption system aluminium oxide-*n*-hexane are compared with those reported for the silica gel system.

MATERIALS AND METHODS

The commercially available PCB and PCN samples investigated were Aroclor 1221, 1242, 1248, 1254, 1260 and 1268 (Monsanto, St. Louis, Mo., U.S.A.) and

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Halowax 1031, 1000, 1001, 1099, 1013, 1014 and 1051 (Koppers, Pittsburgh, Pa., U.S.A.), respectively. The sources of the individual PCBs, PBBs and PCNs have been reported in previous papers¹⁻⁴. Samples of the fluorobenzenes and mixed halogenobenzenes were donated by Dr. J. Visser (I.K.O., Amsterdam, The Netherlands) and Dr. J. J. M. Hageman (University of Amsterdam, Amsterdam, The Netherlands), respectively. Solutions of all mixtures and compounds were prepared in *n*-hexane.

HPLC was carried out on a Siemens S100 liquid chromatograph equipped with a 50- μ l Valco six-port injection valve and a Zeiss PM2 DLC UV detector. The separation columns were 25 cm \times 3 or 4.6 mm I.D. stainless-steel tubes pre-packed with 5- μ m LiChrosorb SI 60 silica gel (Merck, Darmstadt, G.F.R.) or 5- μ m LiChrosorb Alox T5 (Chrompack, Middelburg, The Netherlands). *n*-Hexane (ChromAR grade; Mallinckrodt, St. Louis, Mo., U.S.A.) dried over molecular sieve 5A was used as the mobile phase. The columns were maintained thermostatically at $27 \pm 1^\circ$. Detection was effected at or near the wavelength of maximum absorption of the compound or mixture under investigation, *i.e.*, at ca. 200–215 nm for PCBs and PBBs, 220–275 nm for PCNs and 200–230 nm for halogenated benzenes (PXBes).

RESULTS AND DISCUSSION

Chromatograms of all compounds were run in duplicate at a flow-rate of 2–3 ml·min⁻¹. The capacity factor, k'_i , of component *i* was determined from its retention time, t_{Ri} , and the retention time, t_{R0} , of a non-retarded compound (*n*-pentane):

$$k'_i = (t_{Ri} - t_{R0})/t_{R0} \quad (1)$$

The relative retention, α_{ij} , of components *i* and *j* was calculated from their capacity factors:

$$\alpha_{ij} = k'_i/k'_j \quad (2)$$

Silica gel-n-hexane

Halogenated biphenyls. Chromatograms recorded for the complete series of Aroclor mixtures indicate that the retention times of the PCBs generally decrease with increasing chlorine content. This is illustrated by the chromatograms shown in Fig. 1: the range in which the Aroclors elute shifts to shorter times when going from a mixture containing 21% (w/w) to one containing 60% (w/w) of chlorine. However, from further studies carried out for over 60 individual PCBs, it is obvious that this is not a strict rule: retention varies widely with both the number and position of the chlorine atoms in the biphenyl nucleus. From the results in Table I, and from other previously published compilations of retention time data on PCBs^{1,2}, several conclusions can be drawn, as follows.

(1) Although, in general, retention decreases when an increasing number of chlorine atoms are introduced into the biphenyl nucleus, the introduction of chlorine atoms *ortho* to the phenyl-phenyl bond (2- and 6-positions) promotes retention. To

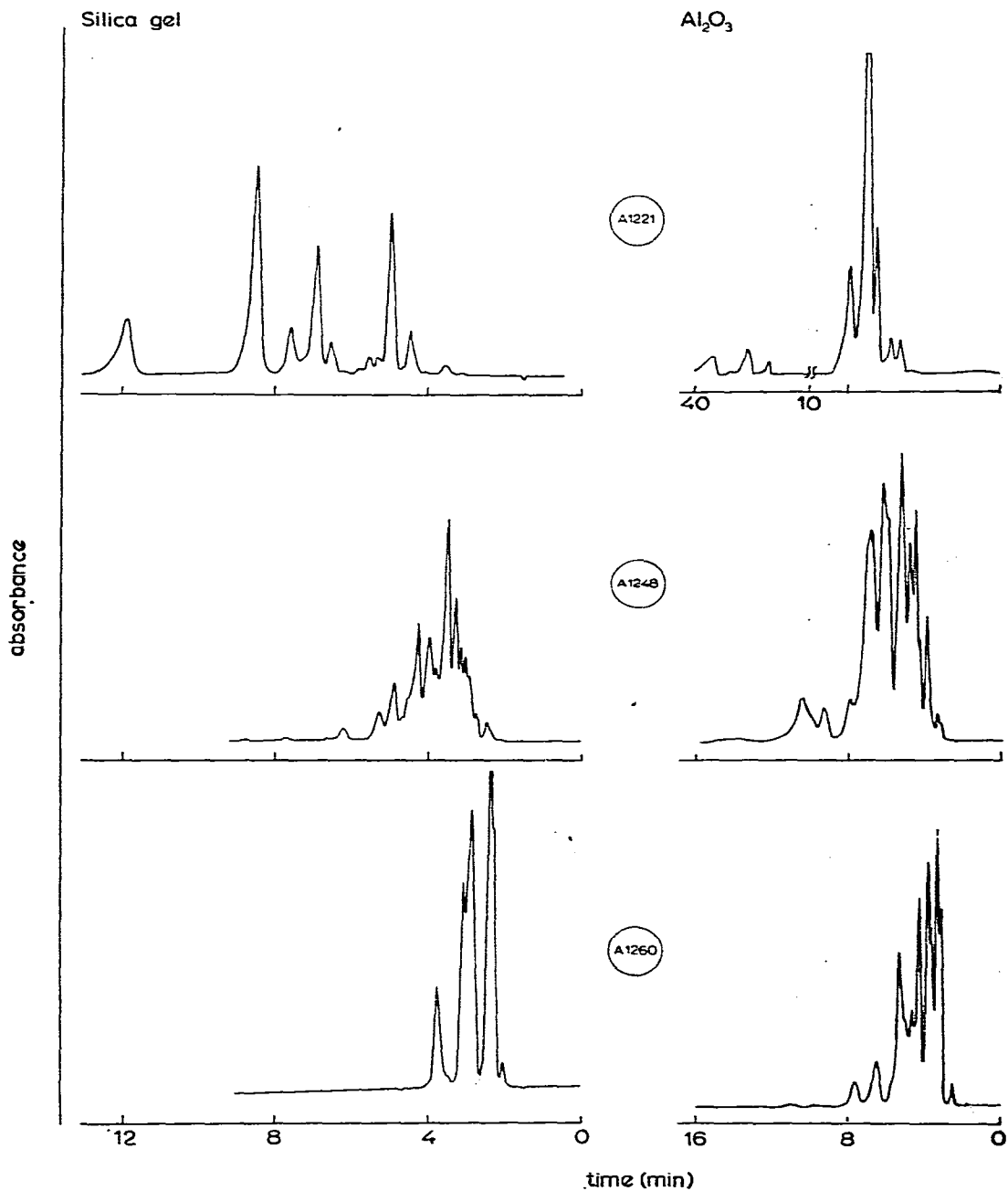


Fig. 1. HPLC of Aroclor 1221, 1248 and 1260 (which contain 21, 48 and 60%, w/w, of chlorine, respectively) on silica gel and aluminium oxide. Mobile phase, dry *n*-hexane; flow-rate, 2 and 3 ml·min⁻¹, respectively.

TABLE I

CAPACITY FACTORS OF PCBs AND PBBs IN THE SYSTEM SILICA GEL-*n*-HEXANE

Substitution	<i>k'</i>		Substitution	<i>k'</i>	
	Cl	Br		Cl	Br
Biphenyl	9.25	9.25	2,3,2',3'	3.50	—
2	6.35	6.90	2,4,2',4'	1.65	—
3	4.60	4.85	2,5,2',5'	2.00	2.90
4	5.05	5.20	2,6,2',6'	3.75	—
2,2'	5.50	6.65	3,4,3',4'	2.35	—
3,3'	2.65	—	3,5,3',5'	0.95	1.55
4,4'	2.90	3.30	2,3,2',4'	2.35	—
2,3	5.25	—	2,4,6,3'	1.70	—
2,4	3.80	—	2,4,6,4'	1.55	—
2,5	3.55	4.15	2,5,3',4'	1.85	—
2,6	5.40	—	2,3,4,2',3',4'	2.15	—
3,4	4.00	—	2,3,6,2',3',6'	2.25	—
3,5	2.60	—	2,4,5,2',4',5'	1.15	2.10
2,5,2'	3.20	4.25	2,4,6,2',4',6'	0.75	—
2,5,3'	2.15	2.75	3,4,5,3',4',5'	1.50	3.05
2,5,4'	2.05	2.65	Deca	0.45	2.25

a lesser extent, the same is true for 2,3-substitution. This conclusion is strikingly demonstrated by such series as:

$$2 > 4 > 3 \quad (3a)$$

$$2,2' > 4,4' > 3,3' \quad (3b)$$

$$2,6,2',6' > 2,3,2',3' > 2,5,2',5' > 2,4,2',4' \quad (3c)$$

and also from the fact that, *e.g.*, 2,3,2',3'-tetra-PCB has a higher capacity factor than has 3,3'-di-PCB, while both 3- and 4-mono-PCB or -PBB move ahead of the corresponding, more highly substituted, 2,2'-di-PCB or -PBB, respectively. For not too highly substituted halogenated biphenyls the *ortho* effect is about equally large for PCBs and PBBs, as shown by the values calculated for the relative retentions of, *e.g.*, the pairs 2-PXB/4-PXB, 2-PXB/3-PXB, 2,2'-PXB/4,4'-PXB and 2,5,2',5'-PXB/3,5,3',5'-PXB, which are 1.27 and 1.32, 1.38 and 1.42, 1.91 and 2.02, and 2.15 and 1.90, for X = Cl or Br, respectively.

(2) Replacement of a chlorine with a bromine atom invariably leads to an increase in capacity factor. For the mono- to tetra-substituted biphenyls, where three or four pairs of compounds are available for comparison, we can conclude that $\alpha_{\text{PBB/PCB}}$ is determined chiefly by the degree of substitution: mono, 1.05; di, 1.2; tri, 1.3; tetra, 1.5. For the remainder, the order of elution in the PBB series is broadly the same as that encountered in the PCB series.

(3) The order of elution of halogenated biphenyls bearing one to four halogen atoms in a single ring as a rule is the same as that of the symmetrically substituted compounds having from two to eight substituents. One example can be read from sequences 3a and b, while further illustrations are given in Table II here and in

Table III in ref. 2. From these tables it is also apparent that the capacity factors of, *e.g.*, a,b,d',e'-tetra-PCBs and a,b,c,d',e',f'-hexa-PCBs are intermediate between those of a,b,a',b'- and d,e,d',e'-tetra-PCBs, and a,b,c,a',b',c'- and d,e,f,d',e',f'-hexa-PCBs, respectively.

TABLE II

COMPARISON OF RETENTION BEHAVIOUR OF DI- AND TETRA-SUBSTITUTED PCBs IN THE SYSTEM SILICA GEL-*n*-HEXANE

PCB	<i>k'</i>	PCB	<i>k'</i>
3,5	2.60	3,5,3',5'	0.95
2,4	3.80	2,4,2',4'	1.65
		2,4,3',4'	1.70
		2,4,2',5'	1.90
2,5	3.55	2,5,2',5'	2.00
3,4	4.00	3,4,3',4'	2.35
		2,3,2',4'	2.35
		2,3,2',5'	2.65
2,3	5.25	2,3,2',3'	3.50
2,6	5.40	2,6,2',6'	3.75

(4) The data in Table III in ref. 2 indicate that within the series of tri- and hexa-substituted PCBs investigated, retention decreases in the order

$$2,3,6 > 2,3,4 > 3,4,5 > 2,4,5 > 2,4,6 \quad (4)$$

The same sequence is observed with the 2,3,4,5,a',b',c'-hepta-substituted PCBs. One immediately notes the extraordinary behaviour of the 2,4,6-substituted compounds. Although these bear two (tri), three (hepta) or even four (hexa) chlorine atoms *ortho* to the central C-C bond, which should promote retention (see above), their capacity factors are seen to be unexpectedly small. Thus, whereas 2,6-substitution promotes retention, 2,4,6-substitution diminishes the interaction between the substituted PCB and the silica gel. Here, we merely note that the halogen atoms in the 2,4,6-substituted ring(s) are in a *meta* position relative to each other (although, admittedly, not to the phenyl-phenyl bond) and that relatively low capacity factors are also observed for the *meta*-substituted compounds 3,5-di- and 3,5,3',5'-tetra-PCB and -PBB in the series of di- and tetra-halogenated biphenyls, respectively.

Halogenated benzenes. In order to augment our knowledge of the relationship between retention and structure of halogenated aromatics, capacity factors were also recorded for the complete series of chlorobenzenes, a large number of bromo- and fluorobenzenes and three iodobenzenes. The results are reported in Table III and Fig. 2. Limiting ourselves to the chloro- and bromo-substituted compounds, we note that again retention generally decreases with an increasing number of substituents. This rule is more strictly adhered to with the chloro- than the bromobenzenes, as shown by the values of $\alpha_{\text{PBBc}/\text{PCBc}}$, which increase continuously (from 1.1 to 2.5) with an increasing number of halogen atoms present in the benzene nucleus. The α values are primarily determined by the number, not the position, of the substituents, as was also observed with the halogenated biphenyls; for, *e.g.*, the three dihalogenobenzenes,

TABLE III

CAPACITY FACTORS OF POLYHALOGENOBENZENES IN THE SYSTEM SILICA GEL-*n*-HEXANE

Substitution	F	Cl	Br	I
Benzene	2.20	2.20	2.20	2.20
Mono	1.40	1.15	1.25	1.30
1,2	1.15	1.00	1.25	—
1,3	0.95	0.70	0.85	1.00
1,4	1.25	0.75	0.90	1.05
1,2,3	—	0.85	—	—
1,2,4	—	0.65	0.85	—
1,3,5	—	0.40	0.50	—
1,2,3,4	0.95	0.70	—	—
1,2,3,5	0.75	0.45	—	—
1,2,4,5	1.00	0.50	0.75	—
Penta	0.70	0.40	0.80	—
Hexa	0.45	0.30	0.80	—

values of 1.23, 1.25 and 1.26 are found. Another noteworthy aspect is that the 1,3,5-tri-substituted benzenes display very small k' values in both the chloro- and bromobenzene series, *i.e.*, *meta*-substitution again decreases retention.

As is readily apparent from Table III, the results obtained for the mono- and diiodobenzene(s) fit in well with those recorded for the chlorinated and brominated compounds. However, this is not so for the fluorobenzenes. Admittedly, capacity

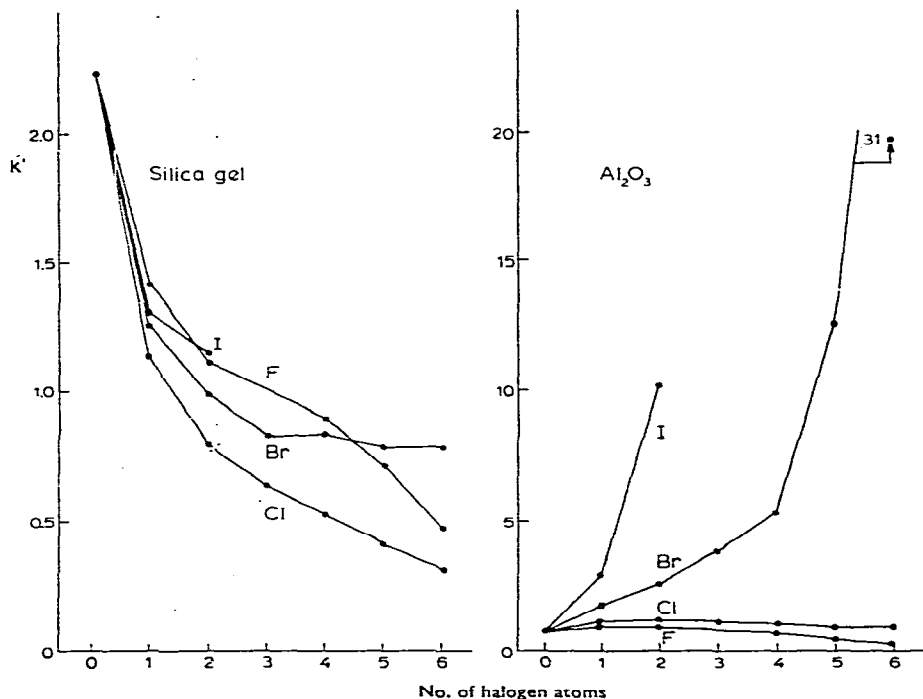


Fig. 2. Dependence of k' values of halogenated benzenes on the number of substituents for the systems silica gel-*n*-hexane and aluminium oxide-*n*-hexane.

factors do decrease with an increasing number of substituents, but the k' values are larger than those reported for the corresponding chloro-, and sometimes even the bromo- and iodobenzenes. Also, the order of elution observed within the series of di- and tetrafluorobenzenes does not parallel that recorded for the other halogens and *ortho*-substitution does not seem to play a very prominent role with the fluorine-containing compounds.

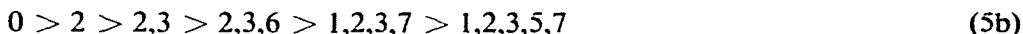
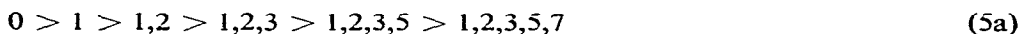
Lastly, attention has been paid to the behaviour of mixed poly-X,Y-halogenobenzenes. As can be seen from Table IV, the capacity factors of the mixed compounds, with a single minor exception (F/Br), are intermediate between those of the poly-X- and poly-Y-halogenobenzenes.

TABLE IV

COMPARISON OF CAPACITY FACTORS OF DI-X-, X,Y- AND DI-Y-BENZENES IN THE SYSTEM SILICA GEL-*n*-HEXANE

<i>Substitution</i>	<i>Di-X</i>	k'	<i>X, Y</i>	k'	<i>Di-Y</i>	k'
1,2	F	1.15	F, Br	1.25	Br	1.25
1,3	F	0.95	F, Br	0.90	Br	0.85
1,4	F	1.25	F, I	1.10	I	1.05
1,2	Cl	1.00	Cl, Br	1.10	Br	1.25
1,3	Cl	0.70	Cl, Br	0.75	Br	0.85
1,4	Cl	0.75	Cl, Br	0.85	Br	0.90
1,4	Cl	0.75	Cl, I	0.85	I	1.05

Halogenated naphthalenes. HPLC of the Halowax mixtures demonstrates that in general the retention times of the PCNs are lower than those of the PCBs and that, again, the introduction of an increasing number of chlorine atoms into a particular substituted naphthalene leads to a decrease in capacity factor (*cf.*, Fig. 3 and Table V). Two illustrative examples are:



where 0 represents naphthalene. A more detailed study of about 30 individual PCNs revealed that the following additional rules hold true:

(1) Introduction of chlorine atoms in non-adjacent α -positions decreases retention to a greater extent than does non-adjacent β -substitution. Examples are the low capacity factors observed for 1-mono-PCN compared with 2-mono-PCN and for 1,4- and 1,5-di-PCN compared with the 2,6- and 2,7-substituted isomers.

(2) The introduction of chlorine atoms in adjacent positions causes an increase in retention, especially if the introduction occurs in such a way that 1,8-substitution results. An impressive demonstration of this rule, and also the first rule, can be seen in Table VI. One should note the relatively high capacity factors of 1,3,5,8- and, especially, 1,4,5,8-tetra-PCN, which have three and four chlorine atoms in the α -position, respectively. Another noteworthy feature is the extremely low k' value recorded for 1,3,5,7-tetra-PCN. Probably the complete absence of substitution in any adjacent position causes this compound to move ahead of all PCNs studied, including the fully substituted octachloronaphthalene.

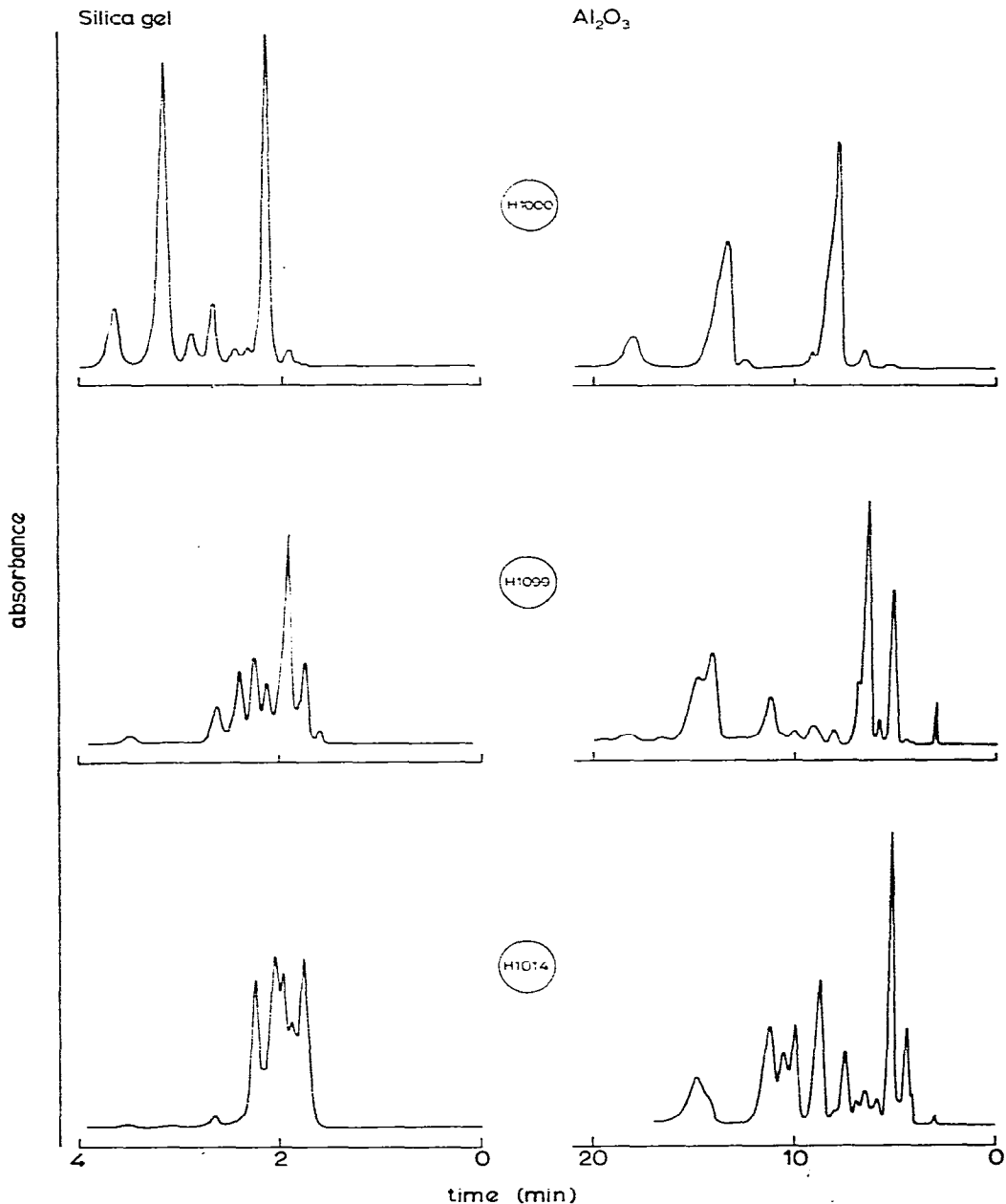


Fig. 3. HPLC of Halowax 1000, 1099 and 1014 (which contain 26, 52 and 62%, w/w, of chlorine, respectively) on silica gel and aluminium oxide. Mobile phase, dry *n*-hexane; flow-rate, 2 and 3 ml·min⁻¹, respectively.

Aluminium oxide-*n*-hexane

Halogenated biphenyls. As a preliminary means of comparison, chromatograms were run for the series of Aroclors. The results, three of which are included in Fig. 1, show that the retention time range in which the PCBs elute is wider than that in the

TABLE V

CAPACITY FACTORS OF PCNs IN THE SYSTEM SILICA GEL-*n*-HEXANE

PCN	<i>k'</i>	PCN	<i>k'</i>	PCN	<i>k'</i>
Naphthalene	3.80	1,2,3	1.30	1,2,3,4	0.75
1	1.80	1,2,5	0.80	1,2,3,5	0.70
2	2.20	1,2,6	1.10	1,2,3,7	1.10
1,2	1.60	1,2,7	1.30	1,2,4,6	0.55
1,3	1.05	1,2,8	2.15	1,3,5,7	0.45
1,4	0.90	1,3,6	0.80	1,3,5,8	0.80
1,5	0.90	1,3,7	0.90	1,3,6,7	0.85
1,8	2.25	1,3,8	1.40	1,4,5,8	1.30
2,3	1.95	1,4,6	0.75	1,4,6,7	0.70
2,6	1.50	2,3,6	1.45	Octa	0.45
2,7	1.50				

TABLE VI

DEPENDENCE OF CAPACITY FACTORS OF DI- AND TETRA-SUBSTITUTED PCNs ON THE POSITION OF THEIR CHLORINE ATOMS IN THE SYSTEM SILICA GEL-*n*-HEXANE

Di-PCN	<i>k'</i>	Position of adjacent Cl atoms	No. of α -Cl atoms	Tetra-PCN	<i>k'</i>	Position of adjacent Cl atoms	No. of α -Cl atoms
1,4	0.90	—	2	1,3,5,7	0.45	—	2
1,5	0.90	—	2	1,2,4,6	0.55	1,2	2
1,3	1.05	—	1	1,4,6,7	0.70	2,3	2
1,6	1.20	—	1	1,2,3,5	0.70	1,2; 2,3	2
1,7	1.35	—	1	1,2,3,4	0.75	1,2; 2,3	2
2,6	1.50	—	0	1,3,5,8	0.80	1,8	3
2,7	1.50	—	0	1,3,6,7	0.85	2,3	1
1,2	1.60	1,2	1	1,2,3,7	1.10	1,2; 2,3	1
2,3	1.95	2,3	0	1,4,5,8	1.30	1,8	4
1,8	2.25	1,8	2			1,8	

silica gel system. As a consequence, resolution is better with aluminium oxide as the stationary phase, especially for the more highly chlorinated biphenyls. For the remainder, retention tends to decrease with increasing halogen substitution in the biphenyl nucleus, but seemingly less so than in the silica gel system.

The behaviour of the individual PCBs and PBBs in the two adsorption systems under discussion will be compared by using the data reported in Tables I and VII.

(1) Without exception, the capacity factors recorded for aluminium oxide as adsorbent are higher than those observed for silica gel. This suggests that peaks may be "lost" from chromatograms such as shown in Fig. 1, as indeed occurred initially with the three late-eluting peaks shown in the chromatogram of Aroclor 1221 (later identified as 3-mono-PCB, biphenyl and 4-mono-PCB, in order of increasing retention). Consequently, in practical applications, the addition of a small percentage of water to the aluminium oxide-*n*-hexane system is to be recommended. However, as halogenated benzenes, which display low retention on aluminium oxide, were included in this study, dry *n*-hexane was preferred as the eluent. For the remainder,

TABLE VII

CAPACITY FACTORS OF PCBs AND PBBs IN THE SYSTEM ALUMINIUM OXIDE-*n*-HEXANE

Substitution	k'		Substitution	k'	
	Cl	Br		Cl	Br
Biphenyl	35	35	2,3,2',3'	9.8	—
2	8.0	10	2,4,2',4'	4.0	—
3	25	30	2,5,2',5'	4.0	14
4	35	54	2,6,2',6'	5.7	—
2,2'	6.5	12	3,4,3',4'	≫	—
3,3'	24	—	3,5,3',5'	8.2	17
4,4'	53	≫	2,3,2',4'	6.6	—
2,3	8.3	—	2,4,6,3'	2.7	—
2,4	5.3	—	2,4,6,4'	3.0	—
2,5	5.0	9.4	2,5,3',4'	5.7	—
2,6	5.7	—	2,3,4,2',3',4'	9.5	—
3,4	33	—	2,3,6,2',3',6'	4.7	—
3,5	—	—	2,4,5,2',4',5'	2.6	15
2,5,2'	4.9	13	2,4,6,2',4',6'	3.9	—
2,5,3'	5.3	12	3,4,5,3',4',5'	34	≫
2,5,4'	5.5	14	Deca	1.4	57

for a considerable number of halogenated aromatics we have verified experimentally that the addition of water does not result in any major changes in the order of elution within each series of compounds tested. Thus, the results presented below remain valid for less active adsorbents.

(2) As with silica gel, retention invariably increases when a chlorine atom is replaced with bromine. Also, the relative retention, $\alpha_{\text{PBB/PCB}}$, increases with increasing number of substituents; however, the extent to which this occurs is much greater than in the silica gel system: mono, 1.2; di, 1.8; tri, 2.4.

(3) Although there is a general tendency for the k' values to decrease with increase in the number of substituents, the positions of the halogen atoms are seen to exert a marked influence. For instance, the halogenobiphenyls, having substituents *ortho* to the phenyl-phenyl bond, display markedly lower k' values than do their non-*ortho*-substituted isomers. For the remainder, substitution *meta* to the central C-C bond affects retention only slightly, whereas substitution *para* to this bond strongly promotes retention, as shown by the high k' values observed for, e.g., 4-mono-, 4,4'- and 3,4-di- and 3,4,3',4'-tetra-substituted halogenobiphenyls. As a consequence, biphenyl itself elutes before several PCBs and PBBs. Therefore, the analysis cannot be terminated automatically as soon as the biphenyl peak emerges, a procedure which has always, and justifiably so¹, been adopted by us in the analysis of PCB mixtures on a silica gel column.

Apart from the fact that, on aluminium oxide, retention increases in the order *ortho* \ll *meta* $<$ *para*, which contrasts sharply with the sequence reported for silica gel, *viz.*, *meta* $<$ *para* $<$ *ortho* (*cf.*, eqns. 3a and b), some parallelism in the behaviour of the halogenated biphenyls on both adsorbents can also be noted. For example, the retention behaviour in the series of a-mono- and a,b-di-PCBs is analogous to that in the series of the symmetrically substituted a,a'-di- and a,b,a',b'-tetra-PCBs, respec-

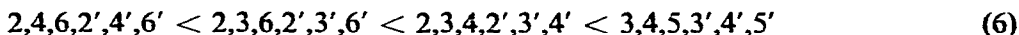
TABLE VIII

CAPACITY FACTORS OF POLYHALOGENOBENZENES IN THE SYSTEM ALUMINIUM OXIDE-*n*-HEXANE

Substitution	F	Cl	Br	I
Benzene	0.75	0.75	0.75	0.75
Mono	1.00	1.15	1.75	2.90
1,2	1.05	1.55	3.40	—
1,3	0.75	0.95	1.85	6.1
1,4	1.05	1.10	2.50	9.5
1,2,3	—	1.90	—	—
1,2,4	—	1.00	3.25	—
1,3,5	—	0.45	1.25	—
1,2,3,4	0.75	1.65	—	—
1,2,3,5	0.50	0.70	—	—
1,2,4,5	0.75	0.75	3.90	—
Penta	0.50	0.90	12.5	—
Hexa	0.25	0.90	31	—

tively. Also, for compounds such as 2,3,2',4'-tetra-PCB and 2,5,3',4'-tetra-PCB, k' values intermediate between those of the appropriately symmetrically substituted tetra-substituted PCBs (*cf.*, Table VII) are observed.

(4) In the series of symmetrically substituted hexachlorobiphenyls, the observed order of elution



is according to expectation: the number of substituents in a position non-*ortho* to the central C-C bond primarily determines retention. However, 2,4,5,2',4',5'-hexa-PCB displays a surprisingly small capacity factor, which cannot readily be accounted for, especially as this PCB has two retention-promoting chlorine atoms in the *para*-position, as has the 2,4,6,2',4',6'-isomer.

Halogenated benzenes. Retention of chloro-, bromo- and iodobenzenes on aluminium oxide is invariably stronger than on silica gel, as shown by the data in Table VIII. In addition, with the heavy halogens (Br, I), the capacity factors do not decrease with increase in the number of substituents (as occurs on silica gel), but show a sharp increase (Fig. 2). As a consequence, the values of *e.g.*, $\alpha_{\text{PBBc/PCBc}}$, are between 1.5 and 34.5 for aluminium oxide as adsorbent, compared with 1.1 and 2.5 (see above) for silica gel. For the remainder, several marked analogies exist between the results obtained in both adsorption systems. For example, with the dihalogenobenzenes, the capacity factors without exception increase in the order *meta* < *para* < *ortho*, while the retentions of 1,3,5-trichloro and -tribromobenzene are surprisingly weak on both silica gel and aluminium oxide. Further, the k' values always increase in the order Cl < Br < I, and the retentions of mixed poly-X,Y-halogenobenzenes are intermediate between those of the corresponding poly-X- and poly-Y-halogenobenzenes (Table IX).

The behaviour of fluorobenzenes on aluminium oxide in comparison with silica gel is markedly different to that observed for the other halogenobenzenes, retention being generally higher in the latter system. Thus the capacity factors for

TABLE IX

COMPARISON OF CAPACITY FACTORS OF 1,4-DI-X, 1,4-X,Y- AND 1,4-DI-Y-BENZENES IN THE SYSTEM ALUMINIUM OXIDE-*n*-HEXANE

<i>di-X</i>	<i>k'</i>	<i>X, Y</i>	<i>k'</i>	<i>di-Y</i>	<i>k'</i>
F	1.05	F, I	2.55	I	9.50
Cl	1.10	Cl, Br	1.60	Br	2.50
Cl	1.10	Cl, I	3.15	I	9.50
Br	2.50	Br, I	4.40	I	9.50

the fluorinated compounds recorded for aluminium oxide fit in well with those reported for the other substituted benzenes, at least as regards the now "normal" order of elution, F < Cl < Br < I.

Halogenated naphthalenes. The *k'* values of the PCNs on aluminium oxide vary more widely than those on silica gel; this parallels the behaviour of the halogenated biphenyls and benzenes. In addition, the *k'* values tend to decrease with the introduction of an increasing number of halogen atoms in the naphthalene nucleus. Surprisingly, this phenomenon, which can easily be seen from the data in Table X, is hardly apparent from the chromatograms shown in Fig. 3, although these do demonstrate that the overall resolution of the more highly chlorinated Halowax mixtures on aluminium oxide is superior to that on silica gel. Obviously, this seeming paradox is due to the ability of aluminium oxide to separate isomers (especially the series of tetra-PCNs), but not mixtures of compounds that differ in the extent of substitution. Possibly this effect is observed more easily with the Halowax than the Aroclor series owing to the smaller number of components present in the former mixtures.

TABLE X

CAPACITY FACTORS OF PCNs IN THE SYSTEM ALUMINIUM OXIDE-*n*-HEXANE

PCN	<i>k'</i>	PCN	<i>k'</i>	PCN	<i>k'</i>
Naphthalene	27	1,2,3	26	1,2,3,4	13
1	19	1,2,5	11.5	1,2,3,5	9.5
2	28	1,2,6	22	1,2,3,7	21
1,2	25	1,2,7	23	1,2,4,6	5.1
1,3	12.5	1,2,8	»	1,3,5,7	2.4
1,4	10.5	1,3,6	11	1,3,5,8	7.2
1,5	11	1,3,7	11.5	1,3,6,7	10.5
1,8	50	1,3,8	25	1,4,5,8	17.5
2,3	33	1,4,6	7.5	1,4,6,7	5.7
2,6	23	2,3,6	38	Octa	3.7
2,7	28				

For the dependence of retention on the position of the halogen atoms, it is remarkable that the order of elution of the PCNs on aluminium oxide is closely analogous to that on silica gel. This is clearly demonstrated by comparing the data in Tables V and X, which, for the sake of simplicity, have been regrouped in Table XI.

TABLE XI

COMPARISON OF RANGE OF k' VALUES OF DI- AND TRI-SUBSTITUTED PCNs IN THE SYSTEMS SILICA GEL- AND ALUMINIUM OXIDE- n -HEXANE

<i>Di-PCNs</i>	k'		<i>Tri-PCNs</i>	k'	
	<i>SiO₂ gel</i>	<i>Al₂O₃</i>		<i>SiO₂ gel</i>	<i>Al₂O₃</i>
1,3, 1,4, 1,5	0.9-1.1	11-12	1,4,6	0.7	7
1,2, 2,6, 2,7	1.5-1.6	23-28	1,2,5, 1,3,6, 1,3,7	0.8-0.9	11-12
1,8, 2,3	2.0-2.3	>30	1,2,3, 1,2,6, 1,2,7, 1,3,8 1,2,8, 2,3,6	1.1-1.4 >1.5	22-26 >35

CONCLUSION

About a decade ago, Snyder published a series of papers on adsorption chromatography, in which a detailed description was given of the major factors that influence the relative adsorption of different solutes on aluminium oxide and silica gel (*cf.*, refs. 9-11 and literature cited therein). Snyder suggested that, on aluminium oxide, the so-called weak localization of aromatic hydrocarbons and their halogen derivatives occurs by accommodation of the adsorbing group(s) on a linear group of active sites on the adsorbent. This explains the preferred retention of *para*- relative to *meta*-substituted benzenes and biphenyls. Further, non-planarity of biphenyls substituted *ortho* to the central C-C bond is accepted as the cause of the relatively weak retention of such isomers. In adsorption on silica gel, weak localization is stated not to play a role and, consequently, the marked tendency towards preferential adsorption of linear over non-linear isomers reported for aluminium oxide is not displayed by silica gel. Adsorption on the latter adsorbent is explained in terms of the presence of "reactive" and "bound" (plus some "free") silanols on the silica gel surface, the preferential adsorption of water and so-called non-localized solutes occurring on the reactive silanol sites. Solute planarity also appears to be important for adsorption on silica gel, although generally less so than on aluminium oxide. Snyder's conclusions were based mainly on experiments performed with fused aromatic ring systems, aromatic compounds bearing one or two, often rather polar, substituents, and mono- and dihalogenobenzenes.

The present retention data and the discussion demonstrate that, qualitatively, adsorption of not too highly halogenated (0-6 halogen atoms), and especially chlorinated and brominated, benzenes, biphenyls and naphthalenes on silica gel and aluminium oxide can be described adequately by means of a limited number of rules. Agreement between Snyder's data and the present results is generally satisfactory. For example, with aluminium oxide as adsorbent, for a series of *ca.* 20 halogenobenzenes, the ratio R°/k' is 2.2 ± 0.3 (standard deviation, S.D.); here, R° , the solute linear equivalent retention volume ($\text{ml}\cdot\text{g}^{-1}$), is the retention parameter used by Snyder, while the k' values were taken from the present paper. Similarly, for silica gel, a constant value of 7.7 ± 0.1 (S.D.) was calculated for R°/k' for benzene and the four monohalogenobenzenes*. Thirdly, calculation shows that in both Snyder's and

* One should bear in mind that different types of adsorbents were used in the work described in refs. 9 and 10 and in the present study, while *n*-pentane was the mobile phase instead of *n*-hexane. In addition, water was added to Snyder's adsorption systems (0.7% with aluminium oxide and 2.5% with silica gel).

the present paper the retention behaviour of poly-X,Y-halogenobenzenes can reliably be predicted from those of the corresponding di-X- and di-Y-halogenobenzenes, using the relationship

$$R_{XY}^2 = (R_{XX}R_{YY})^{0.5} \quad (7a)$$

or

$$k'_{XY} = (k'_{XX}k'_{YY})^{0.5} \quad (7b)$$

Despite such satisfactory agreement, differences do occur, *e.g.*, with the dihalogenobenzenes included in the series of benzenes referred to above. According to Snyder's data, sorption on aluminium oxide increases in the order *meta* < *ortho* \approx *para*, whereas in the present paper *o*-dichloro- and *o*-dibromobenzene are retained far more strongly than are the *para* isomers. The distinct effect, in the polyhalogenobenzene series, of *ortho*-substitution on sorption is also demonstrated by the fact that, in Table VIII, 1,2,3-tri- and 1,2,3,4-tetrachlorobenzene display higher *k'* values than do their respective isomers. This observation partly invalidates Snyder's conclusions concerning the configuration of 1,2,3-tri- and 1,2,4,5-tetrahalogenobenzenes on aluminium oxide (*cf.*, Fig. 5 in ref. 9).

For the other classes of solutes tested, the present results also largely confirm Snyder's earlier conclusions. For instance, with aluminium oxide, the paramount importance of planarity is demonstrated by the relatively low *k'* values recorded for all PCBs and PBBs substituted *ortho* to the phenyl-phenyl bond. In addition, the effect of linearity is clearly demonstrated by the high retention observed for such compounds as the 4-mono- and 4,4'-disubstituted halogenobiphenyls. Here it is noteworthy that substitution in the *para* position no longer exerts a marked influence once two (or more) *ortho* positions have been occupied by halogen atoms: in both the 2,a-di- and 2,a,2',a'-tetra-PCB series, retention is now seen to increase in the order

$$2,5 \approx 2,4 < 2,6 < 2,3 \quad (8)$$

which suggests that the presence of chlorine atoms *ortho* to each other promotes retention. Further, the combined data reported for the halogenated biphenyls and the other classes of solutes tested confirm the fact that aromatic halogen compounds display much higher retentions on aluminium oxide than on silica gel.

On the other hand, the results in Table I do not indicate that solute planarity plays a significant role in adsorption on silica gel. Conversely, substitution *ortho* to the central C-C bond clearly leads to an increase in retention relative to substitution in *meta* or *para* positions. This has recently been confirmed¹² by us for series of terphenyls and also tetradecachloroterphenyls: in both instances the *ortho* isomers display the highest *k'* values.

In Table XII, the retentions of PCBs are compared with those of polymethylbiphenyls. On aluminium oxide, sorption of both classes of compounds appears to be determined by the same effects, as shown by the influence of *ortho versus non-ortho-* and *para- versus meta-*substitution. With silica gel, however, no marked analogy in the retention behaviour of the halogeno- and methylbiphenyls is observed;

TABLE XII

COMPARISON OF CAPACITY FACTORS OF POLYCHLORO- AND POLYMETHYLBIPHENYLS IN THE SYSTEMS ALUMINIUM OXIDE- AND SILICA GEL-*n*-HEXANE

Substitution	$k_{Al_2O_3}^*$		$k_{SiO_2\ gel}$	
	PCB	PMeB	PCB	PMeB
2	8.0	6.0	6.4	12
4	>	>	5.0	12
2,2'	6.5	3.0	5.5	14
3,3'	>	>	2.7	14
4,4'	>	>	2.9	14
3,5	—	>	2.6	13
2,3,2',3'	9.8	5.5	3.5	22
2,4,2',4'	4.0	2.8	1.6	21
2,5,2',5'	4.0	1.4	2.0	17
3,4,3',4'	>	>	2.4	24
2,4,5,2',4',5'	2.6	1.8	1.2	27
2,4,6,2',4',6'	3.9	0.8	0.8	19

* > = $k' > 20-25$.

for example, substitution *ortho* to the phenyl-phenyl bond, which distinctly influences the sorption of PCBs, apparently plays no role with the methylbiphenyls. These results support the idea that there are fundamental differences in the adsorption of solutes on aluminium oxide and silica gel.

For PCNs, literature data appear to be limited to the explanation of the preferential adsorption, on aluminium oxide, of 2- over 1-mono-PCN in terms of better accommodation of the former, more linear, molecule over a group of active adsorbent sites. This may also explain the high capacity factors of, e.g., 2,3-, 2,6- and 2,7-di- and 2,3,6-tri-PCN, or the relatively low values recorded for 1,4- and 1,5-di-PCN. However, the theory does not readily account for the high retention observed for 1,8-di- and 1,4,5,8-tetra-PCN, nor for the very low k' value reported for 1,3,5,7-tetra-PCN. Obviously, other effects, notably substitution in adjacent positions, play a major, although as yet unexplained, role—a statement which also holds true for the silica gel system.

In summary, planarity and linearity of the solute molecule are of primary importance as regards adsorption on aluminium oxide, but play a minor or no role in adsorption on silica gel. This helps to explain the fact that the retention behaviour of the PCBs and PBBs on aluminium oxide relative to silica gel shows more striking differences than does that for the PCNs. Secondly, the presence of substituents *ortho* to each other and, with the biphenyls, to the central C-C bond, promotes retention. This has been amply demonstrated for both adsorbents. However, one should bear in mind that, for aluminium oxide, the planarity-linearity effect outweighs the *ortho* effect. Solute bearing *meta* substituents, such as the 1,3,5-trihalogenobenzenes and 2,4,6,2',4',6'-hexa-PCB, display relatively weak sorption. Possibly, this can be attributed¹³ to deactivation of the aromatic nucleus due to group-ring resonance interaction. Thirdly, with silica gel, the introduction of halogen atoms into an aromatic nucleus decreases sorption; however, it often promotes it with aluminium oxide, especially with the brominated (and iodinated) compounds. As for the latter phenom-

enon, one should again realize that it may be obscured by planarity and other such effects, *e.g.*, with all halogenobiphenyls bearing substituents *ortho* to the central C-C bond. Lastly, irrespective of the adsorbent used, sorption increases in the order $Cl < Br < I$. For fluorine, the data reported for the PXBes indicate the order $F < Cl$ when using aluminium oxide; with silica gel, the behaviour of fluorinated compounds seems to be irregular.

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