STRUCTURAL IDENTIFICATION OF POLYCHLORINATED BIPHENYLS IN COMMERCIAL MIXTURES BY GLC, NMR AND MASS SPECTROMETRY

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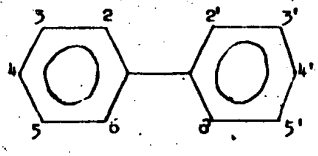
SUMMARY

The major constituent polychlorinated biphenyls in Aroclor 1254 have been characterised by high resolution Nuclear Magnetic Resonance and Mass Spectrometry following separation by liquid-solid and gas-liquid chromatography. The retention indices of these compounds together with those of forty synthesised PCBs have been used to predict the structures of the remaining Aroclor 1254 constituents, together with all those of Aroclor 1242 and 1260.

INTRODUCTION

Polychlorinated biphenyls (PCBs) exhibit a high degree of biological and chemical stability. As they are lipid soluble they tend to accumulate in food chains and, since 1966, have been detected in a wide range of marine organisms, sea birds and even human adipose tissue[1-8]. Particularly high levels have been found in marine samples taken from the Baltic[6] and the Pacific coastal waters of USA[7]. Quantitative analytical data are only approximate because of the large number (210) of PCBs that are theoretically possible - up to 10 chlorine atoms may be substituted in the molecule as indicated below:



Quantitative results are estimated by relating the peak areas obtained from extracts of PCBs in an electron-capture (E.C.) GLC chromatogram to the corresponding peak area(s) produced by a known weight of a commercial PCB mixture or an insecticide such as DDE. Certain authors[9-11] have shown that the pattern of GLC peaks produced by extracts of marine organisms are very similar to those produced by commercial PCB products containing 54% or 60% chlorine. While this is true for species indigenous to rivers and well defined sea-areas, eg. estuaries, the situation may be quite different in large sea areas such as the North Sea. The PCBs that are present in some species of sea fish have been shown in this laboratory to vary widely in type and quantity and bear little resemblance to any commercial product[12].

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In spite of a growing interest in the toxicology of PCBs, relatively little data has been published on this aspect to date. All available information relates to trials involving commercial products[13] although individual PCBs may well have differing toxicities.

Both from the analytical and toxicological viewpoints, therefore, there is a need to think in terms of individual PCBs rather than complex mixtures, in order to progress to a greater understanding of the problems and significance of PCB contamination. As a first step in this direction we have used a combination of analytical techniques to characterise the component PCBs in Aroclors 1242, 1254, and 1260. (Commercial products manufactured by Monsanto Chemicals Ltd. containing 42%, 54% and 60% w/w chlorine respectively). Aroclor 1254 was fractionated on a column of alumina and the major PCBs in the fractions were separated and collected from a gas-liquid chromatograph. The separated PCBs were characterised by mass spectrometry (MS) and high resolution nuclear magnetic resonance spectroscopy (NMR) to determine the number and positions of the chlorine atoms associated with each compound. NMR was chosen in preference to infra-red spectroscopy as it is a more definitive technique although the latter has the advantage of requiring a smaller sample size.

The NMR spectra of chlorinated biphenyls which have been reported previously[14,15,16,17] were unhelpful in this instance. Mayo and Goldstein[18] have analysed biphenyl itself on 60 and 100 MHz spectrometers.

The PCBs characterised by NMR together with forty individually synthesised PCBs were subsequently analysed by high resolution GLC to determine their absolute retention values using the Kovats[19] retention index (RI) system. Previous GLC data on PCBs have been restricted to relative retention times[20]. Attempts to derive accurate retention data

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based on internal standards were unsuccessful due to variations in conditions during the lengthy GLC runs of up to 16 hours duration, and because of the severe limitations placed on the choice of standards due to the complex Aroclor chromatograms. The structures of the minor PCB constituents of Aroclor 1254 and all PCBs in Aroclors 1242 and 1260 have been predicted by a variation of the interpolation method suggested by Evans and Smith[21].

EXPERIMENTAL

1. Fractionation of Aroclor 1254 on an alumina column.

60 mg of Aroclor 1254 was washed onto a 25 × 1 cm column of 20 g activity grade I alkaline alumina (M Woelm Eschwege Germany) with five 1 ml volumes of hexane. All PCBs were eluted in 900 ml of hexane (flow rate of 1 ml/min), collected in 25 ml fractions. This fractionation was repeated with a further 60 mg of Aroclor and the corresponding fractions were added together. After suitable dilution, usually 100 or 1000 fold, each fraction was analysed by EC GLC to determine whether further separation of PCB's was required. Analyses were performed on a Pye model 74 instrument fitted with a nickel 63 E.C. detector and a 7 ft × 1/4 inch 'pyrex' column of 2% Apiezon L + 0.02% Epikote resin on 80-100 mesh Varaport 30 packing material. Of a wide variety of stationary phases that were tested, Apiezon 'L' gave the highest resolution of PCBs in Aroclor 1254. Argon was used as carrier gas at an inlet pressure of 25 psi and a flow of 80 ml/min. The column and injector were maintained at a temperature of 230°C and the detector at a temperature of 325°C.

2. Collection of GLC fractions for NMR.

Alumina column fractions were separated into their component PCB's on a 7 ft × 1/4 inch GLC column similar to that described above but fitted into a Pye 104 chromatograph adapted for fraction collecting. The effluent gas was split in the ratio of 25 parts to the collection outlet and 1 part to the flame-ionisation detector. The temperature was programmed from 220-230°C at 1°C/min. and the carrier gas flow was 40 ml/min of argon measured at 220°C. The outlet to the collection trap was maintained at 250°C. The traps were interchangeable straight metal tubes loosely packed with glass wool. They were screwed onto a threaded outlet tube and maintained at a temperature of approximately 0°C above a tray of solid carbon dioxide. Trapping efficiencies of nearly 90% were obtained with 246-2'4'6' hexachlorobiphenyl, but for other PCBs recoveries were often much lower. Trapped PCBs were eluted with light petroleum ether which was evaporated off and replaced with deutero-chloroform or tetrachloroethylene before NMR analyses.

3. NMR.

Spectra were obtained on a Varian HR 220 spectrometer. The temperature of the probe varied between ~13°C at the beginning of a day to ~18°C over the greater part of the day. This temperature difference was shown to be insignificant in the interpretation of spectra as selected spectra obtained at 55°C showed only slight shifts in some resonance lines.

For most gas chromatographic fractions the sample size was small and several of the spectra were very weak. In these cases, the spectrometric scanning conditions resulted in the spectra being extremely noisy.

4. Combined GLC-Mass Spectrometry.

Aroclor mixtures were separated using a Perkin-Elmer 50 ft x 0.02 in. i.d. support coated open tubular (SCOT) Apiezon L column under the conditions described in the next section. The column effluent was split between the flame-ionisation detector and an AEI MS12 mass spectrometer. Approximately 10% of the column effluent was taken into the mass spectrometer ion source with no removal of the carrier gas. Peak homogeneity was monitored by repetitively scanning over the parent molecular ion region, observing an oscilloscope screen and recording several spectra during the elution of a peak.

The higher chlorinated PCBs in Aroclor 1260, which had very long retention times on the SCOT column, were analysed on the 7 ft x 1/4 inch o.d. column described previously. The larger sample sizes and shorter retention times enabled a higher PCB concentration to be obtained in the carrier-gas stream. The column was connected to the MS12 through a Watson-Biemann type fritted glass separator. The carrier gas flow rate was 40 ml/min of helium and the column temperature was maintained at 205°C. PCBs fractionated from Aroclor 1254 and all the synthesised PCBs were examined either as effluents from the 7 ft column or in the mass-spectrometer direct-inlet system.

5. High Resolution GLC and Retention Indices.

The Aroclors and synthesised PCBs were analysed on the SCOT Apiezon 'L' column fitted in a Pye model 104 . A flame-ionisation detector was necessary as the n-alkanes which were added to samples for the determination of retention indices produced very small and inverted peaks with an electron capture detector. Helium was used as the carrier gas at a flow of 2.5 ml/min. The column was operated at 205°C which was near the maximum temperature permitted (225°C) for this phase. The operating temperature was dictated by the need

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to examine all the PCBs under conditions that allowed both the higher chlorinated PCBs in Aroclor 1260 to be eluted quickly and the lower chlorinated species in Aroclor 1242 to be resolved adequately. Some column deterioration was noted at 205°C and significant variations in the RIs of PCBs were evident after three weeks. The changes were almost entirely due to reduced retention volumes of the n-alkanes relative to those of the PCBs. To overcome this effect the RIs of well characterised PCBs were used as sub-standards, as the retention volumes of PCBs were constant relative to each other. Column efficiences were constant at between 24,000 and 27,000 theoretical plates, depending on the PCB.

Several pairs of peaks eg. 245-2'4'5' and 34-2'3'4' changed relative positions as the column temperature was increased between 190-210°C. For this reason isothermal was preferred to temperature programmed gas chromatography.

Retention Indices of the later PCBs in Aroclor 1260 were determined on the 7 ft × 1/4 inch Apiezon L column and were correlated graphically with the SCOT column RIs.

RESULTS

1. Fractionation on an alumina column

Only twenty-three distinct peaks were apparent in the gas chromatogram of Aroclor 1254 (Fig.1) analysed on the 7 ft packed Apiezon 'L' column at 230°C. Similar GLC analyses of the hexane fractions obtained from the alumina column produced chromatograms containing a total of thirty-five peaks. This increase in the number of resolved peaks was due to the combination of the different el n patterns of the two systems. After characterisation of the PCBs by NMR, the order of elution of the substituted phenyl rings, assuming

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a constant second ring, was shown to be 245-, 25-, 2345-, 24-, 236-, 34-, 23-, 234-, from the alumina column compared to 25-, 24-, 23-, 236-, 34-, 245-, 234-, 2345- from the GLC column.

Three PCBs, corresponding to peaks 39, 44 and 56 in Fig.2, were sufficiently well resolved by alumina column fractionation to be analysed directly by MS and NMR. A further nineteen PCBs, including all the remaining major peaks in the Aroclor 1254 chromatogram, required further separation by G.L.C. before MS and NMR analyses.

2. NMR and Mass Spectroscopy

The structures of the major Aroclor 1254 PCBs that were identified by NMR and Mass spectrometry after their separation and collection from a gas chromatograph are given in Table I. Three further minor peaks are included in Table 2.

The NMR chemical shifts for both these PCBs and the synthesised compounds give a rational gradation of frequencies depending on the substitution patterns of both rings. Full details of the chemical shifts, the line splittings and structural assignments will be published separately[22].

The spectra of all the compounds examined from Aroclor 1254 gave near first order spectra. The 234- and 236- substituted phenyls gave AB $\longrightarrow$ AX systems; the 24-, 25- and 34- substituted phenyls gave ABX $\longrightarrow$ AMX systems; and the 245- and 2345- substituted phenyls gave two and one singlet(s) respectively. Under the spectrometer conditions used for running these weak spectra, and considering the 220 MHz spectrometer linewidth, the splitting due to para-coupling could not be observed.

The majority of the structural assignments in Table I were obtained from NMR data alone - the MS and RI evidence confirmed the results. However, in one or two cases an interplay of all three techniques allowed firm assignments to be made.

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The fraction trapped as peak 24 was shown to be a mixture by GLC. The NMR spectrum of the mixture contained resonance lines compatible with the 23-, 24- and 25- dichlorophenyl groups. A chromatogram of Aroclor 1254 containing added 24-2'4' tetrachlorobiphenyl showed that the retention time of the latter was not coincident with peak 24, although the trapped fraction could have included some 24-2'4' compound from the tail of the peak. The spectrum also suggested that the other alternative RI structure 3-2'4'6' tetrachlorobiphenyl was not present. Using the total NMR, MS and RI evidence, the most likely structure is 23-2'5' tetrachlorobiphenyl.

The fraction trapped as peak 32 gave a very weak spectrum in tetrachloroethylene which contained lines typical of a 236-trichlorophenyl group. Other lines were too weak for certain correlation. However the limited evidence is in agreement with the predicted GLC/RI structure of 23-2'3'6' pentachlorobiphenyl.

Peaks 42 and 43 were always eluted closely to one another on every chromatogram. Two different fractionations of these peaks gave spectra which included lines typical of a 245-trichlorophenyl group in addition to those of 25-2'3'4' pentachlorobiphenyl. This agrees with a GLC/RI predicted structure of 23-2'4'5' pentachlorobiphenyl for Peak 42.

The fraction trapped as peak 68 was shown by a subsequent GLC separation to contain a large number of impurities and, in addition, the spectrum was very weak. However, after the GLC/RI values suggested that the peak might be 245-2'3'4'5' heptachlorobiphenyl it was possible to predict the frequencies of the three singlets in the spectrum from the total PCB NMR data. The three lines were, in fact, present at the predicted f[illegible]ncies.

The mass spectrometric results obtained from the GC/MS runs are quoted in Tables 1, 2, 3 and 4 as the number of chlorine atoms associated with each PCB peak.

Prediction of PCB structures using RIs

Retention indices are a more reproducible form of presenting logarithmic retention volumes of GLC peak maxima and are obtained with reference to the well defined retention values of a series of n-alkanes separated in the same chromatogram. The RI of a compound is directly proportional to its free energy of solution in a stationary phase, which in turn is an approximately additive function of the groups constituting the molecule. Consequently any PCB molecule can be thought of as consisting of two chloro-substituted phenyl groups each with its own $\frac{1}{2}$(RI) value. All PCBs are composed of twenty such basic groups and the RI of any PCB can therefore be estimated by adding together the $\frac{1}{2}$(RI) values of the two component phenyl groups.

The $\frac{1}{2}$(RI) values of all substituted phenyl groups containing up to three chlorine atoms and the RIs of the PCBs from which they were derived are shown in table 5. Certain discrepancies are apparent between the $\frac{1}{2}$(RI)s for the same ring structures calculated from different PCBs. These discrepancies are due to electronic and steric effects which vary according to the substitution patterns in the two rings under consideration. Electronic effects are induced by the introduction of chlorine atoms into the phenyl ring and vary according to the exact position of each substitution. The inclusion of chlorine atoms results in a change in the electronic distribution in each ring and varies the character of the phenyl-phenyl bond. The nett effe is illustrated in table 5 by the increased RI (2 to 7 units) for molecules substituted in both rings compared to those substituted in only one ring.

The steric effect, previously noted in methyl biphenyls,[23,24] is caused by substitution of chlorine atoms in the ortho (2,2',6, and 6') positions in the rings. Increasing substitution in these positions progressively restricts free rotation of the phenyl groups and is accompanied by a reduction in RI. This effect is illustrated in table 5 by the smaller RI for 26- and 246- rings (-2 and -8 units respectively) derived from molecules containing two ortho chlorine compared to no ortho chlorine in the second ring.

Any electronic and steric effects must be allowed for when predicting the RIs of PCBs, by using appropriate $\frac{1}{2}$(RI) values derived from a similar type of PCB molecule. Using the data in table 5, the experimental and predicted RIs for a further range of PCBs of known structure are compared in table 6. With only one exception, for which no obvious explanation can be given, agreement is to within 4 RI units which is consistent with our experimental error of ±2 units in determining RIs. Therefore structural predictions of PCBs in Aroclors 1242 and 1254 could be made with confidence.

Very few standard PCBs containing more than three chlorine atoms per ring were available for analysis but, from the limited data obtained (table 7), it was evident that electronic and steric effects had a much greater influence on their RIs than on those of the lower chlorinated PCBs. This resulted in increased difficulty in predicting the RIs of some of the hexa, and the hepta, octa and nona-substituted molecules. However the relation between structure and RI was apparent when most of the later peaks in the chromatogram of Aroclor 1260 were studied. After allowance was made for electronic and steric effe' particularly in the case of the 2346- and 2356- rings joined to rings containing two, one or no 'ortho' chlorine atoms, the more obvious correlations for the octa- and nona-substituted compounds could be made, and then

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the remaining peak assignments fitted into place. In this way using the inferred ½(RI) values given in table 8 it was possible to allocate structures to all appropriate peaks in the Aroclor 1260 chromatogram. The values in table 8 are conditional on the presence of at least two substituted chlorine atoms in the second phenyl ring. This condition was imposed because it was not clear what effect a greater imbalance in the number of chlorine atoms between the rings would have on RIs. In practice, the MS data giving the number of chlorine atoms present in each PCB enabled potential errors due to the above effects to be greatly reduced.

Typical chromatograms of Aroclors 1242, 1254 and 1260 obtained from the SCOT column are shown in figs.2-4. Tables 1,2,3 and 4 list the RIs and chlorine contents of all PCB peaks present in the Aroclors. The tables also include the alternative PCB structures that have RIs predicted to be within an arbitrary ±5 of each peak. The RIs of synthesised PCBs are used where applicable, and those that were derived from the inferred ½(RI)values given in table 8 are given in brackets in tables 1-4. PCB molecules having a difference of more than two chlorine atoms between rings are not included as these would be unlikely to occur in Aroclor mixtures. For the sake of emphasis the major peaks in the chromatograms are presented first, in tables 3 and 4, in order of increasing RI.

Table 8

Inferred Half RI values for four and five substituted phenyl rings as affected by 'ortho' substitution in the second ring

Substituted phenyl ring	½(RI) values		
	Two ortho	One ortho	No ortho
2345-	1353	1363	1375
2346-	1245	1250	1260
2356-	1219	1228	1238
23456-	1405	1415	1428

The changes in $\frac{1}{2}$(RI) values found in this investigation are in line with changes in the NMR chemical shift data. When considering the protons in one ring of the molecule, the chemical shifts are affected by the overall presence of chlorine atoms in either ring, the substitution position of chlorine atom(s) in the ring being examined, and the number and relative location of the ortho chlorine atoms causing steric hindrance in the molecule.

Allowing for variations in detector response, the SCOT column chromatograms indicate that the predominant species in Aroclor 1254 are pentachlorobiphenyls with smaller amounts of tetra and hexachlorobiphenyls. This agrees well with the chlorine content of this Aroclor which corresponds to a mean value of 5 atoms per molecule. The most abundant chlorine substitutions are those in the 25-, 34-, 234-, 236-, 245- and 2345- positions. When alternative predicted structures are available, therefore, preference should probably be given to those containing these substitution patterns. It is noticeable that certain substitutions, such as 246-, rarely occur and others, such as 3-, 35- and 23-, occur only infrequently.

Aroclor 1242 contains predominately trichlorobiphenyls with a lesser amount of dichloro and tetrachloro-compounds. The mean chlorine value for this Aroclor is 3.1 atoms per molecule. The most frequent substitutions occur in the 2-, 24- and 25- positions. The predominant compounds in Aroclor 1260 were hexachlorobiphenyls with a significant level of heptachlorobiphenyl. The mean chlorine value of this Aroclor is 6.1 atoms/molecule. The most frequent substitution patterns were similar to those found in Aroclor 1254, although 235-, 2346- and 2356- structures were in greater evidence.

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FIGURE LEGENDS

Fig.1. 2 ng of Aroclor 1254 chromatographed on a 7 ft Apiezon L packed column at 230°C. Attenuation setting maintained at 2×10^2 - electron capture detector.

Fig.2. 0.2 µl of 5% Aroclor 1254 solution chromatographed on an Apiezon L SCOT column at 205°C. Attenuation setting maintained at 1×10^2 - flame ionisation detector.

Fig.3. 0.2 µl of 1% Aroclor 1242 solution chromatographed on an Apiezon L SCOT column at 205°C. Initial attenuation setting of 5×10^2 reduced as indicated - flame ionisation detector.

Fig.4. 0.25 µl 10% Aroclor 1260 solution chromatographed on an Apiezon L SCOT column at 205°C. Attenuation setting maintained at 1×10^2 - flame ionisation detector.

Table 1

The Retention Indices, Chlorine numbers, and structures of the major PCB constituents in Aroclor 1254

Peak no:	R.I.	Chlorine no:	NMR determined structure	Alternative predictions structure	R.I.
22	1994	4	25 - 2'5'	2 - 2'3'5'	1996
23	2010	4		2 - 2'4'5'	2012
				24 - 2'5'	2010
24	2022	4	23 - 2'5'	3 - 2'4'6'	2021
				24 - 2'4'	2027
		(5)		26 - 2'3'6'	2017
29	2089	5	25 - 2'3'6'	26 - 2'3'5'	2092
32	2119	5	23 - 2'3'6'		
33	2136	4	25 - 3'4'		
36	2159	5		25 - 2'3'5'	2139
39	2175	5	25 - 2'4'5'	24 - 2'3'5'	2175
				35 - 2'4'6'	2175
		(6)		26 - 2'3'4'6'	(2175)
				236 - 2'3'6'	2172
41	2191	5	24 - 2'4'5'	23 - 2'3'5'	2189
42	2203	5	23 - 2'4'5'		
43	2207	5	25 - 2'3'4'		
44	2228	5	34 - 2'3'6'		
45	2238	-		24 - 2'3'4'	2226
				23 - 2'3'4'	2240
				26 - 3'4'5'	2240
				34 - 3'5'	2238
				235 - 2'4'6'	2240
48	2264	6	236 - 2'4'5'	24 - 2'3'4'6'	(2263)
50	2299	6	234 - 2'3'6'		
52	2321	5	34 - 2'4'5'		
55	2356	5	34 - 2'3'4'		
56	2356	6	245 - 2'4'5'	25 - 2'3'4'5'	(2360)
				35 - 2'3'4'6'	(2357)
59	2390	6	234 - 2'4'5'	23 - 2'3'4'5'	(2391)
				246 - 3'4'5'	2388
		(7)		235 - 2'3'5'6'	(2390)

(RI)- inferred prediction using table 8
(Cl No:) chlorine number of minor constituent not associated with accurate RI.

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TABLE 9

The Retention Indices, Chlorine Numbers and Predicted Structures of the minor peaks in Arvelor 1254

Peak no.	R.I.	Chlorine no.	Structure	Predicted R.I.
1	1490	0	Biphenyl	1491 +
2	1579	1	2 -	1577 +
3	1672	2	2 - 2'	1669 +
4	1688	1	4 -	1687 +
5	1760	2	2 - 5'	1774
6	1758	2	4 -	1756 +
7	1783	2	2 - 3'	1770
8	1774	3	2 - 2'6'	1759
9	1788	2	2 - 4'	1782 +
10	1833	3	2 - 4'5'	1831
11	1849	3	2 - 2'4'	1868
12	1863	3	2 - 2'5'	1869
			3 - 2'6'	1866
13	1879	3	4 - 2'6'	1878
14	1896	-	4 - 4'	1894 +
15	1982	4	2 - 2'4'6'	1986 +
16	1935	-	2 - 3'5'	1932
			2 - 3'5'6'	1938
			3 - 3'5'	1932
17	1943	-	4 - 2'5'	1944
18	1958	3	3 - 2'4'	1949
19	1963	3	3 - 2'5'	1965
			4 - 2'4'	1962 +
20	1966	3	3 - 2'5'	1963
			4 - 2'4'	1962 +
21	1980	3	2 - 3'4'	1973
22	2030	4	4 - 2'5'6'	2031
			23 - 2'4'	2041
24	2051	4	2 - 2'5'4'	2047
			23 - 2'5'	2053
25	2058	4	23 - 2'4'	2053
26	2070	4	24 - 3'4'	2071
27	2097	-	3 - 2'5'5'	2097
			24 - 2'4'6'	2097
			25 - 3'5'	2099
			24 - 2'5'5'	2092
28	2113	5	23 - 2'5'6'	2120
			25 - 2'4'6'	2112
29	2146	4	3 - 2'4'5'	2144
			3 - 2'5'4'	2148
30	2152	- (not 6)	3 - 2'5'4'	2146
			24 - 3'4'	2154
31	2168	-	23 - 3'4'	2158
			234 - 2'4'6'	2160
32	2169	-	23 - 3'4'	2168
			234 - 2'3'6'	2173
33	2186	-	23 - 2'5'5'	2183
			26 - 2'3'6'	2183
34	2244	-	3 - 2'4'5'	2245
			24 - 2'3'5'6'	(2241)
			25 - 2'3'4'6'	(2241)
			234 - 2'5'6'	2248
35	2254	-	3 - 3'4'5'	2257
			23 - 2'3'5'6'	(2256)
			24 - 2'5'5'	2258
			245 - 2'4'6'	2258
36	2283	-	23 - 2'3'4'6'	(2278)
			26 - 2'3'5'5'	(2283)
			34 - 3'4'	2282 +

Peak no.	R.I.	Chlorine no.	Structure	Predicted R.I.
51	2310	5	25 - 3'4'5'	2307
			35 - 2'3'4'	2310
		(7)[a]	236 - 2'3'5'6'	2303
53	2335	5	23 - 3'4'5'	2336
		(7)[a]	256 - 2'3'4'6'	(2329)
54	2340	6	35 - 2'3'5'6'	(2335)
			235 - 2'4'5'	2340 +
57	2372	-	24 - 3'4'5'	2376
			234 - 2'3'5'	2374
58	2375	6	34 - 2'3'5'6'	(2376)
			24 - 2'3'5'6'	(2379)
			234 - 2'3'5'	2374
60	2400	6	34 - 2'3'4'6'	(2401)
			345 - 2'3'5'	2398
61	2413	7	235 - 2'4'5'	(2412)
62	2425	6	234 - 2'3'4'	2426
63	2433	7	235 - 2'3'4'5'	(2434)
			245 - 2'3'4'6'	(2436)
			246 - 2'3'4'5'	(2430)
64	2451	7	234 - 2'3'5'6'	(2450)
65	2466	7	234 - 2'3'4'6'	(2460)
66	2489	8	245 - 3'4'5'	2488 +
67	2519	8	34 - 2'3'4'5'	2522
68	2543	7	245 - 2'3'5'5'	(2541)
			345 - 2'3'5'6'	(2548)
69	2577	7	234 - 2'3'4'5'	2572

+ R.I. of synthesized compound
(RI) Predicted R.I. using Table 8
a smaller peaks eluted earlier than major parts of peaks 51 and 53 respectively.

MONS 067728

TABLE 3

The Retention Indices, Chlorine Numbers and Predicted Structures of Aroclor 1254 peaks

Peak no.[1]	R.I.	Chlorine no.[1]	Structure	Predicted R.I.
3	1672	2	2 - 2'	1589 *
5	1705	2	2 - 3'	1770 *
8	1781	2	2 - 4'	1785 *
9	1833	3	2 - 2'5'	1831
10	1869	3	2 - 2'4'	1848
11	1865	3	2 - 2'3'	1859
			3 - 4'5'	1844
12	1879	3	4 - 2'4'	1878 *
13	1866	2	4 - 4'	1894 *
15	1935	3	2 - 3'5'	1932
			3 - 2'5'	1932
17	1951	3	3 - 2'4'	1949
18	1961	3	3 - 2'3'	1963
			4 - 2'5'	1938 *
19	1966	3	3 - 2'5'	1951
			4 - 2'4'	1966 *
20	1980	3	2 - 3'4'	1973
21	1996	4	2 - 2'3'5'	1996
			25 - 2'5' a	1996 *
22	2011	4	2 - 2'4'5'	2012
			24 - 2'5'	2010
24	2024	4	3 - 2'3'5'	2029
			3 - 2'4'6'	2021
31	2038	3	4 - 3'4'	2038
		4	25 - 3'5'	2034
		(5)	24 - 2'4'6'	2037
			23 - 2'3'6' a	2029
34	2138	4	25 - 3'4' a	2138
35	2148	4	2 - 2'4'5'	2144
			3 - 2'3'4'	2148
1	1490	0	Biphenyl	1491 *
2	1580	-	2 -	1577 *
4	1750	2	23 -	1744
6	1758	-	24 -	1736 *
7	1774	2	2 - 2'6'	1759
14	1983	4	2 - 2'4'6'	1984 *
			25 - 2'6'	1987
16	1944	3	4 - 2'5'	1944
23	2015	4	2 - 2'4'5'	2012
29	2088	4	3 - 2'4'6'	2089
			23 - 2'5'	2083
			24 - 2'6'	2087 *
			24 - 3'5'	2088
			4 - 2'4'6'	2093 *
			4 - 2'3'6'	2081
28	2057	4	23 - 2'6'	2061
27	2061	4	2 - 2'3'4'	2047
33	2098	4	23 - 2'3'	2099

Peak no.[1]	R.I.	Chlorine no.[1]	Structure	Predicted R.I.
28	2061	-	1	
30	2080	3	3 - 3'4'	2075
32	2121	4	23 - 3'5'	2125
			4 - 2'4'5'	2125
33	2123	4	23 - 3'5'	2125
			4 - 2'4'5'	2125
36	2167	4	4 - 2'3'5'	2100
37	2176	4	23 - 3'4'	2155
38	2178	4	24 - 3'5'	2175
			25 - 2'4'5' a	2175 *
			26 - 2'3'5'6'	(2175)
			23 - 2'5'6'	2183
			24 - 2'4'6'	2175
39	2195	5	24 - 2'4'5' a	2191
40	2207	5	23 - 2'4'5'	2206
			23 - 2'5'6' a	2210
41	2212	5	25 - 2'4'6' a	2210
42	2232	5	24 - 2'3'6'	2225
			24 - 2'5'6' a	2228
43	2242	6	24 - 2'4'5'6'	(2241)
			23 - 2'5'6'6'	(2247)
			23 - 2'4'6'6'	2240
44	2258	-	24 - 2'5'6'6'	(2269)
			234 - 2'4'5' a	2258 *
45	2287	-	24 - 2'4'5'5'	(2283)
			24 - 3'6'	2282 *
			25 - 2'4'6'	2291

a Structures found in Aroclor 1254 by NMR determinations.
* RIs of synthesized compound or NMR standard.
(RI) Predicted RIs using Table 6.
(Cl No.) Chlorine number of minor constituent not associated with accurate RI.

MDNS 067729

TABLE 4

The Retention Indices, Chlorine Numbers and Predicted Structures of Arochlor 1250 peaks

Peak no.	R.I.	Chlorine no.	Structure	Predicted R.I.
31	2086	5	25 - 2'3'6' a	2089
			23 - 2'4'6'	2081
32	2108	6	236 - 2'3'6'	2112
34	2174	5	24 - 2'5'3'	2173
			22 - 2'6'3' a	2173 *
			25 - 2'4'3'	2173
43	2179	6	24 - 2'5'3'6'	(2181)
			234 - 2'4'6'	2182
45	2214	6	24 - 2'4'3'6'	(2203)
			234 - 2'4'5' a	2204 *
47	2236	6	234 - 2'3'6' a	2239
48	2300	7	236 - 2'3'4'6'	(2305)
			245 - 2'3'5'6'	(2295)
52	2358	6	25 - 2'3'4'5'	(2360)
			35 - 2'3'4'6'	(2387)
			245 - 2'4'5' a	2358 *
55	2390	6	23 - 2'4'5'6'	(2391)
			234 - 2'4'5' a	2390 *
			345 - 2'4'6'	2388
		(7)	236 - 2'3'4'6'	(2390)
57	2411	7	235 - 2'3'4'6'	(2412)
			245 - 2'3'5'6'	(2405)
58	2428	6	234 - 2'3'4' a	2429 *
		7	245 - 2'3'5'6'	(2428)
59	2432	7	235 - 2'3'4'6'	(2440)
			245 - 2'4'5'6'	(2438)
		8	2345 - 2'3'4'6'	2438 *
60	2445	7	234 - 2'3'5'6'	(2440)
			235 - 2'4'5'6'	(2439)
66	2542	7	245 - 2'3'4'5'	(2543)
68	2575	7	234 - 2'4'5'6	(2575)
			345 - 2'4'5'6'	(2570)
76	2726	8	2345 - 2'3'4'5'	(2726)

Peaks 2 - 21 in Arochlor 1254 chromatogram were also present in Arochlor 1260

22	1994	4	2 - 2'3'5'	1996
			23 - 2'5' a	1994 *
23	2009	4	2 - 2'4'5'	2012
			24 - 2'5'	2010
24	2013	-	2 - 2'4'5'	2012
			24 - 2'5'	2010
25	2022)	4	3 - 2'4'6'	2021
26	2024)		23 - 2'5'	2022
			24 - 2'4'	2021 *
27	2038	-	3 - 3'5'	2033
			3 - 3'5'6'	2035
			3 - 2'4'6'	2032 *
			34 - 2'5'	2031 *
			35 - 3'5'	2038
28	2044	4	4 - 2'5'6'	2041
			23 - 2'5'	2041
29	2058	4	2 - 2'5'6'	2057
			25 - 2'3'	2053
30	2098	4	23 - 2'5'	2098
32	2117	-	3 - 2'4'5'	2115
			23 - 2'5'6'	2116

Peak no.	R.I.	Chlorine no.	Structure	Predicted R.I.
33	2125	-	4 - 2'4'5'	2125
			23 - 2'5'	2125
34	2138	4	25 - 2'4' a	2138
35	2145	4	2 - 2'4'5'	2144
			3 - 2'5'6'	2146
36	2158	5	25 - 2'3'5'	2159
39	2192	-	23 - 2'5'5'	2189
			24 - 2'5'5' a	2191
40	2205)	5	25 - 2'3'6' a	2201 *
41	2209)		25 - 2'4'6' a	2210
42	2289	5	24 - 2'3'6'	2287
			34 - 2'3'6' a	2286
44	2254	6	23 - 2'3'6'5'	(2256)
			245 - 2'4'6'	2254
46	2283	-	23 - 2'3'6'6'	(2285)
			24 - 2'3'5'	(2295)
			25 - 2'3'6' a	2287
49	2321	5	24 - 2'3'5'	2324
			34 - 2'3'5' a	2317
50	2326	7	236 - 2'3'4'5'	(2341)
			246 - 2'3'4'6'	(2331)
51	2340	6	24 - 2'3'5'6'	(2335)
			235 - 2'4'5'	2342 *
53	2372	-	24 - 2'3'4'5'	(2376)
			234 - 2'3'5'	2374
			34 - 2'3'4'5'	(2375)
54	2375	-	24 - 2'3'5'6'	(2379)
			236 - 2'3'5'	2374
55	2408	6	34 - 2'3'4'6'	(2401)
61	2460	7	246 - 2'3'4'6'	(2462)
62	2484	8	2345 - 2'3'4'6'	(2468)
63	2486	8	235 - 2'3'4'5'6'	(2489)
			245 - 2'3'4'5'6'	(2481)
			2346 - 2'3'4'6'	2490 *
		6	25 - 2'4'5'	2488 *
64	2518	8	24 - 2'3'4'5' a	(2519)
65	2528	7	235 - 2'4'5'6'	(2535)
67	2550	-	245 - 2'3'5'6'	(2548)
69	2578	8	235 - 2'3'4'5'6'	(2577)
			2345 - 2'3'5'6'	(2567)
70	2587	8	2345 - 2'3'4'5'	(2586)
71	2597	8	245 - 2'3'4'5'6'	(2593)
72	2605	8	2345 - 2'3'4'6'	(2603)
73	2618	9	2346 - 2'3'4'5'6'	(2624)
74	2640	8	234 - 2'3'5'6'	(2637)
-	-	9	2345 - 2'3'4'5'6'	(2650)
75	2682	-	345 - 2'3'4'5'	(2669)
77	2738	-	345 - 2'3'4'5'6'	(2738)
78	2770	9	2345 - 2'3'4'5'6'	(2768)
-	-	10	23456 - 2'3'4'5'6'	2810 *

a Structures found in Arochlor 1254 by NMR determinations
(RI) Predicted RIs using Table 8
(Cl.No.) Chlorine number of minor constituent not associated with accurate RI
* Peaks observed on most spectrometer oscillograms, but not on G.L.C.

MONS 067730

TABLE 5

Half RI values of phenyl rings containing up to three chlorine atoms

Ring structure	½(RI) value	Derivation	R.I.
phenyl-	747	biphenyl	1494
2-	834	2 - 2'	1669
	829	2 -	1577
3-	935	3 - 3'	1871
	930	3 -	1678
4-	947	4 - 4'	1894
	940	4 -	1687
23-	1028	23 - 2'4'5'	2206
	1025	23 -	1772
24-	1013	24 - 2'4'	2026
	1009	24 -	1756
25-	997	25 - 2'5'	1994
26-	930	26 - 2'6'	1860
	932	26 -	1679
34-	1141	34 - 3'4'	2282
	1134	34 -	1881
35-	1097	35 - 3'5'	2194
	1095	35 -	1842
234-	1213	234 - 2'4'5'	2391
235-	1162	235 - 2'4'5'	2340
236-	1086	236 - 2'4'5'	2264
245-	1178	245 - 2'4'5'	2356
246-	1076	246 - 2'4'6'	2151
	1085	246 - 4'	2033
	1090	246 - 2'	1924
	1084	246 -	1831
345-	1310	345 - 2'4'5'	2488

MCNS 067731

TABLE 6

Comparison of experimental and predicted RIs of PCBs

Structure	RI Experimental	RI Predicted
2 - 4'	1782	1781
245 -	1922	1925
24 - 4'	1960	1956
25 - 2'3'6'	2089	2083
25 - 3'4'	2136	2138
25 - 2'4'5'	2175	2174
24 - 2'4'5'	2191	2191
25 - 2'3'4'	2207	2210
34 - 2'3'6'	2228	2227
234 - 2'3'6'	2299	2299
34 - 2'4'5'	2321	2319
235 - 2'4'5'	2339	2340
34 - 2'3'4'	2356	2354
234 - 2'3'4'	2425	2424

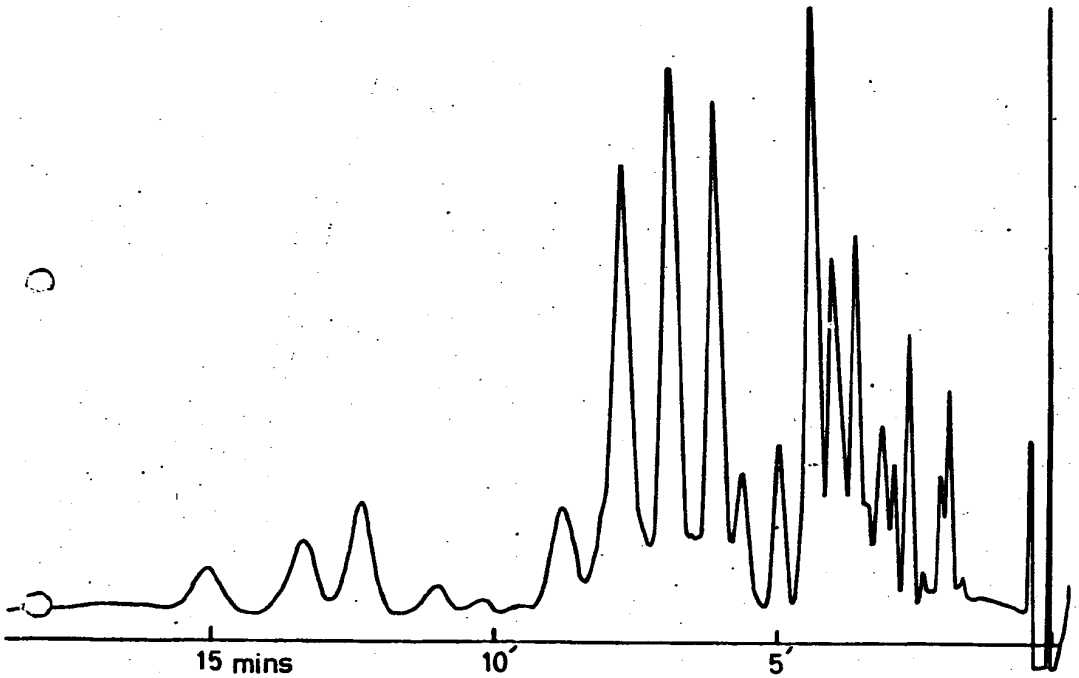
MONS 067732

TABLE 7

Experimental half RI values of phenyl rings containing 4 and 5 chlorine atoms

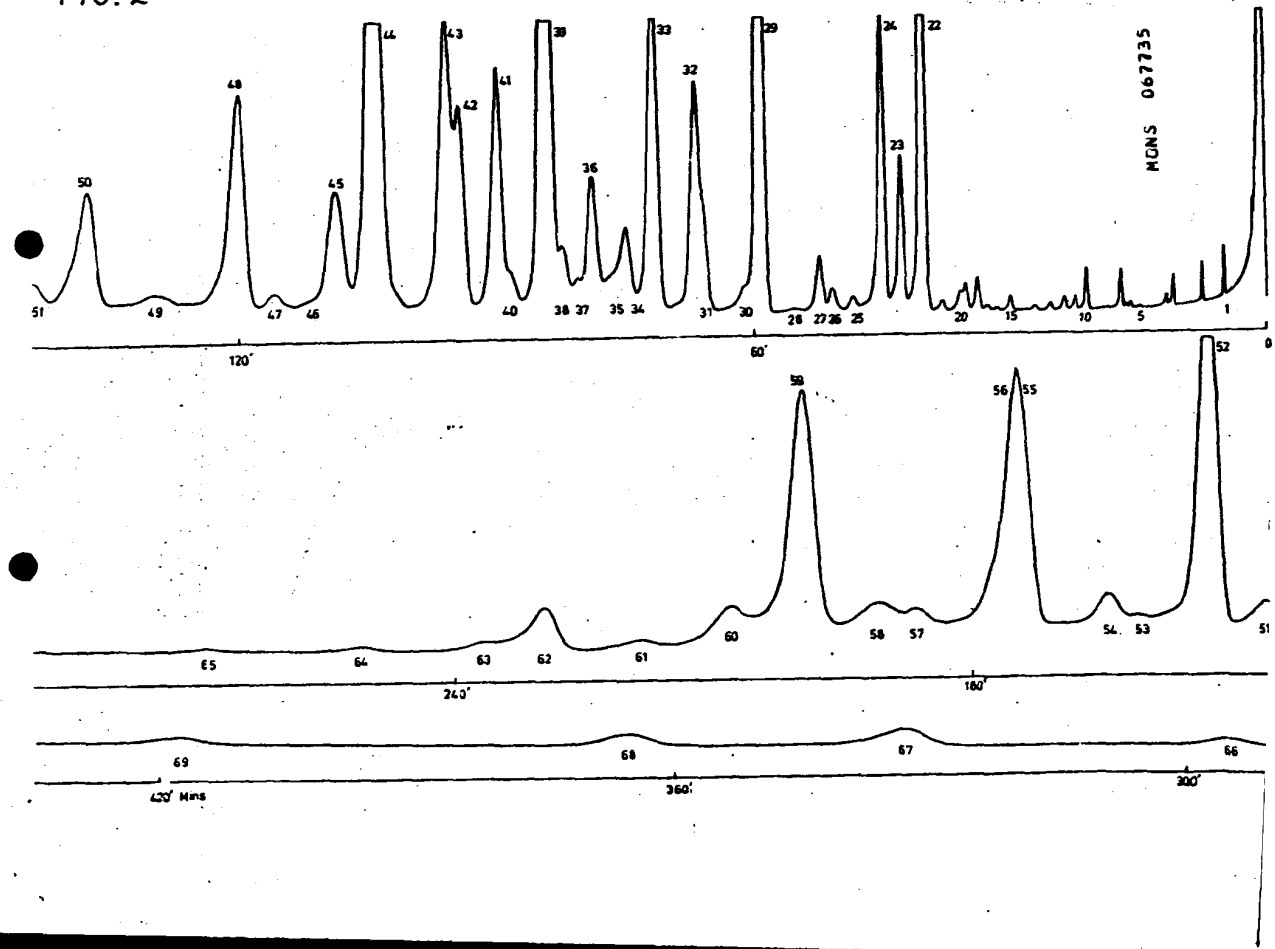
Ring Structure	½(RI) value	Derivation	R.I.
2345-	1364	2345 - 2'3'4'	2577
	1378	2345 - 3'4'	2519
	1380	2345 -	2127
2346-	1245	2346 - 2'3'4'6'	2490
2356-	1219	2356 - 2'3'5'6'	2438
	1273	2356 -	2020
23456-	1405	23456 - 2'3'4'5'6'	2810

MONS 067733



MONS 067734

FIG. 2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120' 60'
65 64 63 62 61 60 59 58 57 56 55 54 53 52 51
240' 180'
69 68 67 66
420' Mins 360' 300'

FIG. 3

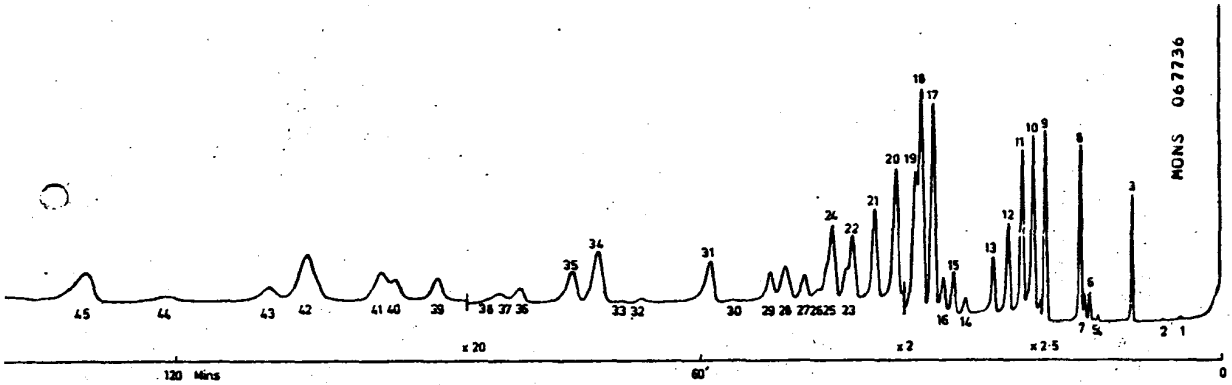
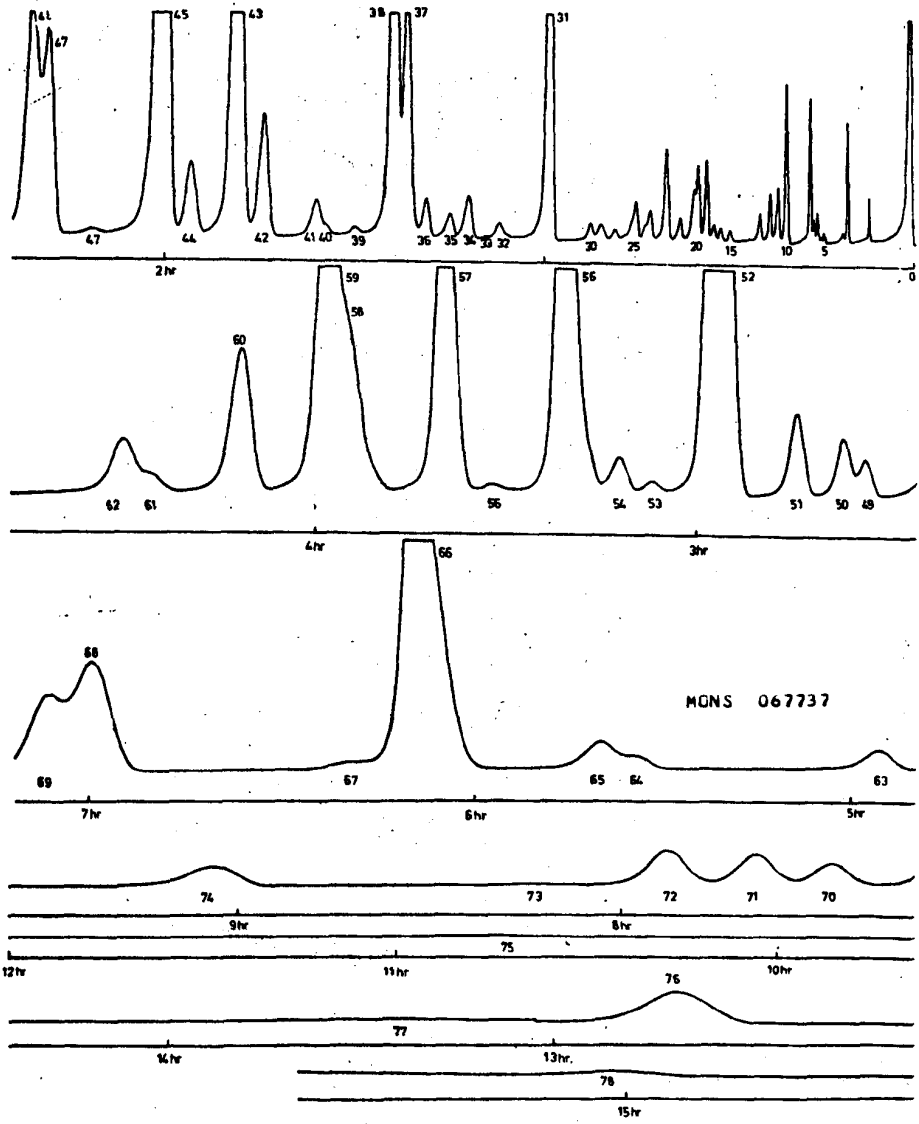

MONS 067736
45
44
43
42
41 40
39
38 37 36
35
34
33 32
31
30
29 28 27 26 25 23
24
22
21
20 19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
x 20
x 2
x 2·5
120 Mins
60'
0

FIG. 4



MONS 067737