STUDIES IN THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND CHROMATOGRAPHIC BEHAVIOUR

PART VI. THE BEHAVIOUR OF SOME ALKYL ETC. PHENOLS CHROMATOGRAPHED BY REVERSED-PHASE THIN-LAYER PARTITION CHROMATOGRAPHY*

L. S. BARK AND R. J. T. GRAHAM

Department of Chemistry and Applied Chemistry, The Royal College of Advanced Technology, Salford, Lancs. (Great Britain)

(Received December 20th, 1965)

INTRODUCTION

In earlier papers[1,2] we have reviewed the use of reversed-phase partition paper chromatography in the separation of phenols and, in particular, the use of such systems in a study of the relationship between molecular structure and chromatographic behaviour.

GREEN and co-workers[3–6] used papers impregnated with ethyl oleate in a study of the MARTIN[7] additivity principle, relating molecular structure to chromatographic behaviour.

Using the same impregnant, ethyl oleate, in reversed-phase thin-layer partition chromatography, we have found that much less was required to impregnate cellulose powder than to impregnate cellulose in the form of paper strips[1].

The chromatographic parameters, R_F and R_M, obtained by chromatographing a series of nitrophenols on thin layers of cellulose impregnated with ethyl oleate[2], under carefully controlled conditions, using polar mobile phases were used to explain the behaviour of these compounds, relative to the physico-chemical processes involved. It was suggested that the mechanism of the chromatographic process was essentially partition of the nitrophenols between the non-polar stationary phase and the polar mobile phase. The phenols, at first dissolved in the non-polar phase, may be dissolved by the mobile phase as a result of:

(i) Solvation of the phenolic group by the proton acceptor (water or ethanol).

(ii) Solvation of the nitro group with the mobile phase.

The non-polar or hydrophobic part of the molecule will be dissolved by the non-polar stationary phase, by the normal processes of dissolution. This factor will be of relatively greater importance in the chromatographic behaviour of the alkylated phenols than it is for the polar nitrophenols.

The overall amount of solvation of the phenolic group will be affected by:

(i) The ability of the phenol group to be solvated. This may be influenced by steric factors.

* For Parts II, III, IV and V of this series, see refs. 8, 1, 2 and 9.

(ii) Altering the polarity of the phenolic grouping.
(iii) Self association of the phenol.

To minimise the polarity effects, homologous series of alkylated phenols were chosen. Such low concentrations of the phenols were present that the relatively large number of hydroxyl groups in the mobile phase caused self association to be improbable.

EXPERIMENTAL

Cellulose (15 g), slurried with 70 ml of a 0.75 % (v/v) solution of ethyl oleate in diethyl ether, was used to coat glass plates using a Shandon thin-layer applicator, as previously described[2].

Aqueous ethanol (25 % v/v and 37.5 % v/v) were the mobile phases[2]. The application of the phenols (1 μl of 0.25 % solutions in suitable solvents) to the layers was done with our multiple-spotting device[8], and the chromatograms were eluted by an ascending technique in our double saturation chamber[8], at a constant temperature of 25° ± 0.5°, for a fixed period of time, until the solvent front had travelled a distance of 14.5 ± 0.5 cm. The phenols were detected as yellow spots on a purple background by spraying the layers with alkaline permanganate.

RESULTS

The results are shown in the various sections of Table I. Where the R_F/R_M values of a given phenol are quoted in more than one section of the Table, this is done to enable comparisons to be made. The results are the mean of 4 runs on plates carrying an internal standard. The values for the internal standard did not differ by more than ± 0.01 R_F units from a pre-determined mean. The results for the individual phenols were also reproducible to ± 0.01 R_F units.

DISCUSSION

For convenience the compounds are divided into arbitrary groups similar to those considered for the adsorption chromatography of these compounds on alumina surfaces[9].

(a) *Methylated phenols*

The R_F/R_M values of these phenols in the eluent systems, aqueous ethanol (25 % v/v) (to be referred to as System 1) and aqueous ethanol (37.5 % v/v) (to be referred to as System 2) are given in Table Ia. From this and from Fig. 1 it can be seen that the migration of the phenols is dependent upon the position of the methyl groups relative to the phenolic group, and that, as was the case for adsorption chromatography[9], they may be divided into three groups according to the number of *ortho*-substituents present in the molecule.

It can be seen from Table Ia and from Fig. 1 that the addition of methyl groups to one or both *ortho*-positions has a smaller effect on the R_F values than was the case in adsorption chromatography[9]. Indeed the addition of the second *ortho*-methyl group has a smaller effect than the addition of the first. This is in accord with

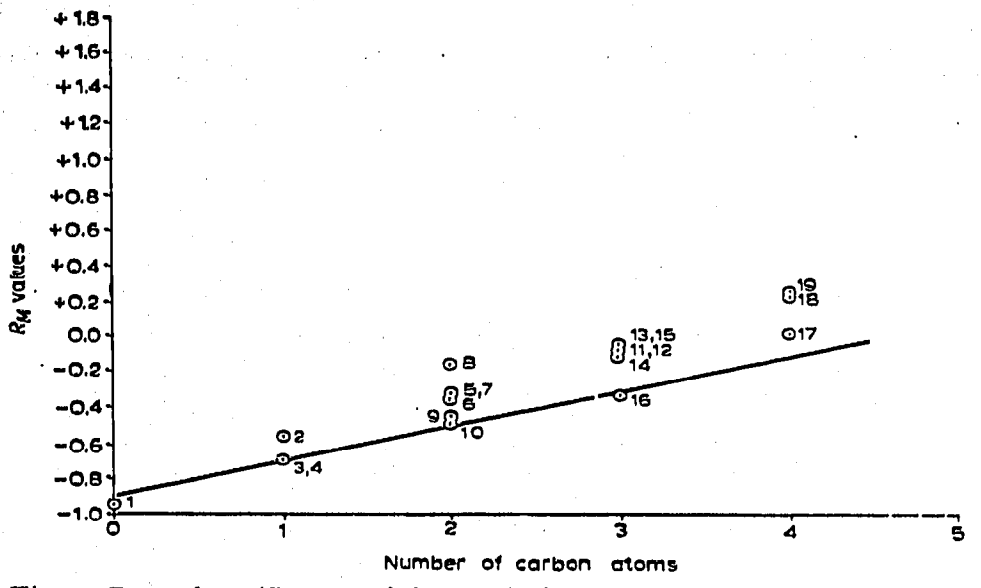

Fig. 1. R_M values (System 2) for methylated phenols *vs.* number of carbon atoms in the side chains.

the observation of GREEN *et al.*[4] from their work on ethyl oleate impregnated paper.

A probable explanation is that in the adsorption system the approach of the phenolic oxygen to the hydroxylated alumina is sterically hindered. In the partition system, however, the steric hindrance is to solvation of the phenolic group by the aqueous eluents. HEINEN[10] has suggested that the acid dissociation constant of 2,6-dimethylphenol shows no steric hindrance of solvation of the phenolic group by water, but that the same molecule does exhibit steric hindrance to hydrogen bonding with acetone. He suggests that this is because of the different sizes of the solvating molecules, the approach of the small water molecule is unhindered while that of the larger acetone molecule is. In the partition system, predominantly aqueous phases are the mobile phases and migration of the phenols is supposed to take place as a consequence of solvation of the phenolic group by this mobile phase. This, by token of HEINEN's suggestion[10], is little affected by steric hindrance.

In each of the three groups, the addition of one or more methyl groups to the ring results in a decrease in R_F values relative to that of the parent phenol. In Group 1 (no *ortho*-substituents), Fig. 1 clearly shows that the MARTIN[7] relation is strictly obeyed, as was found by GREEN *et al.*[4].

For Groups 2 and 3 (1-*ortho*- and 2-*ortho*-substituents respectively), it is clear from Fig. 1 that the MARTIN[7] relation is approximately true. On balance, for nuclear methylated phenols, the MARTIN[7] relation is valid, subject to positional effects as suggested by BARK AND GRAHAM[11] for nuclear methylated phenoxyacetic acids.

(b) 3- and/or 4-straight chain monoalkyl-substituted phenols

GREEN AND MARCINKIEWICZ[3] have pointed out that for R_F values to be meaningful in a study of the MARTIN[7] relation, they must lie in the range 0.20 to 0.80. Bearing in mind this limitation, R_F values outside this range are used to show trends rather than for a strict evaluation of the MARTIN[7] relation.

From Table Ib and Fig. 2 it is obvious that within the imposed limits, the MARTIN[7] relation is strictly valid for *para*-straight chain alkyl-phenols. This confirms

TABLE I

REVERSED PHASE THIN-LAYER CHROMATOGRAPHY USING AS THE STATIONARY PHASE CELLULOSE (15 g) IMPREGNATED WITH ETHYL OLEATE (70 ml) OF ETHYL OLEATE IN DIETHYL ETHER (0.75% v/v)

Key	*Phenol*	*System 1*		*System 2*	
		R_F	R_M	R_F	R_M
(a) Methylated phenols					
1	Phenol	0.795	−0.588	0.900	−0.955
2	2-Methyl-	0.625	−0.221	0.785	−0.562
3	3-Methyl-	0.675	−0.318	0.830	−0.688
4	4-Methyl-	0.660	−0.288	0.830	−0.688
5	2,3-Dimethyl-	0.455	+0.079	0.680	−0.327
6	2,4-Dimethyl-	0.425	+0.131	0.690	−0.348
7	2,5-Dimethyl-	0.475	+0.043	0.680	−0.327
8	2,6-Dimethyl-	0.400	+0.176	0.635	−0.240
9	3,4-Dimethyl-	0.530	−0.048	0.765	−0.513
10	3,5-Dimethyl-	0.515	−0.026	0.740	−0.455
11	2,3,4-Trimethyl-	0.290	+0.389	0.575	−0.131
12	2,3,5-Trimethyl-	0.290	+0.389	0.550	−0.077
13	2,3,6-Trimethyl-	0.280	+0.410	0.540	−0.070
14	2,4,5-Trimethyl-	0.300	+0.368	0.570	−0.123
15	2,4,6-Trimethyl-	0.280	+0.410	0.540	−0.070
16	3,4,5-Trimethyl-	0.430	+0.123	0.680	−0.327
17	2,3,4,5-Tetramethyl-	0.240	+0.501	0.500	0.000
18	2,3,4,6-Tetramethyl-	0.180	+0.659	0.370	+0.231
19	2,3,5,6-Tetramethyl-	0.150	+0.750	0.360	+0.250
(b) 3- and/or 4-straight chain monoalkyl-substituted phenols					
1	Phenol	0.795	−0.588	0.900	−0.955
3	3-Methyl-	0.675	−0.318	0.830	−0.688
4	4-Methyl-	0.660	−0.288	0.830	−0.688
20	3-Ethyl-	0.485	+0.027	0.700	−0.368
21	4-Ethyl-	0.455	+0.079	0.690	−0.348
22	4-*n*-Propyl-	0.240	+0.501	0.550	−0.077
23	4-*n*-Butyl-	0.110	+0.908	0.375	+0.223
24	4-*n*-Amyl-	0.060	+1.195	0.210	+0.550
25	4-*n*-Nonyl-	0.000	—	0.060	+1.195
(c) 3- and or 4-branched chain monoalkyl-substituted phenols					
22	4-*n*-Propyl-	0.240	+0.501	0.550	−0.077
26	4-Isopropyl-	0.250	+0.477	0.585	0.149
23	4-*n*-Butyl-	0.110	+0.908	0.375	+0.223
27	4-*sec.*-Butyl-	0.140	+0.789	0.440	+0.105
28	3-*tert.*-Butyl-	0.215	+0.526	0.480	+0.035
29	4-*tert.*-Butyl-	0.230	+0.867	0.500	0.000
24	4-*n*-Amyl-	0.060	+1.195	0.210	+0.550
30	4-*sec.*-Amyl-	0.055	+1.234	0.180	+0.659
31	4-*tert.*-Amyl-	0.150	+0.750	0.355	+0.259
32	4-(3-Methylbutyl)-	0.075	+1.091	0.390	+0.195
33	4-*tert.*-Octyl-	0.040	+1.380	0.060	+1.195
(d) 4-Monosubstituted phenols containing other structural groups					
1	Phenol	0.795	−0.588	0.900	−0.955
22	4-*n*-Propyl-	0.240	+0.501	0.550	−0.077
34	4-Allyl-	0.350	+0.269	0.670	−0.307
23	4-*n*-Butyl-	0.110	+0.908	0.375	+0.223
35	4-Crotyl-	0.170	+0.689	0.500	0.000
36	4-Cyclopentyl-	0.130	+0.826	0.370	+0.231

(*continued on p. 421*)

TABLE I (*continued*)

Key	*Phenol*	*System 1*		*System 2*	
		R_F	R_M	R_F	R_M
37	4-Cyclopent-2-enyl-	0.190	+0.630	0.470	+0.051
38	4-Cyclohexyl-	0.070	+1.124	0.220	+0.550
39	4-Phenyl-	0.110	+0.908	0.445	+0.096
40	4-Benzyl-	0.105	+0.931	0.420	+0.140
41	4-Cumyl-	0.080	+1.061	0.220	+0.550
(*e*) *Polyalkyl-substituted phenols containing no ortho-substituent*					
22	4-*n*-Propyl-	0.240	+0.501	0.550	—0.077
16	3,4,5-Trimethyl-	0.430	+0.123	0.680	—0.327
42	3-Methyl-5-ethyl-	0.300	+0.368	0.610	—0.195
43	3-Methyl-4-isopropyl-	0.245	+0.489	0.550	—0.077
44	3-Methyl-5-isopropyl-	0.175	+0.673	0.485	+0.027
45	3-Methyl-5-*sec.*-butyl-	0.070	+1.124	0.310	+0.348
46	3,5-Di-*tert.*-butyl-	0.000	—	0.110	+0.908
(*f*) *Substituted alkyl-phenols containing one ortho-group*					
1	Phenol	0.795	—0.588	0.900	—0.955
2	2-Methyl-	0.625	—0.221	0.785	—0.562
47	2-Ethyl-	0.400	+0.176	0.610	—0.195
48	2-*n*-Propyl-	0.200	+0.602	0.460	+0.070
49	2-*sec.*-Butyl-	0.115	+0.886	0.340	+0.288
50	2-*tert.*-Butyl-	0.080	+1.061	0.240	+0.501
51	2-*n*-Octyl-	0.000	—	0.040	+1.380
52	2-Allyl-	0.280	+0.410	0.575	—0.131
53	2-Phenyl-	0.130	+0.826	0.415	+0.149
54	2-Cyclohexyl-	0.060	+1.195	0.195	+0.616
55	2-Methyl-4-*tert.*-butyl-	0.085	+1.032	0.320	+0.327
56	2-Methyl-4-octyl-	0.020	+1.690	0.060	+1.195
57	2-*tert.*-Butyl-3-methyl-	0.040	+1.380	0.120	+0.865
58	2-*tert.*-Butyl-4-methyl-	0.050	+1.279	0.150	+0.750
59	2-Octyl-4-methyl-	0.000	—	0.020	+1.690
(*g*) *Substituted alkyl-phenols containing two ortho-groups*					
1	Phenol	0.795	—0.588	0.900	—0.955
8	2,6-Dimethyl-	0.400	+0.176	0.635	—0.240
60	2,6-Dimethyl-4-*n*-propyl-	0.080	+1.061	0.240	+0.501
61	2,6-Dimethyl-4-allyl-	0.095	+0.979	0.310	+0.348
62	2,6-Di-*tert.*-butyl-	0.030	+1.510	0.050	+1.279
63	2-Methyl-4,6-di-*tert.*-butyl	0.000	—	0.050	+1.279
64	2,6-Di-*tert.*-butyl-4-methyl-	0.000	—	0.020	+1.690
(*h*) *Alkoxy-phenols*					
1	Phenol	0.795	—0.588	0.900	—0.955
65	2-Methoxy-	0.810	—0.629	0.855	—0.780
66	3-Methoxy-	0.685	—0.334	0.835	—0.703
67	4-Methoxy-	0.680	—0.348	0.835	—0.703
68	3,5-Dimethoxy-	0.170	—0.389	0.870	—0.827
69	4-Ethoxy-	0.660	—0.288	0.825	—0.674
70	4-Cyclopentyloxy-	0.315	+0.338	0.620	—0.213
71	4-Heptoxy-	0.000	—	0.120	+0.865
72	4-Dodecyloxy-	0.000	—	0.020	+1.690
73	4-Tetradecyloxy-	0.000	—	0.020	+1.690
74	4-Hexadecyloxy-	0.000	—	0.020	+1.690
75	4-Phenoxy-	0.300	+0.368	0.515	—0.026
76	3,5-Dicarbomethoxy-	0.000	—	0.000	—

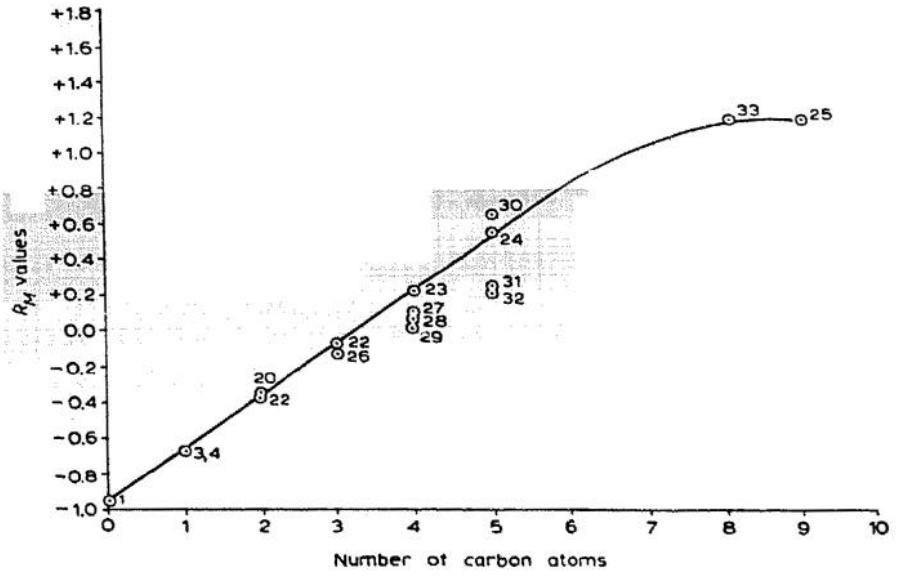

Fig. 2. R_M values for 3- and 4-alkyl-substituted phenols *vs.* number of carbon atoms in the side chains.

the results of GREEN *et al.*[4], for the same phenols in ethyl oleate/aqueous ethanol paper partition chromatography.

Table II shows the R_F values of the C_2 and C_3 alkyl-substituted phenols together with those of GREEN *et al.*[4] It can be seen that the order of R_F values is the same in both cases. They enable an appraisal of the chromatographic system to be made.

TABLE II

R_F VALUES OF C_2 AND C_3 ALKYL-SUBSTITUTED PHENOLS

Phenol	*R_F value*	
	System 2	*From ref. 4*
3,4-Dimethyl-	0.785	0.735
3,5-Dimethyl-	0.740	0.735
3-Ethyl-	0.700	—
4-Ethyl-	0.690	0.700
3,4,5-Trimethyl-	0.680	0.585
4-*n*-Propyl-	0.550	0.450

It is supposed that the hydrocarbon part of the molecule will lie flat in the interface of the two phases, with the phenolic group acting as a solvent hook. Solvation of the phenolic group will remove the molecule from the stationary phase to the mobile phase, so carrying it in the direction of eluent flow. Thus any reduction in solvation will reduce the R_F values. This will be effected in two ways:

(i) By electronic interaction increasing the electron density at the phenolic hydrogen. This will reduce its tendency to protonate the eluent and so give lower R_F values.

(ii) By a steric factor which will enhance the solubility of the hydrocarbon part of the molecule in the stationary phase.

In the case of (i), the inductive and hyperconjugative release of electrons by the methyl group will increase the electron density at the hydrogen atom and so reduce the R_F values. An increase in the numbers of methyl groups will enhance this effect, still further reducing the R_F values.

It would be expected that the electron release will decrease with an increase in the alkyl chain length, and that the R_F values of the polymethylated phenol should be lower than that of a mono-alkylated phenol containing the same number of carbon atoms. This is not so, thus while electronic effects may have some importance, they alone cannot account for the order of R_F values. GREEN *et al.*[4] attempted to account for the differences in terms of differences in the electronic effects of side chain hydrogen atoms held on the α, β, γ, etc. carbon atoms, and calculated ΔR_M parameters for each of these. They did not consider the second possibility. This, however, is done here.

The steric factor (ii) is best considered in terms of the addition of the substituents relative to the direction of flow of the mobile phase. Taking 4-methylphenol as the parent of the series, the addition of methyl groups must be to the 3- and/or 5-positions, *i.e.* the increase in molecular size is at an angle to the direction of solvent flow. The addition of (CH_2)-groups to the 4-methyl group increases the chain length, and extends the molecular axis in the direction of eluent flow. Because each molecule may be regarded as being solvated at the phenolic group only, the force needed to remove the more compact molecule from the stationary phase is probably less than that needed to remove the more extensive molecule. The R_F values of the polymethylated isomers are higher than those of the corresponding long chain isomers. By analogy, it is easier to pick a coil of rope clear of the ground than it is to pick up the same piece of rope when uncoiled.

(c) 3- and/or 4-branched chain monoalkyl-substituted phenols

Branched chain isomers with no *ortho*-substituents (Table Ic) generally have R_F/R_M values which differ from those of the straight chain isomer. GREEN *et al.*[4] have attempted to show that where such differences occurred that they were caused by electronic differences resulting from the different contributions of the α, β, γ, etc. hydrogen atoms. For the large difference between the *tert.*-butyl- and *n*-butylphenols, they suggested a resonance contribution from a non-bonded methyl group. This effectively meant that there was a weakening of the carbon–hydrogen bond for each of the 3-methyl groups, and that these hydrogen atoms could take part in hydrogen bonding with the mobile phase and so increase the R_F values. It is alternatively suggested here that the effect of chain branching is to increase the size of the substituent and so force the hydrocarbon part of the molecule out of the two-dimensional stationary phase into the mobile phase and so increase the R_F values. The results in Table Ic show that, generally, the increase in the bulk of the substituent relative to the straight chain isomer does increase the R_F values.

(d) 4-Monosubstituted phenols containing other structural groups

The presence of a double bond in the molecule has the expected result of increasing the polarity of the molecule and hence the R_F values (Table Id), the increase

being attributed to the formation of a hydrogen bond between the hydrogen atoms of the eluent system and the electrons of the double bond.

The presence of an alicyclic substituent gives the molecule an R_F value equivalent to that of a saturated straight chain hydrocarbon of one less carbon atom. The values for the three aromatic substituted phenols may be explained in part by assuming some eluent–double bond interaction to give increased R_F values. That this is not as large as is expected from the number of such bonds may be due to the increased solubility of these compounds in the stationary phase. The difference between *p*-benzyl- and *p*-cumylphenol may be caused by the branching of the isopropyl part of the molecule between the rings forcing part of the molecule further into the stationary phase.

(*e*) *Polyalkyl-phenols containing no ortho-substituent*

The results for polyalkyl-substituted phenols (Table Ie), confirm the previously expressed view regarding the easier removal of a polysubstituted phenol from the stationary phase compared with an isomeric 4-alkyl straight chain substituted compound. Fig. 3 emphasises this, but shows that the MARTIN[7] relation is still approximately valid.

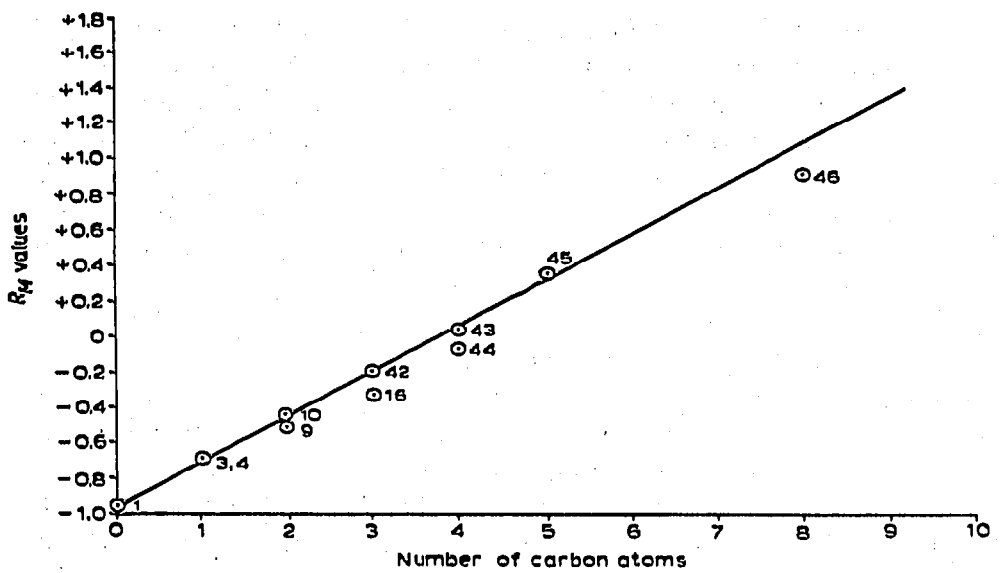

Fig. 3. R_M values (System 2) for polyalkyl-phenols containing no *ortho* groups *vs.* number of carbon atoms in the side chains.

(*f*) *Substituted alkyl-phenols containing one ortho-group*

Here again the results (Table If) indicate the approximate validity of the MARTIN[7] relation. Too few straight chain compounds were available for study to establish the strict validity of the relation for these compounds, though the results for the first three members of the series indicate the probability of this. The effects of other structural features follow the pattern expected of them from the behaviour of groups containing the same structural features when substituted in the 3- and/or 4-positions. The expected increase in R_F value of the 2-allyl group, compared with 2-*n*-propyl is seen. The values of 2-cyclohexyl- and 2-phenylphenol show the same order with respect to each other as did their 4-substituted isomers. The effects of

chain branching and polysubstitution compared with straight chain substitution are also shown in Fig. 4.

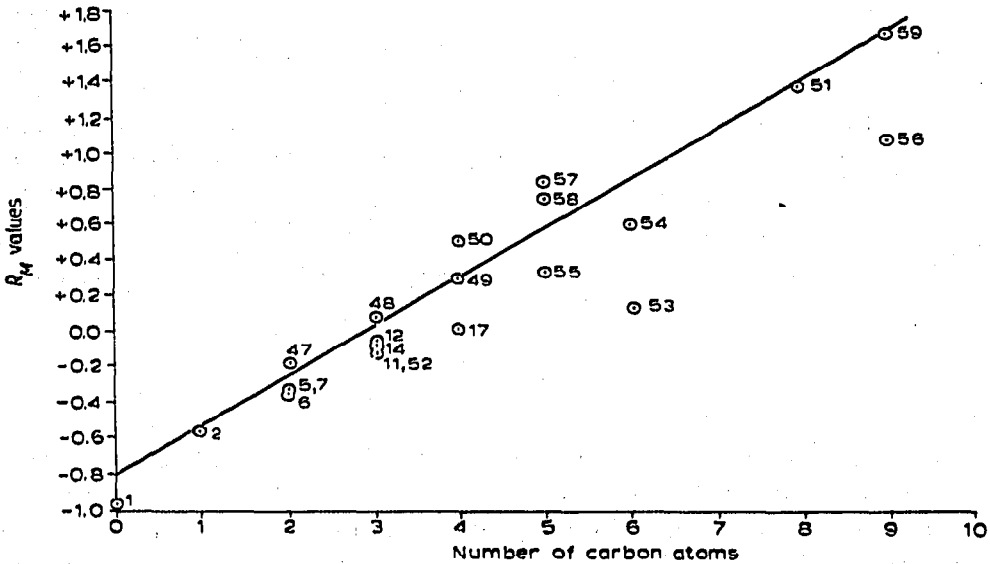

Fig. 4. R_M values (System 2) for alkyl-phenols containing one *ortho*-substituent *vs.* number of carbon atoms in the side chains.

(*g*) *Substituted alkyl-phenols containing two ortho-groups*

The effect of di-*ortho*-substitution with bulky groups is shown in the results for the 2,6-dimethyl- and the 2,6-di-*tert.*-butylphenols (Table Ig). The increased polarity of the molecule caused by the presence of the double bond is seen in the results for the pair 2,6-dimethyl-4-*n*-propyl- and 2,6-dimethyl-4-allylphenol. The isomeric pair, 2-methyl-4,6-di-*tert.*-butyl- and 2,6-di-*tert.*-butyl-4-methylphenol is just separable in System 2; this is accounted for by the replacement of an *o*-methyl group

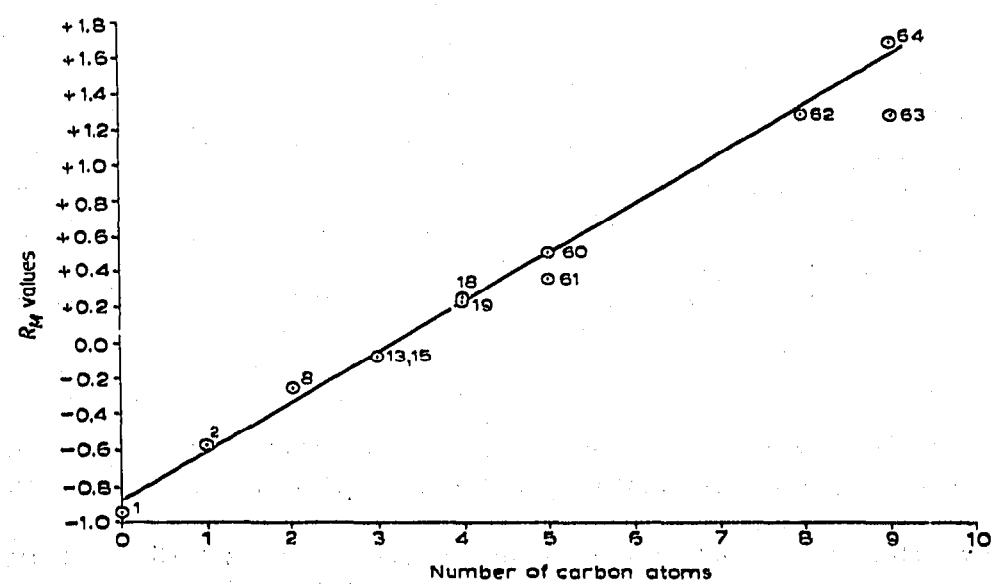

Fig. 5. R_M values (System 2) for alkyl-phenols containing two *ortho*-substituents *vs.* number of carbon atoms in the side chain.

by an *o-tert.*-butyl group. The approximate validity of the MARTIN[7] relation is illustrated by Fig. 5.

(*h*) *Alkoxy-phenols*

The aliphatic ethers (Table Ih) follow the expected pattern of a reduction in R_F values with increasing chain length of the alkyl part of the ether. Some evidence of an *ortho*-effect is seen in the results for the isomeric methoxyl derivatives. The effects of the presence of an alicyclic ring, and an aromatic one are seen in the results for 4-cyclopentyloxyl-, and 4-phenoxylphenol, and are of the expected order. The increase in R_F values for the 3,5-dimethoxyl compound may be a result of solvation of the ether oxygen. The value for 3,5-dicarbomethoxyphenol may be caused by a molecular weight factor.

CONCLUSION

We consider the mechanism of the separation for alkyl-phenols chromatographed by reversed-phase thin-layer chromatography between a non-polar stationary phase and a polar mobile phase to be essentially a partition process. The phenols at first dissolved in the non-polar phase, are removed as a result of solvation of the phenolic group by the polar mobile phase.

Where solvation of the phenolic group is hindered by the presence of substituents in one or both *ortho*-positions, lower R_F values result, the greater the bulk of the substituent the greater the lowering of R_F values.

For phenols substituted in the 3- and/or 4-positions, a single substituent containing a given number of carbon atoms has a greater effect on the R_F values than two substituents containing, in total, the same number of carbon atoms as the single side chain.

The MARTIN additivity principle is shown to be approximately valid, subject to modifications involving *ortho*-effects, chain branching and for polysubstituted phenols, positional effects.

ACKNOWLEDGEMENTS

For the gift of some of the phenols used in this study we thank Dr. J. GASPARIČ, Research Institute for Organic Syntheses, Pardubice-Rybitví, Czechoslovakia, Dr. J. GREEN, Vitamins Research Ltd., Tamworth, Surrey, and Mr. J. YOUNG, Midland Tar Distillers Ltd., Oldbury, Nr. Wolverhampton.

We also thank Professor L. HUNTER, The University, Leicester, for discussion.

SUMMARY

The alkyl etc. phenols have been chromatographed under controlled conditions, by reversed-phase thin-layer chromatography on cellulose impregnated with ethyl oleate as the stationary phase, and aqueous ethanol as the mobile phase.

The results indicate that the mechanism of separation is essentially one of partition between the two phases, the phenols being removed from the stationary phase by solvation of the phenolic group by the polar mobile phase. Steric effects are

considered to be of greater importance than electronic effects in influencing the degree of solvation of the phenolic group.

The MARTIN additivity principle is considered to be approximately valid, subject to modifications as a result of the influence of chain branching, steric effects, and for polysubstituted phenols, positional effects.

REFERENCES

1 L. S. BARK AND R. J. T. GRAHAM, *Talanta*, in the press.
2 L. S. BARK AND R. J. T. GRAHAM, *Proc. S.A.C. Conf., Nottingham, 1965*, W. Heffer and Son, Cambridge, 1966, p. 112.
3 J. GREEN AND S. MARCINKIEWICZ, *J. Chromatog.*, 10 (1963) 35.
4 J. GREEN, S. MARCINKIEWICZ AND D. MCHALE, *J. Chromatog.*, 10 (1963) 42.
5 J. GREEN, S. MARCINKIEWICZ AND D. MCHALE, *J. Chromatog.*, 10 (1963) 158.
6 S. MARCINKIEWICZ AND J. GREEN, *J. Chromatog.*, 10 (1963) 184.
7 A. J. P. MARTIN, *Biochem. Soc. Symp.*, 3 (1950) 4.
8 L. S. BARK, R. J. T. GRAHAM AND D. MCCORMICK, *Talanta*, 12 (1965) 122.
9 L. S. BARK AND R. J. T. GRAHAM, *J. Chromatog.*, 23 (1966) 120.
10 W. HEINEN, *Doctoral Thesis*, University of Utrecht, 1964.
11 L. S. BARK AND R. J. T. GRAHAM, *Analyst*, 85 (1960) 663.