CHROMATOGRAPHIC BEHAVIOUR OF ISOMERIC LONG-CHAIN ALIPHATIC COMPOUNDS

III. THIN-LAYER CHROMATOGRAPHY OF POSITIONAL ISOMERS OF SUBSTITUTED FATTY ACIDS AND ALCOHOLS

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SUMMARY

The migration behaviour of five complete series of disubstituted octadecane derivatives (ketostearates, hydroxystearates, acetoxystearates, octadecanediols and octadecanediacetates) has been studied on five different thin-layer chromatographic (TLC) adsorbents. In addition, nearly complete series of the corresponding derivatives of eicosane and all the positional isomers of two monosubstituted series (hydroxy- and acetoxyoctadecanes) have been studied on one adsorbent. Considerable and systematic variations in the mobilities of the positional isomers of these various compounds were apparent. Similar patterns of migration were obtained for all the disubstituted series and these were more or less constant on the various adsorbents. Some discussion of the possible factors causing migration differences between positional isomers is included.

The fact that positional isomerism of substituted fatty acid derivatives could have appreciable effect on their mobilities on thin-layer chromatography (TLC) has been evident from reports of separations of a few isomers by ourselves[1–5] and others[6–8]. We recently showed that there were considerable and systematic variations in mobilities of positional and/or geometric isomers of a wide range of substituted fatty acids[9]. All the positional isomers of methyl hydroxystearate were then examined and, when chromatographed side by side, these produced a sinusoidal pattern of spots on the thin-layer plate[9]. More recently we have examined a considerable number of positionally isomeric *cis*- and *trans*-octadecenoates on layers impregnated with silver nitrate and we found that similar sinusoidal patterns of spots were obtained for these compounds[10]. These results were not only of obvious practical significance but also posed some interesting questions as to the influence of slight structural differences on the chromatographic process.

In an attempt to gain further insight into the factors involved in these variations

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between positional isomers, we have now investigated the migration behaviour of several series of long-chain keto, hydroxy and acetoxy compounds on a variety of thin-layer adsorbents. The questions are not all answered but the tremendous selectivity of conventional adsorption TLC is demonstrated even more fully than hitherto.

EXPERIMENTAL

Materials

The hydroxystearate positional isomers were obtained as described in our previous paper[9]. All of the positional isomers of ketostearate were most generously provided by A. P. TULLOCH*. The 4- through 16-keto-eicosanoate isomers were obtained from R. A. LUCAS** and the hydroxyeicosanoate series was obtained by reducing a small portion of each of these with sodium borohydride in isopropanol solution. Reduction of a portion of each of the C_{18} and C_{20} keto esters with lithium aluminium hydride in diethyl ether solution gave the two series of diols (hydroxyoctadecanols and hydroxyeicosanols). A portion of each of the C_{18} and C_{20} hydroxy esters and hydroxy alcohols was treated with acetic anhydride–pyridine (2 : 1) at room temperature to give the isomeric series of acetoxy esters and diacetates, respectively. Because almost all of the samples of keto esters which we were given were chromatographically pure and because all of the reactions were quantitative, in only a few cases was purification of the products deemed to be necessary. In those few cases pure compounds were obtained by preparative TLC.

The isomeric octadecanols were synthesised by conventional condensation reactions between aldehydes and the Grignard derivatives of alkyl bromides, of appropriate chain lengths. The products were purified by a combination of preparative TLC and crystallisation from light petroleum and the purity and identity of the individual isomers was checked by TLC and GLC. A portion of each of these was acetylated to provide the isomeric series of acetoxyoctadecanes.

Procedures

Thin layers (*ca.* 250 μ) of adsorbent were applied to glass plates (20 × 20 cm) in the usual way, using the Desaga equipment. The adsorbents investigated were Silica Gel G (Merck), MN-Silica Gel G (Macherey, Nagel), Silica Gel SG41 (Whatman), and acidic alumina and basic alumina for TLC (Woelm).

Plates were activated immediately before use by heating at 110° for 30 min. After the plates had cooled to room temperature, the samples were applied as dilute (*ca.* 1 %) solutions in chloroform in a straight line exactly 1.5 cm from the bottom of the plate. A small amount of pure cholesterol in chloroform solution was applied on top of each sample spot and two or three additional spots of cholesterol were applied on each side of the series of samples. The cholesterol was added in this way for two reasons; to act as internal standard for calculation of relative R_F values, and to ensure that these values were measured only on plates which had developed perfectly evenly, without edge effects or other inconsistencies.

The plates were developed in tanks lined with solvent-soaked filter paper with diethyl ether–light petroleum (b.p. 40–60°) mixtures, of composition suitable for the

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series of compounds being examined. In general, on silica layers the proportions of these solvents were 80:20 for the series of diols, 50:50 for the hydroxy esters and 25:75 for the keto esters, acetoxy esters and diacetates. On the alumina layers higher proportions of diethyl ether were required to obtain migrations similar to those on the silica layers. The solvents were made up freshly for each plate and exactly 100 ml was added to the tank each time, so that the distance from the solvent surface to the line of applied samples was always the same. The layers were scored across with a blunt pencil exactly 10 cm above the origin spots so that solvent development was restricted to that distance. When the solvent front had reached the 10 cm line on the layer, the plates were removed and the spots were located by spraying with 5 *N* sulphuric acid followed by heating at 200° to char the organic materials. Each chromatogram was then photographed by Polaroid Land camera and/or photocopied on blue-line diazo paper and R_F values and relative R_F values (R_c) were measured and calculated from the latter.

RESULTS

All of the series of positionally isomeric bifunctional compounds, when chromatographed as described above, gave patterns of spots which were qualitatively fairly similar to each other and which were more or less constant on the five different adsorbents investigated. Illustrations of the pattern obtained with the series of hydroxystearates on Silica Gel G (Merck) have been reproduced previously[5,9] and in Fig. 1 is shown a chromatogram of the isomeric acetoxystearates on MN-Silica Gel G (Macherey, Nagel).

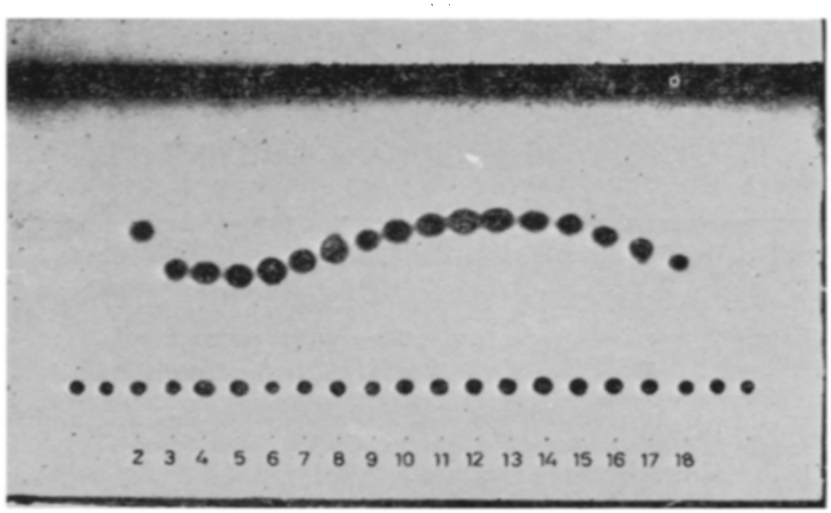

Fig. 1. Thin-layer chromatogram of isomeric methyl acetoxystearates on MN-Silica Gel G (Macherey, Nagel). The position of the acetoxyl group is indicated by the sample number and small amounts of cholesterol internal standard have been applied with each isomer and at each end of the series. Developing solvent was diethyl ether–light petroleum (25:75) and spots were located by spraying with 25% H_2SO_4 and charring.

The complete results of the TLC of each of the five bifunctional C_{18} series on each of the five adsorbents and of the five C_{20} series on Silica Gel G (Merck) are summarised in Table I. R_F values relative to cholesterol (as $R_c \times 100$ values) are tabulated

TABLE I

RELATIVE R_F VALUES ($R_c \times 100$) OF POSITIONALLY ISOMERIC BIFUNCTIONAL LONG-CHAIN COMPOUNDS

Compound class	*Chain length*	*Adsorbent*[a]	*Solvent*[b]	*Position of variable substituent*[c]					
				2	3	4	5	6	7
Hydroxy esters	18	i	A	159	132	109	93.3	101	113
	18	ii	A	161	131	107	93.3	97.9	107
	18	iii	A	186	145	117	103	113	128
	18	iv	B	?	?	d/143	d/114	92.5	112
	18	v	B	76.9	109	D	D	95.7	111
	20	i	A			124	100	106	118
Acetoxy esters	18	i	C	454	371	370	364	381	407
	18	ii	C	391	321	312	308	317	334
	18	iii	C	565	463	449	431	435	468
	18	iv	D	840	586	626	676	734	810
	18	v	D	457	399	409	408	415	437
	20	i	C			393	404	436	455
Keto esters	18	i	C	525	451	344	318	313	339
	18	ii	C	407	373	294	281	281	303
	18	iii	C	591	538	409	376	369	392
	18	iv	D	D	D	597	537	528	560
	18	v	D	D	D	463	438	430	453
	20	i	C			373	352	333	371
Diols	18	i	B	25.8	27.5	21.3	17.9	28.6	38.6
	18	ii	B	28.6	25.8	19.0	17.3	24.3	32.2
	18	iii	B	31.1	24.6	19.1	19.4	28.0	37.3
	18	iv	E	4.0	9.9	7.8	6.7	10.7	17.1
	18	v	E	6.0	12.7	9.6	9.6	13.1	18.9
	20	i	B			20.7	20.7	27.6	38.8
Diacetates	18	i	C	375	320	308	317	342	362
	18	ii	C	331	288	283	295	309	324
	18	iii	C	489	421	398	406	435	468
	18	iv	D	750	618	575	622	653	682
	18	v	D	591	513	493	513	544	579
	20	i	C			386	378	401	420

[a] Adsorbents: (i) Silica Gel G (Merck); (ii) MN-Silica Gel G (Macherey, Nagel); (iii) Silica Gel SG41 (Whatman); (iv) acidic alumina for TLC (Woelm); (v) basic alumina for TLC (Woelm).

[b] Solvents: (A) diethyl ether–light petroleum (50:50); (B) diethyl ether–light petroleum (80:20); (C) diethyl ether–light petroleum (25:75); (D) diethyl ether–light petroleum (40:60); (E) diethyl ether (100).

[c] $R_c \times 100$ values were calculated in the normal way; D denotes total decomposition with no migration; d/143 and d/114 denotes partial decomposition with streaking, above unchanged components of $R_c \times 100$ values of 143 and 114.

[d] R_x = mean actual R_F value of cholesterol internal standard.

under the position of the variable substituent and the mean actual R_F value of cholesterol (R_x), in each case, is included to indicate the extent of its migration and that of the isomeric series on the plates from which these values were computed. It should be pointed out straight away in what sense we feel justified in quoting these values to the third significant figure. We do not consider that the R_F value or R_c value of any individual isomer is necessarily reproducible to within $\pm$ 10 % or so of the value quoted, but we are concerned here with differences between the various members of each series. Because each series was run altogether on a single plate, we

8	9	10	11	12	13	14	15	16	17	18	19	R_x[d]
124	136	145	150	156	155	153	145	132	101	89.6		0.346
119	131	140	146	148	147	144	135	121	90.2	74.9		0.327
144	159	170	179	184	184	180	172	157	110	97.0		0.282
138	162	190	218	239	254	260	256	243	220	145		0.173
131	152	173	184	199	200	194	185	170	112	81.6		0.282
131	143	154	161	165	167	167	164	159			106	0.347
431	454	473	486	491	493	491	480	457	419	379		0.140
355	372	390	400	409	412	407	401	379	357	331		0.145
491	507	528	537	542	546	537	532	528	502	468		0.108
910	970	1040	1066	1086	1094	1080	1060	1014	938	800		0.050
459	472	488	504	508	508	502	500	480	455	430		0.126
480	499	514	527	532	534	533	529	521			462	0.140
370	400	438	462	476	482	475	446	396	265			0.112
327	348	366	379	388	389	386	371	335	230			0.153
421	457	484	500	514	529	530	505	454	301			0.093
611	642	667	697	717	720	722	713	694	553			0.090
485	506	529	539	553	553	551	529	487	372			0.121
409	446	483	515	531	545	545	538	512				0.112
50.1	59.7	66.0	71.6	73.3	74.4	72.7	65.8	60.1	42.5	36.5		0.699
40.9	47.9	54.3	58.9	61.3	61.3	58.6	53.4	46.2	31.9	24.9		0.543
48.3	57.4	66.5	72.7	74.4	74.6	72.2	67.0	59.8	42.6	35.2		0.418
23.3	29.1	36.1	43.3	50.8	53.2	55.4	51.9	45.7	29.4	18.7		0.374
24.6	29.9	36.6	40.6	44.0	44.9	43.0	38.0	32.7	19.6	14.3		0.418
47.9	59.0	66.4	71.8	75.7	77.7	78.3	77.0	75.2			44.0	0.678
390	409	431	442	447	450	442	432	408	385	362		0.120
338	350	359	368	374	375	374	364	347	328	303		0.152
491	507	514	519	520	525	530	528	507	477	449		0.108
733	778	822	855	880	884	880	867	834	708	638		0.060
607	627	650	658	664	665	657	644	609	581	513		0.115
443	467	485	496	508	513	517	515	512			458	0.130

are confident that differences to this order of accuracy are real and are measurable.

The table is subdivided according to compound class so that similarities or variations in the patterns from one adsorbent to another may be most easily distinguished. The patterns obtained with the various series will be discussed and illustrated more fully below; but they are basically of the form illustrated in Fig. 1, with a minimum mobility around the 5-substituted isomer and, for the C_{18} series, a maximum mobility for the 12- and 13-substituted isomers.

From the data in Table I it can be concluded that there are generally only very minor differences in the pattern of a given series from one adsorbent to another. This is particularly true of the three silica gels studied, where the patterns obtained for a given isomeric series are virtually identical. The only exceptions are the three members of the diol series with substitution in the 2-, 3- and 4-positions. These variations in the mobilities of the 1,2-, 1,3- and 1,4-diols relative to the 1,5- and other diols may reflect quite specific 'bidentate' interactions with pairs of active sites which are differently spaced in these different adsorbents.

There are somewhat greater differences in the patterns of the various series on the two alumina adsorbents, as compared to the silica gels, but again these are confined to the 5-, 4-, 3- and 2-substituted isomers. Thus, the 1,2-octadecanediol is substantially less mobile than any of the other isomers on both acidic and basic aluminas, the rest of the diol pattern being normal. This again, we suggest, reflects a rather specific type of interaction with the adsorbent for this isomer. The 2- and 3-keto isomers are decomposed on both aluminas and do not migrate at all, while the 4- and 5-hydroxy esters are also partly or completely decomposed, presumably by hydrolysis and lactone formation, to give more mobile components. In the acetoxy ester series the position of minimum mobility is moved from the 5-substituted isomer on the silicas to the 3-substituted isomer on both aluminas but the pattern of the diacetate series is almost identical on all five adsorbents.

It can be concluded therefore that, apart from a few rather specific variations, the differences in mobilities between positional isomers of these bifunctional long-chain compounds are not due to any specificity in pore size or in arrangement of active sites on the adsorbent surface. The patterns obtained, being basically very similar on quite different adsorbents, must therefore be the result of some more general parameter or parameters, either a varying property of the isomeric compounds themselves, such as polarisability of the functional groups, or a significantly varying interaction between individual isomers and the moving solvent phase.

The data in Table I is made more accessible for comparisons if the relative R_F values are plotted against the position of the variable substituent. This, in effect, reproduces the patterns obtained on the original plates. In Fig. 2 are plotted the data

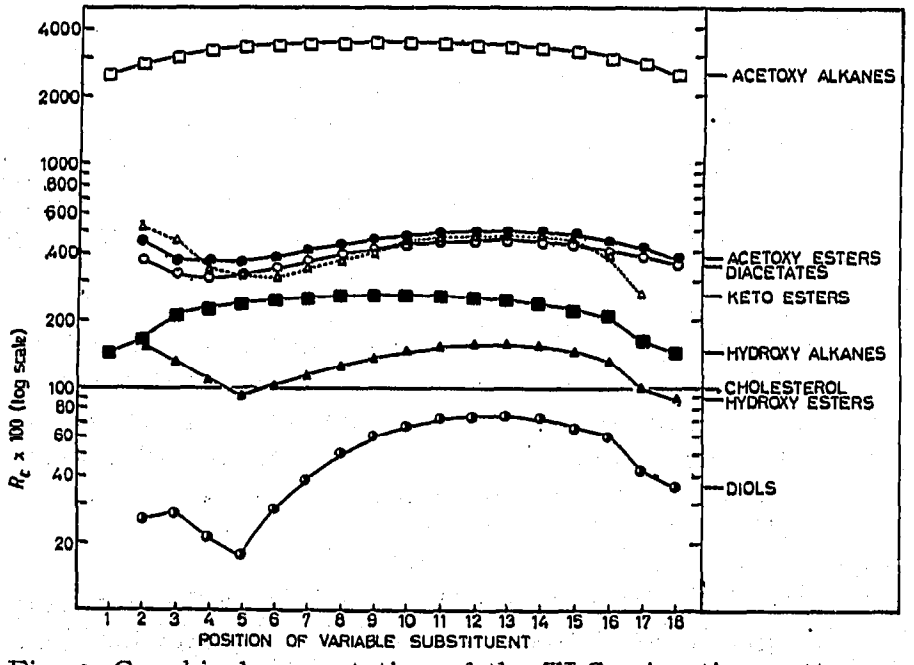

Fig. 2. Graphical presentation of the TLC migration patterns of positional isomers of acetoxyoctadecane (□—□), hydroxyoctadecane (■—■), ketostearate (△- - -△), hydroxystearate (▲—▲), acetoxystearate (●—●), dihydroxyoctadecane (◑—◑), and diacetoxyoctadecane (○—○) on Silica Gel G (Merck). R_F values relative to cholesterol (as R_c × 100 values) are plotted on a logarithmic scale against the position of the variable substituent. The data and the conditions of chromatography for the various bifunctional series are summarised in Table I; the developing solvent for the hydroxyoctadecanes was diethyl ether–light petroleum (40:60) and for the acetoxyoctadecanes was diethyl ether–light petroleum (5:95).

from Silica Gel G (Merck) plates of the five series of C_{18} bifunctional compounds and also of the positionally isomeric hydroxyoctadecanes and acetoxyoctadecanes, to facilitate comparisons of the patterns obtained for these various series on the same adsorbent. The $R_C \times 100$ values have been plotted on a logarithmic scale because the patterns so produced are far more similar to the actual patterns obtained on the plates than if they were plotted on a linear scale. Although, of course, there are only nine positionally isomeric octadecanols and acetoxyoctadecanes (1 through 9), these have been extended in reverse order to provide positions 10 through 18 of the "complete" curve and so allow more ready comparison to be made between these monofunctional series and the various bifunctional series.

From the summary of results provided by Fig. 2, a number of interesting features are apparent. Although the shapes of the curves obtained for the various bifunctional series are roughly similar, there are a number of differences in detail but more obviously there is a considerable difference in the 'amplitude' of the curves. Thus, there are much greater variations in mobilities between the individual diols than between the hydroxy esters and these in turn are greater than the variations between the individual keto esters, acetoxy esters or diacetates. Similarly with the monofunctional series, the amplitude of the octadecanol pattern is considerably greater than that of the acetoxyoctadecanes. These differences in amplitude are not due to differing distances of migration of these series because the developing solvent in each case was chosen to provide migration about halfway up the plate. In the same context, although the three series of keto esters, acetoxy esters and diacetates have approximately the same absolute polarities in this system and were, in fact, all developed with the same solvent mixture, the amplitude of the keto ester curve is considerably greater than those of the other two.

As mentioned earlier, each of the curves of the disubstituted alkanes shows a relatively high mobility for the 2-substituted compound, which decreases to a minimum at around the 5-substituted isomer. The mobilities progressively increase again as the substituent is moved along the chain to a maximum at the 12- and 13-positions and then finally decrease towards the terminally substituted isomer. Although the maximum of each of these curves is consistently established at the 12- and/or 13-position, the substituent position corresponding to the minimum mobility varies somewhat. In the diol series, the hydroxy ester series and the acetoxy ester series, it is the 5-position, whereas in the diacetate series it is the 4-position and in the keto ester series it is the 6-position. The significance, if any, of these differences is at present unknown.

From the data in Table I, particularly if they are plotted out as in Fig. 2, it can be seen that the patterns of the five bifunctional C_{20} series, although incomplete, are virtually identical to the corresponding C_{18} series curves, but at slightly higher relative R_F values due to the increased chain length. The minima of the C_{20} curves are in the same positions as those of the C_{18} series, with the same variation mentioned in the previous paragraph. The maximum of each of the C_{20} series curves, however, has moved not two places along the chain but only one, to the 13- and/or 14-positions compared to the 12- and/or 13-positions of the C_{18} series.

The monofunctional series included in Fig. 2, the hydroxy and acetoxy alkanes, provide much simpler patterns with mobilities decreasing in a smooth curve from the centrally substituted isomers to the (ω-1)-substituted octadecanol and to the termi-

nally substituted acetoxyoctadecane. The terminally substituted octadecanol, however, has rather more mobility than would be predicted by an extrapolation of the smooth curve. The 'kink' so produced at the end of the octadecanol curve is exactly reproduced in the patterns of the hydroxy esters and of the diols. The question of whether the keto ester series would show this effect cannot be answered directly as the terminal carbonyl isomer (17-formylheptadecanoate) was not available. However, by analogy with the results of MARCUSE *et al.*[11] on TLC of alkanals and positionally isomeric alkanones, this 'kink' would probably be even more pronounced and the terminal isomer, indeed, would have greater mobility than 17-keto-octadecanoate. The acetoxy esters and the diacetates, in contrast, show no discontinuity in the curve between the 17- and 18-substituted isomers, but Fig. 2 shows that this again is identical to that found with the corresponding monofunctional series, the acetoxyoctadecanes.

DISCUSSION

From the results reported above, it is evident that positionally isomeric series of bifunctional long-chain alkanes show similar patterns of migration, when subjected to TLC as we have described. It was predicted in an earlier paper[9] that all series of positionally isomeric oxygenated stearate derivatives would give rise to similar migration patterns. This prediction has been verified for the various series described herein and also for the whole series of *cis*- and *trans*-epoxystearates (personal communication from G. MAERKER). We have also shown[10] that, on silica gel impregnated with silver nitrate, the *cis*- and *trans*-series of positionally isomeric octadecenoates also give patterns very similar to those described herein (see also ref. 14).

We have shown that the migration patterns of the various series are remarkably constant on the five different adsorbents examined. There are only a few variations from the standard pattern and these are by some of the isomers whose two functional groups are relatively close together. We have suggested above that these few variations are due to rather specific 'bidentate' interactions with pairs of active sites which are differently spaced on the different adsorbents. Otherwise, it is clear that the patterns obtained are not due to some specific attribute of a particular adsorbent, such as has been shown by KLEIN with isomeric sterol acetates[12,13], but are the result of some more general but variable property of the isomers themselves or of their interactions with a nonpolar mobile phase and/or any polar stationary phase.

We have previously argued[9], on the basis of the results now reported, that the facile explanation that intramolecular hydrogen bonding between the hydroxyl and the carboxyl groups of the 5-, 4-, 3- and 2-hydroxystearates is the cause of their decreasing mobilities cannot be correct, because the keto and acetoxy esters and the diacetates, where such hydrogen bonding is manifestly impossible, show very similar patterns on TLC. We have suggested[10], however, that over this range of positions in isomeric octadecenoates or oxygenated esters an inductive effect of the carboxylic ester group is a predominant factor, becoming more pronounced as the double bond or substituent more closely approaches this terminal group. This inductive effect may operate in different directions for different series (e.g. towards the carboxylate group in the octadecenoates but towards the keto group in the keto esters) but with a similar result in terms of differences in adsorptive affinities and hence mobilities.

Strong similarities are apparent from Fig. 2 between the patterns of analogous monofunctional and bifunctional series in the region from about the 13- or 14-substituted isomer to the 18-substituted compound. We have already noted the smooth curve in this region of the three acetoxy series and the discontinuity between the 17- and 18-substituted isomers in the three hydroxy series. These similarities suggest that the mobilities of these long-chain isomers with substitution at or near the methyl end of the chain are unaffected by the presence of a functional group at the 1-position. Thus, elucidation of the factors causing differences in the mobilities of positionally isomeric monofunctional compounds will, we believe, automatically provide an explanation for this region of the patterns of bifunctional compounds.

The middle regions of these migration patterns, from about the 5- to the (ω-5)-positions, are probably going to be the least easy to explain. The fact that maximum mobility is at the 9-position in the monosubstituted C_{18} series but at the 12- and 13-positions in the disubstituted C_{18} series and at the 13- and 14-positions in the disubstituted C_{20} series indicates clearly that, in this 5- to (ω-5)-substituted region, the presence of a polar substituent on the 1-position does influence the differences in mobilities between individual isomers. This influence of one group on another, relatively distant group, in terms of TLC mobilities, must be accounted for in any rationalisation of the factors causing mobility differences between positional isomers.

We currently believe that these differences in mobilities of positional isomers, apart from the 1,2- to 1,5-disubstituted compounds, are not due to some variable property of the compounds themselves (*e.g.* polarisability) but are the result of the varying sum of attractive interactions between the mobile phase and two alkyl chains of varying length. The sum of the repulsive forces between the stationary phase and these two alkyl chains may also vary and provide a contribution to these differences between isomers on TLC. We are continuing TLC studies of other series of isomeric and homologous oxygenated compounds and from these and from published data on other physical properties of positional isomers we hope soon to be able to describe some verification or otherwise of these ideas and to provide a rational explanation for the differences in chromatographic behaviour of positional isomers described in this series of papers.

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