CHROMATOGRAPHIC BEHAVIOUR OF ISOMERIC LONG-CHAIN ALIPHATIC COMPOUNDS

I. THIN-LAYER CHROMATOGRAPHY OF SOME OXYGENATED FATTY ACID DERIVATIVES

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(Received February 15th, 1965)

Among the first reports of applications of thin-layer chromatography (TLC), using the apparatus and techniques of STAHL, were descriptions of separations of fatty acids and other lipids[1,2]. It was immediately realised that TLC was an ideal tool in the detection, separation and isolation of fatty acids containing oxygenated substituents and as an aid in elucidating the structures of such compounds[3,4]. The introduction of silver nitrate impregnated adsorbents as a means of separating compounds differing in degree or type of unsaturation[5–7] has greatly increased the usefulness of TLC for analytical and structural investigations of oxygenated fatty acids.

Because of the large number and variety of oxygenated fatty acids in our possession and our interest in studies of naturally occurring epoxy and hydroxy acids and their derivatives, we considered it appropriate to investigate the migration characteristics of a wide range of these compounds on thin layers of silica gel and of silver nitrate impregnated silica gel. The results of this investigation reported here demonstrate more fully than hitherto the wide possibilities of these TLC procedures in analytical and structural work in the fatty acid field.

EXPERIMENTAL

Materials

The 18- and 17-hydroxy-oleates and -stearates were isolated from samples sent by A. P. TULLOCH** who also very generously provided samples of the 16-, 15-, 14-, 13-, 11-, 7-, 6- and 5-hydroxystearates. The 10-hydroxystearate was a gift from A. T. JAMES***, the 4-, 3- and 2-hydroxystearates were synthesised by conventional procedures and the 12-, 9- and 8-hydroxy isomers were obtained by hydrogenation of appropriate natural unsaturated hydroxy acids. Most of the unsaturated monohydroxy acids were isolated from seed oils: 12-OH-*cis*-9-octadecenoic (ricinoleic)

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from castor, 9-OH-*cis*-12-octadecenoic from *Strophanthus kombe*, 9-OH-*trans*-10, *trans*-12-octadecadienoic (dimorphecolic) from *Dimorphotheca aurantiaca*, 9-OH-*trans*-10,*cis*-12- and 13-OH-*cis*-9,*trans*-11-octadecadienoic acids from *Artemisia absinthium*, 8-OH-octadec-9-yne-*trans*-11-enoic from *Santalum album* and 8-OH-9,11-octadecadiynoic and 8-OH-9,11-octadecadiyne-17-enoic (plus small amounts of their 13-ene vinylogues) from *Ongoekea gore* (isano oil). From methyl ricinoleate were derived 12-OH-*trans*-9-octadecenoate (ricinelaidate) and 12-OH-9-octadecynoate (ricinstearolate) by elaidinisation and by bromination–dehydrobromination respectively.

The saturated *cis*- and *trans*-epoxy esters were prepared by peracetic acid oxidation of the corresponding *cis*- and *trans*-monoenes. *cis*-12,13-Epoxyoleate was isolated from *Vernonia anthelmintica* seed oil and also from partially epoxidised linoleate whence was also isolated the isomeric *cis*-9,10-epoxy-12-octadecenoate.

The bromohydrins were prepared from the corresponding epoxy esters by reaction with anhydrous hydrogen bromide in diethyl ether, a *cis*-epoxide giving a mixture of *threo*- and a *trans*-epoxide a mixture of *erythro*-bromohydroxy derivatives. Each bromohydrin preparation was a mixture of two positional isomers, *e.g.* 9,10-epoxystearate gave 9-bromo-10-hydroxystearate and 10-bromo-9-hydroxystearate.

The *threo*- and *erythro*-12,13-dihydroxystearates and -oleates were derived from *cis*-12,13-epoxyoleate and the remaining dihydroxy esters were prepared from the corresponding *cis*-monoenes by dilute alkaline permanganate oxidation to give the *erythro*-diols and by performic acid oxidation to give the *threo*-isomers.

Procedures

Thin layers (*ca.* 250 μ) of "Merck" silica gel G were applied to glass plates (20 × 20 cm or 10 × 20 cm) with the Desaga equipment. Silver nitrate impregnated layers were prepared by using aqueous silver nitrate solutions instead of water for mixing with the silica gel. As a standard procedure, 1.25 g of silver nitrate was dissolved in 50 ml of water and mixed with 23.75 g of silica gel G to coat five 20 × 20 cm plates. This gave a standard adsorbent layer with uniform impregnation of 5 % (w/w). Prepared plates were protected from the laboratory atmosphere by storage in a sealed cabinet and were activated for 30 min at 110° just before use.

Samples were applied as dilute (*ca.* 1 %) solutions in chloroform and development was carried out with diethyl ether–hexane mixtures in closed tanks lined with solvent-soaked filter paper. Spots were located by spraying with 50 % aqueous sulphuric acid, 30 % chlorosulphonic acid in acetic acid, or 10 % phosphomolybdic acid in ethanol followed in each case by heating to 200° to char the organic materials, by spraying with 2′,7′-dichlorofluorescein (0.2 % in ethanol) and viewing under ultraviolet light, or by iodine vapours. This last procedure was not effective on impregnated plates.

RESULTS

Monohydroxy esters

The considerable effects of positional isomerism of substituted aliphatic compounds on their mobilities in adsorption chromatography are dramatically demonstrated in Fig. 1 which illustrates a thin-layer chromatogram of all seventeen isomeric

hydroxystearates. As expected, the 18-hydroxystearate which has a primary hydroxyl group is more polar than any of the secondary hydroxyl isomers. The statement by SUBBARAO *et al.*[8] that primary and secondary hydroxyl groups were equivalent in polarity on TLC is obviously erroneous, as two of these authors have since demonstrated without specifically stating it[9]. Their original conclusion was the result of comparing esters and alcohols each having the same number of hydroxyl groups but without considering the obvious increase in polarity conferred by an ester group.

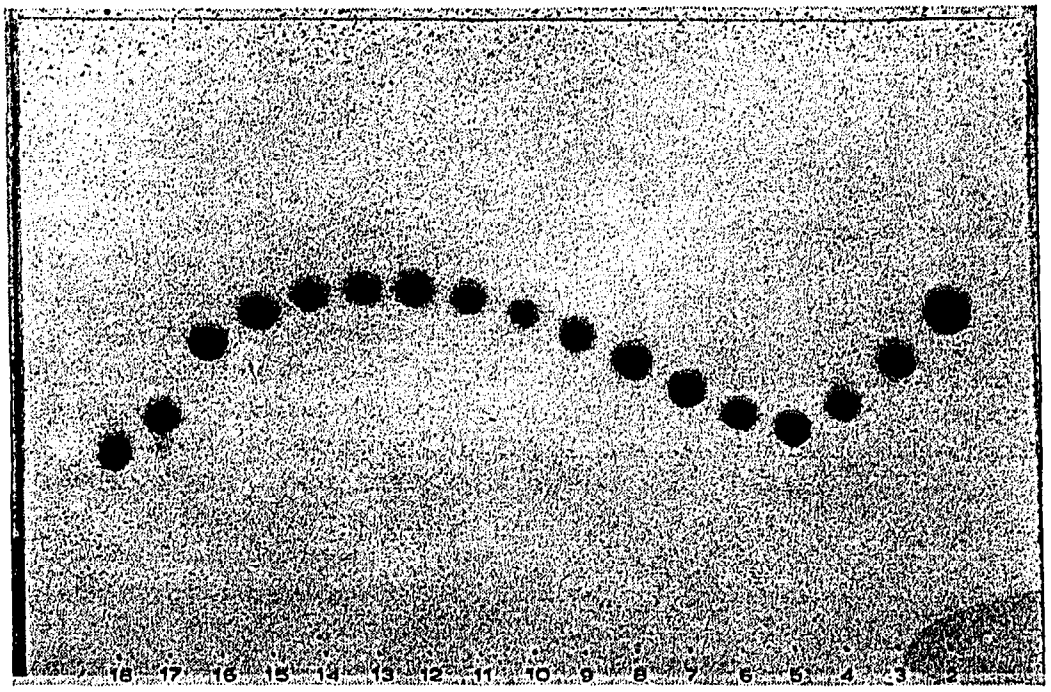
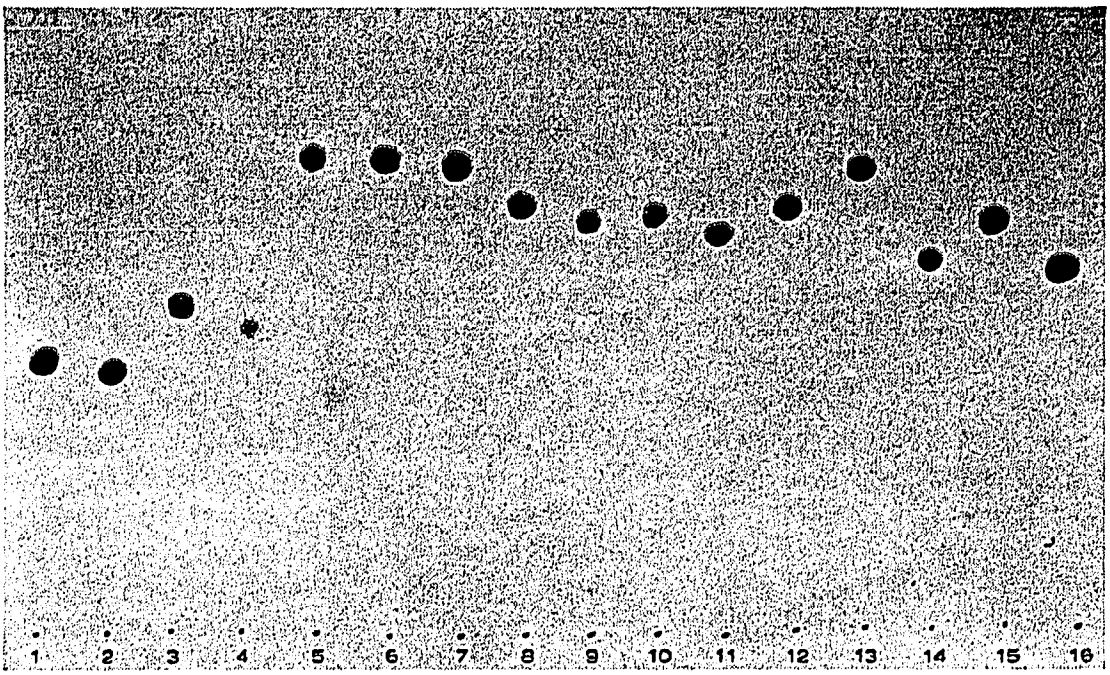


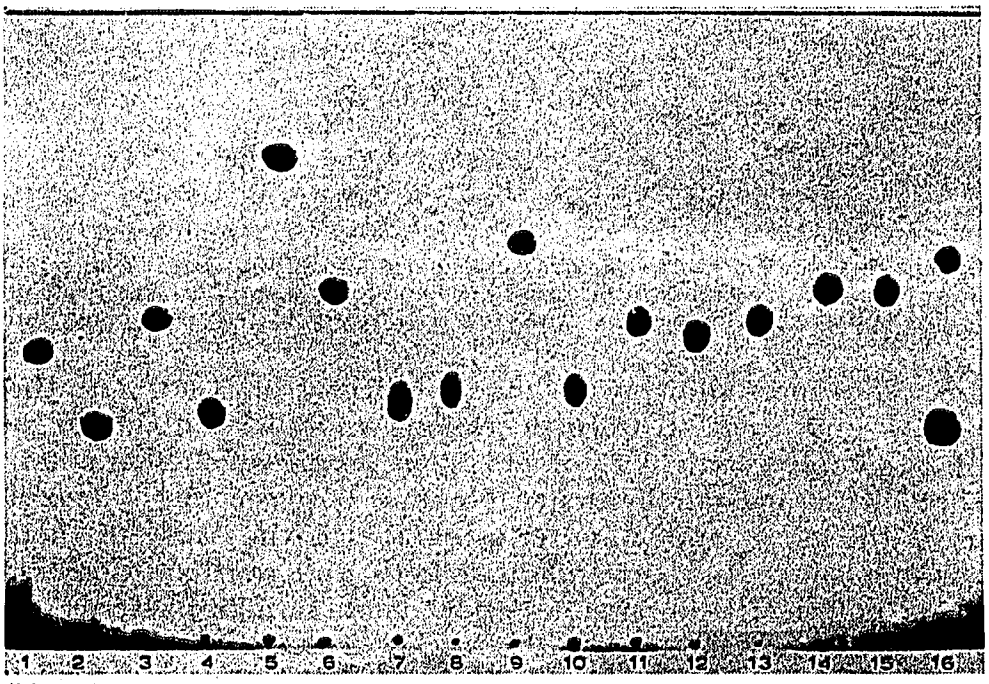
Fig. 1. Thin-layer chromatogram of isomeric methyl hydroxystearates on Silica Gel G. The position of the hydroxyl group is indicated by the sample number. Developing solvent was diethyl ether–light petroleum (1:1) and spots were located by spraying with 50% H_2SO_4 and charring.

The secondary hydroxyl isomers show increasing mobilities from the 17- to the 13- and 12-hydroxystearates and then steadily decreasing mobilities down to 5-hydroxystearate with the 4-, 3- and 2-hydroxy isomers again progressively less polar. The facile explanation that intramolecular hydrogen bonding between the hydroxyl and the carboxyl groups of the 4-, 3- and 2-hydroxystearates is the cause of their decreasing polarities is wrong since the corresponding keto and acetoxy isomers, where hydrogen bonding is manifestly impossible, show very similar patterns on TLC. The migration characteristics of these and other series of isomeric compounds will be described in a later publication in this series, when it is hoped some rational interpretation of the effects of positional isomerism on TLC behaviour will be proposed.

Natural hydroxy acids, particularly those occurring in vegetable oils, are seldom saturated and, valuable as TLC of the saturated esters may be, some knowledge of the migration characteristics of the esters of naturally occurring unsaturated hydroxy acids is also desirable. Fig. 2 illustrates thin-layer chromatograms of a number of natural and synthetic unsaturated hydroxy esters on silica gel and on silver nitrate impregnated silica gel. Appropriate saturated derivatives have been



(a)



(b)

Fig. 2. Thin-layer chromatograms on Silica Gel G (a) and on 5% w/w silver nitrate-impregnated Silica Gel G (b) of methyl esters of the following hydroxy fatty acids: 1 = 18-OH-octadecanoic; 2 = 18-OH-*cis*-9-octadecenoic; 3 = 17-OH-octadecanoic; 4 = 17-OH-*cis*-9-octadecenoic; 5 = 12-OH-octadecanoic; 6 = 12-OH-*cis*-9-octadecenoic; 7 = 12-OH-*trans*-9-octadecenoic; 8 = 12-OH-octadecynoic; 9 = 9-OH-octadecanoic; 10 = 9-OH-*cis*-12-octadecenoic; 11 = 9-OH-*trans*-10,*trans*-12-octadecadienoic; 12 = 9-OH-*trans*-10,*cis*-12-octadecadienoic; 13 = 13-OH-*cis*-9,-*trans*-11-octadecadienoic; 14 = 8-OH-octadecanoic; 15 = 8-OH-octadec-9-yne-*trans*-11-enoic; 16 = 8-OH-octadeca-9,11-diynoic + 8-OH-octadeca-9,11-diyne-17-enoic (+ their two -13-ene vinylogues). Developing solvent was diethyl ether–light petroleum (3:2), spots were located by spraying with 50% H_2SO_4 and reproduced by photocopying.

included so that the effects of degree and type of unsaturation on mobilities can be assessed.

On unimpregnated silica gel (Fig. 2a), isolated double bonds have little or no effect on mobility relative to the corresponding saturated ester (compare sample 2 with 1, 4 with 3, 7 and 6 with 5 and 10 with 9). Conjugated *cis,trans* unsaturation vicinal to the hydroxyl group has similarly little or no effect (compare sample 12 with 10 and 13 with 5; sample 5 is 12-hydroxy rather than 13-hydroxystearate but the two are nearly identical as shown in Fig. 1). Despite the lack of effect of a vicinal *cis,trans*-diene system a *trans,trans*-dienol is more polar than its saturated analogue and may be resolved also from its *cis,trans*-isomer (compare sample 11 with 10 and 12). Although not shown here we have also demonstrated this for 13-hydroxy-*trans,trans*-9,11-octadecadienoate which is similarly separable from its *cis,trans*-isomer (sample 13). Whereas an isolated triple bond appears to confer slightly increased polarity (compare sample 8 with 6, 5 and 7), triple bonds in conjugated systems adjacent to the hydroxyl do not, and such compounds are apparently slightly less polar than the saturated analogue (compare samples 15 and 16 with 14).

On silver nitrate impregnated silica gel (Fig. 2b) the selective retention of many of the unsaturated hydroxy esters due to π-electron complexing with the silver ions permits further differentiation within this class of compounds. Hydroxy esters with isolated unsaturated linkages are all retained preferentially and are clearly separated from their saturated analogues, but the degree of such retention is not always the same (compare sample 2 with 1, 4 with 3, 6, 7 and 8 with 5 and 10 with 9). Thus the 12-hydroxy-*cis*-9-enoic ester is held back relatively more strongly than the 9-hydroxy-12-enoate and the 18- and 17-hydroxy-9-enoates. In the second part of this series we will show that these differences are due partly to the position of the double bond in the chain, which in the case of unsubstituted monoenoic esters are sufficient to permit separation of isomers such as the 6-, 9- and 11-monoenes[10,11], and partly to the relative positions of the double bond and the hydroxyl and carbomethoxyl groups. The resolution of the saturated, *trans*-monoethenoid and *cis*-monoethenoid 12-hydroxy esters is as satisfactory as that obtained with their unsubstituted analogues and, in addition, the isolated triple bond (sample 8) is shown to complex nearly as strongly as does the *cis*-ethylenic bond (sample 7).

The three conjugated dienols (samples 11, 12 and 13) have all been retarded by complexing, to some extent, the two *cis,trans*-dienols relatively more than their *trans,trans*-isomer (sample 11). The degree of complexing of these compounds has been considerably less, however, than that of comparable hydroxy esters with one isolated *cis*-double bond (samples 7 and 10). This may be due to three factors; delocalisation of the π-electrons in conjugated systems, less favourable conformations for complexing of such systems relative to isolated double bond systems, and steric hindrance toward complexing exerted by the adjacent hydroxyl group.

The hydroxy esters with conjugated unsaturation containing acetylenic bonds (samples 15 and 16) have been retarded hardly at all by complexing except for that fraction of sample 16 which has, in addition, an isolated double bond at the end of the chain. This was, indeed, the first procedure by which this isano oil hydroxy ester fraction could be subfractionated and this separation simplified matters sufficiently for the structures of the four hydroxy polyacetylenic acids in this oil to be determined for the first time[12,13].

Epoxy esters

Fig. 3a illustrates the mobilities on silica gel of three pairs of *cis*- and *trans*-isomeric saturated epoxy esters and of two monounsaturated *cis*-epoxy esters. As with the hydroxy esters, the position of the epoxy group in the chain has a considerable effect on mobility whereas the presence of an isolated double bond (compare

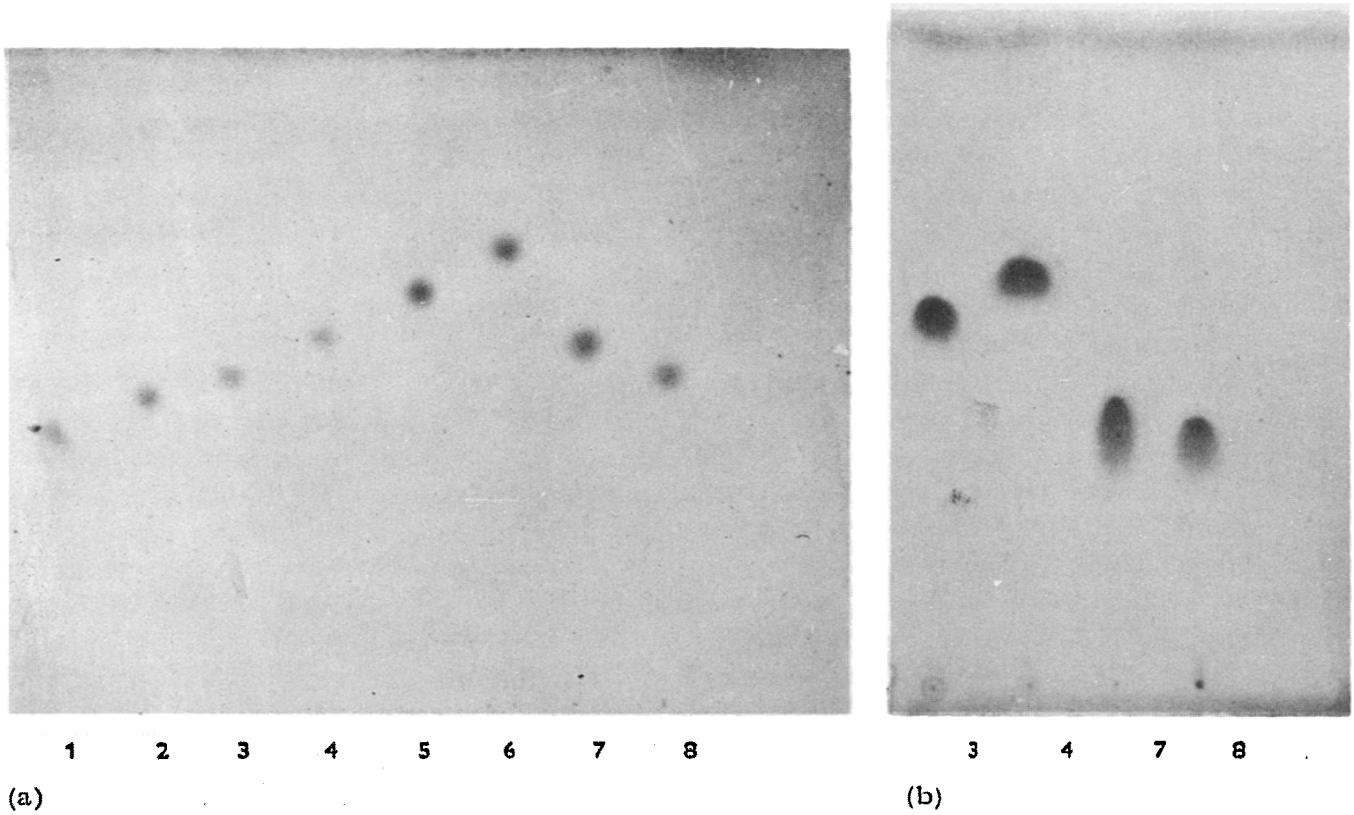


Fig. 3. Thin-layer chromatograms on Silica Gel G (a) and on 5% (w/w) silver nitrate impregnated Silica Gel G (b) of methyl esters of the following acids: 1 = *cis*-6,7-epoxyoctadecanoic; 2 = *trans*-6,7-epoxyoctadecanoic; 3 = *cis*-9,10-epoxyoctadecanoic; 4 = *trans*-9,10-epoxyoctadecanoic; 5 = *cis*-13,14-epoxydocosanoic; 6 = *trans*-13,14-epoxydocosanoic; 7 = *cis*-12,13-epoxy-*cis*-9-octadecenoic; 8 = *cis*-9,10-epoxy-*cis*-12-octadecenoic. Developing solvent was diethyl ether–light petroleum (1:4) and spots were located by spraying with 50% H_2SO_4 and charring.

samples 3 and 8) has none at all. The pattern of migration is the same, considering the four *cis*-epoxy C_{18} esters, with the 6,7-compound least mobile, the 9,10-compounds of intermediate mobility and the 12,13-epoxy ester the most mobile of the series studied.

Besides these effects of positional isomerism on mobilities, geometrical isomerism of epoxy compounds is also shown to have a marked effect. Thus excellent separation of the *cis*- and *trans*-isomers of each pair has occurred, the *trans*-epoxide in each case (samples 2, 4 and 6) being less polar, an observation already made with the 9,10-epoxystearate isomers[14]. This may be the result of the greater inherent polarity of a *cis*-epoxide group coupled to the conformational effect of the more open environment of the *cis*-epoxide which allows a less hindered approach of the group to the surface of the adsorbent than the corresponding *trans*-isomer.

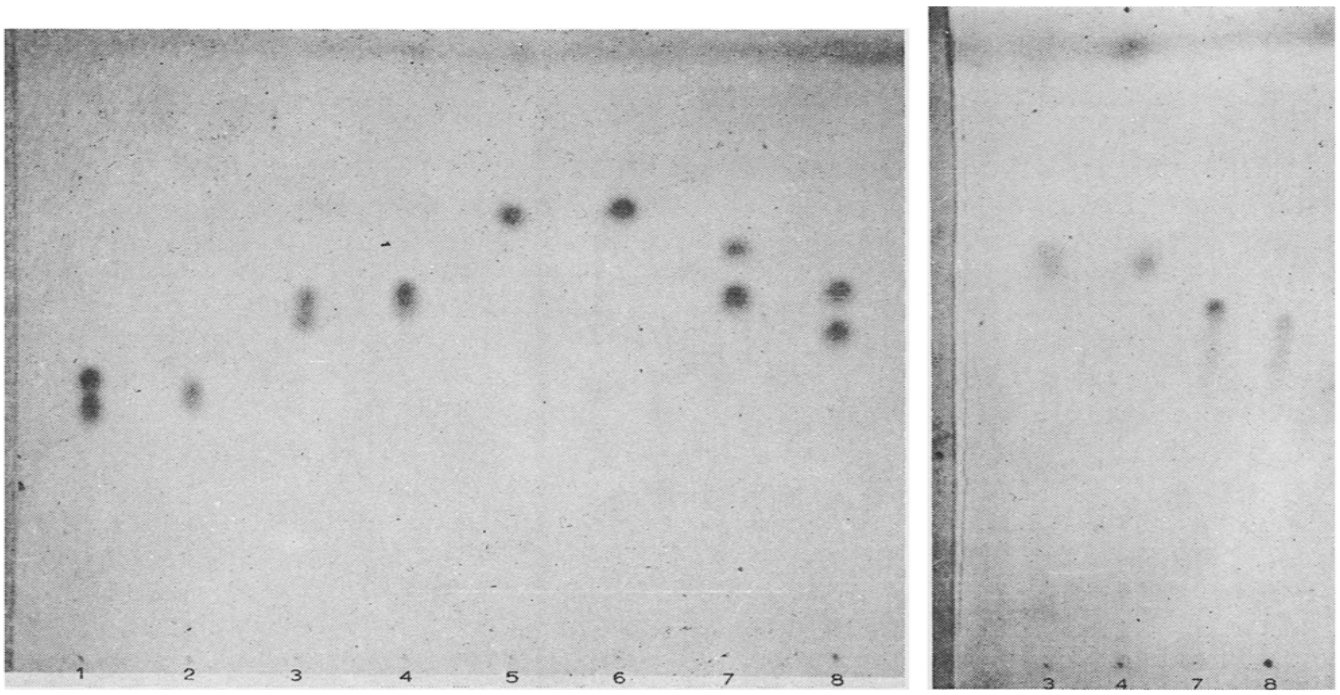
The incorporation of silver nitrate in the adsorbent layer results in specific

retention of the unsaturated epoxy esters so that such compounds can be readily differentiated from their saturated analogues as shown in Fig. 3b. As with the analogous hydroxy esters, the position of the double bond in the chain and relative to the other adsorptive groups has an effect on the degree of such retention. Thus, although the 9,10- and 12,13-epoxy isomers are clearly different in mobilities on silica gel they migrate together on silver nitrate–silica gel.

Halohydroxy esters

One of the simplest and most certain ways of verifying that a given spot on a thin-layer chromatogram corresponds to an epoxy compound is to treat a microsample of the material with anhydrous hydrogen chloride or hydrogen bromide in diethyl ether. Epoxy groups are thereby quantitatively converted to chlorohydrins or bromohydrins and a comparison by TLC of the original and the reacted samples will clearly demonstrate the disappearance of the epoxy component and the appearance of the more polar halohydrins.

The migration characteristics of the bromohydrins derived from the series of epoxy esters described above are illustrated in Fig. 4; the chlorohydrins and iodohydrins behave in an identical manner and cannot be distinguished from analogous bromohydrins by adsorption TLC. Positional isomerism has the same effect on the



(a) (b)

Fig. 4. Thin-layer chromatograms on Silica Gel G (a) and on 5 % (w/w) silver nitrate impregnated Silica Gel G (b) of methyl esters of the following acids: 1 = *threo*-6,7- + -7,6-bromohydroxyoctadecanoic; 2 = *erythro*-6,7- + -7,6-bromohydroxyoctadecanoic; 3 = *threo*-9,10- + -10,9-bromohydroxyoctadecanoic; 4 = *erythro*-9,10- + -10,9-bromohydroxyoctadecanoic; 5 = *threo*-13,14- + -14,13-bromohydroxydocosanoic; 6 = *erythro*-13,14- + -14,13-bromohydroxydocosanoic; 7 = *threo*-12,13- + -13,12-bromohydroxy-*cis*-9-octadecenoic; 8 = *threo*-9,10- + -10,9-bromohydroxy-*cis*-12-octadecenoic. Developing solvent was diethyl ether–light petroleum (3:7) and spots were located by spraying with 50 % H_2SO_4 and charring.

relative mobilities of these compounds as with their parent epoxy esters. Thus the rate of migration is 6,7- < 9,10- < 12,13- for the C_{18} derivatives and these, in turn, all migrate slower than the 13,14-substituted C_{22} esters. With the halohydrins there is no clear differentiation in migration rates between *threo*- and *erythro*-geometric isomers as there was between their parent *cis*- and *trans*-epoxides, although SUBBARAO *et al.*[8] appear to have obtained it between *threo*- and *erythro*-13,14-chlorohydroxydocosanoic acids. Our interpretation of our results is that when the epoxy groups are split to give halohydrins free rotation along the bond between the two substituted carbons is then possible so that the preferred conformations are less different than those of the more rigid epoxides.

The most remarkable aspect of the TLC of bromohydrins, however, is the resolution of each pair of *threo*-positional isomers except for the *threo*-13-bromo-14-hydroxy- and *threo*-14-bromo-13-hydroxydocosanoates. This extremely specific type of separation had been noted previously with the 12(13), 13(12)-chlorohydroxyoleates[4] where some effect of the double bond was considered to be responsible. This explanation, however, is clearly invalidated by the fact of the similar separations of the *threo*-6,7- and the *threo*-9,10-bromohydroxystearate pairs.

On silicic acid impregnated with silver nitrate (Fig. 4b) the monoenoic bromohydrins are again distinguished from their saturated analogues. In this instance there appears to be some interaction between the silver nitrate and the bromohydrin groups as there is some distortion of the spot shapes and the positional isomers of samples 3, 7 and 8 are no longer clearly resolved as on the unimpregnated adsorbent.

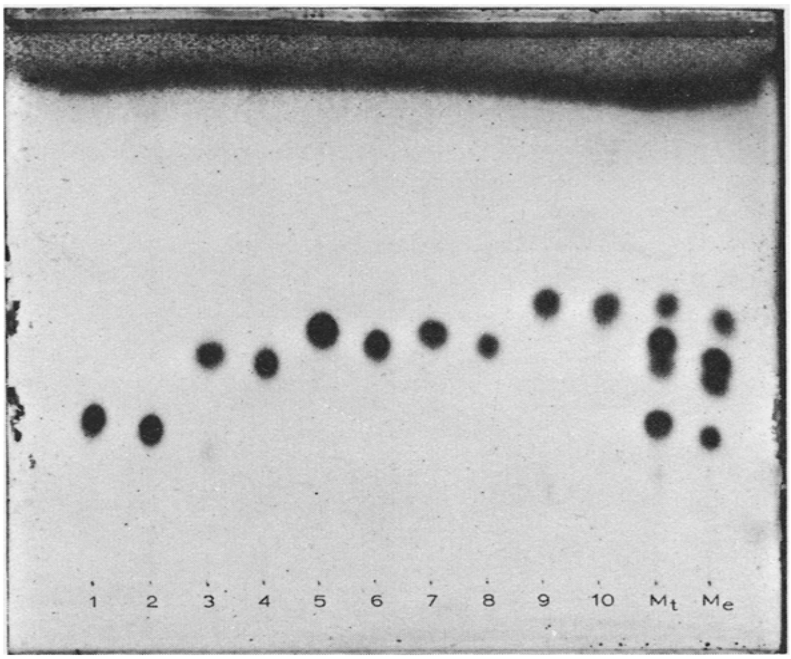


Fig. 5. Thin-layer chromatogram on Silica Gel G of the methyl esters of the following fatty acids: 1 = *threo*-6,7-dihydroxyoctadecanoic; 2 = *erythro*-6,7-dihydroxyoctadecanoic; 3 = *threo*-9,10-dihydroxyoctadecanoic; 4 = *erythro*-9,10-dihydroxyoctadecanoic; 5 = *threo*-12,13-dihydroxyoctadecanoic; 6 = *erythro*-12,13-dihydroxyoctadecanoic; 7 = *threo*-12,13-dihydroxy-*cis*-9-octadecenoic; 8 = *erythro*-12,13-dihydroxy-*cis*-9-octadecenoic; 9 = *threo*-13,14-dihydroxydocosanoic; 10 = *erythro*-13,14-dihydroxydocosanoic; M_t = mixture of 1,3,5 and 9; M_e = mixture of 2,4,6 and 10. Developing solvent was diethyl ether–light petroleum (4:1) and spots were located by spraying with 50% H_2SO_4 and charring.

Dihydroxy esters

The effect of positional isomerism on mobilities is again evidenced by the chromatogram of vicinal dihydroxy esters illustrated in Fig. 5. The pattern of migration is the same as that shown by the monohydroxy, epoxy and halohydroxy analogues of these esters. The differences in mobilities between the isomers either of the *threo*- or of the *erythro*-series of esters shown are sufficient to allow their separation from mixtures of all four (samples M_t and M_e). Since *erythro*-diols are readily and quantitatively prepared by dilute alkaline permanganate oxidation of *cis*-monoenoic acids, this provides a procedure for the separation of some isomeric monoenes from each other. The facile cleavage of such diols either to aldehydic fragments with periodic acid or with lead tetra-acetate or to acidic fragments with permanganate–periodate then allows unequivocal identification of natural monoenes so separated.

Geometrical isomerism has only a marginal effect on the relative mobilities of *erythro*- and *threo*-dihydroxy esters which is, in our hands at least, not sufficient to allow separation of the mixture of a pair of geometrically isomeric esters. SGOUTAS AND KUMMEROW[15] and SUBBARAO *et al.*[8] have apparently achieved separations of isomeric pairs of dihydroxy acids but, in the latter case at least, we consider this to be a result of solubility differences rather than differences in polarity. Substantial differences in mobilities of *threo*- and *erythro*-dihydroxy isomers, however, are obtained by chromatography on silicic acid impregnated with boric acid, sodium borate or sodium arsenite[5,16].

Incorporation of silver nitrate in the layer permits the separation of unsaturated dihydroxy esters from their saturated analogues as with the other series of compounds described here. For even greater selectivity, double impregnation of the silicic acid with both silver nitrate and boric acid permits simultaneous resolution of saturated and unsaturated *threo*- and *erythro*-diols[5].

DISCUSSION

The fact that positional and/or geometrical isomerism could have appreciable effects on migration characteristics on TLC of substituted aliphatic compounds was apparent from previous reports of separations of a few isomers by ourselves[3–5,12,14,16] and by others[8,9,15,17]. The present work, on a large number of isomers of different types, indicates more fully than hitherto the highly selective separations which are possible even on unimpregnated adsorbent layers. This selectivity is on the basis of positional isomerism in most of the series of compounds studied but, in suitable cases, geometrical isomerism of functional groupings is also an important contributory factor. The only complete series of isomers described is the monohydroxystearate series but the patterns of migration of those isomers of the epoxy, halohydroxy and dihydroxy esters which were available parallel that of the hydroxy series. The indication is that approximately the same pattern will be obtained with the isomers of all classes of substituted long-chain derivatives. This we are finding with the isomeric series of keto-, hydroxy- and acetoxy-C_{18} and C_{20} esters and the corresponding alkanediols and diacetates and also with the isomeric C_{18}-monoenoic esters on silver nitrate–silicic acid. The results of these investigations will be the subject of subsequent publications in this journal. Attempts to interpret the factors giving rise

to these variations in the mobilities of isomeric compounds will be deferred until these studies are complete.

These variations in mobilities of isomers are sufficient to allow separation and at least tentative identification of those epoxy esters which are known or may be surmised to occur naturally and of the halohydrins or diols which may be derived from them. The possibility also exists, as indicated above, of separating the commoner naturally occurring isomeric monoenoic acids via their dihydroxy derivatives. A much wider range of monohydroxy C_{18}-esters, namely the 18-, 17-, 13-, 12-, 10-, 9-, 8-, 3- and 2-hydroxy isomers, are known to occur naturally although generally as unsaturated compounds. From the separation pattern of hydroxystearates shown in Figs. 1 and 2 it is clear that TLC may be of considerable value in preliminary identification of such compounds, particularly when by argentation-TLC some indication of the degree and type of unsaturation of the natural unsaturated acid may be obtained.

It has recently been found[18–20] that positionally isomeric hydroxy esters and related compounds exhibit differences in retention volumes on gas–liquid chromatography which are sufficient to allow identification of thirteen of the seventeen possible hydroxystearates[18]. TLC provides a simpler and more rapid procedure but there are clearly instances when the complementary use of these two microanalytical procedures will be necessary for unequivocal identification. GLC is less suitable than TLC and argentation-TLC as a general procedure for characterisation of unsaturated hydroxy esters because of the propensity of some of these compounds to degrade during chromatography at high temperatures[21], a fact which may sometimes be turned to advantage as a further indication of structure[3,13].

These studies by GLC, by TLC and by argentation-TLC may be carried out on only microgram quantities of material so that the main portion of a naturally occurring acid available in possibly very limited amount may be reserved for the final verification of structure by chemical degradation. They are, therefore, of considerable importance in structural investigations and may be expected to be utilised more frequently in the future.

SUMMARY

The migration behaviour on thin-layer chromatograms of a large number of isomeric hydroxy, epoxy, bromohydroxy and vicinal dihydroxy substituted long-chain fatty acids is described. Considerable and systematic variations in mobilities of positional isomers are apparent and geometrically isomeric epoxy and bromohydroxy compounds have differing migration rates. On unimpregnated silica gel the presence of unsaturated groupings seldom affects the migration of these compounds relative to their saturated analogues but incorporation of silver nitrate in the layer permits their differentiation according to the degree and type of unsaturation present. The procedures described are of great value in structural identification of oxygenated fatty acids isolated from natural sources, particularly when used in conjunction with other separatory and analytical techniques.

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