

SEPARATION OF OXYGENATED FATTY COMPOUNDS BY  
THIN-LAYER CHROMATOGRAPHY

R. SUBBARAO, M. W. ROOMI, M. R. SUBBARAM AND K. T. ACHAYA

*Regional Research Laboratory,  
Hyderabad (India)*

(Received March 26th, 1962)

Since thin-layer chromatography (TLC) was evolved by KIRCHNER *et al.*<sup>1</sup> in 1951, its simplicity, quickness and efficiency have led to widespread use of this technique. In the field of fats, MANGOLD AND MALINS<sup>2</sup> applied TLC for the separation and identification of natural lipid components such as hydrocarbons, triglycerides, sterols, fatty acids, etc. MORRIS, HOLMAN AND FONTELL<sup>3</sup> used TLC in conjunction with other methods of separation for the identification of naturally occurring epoxy acids. Mono-, di-, and triglycerides in a mixture were resolved by TLC using various mixtures of ether and petroleum ether for development<sup>4</sup>. MANGOLD<sup>5</sup> has recently reviewed comprehensively the applications of TLC to the separation of lipids.

A number of mono- and polyhydroxy compounds based on ricinoleic and undecylenic acids and the corresponding alcohols, have been prepared by SUBBARAO AND ACHAYA<sup>6,7</sup> for utilization purposes. ROOMI AND SUBBARAM<sup>8</sup> have prepared a series of epoxy, dihydroxy and halohydroxy derivatives, which occur as intermediates in stereochemical transformations, starting from erucic acid and its *trans* isomer brassidic acid. The application of TLC to the separation of these well-defined and pure compounds is described in this paper. These studies have enabled us to make some useful generalizations regarding the effect on the mobility during TLC of the number and position of hydroxy groups, chain length, presence of epoxy and halohydroxy group, stereochemical configuration and degree of saturation.

## MATERIALS AND METHODS

Table I lists the compounds used, their starting materials and method of preparation. The reduction of esters with sodium was carried out according to HANSLEY'S procedure<sup>9</sup>. Hydroxylation to yield *threo* products, and epoxidation with peracetic acid, were performed according to the methods described by SWERN and co-workers<sup>10,11</sup>. Alkaline permanganate oxidation<sup>12</sup> was used to prepare the *erythro* dihydroxy isomers used. Hypohalogenation was conducted according to KING<sup>13</sup>.

Using a thin-layer applicator (Desaga, Heidelberg), glass plates (20 × 20 cm) were coated with a well-stirred suspension of silica gel G (E. Merck, Darmstadt; 30 g in 60 ml water) to give a layer approximately 270  $\mu$  in thickness. The plates were dried at 105–110° for 30 min and preserved in a desiccator until required. The compounds were dissolved in methanol and 10  $\mu$ g was applied with a micropipette on starting points 2–3 cm from the end of the plate. The plate was then placed inside the chamber

TABLE I  
COMPOUNDS USED IN THE EXPERIMENTS,  
THEIR STARTING MATERIALS AND METHOD OF PREPARATION

No.	Compound used and method of preparation	Prepared from
<i>A. Reduction with sodium</i>		
1.	Undecenyl alcohol	Methyl undecylenate
2.	Oleyl alcohol	Methyl oleate
3.	Ricinoleyl alcohol	Methyl ricinoleate
<i>B. Hydroxylation</i>		
<i>(i) with peracetic acid</i>		
4.	<i>threo</i> -9,10-Dihydroxystearic acid	Oleic acid
5.	<i>threo</i> -13,14-Dihydroxydocosanoic acid	Erucic acid
6.	1,10,11-Trihydroxyundecane	Undecenyl alcohol
7.	1,9,10-Trihydroxyoctadecane	Oleyl alcohol
8.	1,9,10,12-Tetrahydroxyoctadecane	Ricinoleyl alcohol
<i>(ii) with cold alkaline permanganate</i>		
9.	<i>erythro</i> -9,10-Dihydroxystearic acid	Oleic acid
10.	<i>erythro</i> -13,14-Dihydroxydocosanoic acid	Erucic acid
<i>C. Formoxylation</i>		
11.	1,10-Dihydroxyundecane	Undecenyl alcohol
12.	1,9(10)-Dihydroxyoctadecane	Oleyl alcohol
<i>D. Esterification</i>		
13.	Methyl <i>threo</i> -9,10-dihydroxystearate	<i>threo</i> -9,10-Dihydroxystearic acid
<i>E. Hydrogenation</i>		
14.	Stearyl alcohol	Oleyl alcohol
15.	1,12-Dihydroxyoctadecane	Ricinoleyl alcohol
16.	Methyl 12-hydroxystearate	Methyl ricinoleate
<i>F. Epoxidation</i>		
17.	<i>cis</i> -9,10-Epoxystearic acid	Oleic acid
18.	Methyl <i>cis</i> -9,10-epoxystearate	Methyl oleate
19.	<i>cis</i> -9,10-Epoxystearyl alcohol	Oleyl alcohol
20.	<i>cis</i> -13,14-Epoxydocosanoic acid	Erucic acid
21.	<i>trans</i> -13,14-Epoxydocosanoic acid	Brassicic acid
<i>G. Hydrohalogenation</i>		
22.	<i>threo</i> -13,14-Chlorohydroxydocosanoic acid	<i>cis</i> -13,14-Epoxydocosanoic acid
23.	<i>erythro</i> -13,14-Chlorohydroxydocosanoic acid	<i>trans</i> -13,14-Epoxydocosanoic acid

for development. The solvent systems consisted of mixtures of ether and petroleum ether (40-60°) in varying proportions (by volume); for the development of fatty acids, the solvent system contained in addition 1% of glacial acetic acid to avoid smearing. Only the proportion of ether in the system is mentioned subsequently. Usually 40-60 min were required for the solvent front to cover a distance of 15 cm. The plates were then sprayed with 50% (v/v) sulphuric acid and kept for 15 min at 105-110°. The organic compounds showed up as brown spots on a white background. The separations were reproducible without difficulty.

## RESULTS AND DISCUSSION

The three sections in Figs. 1 and 2 each depict a single run. For purposes of comparison, the same compound was sometimes included in more than one TLC run.

Fig. 1B shows that a clear separation of mono-, di-, tri- and tetrahydroxyoctadecanes could be achieved by means of 80% ether in petroleum ether. This system was selected after trying a 10% ether system, which caused only the mono-hydroxy compounds to move, and a 30% ether system with which only dihydroxy compounds moved from the starting point. In a later trial (item *q*, Fig. 1C), tetrahydroxyoctadecane separated into two discrete spots when developed with a 90% ether system. Our subsequent work suggests that this may be caused by the presence of both *erythro* and *threo* components.

Separation of a  $C_{11}$  series containing one, two and three hydroxy groups could also be achieved with the 80% ether system. The  $R_F$  value of an undecane was lower

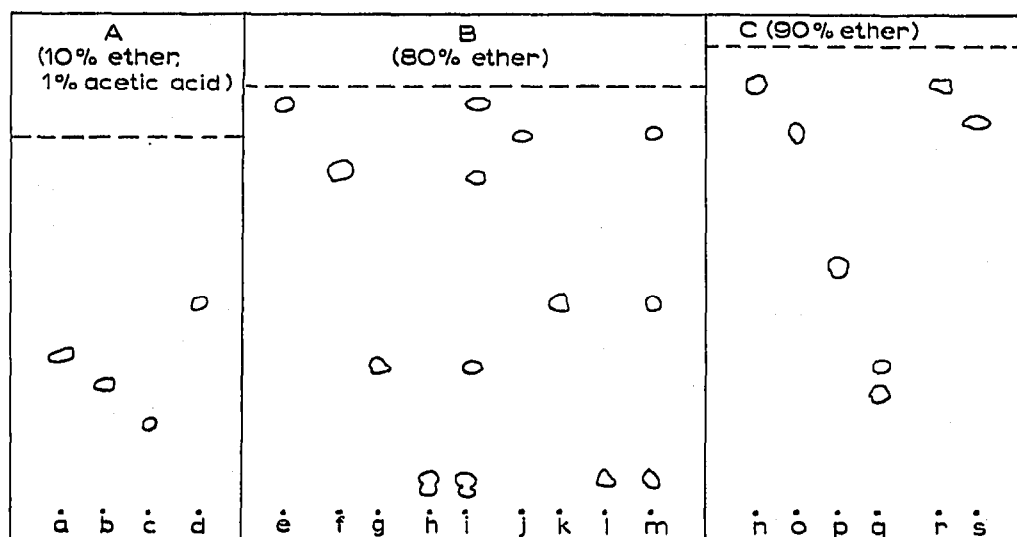


Fig. 1. Separation by thin-layer chromatography of oxygenated derivatives of fatty acids, esters and alcohols, using 10%, 80% and 90% ether-petroleum ether systems (--- solvent front).

		$R_F \times 100$
<i>a</i>	<i>threo</i> -13,14-Chlorohydroxydocosanoic acid	42
<i>b</i>	<i>erythro</i> -13,14-Chlorohydroxydocosanoic acid	34
<i>c</i>	Methyl 12-hydroxystearate	24
<i>d</i>	<i>cis</i> -13,14-Epoxydocosanoic acid	56
<i>e</i>	Stearyl alcohol	96
<i>f</i>	1,12-Dihydroxyoctadecane	81
<i>g</i>	1,9,10-Trihydroxyoctadecane	35
<i>h</i>	1,9,10,12-Tetrahydroxyoctadecane	07
<i>i</i>	Mixture of <i>e</i> , <i>f</i> , <i>g</i> and <i>h</i>	96, 81, 35, 07
<i>j</i>	Undecenyl alcohol	89
<i>k</i>	1,10-Dihydroxyundecane	49
<i>l</i>	1,10,11-Trihydroxyundecane	08
<i>m</i>	Mixture of <i>f</i> , <i>k</i> and <i>l</i>	89, 49, 08
<i>n</i>	Stearyl alcohol	93
<i>o</i>	1,12-Dihydroxyoctadecane	82
<i>p</i>	1,9,10-Trihydroxyoctadecane	53
<i>q</i>	1,9,10,12-Tetrahydroxyoctadecane	34, 26
<i>r</i>	Oleyl alcohol	93
<i>s</i>	Ricinoleyl alcohol	83

than that of an octadecane containing the same number of hydroxy groups; trihydroxyundecane happened to have an  $R_F$  value similar to that of tetrahydroxyoctadecane (Fig. 1B). Small differences in chain length were not distinguishable, e.g. cetyl alcohol was inseparable from stearyl alcohol (item *c*, Fig. 2A).

Unsaturation had no perceptible effect on the mobility of hydroxy compounds; oleyl alcohol and stearyl alcohol had the same  $R_F$  value (items *r* and *n*, Fig. 1C).

The presence of the hydroxy group in the middle or at the end of a chain, i.e. whether the alcohol is secondary or primary, made little difference to the  $R_F$  value, as shown by the similar mobilities of (a) methyl 12-hydroxystearate and stearyl alco-

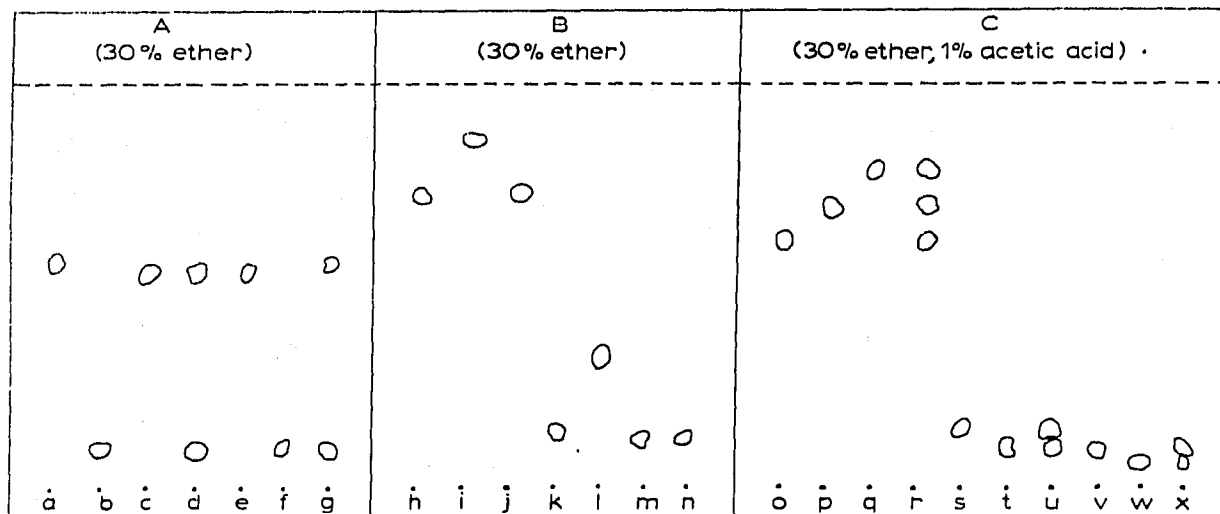


Fig. 2. Separation by thin-layer chromatography of oxygenated derivatives of fatty acids, esters and alcohols, using a 30% ether-petroleum ether system (--- solvent front).

	$R_F \times 100$
<i>a</i> Oleyl alcohol	55
<i>b</i> Ricinoleyl alcohol	10
<i>c</i> Mixture of 35% cetyl and 65% stearyl alcohols	52
<i>d</i> Mixture of <i>a</i> , <i>b</i> and <i>c</i>	52, 10
<i>e</i> Methyl 12-hydroxystearate	52
<i>f</i> Methyl <i>threo</i> -9,10-dihydroxystearate	10
<i>g</i> Mixture of <i>e</i> and <i>f</i>	52, 10
<i>h</i> Stearyl alcohol	72
<i>i</i> Methyl <i>cis</i> -9,10-epoxystearate	86
<i>j</i> Methyl 12-hydroxystearate	72
<i>k</i> 1,12-Dihydroxyoctadecane	12
<i>l</i> <i>cis</i> -9,10-Epoxystearylalcohol	32
<i>m</i> Methyl <i>threo</i> -9,10-dihydroxystearate	11
<i>n</i> 1,9(10)-Dihydroxyoctadecane	11
<i>o</i> <i>cis</i> -9,10-Epoxystearic acid	61
<i>p</i> <i>cis</i> -13,14-Epoxydocosanoic acid	69
<i>q</i> <i>trans</i> -13,14-Epoxydocosanoic acid	79
<i>r</i> Mixture of <i>o</i> , <i>p</i> and <i>q</i>	61, 69, 79
<i>s</i> <i>threo</i> -13,14-Dihydroxydocosanoic acid	14
<i>t</i> <i>erythro</i> -13,14-Dihydroxydocosanoic acid	09
<i>u</i> Mixture of <i>s</i> and <i>t</i>	14, 09
<i>v</i> <i>threo</i> -9,10-Dihydroxystearic acid	09
<i>w</i> <i>erythro</i> -9,10-Dihydroxystearic acid	06
<i>x</i> Mixture of <i>v</i> and <i>w</i>	09, 06

hol (items *j* and *h*, Fig. 2B), (b) ricinoleyl alcohol and methyl 9,10-dihydroxystearate (items *b* and *f*, Fig. 2A), and (c) 1,9 (10)-dihydroxyoctadecane and methyl *threo*-9,10-dihydroxystearate (items *n* and *m*, Fig. 2B).

One epoxy group conferred greater mobility, and hence a higher  $R_F$  value, than one hydroxy group. Thus *cis*-9,10-epoxystearyl alcohol, carrying one epoxy and one hydroxyl group, had a higher  $R_F$  value than either 1,12-dihydroxyoctadecane or methyl *threo*-9,10-dihydroxystearate, which both contain two hydroxyl groups (items *l*, *k* and *m*, Fig. 2B).

The separation of *cis*- from *trans*-13,14-epoxydocosanoic acid was achieved with 30% ether in petroleum ether containing 1% acetic acid. A mixture of *cis*-9,10-epoxystearic acid, *cis*-13,14-epoxydocosanoic acid and *trans*-13,14-epoxydocosanoic acid was clearly resolved into the three components (item *r*, Fig. 2C). *threo*-13,14-Dihydroxydocosanoic acid was separable from the corresponding *erythro* acid, which had a lower  $R_F$  value. Similarly, resolution was achieved of *threo*- and *erythro*-9,10-dihydroxystearic acids (items *s*, *t* and *v*, *w*, Fig. 2C). 13,14-Dihydroxydocosanoic acid had an  $R_F$  value sufficiently different from that of 9,10-dihydroxystearic acid to distinguish the two compounds; in analogy to other separations mentioned in this paper, a solvent system containing a higher proportion of ether will undoubtedly result in further movement and sharper resolution.

Epoxy compounds moved faster than the halohydrins derived from them, and in turn, these halohydrins had a higher  $R_F$  value than the monohydroxy compounds (Fig. 1A). Among the halohydrins, the *threo* derivatives had a slightly higher  $R_F$  value than the corresponding *erythro* derivatives. 13,14-Chlorohydroxy-, 13,14-bromohydroxy- and 13,14-iodohydroxydocosanoic acids, of which the chromatograms are not shown, could not be distinguished from each other by TLC.

TLC therefore offers an excellent method for the rapid identification and separation of compounds of the types described above.

#### ACKNOWLEDGEMENTS

It is a pleasure to thank Dr. G. S. SIDHU and his colleagues, Miss A. BHRAMARAMBA and Mr. MOHD. SWALEH, for help in using the TLC technique.

#### SUMMARY

Thin-layer chromatography was applied to the separation of various types of fatty compounds, using mixtures of ether and petroleum ether, of varying proportions as solvent systems. The following separations were obtained (the values in parentheses indicate the proportion of ether in the solvent system): (a) compounds of the same chain length differing in the number of hydroxy groups (80%); (b) compounds differing in chain length by about four or more carbon atoms (80%); (c) compounds with an epoxy group from the corresponding compound with a hydroxy group or a halohydroxy group; and (d) *cis* isomers from *trans* isomers (30%).

Thin-layer chromatography could not be used to separate (a) compounds differing only in degree of saturation; (b) compounds with primary hydroxyl groups from similar compounds with secondary hydroxyl groups; (c) chloro-, bromo- and iodohydrins from each other.

## REFERENCES

- <sup>1</sup> J. G. KIRCHNER, J. M. MILLER AND G. J. KELLER, *Anal. Chem.*, 23 (1951) 420; see also: E. STAHL, *Pharmazie*, 11 (1956) 633.
- <sup>2</sup> H. K. MANGOLD AND E. C. MALINS, *J. Am. Oil Chemists' Soc.*, 37 (1960) 383.
- <sup>3</sup> L. J. MORRIS, R. T. HOLMAN AND K. FONTELL, *J. Lipid Research*, 2 (1961) 68.
- <sup>4</sup> O. S. PRIVETT, M. L. BLANK AND W. O. LUNDBERG, *J. Am. Oil Chemists' Soc.*, 38 (1961) 312.
- <sup>5</sup> H. K. MANGOLD, *J. Am. Oil Chemists' Soc.*, 38 (1961) 708.
- <sup>6</sup> R. SUBBARAO AND K. T. ACHAYA, *J. Sci. Ind. Research (India)*, 19B (1960) 482.
- <sup>7</sup> R. SUBBARAO AND K. T. ACHAYA, *J. Sci. Ind. Research (India)*, in the press.
- <sup>8</sup> M. W. ROOMI AND M. R. SUBBARAM, *J. Sci. Ind. Research (India)*, in the press.
- <sup>9</sup> V. L. HANSLEY, *Ind. Eng. Chem.*, 39 (1947) 55.
- <sup>10</sup> D. SWERN, G. N. BILLEN, T. W. FINDLEY AND J. T. SCANLAN, *J. Am. Chem. Soc.*, 67 (1945) 1786.
- <sup>11</sup> T. W. FINDLEY, D. SWERN AND J. T. SCANLAN, *J. Am. Chem. Soc.*, 67 (1945) 412.
- <sup>12</sup> A. LAPWORTH AND E. N. MOTTRAM, *J. Chem. Soc.*, (1925) 1628.
- <sup>13</sup> G. KING, *J. Chem. Soc.*, (1949) 1817.

*J. Chromatog.*, 9 (1962) 295-300