SEPARATION OF EPOXY, HYDROXY, HALOHYDROXY AND KETO FATTY ACIDS AND DERIVATIVES BY THIN-LAYER CHROMATOGRAPHY*

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Separation by thin-layer chromatography (TLC) of oxygenated fatty acid derivatives has been reported by SUBBARAO *et al.*[1,2], MORRIS[3–5], SGOUTAS AND KUMMEROW[6] and KAUFMANN AND KO[7]. The use of glycol complexing agents such as boric acid, sodium borate and sodium arsenite for the separation of *erythro* and *threo* isomers of di- and tetrahydroxy fatty acids was first reported by MORRIS[3]. In later work[4,5], he coupled this elegant technique with the use of silver nitrate as an ethenoid complexing agent[3,8,9] to separate methyl esters of various positional and geometrical isomers of oxygenated fatty acids differing in degree of unsaturation. In this paper, the TLC behaviour of a number of hydroxy, epoxy, halohydroxy and keto fatty acids, esters and alcohols prepared as earlier reported[10–13], using direct (D), reversed-phase (RP) and boric acid-coated (B) silica gel plates is described.

MATERIALS AND METHODS

The various epoxy fatty acids, esters and alcohols used in this study were prepared according to the method of FINDLEY *et al.*[14]. *Erythro* and *threo* acids were prepared by hydroxylation of *cis* unsaturated acids with alkaline potassium permanganate[15] and performic acid[16] and the esters were prepared by the usual procedure with methanol and sulphuric acid. *Threo*- and *erythro*-hydroxyacetoxy compounds were prepared by refluxing *cis*- and *trans*-epoxy compounds with acetic acid while the diacetoxy fatty acids were obtained by treating the dihydroxy acids with acetic anhydride and pyridine. *Erythro*- and *threo*-2,3-dihydroxydocosanoic acids were prepared by the method of ROOMI *et al.*[13]. Halohydroxy compounds were prepared according to the method of KING[17]. 9(10)-Hydroxyoctadecanoic and 13(14)-hydroxydocosanoic acids were obtained by catalytic hydrogenation of *cis*-9,10-epoxystearic and 13,14-epoxydocosanoic acids[11] and the 9(10)-ketostearic acid by chromium trioxide oxidation of the 9(10)-hydroxy acid[18]. Both the 9-hydroxy-10-keto and 10-hydroxy-9-ketostearic acids were prepared by neutral permanganate oxidation of oleic acid by KING's method[19]. Permanganate oxidation of 9,10-stearolic acid[20] gave the 9,10-diketostearic acid. Reduction of esters using lithium aluminium hydride in tetrahydrofuran gave the corresponding alcohols.

The direct TLC procedure in use in this laboratory, employing Desaga equipment, 275-μ layers of silica gel G, and ether–light petroleum for development, has

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been described earlier[1]. For reversed-phase TLC, the dried, coated plate was uniformly impregnated with silicone oil (Dow Corning silicone fluid 200) by allowing a 5 % solution in ether to ascend the plate in a developing chamber[21]. Both for acids and esters, only one solvent system was used for reversed-phase TLC, *viz.* acetonitrile–acetic acid–water (70:10:20, v/v). Impregnation with boric acid (2.8 g/25 g silica gel) was carried out by the method of MORRIS[4], and ether–light petroleum mixtures were used for development.

RESULTS AND DISCUSSION

Epoxy compounds

The separation of epoxy acids, esters and alcohols by direct and reversed-phase TLC is indicated in Table I. By DTLC *cis*-epoxy compounds are separated from their *trans* isomers. The monoepoxy has a higher mobility than the corresponding diepoxy compound. Positional isomers of the same carbon chain length are separable (*cis*-6,7- from *cis*-9,10-), and also epoxy compounds of different chain lengths (18 from 22). Esters move faster than either acids or alcohols. By RPTLC also, separations are possible on the basis both of chain length and the number of epoxy groups in the chain.

TABLE I

SEPARATION OF EPOXY ACIDS, ESTERS AND ALCOHOLS BY TLC

R_F values × 100.
D = direct, RP = reversed-phase.

Compound	*Carbon chain length*	*Acids*		*Esters*		*Alcohols*	
		D[a]	*RP*[b]	*D*[a]	*RP*[b]	*D*[a]	*RP*[b]
cis-6,7-Epoxystearic acid	18	41	76	48	50	—	—
trans-6,7-Epoxystearic acid	18	44	76	54	50	—	—
cis-9,10-Epoxystearic acid	18	45	77	54	51	30	71
trans-9,10-Epoxystearic acid	18	48	77	60	51	34	70
cis,cis-9,10,12,13-Diepoxystearic acid	18	32	96	3	81	—	—
cis-13,14-Epoxydocosanoic acid	22	48	64	72	33	42	63
trans-13,14-Epoxydocosanoic acid	22	58	64	80	33	46	63

[a] Ether in light petroleum (30:70, v/v).
[b] Acetonitrile–acetic acid–water (70:10:20, v/v).

Hydroxy acids, esters and alcohols

(a) *Monohydroxy acids.* By DTLC using 30 % ether in light petroleum, 2-hydroxydocosanoic acid was separated from the 13(14)-hydroxy isomer (Table II). MORRIS[5] showed for hydroxystearic esters that two similar maxima occur for the 12(13)-hydroxy and the 2-hydroxy isomers respectively. In the present instance the 2-hydroxydocosanoic acid moves faster than the 13-hydroxy isomer (R_F × 100, 96 and 85 respectively). 6(7)- and 9(10)-Hydroxyoctadecanoic acids and esters are separable from each other, the 6-hydroxy isomer having a lower R_F value. Close positional isomers (6- and 7-, 9- and 10-hydroxy) can be well separated, confirming

TABLE II

SEPARATION OF MONOHYDROXY ACIDS AND ESTERS BY TLC

R_F values × 100.
D = direct.

Compounds	*Acids*	*Esters*
	D[a]	*D*[b]
6(7)-Hydroxyoctadecanoic acid	62, 57	27, 22
9(10)-Hydroxyoctadecanoic acid	71, 66	36, 30
2-Hydroxydocosanoic acid	96	93
13(14)-Hydroxydocosanoic acid	85	78

[a] Ether–light petroleum (30:70, v/v).
[b] Ether–light petroleum (20:80, v/v).

an earlier report[2]. Separation is also effected according to chain length, *e.g.* C_{18} from C_{22}, the latter having greater mobility.

(b) *Dihydroxy acids, esters and alcohols.* Table III shows the separation of dihydroxy compounds by D-, RP- and BTLC. As with epoxy compounds, RPTLC separates according to carbon chain length and number of hydroxy groups, and is ineffective in separating stereoisomers. DTLC on the other hand shows slight separation of *erythro* and *threo* isomers. Incorporation of boric acid as now well established causes the *threo* isomer to move considerably faster than the *erythro*. Positional dihydroxy isomers (6,7- and 9,10-dihydroxyoctadecanoic acids and 2,3- and 13,14-dihydroxydocosanoic acids) are separable by DTLC and BTLC but not by RPTLC. The behaviour of 2,3-dihydroxydocosanoic acids is anomalous. By analogy with the

TABLE III

SEPARATION OF DI- AND TETRAHYDROXY ACIDS, ESTERS AND ALCOHOLS BY TLC

R_F values × 100.
D = direct, B = boric acid-coated, RP = reversed-phase.

	Acids			*Esters*			*Alcohols*	
	D[a]	*B*[b]	*RP*[c]	*D*[a]	*B*[b]	*RP*[c]	*D*[d]	*B*[d]
threo-6,7-Dihydroxystearic acid	8	42	84	24	55	78	—	—
erythro-6,7-Dihydroxystearic acid	5	30	84	20	29	78	—	—
threo-9,10-Dihydroxystearic acid	14	48	85	33	60	78	—	—
erythro-9,10-Dihydroxystearic acid	10	35	85	29	33	78	—	—
threo,threo-9,10,12,13-Tetrahydroxystearic acid	0	10	94	0	17	87	—	—
erythro,erythro-9,10,12,13-Tetrahydroxystearic acid	0	5	94	0	10	87	—	—
threo-2,3-Dihydroxydocosanoic acid	0	0	—	60	64	—	23	45
erythro-2,3-Dihydroxydocosanoic acid	0	0	—	55	56	—	22	44
threo-13,14-Dihydroxydocosanoic acid	25	61	72	46	84	64	55	53
erythro-13,14-Dihydroxydocosanoic acid	22	48	72	39	43	64	54	47

[a] Ether–light petroleum (50:50, v/v).
[b] Ether–light petroleum (40:60, v/v).
[c] Acetonitrile–acetic acid–water (70:10:20, v/v).
[d] Ether–light petroleum (70:30, v/v).

2-monohydroxy acid, the 2,3-dihydroxy acids were expected to move faster than the corresponding 13,14-isomers. However, both the *erythro*- and *threo*-2,3-dihydroxydocosanoic acids did not move from the starting line even when pure ether or methanol was used as solvent. As expected, however, esters of 2,3-dihydroxydocosanoic acids moved farther than the corresponding 13,14-isomers on DTLC. *Erythro* and *threo* isomers were well separated on a boric acid-coated plate. In this instance the 2,3-dihydroxy esters had a lower mobility than the 13,14-isomer.

The 1,2,3- and 1,13,14-docosanetriols were separable both by DTLC and BTLC. Using BTLC, the *erythro* and *threo* isomers of 1,13,14-docosanetriols were separated from each other but not the isomers of 1,2,3-docosanetriols.

A dihydroxy compound can be separated from a tetrahydroxy compound by all three methods. Ester separations were superior, but the actual resolution of acids recorded could be further improved by increasing the volume of ether in the solvent system.

Some aspects of separation of *erythro*- and *threo*-dihydroxy fatty acids and their esters have been discussed by MORRIS[4]. Similar separation of carbohydrates has been attributed to ease of dehydration to form a five-membered acidic complex[22]. When one or both hydroxy groups were acetylated, TLC separation on boric acid plates no longer occurred (Table IV) probably because of the absence of protons from vicinal hydroxy groups necessary for complex formation.

TABLE IV

SEPARATION OF DIHYDROXY, HYDROXYACETOXY AND DIACETOXY ACIDS BY TLC

R_F values × 100.
D = direct, B = boric acid-coated.

Compound	D^a	B^a
threo-9,10-Dihydroxystearic acid	7	34
erythro-9,10-Dihydroxystearic acid	3	28
threo-9(10)-Hydroxy-10(9)-acetoxystearic acid	35, 26	43, 50
erythro-9(10)-Hydroxy-10(9)-acetoxystearic acid	35, 26	43, 50
threo-9,10-Diacetoxystearic acid	52	74
erythro-9,10-Diacetoxystearic acid	52	74

[a] Ether–light petroleum (30:70, v/v).

The diacetoxy acids moved faster than the others, followed by the hydroxyacetoxy and the dihydroxy acids considerably behind. On opening a 9,10-epoxy ring with acetic acid, the two isomers obtained, *viz.* 9-hydroxy-10-acetoxy and 9-acetoxy-10-hydroxy, are well separated by TLC, the spot intensity suggesting roughly equal proportions of the isomers, as during other ring openings of epoxy fatty acids[11].

Halohydroxy acids and esters

The separation of close positional isomers is also possible for halohydroxy acids (Table V). Separation both of positional isomers, *e.g.* 6,7- and 9,10-chlorohydroxystearic acids and by chain length, *e.g.* 9,10-chlorohydroxystearic from 13,14-chlorohydroxydocosanoic acid is possible. Slight separation of even close positional isomers such as 9-chloro-10-hydroxy and 10-chloro-9-hydroxystearic acids is possible.

TABLE V

SEPARATION OF HALOHYDROXY ACIDS AND ESTERS BY TLC

R_F values × 100.
D = direct.

Compound	*Acids*	*Esters*
	D^a	D^a
C_{18} *series*		
threo-6(7)-Chloro-7(6)-hydroxy	25, 22	29, 25
erythro-6(7)-Chloro-7(6)-hydroxy	25, 22	29, 25
threo-9(10)-Chloro-10(9)-hydroxy	31, 28	35, 31
erythro-9(10)-Chloro-10(9)-hydroxy	32, 28	35, 31
C_{22} *series*		
threo-13(14)-Chloro-14(13)-hydroxy	44	67
erythro-13(14)-Chloro-14(13)-hydroxy	40	67

[a] Ether–light petroleum (20:80, v/v).

Esters move faster than acids. Chloro-, bromo- and iodohydroxy acids are not separated from each other. RPTLC failed to give any of these separations except according to chain length.

Hydroxy and keto acids

From Table VI a diketo acid is seen to move faster than a monoketo acid, which in turn moves faster than a monohydroxy acid. A monoketo acid has an R_F value between those of a diketo and a ketohydroxy acid, and similarly a monohydroxy acid lies between a dihydroxy and ketohydroxy acid. Positional ketohydroxy compounds are not separable. When the crude reaction product of peracid epoxidation of an unsaturated acid is separated by DTLC with 30 % ether–light petroleum, the unreacted unsaturated acid moves with the solvent front and the epoxy, dihydroxy and hydroxyacetoxy acids follow the pattern shown in Table VI.

TABLE VI

SEPARATION OF EPOXY, HYDROXY AND KETO ACIDS BY TLC

R_F values × 100.
D = direct.

Compound	*Acids*
	D^a
cis-9,10-Epoxystearic acid	37
9(10)-Hydroxystearic acid	17, 13
9(10)-Ketostearic acid	37
9,10-Diketostearic acid	53
9-Hydroxy-10-ketostearic acid	21
10-Hydroxy-9-ketostearic acid	21
threo-9,10-Dihydroxystearic acid	3
erythro-9,10-Dihydroxystearic acid	1

[a] Ether–light petroleum (20:80, v/v).

The mechanism of separations obtained with various systems is thoroughly discussed by MORRIS[5]. Some further general conclusions may be drawn from the present experimental results. Compounds of the same carbon chain length carrying different groups like epoxy, mono-, di- and tetrahydroxy, keto, diketo and halohydroxy are separable from each other. Slight changes in the position of these groups in the carbon chain alter the polarity sufficiently to enable TLC separation. As is now well established, the use of boric acid-coated plates enables stereoisomers of di- and tetrahydroxystearic acids to be separated. Compounds of different carbon chain lengths can be separated by DTLC, BTLC and RPTLC, the last mentioned indeed effecting only this type of separation.

SUMMARY

Epoxy, halohydroxy, hydroxy, dihydroxy, keto, diketo and tetrahydroxy fatty acids of C_{18} and C_{22} chain lengths are separable by direct thin-layer chromatography. *Erythro* and *threo* di- and tetrahydroxy fatty acids are better separated on a boric acid-coated silica gel plate. Separation both of positional isomers (6,7 from 9,10 compounds) and of close positional isomers (9-hydroxy-10-acetoxy from 9-acetoxy-10-hydroxy) is possible. All the three systems, *viz.* direct, boric acid-coated and reversed-phase thin-layer chromatography, separate according to chain length The first two systems are generally considerably more useful for oxygenated products.

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