Separation of epithio and epoxy fatty acids and their derivatives by thin-layer chromatography

Epithio fatty acids are the sulphur analogues of epoxy fatty acids with sulphur in place of oxirane oxygen. During an investigation on the stereochemical relationships of epithio, halomercapto and hydroxymercapto fatty acids derived from oleic and elaidic acids through the epoxy compounds, it became necessary to separate and identify the individual components from a reaction mixture containing these acids. The separation by thin-layer chromatography (TLC) of epithio fatty acids and their derivatives does not appear to have been reported. In this note, the TLC movements of epithio, halomercapto and hydroxymercapto fatty acids of C_{22} and C_{18} chain lengths have been compared with those reported earlier[1] for epoxy, halohydroxy and dihydroxy fatty acids.

Cis and *trans* epoxy fatty acids used in this investigation were prepared by oxidising the ethylenic acids erucic, brassidic, oleic, elaidic and petroselinic by the peracid method of FINDLEY, SWERN AND SCANLAN[2]. Hypochlorination of the unsaturated acids[3] gave the chlorohydroxy fatty acids. Hydroxylation of *cis* unsaturated acids with performic acid[4] and alkaline potassium permanganate[5] afforded the corresponding *threo* and *erythro* dihydroxy fatty acids. The procedure of KAUFMANN AND SCHICKEL[6] was used to obtain the epithio fatty acids from the corresponding epoxy acids by treatment with thiourea in dioxan solution. The chloromercapto fatty acids were obtained by treating the epithio acids with hydrochloric acid, while treatment of the epoxy acid with potassium hydrosulphide gave the hydroxymercapto fatty acids. Details of their preparation and stereochemical relationships are being reported elsewhere. Melting and mixed melting points indicated purities of 98 % or higher for the compounds.

Experimental procedures for coating of the glass plates with silica gel G, impregnation with silicone oil to yield reversed-phase plates, development and location of spots by charring with sulphuric acid are given in earlier communications[1,7].

Table I gives the separations obtained by direct and reversed-phase systems in terms of $R_F \times 100$ values. In direct TLC epithio compounds are less polar than the corresponding epoxy compounds, but the pattern of resolution is in almost every instance comparable. Thus *cis* and *trans* epithio compounds are separable from each other, with the *trans* having a higher R_F value.

Position of epithio grouping along the carbon chain has a marked effect on the mobility. The farther the epithio grouping from the carboxyl, the higher the R_F value: *cis*-6,7- and *cis*-9,10-epithiooctadecanoic acids have $R_F \times 100$ values of 52 and 58 respectively. Corresponding epoxy compounds have $R_F \times 100$ values of 33 and 39. Both *cis*- and *trans*-13,14-epithiodocosanoic acids are separable from the corresponding C_{18} 9,10-products, chain length and relative position of the substituent being perhaps both contributory.

Chloromercaptooctadecanoic acids occupy an intermediate position between the epithio- and hydroxymercaptooctadecanoic acids, thus resembling their oxygen analogues. In the present instance, however, the 9-hydroxy-10-mercapto acid is separable from 10-hydroxy-9-mercapto acid.

In reversed-phase TLC epoxy compounds move faster than the corresponding

TABLE I

SEPARATION OF EPITHIO AND EPOXY FATTY ACIDS AND THEIR DERIVATIVES BY DIRECT AND REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY

Chain length of acid	*Position in carbon chain*	*Group*	$R_F \times 100$	
			Direct (ether–petroleum ether, 20:80)	*Reversed-phase (acetonitrile–acetic acid–water, 70:10:20)*
22	*cis* 13,14-	Epithio	68	41
22	*trans* 13,14-	Epithio	72	41
18	*cis* 9,10-	Epithio	58	54
18	*trans* 9,10-	Epithio	67	54
18	*cis* 6,7-	Epithio	52	54
22	*cis* 13,14-	Epoxy	48	58
22	*trans* 13,14-	Epoxy	51	58
18	*cis* 9,10-	Epoxy	39	72
18	*trans* 9,10-	Epoxy	42	72
18	*cis* 6,7-	Epoxy	33	72
18	*threo* 9,10(10,9)-	Hydroxymercapto	22, 18	—
18	*erythro* 9,10(10,9)-	Hydroxymercapto	22, 18	—
18	*threo* 9,10-	Dihydroxy	3	85
18	*erythro* 9,10-	Dihydroxy	2	85
18	*threo* 9,10-	Chloromercapto	52	—
18	*erythro* 9,10-	Chloromercapto	52	—
18	*threo* 9,10(10,9)-	Chlorohydroxy	31, 28	64
18	*erythro* 9,10(10,9)-	Chlorohydroxy	31, 28	64

TABLE II

SEPARATION OF EPITHIO AND EPOXY FATTY ALCOHOLS BY DIRECT AND REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY

Chain length of alcohol	*Position in carbon chain*	*Group*	$R_F \times 100$	
			Direct (ether–petroleum ether, 20:80)	*Reversed-phase (acetonitrile–acetic acid–water, 70:10:20)*
22	*cis* 13,14-	Epithio	44	56
22	*trans* 13,14-	Epithio	47	56
18	*cis* 9,10-	Epithio	36	61
18	*trans* 9,10-	Epithio	39	61
22	*cis* 13,14-	Epoxy	40	66
22	*trans* 13,14-	Epoxy	44	66
18	*cis* 9,10-	Epoxy	32	74
18	*trans* 9,10-	Epoxy	35	74

epithio fatty acids. Separation of epithio and epoxy acids are only slightly less satisfactory than by the direct systems. In both epithio and epoxy compounds neither geometrical nor positional isomers are resolved by reversed-phase TLC.

Separation of epithio fatty alcohols are less satisfactory than the corresponding fatty acids both by direct and reversed-phase TLC, as can be seen from Table II.

Thus epithio fatty acids and their derivatives can be well resolved both by direct and reversed-phase TLC in a manner corresponding to their oxygen analogues.

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Dünnschicht-Chromatographie von Lipiden mit Gradienten-Elution auf Kieselgel G

Das Gebiet der Lipide umfasst zahlreiche Substanzklassen, die sich zum Teil sehr erheblich voneinander unterscheiden. Trotzdem gewinnt man sie aus biologischem Material in der Regel mit Hilfe organischer Lösungsmittel als Total-Lipidextrakt[1]. Eine vollständige Auftrennung des Rohextraktes ist daher gegenwärtig fast nie möglich, und man muss sich häufig mit einer Trennung in einzelne Gruppen begnügen. Mit einem normalen Dünnschicht-Chromatogramm ist selbst dieses bescheidene Ziel nicht ohne weiteres erreichbar: Ein beträchtlicher Anteil des Extraktes wandert mit der Fliessmittelfront oder bleibt am Startpunkt zurück. Bessere Resultate erzielt man mit Hilfe der Mehrfachchromatographie (vgl. z.B. Lit. 2). Dieser Technik haftet aber neben dem grossen Aufwand an Zeit vor allem der schwerwiegende Nachteil an, dass sich die chromatographierten Substanzen während der erforderlichen Zwischentrocknungen teilweise verändern können.

Beides wird bei Gradienten-Elution vermieden. Schon mit einem sehr einfachen, konvexen Elutionsgradienten erreicht man unter Umständen befriedigende Trennungen, z.B. von Mono-, Di- und Triglyceriden sowie verschiedenen Pentaerythritestern nach RYBICKA[3]. Eine gute Anpassung an spezielle Probleme wird aber erst möglich, wenn die Form des Elutionsgradienten frei wählbar und nicht mehr an die Versuchsanordnung gebunden ist. Wir haben vor kurzem eine Technik entwickelt[4,5], die praktisch jede beliebige Gradientenform zu verwenden gestattet[6]. Man kann