Separation by thin-layer chromatography of the mercaptoacetic acid addition products of long-chain monounsaturated compounds

Mercaptoacetic acid reacts with monounsaturated fatty acids and esters to form the corresponding carboxymethylthio derivatives[1-3]. Not much information is available regarding their preparation and structural identification. The addition product of oleic acid with mercaptoacetic acid was postulated, without experimental proof, to be a mixture of the 9- and 10-(carboxymethylthio)-stearic acids[1]. In this note, evidence is adduced through TLC separation of isomeric carboxymethylthio derivatives of a number of monounsaturated fatty acids, esters and alcohols. In an earlier publication from this laboratory[4] a similar separation by TLC of isomeric 9(10)-hydroxy-10(9)-mercaptooctadecanoic acids was reported.

Materials and methods

Compounds used for the TLC separation were prepared by reacting the appropriate unsaturated compound with mercaptoacetic acid following the directions of KOENIG AND SWERN[1]. The crude reaction product was purified by urea adduction and repeated low-temperature crystallisation. Desulphurisation studies of these compounds will be reported separately.

The direct TLC procedure in use in this laboratory[5], using Silica Gel G, development with appropriate solvent systems and charring with sulphuric acid, was employed to separate the compounds. For reversed-phase TLC, the Silica Gel G-coated glass plates were first impregnated by ascending development with 5 % silicone oil in ether as described earlier[6].

Results and discussion

TLC separation of mercaptoacetic acid addition products of monounsaturated fatty acids, esters and alcohols by direct (DTLC) and reversed-phase (RPTLC) systems are shown in Table I. Separation was achieved according to chain length by DTLC. Addition products from C_{22} unsaturated acids moved ahead and were resolved from those of the C_{18} and C_{11} acids. Esters have relatively higher R_F values than the corresponding acids, which in turn move faster than the alcohols.

The presence of two components in the addition product, revealed by TLC, lends experimental support to the earlier postulation of KOENIG AND SWERN[1] that both isomers are formed from methyl oleate and mercaptoacetic acid. The 6- and 7-(carboxylmethylthio)-octadecanoic acids are also separable, and had lower R_F values than the respective 9- and 10-isomers. The addition product of erucic acid with mercaptoacetic acid also gave two spots by DTLC. Carboxymethylthioundecanoic acid, apart from having the lowest mobility, gave only a single spot, showing the absence of isomeric forms. This is most probably an anti-Markownikoff addition, whereby 11-carboxymethylthioundecanoic acid is obtained, as observed earlier for terminally-unsaturated short-chain fatty acids[1].

RPTLC gave similar separation patterns except that the polarity was reversed. Separation of 6- and 7- from 9- and 10-isomers was, however, less satisfactory than by DTLC.

Both in DTLC and RPTLC, the respective monounsaturated fatty acids used

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TABLE I

TLC SEPARATION OF MERCAPTOACETIC ACID ADDITION PRODUCTS OF MONOUNSATURATED FATTY ACIDS, ESTERS AND ALCOHOLS
(All values as $R_F \times 100$)

Compound	*Acids from which prepared*	*Direct TLC**			*Reversed-phase TLC***		
		Acids	*Esters*	*Alcohols*	*Acids*	*Esters*	*Alcohols*
11-(Carboxymethylthio)-undecanoic acid	Undecenoic	23	45	12	88	86	93
6(7)-(Carboxymethylthio)-octadecanoic acid	Petroselinic	33, 36	52, 55	22, 25	72, 75	53, 56	81, 83
6(7)-(Carboxymethylthio)-octadecanoic acid	Petroselaidic	33, 36	—	—	72, 75	—	—
9(10)-(Carboxymethylthio)-octadecanoic acid	Oleic	42, 45	61, 64	23, 26	74, 77	51, 54	80, 83
9(10)-(Carboxymethylthio)-octadecanoic acid	Elaidic	42, 45	61, 64	23, 26	74, 77	51, 54	80, 83
13(14)-(Carboxymethylthio)-docosanoic acid	Erucic	46, 49	64, 67	31, 34	61, 64	46, 48	75, 77
13(14)-(Carboxymethylthio)-docosanoic acid	Brassidic	46, 49	—	—	61, 64	—	—

* 40% Ether–light petroleum.
** Acetonitrile–acetic acid–water (70:10:20).

as starting material generally moved to the solvent front, while the unsaturated alcohols, being more polar, had lower R_F values.

Apart from providing useful TLC separation procedures, these results also demonstrate that addition of mercaptoacetic acid to monoene fatty acids, esters and alcohols yields both isomers, with the exception of undecenoic acid which gives rise to a single product probably the 11-carboxymethylthioundecanoic acid.

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Separation and quantitative determination of ^{32}P-labelled lipids from brain particulates by thin-layer chromatography

The separation and isolation of phospholipids by thin-layer chromatography (TLC) on silica gel has been reported by several investigators[1–5]. Although a distinct separation of some phospholipids was achieved by these techniques, they yield separations with overlap among such phospholipids as phosphatidic acid and neutral lipids or phosphatidyl inositol and phosphatidyl serine. During our work on the effect of neurohormones and metabolic inhibitors of phosphate metabolism in nerve-ending particulates of rat brain, it was found that phospholipids were highly labelled when incubated in an oxidative phosphorylation medium containing ^{32}P-orthophosphate[6]. By incorporating certain features of previously described techniques[1,4], nerve endings or mitochondrial ^{32}P-labelled phospholipids, including the highly labelled phosphatidic acid and phosphatidyl inositol, were effectively separated on Silica Gel G by means of two-dimensional TLC, and the radioactive specific activities of the individual phospholipids were determined.

Experimental

Lipid extracts. Total lipids were extracted from rat brain nerve-ending particles or purified mitochondria with chloroform–methanol (2:1) after incubation in an oxidative phosphorylation medium containing 100–150 μc of ^{32}P-orthophosphate for 1 h at 37° as described previously[7]. The extract was filtered, concentrated *in vacuo*