suggested that this fraction might be the 2,4-dinitrophenylhydrazone of a compound with a keto group near the end of a long chain ester. The spectrum showed rather weak hydrazone bands. Its strong carbonyl band at 5.8 μ and the band at 8.5 μ indicated the presence of an ester group. The strength of the C-H stretching band at 3.4 μ suggested considerable aliphatic hydrogen which indicated a long chain attached to the hydrazone. The peaks between 7 and 8 μ showed more splitting than was the case with the 2,4-dinitrophenylhydrazone of a long chain aldehyde, thus leading to the possibility of at least one short chain attached to the hydrazone group.

The mechanisms for the formation of saturated aldehydes and 2-enals by the autoxidation of unsaturated fatty esters have been postulated by various investigators (10,11) based upon the hydroperoxide theory of Farmer et al. (12). The mechanism for the formation of ketones was proposed by Bell et al. (13).

$$R-CH(OOH)-R \longrightarrow R-\underset{\underset{O.}{|}}{CH}-R + .OH$$

$$R-\underset{\underset{O.}{|}}{CH}-R + R'H \longrightarrow R-CHOH-R + R'.$$

$$R-\underset{\underset{O.}{|}}{CH}-R + R'. \longrightarrow R-\underset{\underset{O}{||}}{C}-R + R'H$$

or

$$R-\underset{\underset{O.}{|}}{CH}-R + R'O. \longrightarrow R-\underset{\underset{O}{||}}{C}-R + R'OH$$

It should be noted that a carbonyl compound may contribute to the flavor of an oil at an extremely low concentration, often less than 1 ppm. Modern techniques of flavor chemistry have enabled us to isolate and identify carbonyl compounds which are present in fats and oils at such low concentrations. On the other hand, our knowledge of the numbers and locations of double bonds in unsaturated fatty acids in natural fats and oils is far below this accuracy. Therefore, in order to postulate adequately the mechanism for the formation of all carbonyl compounds, our knowledge of fatty acid composition and location of double bonds must first be significantly enriched.

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Thin-Layer Chromatography of Brain Phospholipids[1,2]

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Abstract

Thin-layer chromatography systems are described for separation of most tissue phospholipids and sphingolipids. Acidic lipids such as phosphatidyl serine and cerebroside sulfate are resolved without streaking on several types of modified silica gel.

Introduction

THIN-LAYER chromatography is especially valuable for the separation of lipids (1,2). However, some phospholipids do not chromatograph well (3) on systems reported to date (4–12). The greatest difficulty is encountered with acidic lipids which show marked tailing on silica gel. Mangold (1,13) has overcome this by adding ammonium sulfate to the stationary phase. Aldehydogenic lipids are split on this stationary phase. In the present investigation, a number of modifications of stationary and mobile phases are described which are designed to minimize streaking of acidic lipids. In addition to previously described chloroform-methanol-ammonia solvents, the effects of impregnation of silica gel with acidic and basic substances are reported. The basic plates give well-defined spots for acidic compounds. An ammonia-containing solvent system was also reported recently by Skidmore and Entenman (14).

Materials and Methods

Thin-layer plates were prepared as described by Stahl (15), using 30.0 g Silica Gel G[3] and 60 ml water. Modified plates were prepared with 27.0 g Silica Gel G and 3.0 g of ammonium sulfate, sodium acetate, oxalic acid, or potassium hydroxide dissolved in 60 ml water, or 20 ml of a saturated solution of sodium borate in 60 ml water.

Lipid samples were obtained by fractionation of rat, monkey, and human brain lipids on combinations of diethylaminoethyl cellulose (16,17), silicic acid-silicate (16–18), and Unisil[4] silicic acid columns. Monkey liver mitochondria lipids were fractionated on a Unisil[4] silicic acid column. Samples were applied to thin-layer plates in a stream of nitrogen using a Hamilton syringe. Plates were developed in unlined, equilibrated tanks until the solvent front was 10 cm above the starting points.

Two indicators have been found to be most useful for phospholipids. Plates are first sprayed with a ninhydrin reagent and heated for 15 min at 110C, then stained with iodine. The plates are exposed to

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[3] C. A. Brinkman and Co., Inc., 115 Cutter Mill Road, Great Neck, Long Island, N.Y. (manufactured by E. Merck, A. G., Darmstadt, Germany).
[4] Clarkson Chemical Co., Inc., Williamsport, Pa.

TABLE I

R_f Values of Brain Lipids on Thin-Layer Plates

Stationary phase / Mobile phase	SGG[a] CMW[g]	SGG[a] CMN[h]	SGG CMN[i]	Borate[b] CMN[h]	Acetate[c] CMN[h]	KOH[d] CMW[g]	Oxalic[e] CMW[g]	$(NH_4)_2SO_4$[f] CMW[g]
Lipid	R_f Value							
Cerebroside	0.89	0.96	0.58	0.44[j]	0.90	0.61	0.82	0.77
	0.85	0.93	0.49	0.35[j]	0.85	0.55	0.79	0.70
Phosphatidyl ethanolamine	0.78	0.90	0.47	0.59	0.92	0.45	0.55	0.65
Phosphatidyl serine	.....[k]	0.29	0.11	0.35	0.57	0.41[k]	0.78[k]	0.65
Lysophosphatidyl ethanolamine	0.55	0.46	0.21	.....	0.70	.....	0.29	0.37
Lysophosphatidyl serine	.....[k]	.....	0.00	.....	0.14	.....	.....	.....
Phosphatidyl choline	0.55	0.63	0.43	0.45	0.77	0.35	0.35	0.45
Lysophosphatidyl choline	0.31	0.28	0.13	.....	0.46	.....	0.10	0.07
Sphingomyelin	0.47	0.47	0.24	0.30	0.58	0.20	0.19	0.30
Cerebroside sulfate	.....	.....	0.41	0.35[j]	0.71	0.28	0.50	0.65
	.....	.....	0.36	0.30[j]	0.67	0.23	0.35	0.55

[a] Silica Gel G.
[b] Impregnated with sodium borate.
[c] Impregnated with sodium acetate.
[d] Impregnated with potassium hydroxide.
[e] Impregnated with oxalic acid.
[f] Impregnated with ammonium sulfate.
[g] 65/25/4 chloroform, methanol, water (v/v/v).
[h] 65/25/4 chloroform, methanol, ammonium hydroxide (v/v/v).
[i] 75/25/4 chloroform, methanol, ammonium hydroxide (v/v/v).
[j] These values vary depending on amount of sodium borate incorporated into the stationary phase.
[k] Streaking observed.

iodine vapor by placing iodine crystals on the bottom of a glass drying tray, then placing the plate face down in a horizontal position over the top of the tray.

Lipids were identified by comparison with synthetic commercial samples, behavior on chromatographic columns, and analysis of purified fractions for cholesterol with Liebermann-Burchard reagent (19), phosphorus (20), ester linkages (21), and galactose (22).

Results

The R_f values obtained for various polar lipids are given in Table I. The values given for each combination of stationary phase and developing solvent were determined on a single plate. R_f values vary considerably from plate to plate depending on the degree of activation.

Two spots were always seen for cerebrosides and cerebroside sulfates. This may be due to separation of cerebrosides containing α-hydroxy fatty acids from those containing unsubstituted fatty acids (9). Sometimes two spots were also seen for phospholipids. In these cases, separations may be due to the nature of the fatty acids (9).

Cerebrosides, phosphatidyl ethanolamine, phosphatidyl choline, sphingomyelin, and lysophosphatidyl choline are separated well on Silica Gel using chloroform-methanol-water combinations. Phosphatidyl serine streaks in this type of system. Addition of acetic acid to the mobile phase did not prevent the streaking. Incorporation of ammonium sulfate or oxalic acid caused phosphatidyl serine to migrate with or ahead of phosphatidyl ethanolamine. Impregnation with these acid materials caused plasmalogens to split. Thus brain phosphatidyl ethanolamine gave three spots corresponding to fatty aldehyde, phosphatidyl ethanolamine, and lysophosphatidyl ethanolamine.

The best separations of cephalins were obtained by addition of ammonium hydroxide to the developing solvent, or by addition of sodium acetate or sodium borate to the stationary phase. Sodium acetate and sodium borate impregnated plates had similar properties. The only difference was that cerebrosides were retarded on the plates impregnated with sodium borate. A spot believed to be cardiolipin (the fraction of monkey liver mitochondria lipids eluted from a silicic acid column with 7/1 chloroform-methanol) is found above phosphatidyl ethanolamine on acidic plates and below on alkaline plates. Cerebroside sulfates form two spots between phosphatidyl choline and phosphatidyl ethanolamine on plates impregnated with sodium acetate, oxalic acid, and ammonium sulfate. Inositol containing phospholipids and phosphatidic acid have not yet been identified. Cholesterol, triglycerides, and fatty aldehydes migrated with the solvent front in all systems.

Discussion

The best results for most phospholipids have been obtained with unmodified Silica Gel G plates and a solvent system containing chloroform, methanol, and ammonium hydroxide. If acidic lipids are known to be absent, a solvent system containing chloroform, methanol and water is just as useful.

Plates impregnated with sodium acetate and sodium borate are valuable for separation of the phospholipids and sphingolipids eluted from silicic acid columns in the neighborhood of cephalins. Comparison of mobilities relative to phosphatidyl ethanolamine permits assignment of acidic or glycolipid character to an unknown spot. Ammonium sulfate and oxalic acid plates are valuable for detection and separation of plasmologens in purified lipid fractions. For example, chromatography of a purified phosphatidyl ethanolamine fraction on an acidic plate would allow separate recovery and radioactivity counting of diacyl phosphatidyl ethanolamine and both the aldehyde and lysophosphatidyl ethanolamine components of the plasmalogen.

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