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THIN-LAYER CHROMATOGRAPHIC BEHAVIOR OF GLYCEROLIPID ANALOGS CONTAINING ETHER, ESTER, HYDROXYL, AND KETONE GROUPINGS

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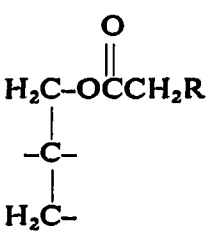
SUMMARY

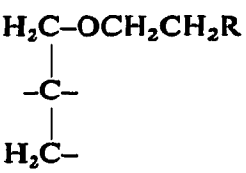
Mixtures of glycerolipid analogs containing ether, ester, hydroxyl, and ketone groupings can generally be separated by thin-layer chromatography on the basis of these functional groups unless phosphate or phosphorylbase groups are present. Usually, similar analogs of glycerophospholipids can be separated only according to their general classes on adsorbent layers. For example, diacylglycerophosphorylethanolamine, alkylacylglycerophosphorylethanolamine, and alk-1-enylglycerophosphorylethanolamine migrate at the same R_F; however, after phospholipase C treatment, the resulting radylacylglycerols and monoradylglycerols can be resolved.

INTRODUCTION

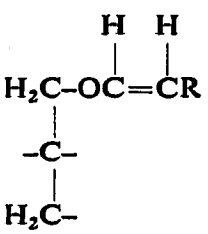
Hydrocarbon chains in glycerolipids occur in ester (acyl)-and ether (alkyl and alk-1-enyl) linkages. The structural similarity between these three types of glycerolipids is depicted by the formulas:



Acyl type



Alkyl type



Alk-1-enyl type

In mammalian systems, the O-alkyl and O-alk-1-enyl chains have only been found at the *sn*-1 position of the glycerol moiety, whereas the groupings at the 2- and 3-positions vary; the types that are found naturally are listed in Table I. Information on

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TABLE I

ETHER ANALOGS OF GLYCEROLIPIDS FOUND IN MAMMALIAN CELLS*

Acyl lipid class	*Types of ether analogs found in nature*	
	Alkyl	*Alk-1-enyl*
Triacylglycerol	+**	+
Diacylglycerol	+	+
Monoacylglycerol	+	−
Acyldihydroxyacetone	+	−
Acyldihydroxyacetone phosphate	+	−
Acylglycerophosphate	+	−
Phosphatidic acid	+	−
Phosphatidylethanolamine	+	+
Phosphatidylcholine	+	+
Phosphatidylserine	+	+

* The ether moieties are at the *sn*-1 position of the glycerol moiety.
** Plus (+) indicates presence, and minus (−) indicates absence.

the biological importance of the ester[1] and ether[2] glycerolipids has been reviewed in two recent books.

In this paper, I have described some thin-layer chromatographic (TLC) systems that our laboratory has used successfully to separate specific classes of the ester-containing and ether-containing glycerolipid analogs. Various solvent systems and literature references to typical examples of their actual applications are listed in Table II. Illustrations of the migration patterns for the glycerolipid analogs in each solvent system are depicted in Fig. 1 (Solvent systems I–III), Fig. 2 (Solvent systems IV–VI), Fig. 3 (Solvent systems VII and VIII) and Fig. 4 (Solvent systems IX–XI). The close structural similarity of certain of these molecular analogs makes their

TABLE II

SOLVENT SYSTEMS (BY VOLUME) USED TO SEPARATE GLYCEROLIPID ANALOGS BY TLC

*Solvent system**	*Hexane*	*Diethyl ether*	*Chloroform*	*Methanol*	*Ethanol*	*Water*	*Acetic acid*	*Ammonium hydroxide*	*Reference*
I	95	5	—	—	—	—	—	—	10
II	80	20	—	—	—	—	1	—	11
III	70	30	—	5	—	—	1	—	12
IV	—	—	98	2	—	—	1	—	13
V	—	100	—	—	—	—	0.5	—	3
VI	40	60	—	—	—	—	1	—	14
VII	40	60	—	—	—	—	—	1	unpublished
VIII	—	—	90	—	10	—	—	—	5
IX	—	—	50	25	—	4	8	—	15
X	—	—	60	35	—	—	—	8	15**
XI	—	—	90	10	—	—	10	—	unpublished

* All systems were equilibrated in chambers lined with paper except Systems I and XI.
** Ref. 15 depicts data obtained with a similar solvent system in which the volume of chloroform is 65 instead of 60.

chromatographic resolution extremely difficult, especially when isomeric forms are encountered.

Ether analogs of triacylglycerols, diacylglycerols, and monoacylglycerols

Ether analogs of triacylglycerols, diacylglycerols, and monoacylglycerols are easily separated from the corresponding ester classes. The migration pattern on Silica Gel G for trisubstituted molecular species (Solvent systems I and II, Fig. 1), for disubstituted molecular species (Solvent systems II–VII, Figs. 1–3), and for monosubstituted molecular species of glycerolipids (Solvent systems III–VIII, Figs. 1–3) always shows that the alk-1-enyl type has a higher R_F value than the alkyl type and that the alkyl type has a higher R_F value than the acyl type. The situation is more complicated when isomeric forms of the disubstituted and monosubstituted types are present.

We have found that Solvent system IV (Fig. 2) is capable of providing some resolution of the isomeric diradylglycerols. Addition of NH_4OH to these systems decreases their ability to resolve the isomeric forms of the diradylglycerols, and when desirable, Solvent system VII (Fig. 3) can be used to prevent resolution of the isomeric diradylglycerols. It is always possible to determine the presence of ether groupings

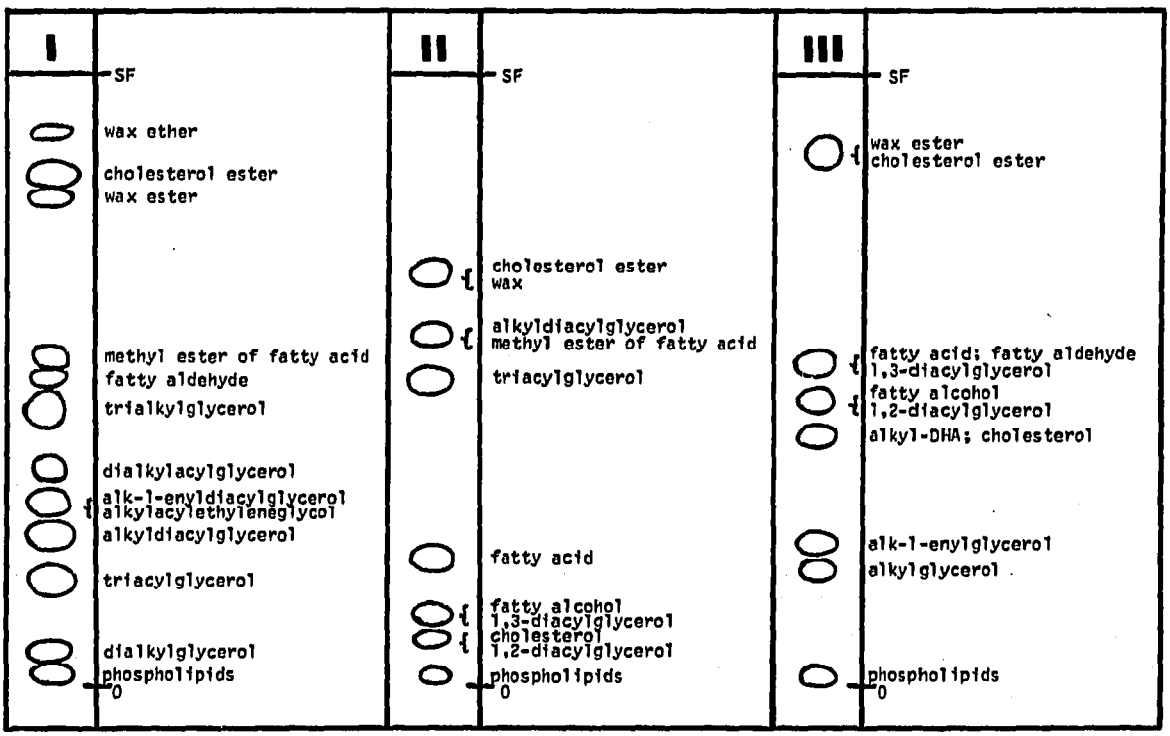

Fig. 1. Typical TLC migration patterns of lipids in Solvent systems I, II, and III. System I is non-equilibrated, whereas Systems II and III are equilibrated in chambers lined with paper. The composition of each solvent system is listed in Table II. O designates origin and SF designates solvent front.

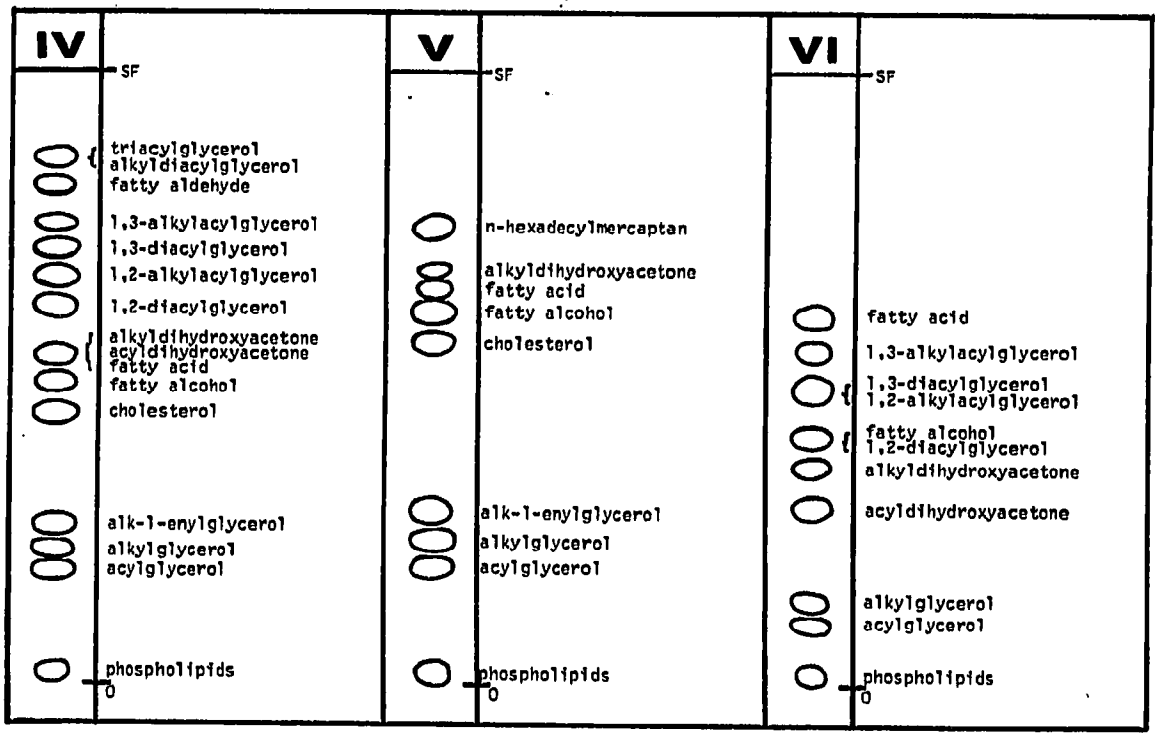

Fig. 2. Typical TLC migration patterns of lipids in Solvent systems IV, V, and VI; all systems are equilibrated in chambers lined with paper. The composition of each solvent system is listed in Table II. O designates origin and SF designates solvent front.

in any glycerolipid mixture by using chemical reduction procedures such as $LiAlH_4$ (ref. 3) or Vitride [$NaAlH_2(OCH_2CH_2OCH_3)_2$] (ref. 4) to remove the ester groupings. Thus the alkylglycerols and alk-1-enylglycerols liberated by these reducing agents can then be separated satisfactorily from each other in Solvent systems III–VI (Figs. 1 and 2).

A mixture of the 1- and 2-isomers of alkylglycerols or alk-1-enylglycerols is not easily separated by TLC except under conditions where anionic complexes can be formed with the adjacent hydroxyl groups of the 1-isomers; this has been accomplished by incorporating borate or arsenite ions[5] into layers of Silica Gel G and then developing the chromatogram in Solvent system VIII. On the arsenite layers (VIII-A), the 1-isomer has a higher R_F value than the 2-isomer, whereas on the borate layers (VIII-B), the 1-isomer has a lower R_F value than the 2-isomer (Fig. 3). Similar chromatographic results could be expected with the two isomeric forms of the acylglycerols and alk-1-enylglycerols.

Ether analogs of acyldihydroxyacetone

Acyldihydroxyacetone and alkyldihydroxyacetone can be resolved on Silica Gel G layers developed in Solvent system VI (Fig. 2). However, if other components,

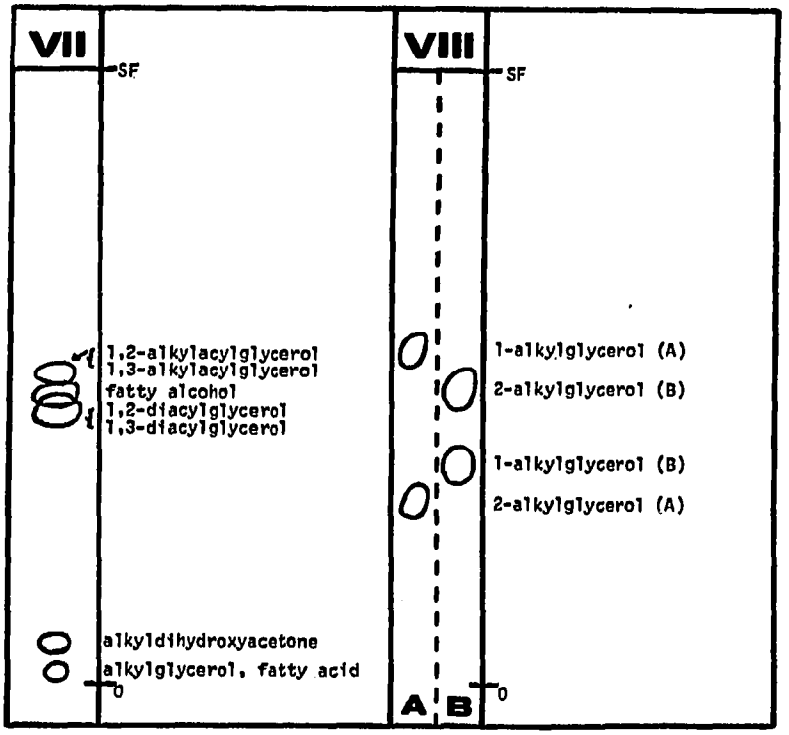

Fig. 3. Typical TLC migration patterns of lipids in Solvent systems VII and VIII; both systems are equilibrated in chambers lined with paper. The composition of each solvent system is listed in Table II. O designates origin and SF designates solvent front. At the bottom of lane VIII, the A designates arsenite-containing adsorbent and the B designates borate-containing adsorbent.

such as fatty alcohols, free sterols, and diradylglycerols, are present, care must be taken to check also the R_F values of these compounds in Solvent systems II–VII (Figs. 1–3). Reduction of the ketone groups in these molecules by $NaBH_4$ or enzymically by NADPH- or NADH-linked reductases yields acylglycerols and alkylglycerols. These monoradylglycerols can be easily separated in Solvent systems III–VI (Figs. 1 and 2).

When NH_4OH is present (Solvent system VII), the acyldihydroxyacetone and alkyldihydroxyacetone barely migrate from the origin; this can be a useful system for isolating either acyldihydroxyacetone or alkyldihydroxyacetone from contaminating long-chain fatty alcohols and diradylglycerols. However, it is important to note that free fatty acids also will migrate barely from the origin in Solvent system VII. Although it is obvious that identifications based solely on TLC behavior are impossible, the TLC patterns obtained in several different solvent systems can be useful in minimizing multiple choices.

Ether analogs of diacylglycerophosphorylcholine, diacylglycerophosphorylethanolamine, diacylglycerophosphorylserine, and their lyso derivatives

The effect of ether groupings *versus* acyl groupings on the TLC behavior of glycerophospholipids is essentially nil. Renkonen[6] obtained only enrichment of the acyl, alkyl, and alk-1-enyl molecular species of phospholipids even after chemically

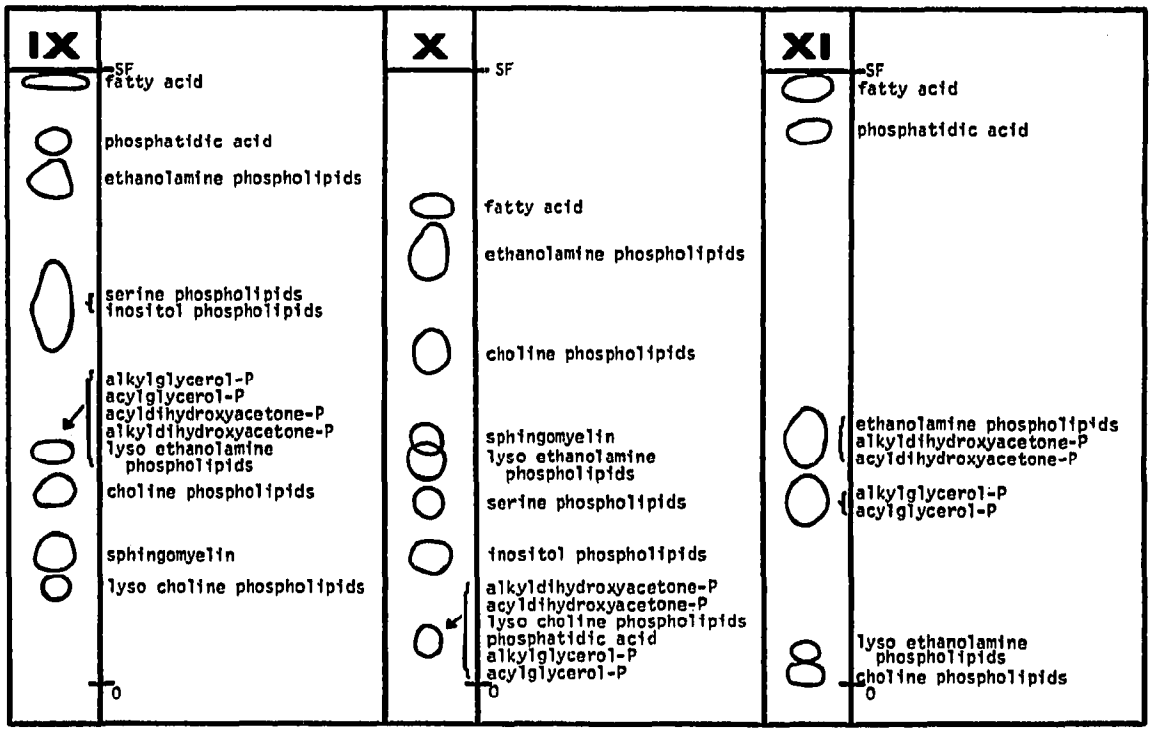

Fig. 4. Typical TLC migration patterns of lipids in Solvent systems IX, X, and XI; Systems IX and X are equilibrated in chambers lined with paper, whereas System XI is nonequilibrated. The composition of each solvent system is listed in Table II. O designates origin and SF designates solvent front.

masking the phosphate and nitrogen-base groupings and after multiple chromatographic developments. Although the masking of the phosphate moiety as the methoxy derivative and the masking of the nitrogen as the dinitrobenzene derivative represent a useful approach, it does not completely resolve the ester and ether glycerophosphatides.

At present, the most practical method for the chromatographic analyses of the phospholipid analogs requires that the phosphorylbase groupings be removed by phospholipase C or by chemical procedures after the choline-, ethanolamine-, and serine-containing glycerophosphatides have been separated from each other in Solvent systems IX and X (Fig. 4). Since lyso forms of these lipids also are encountered, it is generally necessary to carry out the isolation of complex mixtures of glycerophosphatides for subsequent analysis by the two-dimensional chromatography in which Solvent systems IX and X can be used. The isolated lipid fractions can then be analyzed after the phosphorylbase grouping is removed by phospholipase C to yield diradylglycerols or monoradylglycerols (derived from the lyso glycerophosphatides). Each molecular species can then be chromatographed in the solvent systems outlined under *Ether analogs of the triacylglycerols, diacylglycerols, and monoacylglycerols.* The presence of O-alk-1-enyl moieties in phospholipids can be detected by acid hydrolysis directly on adsorbent layers[7] or by chemical reduction[3,4]; the latter also liberates alkylglycerols which are easily isolated.

Ether analogs of phosphatidic acid, acyldihydroxyacetone phosphate, and acylglycerophosphate

The acidic nature of glycerolipids that possess a free phosphate moiety causes them to migrate differently in acidic and alkaline solvent systems. Those lipids possessing free phosphate groups have relatively high R_F values in acidic systems (Solvent system IX), but remain very close to the origin when alkaline solvent systems (Solvent system X) are used (Fig. 4). With acidic conditions, it is possible to separate phosphatidic acid and its ether analogs from acyldihydroxyacetone phosphate or alkyldihydroxyacetone phosphate. Alkyldihydroxyacetone phosphate and alkylglycerophosphate are extremely difficult to separate from each other; Solvent system XI (Fig. 4) can give fair results if humidity conditions are stable. However, good separations of these lipids were obtained[8] if a 1 % solution of *p*-nitrophenylhydrazine is added to the lipid mixture when it is in solvent or after it has been spotted to the adsorbent before chromatography in Solvent system XI. The same procedure would presumably be applicable for the resolution of acyldihydroxyacetone phosphate and acylglycerophosphate by TLC.

The most effective way to obtain adequate separations of these polar glycerolipids is to remove the phosphate, enzymically or chemically, before chromatography. The enzymic method is best since it does not alter other portions of the molecule. We have found that alkaline phosphatase is highly specific for hydrolyzing the phosphate moiety from 1-radylglycerolipids[9]; thus alkaline phosphatase can utilize alkyldihydroxyacetone phosphate and acyldihydroxyacetone phosphate as substrates, and the dephosphorylated products are then easily separated by TLC as described under *Ether analogs of acyldihydroxyacetone*. The free phosphate group of phosphatidic acid and its ether analogs must be removed by phospholipase C or acid phosphatase. Chemical reduction by $LiAlH_4$ (ref. 3) or Vitride[4] and saponification can also be used in some instances to remove the phosphoric acid residue, but these reactions entail the risk of altering other functional groupings, *e.g.*, ketone and acyl groups.

CONCLUSION

The TLC procedures outlined in this paper are based on our laboratory experience with a wide variety of glycerolipid analogs encountered in biological systems. Absolute identifications require more rigorous chromatographic analyses (*e.g.*, gas–liquid chromatography), formation of chemical derivatives, physical measurements, and organic syntheses.

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