Preparative isolation of monogalactosyl and digalactosyl diglycerides by thin-layer chromatography

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SUMMARY Monogalactosyl and digalactosyl diglycerides were separated from leaf and *Chlorella vulgaris* lipid extracts by thin-layer chromatography with Silica Gel G as the stationary phase and acetone–acetic acid–water as the mobile phase. Phospholipids are completely removed and with two-dimensional development can themselves be fractionated.

KEY WORDS thin-layer chromatography · monogalactosyl diglyceride · digalactosyl diglyceride · isolation · phospholipids · fractionation

SEPARATION BY TLC of galactolipids from other lipids, especially phospholipids, is rather difficult in solvent systems such as chloroform–methanol–water or diisobutyl ketone–acetic acid–water (1, 2). Since some phospholipids migrate with or near the galactolipids in these solvent systems, isolation and identification of the galactolipids are almost impossible unless the lipid is separated by two-dimensional chromatography. However, Nichols (3) apparently did obtain good separation of the galactolipids from phospholipids with chloroform–methanol–acetic acid. A disadvantage encountered with some systems, such as diisobutyl ketone–acetic acid–water or butanol–acetic acid–water, is the effort required to evaporate the solvents from the plates, which introduces the danger of oxidizing the highly unsaturated galactolipids and contaminating the isolated lipids with impurities.

This report describes new solvent systems capable of separating galactolipids completely from the phospholipids, and thus of simplifying the isolation and identification of these glycolipids.

TLC Procedure. Plant lipid extracts were prepared for TLC by the method of Folch, Lees, and Sloane Stanley (4). The thin-layer plates were spread with Silica Gel G (Warner-Chilcott Laboratories Instrument Division, Richmond, Calif.) to a thickness of 250 μ and were activated for 1 hr at 110°C. The lipids were chromatographed either with acetone–acetic acid–water 100:2:1 or with acetone alone. Two-dimensional chromatography consisted of a primary development in chloroform–methanol–water 65:25:4 followed by a brief drying period in a vacuum desiccator and development in the second dimension with acetone–acetic acid–water 100:2:1. All separations were carried out in a tank lined with Whatman No. 3 MM filter paper. The lipids were made visible with iodine vapor.

Preparative TLC. Large quantities of galactolipids were isolated from layers 1 mm thick. The thick layers were activated for 4–6 hr at 110°C before use. The lipid

Abbreviations: TLC, thin-layer chromatography; GDG, monogalactosyl diglyceride; DGDG, digalactosyl diglyceride.

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extract was usually separated into crude fractions by stepwise elution of a silicic acid column first described by Vorbeck and Marinetti (5) and later used to obtain galactolipid fractions by Rosenberg, Gouaux, and Milch (6). This preliminary column separation was found to improve subsequent separation by preparative TLC inasmuch as there was less opportunity for interference from pigments and other lipids when larger quantities of galactolipids were isolated. The two galactolipid fractions obtained from the column were each applied to 20 × 20 cm preparative plates in a single streak. Acetone was used as the mobile phase for purification of monogalactosyl diglyceride (GDG) and acetone–acetic acid–water for digalactosyl diglyceride (DGDG). The lipids were detected by spraying the plates with water.

The major fraction, which appeared less translucent than the rest of the plate, was scraped off and eluted in a microcolumn by the method of Rosenberg et al. (6) without the preliminary chloroform wash, since the water spray deactivates the silica gel. However, introducing water into the gel and eliminating the chloroform wash increases the possibility of obtaining nonlipid impurities from the gel. Occasionally small amounts of these impurities precipitated when the eluting solvent was concentrated, but they did not interfere with any of the analyses used in this study.

Analytical Methods. The identity of the galactolipids was confirmed by deacylation (7) and acid hydrolysis (8) followed by identification of the glycose moiety by paper chromatography. The galactose content of the lipids was quantitatively analyzed by the method of Radin, Lavin, and Brown (9) with the aid of a galactose standard curve. The fatty acid composition of the galactolipids was determined by gas–liquid chromatography of the methyl esters (10). The degree of ester unsaturation was determined by hydrogenation with H_2 and Pt.

Separations. The result of a typical TLC separation is shown in Fig. 1. The migration of galactolipids away from phospholipids depends on the insolubility of phospholipids in acetone. It should be pointed out that overloading the origin with too much lipid can hinder separation, as demonstrated by separations *1* and *6* (Fig. 1). This difficulty is more pronounced than with conventional systems as the insoluble phospholipids tend to impede free flow of the solvent. The usual limit of a 250 μ plate was found to be 0.75 mg of the total lipid extract applied in a strip 1 cm in width, but this limit will vary somewhat according to the relative percentage of phospholipid in the extract. Acetone as a developing solvent improves the separation of GDG, R_f 0.60–0.63, from the pigments that migrate in front of it, but then separation of DGDG, R_f 0.11–0.12, from the phospholipids at the origin is not satisfactory.

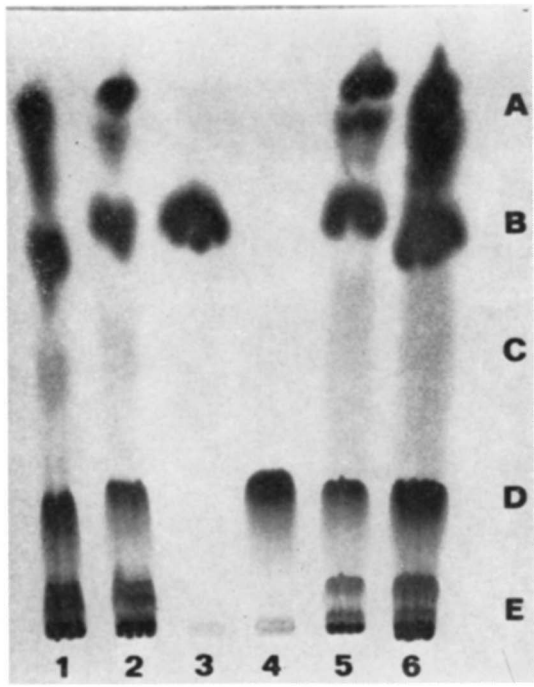

FIG. 1. TLC of plant lipids on Silica Gel G, 250 μ, in acetone–acetic acid–water 100:2:1 (lined chamber). *1* and *2*, barley leaf extract; *3*, isolated GDG (R_f 0.68); *4*, isolated DGDG (R_f 0.23); *5* and *6*, *Chlorella vulgaris* extract. *A*, pigments; *B*, GDG; *C*, unknowns and degraded chlorophyll; *D*, DGDG; *E*, phospholipids.

By using the two solvent systems described, fairly pure galactolipids can be isolated on preparative plates, especially if they are already partially purified by column chromatography. Up to 20 mg of each galactolipid was readily isolated from a single preparative plate after preliminary silicic acid column separation. This usually amounted to an application of about 25 mg of the partially purified galactolipid per plate. The fatty acid composition of spinach leaf galactolipids isolated in this way is shown in Table 1. The composition is similar to that

TABLE 1 FATTY ACID COMPOSITION OF SPINACH LEAF GALACTOLIPIDS

	14:0	15:0	16:0	16:2	16:3	18:0	18:1	18:2	18:3
	wt % of methyl esters								
GDG	tr.*	tr.	0.8	0.4	21.8	tr.	0.5	1.4	75.0
DGDG	tr.	tr.	7.5	tr.	5.4	tr.	1.0	2.0	84.0

Fatty acids designated by chain length: no. of double bonds.
* tr., trace (less than 0.1%).

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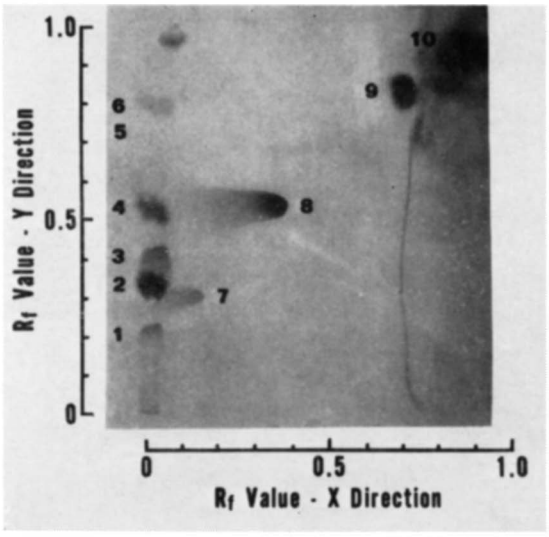

FIG. 2. Two-dimensional TLC of spinach leaf extract on Silica Gel G (250 μ thick). Solvent in the Y direction, chloroform–methanol–water 65:25:4; solvent in the X direction, acetone–acetic acid–water 100:2:1. All separations were in a lined chamber. *1*, phosphatidyl inositol; *2*, phosphatidyl choline; *3*, phosphatidyl glycerol; *4*, phosphatidyl ethanolamine; *5* and *6*, unknowns, one of which is probably phosphatidic acid; *7*, probably sulfolipid; *8*, DGDG; *9*, GDG; *10*, pigments.

given by Allen, Good, Davis, and Fowler (11), except that these investigators found about 8% more 16:3 and 8% less 18:3. Galactolipids isolated from green and etiolated barley leaves were also analyzed for fatty acid and galactose content as shown in Table 2.

On two-dimensional TLC the phospholipids can be fractionated by the first development in chloroform–methanol–water 65:25:4, after which the galactolipids are made to migrate away from them in acetone–acetic acid–water 100:2:1 (Fig. 2). Identification of the phospholipids is simplified by their failure to migrate in the second dimension. Most of the phospholipids were identified by their mobilities in chloroform–methanol–water as determined previously by Lepage (2), and also through isolation of the phospholipids from the thin-layer plate

TABLE 2 FATTY ACID AND GALACTOSE ANALYSES OF GALACTOLIPIDS ISOLATED FROM ETIOLATED AND GREEN BARLEY LEAVES

	Green Leaves		Etiolated Leaves	
	GDG	DGDG	GDG	DGDG
	μmoles/g fresh tissue			
Galactose	1.48	2.18	0.658	0.970
Fatty acids	3.04	1.86	1.28	0.918

followed by deacylation (7) and identification of the corresponding phosphate esters by paper chromatography. A ninhydrin spray was used to confirm the identity of phosphatidyl ethanolamine on the thin-layer plate.

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