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Note

Ascending dry-column chromatography as an aid in the preparative isolation of glycolipids

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The first silicic acid column chromatographic procedure found to be generally useful for the separation of non-polar lipids from polar lipids by elution with chloroform[1] was modified by Lea *et al.*[2], who isolated individual polar lipid classes by stepwise or gradient elution with chloroform containing increasing amounts of methanol. Further improvements in the fractionation procedure of complex lipid mixtures resulted when acetone was introduced as a specific eluting solvent for glycolipids on silicic acid columns[3–5]. Thus the sequential use of chloroform, acetone and methanol on silicic acid columns yielded non-polar lipids, glycolipids and phospholipids respectively, which were then further sub-fractionated into individual classes by the use of other chromatographic techniques[6].

Open-column chromatography, otherwise known as thin-layer chromatography (TLC), because of its high resolving capacity is often used to isolate highly purified lipids from crude lipid fractions obtained by other preparative chromatographic techniques (columns with different types of adsorbents[6], thick layers[7]) or by non-chromatographic techniques (alkaline hydrolysis[8], solvent precipitation[9]). Although thick-layer chromatographic plates have been used as a preparative tool in the fractionation of complex lipid mixtures[7], their limitations have been overcome by using the technique of ascending dry-column chromatography[10]. The application of the latter technique in the preparative fractionation of complex phospholipids of the protozoon *Tetrahymena pyriformis* has been reported[11].

The superiority of this technique over the conventional column chromatographic technique was shown by its higher sample load capacity, better resolving power and lower consumption of solvents. This technique was also superior to the preparative thick-layer technique in that it avoided the repeated spotting and scraping of zones from a number of plates. Hence the technique of ascending dry-column chromatography was further investigated for application to the preparative isolation of glycolipids.

Our interest in the analysis of the molecular species of glycosyl diglycerides by using the combined gas chromatographic–mass spectrometric technique, necessitated

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their isolation in larger amounts for initial chemical characterization, followed by a study of the distribution of their molecular species. Preparative TLC was used by Schwertner and Biale[12], who employed the solvent system of Gardner[13] for the isolation of monogalactosyl diglycerides, digalactosyl diglycerides and sulpholipids from plant mitochondria and chloroplasts. Although the above authors[12] observed appreciable differences in the R_F values of chlorophyll and monogalactosyl diglycerides in the solvent system acetone–acetic acid–water (100:2:1), results of the present work on thin layers of silica gel concur with the observations of Gardner[13] in that the separation of monogalactosyl diglycerides from chlorophyll can be effected by use of acetone alone as the developing solvent.

In spite of the stress laid by Schwertner and Biale[12] on the importance of using acetone–acetic acid–water (100:2:1) as the solvent system for the separation of digalactosyl diglycerides from sulpholipids, the present experiments showed that the use of acetone alone could not only achieve this separation, but could also separate the accompanying phospholipids as a class, when dry-column chromatography was used. The results obtained and the behaviour of sphingoglycolipids in this system are reported here. For the latter investigation, use was made of animal brain as the raw material from which the total lipids were extracted. The choice of brain tissue is of special significance for the present authors, owing to their interest in the molecular species of aldehydogenic lipids occurring in this tissue, the analysis of which is easily carried out by application of the combined gas chromatographic–mass spectrometric technique.

MATERIALS AND METHODS

With the exception of the lipid extracts, which were obtained by the Folch procedure[14], the materials and methods used in the present work have been described

TABLE I

FRACTIONS OF OX-BRAIN LIPIDS* ISOLATED BY ASCENDING DRY-COLUMN CHROMATOGRAPHY

Fraction No.	*Qualitative analysis** of the fraction*	*Weight of lipid fraction (g)*
1	90% phospholipid*** and 10% sulphatide§	0.5
2	90% phospholipid§§ and 10% sulphatide§	1.2
3	80% sulphatide§ and 20% ceramide monohexoside§§§	0.18
4	20% sulphatide§ and 80% ceramide monohexoside§§§	0.26
5	neutral lipids contaminated with 10% glycolipid	0.04
6	neutral lipids contaminated with 10% glycolipid	0.03

* 86% recovery of total lipid loading.

** Phospho-, amino- and aldehydrogenic lipids indicated by Dittmer's spray[15], ninhydrin and 2,4-dinitrophenylhydrazine[16] reagents, respectively.

*** Mostly phospholipids (phosphatidylcholine, phosphatidylserine and sphingomyelin) other than phosphatidylethanolamine.

§ Two spots (one with normal and the other with hydroxy fatty acids).

§§ Mostly phosphatidylethanolamine.

§§§ Two spots (one with normal and the other with hydroxy fatty acids).

TABLE II

FRACTIONS OF SPINACH-LEAF LIPIDS* ISOLATED BY ASCENDING DRY-COLUMN CHROMATOGRAPHY

Fraction No.	*Qualitative analysis of the fraction*	*Weight of the lipid fraction (g)*
1	70% phospholipid** and 30% sulpholipid***	0.298
2	30% phospholipid** and 70% sulpholipid***	0.062
3	5% phospholipid** and 95% sulpholipid***	0.058
4	mainly digalactosyl diglyceride*** contaminated with 5% of monogalactosyl diglyceride*** and colour	0.328
5	mainly monogalactosyl diglyceride*** contaminated with 5% of chlorophyll degradation products	0.164
6	mainly monogalactosyl diglyceride*** contaminated with 5% of chlorophyll degradation products	0.302
7	coloured compounds and neutral lipids§	1.323
8	column wash with methanol and refluxing with previously extracted silica gel from fractions 1–7 (mainly pigments)	0.250

* 90% recovery of total lipid loading.
** Indicated with Dittmer's reagent[15].
*** Indicated with naphthol spray reagent[17].
§ Charring with 50% H_2SO_4.

previously[11]. The lipids of ox brain were subjected to the Folch washing procedure[14] before column loading, in order to free the lipid extracts from gangliosides.

RESULTS AND DISCUSSION

Ox-brain lipids (2.5 g) and spinach-leaf lipids (3.1 g) were loaded on dry columns of 100 and 150 g of silica gel G, respectively, and were developed for 4 and 6 h respectively, as described earlier[11].

The extruded and cut fractions, after TLC, were pooled according to their chemical groups. The major fractions obtained are given in Tables I and II, respectively.

The glycolipid fractions isolated from both sources were further identified by infrared spectroscopy. The infrared spectra were determined on the preparatively isolated glycolipids from ox-brain lipids after being purified by preparative TLC in the system silica gel G/chloroform–methanol–water (65:25:4), and on those from spinach-leaf lipids being purified in the system silica gel G/acetone–acetic acid–water (100:2:1).

The infrared characteristics of the purified lipid fractions are given briefly as follows.

Ceramide monohexoside isolated from ox brain showed typical absorptions at 1650 cm^{-1} (amide I), 1550 cm^{-1} (amide II), 3450 cm^{-1} and 1060 cm^{-1}, both representing the hydroxyl group[18]. The sulphatide isolated from the same source showed, in addition to absorptions similar to those of ceramide monohexoside, absorptions at 1210–1260 cm^{-1} (S→O, asymmetric stretching) and 1040–1080 cm^{-1} (S→O, asymmetric stretching)[19].

The monogalactosyl and digalactosyl diglycerides isolated from spinach leaves

showed typical ester absorption at 1740 cm^{-1} and hydroxyl-group absorptions at 3450 cm^{-1} and 1060 cm^{-1}. The sulpholipid from the same source possessed all the absorptions detected for monogalactosyl and digalactosyl diglycerides, and, in addition, showed typical absorptions at 1350 cm^{-1} (asymmetric S=O), at 1160 cm^{-1} (symmetrical S=O) and at 900 cm^{-1} (S→O), thus characterizing the sulphonic acid nature of the lipid[20].

The present preparative chromatographic method is, however, not suitable for quantitative determinations. Its utility, on the other hand, lies in enabling substantial amounts of glycolipids to be prepared for the purpose of studies relating to lipid–protein interactions, lipidoses, etc.

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