Monogalactosyl Diglyceride and Digalactosyl Diglyceride From Commercial Rapeseed "Lecithin"

LARS-ÅKE APPELQVIST, Division of Physiological Chemistry, Chemical Center, University of Lund, Lund, Sweden

ABSTRACT

By silicic acid column separation small quantities of glycolipid-rich fractions have been isolated from commercial Swedish rapeseed "lecithin." Major components in these fractions are monogalactosyl diglyceride and digalactosyl diglyceride which have been identified by R_f on thin layer chromatography specific color reactions and IR spectrometry. The fatty acid patterns of the galactolipids are different from those of the major phospholipids of the same product, with less of palmitic and oleic and considerably more of linolenic acid.

INTRODUCTION

The major phospholipid components of rapeseed lecithin of Canadian, Polish and Swedish origin and their fatty acid spectra have recently been determined (1-3). Since mono- and digalactosyl diglycerides have been detected in extracts from both immature and fully mature rapeseed (4,5) it was considered of interest to search for these compounds in commercial rapeseed "lecithin," which is manufactured by aqueous precipitation from crude rapeseed oil. If these galactolipids were present in considerable quantities in rapeseed "lecithin" they could have significance for its technological properties which are somewhat different from those of soybean "lecithin." Furthermore, rapeseed "lecithin" was regarded as a possible source of pure galactolipids to be used as markers in analytical thin layer chromatography (TLC).

EXPERIMENTAL PROCEDURES

Commercial rapeseed lecithin was obtained from the Swedish Extraction Association, Karlshamn. A portion was dissolved in chloroform and filtered to remove solids. A 5 ml aliquot containing 1.2 g "lecithin" was applied to a 5 cm wide column packed to a height of 15 cm with silicic acid (Unisil, 100-200 mesh, Clarkson Chemical Co., Ltd., Williamsport, Pa.). The column was eluted with chloroform, chloroform-acetone (1:1, v/v), acetone and methanol as described by Rouser et al. (6). Aliquots of fractions A and D and the entire fractions B and C were evaporated and weighed (Table I). No further analysis was performed on fractions A and D and thus the possibility cannot be excluded that traces of galactolipids were present in these fractions. The total recovery was 81%.

Fractions B and C were subjected to preparative TLC in toluene-ethyl acetate-ethanol (2:1:1 v/v). See (7) for the separation characteristics of this solvent system. The thin layer plates were sprayed with 0.2% ethanolic dichlorofluorescein and appropriate bands eluted after inspection under UV light. To aliquots of the major fractions from the preparative thin layer chromatograms were added known amounts of heneicosanoic acid. Fatty acid methyl esters were prepared by successive treatments with sodium methylate and boron trigluoride in methanol as described elsewhere (8). The fatty acid composition was analyzed on butanediol succinate (BDS) columns in a Varian gas chromatograph, model 2100 (5). Peak areas were determined on a 1 mV recorder (Varian model 20) equipped with a Disc integrator. Percentage composition was determined after internal normalization, since appropriate standard mixtures gave small deviations from stated composition. The results shown in Table II are the mean of two determinations for monogalactosyl diglyceride (MGDG) and four for digalactosyl diglyceride (DGDG), since the latter compound was present in two of the TLC bands from fraction C, a probably consequence of trailing.

TABLE I

Fractionation of Rapeseed "Lecithin" on a Silicic Acid Column

Fraction	Eluting solvent	Weight, mg	Principal components
A	Chloroform	293	"Neutral lipids"
B	Chloroform-acetone (1:1)	(66)[a]	(ESG), MGDG, SG[b,c]
C	Acetone	27	SG, DGDG[b,c]
D	Methanol	588	Phospholipids

[a]The weight data for fraction B probably do not entirely represent lipid material since the recovery from subsequent preparative thin layer chromatography of fraction B was only 35%. On the other hand fraction C gave upon preparative TLC a recovery of 80%.

[b]Arranged in order of increasing polarity.

[c]Abbreviations: ESG, esterified steryl glucoside; MGDG, monogalactosyl diglyceride; SG, steryl glucoside; DGDG, digalactosyl diglyceride.

TABLE II

Amounts and Fatty Acid Spectra of Monogalactosyl Diglyceride and Digalactosyl Diglyceride Isolated From Rapeseed "Lecithin"

Compound	Yield from lecithin, %[a]	Fatty acid composition, %[b]										
		16:0	16:1	16:2, 17:0	16:3	18:0	18:1	18:2	18:3	20:0	20:1	22:1
MGDG[c]	0.16	6.2	0.5	4.6	14.5	1.0	8.6	29.1	27.1	0.6	1.6	4.3
DGDG[c]	0.65	5.0	0.6	2.8	6.3	0.7	5.8	37.4	39.3	0.6	0.8	0.2

[a]Calculated via the internal standard technique in gas chromatographic analysis. The figure 280 (= 18:2) was used as mean molecular weight for the fatty acids of MGDG and DGDG in the transformation of amounts of fatty acid methyl esters to amounts of acyl lipids. The figures thus derived are lower than those derived by weighing of fractions from preparative thin layer chromatograph.

[b]Small amounts of other fatty acids, mainly 12:0 and 14:0, were included in the calculations but excluded from the tabulation.

[c]Abbreviations as in Table I.

The figures given represent the means of the two TLC bands. Other aliquots were analyzed by analytical TLC on precoated silica gel plates (Merck) using toluene-ethyl acetate-ethanol and chloroform-methanol-water (65:25:4).

Comparison of the R_f of the various compounds in the fractions studied with those for known markers MGDG, DGDG, esterified steryl glucoside (ESG) and steryl glucoside (SG) (see text to Table I) and the specific color development with 50% H_2SO_4 after brief heating at ca. 110 C supplied evidence for the identifications presented in Tables I and II. Further proof of the identification of MGDG and DGDG was arrived at by running IR spectra of appropriate fractions in KBr-pellets (Unicam Instruments, Cambridge, U.K., model SP 200 and Perkin Elmer model 521) of the fractions under discussion. The spectra for the two compounds isolated from rapeseed "lecithin" were very similar to those published earlier for MGDG and DGDG from spinach chloroplasts (9) and alfalfa leaves (10).

It may be noted that a trace of ESG appeared in the silicic acid fraction B. From other studies at this laboratory it is known that the major portion of ESG elutes with the "neutral" lipids in chloroform. Both fractions B and C appeared to contain SG with the major portion recovered in fraction C. The amount of SG was approximately half that found for DGDG; this point was not, however, further investigated.

DISCUSSION

The results presented in this paper indicate that monogalactosyl diglycerides and digalactosyl diglycerides are present in rapeseed "lecithin," but only in very small quantities. In view of the low concentrations of galactolipids it is understandable that previous authors have not reported data for these compounds in rapeseed "lecithin" (1-3). On the other hand the presence of DGDG in soybean "lecithin" was recently reported (11).

Whether the galactolipids are of significance for the technological utilization of rapeseed and soybean "lecithin" remains to be investigated. However, it appears to be clearly established that glycolipids play a role in bread making (12) and hence the galactolipids of "lecithin" might have significance in some applications, although they are present in small quantities. The low yield seems, however, to preclude rapeseed "lecithin" as a convenient source of pure galactolipids, necessary as markers for TLC. The presence of about four times as much DGDG compared to MGDG is consistent with other data concerning the total lipid extracts of slightly immature rapeseed (13). In actively photosynthesizing tissues on the other hand there is often more of MGDG than of DGDG; see, e.g., (5) and *loc. cit.* for information on the galactolipids of various organs of *Brassica napus.*

The fatty acid spectra of the galactolipids are very different from those of the major phospholipids of rapeseed "lecithin" (1,2), with less palmitic and oleic and considerably more linolenic acid (Table II). Furthermore, a hexadecatrienoic acid (16:3) is present in considerable amounts, with comparatively more in MGDG than in DGDG. In this context no detailed studies were made on the fatty acid ester with the retention time of 16:3 but it is probably identical to the 7,11,14-hexadecatrienoic acid isolated long ago from rapeseed leaves (14). Other studies in this laboratory have shown that the gas liquid chromatography (GLC) peak designated 16:3 is indeed a tri-ene and that the 16:3 is predominantly but not exclusively associated with the MGDG (5). Finally there is some erucic acid in MGDG but only a trace in DGDG. A definite comparison on this point with the phospholipids is difficult since one recent paper (2) shows no figure for 22:1 in the phospholipids whereas another reports 4-7% (3).

All major compositional differences between MGDG and DGDG as well as between the two galactolipids and phospholipids are similar to those found from other recent studies in our laboratory of the phospho- and galactolipids of different organs of *Brassica napus* (5).

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