Main Constituents of Rapeseed Lecithin

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Abstract

Commercial rapeseed lecithin has been analyzed after separation by silicic acid column chromatography. Besides neutral oil (40%), four major constituents have been found, viz., phosphatidyl ethanolamine (18%), phosphatidyl inositol (8%), phosphatidyl choline (16%) and sterol glycosides (8%). Among the minor fractions lysophosphatidyl ethanolamine accounts for about 2%. The phosphatides are characterized by low erucic acid content and the major fatty acids are palmitic, oleic and linoleic acids.

Introduction

PREVIOUS ANALYSES OF VEGETABLE lecithin gums have mostly been based upon ethanol fractionation. By this procedure the gums were separated in a soluble fraction, lecithins, and an insoluble fraction, cephalins. Generally the cephalins, which include phosphatides such as phosphatidyl ethanolamine and phosphatidyl inositol, have been reported to be the major constituents of rapeseed lecithin (1–3). By means of paper chromatography Sulser (4) later identified serine, ethanolamine, choline and inositol from hydrolyzed rapeseed phosphatides. Weenink and Tulloch (5) fractionated rapeseed lecithin on DEAE-cellulose/silicic acid columns and reported 22% phosphatidyl ethanolamine, 18% phosphatidyl inositol, 15% phosphatidyl choline and 16% of other acidic compounds.

Experimental Procedures

About 2 g of commercial rapeseed lecithin,[1] free from the major part of neutral oil by defatting in cold acetone (6), were dissolved in chloroform. The solution was transferred onto a 150 g silicic acid (Mallinckrodt, 100 mesh) column (Ø = 4 cm). The sample was allowed to settle on top of the column and eluted by gradient elution with methanol-chloroform.[2] The eluate was continuously collected in 10 ml fractions with an automatic fraction collector. A total of 200 fractions was collected and each analyzed by TLC (Fig. 1). Fractions belonging to identical components according to TLC were pooled together. Due to overlapping of adjacent components (Fig. 1) several intermediate fractions containing two or more components had to be separately combined. In this way the number of fractions was reduced to 17, the solvent evaporated, the residue weighed and rechecked by TLC. Each of these fractions was then successively and repeatedly rechromatographed on columns, until complete separation of the final nine components-fractions, pure according to TLC, had been achieved (Fig. 2 and Table I).

TLC was performed on 0.25 mm layers of silica gel G (Merck, Darmstadt, Germany). The plates were eluted with chloroform-methanol-water (65:25:4) and after drying visualized with 2′,7′-dichlorofluorescein, ninhydrin and Dragendorffs' reagent (7), respectively.

Phospholipids were hydrolyzed in 6 N HCl according to Sulser (4). Choline was identified by paper chromatography (4), ethanolamine and inositol by TLC (8). Methanolysis of sterol glycosides was performed according to Dawson (9). Carbohydrate content was estimated colorimetrically (10). The sterols were analyzed by gas chromatography on 6 ft × ⅛ in. glass column with 3% SE-30 Anakrom ABS 100/110, isothermally at 230 C. The phospholipids were transesterified (11) and the methyl esters gas chromatographed on 6 ft × ⅛ in. column with 12% DEGS on Anakrom ABS 70/80, isothermally at 180 C.

[1] Rapeseed lecithin mainly of the species *Brassica napus* manufactured by Svenska Extraktionsföreningen, Karlshamn, Sweden.
[2] Exponential increase of methanol to chloroform; apparatus according to Alm et al. (20).

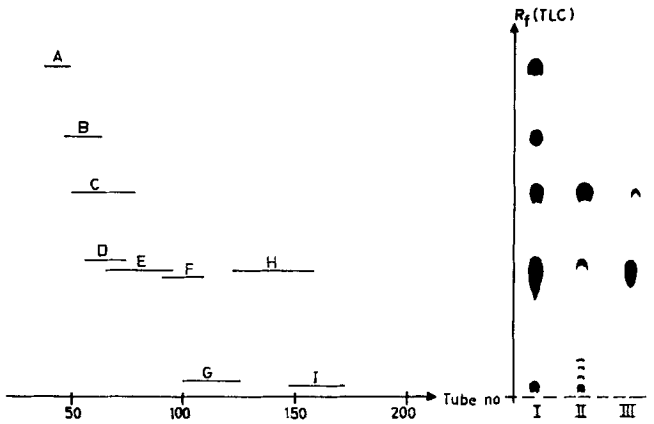

FIG. 1. Elution pattern of rapeseed lecithin on silicic acid column eluted with chloroform-methanol (gradient). Positions of components-fractions according to fraction (tube) number and Rf (TLC)-value after the first column chromatographic separation. To the right: TLC of starting material spotted with dichlorofluorescein (I), ninhydrin (II) and Dragendorffs' reagent (III).

Results and Discussion

The composition of rapeseed lecithin is given in Table I. Fraction A consisted of nonpolar lipids, mainly triglycerides. Fraction B was a solid white substance, melting point about 290 C. Strong IR-absorption bands at 3500–3300 cm^{-1} and 1100–1000 cm^{-1} indicated sterol glycosides (12). Carbohydrate amounted to about 30% expressed as glucose and the

TABLE I

Composition of Rapeseed Lecithin as Determined by Separation on Silicic Acid Column

Fraction	Component	Weight[a] %	N %	P %	N/P
A	Nonpolar lipids (neutral oil)	38.1	....	....	
B	Sterol glycosides[b]	7.9	....[c]	....	
C	Phosphatidyl ethanolamine	17.5	1.6	3.6	0.99
D	Phosphatidyl inositol	7.6	0.1	3.6	
E	Phosphatidyl choline[d]	6.0	1.6	3.8	0.94
F	Lysophosphatidyl ethanolamine	2.0	1.8	4.1	0.97
G	Unidentified	2.3			
H	Phosphatidyl choline[d]	10.2	1.8	3.9	1.02
I	Unidentified	2.6			
		94.2[e]			

[a] Mean value of triplicate runs.
[b] Brassicasterol (11%), campesterol (28%) and sitosterol (61%).
[c] C: 70.4%; H: 9.5%.
[d] See text.
[e] Residue: minor and not isolated components.

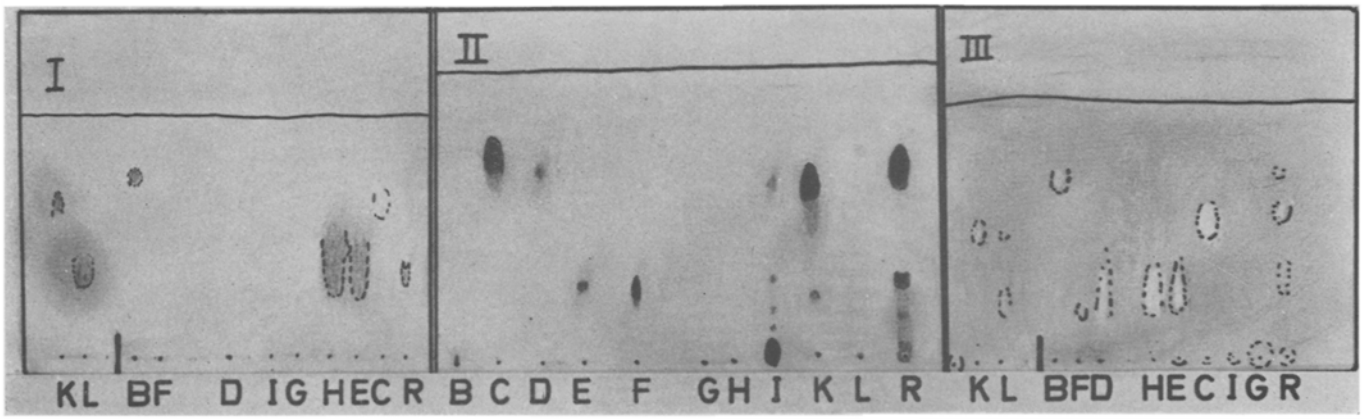

FIG. 2. TLC of rapeseed lecithin components (final fractions) spotted with Dragendorffs, reagent (I), ninhydrin (II) and dichlorofluorescein (III). B–I. Fractions according to text; R. Rapeseed lecithin (original); K. Phosphatidyl ethanolamine (synthetic); L. Phosphatidyl choline (synthetic).

sterol composition agreed with reported data of rapeseed sterols (13,14).

The ninhydrin positive fraction C gave clear evidence of the presence of phosphatidyl ethanolamine which was further established by hydrolysis and elemental analysis. The inositol content of fraction D could not be settled directly in the original phospholipid by $AgNO_3$ reagent as has been reported (7). After hydrolysis, however, the carbohydrate was clearly established by TLC. Fractions E and H were eluted as two separated bands on the silicic acid column (Fig. 1). Their identity as phosphatidyl choline was verified by NMR (15) and by their choline content after hydrolysis. The separation of the phosphatidyl choline into two fractions was first accounted for as an artifact during the column chromatography. Repeated runs gave, however, the same results. Later on the same phenomenon but to a minor degree was observed in the case of phosphatidyl ethanolamine. Fraction F was eluted between the above phosphatidyl choline fractions. It reacted with ninhydrin, and ethanolamine was proved after hydrolysis. Other minor fractions G and I have not been identified and are probably not uniform components. Fraction G was first tentatively assumed to be lysophosphatidyl choline, but the assumption was disproved, since choline could not be established after hydrolysis. Silicic acid chromatography gave far from satisfying separation (Fig. 1); especially fractions B, C and D contaminated each other considerably. By using gradient elution the formation of "ghost"-bands, which are rather frequent in stepewise elutions, were avoided. This did not, however, increase the chromatographic efficiency; probably better separations can be obtained by manually adjusting the chloroform-methanol ratios or by combining the silicic acid column with other types of columns (16–18).

The fatty acid composition of the phospholipids is given in Table II. The most outstanding difference compared to rapeseed oil (fraction A) is the low erucic acid content, which agrees with statements by others who, however, have reported complete absence of this acid (5,19).

TABLE II

Fatty Acid Composition of Rapeseed Lecithin

Fraction-component	Fatty acid (wt %)[a]								
	C16	C16:1	C18	C18:1	C18:2	C18:3	C20:1	C22:1	C24:1
A-Neutral oil	3	..	1	11	14	8	10	50	1
C-Phosphatidyl ethanolamine	16	1	1	28	38	11	1	4	..
D-Phosphatidyl inositol	34	1	1	18	33	10	..	3	..
E-Phosphatidyl choline	10	1	1	32	41	7	1	7	..
H-Phosphatidyl choline	14	2	2	28	39	11	1	4	..

[a] Fatty acids less than 1% have been omitted.

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REFERENCES

1. Heiduschka, A., and W. Neuman, J. Prakt. Chem. *151*, 1 (1938).
2. Hilditch, T. P., and W. H. Pedelty, Biochem. J. *31*, 1964 (1937).
3. Rewald, B., Soc. Chem. Ind. (London) *56*, 403 T (1937).
4. Sulser, H., Mitt. Lebensm. Hyg. *45*, 251–294 (1954).
5. Weenink, R. O., and A. P. Tulloch, JAOCS *43*, 327–329 (1966).
6. Pardun, H., Fette, Seifen, Anstrichmittel *64*, 536–541 (1962).
7. Skidmore, W. D., and C. Entenman, J. Lipid Res. *3*, 471–475 (1962).
8. Stahl, E., ed., "Dünnschicht-Chromatographie," 2nd ed., Springer-Verlag, Berlin-Heidelberg-New York 1967.
9. Dawson, R. M. C., Biochem. J. *75*, 45–53 (1960).
10. Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, Anal. Chem. *28*, 350–356 (1956).
11. Bowyer, D. E., W. M. F. Leat, A. N. Howard and G. A. Greshamn, Biochem. J. *89*, 24 P (1963).
12. Kiribuchi, T., T. Mizunaga and S. Funghashi, Agr. Biol. Chem. *30*, 770–778 (1966).
13. Karleskind, A., F. Audiau and J. P. Wolff, Rev. Franc. Corps Gras *12*, 399–403 (1965); *13*, 165–173 (1966).
14. Fedeli, E., A. Lanzani, P. Capella and G. Jacini, JAOCS *43*, 254–256 (1966).
15. Chapman, D., and A. Morrison, J. Biol. Chem. *241*, 5044–5052 (1966).
16. Rhodes, D. N., and C. H. Lea, Biochem. J. *65*, 526–533 (1957).
17. Rouser, G., A. J. Bauman and G. Kritchewsky, Am. J. Clin. Nutr. *9*, 112–123 (1961).
18. Rouser, G., G. Kritchewsky, D. Heller and E. Lieber, JAOCS *40*, 425–454 (1963).
19. Rao, S. Venkob, M. M. Paulose and B. Vijayalakshmi, Lipids *2*, 88 (1967).
20. Alm, R. S., R. J. P. Williams and A. Tiselius, Acta Chem. Scand. *6*, 826 (1952).

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