# MINOR PHOSPHOLIPIDS OF Dipthychocarpus strictus AND Crambe schugnana

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Continuing a study of the phospholipids of the seeds of plants of the family Cruciferae [1, 2], we have investigated the minor fractions of the total phospholipids of the seeds of Dipthychocarpus strictus (Fisch.) Trautv. and Crambe schugnana Korsh. When the total phospholipids of the seeds of D. strictus were separated on a column of silica gel, the chloroform–methanol (9:1) fraction yielded a phospholipid with $R_f$ 0.9 in system 1 ($X_1$-PL, making up 45% of the total), which was freed from pigments by preparative TLC. This phospholipid is not revealed with ninhydrin and does not react with phospholipase $A_2$; its IR spectrum contains, in addition to bands characteristic for the glycerophospholipids [3, 4], absorption bands in the 1650 and 1540 $cm^{-1}$ regions which do not disappear on hydrogenation. On the basis of the facts given above and also of the results of a comparison of the chromatographic mobility of $X_1$-PL with the product of the reaction of acetyl chloride with phosphatidylethanolamine, $X_1$-PL was shown to be identical with the monosubstituted amide of phosphatidylethanolamine (N-acyl-PE) [5-7]. The total fatty acids and also those corresponding to the O- and N-acyl groups of the phospholipid investigated were isolated and analyzed by known methods [6, 7] (Table 1).

The total phospholipids of the seeds of C. schugnana according to two-dimensional chromatography, contained three minor components: $X_1$, $X_2$, and $X_3$. On column chromatography of the total phospholipids on silica gel, $X_1$-PL was eluted in the homogeneous state by a mixture of chloroform and methanol (95:5), chloroform–methanol (4:1) eluted $X_2$-PL in admixture with phosphatidylethanolamine (it was purified preparatively), and $X_3$-PL was eluted by methanol in the pure form.

$X_1$-PL was identified as N-acylphosphatidylethanolamine by the method indicated above. Information on its fatty-acid composition is given in Table 1.

On the basis of the results of IR spectroscopy, analysis for N and P (2.5 and 5.3%, respectively), and also from the water-soluble products of acid hydrolysis (glycerol and choline), $X_3$-PL was identified as lysophosphatidylcholine. The fatty-acid composition of the lysophosphatidylcholine is given in Table 1.

In system 1 and 2, $X_2$-PL (4.6% of the total phospholipids) has $R_f$ 0.7 and 0.33, respectively, and is shown up by the Vas'kovskii and ninhydrin reagents, and its IR spectrum agrees with that given in the literature for glycerophospholipids [3, 4, 8]. Glycerol and serine were identified (system 3) among the products of acid hydrolysis. The results obtained show that $X_2$-PL is phosphatidylserine. According to the literature, phosphatidylserine is either absent or is present only in very small amounts (1.2-5% of the total phospholipids) in plant seeds [9].

The total fatty-acid composition of the phosphatidylserine was determined by alkaline saponification. To determine the position distribution of the fatty acids, the phosphatidylserine was cleaved with phospholipase $A_2$ (snake venom), and the fatty acids from positions 1 and 2 of the glycerol moiety of the molecule were analyzed as described previously [1, 2]. The results of the GLC analysis of the fatty acids are given in Table 1.

The qualitative set and quantitative composition of the fatty acids in the N-acylphosphatidylethanolamines obtained from D. strictus and C. schugnana are not identical: in the first case lauric acid ($C_{12:0}$) and Erucic acid ($C_{22:1}$) are absent, and linolenic acid predominates among the unsaturated acids. In the second case, the $C_{12:0}$ and $C_{22:1}$ acids are present, and linoleic acid predominates among the unsaturateds. The erucic acid is found mainly in the amide-bound form. In both cases palmitic acid predominates among the saturateds, and the N-

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TABLE 1. Fatty-Acid Compositions of the Minor Phospholipids of the Seeds of D. strictus and C. schugnana

| Fatty acid | D. strictus | | | C. schugnana | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N-acylphosphatidylethanolamine | | | N-acylphosphatidylethanolamine | | | phosphatidylserine | | | lysophosphatidylcholine |
| | total | O-acyl | N-acyl | total | O-acyl | N-acyl | total | position 1 | position 2 | |
| $C_{12:0}$ | — | — | — | 8,0 | 3,1 | 14,0 | 1,6 | — | 4,9 | 1,2 |
| $C_{14:0}$ | 5,6 | 0,9 | 12,2 | 4,4 | 3,1 | 11,7 | 2,5 | — | 5,1 | 1,2 |
| $C_{16:0}$ | 16,8 | 23,8 | 10,0 | 10,8 | 16,0 | 5,5 | 54,1 | 85,7 | 20,3 | 13,0 |
| $C_{16:1}$ | 6,8 | 1,6 | 13,1 | 6,0 | 5,0 | 8,0 | 2,9 | — | 6,7 | 2,5 |
| $C_{18:0}$ | 6,6 | 1,8 | 5,0 | 7,3 | 3,7 | 5,0 | 3,0 | — | 4,1 | 1,5 |
| $C_{18:1}$ | 18,2 | 22,0 | 16,5 | 16,2 | 20,0 | 11,2 | 16,0 | 11,3 | 20,9 | 25,0 |
| $C_{18:2}$ | 20,4 | 18,6 | 23,2 | 23,0 | 30,0 | 11,1 | 16,0 | 3,0 | 33,0 | 27,2 |
| $C_{18:3}$ | 25,6 | 31,3 | 20,0 | 16,1 | 16,1 | 15,5 | 3,9 | — | 5,0 | 13,0 |
| $C_{20:0}$ | — | — | — | — | — | — | — | — | — | 7,8 |
| $C_{22:1}$ | — | — | — | 8,2 | 3,0 | 18,0 | — | — | — | 7,6 |
| ΣS | 29,0 | 26,5 | 27,2 | 30,5 | 25,9 | 36,2 | 61,2 | 85.7 | 34,4 | 24,7 |
| ΣU | 71,0 | 73,5 | 72,8 | 69,5 | 74,1 | 63,8 | 38,8 | 14,3 | 65,6 | 75,3 |

acyls have a more saturated nature than the O-acyls, which is particularly pronounced in the N-acylphosphatidylethanolamine obtained from C. schugnana.

The phosphatidylserine is the most highly saturated fraction of the total phospholipids of C. schugnana [2], where more than half the fatty acids consist of palmitic acid (54.1%). Erucic acid is absent from the phosphatidylserine, and the main unsaturated acids are oleic and linoleic. Only one saturated acid (palmitic) and two unsaturated acids are esterified in position 1 of the glycerol moiety of the phosphatidylserine molecule. The comparatively saturated nature of the fatty acids in position 2 (34.4%) is explained by the saturated nature of the whole phosphatidylserine molecule.

On the basis of the results of the position distribution of the fatty acids, 24 possible molecule forms of phosphatidylserine have been calculated, and in relation to saturation these can be arranged in the following way (%): disaturateds 29.5; diunsaturated 9.4; saturated–unsaturateds 56.2; unsaturated–saturateds 4.9.

## EXPERIMENTAL

For chromatography we used type KSK silica gel (up to 100 μm for a thin layer, and 160-250 μm for a column) and the following solvent systems: 1) chloroform–methanol–water (65 : 35 : 5); 2) chloroform–methanol–25% ammonia (65 : 35 : 5); and 3) 2% ammonia–methanol (2 : 3). The acid hydrolysis of the phospholipids was performed in sealed tubes with 10% hydrochloric acid at 100°C. The total fatty acids of the phospholipids were isolated by alkaline saponification (5% KOH in methanol, room temperature, 15-18 h). The fatty acid composition was determined by the GLC method on a UKh-2 chromatograph at 197°C (column 2.5 m long); the stationary phase was 17% of polyethyleneglycol succinate on Celite-545 (60-80 mesh).

The IR spectra were taken on a UR-20 instrument in the form of films.

Enzymatic Hydrolysis of the Phosphatidylserine. To 60 mg of a sample of phosphatidylserine in 20 ml of ether was added 4 mg of the venom of the Azerbaidzhan kufi dissolved in 0.7 ml of 0.1 M tris buffer (pH 8.5). The mixture was stirred at 37°C (water bath temperature). The reaction was complete after 3 h. The hydrolysis products were separated preparatively in system 1: the zone with $R_f$ 0.9 consisted of the fatty acids from position 2, and that with $R_f$ 0.33 consisted of lysophosphatidylserine.

## SUMMARY

From the combined phospholipids of the seeds of Dipthychocarpus strictus (Fisch.) Trautv. the less polar phospholipid with $R_f$ 0.9 has been isolated, and on the basis of spectra and chemical information it has been identified as N-acylphosphatidylethanolamine. The total fatty-acid composition and also the O- and N-acyls have been determined. From the combined phospholipids of the seeds of Crambe schugnana Korsh. three minor phospholipids have been isolated: N-acylphosphatidylethanolamine, lysophosphatidylcholine, and phosphatidylserine.

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# PHOSPHOLIPIDS OF THE MEDIUM-FIBER COTTON PLANT VARIETY 159-F


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We have investigated the phospholipids (PLs) of the seed kernels of the industrial medium-fiber cotton plant variety 159-F of introduction 2, 1972 harvest (sample 1), and "Élite" of the same variety, 1974 harvest (sample 2), collected in the Pakhtaabad region of Fergana oblast.

The seed kernels of the variety under consideration were characterized by the following indices [1] (%):

| Index | Sample 1 | Sample 2 |
|---|---|---|
| Proportion of kernels | 59.55 | 60.43 |
| Moisture content | 5.3 | 5.2 |
| Oil content | 38.42 | 39.03 |
| Phosphorus content | 0.98 | 0.98 |
| Gossypol content | 0.76 | 0.75 |

As can be seen from the figures given above, the main indices of the seed kernels of the samples studied are practically identical.

The ground seed kernels were defatted with petroleum ether and were freed from gossypol with acetone as described previously [2]; phospholipids were exhaustively extracted with a mixture of ether and methanol (2 : 1) [3]. The crude PL fraction was precipitated with acetone (0°).

To determine the distribution of the PLs in their isolation, all the lipid fractions (for sample 1) were checked with respect to yield and lipid phosphorus content (Table 1) and subjected to qualitative chromatography in a thin layer of silica gel (systems 1 and 2). The acetone-insoluble part was enriched with PLs, 2.57% P, and with carbohydrates (25%), while 12.3% of the lipid phosphorus passed into the acetone-soluble part. The carbohydrates were separated from the acetone-insoluble part and were determined by quantitative gel filtration on "Mol-Selekt" G-25 [4], the PLs being eluted with the solvent system chloroform–methanol–water (90 : 10 : 1), and the carbohydrates with water. The PLs of the acetone-soluble part were regenerated in a column containing silica gel, the neutral lipids being eluted with chloroform and the PLs with methanol. This part of the PLs was added to the bulk of the PLs from the acetone-insoluble part (see Table 1).


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