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# N-ACYLATED PHOSPHOLIPIDS AND LYSOPHOSPHOLIPIDS OF KENAF SEEDS

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In the separation of the total phospholipids (PLs) of kenaf seeds of the variety Kuban'-333 (*Hibiscus cannabinus*, family Malvaceae) [1, 2] in a column of silica gel, the chloroform–methanol (9:1) fraction yielded a mixture of two PLs — $X_1$ and $X_2$ — amounting to 12.3% of the total. These minor components of the PLs were separated preparatively in system 1. The $R_f$ value of the $X_1$-PL in this system was 0.9 and that of the $X_2$-PL 0.65. Both compounds were revealed with the Vas'kovskii reagent and gave a negative ninhydrin reaction. In chromatographically homogeneous $X_1$- and $X_2$-PLs the N:P ratio was ∿1. Their IR spectra contained, in addition to the absorption bands characteristic for glycerophospholipids [3, 4] the bands of an amide carbonyl at 1650 and 1540 $cm^{-1}$. The fatty-acid compositions of the $X_1$- and $X_2$-PLs were determined by alkaline hydrolysis and methylation of the fatty acids followed by GLC analysis (Table 1). The acid hydrolysis of the $X_1$- and $X_2$-PLs showed that their aqueous hydrolyzates contained fatty acids, glycerol (system 2), and ethanolamines (system 3). The spectral characteristics, and also the results of analyses of the products of acid hydrolysis permit the assumption that the $X_1$- and $X_2$-PLs are possibly N-acylated derivatives of phosphatidylethanolamine. On mild alkaline hydrolysis the $X_1$-PL gave fatty acids (see Table 1, O-acyl) and a partially deacylated product which gave a positive reaction for phospholipids (Vas'kovskii reagent), was not revealed by ninhydrin, and was more polar ($R_f$ 0.34, system 1) than $X_2$-PL. The fatty acids of the de-O-acylated PL were isolated by 10% methanolic alkali (see Table 1, N-acyls) [5]. Like the initial $X_1$-PL, the product of its partial deacylation on acid hydrolysis gave fatty acids, glycerol, and ethanolamine. When homogeneous $X_1$-PL was adsorbed from $CHCl_3$ on to alkaline alumina (room temperature, 24 h) in addition to the initial spot a spot of $X_2$-PL was observed. The facts given confirm the structure of a N-acylphosphatidylethanolamine for $X_1$-PL. On the basis of the nonidentity of $X_2$-PL with partially deacylated $X_1$-PL and of the formation of $X_2$-PL from $X_1$ ($Al_2O_3$), the structure of a N-acyllysophosphatidylethanolamine can be suggested for $X_2$-PL. However, the $L_1$-PL did not undergo enzymatic hydrolysis with phospholipase A and we have therefore been unable to make a direct comparison of $X_2$-PL with 1-acylglycerylphosphoryl-N-acylethanolamine.

The reaction of acetyl chloride with phosphatidylethanolamine gave N-acetylphosphatidylethanolamine, the chromatographic behavior of which (system 1) was identical with that of $X_1$-PL. The IR spectrum of the product obtained also showed absorption bands of an amide carbonyl at 1660 and 1560 $cm^{-1}$.

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TABLE 1. Fatty-Acid Compositions of the Minor Components of the Total Phospholipids (PLs) of Kenaf, %

| Fatty acid | N-Acyl-lysophos-phatidyl-ethanol-amine ($X_2$-PL) | N-Acylphosphatidyl-ethanolamine ($X_1$-PL) | | | Lysophos-phatidyl-choline |
|---|---|---|---|---|---|
| | | total | O-acyl | N-acyl | |
| $C_{12:0}$ | 1,9 | 1,5 | 5,4 | — | 1,5 |
| $C_{14:0}$ | 1,4 | 1,4 | 2,6 | 8,5 | 0,9 |
| $C_{16:0}$ | 15,2 | 26,7 | 24,0 | 25,2 | 22,6 |
| $C_{16:1}$ | 2,1 | 2,7 | 1,8 | 8,5 | 1,4 |
| $C_{18:0}$ | 2,9 | 4,3 | 1,1 | 4,6 | 1,8 |
| $C_{18:1}$ | 11,4 | 19,1 | 17,5 | 17,8 | 31,5 |
| $C_{18:2}$ | 64,3 | 42,6 | 45,4 | 32,0 | 39,7 |
| $C_{18:3}$ | 0,8 | 1,7 | 2,2 | 3,4 | 0,6 |
| Saturated acids | 21,4 | 33,9 | 33,1 | 38,3 | 26,8 |
| Unsaturated acids | 78,6 | 66,1 | 66,9 | 61,7 | 73,2 |

N-Acylphosphatidylethanolamines and N-acyllysophosphatidylethanolamines have been detected previously in the seeds of several plants [6, 7], but we are the first to have found them in kenaf seeds.

In all the phospholipids investigated, among the saturated acids palmitic predominates and among the unsaturated acids, linoleic (see Table 1).

The fatty acids of the N-acyllysophosphatidylethanolamine are more unsaturated than those of the N-acylphosphatidylethanolamine. The fatty acids bound to nitrogen are more saturated than those of the glycerol moiety of the molecule.

When the total PLs were eluted from a silica gel column with chloroform—methanol (2:1), lysophosphatidylcholine was eluted in addition to phosphatidylcholine. They were separated preparatively in systems 1 and 4. Lyso-PC has $R_f$ 0.15, N 2.4%, P 5.2%. Its IR spectrum shows, in addition to the bands commonly known for PLs, a band at 975 $cm^{-1}$ which is characteristic for $\overset{+}{N}(CH_3)_3$ groups. A hydrolyzate from the acid hydrolysis of lyso-PC was found to contain glycerol and choline (TLC).

The fatty acids were obtained by the saponification of the lyso-PC with alcoholic alkali (without heating) and were analyzed by GLC (see Table 1).

## EXPERIMENTAL

For column chromatography we used type KSK silica gel, 160-250 μ, and for thin layer chromatography the same material with a grain size of 125 μ. The following solvent systems were used: 1) chloroform—methanol—ammonia (65:35:5); 2) isopropanol—ammonia—water (7:1:2) [8]; 3) 2% $NH_4OH$—$CH_3OH$ (2:3) [9]; 4) chloroform—methanol—water (65:35:5).

The acid hydrolysis of the PLs was carried out as described previously [1, 2], and alkaline hydrolysis according to Stahl [5].

Partial Deacylation of N-Acylphosphatidylethanolamine. A mixture of 80 mg of the substance and 10 ml of 0.1 M methanolic NaOH was incubated at 37°C for 80 min. Then the mixture was neutralized with 20 ml of ethyl formate and evaporated to dryness, and the residue was dissolved in 10 ml of dilute ethanol (1:1) and shaken with petroleum ether (40-60°C; 20 ml). The petroleum ether extract was washed with 50% ethanol (2 × 15 ml). The combined aqueous ethanolic extracts were treated with chloroform (3 × 15 ml), and the chloroform solution was evaporated. The yield of the petroleum ether fraction was 20 mg (fatty acids from the glycerol part of the molecule), and that of the chloroform fraction 55 mg (traces of FAs and glycerylphosphoryl-N-acetylethanolamine). The hydrolysis products were separated preparatively in system 1. The fatty acids were methylated and were analyzed by GLC.

Preparation of N-Acetylphosphatidylethanolamine. To 40 mg of PE in 5 ml of chloroform was added 0.5 ml of acetyl chloride. The mixture was left at room temperature for 2 h. TLC showed the presence of one phosphorus-containing compound with $R_f$ 0.9 ($R_f$ of the initial PE 0.5; system 1), which was not revealed by ninhydrin. The solution was shaken with a satu-

rated solution of sodium bicarbonate, the upper layer was separated off, and the lower — chloroform — layer was distilled to small volume. After purification by preparative TLC the IR spectrum of N-acetylphosphatidylethanolamine was recorded.


## SUMMARY

Three minor components have been isolated from the total PLs of kenaf seeds of variety Kuban'-333 by means of column and preparative thin-layer chromatography and they have been characterized by lysophosphatidylcholine and N-acylphosphatidylethanolamine and its lyso analog.


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# NAPHTHOQUINONES OF *Lithospermum erythrorhizon*


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In recent years, investigations of the chemical composition of plants of the family Boraginaceae have been carried on intensively in view of the discovery of physiologically active compounds in them. Naphthoquinones from the roots of species of *Lithospermum* and *Arnebia* possess an antimicrobial action [1], and they have been reported to have an antitumoral activity [3].

The roots of *Lithospermum erythrorhizon* Sieb. et Zucc., collected in Maritime Territory in July, 1975, contained about 3% of naphthoquinone pigments (on the dry weight of the roots). The aim of the present work was to determine their structure. From the air-dry roots of *L. erythrorhizon* by extraction with petroleum ether (40-70°C) followed by chromatography on columns of silica gel and Sephadex LH-20 we isolated seven pigments. Substance (IV) is a quinoid pigment of nonnaphthazarin structure with mp 72-74°C. Its structure is being determined. We did not detect in the plants from the Maritime Territory the β,β-dimethylacrylate of shikonin [R = $OCOCH{=}C(CH_3)_2$] found by Japanese workers in the roots of *L. erythrorhizon* [6]. The composition and physicochemical properties of the six naphthazarin pigments correspond to those given in the literature [6-9] and completely confirm the structures given. We have recorded the $^{13}C$ NMR spectra of these compounds for the first time (Table 1). It follows from an analysis of the spectrum that a change in the substituents at $C_{11}$ does not substantially affect the chemical shifts of the carbon atoms of the naphthoquinone moiety and of the isopentenyl part of the chain. The assignment of the signals of the carbon atoms was performed by the method of selective decoupling from the protons.


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