A COMPARATIVE STUDY OF THE PHOSPHOLIPIDS OF KENAF SEEDS

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Continuing an investigation of the phospholipids of various types of kenaf (*Hibiscus cannabinus* L.) we have studied the phospholipids of kenaf seeds of the variety "Opytnyi-1961." The isolation and purification of the total phospholipids was carried out by methods described previously. The yield of purified total phospholipids was 1.1% of the weight of the air-dry seeds. The qualitative and quantative compositions of the total phospholipids were determined by two-dimensional chromatography. Six phosphorus-containing compounds were detected, and these were separated quantitatively in the following sequence (%): 47.1, phosphatidylcholines (PCs, R_f 0.40); 20.2, phosphatidylinositols (PIs, R_f 0.67); 17.0, phosphatidylethanolamines (PEs, R_f 0.72); 8.4, N-acylphosphatidylethanolamines (N-acyl-PEs, R_f 0.76); 4.8, N-acyllysophosphatidylethanolamines (N-acyllyso-PEs, R_f 0.81); and 2.5, unidentified phospholipid (X, R_f 0.90).

It follows from the facts given that the combined phospholipids of the seeds of different varieties of kenaf [3-5] differ in the quantitative distribution of the individual phospholipids and, in some varieties, also in qualitative composition: The variety "Kuban'-333" contains lyso-PCs [2], which are not present in the other varieties of kenaf that have been studied. By combining the methods of column, thin-layer, and preparative chromatography, we obtained homogeneous fractions of the combined phospholipids the structures of which were confirmed by IR spectroscopy and by the results of acid, alkaline, and enzymatic hydrolysis [1, 2].

The fatty acid compositions of the phospholipids, the triglycerides, and the individual fractions, and also the position distribution of the fatty acids in the main fractions are given in Table 1. The results of the GLC analysis of the fatty acids shows that the combined phospholipids of this variety contain trace amounts of the 10:0 acid, which was localized in the PIs and N-acylated phospholipids, in the latter predominantly in the amide-bound form.

The possible molecular compositions of the main fractions of the phospholipids were calculated from the results of the position distribution of the fatty acids: in the PCs 43, in the PEs 50, in the PIs 52. In relation to unsaturation, these types can be represented in the following way:

	PCs	PEs	PIs
Disaturated	9.8	11.7	20.4
Saturated—unsaturated	38.5	49.5	56.3
Diunsaturated	41.0	30.6	17.5
Unsaturated—saturated	10.7	8.2	5.8

As can be seen from the figures given, the number of disaturated (SS) and saturated—unsaturated (SU) species increases in the sequence PCs → PEs → PIs, and the number of diunsaturateds in the opposite order: PIs → PEs → PCs. A comparison of the results obtained in this work and those given previously for other varieties showed that there are also differences in the molecular compositions of these fractions: in the main phospholipids of the total phospholipids of kenaf seeds of variety "Opytnyi-1961" there is a considerably larger amount of saturated—saturated and unsaturated—saturated species than for other varieties. This difference in the molecular species is due to the initial composition of the fatty acids and the selectivity of their pairing in the individual phospholipids. The investigation of the N-acyl-PEs and their lyso analogs was performed as described by Bomstein [6]. As compared with the preceding varieties, in the variety of kenaf investigated the molecules of the N-acyl-PEs are considerably more saturated, mainly through the amide-bound fatty acids. The

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TABLE 1. Composition and Position Distribution of the Fatty Acids in the Phospholipids of Kenaf Seeds of Variety "Opytnyi-1961"

Fatty acids	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	ΣП	ΣН
Total phospholipids	Tr.		0,2	0,3	22,7	0,9	3,7	28,8	41,3	2,1	26,9	73,1
Triglycerides			0,2	0,4	23,8	0,9	4,7	29,1	39,5	1,4	29,1	70,9
Phosphatidylcholines												
Total			1,2	0,9	28,8	1,3	4,2	32,9	29,5	1,2	35,1	64,9
Position 1			3,8	1,5	35,6	1,4	6,6	30,1	21,0	—	47,5	52,5
Position 2			0,6	0,9	15,1	1,6	2,6	32,5	44,6	2,1	19,2	80,8
Phosphatidylethanolamines												
Total			1,0	1,1	30,4	2,4	2,3	23,7	39,1	Tr.	34,8	65,2
Position 1			3,5	1,6	51,7	1,4	4,8	11,8	25,2	—	61,6	38,4
Position 2			2,1	3,2	11,1	4,4	3,0	27,7	44,5	4,0	19,4	80,6
Phosphatidylinositols												
Total		1,0	1,7	0,6	38,3	1,4	3,1	15,9	36,2	1,8	44,7	55,3
Position 1		5,1	5,0	2,0	61,5	3,1	6,7	8,8	7,8	Tr.	80,3	19,7
Position 2		1,9	2,3	1,2	21,3	1,1	3,3	21,7	43,9	3,3	30,0	70,0
N-Acylphosphatidylethanolamines												
Total	Tr.	2,5	6,0	3,0	32,6	2,4	6,3	23,6	19,4	4,2	50,4	49,6
O-acyl	Tr.	0,5	1,0	0,7	30,6	1,4	4,5	23,7	35,8	0,8	37,3	62,7
N-acyl	Tr.	12,6	38,5	8,5	14,0	4,8	2,4	7,6	8,4	3,2	76,0	24,0
N-Acyllysophosphatidylethanolamines												
Total	Tr.	0,9	0,9	1,1	14,9	2,3	3,7	15,4	58,7	2,1	21,5	78,5
O-acyl	Tr.	0,7	1,0	0,3	4,4	1,0	1,3	10,1	79,1	2,1	7,7	92,3
N-acyl	Tr.	9,0	10,1	6,2	29,1	10,8	2,3	9,2	17,3	6,0	56,7	43,3

NMR spectrum (δ scale) of the N-acyllyso-PEs has signals in the form of a poorly resolved triplet at 0.8 ppm ($-CH_3$), a broadened singlet at 1.2 ppm (protons of methylene groups), and a multiplet at 5.3 ppm (>CHOCOR'). The presence of the latter signal confirmed the considerable degree of unsaturation of the O-acyls and the structure of the phospholipid under investigation as a 2-acylglycerophosphoryl-N-acetylethanolamine [7].

EXPERIMENTAL

For chromatography we used type-KSK silica gel; for column chromatography: 160-250 μ, and for thin-layer chromatography, 125 μ. The solvent systems were the same as in previous work [1, 2]. The GLC of the samples was performed on a "Khrom-41" instrument with a flame-ionization detector in a steel column (2500 × 3 mm) filled with PÉGS on Celite-545. The carrier gas was helium and its rate of flow 30 ml/min. The total phospholipids were obtained by Folch's method [8]. Acid, alkaline, and enzymatic hydrolyses were carried out as described previously [1]. The fatty acids of the total phospholipids, of the total triglycerides, and of the individual fractions were determined by their saponification with ethanolic alkali [9]. Fatty acids of the glycerol moieties of the molecule and of those bound by the amide bonds in the N-acyl-PEs and their lyso analogs were determined by a method described previously [2]. The NMR spectrum of the N-acyllyso-PEs was taken on a JNM-4H 100/100 MHz instrument in deuterochloroform solution.

SUMMARY

The qualitative and qualitative compositions of the phospholipids of kenaf seeds of the variety "Opytnyi-1961" have been investigated. It has been established that the main components of the total phospholipids are phosphatidylcholines, phosphatidylethanolamines, and phosphatidylinositols, and minor components are N-acyl-PEs and their lyso analogs The structural analysis of these homogeneous fractions has been performed, and the molecular compositions of the main fractions have been calculated. On comparing the results obtained with those of other varieties of kenaf investigated previously, some differences have appeared in the qualitative, quantitative, molecular, and fatty-acid compositions of the total phospholipids and of individual fractions of them.

The differences found are possibly due to the variety of characteristics of the plant. For the N-acyllyso-PEs the structure of 2-acylglycerophosphoryl-N-acylethanolamines has been established.

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THE TRANSFORMATION PRODUCTS OF SOME NATURAL COUMARINS

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The natural coumarins osthole (I) and oxypeucedanin hydrate (II) are the main components of the species of the genus *Prangos* and possess a high hypotensive activity [1, 2]. Consequently, a chemical and pharmacological study of these compounds and also of their analogs is of great interest for practical medicine. The present paper gives the results of a study of new transformation products of (I) and (II) and of decursinol (III).

We have established that when (I) reacts with a mixture of concentrated acetic and hydrochloric acids at room temperature, the main products are two substances: (IV), $C_{15}H_{17}O_3Cl$, mp 89-91°C, M^+ 280; and (V) $C_{15}H_{16}O_4$, mp 69-70°C, M^+ 262.

On the basis of a study of IR, PMR, and mass spectra it has been shown that (IV) is 8-(3'-chloroisopentyl)-7-methoxycoumarin, and (V) is 8-(3'-hydroxyisopentyl)-7-methoxycoumarin. Consequently, under the reaction conditions hydrogen chloride adds to the double bond in the side chain of (I) in accordance with Markownikoff's rule [3]. On reaction with ethanol, compound (IV) formed the ethoxy derivative (VI), $C_{17}H_{22}O_4$, M^+ 290, and on acetylation with acetic anhydride in the presence of pyridine, (V) gave the acetate (VII), $C_{17}H_{20}O_5$, mp 61-62°C, M^+ 304.

We have shown previously [4] that when (II) is treated with 20% sulfuric acid in ethanol, in addition to other coumarin derivatives a new compound is formed, namely: the ethyl ether of (II). It was later shown that the reaction of (II) with acetone in an acid medium forms an acetonide (VIII) with the composition $C_{19}H_{20}O_6$, mp 161-163°C, M^+ 344, and the dehydration of (II) with thionyl chloride gives its 2",3"-sulfinyldioxy derivative (IX) ($C_{16}H_{14}O_7S$, mp 181-183°C, M^+ 350), isoimperatorin (X), and gosferol (XI). Details of the PMR spectra of some of the compounds investigated are given in Table 1.

Decursinol (III) is one of the components of the roots of *Seseli grandivittatum* and contains one secondary hydroxy group in position 3', which was confirmed by the production of the monoacetate [5]. Likewise, on dehydration with 20% H_2SO_4, compound (III) gives xanthyletin. However, the oxidation of (III) with chromium trixoide in acetic acid and in acetone took place in an unusual fashion, and instead of the expected ketone (XII) the dicarboxylic acid (XIII), $C_{14}H_{12}O_7$, mp 225-227°C, M^+ 292, and substance (XIV), with mp 205-207, M^+ 304, were formed. The PMR spectrum of (XIII) taken in dimethyl sulfoxide showed signals with chemical shifts of 6.31 and 7.98 ppm (d, J = 10 Hz, 1H each) and of 8.02 and 6.65 ppm (s, 1H each) due to the protons in positions 3, 4, 5, and 8 of the coumarin nucleus, respectively. A

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