PHOSPHOLIPIDS OF THE SEEDS OF *Phaseolus vulgaris*

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Six homogeneous fractions have been isolated from the total phospholipids of the seeds of the kidney bean, and their ratios and fatty acid compositions and the position distributions of the fatty acid radicals in the main fractions have been determined.

The seeds of Leguminosae are distinguished from the seeds of other higher plants by a high content of polar lipids, especially phospholipids [1]. The phospholipids of the soybean [2-4] have been widely studied; their composition and amount depend on the phase of ripening of the seeds [5, 6]. Other representatives of this family have been studied in less detail.

The change in the phospholipid composition of kidney bean leaves has been considered [7, 8]. It has been established that in the course of the development of the leaves the amounts of PGs and PCs vary most considerably.

We have investigated the phospholipid complex of ripe seeds of the kidney bean (*Phaseolus vulgaris* L., family Leguminosae), of the "Altyn" variety obtained from an experimental plot of UzNIIRis [Uzbek Scientific-Research Institute of Rice]. The comminuted kidney bean seeds were extracted with a chloroform—methanol—water (3:2:0.17) system containing 0.01% of ionol [9]. The total phospholipids, freed from carbohydrates (by gel filtration on Sephadex G-25), from neutral lipids, pigments, and substances of steroid nature (CC on silica gel with elution by chloroform and acetone), and from amino acids [10] (2% of the weight of the seeds), consisted of a transparent glassy mass with a faint yellow color.

On a two-dimensional chromatogram of the combined phospholipids in system 1 (direction I), and 2 (direction II) showed the presence of eight phosphorus-containing spots with the following R_f values (for direction II): 0.07 (lyso-PCs); 0.1 (PAs); 0.2 (PIs); 0.5 (PCs); 0.6 (PEs); 0.7 (X-PLs); 0.85 (N-acyl-lyso-PEs); and 0.9 (N-acyl-PEs). The quantitative distribution of the individual phospholipids in the total material was as follows (%): Lyso-PCs, 3.4; PAs, 0.5; PIs, 20.4; PCs, 37.5; PEs, 25.7; X-PLs, 1.4; N-acyl-lyso-PEs, 3.4; N-acyl-PEs, 7.7; the X-PLs were revealed with the Vas'kovskii reagent and with ninhydrin. From its chromatographic behavior (R_f values in various solvent systems), this component is possibly a N-methyl-PE [11], an intermediate in the metabolic transformation of PEs, and usually found in small amounts [12] or not at all in tissues. This phospholipid has been detected chromatographically previously in soybeans [13] and as a minor component in the total phospholipids of barley [14].

The individual groups of phospholipids were isolated by column chromatography on silica gel using as eluents mixtures of chloroform and methanol in various ratios and pure ethanol. After the repeated preparative TLC of narrow fractions in solvent systems 1 and 3, six components were obtained in homogeneous form.

The fatty acids of the combined material and also those of the individual fractions of phospholipids were cleaved by saponification according to Stahl [15] and were analyzed by GLC in the form of their methyl esters. The results are given in Table 1.

Characteristic for the phospholipids of plants of the family Leguminosae is the absence, or the presence of only trace amounts, of acids with more than 20 carbon atoms in the total material [16-18]. In contrast to other representatives of the family Leguminosae [16-18], about half the fatty acids of the phospholipids of the kidney bean proved to be

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TABLE 1. Compositions and Position Distributions of the Fatty Acids in the Phospholipids of the Kidney Bean of the "Altyn" Variety

Fraction	Fatty acid, wt.%																
	10:0	12:0	14:0	15:0	15:1	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3	21:0	22:0	ΣS	ΣU
Total PLs	3 1	2,5	1.4	1,1	1,0	37,5	1,6	2,0	1,2	4,8	17,0	19.7	7,1	Tr.	Tr.	52,4	47,6
PCs																	
Total	3 8	2,0	1,8	Tr.	—	23,7	Tr.	4,5	—	5,2	28,0	24.6	6,4	—	—	41,0	59,0
Position 1	2,9	1,5	1.7	Tr.	—	46,1	Tr.	Tr.	—	6,3	17,1	17,1	7,3	—	—	58,5	41,5
Position 2	1,8	1 1	1,7	Tr.	—	6,2	2,0	5,5	—	2,0	35,6	34,6	9,5	—	—	18,3	81,7
PEs																	
Total	7,2	4,3	1,6	1,3	1.5	29.0	1,7	2,5	1,7	4.1	20,8	17,5	6,8	—	—	50.0	50.0
Position 1	11,5	8,0	5,8	2,6	3.0	46,5	Tr.	3,0	Tr.	Tr.	6,3	8,4	4,9	—	—	77.4	22,6
Position 2	5.5	1.6	1,2	1,1	1,3	11.5	2,8	2,3	2,1	4,8	37,0	22,4	6.4	—	—	28,0	72,0
PIs																	
Total	2,9	3.7	1,5	1,6	Tr.	40,9	Tr.	2,9	1,2	8,0	12,0	17,6	7.7	Tr.	Tr.	61,5	38,5
Position 1	2,0	3.8	0,6	0,8	Tr.	51,4	Tr.	1,2	1,7	6,6	14,2	13.8	3.9	Tr.	Tr.	66,4	33,6
Position 2	2,3	2,0	1,0	0 9	Tr.	31,8	Tr.	3,3	1,0	6,2	13,6	24,1	13,8	Tr.	Tr.	47,5	52,5
N-Acyl-PEs																	
Total	1,3	1.2	0,8	1.1	Tr.	32.5	Tr.	2,1	1,9	1.4	13,8	18,6	17,7	Tr.	4,6	48,0	52.0
O-Acyls	Tr.	Tr.	0,8	1,0	Tr.	35,3	Tr.	Tr.	1.4	4,8	16,9	25,6	14,2	Tr.	Tr.	41.9	58,1
N-Acyls	Tr.	Tr.	2,3	2 5	Tr.	22,6	Tr.	3,1	Tr.	5,5	15,1	26,5	12,9	2.4	7,1	45.5	54,5
N-Acyl-lyso-PEs	0.6	0,5	0.8	1 5	2,6	16,0	Tr.	3,8	Tr.	4,0	28,3	31,8	10,1	Tr.	Tr.	27,2	72.8
Lyso-PCs	3,3	Tr.	Tr.	Tr.	Tr.	32,6	Tr.	1,0	—	4,2	15,6	32,6	10,7	Tr.	Tr.	41,1	58,9

saturated. The most unsaturated fraction proved to be the N-acyl-lyso-PEs, and the most saturated fraction the PIs. The saturation of the phospholipid molecules increases in the following sequence: N-acyl-lyso-PEs → PCs → lyso-PCs → N-acyl-PEs → PEs → PIs.

The same main acids appear in the fatty acids of the individual fractions as in the phospholipid complex as a whole, with the exception of the minor acids (15:1, 17:1, 21:1, and 22:0), which are absent from certain fractions. In all cases, the 16:0 acid predominates among the saturated acids and one of the C_{18} among the unsaturated acids.

The position distributions of the fatty acids in the molecules of the main groups of phospholipids were determined by enzymatic hydrolysis using snake venom as a source of phospholipase A_2. The table shows that the distributions of the medium-chain-length acids among the classes of phospholipids are dissimilar: the 10:1, 14:0, 15:0, and 17:0 acids esterify positions 1 and 2 differently in the PCs, PEs, and PIs; the 17:0 acid in the PCs and the 18:0 acids in the PEs are bound almost entirely in position 2.

In determining the molecular compositions of the groups of phospholipids studied, we based ourselves on the experimental results on the position distribution of the fatty acid radicals in the PC, PE, and PI molecules, and a mathematical method [19]. As a result, the following group compositions were calculated (%):

	PCs	PEs	PIs
SS	10,5	21,8	31.7
SU	47,9	55 8	35,4
US	7,8	6,4	15,8
UU	33.8	16,0	17,1

Thus, the PCs contain a considerably larger amount of diunsaturated and a small amount of disaturated PCs from the PEs and PIs, which is connected with the fatty-acid composition of the initial molecule and also with the position distribution of the radicals of the acids in the molecules of the initial phospholipids. Among the individual species, the following predominate in the PCs: 16:0/18:2, 16:0/18:1, 18:1/18:1, 18:2/18:1, 18:1/18:2, 16:0/18:3; in the PEs: 16:0/16:0, 16:0/18:1, 16:0/18:2; and in the PIs: 16:0/16:0, 18:1/16:0, 18:2/16:0, 16:0/18:1, 16:0/18:2.

The analysis of the fatty acids of the N-acyl-PEs was carried out by a method described in the literature [20, 21]. This showed that the N-acyls were more saturated than the acids esterified in the glycerol moiety of the molecule. It is interesting to note that the 21:0 and 22:0 acids are bound completely to the nitrogen atom. We observed a similar phenomenon previously in the seeds of the mung bean [18], where the 21:0 acid was found only in the N-acyl-PEs and this exclusively in amide-bound form.

EXPERIMENTAL

For analytical TLC we used plates coated with type KSK silica gel having particle dimensions of about 125 μ in combination with 5% gypsum, and for column chromatography the same silica gel with particle dimensions of 160-250 μ. The following solvent systems were used for TLC: 1) chloroform—methanol—ammonia (65:35:5); 2) chloroform—acetone—acetic acid—methanol—water (10:4:2:5:1); and 3) chloroform-methanol—water (65:35:5).

The fatty acid methyl esters were analyzed on a Chrom-41 instrument at 198°C in a column 2.5 m long filled with 17% of PEGS on Celite-545. The peaks of the fatty acids in GLC were identified from their relative retention times using the linear dependence of the logarithm of this magnitude in the number of carbon atoms.

Elimination of Amino Acids from the Total Phospholipids. The total phospholipids were dissolved in a small amount of water and the solution was extracted several times with chloroform in a separatory funnel. The chloroform extract was evaporated in a rotary evaporator. On TLC (in systems 1 and 3), the residue did not show the presence of amino acids (using ninhydrin as revealing agent).

Enzymatic Hydrolysis of the Phospholipids. The PCs (90 mg) in 15 ml of ether were stirred in the presence of 2 mg of venom and 0.5 ml of Tris buffer (pH 10.0) at 37°C. The reaction was monitored by TLC in system 1. The time of enzymatic hydrolysis was 1 h. The hydrolysis products were separated by TLC in system 1. The free acids from positions 2 were desorbed from the TLC, the lyso-PCs (R_f 0.1) were subjected to alkaline saponification [15], and the acids of positions 1 and 2, in the form of their methyl esters, were analyzed by GLC.

The enzymatic hydrolysis of 80 mg of PEs and 95 mg of PIs was performed similarly. The times of enzymatic hydrolysis were 12 and 18 h, respectively.

SUMMARY

Information has been obtained on the composition of the phospholipids of kidney beans of the variety "Altyn." It has been established that total phospholipids include PCs, PEs, PIs, N-acyl-PEs, N-acyl-lyso-PCs, lyso-PCs, and PAs, and possibly N-methyl-PEs.

It has been established that the phospholipids of kidney bean seeds differ markedly from the phospholipids of representatives of the family Leguminosae investigated previously with respect to the sum of the saturated fatty acids.

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COUMARINS OF *Hapolophyllum obtusifolium*. STRUCTURES OF TWO NEW COUMARIN GLYCOSIDES.

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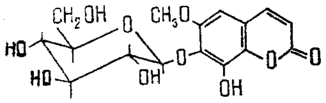
Two coumarin glycosides have been isolated from an aqueous ethanolic extract of the epigeal part of *Haplophyllum obtusifolium*: (I) – $C_{16}H_{16}O_{10}$, mp 164–166°C, $[\alpha]_D$ –52.4° (dimethylformamide); and (II) – $C_{26}H_{26}O_{12}$, mp 206–208°C, $[\alpha]_D$ –110.5° (pyridine). It has been established on the basis of chemical transformations and spectral characteristics that (I) has the structure of fraxetin 7-O-β-D-glucopyranoside and (II) that of scopoletin 7-O-(6'-O-feruloyl-β-D-glucopyranoside).

Continuing a study of *Haplophyllum obtusifolium*, by chromatography of an aqueous ethanolic extract [1] on a column of silica gel with elution by chloroform–methanol we have isolated two new coumarin glycosides.

Glycoside (I) has mp 164–166°C and the composition $C_{16}H_{18}O_{10}$. Its UV spectrum ($\lambda_{max}^{ethanol}$ 228, 257, 306 nm; lg ε 4.02, 3.54, 3.86) is similar to those of capensin and obtusicin [2]. When the UV spectrum was taken in an alkaline medium, a bathochromic shift of the long-wave maximum (Δλ + 28 nm) was observed. Consequently, compound (I) contains a free phenolic hydroxyl.

The IR spectrum of (I) shows absorption bands at (cm^{-1}) 3130–3475 (hydroxy groups), 1732 (carbonyl of an α-pyrone), and 1626, 1585, and 1500 (C=C bonds of an aromatic system).



I

The acid hydrolysis of glycoside (I) formed D-glucose and an aglycone identical with fraxetin, in equimolar proportions. This means that (I) is a monoglucoside of fraxetin. To establish the position of the glucose to the aglycone, glycoside (I) was methylated with dimethyl sulfate in the presence of potassium carbonate. Subsequent hydrolysis of the methylation product led to the formation of a substance $C_{11}H_{10}O_5$ with mp 144–145°C, which was identified by direct comparison with an authentic sample as isofraxidin [3, 4]. Thus, the glucose is attached to the hydroxyl in the C-7 position of fraxetin. The acetylation of (I) gave a pentaacetyl derivative the PMR spectrum of which showed the signals of the protons of four aliphatic and one aromatic acetyl groups, and also those of H-3, H-4, H-5, and CH_3O. The signals of the hemiacyl methine protons and of the anomeric protons of the glucose residue appear at 4.96–5.24 ppm (4 H, multiplet). The signal of the H-5' atom (1 H, m) appears at 3.58 ppm, and the signals of the $-\underline{CH_2}-OAc$ protons at 3.91 ppm (J_{gem} = 12 Hz, $J_{5',6'}$ = 2 Hz) and 4.26 ppm (J_{gem} = 12 Hz, $J_{5',6'}$ = 4.5 Hz), in the form of two

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