paper chromatography (on FN-15 paper) [8]. Viscosities were measured in an Ostwald viscometer with a capillary having a diameter of 0.54 mm at 25°C.

SUMMARY

The optimum conditions for the isolation of the pectin substances from the pulp of mountain ash fruit freed from lipophilic substances have been found. It has been established that the isolation of the pectin substances is best performed with a 0.3 N solution of hydrochloric acid or a 0.5% solution of oxalic acid, and precipitation at a ratio of extract obtained to 96% ethanol of 1:2.

The characteristics of the pectin substances are given. Their ash content, monosaccharide composition, viscosity, and jellying capacity have been studied.

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MOLECULAR COMPOSITION OF THE N-ACYLPHOSPHATIDYLETHANOLAMINE OF THE COTTON PLANT OF VARIETY S-6029

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An N-acylphosphatidylethanolamine has been isolated from the total phospholipids of the cotton plant of variety S-6029. The composition and the position distribution of the fatty-acid radicals has been studied with the aid of enzymatic hydrolysis with phospholipase A_1 (from the fungus *Rhisophus microsporus* UzLT-1) and by alkaline and acid hydrolyses. From the results obtained, the possible molecular composition of the N-acylphosphatidylethanolamine has been calculated by Coleman's method. The total number of all the molecular species amounted to 720.

We have reported previously that unidentified phospholipids have been found in the total phospholipids of the cotton plant of variety S-6029 [1], and one of them was obtained in the homogeneous form. It had an N:P:RCOO ratio of 1:1:3 and gave a negative ninhydrin reaction. Its structure as an N-acylphosphatidylethanolamine (N-acyl-PE) was confirmed by its chromatographic behavior, by IR spectroscopy, and from the products of hydrolysis [2-6].

In view of its unusual structure, an N-acyl-PE does not form the enzyme-substrate complex that is necessary for enzymatic hydrolysis and is not a specific substrate for the phospholipase A_2 from the venom of *Vipera lebatina obtusa*. Consequently, structural studies of native N-acyl-PEs lag behind those of other phospholipids, particularly in the position distribution of the acyl radicals in the molecule.

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We have now used phospholipase A_1 from the fungus Rhizophus microporus UzLT-1 [7], which exhibited strict specificity for the first ester bond, as in the case of PC. It was found that the reaction takes place easily under the conditions of the previous work [7].

Enzymatic hydrolysis yielded only two products (monitoring by TLC in systems 1 and 2), one of which proved to be fatty acids and the other a substance with R_f 0.75 giving a positive reaction for phosphorus and a negative reaction to ninhydrin and being close in chromatographic behavior to N-acyl-lyso-PE. To confirm this, the N-acyl-lyso-PE was deacylated with 0.1 M KOH in methanol at 37-40°C for 40 min. We then identified two products — fatty acids and a substance giving a positive reaction for phosphorus and a negative reaction to ninhydrin. Fatty acids, glycerol, and ethanolamine were found in the products of acid hydrolysis (TLC in systems 3, 4, and 5).

In previous work [5], position specificity was determined after dephosphorylation under severe conditions [8, 9] which did not exclude the possibility of the migration of acyl groups in the molecule and of changes in the unsaturated fatty acids [10], i.e., the complete structure characteristic for the N-acyl groups was not given. This is confirmed by a calculation of the amount of fatty acids in the molecule of the N-acyl-PEs of varieties 5904-I and Termez-7.

The fatty-acid composition and the position-distribution of the acyl radicals in the molecule of the N-acyl-PE that have been found are given below:

Acid	Total fatty-acid composition, %	Composition of the individual acyl radicals, %		
		1	*2*	N-acyl
8:0	1,8	2,6	1,3	—
10:0	1.1	1,4	0.8	0,8
12:0	2,6	2,8	2.5	0,8
14:0	2,6	3,1	2,9	0,6
16:0	28,7	31,9	18,2	30,8
16:1	3,0	4,5	3,7	1,0
17:0	2,0	4.1	—	—
18:0	6,3	9,7	—	6,4
18:1	18,1	17,8	22,9	10,7
18:2	32,2	22,1	47,7	41.1
18:3	1,6	—	—	7,8
ΣS	45,1	55,6	25,7	39,4
ΣU	54,9	44,4	74,3	60,6

As follows from the results obtained, unsaturated fatty acids predominate in the second position of the glycerol part of the molecule (U, 74.3%); in the first position the amounts of unsaturated and saturated acids are approximately the same (S—U 55.6—44.4%), and in the N-acyl part some predominance of unsaturated acids (60.6%) is observed.

The amounts of palmitic acid in the first position and in the N-acyl moiety are approximately the same, while the low-molecular weight 8:0—14:0 acids predominate in the glycerol moiety. The 18:2 acid predominates in the second position and in the N-acyl moiety, while it must be observed that as in the main phospholipids [1], the 18:0 acid is concentrated in the second position, the 18:3 acid is located in the N-acyl moiety, and the 17:0 acid only in the first position.

The high amount of the 16:0 and 18:2 acids in the N-acyl moiety does not exclude the possibility that the N-acyl radicals are precursors of the acyl radicals in the biosynthesis of the lipids and play a decisive role in the functions of biomembranes.

On the basis of the results obtained we have succeeded in calculating the possible molecular composition of the N-acyl-PE by the method of Coleman and A. L. Markman [11].

The total number of all the molecular species amounted to 720 for the main PLs (36, 42, 48). To characterize the species best, we have separated them into the individual types: S*—S**—S***; U—U—U; S—S—U; U—S—S; S—U—S; U—S—U; and U—U—S [*) acids localized in the first position of the glycerol moiety; **) in the second position; ***) N-acyl]:

	SSS	UUU	SSU	SUS	USS	USU	SUU	UUS
No. of species	175	36	140	105	75	60	84	45
Amt. of species, %	5,2	19,8	8,1	16,1	4,1	6,6	26,5	13,6

The S—S—S is represented most widely in the qualitative set — 175 species — although quantitatively it makes up only 5.2% of the total, while the U—U—U type includes 36 species and amounts to 19.8%. At the same time the solid PLs amount to 390 species, 17.4%, and the liquid PLs to 165 species, 59.9%, respectively. It is not excluded that the N-acyl-PEs are present in a more liquefied state, which indicates the complexity and diversity of their role in plant biomembranes.

Thus, we see that the minor components of the total phospholipids increase the complexity of their structure, which, in its turn, gives rise to the necessity for changing the experimental procedure. Their role is understanding the functions of the membranes of the cotton plant, which has scarcely been studied at the present time, remains undisclosed. All this complicates the elucidation of many facts obtained experimentally.

EXPERIMENTAL

The phospholipids were isolated as described previously [1]. The following solvent systems were used: 1) chloroform—methanol—25% ammonia (70:30:2); 2) chloroform—methanol—25% ammonia (70:30:5); 3) petroleum ether—diethyl ether (4:1); 4) isopropanol—ammonia—water (7:1:2); and 5) 2% ammonia—methanol (2:3).

Acid hydrolysis was carried out in 3 N HCl in sealed tubes at 100°C for 24 h.

The phospholipids were purified by TLC on silica gel containing 5% of gypsum or with the addition of 0.01% of sodium carbonate. IR spectra were taken on a UR-20 instrument with the substances in the form of films.

Enzymatic Hydrolysis of the N-Acyl-PE. A solution of 20 mg of the substance in 20 ml of diethyl ether was treated with 1.5 ml of Tris-HCl buffer, pH 8.3, 0.3 ml of 22% $CaCl_2$, and 10 mg of the lipase from the fungus *Rhizophus microsporus* UzLT-1. With constant stirring, the mixture was incubated at room temperature for 2 h. Monitoring was carried out with the aid of TLC in system 1. The products obtained were purified by TLC in systems 2 and 3.

The N-acyl-lyso-PE obtained (15 mg) was incubated in 3 ml of 0.1 M methanolic caustic soda at 37-40°C for 40 min. The subsequent working up was carried out by a method described previously [4].

The acids obtained were methylated with the aid of diazomethane.

Gas—liquid chromatography was performed on a Khrom-41 instrument with a flame-ionization detector at 205°C using as the stationary phase polyethylene succinate (17%) on Celite-545 (60-80 mesh) and polyethylene succinate (17%) on Celite-535 (60-80 mesh) on columns 2.5 and 2 m long, respectively, with internal diameters of 0.3 cm.

SUMMARY

The position-distribution of the fatty acid radicals of an N-acyl-phosphatidylethanolamine has been studied with the aid of enzymatic, alkaline, and acid hydrolyses, on the basis of which its possible molecular composition has been determined.

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3,7,7-TRIMETHYLCYCLOHEPTA-1,3,5-TRIENE — A COMPONENT OF THE TURPENTINES FROM *Pinus sylvestris*

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A previously unknown component has been isolated from the oleoresin turpentine of *Pinus sylvestris* L. It has been established by chemical and spectral methods that it is 3,7,7-trimethylcyclohepta-1,3,5-triene. It has been shown by the GLC method that this hydrocarbon is present in all the main types of turpentines in amounts of from 0.1 to 0.7%.

In industrial samples of the turpentines obtained from the pine *Pinus sylvestris* L. the presence of 15 terpene hydrocarbons, of one oxide, and of five to 10 components of undetermined nature have been established [1-4]. In the present paper we consider the establishment of the structure of an unknown component having a boiling point close to that of β-pinene, which made its isolation by fractional distillation difficult. When GLC analysis is performed on the widely used liquid phase (LP) tricresyl phosphate (TCP) this component has a relative retention time (RRT) with respect to car-3-ene of 1.04 [3], and appears indistinctly when the concentration of the latter is appreciable.

We succeeded in achieving a good separation of this component from the others of the turpentine by using as LP diethyleneglycol adipate (DEGA). Under these conditions, the RRT of the component in relation to that of car-3-ene was 1.28. The amount of the component under investigation in industrial samples of pine oleoresin turpentine [5] does not exceed 0.1%.

As a result of two successive fractional distillations of the turpentine we obtained a concentrate containing 16.1% of the component. By preparative GLC we isolated from the concentrate in 99.7% purity a substance with the composition $C_{10}H_{14}$, bp 62-63°C (20 mm); d_4^{20} 0.8530; n_D^{20} 1.4962; $[\alpha]_D^{20}$0°.

The UV spectrum [$\lambda_{max}^{C_2H_5OH}$ 270 nm (log ε 3.70)] was characteristic for a conjugated system of endocyclic double bonds.

The IR spectrum had the following characteristic absorption bands (cm^{-1}): 1358, 1375 ($-CMe_2$); 1440, 1452, 1469 ($-CH_3$); 1557, 1613, 1630 (three conjugated double bonds).

In its properties, the compound under investigation corresponded to 3,7,7-trimethylcyclohepta-1,3,5-triene (I). The following properties are given for this compound in the literature: bp 53-55°C (13 mm); d_4^{20} 0.8559; n_D^{20} 1.4965. UV spectrum: λ_{max} 269 nm (log ε 3.577) [6].

To confirm the structure of (I) we studied its PMR spectrum, including spectra taken with the use of double resonance. The spin—spin coupling constants for (I) were: $J_{1-2} = J_{5-6} = 10$ Hz, $J_{4-5} = 6.8$ Hz, which agrees well with literature information given for cycloheptatriene derivatives [7]. The constants mentioned permit the doublet of doublets to be assigned to the protons at C_1 and C_6, the low-field doublet to the proton at C_4, and the unresolved multiplet to the protons at C_2 and C_5. The chemical shifts of the signals of the individual protons obtained with the use of double resonance were as follows: 4.90 (H-1), 4.85 (H-6), 5.67 (H-2), 5.71 (H-5), 6.00 (H-4), which corresponds to literature information [8].

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