PHYTOGLYCOLIPIDS OF THE GRAPE

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The qualitative and quantitative compositions of the grape and its component parts — flesh, skin, seeds — have been studied. Ten fractions have been identified, the main ones being cerebrosides and monogalactosyldiglycerides. The compositions of the fatty acids, of the sphingosine bases, of the sterols, and of the carbohydrates of the individual fraction of phytoglycolipids have been determined.

Phytoglycolipids (PGLs) are important components of the photosynthetic apparatus of green plants. Their concentration in the chloroplasts is extremely high, being five times greater than that of the phospholipids [1]. In spite of their wide distribution in the plant world, the PGLs of the grape have scarcely been studied [2]. Nevertheless, at the present time the important technological role of lipids, a considerable part (∿40%) of which consists of glycolipids, in the products of the processing of the grape and, in particular, in wines, has been shown [3].

In this connection, the aim of the present investigation was to study the chemical composition and structural features of the PGLs of the grape and its component parts.

The PGLs of the component parts and of the whole berry of the cultivated grape *Vitis vinifera* L. were isolated from the total lipids by column chromatography on silica gel [4] and were subfractionated by two-dimensional TLC in systems I (1st direction) and II (2nd direction).

The amount of PGLs in the total lipids of the seeds was 15.5%, in the flesh 35.9%, and in the skin 6.4%. TLC distribution permitted us to detect ten glycolipid fractions with the following R_f values (in the 2nd direction): 0.81 (acylmonogalactosyldiglycerides — acyl-MGDGs); 0.78 (monogalactosyldiglycerides — MDGDs); 0.71 (esterified sterol glycosides — ESGs); 0.63 (sterol glycosides — SGs); 0.55 (cerebrosides — CSs); 0.47 (ceramide oligosides — COs); 0.44 (diglactosyldiglycerides — DGDSs); 0.26 (ceramide phosphate inositol oligosides I — CPIO I; 0.18 (ceramide phosphate inositol oligosides II — CPIO II), and 0.10 (sulfoquinovosyldiglycerides — SQDGs). All the above-mentioned fractions gave the specific color reactions characteristic for PGLs on treatment with α-naphthol, periodate—Schiff reagent, and diphenylamine [5]. The spots with R_f 0.18 and 0.26, unlike the others, gave the coloration characteristic for phosphorus-containing lipids on treatment with the Dittmer—Lester reagent in the Vaskovsky—Kostetsky modification [6].

To establish the structures of the PGL fractions, severe acid hydrolysis was carried out with a methanolic solution of hydrochloric acid [5]. The quantitative analysis of the water-soluble and liposoluble fragments permitted us to determine the molar ratio between the main components of the molecules (glycerol, monosaccharides, fatty acids, sphingosine bases), on the basis of which the GPL fractions were identified.

The composition and the amounts of the fractions of the PGLs of the grape and its component parts were as follows (%):

	SQDGs	CPIOs I	CPIOs II	DGDGs	COs	CSs	SGs	ESGs	MGDGs	Acyl-MGDGs	Total amount of PGLs, mg/kg
Flesh	5,1	8,9	9,7	7,2	8,3	15,7	12,0	9,1	19,2	4 8	237,8
Skin	12.0	6,1	5.4	11,4	5 9	8,8	9,4	6.7	25.7	8,6	1456,5
Seeds	7,3	5,8	6,8	5,5	9,1	38,5	7.0	6,4	10,1	3,5	15802,9
Berry	8,1	6,6	7,3	7,8	8,0	22,2	9.7	7,7	16,9	5,7	853.4

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The compositions of the PGLs in the berry and its structural elements were identical and the group distributions were similar, on the whole, in spite of some differences. The predominating fractions were cerebrosides and the monogalactosyldiglycerides.

The fatty acid compositions of the PGLs were determined by the GLC method [4]. The predominating acids in the majority of cases were oleic, linoleic, linolenic, palmitic, and stearic. The PGLs of the seeds were distinguished by the largest amount of unsaturated fatty acids and those of the skin by the smallest amount (Table 1).

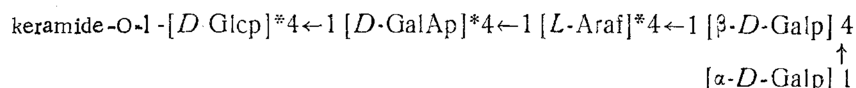
The unsaturated acids predominated quantitatively, which is characteristic for plant glycolipids [7].

It was found by paper chromatography (PC) that the main carbohydrate fragment of the PGL was galactose (∿70% of all the monosaccharides). However, only glucose was found in the SG and ESG fractions. The carbohydrate fragments of the cerebrosides consisted of galactose (67%) and glucose (33%).

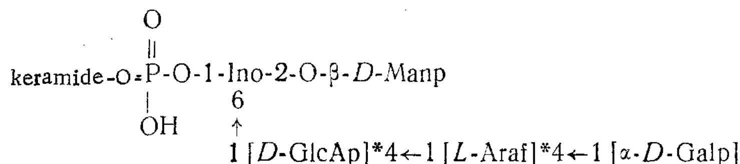
As a result of the analysis of the sphingosine base by GLC, phytosphingosine (47%), dehydrosphingosine (24%), and dihydrosphingosine (29%) were detected in the cerebrosides. In the ceramide oligosides the sphingosine bases were represented by phytosphingosine (81%) and dihydrosphingosine. In the CPIOs I, the composition of the bases was as follows — phytosphingosine (63%), dehydrosphingosine (37%); in the CPIOs II they were phytosphingosine (54%) and dehydrosphingosine (46%).

Analysis of the sterol components of the GSs showed that they consisted predominantly of β-sitosterol (86.1%) with smaller amounts of other sterols — stigmasterol (5.9%) and campesterol (8.0%). The sterol fragments of the ESGs consisted of β-sitosterol (70%), stigmasterol (9.6%), campesterol (16.5%), and ergosterol (4.9%).

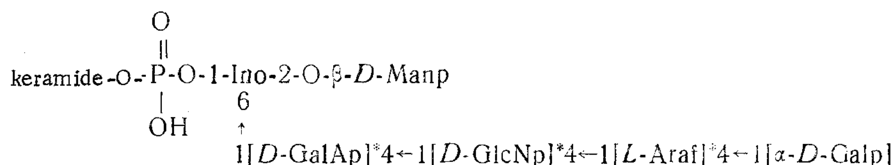
On the basis of the results of IR spectroscopy, GLC, periodate oxidation, methylation, and hydrolysis by specific glycosidases the structures of the oligosaccharide chains of the CO, CPIO I, and CPIO II fractions were established in part. The proposed structure for the ceramide oligosides we represent in the following way:



The structures of the ceramide phosphate inositol oligosaccharides are: CPIOs I:



CPIOs II:



In the fragments denoted by asterisks, the configurations of the glycosidic bonds and the sequence of addition of the monosaccharides were not determined.

Phosphorus-containing glycolipids with similar structures have been detected previously in various plants (wheat, maize, soybeans, etc.) [7, 8].

EXPERIMENTAL

The TLC separation of the PGLs was carried out in the following solvent systems: 1) acetone—toluene—acetic acid—water (60:60:2:1) and 2) chloroform—acetone—ethanol—acetic acid—water (6:8:2:2:1). The quantitative estimation of the PGL fractions was carried out with respect to the carbohydrate component using the orcinol method [9]. The glycerol contents were found by the periodate-chromotropic acid method [5]. The SQDGs were determined by Kine's

TABLE 1. Fatty Acid Compositions of the Phytoglycolipids of the Grape and Its Component Parts, %

Fatty acid	Total PGLs				PGL fractions of the whole berry								
	flesh	skin	seeds	berry (as a whole)	SQDGs	CPIOs II	CPIOs I	DGDGs	COs	CSs	ESGs	MGDGs	acyl-MGDGs
11:0	Tr.	0,9	0,2	0,4	0,5	Tr.	Tr.	0,3	—	Tr.	0.1	0,9	0.6
12:0	6,1	3,0	1,4	3.7	3,9	2,1	1,3	1,7	4,4	2,2	5,5	4,9	0,5
12:1	0,9	0,9	0,5	0,8	0,3	0,3	0,8	0,9	0,8	3 0	0,9	0,1	0,1
13:0	2.7	2,2	0.2	1,8	1,0	2,9	2,0	2,2	2,7	0,3	4,4	1,1	1.5
14:0	4,0	11 4	1,1	2,7	2,3	9,7	7,4	3,9	3,9	2,3	5,0	4,7	6.6
14:1	2,4	4,6	0,6	2,1	0,0	0.3	0,2	4 4	5 6	4,0	6,7	4 7	4,2
16:0	10 8	21,0	12,2	12,7	13.1	12.8	9,9	18,2	18,7	9,5	16,8	8,0	9,7
16:1	7.6	5.5	0,8	6,0	5,3	8 0	5,8	5,2	4,2	5,8	3.3	5,1	4,0
17:0	0,5	0.9	0,3	0,4	0,9	0,2	0,1	0,1	0,5	0,1	0,6	0.2	3,0
18:0	8,7	11,0	2,2	7,2	9,6	5,9	8,3	5,1	7 7	5,7	12,2	4,8	8,7
18:1	29,4	16,7	19.8	23,7	11,7	17,7	22 0	19.8	15,4	15,1	17,9	12,2	11,5
18:2	8 6	[illegible].8	46.7	23,8	33,5	30,4	27.3	24.6	20.1	25.6	8,7	39,4	27,3
18:3	10,9	6,2	11,8	10,0	4.9	5.1	11,2	7.7	12,0	17.8	13.1	11,7	17,8
20:0	0.4	0,8	0,3	0,5	1,9	0,2	0,4	0,8	0.1	0 5	0,7	0,3	0,4
23:0	0,2	1,0	0,1	0.4	0.7	0,6	Tr.	0,5	Tr.	Tr.	0,2	Tr.	0,1
24:0	6,8	4,1	1.8	3,8	9,5	3,8	3.3	5.2	3,9	8,1	3,9	1,9	4.0
Σ saturated	40,2	56,3	19,8	33,6	43,4	38,2	32,7	38,0	41,9	28,7	49,4	26,8	35,1
Σ unsaturated	59,8	43,7	80,2	66,4	56,6	61,8	67,3	62,0	58,1	71,3	50,6	73,2	64,9

method with toluidine dye [9]. The PC of the carbohydrate components was performed by the descending method in the benzene–butan-1-ol–pyridine–water (1:5:3:3, upper phase) system. The spots were revealed with the aniline phthalate reagent [10]. The sugars were methylated by Kuhn's method [11].

The products obtained were converted into their trimethylsilyl (TMS) derivatives by the action of N-(trimethylsilyl) diethylamine (25°C, 12 h). The GLC of the TMS derivatives was performed on a 6.4 mm × 1.8 m column containing 3% of SE-30 on Chromosorb W, with programming of the temperature in the interval from 80 to 130°C at a gradient of 5-10 deg/min and isothermal conditions (130°C) until the end of separation. The rate of flow of gas was 50 ml/min. The temperature of the detector was 300°C. The periodate oxidation and decomposition of the glycoside was carried out by a modified Smith method [12]. A solution of 10 mmole of an individual glycoside was treated with 4 ml of a 0.25 M solution of $NaIO_4$ in the dark at room temperature until the consumption of periodate ceased, which was determined colorimetrically [13]. The excess of periodate was decomposed with ethylene glycol and then 10 mg of $NaBH_4$ was added to the solution and the mixture was left to stand for 24 h (20°C). It was then acidified with acetic acid to pH 5.0 and evaporated with the addition of ethanol. The residue was hydrolyzed with 1.5 ml of 2 N HCl at 100°C for 2 h.

The formic acid formed in the periodate oxidation was determined spectrophotometrically with 2-thiobarbituric acid [14]. The phosphorus in the CPIO I and CPIO II fractions was determined with ammonium molybdate and ascorbic acid [5]. The PGLs were subjected to enzymatic hydrolysis with specific glycosidases — α-D-galactoside galactohydrolase (EC 3.1.20), β-D-glucoside glucohydrolase (EC 3.2.1.21), and β-D-mannosidase (EC 3.2.1.25) with an activity of 4000 units/g.

The hydrolysis of the CO and the CPIOs I and II was carried out in acetate buffer at pH 5.0 with the addition of emulsifying agents — Triton X 100, Tween-20, and sodium cholate. The concentration of enzyme was 1.5 mg/ml. The mixture was kept at 30°C for 24 h. The methanol was added and it was heated. The precipitated enzyme was separated off by filtration [15]. The sugars in the hydrolysate were determined by PC. Analysis of the sphingosine bases was performed by GLC (the phase SE-30 on the support Gas-Chrom S, 210°C) [5].

The sterol components of the hydrolysates of the SG and ESG fractions were isolated by TLC [16]. The combined sterols were then separated by TLC on a 1.8 m × 0.06 m column filled with Chromaton N-AW-DMCS with 5% of the liquid phase SE-30. The rate of flow of gas was 50 ml/min and the column temperature 248°C.

CONCLUSION

The composition and amounts of the phytoglycolipids in the grape and its component elements have been determined. Ten fractions have been isolated and characterized, the main ones being the monogalactosyldiglycerides and the cerebrosides. The predominant carbohydrate component of the phytoglycolipids is galactose (~70%). The fatty acid compositions of the phytoglycolipid fractions have been studied. The structures of the oligosaccharide chains of the ceramide-containing glycolipids have been determined partially.

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GROUP COMPOSITION OF THE NEUTRAL LIPIDS IN THE OIL OF THE FRUIT OF *Hippophaë rhamnoides*

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The composition of the neutral lipids of the fruit flesh and seeds of two forms of sea buckthorn have been determined. The presence of more than 20 groups of lipids has been shown. The two oils differed in the amounts and compositions of their carotenoids and acyl glycerols and the amounts of alcohols, free fatty acids, and esters.

The oil of the fruit flesh of the sea buckthorn, family Elaeagnaceae, is used for medicinal purposes but its lipids have so far been studied inadequately. The total composition of the seeds and fruit flesh in relation to classes of lipids and the composition of the individual groups of lipids such as waxes, esters of polycyclic alcohols, free alcohols, etc., are unknown.

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