Chemistry of Natural Compounds, Vol. 29, No. 5, 1993

GLYCOLIPIDS AND PHOSPHOLIPIDS OF THE FRUIT OF *Elaeagnus angustifolia*

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UDC 547.915.5

The glycolipids and phospholipids of the fruit of Russian olive have been studied. The seeds yielded nine glycolipids and seven phospholipid components, and the pericarp eight glycolipids and three phospholipid components. Their fatty-acid compositions have been determined.

We have previously recorded the neutral lipids of three forms of Russian olive, *Elaeagnus angustifolia* L. [1]. Continuing a study of plants of this family we have investigated the glycolipids (GLs) and phospholipids (PLs) of the seeds and pericarp of Russian olive. With the aid of preparative thin-layer chromatography (PTLC) the total GLs isolated from the seeds were separated into nine, and the GLs from the pericarp into eight, chromatographically pure fractions. A preliminary identification of the fractions separated by GLC was made by comparing their R_f values with those of model components, by means of specific revealing agents, and from the products of alkaline hydrolysis. We then drew up a scheme for identifying the fractions based on the GLs of the seeds. The analysis of the GLs of the pericarp was carried out similarly. On the basis of their chromatographic mobilities, fractions 2 and 4 (R_f 0.15 and 0.54) were assigned to the sulfoquinovosyl glycerides (SQVDGs) and the ceramide oligosides (COs), respectively. Furthermore, the presence of the SQVDGs was confirmed by a qualitative reaction for sulfatides with Bromothymol Blue in KOH. The IR spectrum of fraction 2 showed absorption bands at (cm^{-1} 1420-1350 s, 1260 s, 1060 s, and 800-670 w, which are characteristic for the R−O−SO_2−R group of SQVDGs [2]. The IR spectrum of fraction 4 contained absorption bands at (cm^{-1}) 1550 s, 1620 s, and 3430 w, confirming the presence of a secondary amide group R−CO−R [3]. When the TLC plates were sprayed with anthrone in the presence of H_2SO_4 and were heated, fraction 3 (R_f 0.44) assumed the blue color characteristic for digalactosyldiglyceride (DGDGs), while fraction 7 (R_f 0.78) became cherry red, which showed the presence of monogalactosyldiglycerides (MGDGs).

From its chromatographical mobility (R_f 0.90), fraction 9 was assigned to the acyl-MDGDs (AcMDGDs), The spectral characteristics of fractions 3, 7, and 9 (presence of stretching vibrations of a free hydroxyl OH with a frequency (cm^{-1}) of 3630 w, of −C−O−C− functional groups at 1060-1150 s, 1470 m 1390 m, and 830 m, and of an ester grouping RCO−OR at 1470 s, 1140 m) confirmed the identification of the structures mentioned.

On being sprayed with a solution of diphenylamine, fraction 5 gave a blue spot of cerebrosides (CBs). Their R_f value (0.62) was close to that given in the literature for CBs [4].

On TLC, fraction 6 migrated at the same level and as a standard sterol glycoside (SG) (R_f 0.60); on spraying with 20% perchloric acid followed by heating a bright red spot changing to cherry-red appeared, which is characteristic for SGs. The same qualitative reaction with perchloric acid was given by fraction 8, the R_f value of which (0.66) indicated the presence in this case of esterified SGs (Ac-SGs). This was also confirmed by the severe hydrolysis of fraction 8 with a solution of KOH in methanol; the unsaponifiable part of the hydrolysate had the same mobility as fraction 6, while in the IR spectrum there were absorption bands of aryl alkyl ethers, cyclic compounds (C−O — stretching, 1270 cm^{-1}, s, and =C−H — stretching, 3030 cm^{-1}, s).

Tables 1 and 2 give the amounts of the fractions of GLs in the seeds and pericarp and their fatty acid (FA) compositions. In addition to the classes of compounds mentioned, the seeds contained one, and the pericarp two, unidentified classes of GL nature (qualitative reaction for GLs).

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 646-651, September-October, 1993. Original article submitted May 4, 1993.

0009-3130/93/2905-0569$12.50

TABLE 1. Amounts of GLs in the Seeds of Russian Olive and Their FA Compositions

No. of the fraction, class of GLs, amount %	Acid, %, GLC									
	14:0	15:0	16:0	16:1	18:0	18:1	18:2	Σ_{sat}	Σ_{unsat}	Other acids *
1. Unident. (10.0)	0,6	0,9	21,3	—	34.4	3.5	—	94.2	5.8	[a]39,3
2. SQVDGs (12.3)	2.2	1.4	30.0	2.3	11.8	17.1	15.5	57.0	43.0	19.7
3. DGDGs (25.3)	0.8	1.6	69.2	2.7	9.1	6.6	—	89.5	10.5	10.0
4. COs (1.3)	1.3	1.9	55.7	3.3	18.4	7.2	—	86.6	13.4	12.2
5. CBs (2.5)	2.3	1.3	44.2		21.9	16.2	—	72.2	27.8	14.1
6. SGs (6.7)	—	—	—	—	—	—	—	—	—	—
7. MGDGs (34.9)	0.5	0.5	36.1	4.9	31.8	15.4	5.9	72.9	27.1	4.9
8. Ac-SGs (4.2)	—	0.5	47.4	—	15.2	17.9	—	68.1	31.9	[b]19.0
9. Ac-MGDGs (2.8)	3.4	1.1	59.7	2.2	7.9	7.7	8.6	81.5	18.5	9.4

*Other acids: 9:0, 10:0, 11:0, 12:0, 13:0, 13:1, 14:1, 162, 17:0, 171, 18:3, 19:0, 19:1, 20:0, 20:1, 21:0, 22:0, 23:0.

TABLE 2. Amounts of GLs in the Pericarp of Russian Olive and Their FA Compositions

Class of GLs, % of the total	Acid, %, GLC									
	15:0	16:0	16:1	18:0	18:1	18:2	20:0	Σ_{sat}	Σ_{unsat}	Other acids *
SQVDGs (8.4)	1.8	58.8	—	—	11.7	6.5	4.1	71.6	28.4	17.1
DGDGs (11.6)	—	61.5	—	5.5	15.9	3.0	2.8	74.9	25.1	11.3
X_1 (2.2)	1.1	61.9	3.4	6.4	9.3	3.2	12.4	83.2	16.8	2.3
X_2 (4.1)	1.2	33.6	—	7.8	35.8	6.6	3.0	45.6	54.4	12.0
SGs (18.1)	—	—	—	—	—	—	—	—	—	—
MGDGs (36.1)	1.1	43.9	4.3	1.9	22.3	22.5	—	50.9	49.1	4.0
Ac-SGs (11.6)	0.5	41.4	3.1	1.6	42.8	9.5	—	44.2	55.8	1.1
Ac-MGDGs (7.9)	—	40.2	3.4	—	25.9	25.1	0.5	42.2	57.8	4.9

*Other acids: the 11:1, 12:1, 13;0, 13:1, 14:0, 14;1, 15:1, 16:2, 17:0, 17:1, and 19:0 species were present in all the fractions in various amounts.

It is known [5] that galactolipids in photosynthesizing tissues are usually highly unsaturated because of a high level of trienic acids. Reserve lipids have been little studied in this respect, but, at least among the species that have been studied [5, 6], unsaturated FAs predominate over saturated ones in seed glycosides. In the GLs of Russian olive seeds, in contrast to existing information on the seeds of other plants, a large degree of unsaturation of the FAs esterifying them was detected, particularly in relation to the DGDGs, where the 16:0 acid predominated. Furthermore, the GLC of the total FAs of the glyco-

TABLE 3. Amounts of PLs in Russian Olive Seeds and Their FA Compositions

FLs, % of the total	Acid, %, GLC						
	16:0	18:0	18:1	18:2	18:3	Σ_{sat}	Σ_{unsat}
PCs (52.1)							
total	7.3	3.8	30.3	50.4	8.2	11.1	88.9
sn-1	14.3	7.6	31.9	40.9	5.3	21.9	78.1
sn-2	0.3	—	28.7	60.0	11.0	0.3	99.7
PEs (16.4)							
Total	12.4	5.2	25.2	51.1	6.1	17.6	82.4
sn-1	23.8	10.4	29.8	33.6	2.4	34.2	65.8
sn-2	1.1	—	20.5	68.5	9.9	1.1	98.9
PIs (19.2)							
Total	30.2	5.1	15.9	34.0	14.8	35.3	64.7
sn-1	54.6	10.1	11.0	18.6	5.7	64.7	35.3
sn-2	5.8	—	20.8	49.4	24.0	5.8	94.2
PAs (3.5)	20.7	6.0	26.0	36.8	10.5	26.7	73.3
N-Acyl-PEs (1.7)	35.5	6.4	28.2	29.9	Tr.	41.9	58.1
Lyso-N-acyl-PEs (3.2)	65.2	2.4	17.7	14.7	Tr.	67.6	32.4
X1 (3.9)	77.2	10.9	9.3	2.6	Tr.	88.1	11.9

In all the PLs there were trace amounts of the 12:0, 14:0 and 16:0 acids.

TABLE 4. Amounts of PLs in the Pericarp of Russian Olive and Their FA Compositions

PLs	Acid, %, TLC								
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	Σ_{sat}	Σ_{unsat}
PCs (45.8)									
total	Tr.	12.0	0.2	8.1	29.2	42.7	7.8	20.1	79.9
sn-1	Tr.	23.2	—	16.2	24.8	29.3	6.5	39.4	60.6
sn-2		0.8	0.5	—	33.5	56.1	9.1	0.8	99.2
PEs (25.0)									
Total	—	22.0	1.3	30.6	9.4	29.1	7.4	52.8	47.2
sn-1	—	38.8	—	61.2	—	—	—	100.0	—
sn-2	—	5.6	2.7	—	18.7	58.2	14.8	5.6	94.4
PIs (29.2)									
Total	1.0	42.2	0.5	3.3	10.6	25.1	17.3	46.5	53.5
sn-1	2.0	79.6	—	6.6	4.1	4.1	3.6	88.2	11.8
sn-2	0.1	4.8	0.9	—	17.1	46.1	31.0	4.9	95.1

lipids of the seeds showed the presence of long-chain acids from 20:0 to 23:0 which were not present in the neutral acyl-containing classes of lipids of the seeds, where the main esterifying acids were the 18: 1 and 18:2 types [1].

With respect to the amounts of the main GL classes, the pericarp differed somewhat from the seeds. While in the seeds three classes of GLs (MGDGs, DGDGs, and SQVDGS) predominated, in the flesh of the fruit the dominating classes were the MGDGs and SGs, and the third place was shared by the DGDGs and Ac-SGs. The 16:0 acid was again the main one, while the amount of the 18:0 acid was considerably smaller than in the seeds and the proportion of the 18:1 acid had somewhat increased.

The FA composition of the glycolipids of the nonphotosynthesizing parts of plants is characterized by a high level of the 18:2 acid [5]. However, the plant that we studied deviates from this rule in relation to the FA composition (the main acid was the 16:0 species), although this is not the only case known in the literature. For example, another exception from this general rule is formed by turnip roots in which a high content of the 18:3 acids, which is usually characteristic for the photosynthesizing tissues of plants [7], has been detected.

Hydrolysates of GL fractions freed from acyl groups after severe acid hydrolysis were investigated by descending paper chromatography (PC). Standard glycerol, inositol, glucose, galactose, rhamnose, xylose and galacturonic acid were used as markers. In this way it was found that the sugar part of the GL fractions consisted exclusively of galactose.

The total PLs isolated from a chloroform–methanol extract by column chromatography (CC) and PTLC were separated by rechromatography into three classes in the case of the pericarp and seven classes in the case of the seeds. The PL classes

isolated were identified by comparing their chromatographic mobilities with those of model compounds and by qualitative reactions for functional groups.

The qualitative compositions of the PLs of the seeds and pericarp of Russian olive and the FAs composing them are given in Tables 3 and 4. The main PLs were phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), and phosphatidylinositols (PIs), with the PCs predominating in both cases. It must be mentioned that in the qualitative respect the Russian olive seeds were distinguished by the presence of phosphatidic acids (PAs) and N-acyl-PEs and N-acyl-lyso-PEs, and also an unidentified PL giving a positive reaction for sugars which was not detected in the pericarp. It is possible that the tissue of the pericarp did not contain highly active enzymes catalyzing the hydrolysis of the PLs.

On comparing the FA compositions of the PLs of the pericarp and of the seeds it can be seen that they were qualitatively identical. Predominating among the saturated acids was the 16:0 species, and among the unsaturated acids the 18:2 and 18:1 species. In the main PL classes the proportion of unsaturated acids predominated.

On investigating the position distribution of the FAs in the structures of the PLs, we observed a high specificity of the esterification of the unsaturated FAs in the sn-2 position of the glycerol moiety of the molecule. It is obvious that the total unsaturation of the PLs of the seeds was higher than that of the PLs of the pericarp, which indicates the specificity and functionality of the membranes of the different parts of the plant.

The minor PLs, apart from the PAs, identified in the seeds of Russian olive were distinguished by a high content of saturated acids. In all cases, linolenic acid mainly esterified the sn-2 position of the glycerol moiety.

EXPERIMENTAL

To isolate the total GLs from the seeds, the latter were defatted with hexane and were ground in an electric mill, and the total polar lipids were extracted with a mixture of chloroform and methanol (2:1). The extract was purified on a column of Molselekt G-25, and the total GLs were separated from the neutral lipids by TLC in the acetone–toluene–acetic acid–water (60:60:60:20:1) system (system 1).

The total GLs were separated into individual classes by rechromatography in a thin layer in system 1.

To isolate the FAs, all the GL fractions were subjected to the action of alkaline hydrolysis with a 10% methanolic solution of KOH. Hydrolysis was conducted for 1 h with heating in the water bath. After the acidification of the hydrolysate with HCl, the FAs were extracted with ether and were methylated with diazomethane.

To determine the carbohydrate components of the GLs, hydrolysates of the fractions freed from the acid moieties were subjected to severe acid hydrolysis by boiling with 2 N HCl for several hours. The compounds obtained were investigated by descending paper chromatography (PC) on Whatman paper in the butanol–pyridine–water (6:4:3) system (system 2). After the chromatograms had been run in a chamber, they were revealed with acid aniline phthalate (aqueous solution of phthalic acid + butanol + a few drops of aniline, 10 minutes' heating in a chest at 100°C).

To isolate the total PLs of the seeds, the ground seeds were extracted with a mixture of chloroform and methanol (2:1), and the extract was evaporated in a rotary evaporator. After the elimination of the neutral lipids and Pls with acetone, the total PLs were isolated by CC with elution by chloroform, with mixtures of chloroform and methanol, and with methanol.

The total PLs were separated into individual classes by preparative TLC in the chloroform–methanol–ammonia (70:30:5) system (system 3). The individual PL classes were identified by determining the R_f values of the components in comparison with authentic samples and by the performance of qualitative reactions for functional groups, as in [8].

Alkaline and enzymatic hydrolysis of the PLs were carried out as in [8].

GLC was conducted on a Chrom-41 chromatograph with a flame-ionization detector using a 2-m stainless steel column filled with Chrom W (60–80 mesh) with 17% of polyethyleneglycol succinate, t_{evap} 250°C, t_{therm} 196-200°C.

IR spectra were taken on a UR-20 instrument.

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