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LIPIDS OF *Phellodendron lavalei* SEEDS

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The chemical composition of neutral lipids and phospholipids from seeds of Phellodendron lavalei *cork is studied. Characteristic features of the fine structure of the separate classes are found. Significant quantities of eicosatrienoic acid and neutral lipids are found. The polar lipids are biologically active.*

Key words: *Phellodendron lavalei*, seeds, neutral lipids, phospholipids.

The cork *Phellodendron lavalei* Dode (Rutaceae) is a Japanese species. Its fruit and leaves are used in folk medicine of many countries for various ailments [1, 2]. A preparation (amodene) from the leaves of *P. lavalei* and *P. amurense* at the All-Union Institute of Medicinal Plants (Moscow) contains the pure flavanoid glycoside flavin 8-(3-methylbut-2-enyl)-5,4-dihydroxy-7-O-β-D-glucopyranosylflavanonol, an effective antihepatotoxic and antiviral agent [3].

Cork grows in the Caucuses and Crimea. Recommendations for cultivating this plant were developed at the experimental station for medicinal plants of the Institute of Pharmacochemistry of the Academy of Sciences of Georgia in Kobuleti, where a plantation was also established. The plant forms copious quantities of fruit that remain unused. Therefore, we considered it advisable to study the lipids of the seeds.

Neutral lipids of air-dried and ground material were obtained by exhaustive extraction with boiling *n*-hexane. The total neutral lipids were obtained as a yellow oily transparent liquid (16%).

TLC in system 1 of the neutral lipids revealed bands corresponding to [4]: hydrocarbons (HC), sterol esters (SE), methyl esters of fatty acids (MEFA), triacylglycerines (TAG), free fatty acids (FFA), free sterols (FS), diacylglycerines (DAG), monoacylglycerines (MAG), and phospholipids (PL). Adsorption chromatography of the neutral lipids on a silica-gel column with subsequent preparative TLC of the crude fractions isolated the separate classes of lipids (mass %): HC, 3.1; SE, 2.6; MEFA, 1.2; TAG, 87.2; FFA, 3.1; FS, 1.7; DAG, 0.4; MAG, 0.3; PL, 0.4. The ratio of the separate classes of neutral lipids was typical for oils of higher plants.

Fatty acids (FA) of neutral lipids from cork consist of ten components that include the usual FA in addition to lauric, myristic, and isopalmitic. It is especially important that eicosatrienoic acid was found in a significant quantity.

The sterol composition was studied by analyzing the free sterols and sterol esters. GLC analysis showed that free sterols of cork seeds contain (%): stigmasterol, 30.46; cholesterol, 28.80; ergosterol, 18.58; and β-sitosterol, 22.16. Sterol esters consist of 31.96% stigmasterol and 68.04% β-sitosterol [5].

Polar lipids of cork were extracted by the Folch method [6]. After purification from accompanying substances [7], PL (0.04%) were obtained from the air-dried material. Two-dimensional TLC on silica gel in systems 2 and 3 produced PL that were detected spectrophotometrically [8] (%): phosphatidylcholine (PC), 44.0; phosphatidylinosite (PI), 36.8; phosphatidylethanolamine (PE), 5.5; phosphatidyl acids (PA), 6.6; N-acylphosphatidylethanolamine (N-PE), 4.6; phosphatidylglycerine (PG), 2.3; lyso-phosphatidylcholine (lyso-PC), 0.2; and N-acyl-lyso-phosphatidylethanolamine (N-acyl-lyso-PE), trace.

*Deceased.

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TABLE 1. Fatty Acids of Total and Separate Classes of Neutral Lipids (%, GLC)

Acid	Total	TAG	FFA	SE
12:0	1.5	0.5	-	-
14:0	0.8	0.2	-	-
iso-16:0	2.1	1.2	-	-
16:0	9.1	8.4	31.7	25.9
16:1	2.7	1.7	-	-
18:0	2.0	1.8	5.5	3.9
18:1	26.5	29.2	26.6	33.0
18:2	28.1	43.4	18.1	24.6
18:3	18.7	10.3	2.6	12.6
20:3	8.5	3.3	15.5	-

TABLE 2. Composition and Distribution of FA in PC and PI of Cork Seeds

Phospholipids	Fatty acids, % GLC						
	16:0	18:0	18:1	18:2	18:3	Σ_{sat}	Σ_{unsat}
Total PL	29.0	9.8	27.5	24.4	9.3	38.8	61.2
PC							
Total	47.2	13.9	27.8	11.1	-	61.1	38.9
sn-1	57.6	20.7	16.8	4.9	-	78.3	21.7
sn-2	36.8	7.0	38.7	17.5	-	43.8	56.2
PI							
Total	23.0	27.0	46.3	3.7	-	50.0	50.0
sn-1	32.5	35.0	27.5	5.0	-	67.5	32.5
sn-2	13.5	19.0	65.1	2.4	-	32.5	67.5

It can be seen that the dominant components of PL are PC and PI. The distribution is regular with PC > PI > PE. The high PI content is interesting. Column chromatography and preparative TLC on silica gel yielded chromatographically pure PC and PI, for which structural analysis was performed [9, 10]. IR spectra agree with those in the literature for glycerophospholipids and give the following picture. For PC: 980 $[-N(CH_3)_3]^+$, 1750 (C=O), 1260 (P=O), 3400 (OH), 2940, 2870, 1470 (CH, CH_2, CH_3), 1090 (P–O–C), 3020 (C=C); for PI: 1750 (C=O), 1250 (P=O), 1080 (P–O–C), 3500-3200 (OH), 2865, 2930, 1480 (CH, CH_2, CH_3), 3015 cm^{-1} (C=C).

Alkaline hydrolysis of FA was performed by methylation with diazomethane and GLC analysis. The positional specificity of the FA in the PL was determined by enzymatic hydrolysis by phospholipase A_2 [11] in TRIS-buffer (pH 7). The released sn-2 FA were methylated by diazomethane. The lyso-compounds were hydrolyzed by alkali. The sn-1 FA were methylated. The resulting MEFA were analyzed by GLC.

Table 2 shows that palmitic acid dominates the saturated acids in the PC; stearic acid, the PI. This is rarely encountered in plant PL. PC are more saturated than PI. The even distribution of saturated (50%) and unsaturated (50%) acids in the PI is interesting. The similar content of 16:0 and 18:0 acids in the sn-1 is also interesting. The sn-1 contain 78.3% PC and 67.5% PI of saturated acids. The unsaturated acids in sn-2 are 56.2 and 67.5%, respectively.

The probable molecular compositions of the PC and PI were calculated using data for the positional distribution of FA [12].

The molecular-type composition of the studied PL contains 16 types. The principal types for the PC are 16:0—16:0 (21.2%), 16:0—18:1 (22.3%), 16:0—18:2 (10.1%); for PI, 16:0—18:1 (21.2%), 18:0—18:1 (22.8%), 18:1—18:1 (18.9%).

TABLE 3. Molecular Composition of PC and PI of Cork Seeds, mass %

Molecular composition	PC	PI	Molecular composition	PC	PI
16:0 - 16:0	21.2	4.4	18:1 - 16:0	6.2	3.7
16:0 - 18:0	4.0	6.2	18:1 - 18:0	1.2	5.2
16:0 - 18:1	22.3	21.2	18:1 - 18:1	6.5	18.9
16:0 - 18:2	10.1	0.8	18:1 - 18:2	2.9	0.6
18:0 - 16:0	7.6	4.7	18:2 - 16:0	1.8	0.7
18:0 - 18:0	1.4	6.7	18:2 - 18:0	0.3	0.9
18:0 - 18:1	8.0	22.8	18:2 - 18:1	2.0	3.3
18:0 - 18:2	3.6	0.8	18:2 - 18:2	0.9	0.1

TABLE 4. Composition of PC and PI of Cork Seeds

Type	PC	PI
SS	22.6	11.1
SS	11.6	10.9
SU	44.0	45.6
US	9.5	10.5
UU	7.4	19.0
UU	4.9	3.9

The positional-type composition of the PC and PI were calculated from their type composition (Table 4). The data show that the principal ones are the saturated—unsaturated (SU) type (44% in PC, 45.6% in PI). Saturated—saturated (SS), 22.6%, are also characteristic of PC; unsaturated—unsaturated (UU), 19.0%, of PI.

Polar lipids of cork seeds inhibit growth of intergrown tumors in animal experiments. The evaluation of their use in medicine will continue.

EXPERIMENTAL

TLC methods for observing separate classes of lipids and column chromatography for isolating and identifying FA have been described [13].

TLC was performed on Silufol plates and on silica gel with added $CaSO_4$ (10%) in systems C_6H_{14}—$(C_2H_5)_2O$—CH_3CO_2H (86:14:1, 1), $CHCl_3$—CH_3OH—NH_4OH (25%) (65:30:4, 2), and $CHCl_3$—CH_3OH—CH_3CO_2H—H_2O (glacial) (170:25:25:6, 3).

IR spectra were recorded on a UR-20 instrument as films.

Sterols were analyzed by GLC on a Varian instrument with a flame-ionization detector. The column (2.0×0.25 cm) was packed with 3% SE-30, Chromosorb W, temperature 268° C, carrier-gas (He) flow rate 25 ml/min, H_2 50 ml/min.

GLC was performed on a Khrom-41 chromatograph using a stainless-steel column (2.5 cm × 4 m) packed with 17% PEGS on Chrom W (60-80 mesh) at 200° C.

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