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LIPIDS OF THE SEEDS OF *Paliurus spina-christi*

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The composition of the unsaturated and oxidized fatty acids and steroids of the lipids of the seeds of *Paliurus spina-christi* has been determined. Among the unsaturated acids the 20:1 (11) was detected, while the 18:1 and 18:2 acids predominate. The oxidized acids are represented by the sum of the 9,10(12,13)-epoxy-12(9)-18:1, the 9,10-epoxy-18:0, the oxo-18:1, the oxo-18:2, the 12-hydroxy-9-18:1, and the 9-hydroxy-10,11-18:2 acids. The main component of the sterols is β-sitosterol. It has been found that the composition of the acylglycerols of the lipids of the seeds of *P. spina-christi* gathered in different years is variable.

Paliurus spina-christi Mill. (Christ-thorn paliurus) (family Rhamnaceae) grows throughout the territory of the Georgian SSR and other regions of the Soviet Union [1]. The fruit of this plant has long been used in folk medicine for the most diverse diseases. Recently, it has been found in the Institute of Pharmacochemistry of the Academy of Sciences of the Georgian SSR that the lipids of the seeds of *P. spina-christi* exhibit an interesting biological activity in experiments on animals.

In the literature there are only general references to the lipids of the seeds of *P. spina-christi* [3], and we therefore set ourselves the aim of making a detailed analysis of the composition and structure of the lipid components of the seeds of this plant growing in Georgia.

The yield of total neutral lipids from the seeds amounted to 20%. A number of physicochemical indices of the total lipids and of the fatty acids isolated from them were determined by methods generally adopted:

Index	Total lipids	Acids
Density, d_4^{20}m g/cm^3	0.9227	0.9100
Refractive index, n_D^{20}	1.4778	1.4662
Iodine No., % I_2	102.6	109.2
Acid No., mg KOH	4.5	–
Amount of unsaponifiables, %	0.2	–

The IR spectra of the lipids and of the methyl esters (MEs) of the fatty acids had absorption bands in the region of conjugated double bonds at 230-234 nm and of a carbonyl group at 265-280 nm. The IR spectrum showed absorption bands of an epoxy group at 850, 875, 920, and 1280 cm^{-1} and of a trans-olefinic bond at 970 cm^{-1}, a broadened band of an ester carbonyl at 1750 cm^{-1} with inflections at 1670, 1690, and 1705 cm^{-1}, and the absorption of hydroxy groups at 3480 cm^{-1}.

On TLC analysis (in system 1) of the total lipids, they were found to contain hydrocarbons (HCs), sterol esters (SEs), triacylglycerols (TAGs), epoxyacylglycerols (EAGs), free fatty

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TABLE 1. Fatty Acid Composition of the Lipids of Paliurus spina-christi

Acid	Total lipids		TAGs	FFAs	DAGs
	I	II			
12:0	0,8	0,4	1.0	—	0,8
14:0	0,2	0,4	0,5	—	0,8
16:1	0,4	0,3	Tr.	—	Tr.
16:0	8,0	7,7	12 0	22.5	12,4
18:0	10,3	3.5	11,2	10,3	8,3
18:1	35,3	36,9	30,4	29.2	32,7
18:2	38.2	43,0	41 2	29,2	42.6
18:3	2,6	3,4	1,7	8.8	2,4
20:0	—	Tr.	—	—	—
20:1	0,3	2,9	1,1	—	Tr.
Sum of the unidentified compounds	3.9	0,6	0,9	—	—
Σ sat	19,3	12,0	24.7	32.8	22,3
Σ unsat	76.8	87,4	74,4	67,2	77,7

Note. Taken on polar, and the remainder on moderately polar phases.

acids (FFAs), and oxo- (oxo-AGs), hydroxy- (hydroxy-AGs), and diacylglycerols (DAGs), free sterols (FSs), and unidentified classes with R_f 0.32, 0.22, 0.21, and 0.13.

The total lipids were separated into individual classes by chromatography on a column of silica gel with the use as mobile phases of mixtures of petroleum ether with diethyl ether, diethyl ether alone, chloroform, and methanol, and in this way the following fractions were obtained: HCs with ESs, 2.1%; TAGs, 89.9%; EAGs, FFAs, and oxo-AGs, 1.3%; DAGs and hydroxy-AGs, 0.4%; while the chloroformic eluate contined 3.5% and the methanolic eluate 2.8% (of the weight of the total lipids deposited on the column).

The compositions and structure of the fatty acids and sterols were investigated in detail.

The fatty acids were isolated from the lipids by alkaline hydrolysis and were converted into their MEs. The fraction contining the FFEs was methylated and the MEs formed were separated from the accompanying oxidized lipids by preparative TLC in system 2. The MEs of the fatty acids obtained from the total lipids were purified similarly. The composition of the fatty acid MEs were determined by GLC, and the results are given in Table 1.

It can be seen from Table 1 that in all the fractions of lipids from the seeds of P. spina-christi the 18:1 and 18:2 unsaturated acids predominated, while the FFAs amounted to more thn 30% of the saturated components. A difference was observed in the amounts of the 18:0, 18:1, and 18:2 acids according to the conditions of GLC analysis. This fact was the basis for a more detailed analysis of the structure of the fatty acids, and consisted in the separation of their MEs into individual fractions according to degree of unsaturation followed by the oxidation of these fractions with the periodate–permanganate reagent.

The MEs of the acids from the total lipids were analyzed. They were separated into fractions by means of Ag^+-TLC in system 3, as a result of which the following fractions were obtained: 19.2% of the MEs of saturated, 36.2% of those of monoenoic, 37.1% of those of dienoic, 2.1% of those of trienoic, and 1.6% of unidentified acids (the losses amounting to 3.8%). In the fraction of the MEs of saturated acids the following were detected by the GLC method: 12:0 (1.4%), 14:0 (0.7%), 16:0 (59.7%), 18:0 (29.2%), 18:1 (3.1%), and 20:1 (0.7%). The fraction of monoenoic MEs consisted mainly of the 18:1 compound (96.2%) with small amounts of the 16:1 (2.3%) and 20:1 (1.4%) representatives.

The destructive oxidation of this fraction gave the dimethyl ethers of the 9:0 and 11:0 dicarboxylic acids (94.4% and 5.6%, respectively), and the MEs of the 9:0 and 7:0 monocarboxylic acids (99.2%, and 0.8%, respectively).

The fraction of dienic MEs contained an 18:2 ME in the products of the oxidation of which the dimethyl esters of the 9:0, 7:0, and 5:0 acids were identified (97.9, 1.0, and 1.0%, respectively), and the 6:0 ME.

The fraction of the trienic MEs contained an 18:3 compound the oxidation of which gave the dimethyl esters of the 9:0, 7:0, and 5:0 acids (91.6, 5.1, and 3.3%, respectively). Of

the monocarboxylic fragments in the degradation products, only propionic acid (3:0) was detected by TLC on cellulose in system 4.

Thus, the mixture of unsturated fatty acids from the lipids of the seeds of P. spina-christi includes the 16:1(9), 18:1(9), 18:2(9, 12), 18:3(9, 12, 15), and 20:1(11) acids and, in minor amounts, possibly the 18:2(5, 12) and 18:3(5, 12, 15) acids. This is the first time that the eicosenoic acid (20:1) has been detected in the lipids of plants of the family Rhamnaceae [4].

As mentioned above, in the mixture of fatty acids isolated from the total lipids, in addition to unsaturated acids there were also oxidized forms. When the MEs were chromatographed in a thin layer of silica gel in system 2, they appeared in the form of the spots of the MEs of epoxy (R_f 0.66), oxo (R_f 0.63), and hydroxy (R_f 0.41) acids. The epoxy compounds gave a positive reaction with picric acid and the keto compounds one with 2,4-dinitrophenylhydrazine. The MEs of the oxidized forms were isolated in the process of purifying the MEs of unsubstituted acids. In view of their very small amount, the oxo acids were not separated from the epoxy acids. The IR spectrum of this combined material showed that the absorption bands of an epoxide ring at 830, 850, 890 (cis and trans forms), 920, and 1280 cm^{-1}, of cis,trans-conjugated olefinic bonds at 950 and 985 cm^{-1}, and absorption bands at 1610, 1645, 1655, 1690, 1710, and 1720 cm^{-1} belonging to COCH=CH and $CO(CH=CH)_2$ groups.

To determine their structures, the MEs of the epoxy acids were converted into dihydroxy derivatives which were then analyzed by mass spectrometry in the form of their bis(trimethylsilyl) ethers (TMS derivatives). The mass spectrum contained the peaks of fragments with m/z 457 $[M - 15]^+$, 441 $[M - 31]^+$, 299, 259, 213, and 173, which are characteristic for the fragmentation of the TMS derivatives of the MEs of the isomeric 9,10(12, 13)-dihydroxyoctadec-12(9)-enoic acids [5]. Of them, the peaks of ions with m/z 259 and 213 assigned to the derivative of the 9,10-dihydroxyoctadec-9-enoate had the greatest intensity. The strong peaks of ions with m/z 215, 459 $[M - 15]^+$, and 443 $[M - 31]^+$ observed in the spectrum were formed in the fragmentation of the TMS derivative of the ME of the 9,10-dihydroxy-18:0 acid. According to these results, the set of epoxy acids consisted of 9,10-epoxyoctadec-12-enoic, 12,13-epoxyoctadec-9-enoic, and 9,10-epoxyoctadecanoic.

From fragments with m/z 310 $[M^+]$, 295, 297 $[M - 15]^+$, 279, 281 $[M - 31]^+$, 280, 282 $[M - 32]^+$, 267, 269 $[M - 43]^+$, and 261, 263 $[M - 49]^+$ not containing Si it was established that together with the TMS derivatives there were the MEs of oxo-18:1 and oxo-18:2 acids. The IR spectrum showed that the oxo-18:2 acid contained a $-CO(CH=CH)_2$ group.

The UV spectrum of the MEs of the hydroxy acids showed an absorption maximum at 234 nm, and the IR spectrum absorption bands of a hydroxyl at 3300-3600 cm^{-1}. The mass spectrum of the TMS derivatives of the MEs of the hydroxy acids contained peaks of ions with m/z 382, 369, 367, 353, 351, 311, 299, 225 and 187.

The mass numbers and ratios of the intensities of the main fragments show that the main components in this group were 12-hydroxyoctadec-9-enoic and 9-hydroxyoctadeca-10,11-dienoic acids [6].

To isolate the sterols, the combined lipids were subjected to severe alkaline hydrolysis, the unsaponifiable fraction was separated off, and the sterols were precipitated from methanolic solution at low temperature. The resulting mixture was finally freed from impurities by preparative TLC on silica gel in system 5.

GLC analysis showed that the sterols of the lipids of the seeds of P. spina-christi contained 66% of β-sitosterol, 13% of stigmasterol, 11% of campesterol, and 10% of unidentified compounds.

When the lipids of the seeds of P. spina-christi of the 1983 harvest collected from the same plot were analyzed, no hydroxy lipids were found in them. The set of unsaturated fatty acids consisted of the 9:0 (0.1%), 10:0 (0.1%), 12:0 (0.8%), 13:0 (0.7%), 14:0 (0.2%), 16:0 (14.9%), 18:0 (10.3%), 18:1 (43.3%), 18:2 (22.9%), 18:3 (3.7%), 20:1 (0.9%), 20:2 (1.3%), 20:3 (0.8%), and 16:1 (traces) acids, i.e., in comparison with the 1980 harvest (see Table 1) the amount of 18:2 acid had fallen and the sum of the 18:1 + 16:0 acids had increased by 15%.

The prolonged storage of a lipid extract of the seeds under laboratory conditions (5-6 months) did not cause the formation of oxidized forms of the lipids in it. These facts permit the assumption that the appearance of oxidized lipids in the seeds of P. spina-christi of the 1980 harvest may be a consequence of a disturbance of the lipid metabolism under the influence

of unfavorable environmental factors. A comparative evaluation of the biological activities of the lipids of the seeds of the two harvests show that the oxidized lipids did not affect the specific acitivity of the total lipid material.

EXPERIMENTAL

UV spectra were taken on a Hitachi spectrometer in hexane, IR spectra on a UR-10 instrument in a film, and mass spectra on a MKh-1303 instrument (140-160°C; ionizing energy 50 eV).

GLC conditions: the MEs of the fatty acids were chromatographed on a Pye-105 instrument with a flame-ionization detector using a 8-m glass capillary column containing OV-101 at 200°C with a rate of flow of the carrier gas (nitrogen) of 40 ml/min, and also on a Chrom-4 chromatograph with a flame-ionization detector using a 2.5 m × 3 mm stainless steel column filled with Chromaton N-AW-DMCS impregnated with 17% of ethylene succinate. The temperature of analysis for the MEs of the high-molecular-weight acids and di-MEs of the dicarboxylic acids was 192°C, and for the MEs of the low-molecular-weight acids 132°C, the rates of flow of the carrier gas (helium) being 100 and 75 ml/min, respectively.

The GLC analysis of the sterols was performed on a Varian chromatogrphy with a flame-ionization detector using a 2 m × 4 mm column filled with Chromosorb W impregnated with 3% of SE-30 at 200°C at a rate of flow of helium of 50 ml/min.

Column chromatography was performed on silica gel L 100/160 [7] and thin-layer chromatography on Silufol and silica gel L 5/40 with the addition of 15% of $CaSO_4$ in the following solvent systems: 1) heptane–methyl ethyl ketone–CH_3COOH (43:7:0.5), two runs, hexane–diethyl ether – 2) (7:3) and 5) (9:1); and 3) benzene (amount of $AgNO_3$ added to the sorbent 20%); and TLC on cellulose in system 4: t-butanol–ammonia–H_2O (20:1:4).

Ripe seeds of P. spina-christi were collected on the territory of the experimental field for medicinal plants of the Institute of Pharmacochemistry of the Academy of Sciences of the Georgian SSR in Tbilisi in December, 1980, and in December, 1983.

The lipids were extracted from the air-dry freshly ground seeds three times with petroleum ether (bp 40-70°C) at room temperature by the steeping method. The time of each extraction was 6 h. The extracts obtained were combined and filtered, and the solvent was evaporated off in vaccum in a rotary evaporator.

The physicochemical indices of the total lipids were determined by standard methods [8].

Qualitative rections, the treatment of the epoxy acids, the preparation of the TMS derivatives, and oxidation with the periodate–permanganate reagent were performed as described previously [7].

SUMMARY

The composition of the unsaturated and oxidized acids and the sterols of the lipids of the seeds of Paliurus spina-christi has been determined. In the unsaturated acids the 20:1-(11) compound was detected, while the 18:1 and 18:2 acids predominated. The oxidized acids were represented by the sum of the 9,10(12,13)-epoxy-12(9)-18:1, the 9:10-epoxy-18:0, the oxo-18:1, the oxo-18:2, the 12-hydroxy-9-18:1, and the 9-hydroxy-10,12-18:2 acids. The main component of the sterols was β-sitosterol.

A variability of the composition of the acylglycerols of the seeds of P. spina-christi of different harvest years has been found.

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