LIPIDS OF THE FRUIT OF Acanthopanax sessiliflorus

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In the lipids of the seeds and pericarp of the introduced species Acanthopanax sessiliflorus (Rupr. et Maxim.) Seem (family Araliaceae), 23 classes of compounds have been detected, of which 19 have been identified. Sixteen of the classes have been studied. The lipids of the pericarp contain a more complex set of components than the seeds. Hydroxyacyldiacylglycerides, fatty acid methyl esters, and a complex group of pthalic acid esters have been detected in the family Araliaceae for the first time. The fatty acid compositions of the seed and pericarp lipids are characterized by a high level of petroselinic acid.

Many species of the family Araliaceae belong among the medicinal plants. Galenical preparations made from them possess tonicizing, stimulating, adaptogenic, and other types of action [1]. There is little information on the lipids of the plants of this family, although the glycosides, saponins, essential oils, and lignins of various organs of the Araliaceae have been subjected to chemical analysis. It has been found that a characteristic feature of the family is the presence in the seed lipids of petroselenic acid (18:1(6)), the amount of which varies from 1.5 to 83% of the total fatty acids [2].

The object of our investigation was the dry fruit of Acanthopanaz sessiliflorus (Rupr. et Maxim) Seem. (family Araliaceae) introduced into the Tashkent Botanical Garden. The natural habitat of the wild species is the Far East [1]. Preparations from the fruit and leaves of this plant are of interest because of their pharmacological action [3].

We studied the lipids of the dry fruit (I), the seeds (II), and the pericarp of the fruit (III). The seeds amounted to 10.4% of the weight of the dry fruit and the yields of hexane extracts from (I), (II), and (III) were 7.2, 30.3, and 4.40, respectively. The extracts differed in color and consistency: (II) consisted of a light yellow liquid, (I) and (III) had a brown-green color and a specific pleasant smell, while extract (III) was a solid at room temperature and (I) acquired a high viscosity and formed a mat film on the walls of the flask only on cooling to −10°C.

Preliminary information on the composition of the lipids of the extract was obtained from the results of TLC on Silufol in systems 1-4. Then extract (I) was separated on a column of silica gel (CC) and narrow fractions of the lipids were analyzed by TLC using systems appropriate to the polarities of the fractions. For a detailed analysis, homogeneous classes of the main lipids were isolated from the narrow fractions by subsequent preparative TLC. The quantitative compositions of lipids (I)-(III) were judged from the results of the preparative chromatographic mass (CC, TLC). Model samples of plant lipids, literature information on the chromatographic mobilities of individual classes of lipids, and the capacity of some lipophilic compounds for giving a characteristic coloration of the spot when the chromatograms were treated with 50% H_2SO_4 were used to identify the homogeneous classes.

The following were found in extract (I): paraffinic and isoprenoid hydrocarbons (HCs, 1% of the weight of the extract), carotenes (tr.), 2.6% of fatty acid ester (FAEs) with aliphatic fatty alcohols, with triterpenols (TEs), and with sterols (SEs), and with three unidentified alcohols (XE_1, XE_2, and XE_3), 0.5% of fatty acid methyl esters (FAMEs) and phthalic acid esters (PthEs), triacylglycerols (TAGs) (84.6%), free fatty acids (FFAs, 1.3%), fatty alcohols (Falcs), 1.8%), acetates of triterpene acids (0.2%), 1.3% of hydroxyacyldiacylglyerosl (OH-TAGs) and 1,3- and 1,2(2,3)-diacylglycerols (DAGs), triterpenols

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(Trs, 0.2%), sterols (STs, 4.5%), an unidentified class (X, tr.), chlorophyll (Chl, 0.5%), 1(3)- and 2-monoacylglycerols (MAGs, tr.), and glycolipids (GLs, 1.5%).

In extract (II), according to the results of TLC, of the above-mentioned set, XE_1, FAlcs, the acetates of triterpene acids, and Chl were absent, the main component being the TAGs.

Extract (III) contained no OH-TAGs, DAGs, MAGs, and GLs but was rich in pigments, PthEs, and esters of FAs with monohydric alcohols. Two components of this extract (R_f 0.48 and 0.45, system 4) were not identified.

It appeared of interest to localize the 18:1(6) acid in different parts of the fruit of Ac. sessiliflorus. With this aim, in order to analyze their fatty-acid compositions, we isolated the TAGs (58.7% on the weight of the extract) and the FFAs (2.9%) from extract (III) by the CC method. Part of extracts (I), (II), and (III) and the acyl-containing classes (I) and (III) were hydrolyzed with ethanolic caustic soda and the FAs were obtained and then these were analyzed in the form of their methyl esters (MEs) by the Ag^+-TLC (system 8) and GLC methods.

According to the results of Ag^+-TLC, almost all the samples contained, together with oleic acid (18:1(9), R_f 0.4), its 18:1(6) isomer, with R_f 0.68, the latter predominating quantitatively. An exception was formed by the FFAs of extract (III), where the amounts of the 18:1(9) and 18:1(6) acids were approximately equal. Of the other unsaturated acids, dienic (R_f 0.48) and trienic (R_f 0.28) components were detected.

For a more accurate quantitative estimate of their isomeric compositions, the MEs of the acids from the TAGs and the FFAs of extract (I) were separated by preparative Ag^+-TLC in the same system and the monoenes isolated were oxidized with the periodate-permanganate reagent. The amount of the 18:1(9) acid was calculated from the GLC results on the basis of the ratio of the peaks of the 9:0 acid formed on the oxidative degradation of the 18:1(9) component and the 12:0 acid produced by the degradation of the 18:1(6) component. The results of the analyses of the fatty acid compositions are given in Table 1.

It can be seen from Table 1 that not only the reserve lipids (II) but also the surface lipids (III) of the fruit of Ac. sessiliflorus contain the 18:1(6) acid. However, this acid is not present in all the classes of lipids. It was not detected in the MAGs, the GLs, and the esters of FAs with monohydric alcohols. With the same qualitative composition of the FAs, the lipids of extracts (III) contained larger amounts of saturated and polyenic (18:2 and 18:3) components.

The 22:0 and 24:0 acids are probably present only in the FFAs of the seeds, the total degree of unsaturation of this class being lower in the seeds than in the pericarp. We have previously reported the structure of the TAGs of the fruit of Ac. sessiliflorus from the results of a stereospecific analysis [4].

In the saponification products of the fraction of extract (I) consisting of the OH-TAGs and DAGs spots of unsubstituted acids (R_f 0.51) and of hydroxy fatty acids (R_f 0.28) were detected. After the separation of the acids in the form of their MEs by preparative TLC in system 7, the weight ratio of unsaturated and hydroxy acids amounted to 10:1 (instead of 2:1 in the case of the individual OH-TAGs). Thus, it was found that the amount of DAGs in this total was four times higher than that of the OH-TAGs. Table 1 gives the composition of the unsubstituted acids of this fraction.

The IR and UV spectra of the MEs of the hydroxy acids from Ac. sessiliflorus were similar to those described previously [5]. The compositions and structures of the main components were established from the products of the periodate-permanganate degradation of the hydroxy fatty acid MEs and from the results of the mass spectrometry of their trimethylsilyl (TMS) derivatives. The most intense peaks of fragments from the breakdown of the TMS derivatives corresponded to the known 12-hydroxy-18:0 (m/z 187, 301), 9-hydroxy-11-18:1 (227, 259), 12-hydroxy-9-18:1 (187, 299, 270), and 9(13)-hydroxy-10, 12(9, 11)-18:2 (225, 311) acids [6].

According to their UV spectra, in the α-hydroxy acids the double bonds were in cis-trans conjugation.

This is the first time that hydroxy acids have been detected in lipids of plants of the family Araliaceae.

TABLE 1. Fatty Acid Compositions of the Acyl-Containing Lipids of the Fruit of Acanthopanax sessiliflorus (GLC, %)

Acid	I							II	III		
	extract	FAEs	FAMEs	TAGs	FFAs	OH-TAGs + DAGs	MAGs + GLs	extract	extract	TAGs	FFAs
12:0	tr.	tr.	11.8	tr.	tr.	—	3.9	tr.	0.4	tr.	tr.
13:0	tr.	tr.	tr.	tr.	tr.	—	tr.	tr.	0.6	tr.	tr.
14:0	tr.	1.6	1.1	tr.	0.6	0.7	1.6	tr.	0.6	0.5	0.9
15:0	tr.	tr.	tr.	tr.	tr.	—	1.7	tr.	tr.	tr.	tr.
16:0	4.6	1.8	21.0	3.1	20.7	10.5	36.0	3.2	11.0	9.1	20.2
16:1	1.1	tr.	—	tr.	0.6	tr.	—	0.9	tr.	1.5	0.5
17:0	tr.	tr.	tr.	tr.	tr.	—	1.2	tr.	1.6	tr.	0.6
18:0	0.4	2.1	3.7	tr.	2.9	tr.	3.8	tr.	1.6	1.4	1.1
18:1(6)	57.0	—	—	52.8	15.4	43.3	—	62.1	32.5	41.5	5.1
18:1(9)		12.6	30.0	8.9	2.5		17.4				
18:2	33.8	66.9	29.7	34.1	38.9	40.1	34.4	33.8	42.9	40.8	53.9
18:3	3.1	—	2.7	1.1	8.3	5.4	tr.	tr.	8.8	5.2	16.5
20:0	tr.	—	tr.	tr.	tr.	—	—	tr.	tr.	tr.	1.2
22:0	—	—	—	—	7.5	—	—	—	—	—	—
24:0	—	—	--	--	2.6	—		—	—	—	—
Σ_{sat}	5.0	20.5	37.6	3.1	34.3	11.2	48.2	3.2	15.8	11.0	24.0
Σ_{unsat}	95.0	79.5	62.4	96.6	65.7	88.8	51.8	96.8	84.2	89.0	76.0

On Silufol in system 3, the FA ester present in each extract has the form of an extended spot (R_f 0.80-0.85) with a red-brown coloration after treatment with $H_2SO_4/t°$. After a parallel chromatogram had been treated with I_2 vapor and had been kept briefly in the air, an additional white spot was detected which showed the presence of esters of fatty alcohols with saturated acid and alcohol moieties.

The products of the severe hydrolysis of this total material (TLC, system 4) consisted of FAs, FAlcs, Trs, Sts, and three unidentified alcohols with R_f 0.43, 0.36, and 0.26.

A feature of the acid fraction of the esters was the high proportion of the 18:2 acid in them and the absence of the 18:1(6) acid. The composition of the acids was confirmed by the mass numbers and intensities of the peaks of the fragments $[RCO]^+$ and $[RCO - 1]^+$ in the mass spectrum of the initial esters [7].

The peaks of ions characteristic for fatty alcohols, phytosterols [8], and triterpenols [9] were observed in the spectrum. The composition of the alcohols were as follows (GLC, %): aliphatic C_{18} (4.7), C_{20} (11.4), C_{22} (4.8), C_{24} (2.0), C_{26} (3.6); stigmasterol (10.2), β-sitosterol, β-amyrin (48.8), and four unidentified peaks with relative retention times (RRTs) 0.65 (4.1), 1.14 (tr.), 1.33 (7.1) and 4.11 (3.7).

The phthalic acid esters appeared on TLC in the form of a lilac spot after treatment of the layer with $H_2SO_4/t°$ and the prolonged keeping of the chromatogram in the air. The UV spectrum of these compounds corresponded to that described in [10], and in the mass spectrum intense peaks with fragments m/z 149, 167, and 279 were observed. On GLC, 13 peaks were obtained which were identified by a consideration of the characteristics of the mass spectrum given in [11]. According to the results of the analysis, more than half the total PthEs consisted of butyl octyl phthalate (RRT – relative retention time with respect to dibutyl phthalate):

Phthalate	RRT	X	Phthalate	RRT	X
Dimethyl	0.26	Tr.	Propyl octyl	2.88	Tr
Diethyl	0.40	0.7	Butyl octyl	4.00	62.2
Dipropyl	0.45	0.9	Pentyl octyl	4.76	2.0
Butyl propyl	0.78	0.6	Hexyl octyl	5.35	Tr.
Dibutyl	1.00	5.9	Dioctyl	6.50	2.5
Methyl octyl	1.32	Tr.	Decyl octyl	9.08	8.8
Ethyl octyl	1.65	16.4			

It must be mentioned that 8 out of the 13 PthEs were heterogeneous, i.e., they contained two different alkyl residues, one of which was octyl.

In the mass spectra of the PthEs, in addition to fragments from the decomposition of the phthalates, ions were present which were assigned to natural FAMEs as impurities. It was impossible to separate them from the phthalates. When this fraction was analyzed by GLC under conditions in which phthalates were not desorbed from the phase, the composition of the natural FAMEs was obtained (Table 1).

We then investigated the classes containing no fatty acyl residues.

When the HCs were chromatographed (TLC, system 1), paraffins (R_f 0.90) and three spots with R_f 0.58, 0.36, and 0.15, revealed by I_2 were detected. When the chromatogram was treated with $H_2SO_4/t°$, the spots gave pink, scarlet, and red colorations, respectively, changing to various shades of lilac when the chromatogram was kept in the air for a day. The spot with R_f 0.15 corresponded in mobility and color to standard squalene. Taking into account the results of IR spectroscopy, and with the aid of GLC, among the components of this class were identified paraffins – C_{21} (tr.), C_{23} (tr.), C_{25} (2.9), C_{29} (9.7), and C_{31} (tr.) – and isoprenoids – C_{24} (tr.), C_{28} (0.90), C_{30} (squalene 84.3).

The set of fatty alcohols included (%, GLC): C_{18}(tr.), C_{22} (5.7), C_{24} (13.4), C_{26} (44.5), C_{28} (36.4), and C_{30} (tr.).

The free Sts consisted, according to GLC, of stigmasterol (31.8%) and β-sitosterol (68.2%).

In the free triterpenols, on the basis of their GLC behavior and their mass spectra [12], β-amyrin and lanosterol (one peak with RRT 1.06, 16.7%), 24-methylenecycloartenol (RRT 1.68, 62.4%), and three unidentified components with RRTs of 0.65 (13.1%), 0.88 (4.0%), and 1.33 (4.0%) were detected.

The carotene of Ac. sessiliflorus corresponded in its UV absorption to β-carotene [13].

The chlorophylls were represented by four components with R_f 0.51, 0.44, 0.40, and 0.38 in system 6. The main component with R_f 0.51 corresponded, according to its UV spectrum, to chlorophyll a.

EXPERIMENTAL

UV spectra were taken on a Hitachi spectrophotometer in hexane, IR spectra on a UR-10 instrument in a film, and mass spectra on MKh-1303 and MKh-1310 instruments.

GLC was performed on a Chrom-4 instrument with a flame-ionization detector. A 4 × 2500 mm column filled with 17% of ethylene succinate (ES) and a 4 × 1200 mm column filled with 5% of SE-30 were used. In both cases the support for the phases was Chromaton N-AW-DMCS.

The ES column was used for the analysis of the MEs prepared from acids and those naturally present. The column temperature for the FAMEs was 196°C, for the dicarboxylic acid MEs 180°C, and for the MEs of low-molecular-weight acids 132°C.

The HCs, FAlcs, Trs, and Sts were chromatographed on SE-30 under isothermal conditions at 240, 250, and 270°C. The PhtEs wee analyzed on SE-30 in the temperature interval of 180-280°C with programming at 2°C/min.

RRTs were calculated for the Trs and Sts in relation to β-sitosterol, and for the phthalates in relation to dibutyl phthalate.

CC was performed on silica gel L 100/160 [5] and TLC on Silufol and silica gel L 5/40 with the addition of 6.5% of $CaSO_4$ in the following solvent systems: 1) hexane; 2) hexane–diethyl ether (95:5); 3) hexane–diethyl ether–CH_3COOH (90:10:1) and 4) (70:30:1); 5) chloroform–CH_3OH–H_2O (65:25:4); and 6) benzene–acetone–petroleum ether–hexane (10:10:3:10), and preparative TLC in 7) hexane–diethyl ether (7:3).

Ag^+-TLC was performed on silica gel 5/40 with the addition of 10% of $CaSO_4$ and 20% $AgNO_3$ in benzene (system 8).

As the markers for GLC and TLC we used a set of paraffins and the acetate of ursolic acid from Lavandula vera [14], ceryl alcohol, β-sitosterol and triterpenols, cottonseed oil monogalactosyldiacylglycerols [8], Galeopsis bifida squalene [5], and phthalates:dibutyl

phthalate, butyl octyl phthalate, diheptyl phthalate, and di(2-ethyl hexyl) phthalate. The homologous series of HCs, FAlcs, and PthEs were identified as described in [15, p. 177].

The fruit of Ac. sessiliflorus was collected by L. N. Ismagilova of the Tashkent Botanical Garden in 1981 in the stage of ripeness. It was dried to the air-dry state at room temperature and 16.0 g of the dried fruit was separated manually nto seeds (1.7 g) and pericarp (14.3 g). The materials were ground in a coffee mill and the lipids were extracted with hexane by steeping at room temperature.

The alkaline hydrolysis of the lipids and the oxidation of the MEs with con Rudloff's periodate-permanganate reagent were performed as described in [5], and severe acid hydrolysis by a handbook method [15, p. 87].

Acids were methylated with a solution of CH_2N_2 in diethyl ether.

The hydroxy acid MEs were silylated with a mixture of chlorotrimethylsilane and hexamethyldisilizane in pyridine (6:1:2, v/v/v) [5]. The TMS derivatives were extracted with diethyl ether, and the last traces of the reagent were evaporated off at a temperature not exceeding 40°C.

Hydroxy Acid Methyl Esters (R_f 0.28 in System 4). IR spectrum, ν_{film}^{KBr}: 3640-3200, 980 cm^{-1} (cis-trans conjugation). UV spectrum, λ_{max}^{hexane}: 234 nm. Mass spectrum of the TMS derivatives (160°C, 40 eV, 0.5 mA): m/z: 386, 384, 382 M^+, 371, 369, 367 $[M-15]^+$, 355, 353, 351 $[M-31]^+$, 311, 301, 299, 270, 250, 225, 187.

Fatty Acid Esters (R_f 0.80-0.85 in system 3). Mass spectrum (100°C, 40 eV, 0.5 mA): m/z: 211, 239, 267, 265, 263 $[RCO]^+$, 210, 238, 266, 264, 262, $[RCO-1]^+$ for the acid fraction; 252, 280, 308, 336, 364, and 392 $[M-18]^+$ for the alcohol fraction; 396, 394 $[M-18]^+$, 273, 255, and 213 for the phytosterols; and 412, 410, 408 $[M-18]^+$, 218, 203, and 189 for the triterpinols.

Phthalic Acid Esters (R_f 0.22 in System 2). UV spectrum, λ_{max}^{hexane}: 225, 252, 260, 280 nm. Mass spectra of model samples (160°C, 40 eV, 0.5 mA), m/z:

dibutyl phthalate: 278 M^+, 223, 205 (butyl-), 167, 149, 105, 104, 83, 71, 69, 65, 60, 57, 56, 55, 43, 41.

butyl octyl phthalate: 334 M^+, 279, 261 (octyl-), 223, 205 (octyl-), 167, 149, 113, 112, 105, 83, 77, 71, 70, 57, 56, 55, 45, 43, 41;

diheptyl phthalate: 362 M^+, 265, 247 (heptyl-), 167, 149, 70, 69, 57, 56, 55, 43, 41;

di(2-ethyl hexyl) phthalate: similar to that described in [10].

Mass spectrum of the phthalates from Ac. sessiliflorus, m/z: 418, 390, 362, 348, 334, 320, 306, 292, 278, 264, 250, 222, 194 M^+, 307, 289 (decyl-), 279, 261 (octyl-), 251, 233 (hexyl-), 237, 219 (pentyl-), 223, 205 (butyl-), 209, 191 (propyl-), 195, 177 (ethyl-), 181, 163 (methyl-), 167, 149, 135, 133, 105, 104, 84, 83, 77, 76, 51, 50, 43. 41.

Natural Fatty Acid Methyl Esters (R_f 0.40 in System 2). Mass spectrum (160°C, 40 eV, 0.5 mA), m/z: 298, 296, 294, 292, 284, 270, 268, 256, 242, 228, 214 M^+, 267, 265, 263, 261, 253, 239, 237, 225, 211, 197, 183 $[M-31]^+$, 266, 264, 262, 260, 252, 238, 236, 224, 210, 196, 182 $[M-32]^+$, 74.

The glycolipids (R_f 0.82 in system 5) were revealed with α-naphthol and in their chromatographic mobility corresponded to monogalactosyldiacylglycerols [15].

Hydrocarbons: white oily liquid. IR spectrum, ν_{film}^{KBr}, cm^{-1}: 840, 1675, 1380 (isoprenoids), 2960, 2930, 2860, 1470, 730 (paraffins).

Fatty alcohols (R_f 0.48 in system 4) were detected in the form of a white spot after the treatment of the chromatogram with I_2 and its brief residence in the air.

The acetates of the triterpene alcohols, the free triterpinols, and the sterols were revealed on TLC in system 4 by spraying the chromatogram with 50% H_2SO_4 followed by heating, in the form of pink, orange, and red spots with R_f 0.48, 0.40, and 0.31, respectively. IR spectrum of the acetates of the triterpene acids, ν_{film}^{KBr}, cm^{-1}: 1705, 1730, 1610, 1550, 1520, 1420, 1390, 1358, 1312, 1250. Mass spectrum of the Trs (70-80°C, 50 eV, 0.5 mA), m/z: 440, 428, 426, M^+, 425, 422, 411, 407, 379, 353, 300, 297, 295, 273, 270, 259, 255, 241, 218, 203, 189, 175, 95.

β-Carotene (R_f 0.95 in system 6); UV spectrum, λ_{max}^{hexane}: 400, 420, 448, 478 nm.

Chlorophyll a; UV spectrum, λ_{max}^{hexane}: 441, 417, 428, 534, 617, 665 nm.

SUMMARY

In the lipids of the seeds and pericarp of the introduced species Acanthopanax sessiliflorus (Rupr. et Maxim) Seem. (family Araliaceaea), 23 classes of compounds have been detected, of which 19 have been identified. The compositions of 16 classes have been studied.

The lipids of the pericarp contain a more complex set of components than those of the seeds.

Hydroxyacyldiacylglycerols, fatty acid methyl esters, and a complex combination of phthalic acid esters have been detected in the family Araliaceae for the first time.

The fatty acid composition of the lipids of the seeds and of the pericarp is characterized by a high content of petroselenic acid.

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