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UNSAPONIFIED COMPOUNDS AND UNSATURATED FATTY ACIDS OF LIPIDS FROM *Mediasia macrophylla* LEAVES

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Unsaponified compounds of lipids from Mediasia macrophylla *were investigated. They were found to contain about 50% essential oil. The content of petroselinic acid in the mixture of acids isolated from the neutral lipids and glycolipids of the leaves was determined by TLC, GC, and destructive oxidation.*

Key words: *Mediasia macrophylla*, unsaponified compounds, essential oil, neutral lipids, glycolipids, petroselinic acid.

We collected *Mediasia macrophylla* (Apiaceae) when the fruit started ripening in the village Kaltakur of Kashkadarinsk district. We studied dry leaves with 8.4% moisture content.

In continuation of a study of the lipids in leaves of this plant species [1], we saponified neutral lipids with alcoholic KOH (10%) and isolated from them the unsaponified compounds (US) (39.4%). This is 9.6% greater than those extracted from the total lipids. Then, TLC separated the US using system 1 and identified six classes of lipophilic components, two classes of essential-oil components, and anthocyans. The individual fractions and classes of US were separated by PTLC using the same solvent system (Table 1). The content and composition of carotinoids have been reported [1]. The essential-oil components with R_f values 0.64 and 0.32 did not change chromatographically after saponification relative to the essential-oil components obtained from the leaves by steam distillation. This is consistent with the lack of carboxylic acids in them.

The tocopherol composition was identified and determined by TLC using system 2 with development by a specific reagent. We detected α- and β-tocopherols and their dimers. Triterpenes, sterols, and anthocyans were identified by chromatographic mobility and qualitative reactions. Table 1 shows that the essential-oil fraction dominates in US of the leaves.

The main components are *p*-cumene (27.2%), cymene (15.1%), and carvacrol (12.5%) [2].

The lipid classes include a high content of triterpenols and anthocyan and xanthophyll pigments.

A systematic investigation detected unusual unsaturated fatty acids in lipids from all plant organs. However, the acids are most characteristic for reserve plant tissues and, as a rule, are localized in the triacylglycerides [3].

Some acids with an unusual position for the olefinic bonds are specific to the individual lipid classes. Thus, the high content (20%) of *cis*-vaccenoic acid 18:1(11) in glycolipids of mango in which the total lipid content is only 0.5% was reported [4]. Monogalactosyldiacylglycerides of spinach leaves contain 30% hexadecatrienoic acid 16:3(7,10,13) whereas this acid is absent in the phospholipids [5].

Petroselinic acid 18:1(6) is often found in lipids from various organs of representatives of the Apiaceae family. Its content is significant (>80%) in stems and roots of *Anethum graveolens* [6]. Lipids of *M. macrophylla* seeds were demonstrated [7] to contain 18:1(6) and petrosolinoleic acid 18:2(6,12).

We isolated the total acids from the neutral lipids in order to determine the isomeric composition of the unsaturated fatty acids of *Mediasia* after removing the US. They were purified first by column chromatography as the methyl esters and then separated according to the degree of unsaturation by PTLC over silica gel impregnated with $AgNO_3$ (20%) using system 3. Four bands corresponding to the methyl esters of saturated (R_f 0.84), monoene (R_f 0.62), diene (R_f 0.48), and triene (R_f 0.36) acids were detected after spraying the plates with H_2SO_4 (50%) and heating. Each fraction was eluted from the plates by diethylether. The composition was determined by GC (Table 2).

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TABLE 1. Composition of US in Lipids from *Mediasia macrophylla* Leaves

US components	In system I	Component composition in mass %		
		US	lipids	leaves
Carbohydrates	0.96	3.5	1.2	0.07
Carotinoids	0.90	0.6	0.2	0.01
Essential oil + tocopherols	0.64	7.9	2.6	0.15
Essential oil	0.32	49.2	16.4	0.93
Triterpenes	0.20	11.3	3.8	0.22
Sterols	0.12	8.7	2.9	0.16
Unidentified compounds	0.08	5.5	1.8	0.10
Anthocyans + xanthophylls	0.02	13.3	4.4	0.25

TABLE 2. Fatty-Acid Composition of Fractions Isolated by PTLC and GC, %

Methyl esters of acids	Acid fractions			
	saturated	monoenic	dienic	trienic
12:0	2.5	-	-	-
14:0	17.4	-	-	-
15:0	1.5	-	-	-
16:0	64.5	3.7	-	-
17:0	1.5	-	-	-
18:0	7.6	2.4	-	-
16:1	2.7	-	-	-
18:1	2.3	93.9	6.6	-
18:2	-	-	88.1	4.2
18:3	-	-	5.3	95.8

It can be seen that the fraction of saturated methyl esters contains impurities of 16:1 and 18:1 acids. The monoene fraction contains practically only (93.9%) the octadecenoic acid. The diene and triene methyl esters contain linoleic (88.1%) and linolenoic acids (95.8%), respectively.

Destructive oxidation using periodate—permanganate method on [8] was used to determine the presence of isomeric fatty acids in the monene and diene fractions. The triene fraction was isolated in a small amount so that it could not be analyzed further.

The mixture of methyl esters isolated from *M. macrophylla* glycolipids was oxidized in the same way. The resulting low-molecular-weight mono- and dicarboxylic acids were treated with conc. NH_4OH solution. The ammonium layer was separated over a thin layer of cellulose using system 4 for monocarboxylic acids [9] and system 5 for dicarboxylic acids [10].

The oxidation products of the monoene-acid methyl esters included the monocarboxylic acids, pelargonic (9:0) and laurinic (12:0), and the dicarboxylic acids, adipinic (6:0 di) and azelainic (9:0 di).

The oxidation products of the diene-acid fraction contained capronic (6:0), malonic (3:0 di), and azelainic (9:0 di) acids. The 9:0, 12:0, 6:0 (di), and 9:0 (di) acids were also found in the oxidized mixture of glycolipid methyl esters.

The fragmentation indicates that the monene acids of the neutral lipids (NL) and glycolipids (GL) consist of two components: oleic 18:1(9) acid and its isomer petroselinic 18:1(6) acid.

After oxidation of the diene-acid fraction, ordinary linoleic 18:2(9,12) acid was found in it. We present data for the content of 18:1(9) and 18:1(6) in the NL and GL of *M. macrophylla* leaves.

	GC, %	Cald. from fragments, %	
	18:1(9)+18:1(6)	18:1(9)	18:1(6)
NL	7.3	2.9	4.4
GL	7.7	2.0	5.7

Research results of NL and GL in *M. macrophylla* leaves showed that they contain the 18:1(6) and 18:1(9) isomers with the predominance of the former.

EXPERIMENTAL

GC of methyl esters of low-molecular-weight monocarboxylic acids was performed on a Chrom-5 instrument using a column packed with 5% Reoplex on N-AW at 100°C (thermostat) and N_2 flow rate 30 mL/min.

Methyl esters of dicarboxylic acids were examined at 175°C. Peaks of the methyl esters of low-molecular-weight fatty acids were identified by comparison with model compounds and acids obtained after destructive oxidation of pure oleic acid.

Oleic and petroselinic acids have the same GC retention times. Therefore, their quantitative contents were calculated from the content of laurinic and pelargonic acids produced by oxidation [7].

TLC and PTLC were carried out on silufol and silica-gel plates using the solvent systems: C_6H_{12}—$(C_2H_5)_2O$ (8:2) (1), C_6H_6—CH_3OH (98:2) (2), C_6H_6—C_6H_{12} (4:1) (3), C_2H_5OH—$CHCl_3$ (1:3) (4), and cellulose; *n*-$HO(CH_2)_2CH_3$—NH_4OH—H_2O (9:1:2) (5) and C_2H_5OH—NH_4OH—H_2O (20:3:2) (6).

Tocopherols were separated using system 2. The developer was a mixture of α,α'-dipyridyl (0.5%) and $FeCl_3$ (0.2%) in ethanol. The standards were α-, δ-, and γ-tocopherols isolated from soy oil.

Anthocyans had R_f values 7.4 in system 4 and were colored red upon development by H_2SO_4 (50%). Methyl esters of fatty acids were prepared using diazomethane. They were purified using a silica-gel column 1-cm in diameter and 5-cm in height. The column was eluted successively with hexane and 5% diethylether in hexane. Chromatograms of low-molecular-weight fatty acid salts were sprayed with bromphenol blue solution (0.025%) in acetone:water (9:1) and exhibited blue spots on a yellow background.

Oxidation by periodate—permanganate was performed in a *t*-butanol:water (20:30 mL) mixture containing K_2CO_3 (25 mg) by refluxing on a water bath for 1 h. The KIO_4—$KMnO_4$—methyl-ester ratio was 10.7:0.1:1. The reaction mixture was cooled and acidified by H_2SO_4 (10%). The excess of oxidant was destroyed by dry $Na_2S_2O_5$. The acidic fragments were saponified by treatment with KOH (10%). The alcohol was distilled off. The esters were decomposed by HCl (15%). The acids were extracted by ether.

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