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LIPIDS OF *Maclura aurantiaca**

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The lipids of Maclura aurantiaca *seeds have been investigated. The compositions of the neutral and the phosphorus-containing compounds have been determined.*

Maclura aurantica (osage orange), fam. Moraceae, is a dioecious deciduous tree 10-20 m high with a dense crown and thorny branches. Its collective fruits are spherical, golden yellow, and wrinkled [1].

The oil of osage orange seeds are assigned to the semidrying type and the following characteristics have been determined: specific gravity (0.9234), refractive index (1.4769), saponification No. (194.3), iodine No. (144.37%), thiocyanogen No. (79.65%) and its contents of unsaponifiables (2.21%) and of phosphatides (0.29%) [2].

Macluraxanthone, C_{10}—C_{29} hydrocarbons, lupeol, and fatty acids (FAs) with chain lengths from C_9 to C_{18} and various degrees of unsaturation have been isolated from a petroleum ether extract of the roots of this plant [3].

The composition of the neutral lipids of the seeds of the species *M. pomifera* Nutt. has been studied [4].

We have investigated the composition of the lipids of a chloroform—methanol extract of the seeds of *M. aurantiaca* previously separated into neutral and polar components. The classes of neutral lipids obtained were the usual ones for the seed lipids of higher plants, only the native FAs methyl esters (FAMEs) being found comparatively rarely in the vegetable seldom (Table 1).

According to the results of MS, the hydrocarbons included a homologous series of the C_{18}—C_{31} components, with the main peak of a molecular ion M^+ 408, which corresponds to nonacosane, C_{29}.

We determined the fatty acid compositions of the main acyl-containing classes of the neutral lipids but did not carry out such analysis for the minor components (diacylglycerols and esters of FAs with aliphatic and cyclic alcohols) (Table 2).

The results obtained permit *Maclura* seeds oil to be assigned to the typical linoleic-acid-containing oils. The level of linoleic acid in the TAGs was about 70%.

To confirm the structures of the acids, the total FAs from all three fractions were separated preparatively in the form of their MEs on an argentized layer of silica gel. Four types of acids were obtained: saturated and mono-, di-, and trienic.

The unsaturated acids were oxidized by the periodate—permanganate method [4]. The products of oxidative degradation were identified by TLC and MS in the form of methyl esters. Among the products of the oxidative degradation of the monoenes we identified the monocarboxylic acids pelargonic (nonanoic), $C_9H_{18}O_2$, and enanthic (heptanoic), $C_7H_{14}O_2$, and the dicarboxylic acids azelaic (nonanedicarboxylic), $C_9H_{16}O_4$, and undecanedicarboxylic, $C_{11}H_{20}O_4$. This shows the presence among the monoenic acids of oleic, 18:1(9), and vaccenic, 18:1(11) (the fragments $C_3H_6O_2$, $C_3H_4O_4$, and azelaic and undecanedicarboxylic acids).

The presence in MS of the peaks of molecular, M^+, and fragmentary, $[M - 31]^+$, ions with *m/z* 294 and 263, and also the identification of the degradation products ($C_6H_{12}O_2$, $C_3H_4O_4$, and $C_9H_{16}O_4$) confirmed that the dienic acid fraction was represented by linoleic acid.

In the trienic fraction it was possible similarly to identify the 18:3(9,12,15) acid, α-linoleic, and the 20:3(11,14,17) acid, eicosatrienic.

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TABLE 1. Neutral Lipids of *Maclura aurantiaca* Seeds

Lipid	Content, % by weight
1. Hydrocarbons	1.6
2. Esters of FAs with aliphatic and cyclic alcohols	0.9
3. Fatty acid methyl esters	2.1
4. Triacylglycerols (TAGs)	85.0
5. Free fatty acids	4.8
6. Diacylglycerols	0.5
7. Sterols	2.5
8. Unidentified	2.6

TABLE 2. Fatty Acid Compositions of Individual Lipids of *Maclura* Seeds, % GLC

Lipid	Acid							
	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:3
FAMEs	-	-	25.2	-	28.3	36.1	5.5	4.9
TAGs	0.1	-	10.2	2.1	12.3	68.4	6.9	-
FFAs	0.9	3.4	15.8	1.7	15.5	35.6	10.2	5.6

The sterols were separated by the Ag^+/TLC method into two zones, of substances with R_f 0.42 and 0.21. Each zone was isolated preparatively, and their MSs were recorded. The substances from the lower zone proved to be a mixture of two dienic phytosterols: stigmasterol (M^+ 412) and 24-ethylidenelophenol (M^+ 426).

In MS, the less polar substances showed three peaks of molecular ions, M^+ 386, M^+ 400, and M^+ 414, relating to sterols with one double bond in position 5: cholesterol (cholest-5-en-3β-ol), campesterol (24-methylcholest-5-en-3β-ol), and β-sitosterol (stigmast-5-en-3β-ol).

On comparing the results obtained with the composition of the lipids given for the seeds of the species *M. pomifera* [5], one may observe a qualitative identity of the lipids of the two species. Differences are seen only in the quantitative levels of individual classes. In the lipids of *M. pomifera* there are 8% of hydrocarbons, as compared with 1.6% in *M. aurantica*. It is quite possible that Bitadze [5] failed to separate the hydrocarbons from the waxy compounds covering the seed coat. In addition, *M. pomifera* is distinguished by a higher level of FA methyl esters and of free FAs (5.5 and 6.8%, respectively).

Among the phospholipids we detected (% on the total) phosphatidylcholine, 45; phosphatidylinositol, 28; phosphatidylethanolamine, 15; lysophosphatidylcholine, 3.5; an N-acylphosphatidylethanolamine, 2.5; and unidentified components, 6.

EXPERIMENTAL

The moisture content of the fresh fruit was 82% [6]. The seeds were separated from the flesh by hand, and their ratio in the fruit was determined gravimetrically as 1.5:1. The seeds and the pericarp were dried in the air, comminuted, and extracted with chloroform—methanol (2:1). The yields of total lipids after the extracts had been washed with water and NaCl solution were, as percentages of the weight of the air-dry material, 17.3% from the flesh and 23% from the seeds. The extracts were then deposited on columns of silica gel, and the neutral lipids were eluted with chloroform. The yields of neutral lipids as percentages of the weight of the chloroform—methanol extract were: 1.5% from the flesh and 96% from the seeds. The polar components of the seeds remaining after the removal of the neutral lipids were separated into phospholipids and glycolipids [7]. The amounts of the latter in the chloroform—methanol extract of the seeds were determined gravimetrically

as 3.5 and 0.5% on the weight of the extract, respectively.

The neutral lipids of the seeds were separated into classes by CC, using mixtures of hexane and diethyl ether as eluents. To obtain homogeneous fractions we used PTLC on silica gel in various systems.

Conditions for the Preparative Separation of the Total FAMEs: 20 × 20 cm glass plates, layer of KSK silica gel for TLC with 20% of $AgNO_3$, solvent system chloroform—ethanol (99:1).

TLC of the Products of the Oxidative Degradation of the Unsaturated FAs: 10 × 20 cm glass plates with a non-fixed layer of cellulose, solvent system *tert*-BuOH—NH_4 OH—H_2O (20:1:4).

Separation of the Sterol Fraction. Analytical TLC: silica gel impregnated with 5% of $AgNO_3$; solvent system hexane—benzene (1:1). Preparative TLC: silica gel impregnated with 20% of $AgNO_3$: solvent system similar to that used in analytical TLC.

TLC of the Phospholipids. The phospholipids were identified by two-dimensional TLC: first direction — chloroform—MeOH—25% NH_4OH (70:30:5); second direction — chloroform—MeOH—acetic acid—H_2O (10:5:1:1).

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