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NEUTRAL LIPIDS OF *Xanthium strumarium* SEEDS

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Chemical, chromatographic, and spectral methods are used for the first time to analyze neutral lipids, ordinary and hydroxylated fatty acids, and lipophilic components of Xanthium strumarium *L. seeds.*

Key words: *Xanthium strumarium*, neutral lipids, epoxyacids, hydroxyacids.

The *Xanthium* genus of the Asteraceae family comprises up to 70 plant species [1]. Two of these are described in the flora of Uzbekistan: *Xanthium strumarium* L. (common) and *X. spinosum* L. (thorny) [2]. We investigated lipids from seeds of the common species. All parts of this plant are known to contain iodine. The leaves and roots are used to stain tissues yellow. Oil from seeds is suitable for preparing lacquers [2].

The total neutral lipids, which make up 15.3% of the seed mass, were separated into individual classes by column chromatography (CC) on silica gel with subsequent rechromatography of the fractions consisting of two or more components by preparative TLC.

The compounds were identified by qualitative reactions, TLC mobility, spectral characteristics, and chemical transformations. The content of lipid classes was estimated by gravimetry (Table 1). The contents of DAG, MAG, FFA, and unidentified components are elevated in the lipids of this plant. The fraction of the last includes several compounds that give a bright color upon spraying the plates with H_2SO_4 solution. The IR spectra showed that these components are nonlipid in nature. Therefore, they were not investigated further.

Diacylglycerides were separated into two groups of isomers. The IR spectrum of 1,3-DAG differed from that of 1,2(2,3)-isomers by the lack of strong bands for stretching vibrations of primary OH groups at 1060 cm^{-1}. Table 1 also shows that the oxygenated triacylglycerides are mainly epoxy- and hydroxyacylglycerides. The OADAG content was only 0.3%.

TABLE 1. Neutral Lipids of *X. strumarium* Seeds

Lipid	Content, mass %
Hydrocarbons (CH)	0.2
Fatty-acid esters of aliphatic and cyclic alcohols (FAE)	0.3
Triacylglycerides (TAG)	88.0
Epoxyacyldiacylglycerides (EADAG)	0.8
Oxoacyldiacylglycerides (OADAG)	0.3
Free fatty acids (FFA)	1.6
Hydroxyacyldiacylglycerides (HADAG)	1.4
Triterpenes (TT)	Tr.
1,3-Diacylglycerides (1,3-DAG)	1.4
1,2 (and/or 3,2)-Diacylglycerides [1,2(3,2)-DAG]	1.6
Sterols (ST)	0.1
Monoacylglycerides (MAG)	1.0
Unidentified components	3.3

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TABLE 2. Fatty Acids of *X. strumarium* Seed Lipids

Lipid	Acid, % of mass									
	12:0	13:0	14:0	15:0	16:0	16:1	18:0	18:1	18:2	20:0
TAG	-	-	-	-	3.1	0.4	1.0	15.6	79.9	-
EADAG	0.5	0.1	0.4	0.2	8.5.	0.3	4.4	28.2	57.4	-
OADAG	-	-	0.2	-	15.2	0.5	7.4	38.5	38.2	-
FFA*	0.1	0.1	0.3	0.5	17.2	0.6	4.5	31.5	39.7	1.4
HADAG	-	-	-	-	7.1	0.2	1.7	28.1	62.9	-
1,3-DAG	0.1	-	0.2	0.1	6.9	0.4	2.6	20.4	68.5	0.8
1,2(2,3)-DAG	-	-	-	-	3.6	0.2	0.5	7.6	88.1	-
MAG	-	-	0.1	-	3.7	0.3	1.2	13.9	80.8	-

*22:0 (0.6%) and unidentified fatty acids (3.4%) are also present.

TABLE 3. Epoxyacids of *X. strumarium* Seed Lipids

Acid	Mass number of characteristic ions from TMS-derivatives of dihydroxyacid methyl esters, *m/z*					
	M^+	$[M-15]^+$	$[M-31]^+$	A*	B**	addtl.
9,10-epoxy-18:0	-	459	443	215^1, 372^2	259^1, 361^2	332
9,10-epoxy-18:1 (12)	472	457	441	213^1, 315^2	259^1, 361^2	332
12,13-epoxy-18:1 (9)	"	"	"	173^1, 275^2	299^1, 401^2	270

*Fragments: 1) CH_3...CHOTMS; 2) CH_3...$(CHOTMS)_2$.
**Fragments: 1) CHOTMS...$COOCH_3$; 2) $(CHOTMS)_2$...$COOCH_3$.

The content of ordinary fatty acids of the acyl-containing lipids was found by GLC and is presented in Table 2. The principal components of the total acids are 18:1 and 18:2. The content of 16:0 is elevated only in the FFA and OADAG.

The epoxy- and hydroxyacids (EFA and HFA) were isolated as the methyl esters (ME) by CC on silica gel from the hydrolysis products of the corresponding acylglycerides. According to GLC (PEGS), the EFA contained 12.6% epoxyoctadecanoic and 87.4% epoxyoctadecenoic acids. The structures of these were found by mass spectrometry of the trimethylsilyl derivatives (TMS) of the ME of the dihydroxyacids (Table 3). The EFA were identified from the mass spectra using literature data [3] and results of our own investigations [4]. Thus, the epoxyacids include 9,10-epoxy-18:0, 9,10-epoxy-18:1 (12), and 12,13-epoxy-18:1 (9) acids, which are very characteristic of plants from the Asteraceae family [5].

The UV spectrum of the total hydroxyacids exhibits an absorption band at λ_{max} (C_6H_{12}) = 233 nm, which is characteristic of *cis,trans*-conjugated dienes. This was confirmed by the presence in the IR spectrum of strong bands at 960 and 990 cm^{-1} [6]. The content of HFA with a conjugated system of ethylene bonds was 37.0% according to the UV spectrum [7]. The mass spectrum of the HFA TMS ethers (Table 4) contained peaks for the molecular ions and characteristic fragments that belong to saturated, monoenic, and dienic components of the C_{18} acid. The strongest peaks in the mass spectrum were those with *m/z* 225 and 311, which belong to two isomers of the conjugated dienic acids 9-OH-18:2 (10,12) and 13-OH-18:2 (9,11). Analysis of the intensity ratios of these fragments suggested that their contents are equal [8]. The peak intensity for the fragment with *m/z* 227 compared with that for the one with *m/z* 259 suggested that the allyl isomer of 9-OH-18:1 (10) was present along with 9-OH-18:1 (12) [8]. Fragments with *m/z* 185 and 325 in addition to 239 and 271 could have formed from degradation of two isomers of the hydroxylinoleic acids 12-OH-18:2 (9,13) and 10-OH-18:2 (8,12). It is also possible that the allyl isomer of the hydroxyoleinic acid 10-OH-18:1 (8) is present. This is consistent with the intensity of the ion with *m/z* 271 [9, 10].

TABLE 4. Hydroxyacids of *X. strumarium* Seed Lipids

Acid	Mass number of characteristic ions from TMS-derivatives of dihydroxyacid methyl esters, *m/z* (rel. %)						
	M^+	$[M-15]^+$	$[M-31]^+$	$[M-47]^+$	A*	B**	addtl.
10-OH-18:0	-	371(4)	355(1)	339(3)	215(8)	273(8)	169(10)
12-OH-18:0	-	"	"	"	187(46)	301(4)	-
8-OH-18:1 (9)	384(4)	369(5)	353(3)	337(4)	241(13)	-	-
9-OH-18:1 (12)	"	"	"	"	227(38)	259(13)	230(3), 294(4)
9-OH-18:1 (10)	"	"	"	"	227	-	-
10-OH-18:1 (8)	"	"	"	"	-	271(46)	-
11-OH-18:1 (9)	"	"	"	"	-	285(13)	-
11-OH-18:1 (12)	"	"	"	"	199(10)	-	-
12-OH-18:1 (9)	"	"	"	"	187	299(7)	270(13)
13-OH-18:1 (9)	"	"	"	"	173(10)	313(9)	284(3)
9-OH-18:2 (10, 12)	382(58)	367(8)	351(6)	335(4)	225(100)	311(71)	130(35), 292(6)
13-OH-18:2 (9, 11)	"	"	"	"	"	"	"
10-OH-18:2 (8, 12)	"	"	"	"	239(8)	271	-
12-OH-18:2 (9, 13)	"	"	"	"	185(30)	325(5)	-

*Fragment: CH_3...CHOTMS; **Fragment: CHOTMS...$COOCH_3$.

The composition of the lipophilic components was also established using mass spectrometry. The mass spectrum of the hydrocarbons contained peaks for the molecular ions of saturated compounds in the series $C_{29}H_{60}$-$C_{22}H_{46}$, monoenic, dienic, trienic, tetraenic, and pentaenic components with a carbon chain length from C_{36} to C_{22}. The strongest peaks were those with *m/z* 408 and 380, 322 and 308, and 320 and 306, which belong to saturated $C_{29}H_{60}$ and $C_{27}H_{56}$, monoenic $C_{23}H_{46}$ and $C_{22}H_{44}$, dienic $C_{23}H_{44}$ and $C_{22}H_{42}$ hydrocarbons, respectively.

The total sterols contained two components with *m/z* 414 and 412. Their fragmentation was consistent with β-sitosterol and stigmasterol. According to GLC (SE-30), the content of β-sitosterol was 52.0%; of stigmasterol, 48.0%.

The mass spectrum of the esters (RCO_2R') contained peaks of molecular ions with *m/z* 804, 790, 776, 760, 748, 746, 732, 730, 728, 718, 716, 714, 704, 702, 700, 690, 688, 686, 676, 674, 672, 662, 660, 658, 648, 646, 644, 634, 632, 630, 620, 618, 606, 592, 578, 564, 562, 550, 536, 522, 508, and the group of fragments $[RCO_2H_2]^+$, $[RCO]^+$, and $[RCO - 1]^+$, which belong to the series of saturated 10:0-34:0 and unsaturated 18:1 and 18:2 acids. The strongest peaks were from fragments of 16:0, 18:0, 20:0, and 22:0 acids. Fragments $[R' - 1]^+$ with *m/z* 448-224 come from aliphatic alcohols of the C_{16}-C_{32} series; with *m/z* 396 and 394, from sterols. Therefore, the molecular ions with *m/z* 676 and 674 can be assigned to esters of sterols and 18:2 acid; with *m/z* 804, 790, 776, and 748, to esters of stigmasterol and high-molecular-weight saturated acids. The remaining peaks of molecular ions correspond to esters of saturated alkanols and fatty acids [11].

EXPERIMENTAL

UV spectra of ME of oxygenated acids were recorded on a Hitachi spectrophotometer in hexane. IR spectra of lipids were recorded on a UR-10 instrument from films. Mass spectra were measured on an MX-1310 instrument with 40-50 eV ionizing-electron energy and ionization chamber temperatures of 120-180°C for hydrocarbons, 150-180°C for sterols, 150-100°C and 80-70°C for silyl derivatives of ME of oxygenated fatty acids.

GLC was performed on a Chrom-4 instrument with a flame-ionization detector. A 2000×4 mm column packed with PEGS on Chromaton W at 198°C was used for ME of fatty acids; a column of the same size but packed with SE-30 at 280°C, for sterols.

Neutral lipids were extracted from previously ground seeds by hexane during standing at room temperature. Column chromatography of neutral lipids and ME was performed on L 100/250 silica gel with elution by hexane with added ether (0,

10, 20, 30, 40, 50, and 100%). Preparative TLC was performed on L 5/40 silica gel with 10% gypsum.

EADAG and ME of epoxyacids were identified using 0.05 M picric acid in ethanol [12]; OADAG, 0.4% 2,4-dinitrophenylhydrazine in 2 N HCl [13]; sterols, 50% aqueous H_2SO_4.

ME of epoxyacids were converted to dihydroxy derivatives by the literature method [14]. Trimethylsilyl derivatives of hydroxyacids were prepared as before [13]. Acylglycerols were hydrolyzed by 8% KOH in methanol with stirring for 15 min using 1 g TAG per 10 ml of base solution. Fatty acids were methylated with CH_2N_2 in diethylether. Diazomethane was prepared by the literature method [15].

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