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NEUTRAL LIPIDS OF THE SEEDS OF *Oenothera biennis*

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The lipids and fatty acids of the seeds of Oenothera biennis *L., fam. Onagraceae, have been studied with the aid of chemical, chromatographic, and spectral analyses. In the lipids we identified the usual and oxygenated acylglycerides and five classes of lipophilic substances. The structures of four epoxy acids and of 22 hydroxy acids have been determined. The physiologically active 18:3(6,9,12)- acid was found in all the acyl-containing classes.*

The oil of the seeds of *Oenothera biennis* L.(common evening primrose), fam. Onograceae, contain the highly active essential 18:3(6,9,12) fatty acid, or γ-linolenic acid, in view of which it is used widely in medicine. This rare acid is found in the seed lipids of such families as Boraginaceae [1] and Asteraceae [2], in the marine algae Spirulina [3], and in fungi [4], etc. Only the composition of the fatty acids and some groups of unsaponifiable lipophilic substances of the oil under study were known from the literature [5].

We have made a full investigation of the neutral lipids of *O. biennis* seeds gathered from plants introduced into the Tashkent Botanical Garden. The neutral lipids were extracted from the previously ground seeds with hexane. Their yield amounted to about 23% of the weight of the seeds.

The total lipids were separated into classes by CC and PTLC on silica gel. The substances were assigned to definite classes on the basis of qualitative reactions, chromatographic mobilities in a thin layer of silica gel in comparison with models, and spectral characteristics and chemical transformations. The amounts of the lipid classes were as follows (% by weight): hydrocarbons (HCs) — 0.1; esters of fatty acids and cyclic alcohols (CEs) — 0.5; triacylglycerides (TAGs) — 94.9; epoxyacyldiacylglycerides (epDAGs) — 0.8; tocopherols — 0.1; free fatty acids (FFAs) — tr.; hydroxyacyldiacylglycerides (hDAGs) — 1.1; triterpene alcohols (TTs) together with aliphatic alcohols (AAs) — 0.2; methylsterols (MSs) — 0.1; diacylglycerides (DAGs) — 1.2; sterols (STs) — 0.4; monoacylglycerides (MAGs) — 0.4; unidentified components — 0.2.

The fatty acid (FA) compositions of the acyl-containing classes of lipids were determined by GLC (Table 1). The distribution of the double bonds in the unsaturated acids was established by oxidative degradation using the periodate-permanganate method [6] after preliminary separation of the acids according to their degree of unsaturation by preparative TLC/$AgNO_3$ in solvent system 1. This showed that the saturated acid fraction included the following components (GLC, % by weight): 14:0 — 0.3; 16:0 — 76.5; 18:0 — 19.8; 20:0 — 0.6; 22:0 — 2.7. In the monoenoic acid fraction we detected the 18:1(9) acid (96.5%), as was confirmed by its degradation fragments — the 9:0 monocarboxylic and 9:0 dicarboxylic acids, and the 16:1(9) acid (3.5)%. The dienic acid fraction was represented only by the 18:2(9,12) acid, as was deduced from its degradation fragments — the 6:0 monocarboxylic and the 9:0 dicarboxylic acids. The trienic fraction consisted solely of the 18:3(6,9,12) acid, the degradation fragments of which were the 6:0 monocarboxylic and 6:0- dicarboxylic acids.

The figures of Table 1 show that the dominating acids of all the classes of lipids were the 18:1 and 18:2 types, the level of which was lower only in the MAGs because of the increased proportion of 14:0 and 16:0 acids. γ-Linolenic acid was present among the FAs of all the lipid classes, while the amount of this acid in the sn-2 position of the TAGs was half its weight among the FAs of the TAGs. In addition, the results given in Table 1 show the absence of the usual 18:3(9,12,15) acid from the FAs, which agrees with literature reports [5].

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TABLE 1. Fatty Acid Composition of the Acyl-containing Classes of the *O. biennis* Lipids

Acid	Content, GLC % by weight					
	TAGs	2-MAGs	Ep-TAGs	H-TAGs	DAGs	MAGs
10:0	-	-	-	2.7	2.5	3.5
11:0	-	-	-	2.6	1.7	3.7
12:0	-	-	-	4.9	4.5	8.8
14:0	0.3	-	0.4	6.3	4.5	12.3
16:0	6.1	1.0	6.3	11.8	13.2	15.5
16:1(9)	0.3	-	0.8	1.5	0.9	1.0
17:0	-	-	-	1.1	0.9	4.6
18:0	1.9	0.7	2.1	2.9	2.7	3.0
18:1(9)	25.9	26.8	18.8	20.6	24.9	19.6
18:2(9, 12)	60.5	64.5	63.7	40.7	39.9	26.3
18:3(6, 9, 12)	5.0	7.0	7.9	4.9	2.7	1.3
20:0	-	-	Tr.	Tr.	1.6	0.4
22:0	-	-	Tr.	Tr.	Tr.	Tr.
Σ_{sat}	8.3	1.7	8.8	32.3	31.6	51.8
Σ_{unsat}	91.7	98.3	91.2	67.7	68.4	48.2

*The 2-MAGs were obtained by enzymatic hydrolysis of the TAGs.

TABLE 2. Molecular Species of TAGs of the *O. biennis* Seed Lipids with Levels > 1%

TAG species		% by weight
sn-1-sn-2-sn-3	sn-1-sn-2-sn-3	
S-18:1-18:1+18:1-18:1-S		1.6
S-18:1-18:1+18:1-18:1-S		3.6
18:1-18:1-18:2+18:2-18:1-18:1		8.2
18:2-18:1-18:3+18:3-18:1-18:1		1.3
18:1-18:3-18:2+18:2-18:3-18:1		2.1
18:3-18:3-18:2+18:2-18:3-18:3		2.4
18:1-18:1-18:1		1.7
18:2-18:1-18:2		9.2
S-18:2-18:1+18:1-18:2-S		4.0
S-18:2-18:2+18:2-18:2-S		8.9
18:1-18:2-18:2+18:2-18:2-18:1		19.6
18:1-18:2-18:3+18:3-18:2-18:1		1.3
18:2-18:2-18:3+18:3-18:2-18:2		2.8
18:1-18:2-18:1		4.3
18:2-18:2-18:2		22.1

S — 16:0; 18:0.

The species composition of the TAGs was determined by pancreatic hydrolysis [7] (Table 2). The trilinoleoyl- and monooleyldilinoleoyl-TAGs made up almost 42.0%, and the linolenoyl-containing species about 10% of the total weight of the TAGs. The epoxy and hydroxy acids were isolated for investigation from the products of the hydrolysis of the neutral lipids and were separated in the form of MEs by CC on silica gel. On analyzing the methyl esters of the epoxy acids (MEEAs) on TLC/$AgNO_3$ in solvent system 2, we detected three zones of substances, with R_f 0.8, 0.6, and 0.2, that gave a positive qualitative reaction for an epoxy ring and corresponded to saturated and mono- and dienic components. According to GLC (PEGS) results, the content of 18:1 MEEAs was 93.0%, of the 18:0 species 3.7%, and of the 18:2 species 3.3%.

TABLE 3. Epoxy Acids of the *O. biennis* Seed Lipids

Acid	Mass numbers of characteristic fragments of the di-OTMS derivatives, *m/z* (rel. %)					
	M^+	$[M-15]^+$	$[M-31]^+$	others	A*	B**
9,10-Epoxy-18:0	OTS	459(3)	443(3)	332(3)	215^1(11)	259^1(25)
					317^2(1)	361^2(4)
9,10-Epoxy-18:1(12)	471(1)	457(6)	441(12)	332	213^1(7)	259^1
					315^3(2)	361^2
12,13-Epoxy-18:1(9)	"	"	"	270(5)	173^1(79)	299^1(95)
					275^2(10)	401^2(6)
15,16-Epoxy-18:2(9,12)	470(0.5)	455(0.2)	439(0.2)	412(0.3)	131^1(7)	339^1(0.5)
				310(0.3)	233^2(2)	441^2(12)

*The fragments: 1) CH_3...CHOTMS; 2) CH_3...$(CHOTMS)_3$.
**The fragments: 1) CHOTMS...$COOCH_3$; 2) $(CHOTMS)_2$...$COOCH_3$.

TABLE 4. Hydroxy Acids of *O. biennis* Seeds

Acid	Mass numbers of characteristic fragments of the di-OTMS derivatives, *m/z* (rel. %)						
	M^+	$[M-15]^+$	$[M-31]^+$	$[M-47]^+$	A*	B**	others
9-OH-16:0	-	343(1)	327(2)	311(28)	201(4.5)	259(22)	
9-OH-16:1(10)	356(0.5)	341(1.5)	325(1.5)	309(1)	199(19)	285(6)	
7-OH-16:1(8)	"	"	"	"	227(20)	257(3)	
13-OH-16:2(9,11)	354(1)	339(1)	323(1)	307(1.5)	197(6)	311(28)	
11-OH-17:0	-	357(1)	341(1)	325(1.5)	187(66)	287(2)	
12-OH-17:0	-	"	"	"	173(28)	301(2)	
9-OH-17:1(12)	370(1)	355(1)	339(2)	323(1)	213(8)	259(22)	
9-OH-17:2(10,12)	168(1)	353(1)	337(2)	321(1)	211(4)	311(28)	
13-OH-17:2(9,11)	"	"	"	"	"	"	
8-OH-18:0	-	371(1)	355(1)	339(1.5)	243(1)	245(2)	
9-OH-18:0	-	"	"	"	229(5)	259(22)	
10-OH-18:0	-	"	"	"	215(7)	273(3)	
8-OH-18:1(9)	384(2)	369(1)	353(1)	337(1.5)	241(6)	-	
10-OH-18:1(8)	"	"	"	"	-	217(7)	
11-OH-18:1(9)	"	"	"	"	-	285(6)	
9-OH-18:1(9)	"	"	"	"	227(20)	259	230(2)
12-OH-18:1(9)	"	"	"	"	187(66)	299(6)	270(5)
13-OH-18:1(9)	"	"	"	"	173(28)	313(3)	
9-OH-18:2(10,12)	382(10)	367(1.6)	351(2)	335(1)	225(100)	311(28)	292(6), 130(66)
13-OH-18:2(9,11)	"	"	"	"	"	"	
8-OH-18:2(9,12)	"	"	"	"	239(9)	-	
12-OH-18:2(9,130	"	"	"	"	185(50)	-	

*The fragment CH_3...CHOTMS.
**The fragment CHOTMS...$COOCH_3$.

TABLE 5. Lipophilic Components of *O. biennis* Seeds

Components	Mass numbers of characteristic ions, *m/z* (intensity)
Hydrocarbons	Molecular ions:
$C_{31}H_{64}$-$C_{20}H_{42}$	436-282 (strong)
$C_{31}H_{60}$-$C_{17}H_{34}$	420-238 (medium)
Esters	$[RCO_2R']$:$[RCO]^+$, $[RCO_2H_2]^+$
acyl part:	
12:0, 14:0, 16:0-20:0,	183, 201; 211, 229; 239, 257-295, 313;
22:0-26:0, 16:1, 18:1,	323, 341-379, 397; 237, 255; 265, 283; 263,
18:2, 18:3	281; 261, 279
alcohol part:	$[R'-1]^+$ and others
$C_{29}H_{50}O$ - β-sitosterol	396, 329, 303 (strong)
$C_{28}H_{48}O$ - campesterol	382, 367, 315 (weak)
$C_{29}H_{48}O$ - stigmasterol	394, 379, 351 (weak)
$C_{30}H_{50}O$ - α- and β-amyrins	408, 393, 218, 207, 203, 189
Cycloartenol	
Tocopherols:	Molecular ions:
(α-, γ-, and β-)	430, 416, 402
Free alcohols:	
aliphatic:	$[M-18]^+$
$C_{16}H_{34}O$-$C_{20}H_{42}O$	224-280 (medium)
$C_{28}H_{58}O$, $C_{22}H_{46}O$	392 (medium), 308 (strong)
$C_{24}H_{50}O$, $C_{26}H_{54}O$	336 (strong), 364 (strong)
$C_{21}H_{44}O$-$C_{27}H_{56}O$	294, 322, 350, 378 (weak)
cyclic:	M^+, $[M-15]^+$, $[M-18]^+$ and others
campesterol	400, 385, 382, 367, 315
stigmasterol	412, 397, 394, 379, 351, 300
β-sitosterol	414, 399, 396, 329, 303
α- and β-amyrins	426, 411, 408, 393, 218, 207, 204
cycloartenol	203, 189 (strong)
$C_{31}H_{52}O$-24-methylenecycloartenol	440, 425, 422, 407, 315, 300
4-Metylsterols:	M^+, $[M-15]^+$, M $[M-98]^+$ and others
obtusifoliol	426, 411, 328 (100%), 285 (95%)
gramisterol	
citrostadienol	

The structures of the epoxy acids were determined by the mass spectrometry of the trimethylsilyl (TMS) derivatives of the dihydroxy acids obtained from the total MEEAs. The mass spectra of the total MEEAs (Table 3) showed peaks of the molecular ions of the 18:1 and 18:2 components and of fragments from the breakdown of the TMS derivatives of the 9,10-epoxy-18:0, 9,10-epoxy-18:1(12), 12,13-epoxy-18:1(9), and 15,16-epoxy-18:2(9,12)acids. The first three acids are the most widely distributed in the lipids of higher plants [8], while the 15,16-epoxy-18:2(9,12) acid has been found in a limited number of plants [9—11].

In a thin layer of silica gel/$AgNO_3$ with solvent system 3, the methyl esters of the hydroxy acids (MEHAs) were separated into three zones of substances with R_f 0.8, 0.6, and 0.4, corresponding to saturated, monoenic, and dienic components. In the GLC analysis of silyl derivatives of the MEHAs on a nonpolar phase (SE-30), the main peak (more than 90%) was a poorly separated one of C_{18} acids with different degrees of unsaturation.

In the mass spectra of silyl derivatives of the MEHAs (Table 4) we observed the peaks of molecular ions and of characteristic fragments relating to the structures of saturated, monoenic, and dienic acids of the C_{16}—C_{18} series [11]. It can be seen from Table 4 that the lipids of *O. biennis* most probably contain the 22 hydroxy acids previously found in the seed lipids of plants of other families [8].

Analysis of the lipid composition of the material under investigation showed that the proportion of lipophilic components was 1.4% of the weight of the lipids, and the main ones were esters (0.5%)and free sterols (0.4%).

The lipophilic components were identified by mass spectrometry (Table 5). Hydrocarbons were represented by saturated and monoenic components. The most intense peaks were the molecular peaks of the saturated homologs. Among the acids of the esters we detected saturated medium- and high-molecular-weight components and also isologs of the C_{18} series, including γ-linolenic acid. The alcohol components of esters were represented by cyclic alcohols, which were detected both in the lipids and in the free form.

The aliphatic alcohols included components from C_{16} to C_{27}, the most intense peaks being those of the C_{22}, C_{24}, and C_{26} molecular ions. Among the free cyclic alcohols and methylsterols and tocopherols we detected the same components as Hudson [5].

EXPERIMENTAL

The conditions for taking mass spectra, for GLC analysis of the MEs of high- and low-molecular-mass fatty acids, and for CC and PTLC have been described in [12]. The alkaline hydrolysis of the acylglycerols and the separation of the MEFAs by CC into those of epoxy and hydroxy acids were performed as shown in [13].

The preparative separation of the total MEs of the common FAs according to their degrees of unsaturation and the analysis of the epoxy and hydroxy acids were carried out in a thin layer of silica gel with 20% of $AgNO_3$.

Solvent systems: 1) hexane—benzene—diethyl ether (6:4:0.5); 2) benzene—chloroform—diethyl ether (50:50:2); 3) benzene—chloroform—diethyl ether (50:50:15).

Epoxy-containing lipids were identified with a 0.05 M ethanol solution of picric acid [14], and cyclic alcohols with a 50% aqueous solution of H_2SO_4, while the MEs of epoxy acids were converted into dihydroxy derivatives by the method of Gunstone and Schuler [15]. The TMS derivatives were obtained by the procedure of [16]. The FAs were methylated with diazomethane in diethyl ether.

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