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THE LIPIDS OF *Hibiscus* SEEDS

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The composition and amounts of various classes of lipids of the seeds of Hibiscus sp. *have been investigated. Seven classes of neutral lipids, five of phospholipids, and five of glycolipids have been identified and their fatty-acid and triglyceride compositions have been established. The presence has been shown of 16 main types of triacylglycerols and 25 types of epoxyacyldiacylglycerols. An epoxy-18:0 and an epoxy-18:1 acid in a combined amount of 1.4% have been detected among the acids of the free lipids, together with 8.1% of cyclopropenoid acids.*

The genus *Hibiscus*, fam. Malvaceae, numbers more than 600 species of plants [1]. The majority of them have medicinal properties and are used for the preparation of drugs possessing antiphlogistic, cholagogic, antisclerotic, and antitumoral effects [2]. About 136 species of this family have been studied for the presence of flavonoids, coumarins, and anthocyanins [3].

However, there have been few studies of their lipids, and these have been mainly devoted to the cyclopropenoid acids (CPAs) that are characteristic of the Malvaceae family [4-6]. We have now investigated the lipids of the seeds of *Hibiscus sp.* introduced into the Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan. The free lipids (FLs) amounted to 20.1% of the weight of the seeds, and the bound lipids (BLs) to 2.1%. The latter consisted to the extent of 40.7% of neutral lipids (NLs) and 59.3% of polar lipids (PLs), in which there were 59.1% of phospholipids (PhLs) and 40.9% of glycolipids (GLs).

The free lipids were separated into fractions by CC on silica gel with solvent systems 1-6. Fractions consisting of two or more classes of lipids were separated further by preparative TLC. The substances were assigned to definite classes on the basis of their chromatographic mobilities in a thin layer of silica gel in comparison with those of model specimens of lipids, by qualitative reactions, from their spectral characteristics, and by chemical transformations.

The following set of main classes of lipids was revealed (% by weight): hydrocarbons — 0.7; esters of aliphatic alcohols, sterols, and triterpenols with fatty acids (FAEs) — 1.2; triacylglycerols (TAGs) — 87.5; epoxyacyldiacylglycerols (epTAGs) — 3.4; free fatty acids (FFAs) — 2.9; hydroxyacyldiacylglycerols (hTAGs) — 1.6; diacylglycrols (DAGs) — 0.8; sterols — 0.8; polar lipids and unidentified components — 1.1.

In the free lipids the usual TAGs predominated, while oxygenated TAGs (epTAGs and hTAGs) amounted to 5% of the weight of the lipids. The acyl-containing classes of lipids were hydrolyzed, and the fatty acids obtained were analyzed in the form of their methyl esters (FAMEs) by GLC (Table 1). Among the common fatty acids the 16:0, 18:1, and 18:2 species predominated, and among the epoxy acids monenic components of the C-18 series. The total degrees of saturation of the FFAs and FAEs were almost the same and were considerably higher than those of the TAGs and the epTAGs, mainly through an increased level of the 16:0 acid. At the same time, 40.3% of the unsaturation of the FFAs was due to the 18:1 acid, and 41.4% of that of the FAEs to the 18:2 acid.

The neutral lipids from the BLs included the same proportion of the 16:0 acid as the FFAs of the FLs, but the amount of the 18:2 acid in them was 1.9 times greater. The GLs in the bound lipids were more saturated than the FLs because of a high level of the 16:0 and 18:0 acids.

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TABLE 1. Fatty Acids of the Seeds of *Hibiscus sp.*, GLC, % by weight

Acid	Free lipids					Bound lipids		
	TAGs	epTAGs	FFAs	FAEs	DAGs	NLs	PhLs	GLs
12:0	Tr.	0.5	Tr.	0.9	Tr.	Tr.	Tr.	Tr.
13:0	Tr.	0.3	Tr.	0.6	–	Tr.	Tr.	Tr.
14:0	0.2	0.2	0.5	0.9	–	0.6	0.1	0.8
15:0	–	Tr.	0.8	1.5	–	Tr.	Tr.	Tr.
16:0	15.8	13.9	28.7	23.4	26.3	28.1	15.4	24.8
16:1	0.4	0.5	1.3	0.8	4.7	Tr.	Tr.	2.7
18:0	2.7	2.8	7.8	5.2	5.6	4.8	3.1	11.4
18:1	27.2	17.0	40.3	21.4	20.6	36.4	23.6	16.4
18:2	52.7	21.8	16.4	41.4	42.8	30.1	57.8	43.9
18:3	1.0	1.2	3.3	2.3	–	Tr.	–	–
20:0	Tr.	2.4	0.9	1.6	–	–	–	–
ep18:0	–	3.2	–	–	–	–	–	–
ep18:1	–	36.2	–	–	–	–	–	–
Σ_{sat}	18.7	23.3	38.7	34.1	31.9	33.5	18.6	37.0
Σ_{unsat}	81.3	76.7	61.3	65.9	68.1	66.5	81.4	63.0

TABLE 2. Main Molecular Species of the TAGs of *Hibiscus sp.* from Their Mass Spectra

Species	Mass numbers, M^+, of the ions and characteristic fragments, *m/z*			
	M^+	$[M-18]^+$	$[M-R_1COO]^+$	$[M-R_3COO]^+$
16:0–18:2–18:2	854	836	599	575
16:0–18:2–18:1	856	838	601	575
16:0–18:1–18:2	"	"	"	577
16:0–18:1–18:1	858	840	603	"
18:2–18:2–18:2	878	860	599	599
18:2–18:2–18:1	880	862	601	599
18:2–18:1–18:2	"	"	"	601
18:1–18:2–18:2	"	"	599	"
18:1–18:2–18:1	882	864	601	601
18:2–18:1–18:1	"	"	603	"
18:1–18:1–18:2	"	"	"	603
18:0–18:2–18:2	"	"	599	"
18:1–18:1–18:1	884	866	603	603
18:0–18:2–18–1	"	"	601	"
18:0–18:1–18:2	"	"	"	605
18:0–18:1–18:1	886	868	603	"
19:0–18:2–18:2	896	878	599	617
19:0–18:2–18:1	898	880	601	"

The presence of cyclopropenoid acids in the free lipids was shown by the qualitative Halphen reaction and from the IR spectrum of the FAMEs, in which contained absorption bands characteristic of the cyclopropene ring at 1010 and 1869 cm^{-1} [7]. The amount of CPAs in the free lipids was determined by titration with HBr in glacial acetic aid [8]; it proved to be 8.1%.

To find the main molecular species of TAGs, epTAGs, and FAEs, they were subjected to mass spectrometric analysis. The mass spectrum of the ordinary TAGs (Table 2) showed eight intense peaks of molecular ions, corresponding to 16 main molecular species of TAGs, and two low-intensity peaks of molecular ions with *m/z* 898 and 896 corresponding to TAGs including the 19:0 acid. The mass spectrum of the epTAGs (Table 3) contained 11 peaks of molecular ions, corresponding to 25 species. The most characteristic breakdown fragments of each species are also given in Tables 2 and 3. Species with epoxyacyl residues in the sn-2 position were detected from the fragments $[R_2CO + 74]^+$ and $[R_2CO + 128]^+$.

Although mass spectrometry does not enable the set of acyl residues in the sn-1 and sn-3 positions of the molecules to be determined, common features of the structure of the TAGs of higher plants have permitted us to suggest the most probable set of individual TAG species of the hibiscus, with the saturated acyls predominantly in the sn-1 position and the epoxyacyls in the sn-3 position.

The mass spectrum of the esters of alcohols and fatty acids showed a series of peaks of molecular ions, with *m/z* 846, 844, 830, 816, 788, 744, 760, 746, 732, 718, 704, 690, 688, 678, 676, 674, 672, 664, 662, 652, 648, 646, 644, 638, 634, 632, 620, 618, 616, 606, 604, 602, 592, 590, 588, 580, 578, 564, 562, 560, 550, 536, 534, 532, 522, 508, 494, 480. The most intense were those with *m/z* 678, 676, and 664. The first two were assigned to esters of β-sitosterol (*m/z* 414) with the 18:1 and 18:2 acids, and the third to esters of triterpenols having *m/z* 426 with the 16:0 acid. The other molecular-ion peaks corresponded to esters of saturated alkanols having from 16 to 32 carbon atoms with 10:0-30:0 acids.

TABLE 3. Main Molecular Species of the epTAGs of *Hibiscus sp.* from Their Mass Spectra

Species	Mass numbers, M+, of the ions and characteristic fragments, *m/z*					
	M^+	$[M-18]^+$	$[M-R_1COO]^+$	$[M-R_3COO]^+$	$[R_2CO+74]^+$	$[R_2CO+128]^+$
16:0–18:3–ep18:1	868	850	613	573	335	389
16:0–ep18:1–18:3	"	"	"	591	353	407
16:0–18:2–ep18:1	870	852	615	575	337	391
16:0–ep18:1–18:2	"	"	"	591	353	407
16:0–18:1–ep18:1	872	854	617	577	339	393
16:0–ep18:1–18:1	"	"	"	591	353	407
18:2–18:3–ep18:1	892	874	613	597	335	389
18:3–18:2–ep18:1	"	"	615	"	337	391
18:2–18:2–ep18:1	894	876	"	599	"	"
18:2–ep18:1–18:2	"	"	"	615	353	407
18:1–18:3–ep18:1	"	"	613	599	335	389
18:1–ep18:1–18:3	"	"	"	617	353	407
18:1–18:2–ep18:1	896	878	615	601	337	391
18:2–18:1–ep18:1	"	"	617	"	339	393
18:2–18:2–ep18:0	"	"	617	"	351	409
18:1–ep18:1–18:2	"	"	615	617	353	407
18:2–ep18:0–18:2	"	"	617	"	351	409
18:1–18:1–ep18:1	898	880	"	603	339	393
18:1–18:2–ep18:0	"	"	"	605	337	391
18:2–18:1–ep18:0	"	"	619	"	339	393
18:1–ep18:1–18:1	"	"	617	617	353	407
19:0–18:2–ep18:1	912	894	615	621	337	391
19:0–18:1–ep18:1	914	896	617	623	339	393
20:0–18:2–ep18:1	926	908	615	635	337	391
20:0–18:1–ep18:1	928	910	617	637	339	393

The phospholipids were investigated by two-dimensional TLC in systems 7. Spots of the following PhLs were identified: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid, and lysophosphatidylcholine. The main ones were PC, PE, and PI.

The glycolipids were isolated from the BLs by PTLC, using solvent system 8, in which PhLs remain at the start and GLs rise to the top. The total GLs were separated into individual components by TLC on silica gel in system 9. Their amount was estimated via the carbohydrate component with anthrone after severe acid hydrolysis [9]. The composition of the hibiscus GLs was as follows (% by weight): monogalactosylglycerols — 3.3; esterified sterol glycosides — 10.8; sterol glycosides — 51.5; digalactosyldiglycerides — 16.3; and cerebrosides — 9.8. The predominating GLs were sterol glycosides, digalactosyldiglycerols, and esterified sterol glycosides.

EXPERIMENTAL

For general observations, see [10].

Solvent systems: 1-6) hexane—diethyl ether (10.0, 9:1, 8:2, 6:4, 5:5, 0:10); 7) $CHCl_3$ — MeOH — 28% NH_4OH (10:4:1) and $CHCl_3$ — MeOH — CH_3COOH — H_2O (10:4:1:1); 8) CH_3COCH_3 — CH_3COOH — H_2O (100:2:2); 9) $CHCl_3$ — CH_3COCH_3 — MeOH — CH_3COOH — H_2O (65:20:10:10:3).

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