

LIPIDS OF THE VEGETATIVE AND GENERATIVE ORGANS OF *Hibiscus* sp.

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The lipids of the leaves, stems, and roots of Hibiscus sp. have been studied. Their fatty acid compositions have been determined. The greatest amount of unsaturated fatty acids was present in the glycolipids of the leaves and the stems. More than 25% of low-molecular-mass C₁₀-C₁₂ fatty acids have been found in the neutral lipids of the roots.

The majority of plants of the *Hibiscus* genus (fam. Malvaceae) are used in medicine since they contain biologically active substances [1, 2].

The lipids form one of the important components of these substances, and the study of their composition is therefore a matter of interest. Thus, the leaves of *Hibiscus manihot*, which are used in food, are rich in phospho- and glycolipids, the total amount of which is more than 60%. The fatty acids include 12 components, the main ones being the 16:0, 18:2, and 18:3 species [3].

Earlier, in an investigation of the seed lipids of *Hibiscus syriacus*, we detected the presence of malvic and sterculic acids in them [4]. In the present paper we give the results of a study of the lipids of the leaves, stems, and roots of *Hibiscus* sp. (Table 1).

The stems formed the bulk of the plant, while the largest amount of lipids was present in the leaves. The total lipids isolated from the various organs had different colors: from the leaves, dark green; from the stems, pink-green; and from the roots, light brown.

A considerable amount of crimson pigments (anthocyanins, according to qualitative reactions) was detected in the lipids of the leaves and stems. The lipids were separated by CC on silica gel into neutral lipids (NLs), glycolipids (GLs), and phospholipids (PLs): their amounts are given in Table 2.

The NLs proved to be the predominant fraction in all the organs. The second fraction, quantitatively, was that of the GLs. In none of the plant organs did the level of PLs exceed 13.2%.

As we have noted previously [5], the quantitative compositions of the lipid classes are greatly affected by the levels of pigments in them. It has been reported in the literature that chlorophylls predominate over other groups of compounds in the NLs of spinach [6], while 7.5% of chlorophyll has been found in the leaf NLs of *Mentha arvensis* and 8.2% in the stem NLs [7].

By the method of [8], from the UV spectra of the total lipids of the leaves and of the NLs and GLs of the stems we determined quantitatively the levels of chlorophylls *a* and *b* in them (mg/g):

	Chlorophyll	
	<i>a</i>	<i>b</i>
Total leaf lipids	29.2	1.3
Stem NLs	0.048	0.26
Stem GLs	9.05	7.7

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TABLE 1. Indices of the Leaves, Stems, and Roots of *Hibiscus sp.*, % by Weight

Index	Leaves	Stems	Roots
Weight of the plant	41.2	49.9	8.9
Moisture content	77.6	52.7	35.8
Amount of lipids on the a.d.w.	3.58	1.02	3.47

TABLE 2. Quantitative Compositions of the Lipid Classes, % on the Total Lipids

Class of lipids	Leaves	Stems	Roots
NLs+pigments	53.3	52.3	48.4
GLs+pigments	39.1	34.5	41.4
PLs+pigments	7.6	13.2	10.2

In the leaf lipids there was 22.5 times as much chlorophyll *a* as *b*, while in the stems the pigments predominated in the GL fraction and the ratio of chlorophylls *a* and *b* was 1.1:1.0.

The group compositions of the NLs of the leaves, stems, and roots were determined by TLC in systems 1 and 2, those of the GLs in systems 3 and 4, and those of the PLs in system 5. The lipids were identified from their chromatographic mobilities, by qualitative reactions, and by comparison with authentic specimens.

The NLs contained hydrocarbons, sterol esters, triacylglycerols, FFAs, triterpenols (TTs), and sterols. It was found visually that in the root lipids the TTs were present in insignificant amount in comparison with the other classes of NLs. Their amount in the leaf lipids, however, was considerably greater than that of the sterols. Isoprenols were detected in the leaf NLs.

As components of the GLs we identified sterol glycoside esters, monogalactosyldiacylglycerols, sterol glycosides, cerebrosides, digalactosyldiacylglycerols, and sulfolipids.

The PLs were separated by TLC in system 5: five components were detected, of which we identified three: phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol.

After the elimination of nonlipid components and concentration, a chloroform solution of the root lipids deposited a precipitate (12% of the weight of the lipids) which was separated off and analyzed by TLC in system 1. The R_f value of the substance isolated coincided with that of TAGs, and, after saponification of the precipitate with a 10% alcoholic solution of KOH and decomposition with 20% H_2SO_4 , fatty acids were obtained with a 67.3% content of saturated acids, which was apparently the reason for the separation of the TAGs as a precipitate.

Table 3 gives the compositions of the fatty acids of the lipid classes that were isolated. The lipids of the organs studied contained a set of 14 fatty acids, which were present in various proportions in the individual classes. A high concentration of the 16:0 acid was observed in the PLs of the leaves, stems, and roots, while the 18:3 acid was concentrated predominantly in the GLs of the leaves, and to a smaller extent in the stems and in the roots. The 18:1 acid was present in all classes in fairly appreciable amounts, while there was 21.7% of the 18:2 acid in the root PLs and considerably smaller, although still appreciable, amounts in the other classes.

It must be mentioned that the NLs of the roots contained the low-molecular-mass 10:0 and 12:0 fatty acids, the sum of which exceeded 25%. The composition of the precipitate, including 28.2% of lauric acid, is also of interest.

We have shown previously [1] that the seeds of *Hibiscus sp.* contain 8.1% of cyclopropanoid acids (CPAs). To determine the presence of CPAs in the lipids of the materials studied, we first freed them from pigments by Khor's method [9] and then, by means of the qualitative Halphen reaction, we established the presence of CPAs in the root lipids and their absence from the leaf and stem lipids. By titration with 0.1 N HBr in glacial acetic acid we detected 5.34% of CPAs in the root lipids. These results agree with literature information on the lipids of some species of plants of the Malvaceae family [10], although there are also reports on the presence of CPAs in leaf and stem lipids [11].

EXPERIMENTAL

The UV spectra of the pigments were recorded on a Hitachi spectrometer in hexane.

TABLE 3. Fatty Acid Compositions of the Lipid Classes from the Leaves, Stems, and Roots of *Hibiscus sp.*, % GLC

FA	Leaves			Stems			Roots			
	NLs	GLs	PLs	NLs	GLs	PLs	NLs	GLs	PLs	ppte
10:0	tr.	-	-	-	0.7	tr.	10.2	tr.	tr.	-
12:0	2.1	0.3	tr.	0.9	0.8	tr.	15.6	1.6	tr.	28.2
13:0	tr.	0.4	-	tr.	0.8	-	-	-	-	-
13:1	-	-	-	-	-	-	11.5	4.6	-	-
14:0	1.7	0.9	1.4	1.4	0.8	0.5	0.7	3.7	1.6	2.8
15:0	tr.	-	-	0.3	-	-	0.4	-	-	-
16:0	39.3	31.3	62.5	43.6	36.3	64.1	22.8	34.7	53.0	19.5
16:1	2.0	2.3	3.7	0.9	3.1	1.3	12.2	1.8	tr.	11.3
17:0	tr.	tr.	-	1.5	0.6	tr.	1.2	-	tr.	Cl.
18:0	3.4	1.3	2.2	0.7	1.8	1.5	6.0	7.4	3.9	7.5
18:1	9.4	12.8	11.8	26.9	14.5	12.2	8.9	18.9	11.7	13.7
18:2	11.2	4.0	10.3	15.8	19.9	17.1	6.5	14.4	22.0	7.7
18:3	30.9	16.7	8.1	6.8	20.7	3.3	4.0	12.9	7.8	-
20:0	-	-	-	1.2	-	tr.	tr.	-	-	9.3
Σ_{sat}	46.5	34.2	66.1	49.6	41.8	66.1	56.9	47.4	58.5	67.3
Σ_{unsat}	53.5	65.8	33.9	50.4	58.2	33.9	43.1	52.6	41.5	32.7

GLC was conducted on a Chrom 4 instrument under the conditions of [5].

The plant *Hibiscus sp.* was provided by workers at the Institute of Botany, Academy of Sciences of the Republic of Uzbekistan at the time of flowering (end of August). The fresh leaves were treated with boiling isopropanol, and the lipids were isolated with a mixture of chloroform and methanol (2:1, v/v). Nonlipid components were eliminated with a 0.04% solution of CaCl_2 .

The chlorophyll pigments were separated in the following way: the total lipids of the leaves or stems were added to a glass column with a diameter of 1.2 cm containing 1.5 g of a mixture of activated carbon and Celite 545 (2:1, w/w). The lipids were eluted with chloroform, the chlorophyll pigments remaining on the column.

The total lipids were separated by CC on silica gel, the NLs being eluted with chloroform, the GLs with acetone, and the PLs with methanol.

Solvent systems: 1) $\text{CH}_3\text{COCH}_3-\text{C}_6\text{H}_{14}$ (3:7); 2) $\text{CH}_3\text{COC}_2\text{H}_5-\text{C}_6\text{H}_{14}-\text{CH}_3\text{COOH}$ (43:7:1); 3) $\text{CHCl}_3-\text{CH}_3\text{OH}-\text{CH}_3\text{COCH}_3-\text{CH}_3\text{COOH}-\text{H}_2\text{O}$ (65:10:20:10:3); 4) $\text{CH}_3\text{COCH}_3-\text{toluene}-\text{CH}_3\text{COOH}-\text{H}_2\text{O}$ (60:60:2:1); 5) $\text{CHCl}_3-\text{CH}_3\text{OH}-25\% \text{NH}_3$ (65:35:5).

GLs were revealed with α -naphthol, PLs with ninhydrin and with the Dragendorff and Vaskovskii reagents.

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