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UNSAPONIFIABLE SUBSTANCES OF THE LIPIDS OF THE LEAVES OF *Hibiscus sp**

T. V. Chernenko, S. D. Gusakova, and A. I. Glushenkova

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The unsaponifiable substances of the lipids of the leaves of Hibiscus sp. *have been studied. The compositions have been found of seven classes of compounds: hydrocarbons, carotenoids, tocopherols, isoprenols, triterpenols, and phytosterols and their esters.*

Unsaponifiable lipophilic substances compose an important group of compounds accompanying lipids and possessing a supplementary biological action. Hypolipemic properties are known for the triterpenols and sterols — the main components of the usaponifiable substances [1, 2]. The high abortive and contraceptive activity of the unsaponifiable fraction of an extract of the flowers of *Hibiscus rosa-sinensis* (fam. Malvaceae) is explainewd by the presence of substances of triterpenoid and steroid natures [3]. Substances repelling boll weevils have been detected in the flowers of *Hibiscus furcatus*. The active fractions were fatty acids (FAs), FA methyl esters, and unsaponifiables, including hydrocarbons, tocopherols, triterpene alcohols, and sterols [4].

Previously, in studying the lipids of representatives of the Malvaceae family [5-7], we detected in the unsaponifiable fraction of the seeds of *Hibiscus syriacus* 0.21% of tocopherols (16% being biologically active, and 0.05% antioxidant) and, in addition 0.27% of free sterols and 0.24% of sterol esters with FAs.

In the present paper we give the results of an investigation of the unsaponifiable substances (USs) of the leaves of *Hibiscus sp.* introduced into Uzbekistan. The lipids were isolated from the leaves in an amount of 3.75%, calculated on the dry mass. The amount of USs in the lipids was 33.8%, or 1.27% on the absolutely dry mass of the plant material. The total USs were were separated by CC into individual fractions, using solvent systems 1-6, although 12.5% of the USs deposited on the column was not desorbed. As a result of the rechromatography of the fractions by preparative TLC, the following classs of substances were isolated in homogeneous form: hydrocarbons, phytosterol esters with FAs, aliphatic isoprenoid alcohols, triterpene alcohols, and free phytosterols. The amounts of carotenoids and tocopherols in the USs were determined by colorimetric methods. The composition of the USs according to the combined results is given in Table 1.

As can be seen from Table 1, in the total USs triterpene and isoprenoid alcohols predominated. By TLC in system 7, the hydrocarbons were separated into two spots with R_f 0.95 and 0.9, relating to paraffins and alkenes. The spot of the alkenes was colored when the chromatogram was treated with iodine vapor. According to their mass spectrum, the alkanes were represented by a mixture of homologs with chain lengths from C_{19} to C_{28} (M^+ 268-394) in which tricosane $C_{23:0}$ and tetracosane $C_{24:0}$ predominated. The alkenes formed the homologous series $C_{14:1}-C_{28:1}$ (M^+ 196-392) with $C_{17:1}$ and $C_{18:1}$ predominating. The spectrum also showed weak peaks of M^+ for the $C_{26:2}-C_{28:2}$ alkadienes (M^+ 362-390).

When the total USs were chromatographed by TLC in system 8, the carotenoid pigments appeared in the form of four spots with R_f 0.95, 0.34, 0.27, and 0.18, but when the USs were separated on a column, only two zones were eluted successively: yellow and orange. The UV spectrum of the yellow pigment showed absorption bands at λ_{max} (ethanol) 421, 442, 472 nm, which are characteristic for cryptoxanthin epoxide. The spectrum of the orange pigment — λ_{max} (ethanol) 421, 443, 473 nm — corresponded to dihydroxy-β-carotene epoxide [8].

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Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 40 64 75. Translated from Khimiya Prirodnykh Soedinenii, Vol. 33, No. 1, pp. 49-51, January-February, 1997. Original article submitted May 29, 1996.

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TABLE 1. Composition of the Unsaponifiable Substances of the Leaves of *Hibiscus sp.*

Class of substances	Content		
	% on the weight of the USs	% on the weight of the lipids	mg/g a.d.w. of the leaves
Hydrocarbons	4.3	1.5	0.5
Carotenes and xanthophylls	1.1	0.4	0.2
Phytosterol esters	2.5	0.8	0.3
Tocopherols	0.3	0.1	0.03
Isoprenoid alcohols	16.8	5.7	2.1
Triterpene alcohols	20.8	7.0	2.6
Phytosterols	9.8	3.3	1.2
Unidentified	44.4	15.0	5.9

On TLC in system 2, the phytosterol esters had R_f 0.47. The products of the hydrolysis of the esters were separated into three spots by TLC in system 3. Two spots, with R_f 0.36 and 0.30, were identified as FAs and sterols, respectively. The FAs of the phytosterol esters included the following acids (% GLC): 12:0 (9.8), 13:0 (4.9), 14:0 (2.7), 16:0 (27.9), 17:0 (1.9), 18:0 (2.3), 18:1 (26.9), 18:2 (23.6).

According to its mass spectrum, the sterol moiety of the esters consisted of β-sterol (M^+ 414, 100%), stigmasterol (M^+412; 40%), campesterol (M^+ 400; 20%), and cholesterol (M^+ 386; 6.5%) [9].

Thus, the main components of the class under discussion were esters of FAs — mainly the 16:0, 18:1, and 18:2 acids — with β-sitosterol. It is known that such esters are more difficult to hydrolyze with alcoholic alkali than esters of glycerol and of acyclic alcohols, which explains their presence as components of the USs.

The compositions of the tocopherols, the aliphatic isoprenoid alcohols, the triterpenoid alcohols, and the free phytosterols were studied by mass spectrometry. In the mixture of tocopherols we found α-tocopherol (M^+ 430; 100%), and β- or γ-tocopherol (M^+ 416; 18%) [10].

The fraction of isoprenoid alcohols, eluted from the column together with the FAs, was separated by preparative TLC in system 5, giving homogeneous isoprenols (R_f 0.59). According to mass spectrometry, the isoprenols consisted of five main components with numbers of isoprene units, *n*, from 9 to 13 (M^+ 902-630) [sic]. The highest intensities of the peaks of the M^+ and $(M - 18)^+$ ions were shown by undecaprenol, $C_{55}H_{90}O$ (*m/z* 766, 748) and dodecaprenol, $C_{60}H_{98}O$ (*m/z* 834, 816) [11]. The spectrum also showed the presence of ions of an analogous type, but with lower intensity, for the C_9-C_{13} isoprenols with *m/z* 2 mass units greater (dolichol family) [12], and 2 and 4 mass units smaller, than *m/z* 902-630.

Of the triterpene alcohols with M^+ 426, the following were identified from their characteristic ions: β- and/or α-amyrin (*m/z* 218, 203, 189), cycloartenol (*m/z* 408, 286, 339), lupeol (*m/z* 207), and of those with M^+ 440 — 24-methylenecycloartenol (*m/z* 407, 353, 300) [9]. The ion with *m/z* had the strongest peak in the mass spectrum. The composition of the free phytosteols was identical with that of those bound with FAs.

EXPERIMENTAL

UV spectra were taken on a MKh-1303 spectrophotometer in ethanol, and mass spectra on a MKh-1310 instrument at an ionizing energy of the electrons of 40/50 eV, and at a temperature of the ionization chamber of 100°C and of the evaporator bulb of 80°C.

TLC was conducted on silica gel with 10% of gypsum and on Silufol in the following systems: 1) C_6H_{14}; 2) $C_6H_{14}-CH_3COCH_3$ (9.5:0.5), 3) (8:2), 4) (6:4), 5) (5:5); 6) CH_3COCH_3; 7) $C_7H_{16}-C_6H_6$ (9:1); 8) $C_6H_{14}-CH_3COCH_3-C_6H_6-$isopropanol (69.5:25:4:1.5).

The lipids were extracted with chloroform–methanol (2:1, v/v) from fresh leaves that had previously been fixed with hot isopropanol. The saponification of the lipids and the isolation of the unsaponifiable substances were carried out by known methods [13].

REFERENCES

1. Yu. K. Vasilenko, L. I. Lisevitskaya, L. M. Frolova, and E. P. Parfent'eva, Farmakol. Toksikol., No. 5, 66 (1982).
2. V. V. Sokirka, V. V. Panina, and B. V. Shemeryanskii, Khim.-Farm. Zh., No. 9, 1102 (1987).

3. M. P. Singh, R. H. Singh, and K. N. Udupa, Planta Med., No. 3, 171 (1982).
4. C. S. C. Rao and S. S. Nigam, J. Indian Chem. Soc., No. 7, 747 (1979).
5. T. V. Chernenko and A. U. Umarov, Khim. Prir. Soedin., 652 (1974).
6. T. V. Chernenko, A. U. Umarov, and A. L. Markman, Khim. Prir. Soedin., 719 (1973).
7. T. V. Chernenko, N. T. Ul'chenko, A. I. Glushenkova, U. P. Pratov, and E. M. Érkenova, Khim. Prir. Soedin., 40 (1995).
8. F. Foppen, Chromatogr. Rev., No. 3, 1 (1971).
9. T. Iton, T. Tamura, and T. Matsumoto, Lipids, 173 (1974); J. Am. Oil Chem. Soc., 300 (1973).
10. S. E. Scheppele, R. K. Mitchum, C. J. Rudolph, K. F. Kinneberg, and G. V. Odell, Lipids, No. 5, 297 (1972).
11. A. R. Wellburn, J. Stevenson, F. W. Hemming, and R. A. Morton, Biochem. J., **102**, 313 (1967).
12. J. W. Rip, C. A. Rupar, K. Ravi, and K. K. Caroll, Progr. Lipid Res., No. 4, 270 (1985).
13. Handbook on Methods of Investigation, Technical, and Chemical Control, and the Accounting of Production in the Oils and Fats Industry [in Russian], Leningrad, Book 2 (1967).