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SEED LIPIDS OF SOME SPECIES OF THE GENUS *Althaea*

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We have determined the fatty acid composition, terpenoids, phthalates, alcohols, esters, and nitrogen-containing compounds in the seeds of Athaea *L. species:* A. officinalis *L.*, A. armeniaca, *Ten.*, A. taurinensis *DC., and* A. broussonetiifolia. *All the species studied contained considerable amounts of palmitic, linoleic, petroselinic, and stearic acids. Terpenoids were represented by aldehydes and ketones of the monoterpene series, phthalates by dibutyl phthalate, dihydric alcohols and ethers by compounds with the composition $C_{10}H_{22}O_2$, and nitrogen-containing substances by 4-morpholinylbutylamine.*

Of the various species of *Althaea* L., wide use is made of *A. officinalis* L. (marshmallow) and *A. armeniaca* Ten. The roots are used as expectorant, coating, and antiinflammatory agents mainly in diseases of the respiratory passages. A mixture of polysaccharides from the herbage of marshmallow in the form of Mukaltin tablets is prescribed in cases of bronchitis, pneumonia, bronchoectasis, etc. [1]. In folk medicine, marshmallow seeds are used for the prophylaxis and treatment of atherosclerosis and other diseases [2].

Little is known about the chemical composition of the seeds of various species of *Althaea*. There is only information for *A. rosea* L. (hollyhock) [3]. The seed oil of this species contains hydrocarbons of the C_{14}-C_{33} series, C_{14}-C_{22} fatty acids, and C_{20}-C_{28} saturated aliphatic alcohols. Sterols are represented by β-sitosterol, cholesterol, campesterol, and stigmasterol. There is no information on the chemical composition of the seeds of other *Althaea* species, including marshmallow. The investigation that we have performed is of interest from the point of view of the biochemical systems of plants and for revealing chemotaxonomic characteristics within this genus, and also for determining the authenticity of seed material in introduction work. We have studied the chemical composition of the seeds of four species of *Althaea* growing on Ukrainian territory: *A. officinalis, A. armeniaca, A. taurinensis* DC, and *A. broussonetiifolia*, gathered in August 1993 in the Dnepropetrovsk province.

Fatty acids were identified by the method of selective detection using *m/z* 74, 88, which are specific for fatty acid methyl esters. Then the time of retention on the column was taken into consideration (Kovac's chromatographic indices were used) [4]. In addition, in the assignment of the acids we determined the peaks of the molecular ions (M^+) and of the $(M - OCH_3)^+$ ions. This course of identification has been tested in a study of the lipid compositions of a number of samples of natural origin [5].

Characteristic for the seeds of the above-mentioned *Althaea* species was the presence of considerable amounts of four fatty acids: palmitic, linoleic, petroselenic, and stearic (Table 1). Their quantitative composition varied from species to species. It is known [6] that the reproducibility of electron-impact mass spectra under the conditions of a chromato-mass spectrometric experiment averages 20 rel.-%, which permits a concrete assignment of a species to be made even at this stage. For example, the quantitative ratio of petroselenic, palmitic, and stearic acids in the fatty oil of the seeds of *A. taurinensis* was 16:5:1, in *A. broussonetiifolia* 9:4:1, and in *A. officinalis* and *A. armeniaca* 11:6:1 and 19:5:1, respectively.

The ratios found are reliable criteria for the identification of a species. In a concrete case it is possible to be limited to the choice of two of the fatty acids mentioned or to introduce a fourth parameter — the quantitative characteristics of lineolic acid.

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TABLE 1. Composition of the Fatty Acids of the Seeds of *A. offinicialis* (sample 1), *A. armeniaca* (sample 2), *A. taurinensis* (sample 3), and *A. broussonetiifolia* (sample 4)

Acid	Amount in the sample, %				M^+	Retention time on the column, t_{min}
	1	2	3	4		
Pelargonic	0.31	1.24	–	2.87	172	6′18″
Myristic	0.44	–	–	–	242	10′03″
Hexadecenoic	0.19	–	–	–	268	11′25″
Palmitic	19.95	22.43	19.93	17.43	270	11′35″
Margaric	0.40	–	–	–	284	12′25″
Linoleic	6.06	5.28	8.33	6.41	294	13′11″
Petroselenic	54.90	42.01	60.17	40.43	296	13′20″
Stearic	6.64	3.49	3.73	4.66	298	13′36″
Dodecanoic	0.92	–	–	0.32	312	14′11″
Erucic	–	–	1.24	–	324	15′31″
Arachidic	0.32	–	–	–	326	15′37″
Behenic	0.39	1.12	–	–	354	16′25″

In addition to the characteristics mentioned, a number of species can be characterized unambiguously by the presence of one or two acids specific for the seeds of the particular *Althaea* species. For example, the seeds of *A. taurinensis* lack pelargonic acid (at a sensitivity for its detection of 0.01%), while the seeds of marshmallow contain margaric acid, which is specific for this species. The seed oils of marshmallow and *A. brussonetiifolia* contain nonadecanoic acid, which has not been found in the seeds of *A. armeniaca* and *taurinensis*. The seed oil of marshmallow contains the greatest amount of nonadecanoic acid. The presence of erucic acid is characteristic only for *A. taurinensis*, while arachidic acid is present only in marshmallow seed oil. Behenic acid has been identified in the seeds of marshmallow and *A. armeniaca*. Hexadecanoic acid is found only in the oil of marshmallow.

Thus, the composition of the fatty acids permits the identification of *Althaea* species with a considerable degree of reliability (from several parameters).

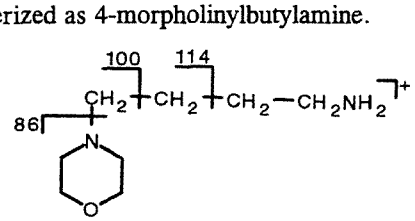
In addition to fatty acids, we established the presence of a small amount of terpenoids predominantly belonging to the class of aldehydes and ketones of the monoterpene series. The terpenoids were identified by the procedure of [6], with the supplementary use of a catalog [7]. In all the samples, with the exception of the fatty oil of *A. broussonetiifolia* seeds, we identified camphor (M^+ 152, t_{min} = 7′10″), the amount of which ranged from 0.80% (*A. taurinensis*) to 6.13% (*A. armeniaca*). Marshmallow seeds contained 2.05% of it.

Another terpene, with t_{min} = 7′02″, we took to be neral (*A. armeniaca*), its amount being 3.39%. In *A. taurinensis* seeds, in addition to camphor, we detected the presence of vanillin (t_{min} = 8′04″, amount 0.83%). Marshmallow seeds contained 1.30% of neral and 0.19% of geranial (t_{min} = 8′18″) [8].

The presence of phthalates, identified in practically all the *Althaea* species studied ((t_{min}) = 12′05″), was established from the characteristic ion with *m/z* 149 [9]. Thus, the concentration of dibutyl phthalate ($C_{16}H_{22}O_4$) in marshmallow seeds was 1.65%, in the seeds of *A. armeniaca* 7.70%, in those of *A. taurinensis* 1.39%, and in those of *A. brussonetiifolia* 5.99%.

Dihydric alcohols or ethers having the composition $C_{10}H_{22}O_2$ (M^+ 174, t_{min} = 8′43″) were present in the seeds of marshmallow and of *A. broussonetiifolia*. The presence of dihydric alcohols and, possibly, trihydric alcohols was detected in marshmallow seeds (M^+ 174, 176, t_{min} = 4′26″ and 5′59″, respectively). The total amount did not exceed 1%. In samples of marshmallow and *A. armeniaca* we detected the presence of a phenyl alkyl ketone (M^+ 204, t_{min} = 11′06″) represented by the structure $C_6H_5COC_7H_{15}$, in amounts of 0.3 and 1.9%, respectively.

In addition, we have shown for the first time that the seeds of *A. broussonetiifolia* contain a compound including two nitrogen atoms. This compound had M^+ 185, t_{min} = 6′40″, its amount being 3.9%. Its structure was established with the aid of a computer library, and it was characterized as 4-morpholinylbutylamine.



A similar compound with the same characteristics was observed in the seeds of *A. armeniaca*, in an amount of 4%. Furthermore, the same species was found to contain acyclic hydrocarbons with the provisional structure of 2-methyl-1-propenylcyclopentane. The assignment of this compound was made on the basis of the peaks observed in the mass spectrum and the results of a library search.

EXPERIMENTAL

The seeds were ground in an electric mill and were extracted with ethyl ether. The solvent was distilled off and the substance obtained was treated with diazomethane. The extracts of *A. officinalis*, *A. armeniaca*, *A. taureninesis*, and *A. brussonetiifolia* were analyzed in a chromato-mass spectrometric system consisting of a HP-5890 A chromatograph, a Finnigan MAT ITD-700 ion-trap mass-spectrometric detector, and systems for controlling the instrument and for processing the results based on a IBM PC-AT personal computer with a library of mass spectra.

This system, working at a relatively high pressure in the analyzer (0.01-0.1 Pa), allows the injection of a comparatively large sample (about 1-5 μl), which permits a considerable increase in the overall sensitivity of the analysis. The limit of detection of compounds of various classes is 0.1-1 ng of substance injected into the column, or 1-10 ng/liter.

Separation was carried out in a quartz capillary column 0.20 mm $\times$ 25 m with the cross-grafted stationary phase SE-54. Temperature regime: initial temperature of the thermostat 100°C with a holding time of 1 min: programmed regime of the temperature rising by 20°C/min to a final temperature of 260°C, with holding in the isometric regime for 30 min. The size of sample injected was 1-2 μl, the temperature of the injector 250°C and that of the interphase 240°C, rate of flow of carrier gas (helium) 1 ml/min.

The quantitative evaluation of the ingredients of the fatty acids and the other natural components was made in the basis of the contribution of the ion currents corresponding to each definite component to the total ion current.

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