3. For each branch in the polymer chain there are no less than 7 xylose residues, which indicates a block structure of the macromolecule.

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COMPOSITION OF THE COATS AND KERNELS OF THE SEEDS OF *Nepeta pannonica* AND *Lavandula vera*

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The lipid compositions of the coats and kernels of the seeds of *Nepeta pannonica* and *Lavandula vera* have been studied. It has been established that the lipids of the seed coats of the two species of plants differ substantially in their composition. The lipids of the kernels of *Nepeta* have been found to contain free fatty acids with chain lengths of from C_{20} to C_{35}. Ursolic acid and its acetate have been isolated from extracts of the seed coats of *Lavandula*, and dimethyladipic acid from the seed oil of this species.

Recently, a considerable number of publications devoted to the surface lipids of various plant organs, and, in particular, the stems and leaves, has appeared [1]. The surface lipids play an important role in the interaction of the plants with the environment, controlling their water balance and protecting them from pathogens. These lipids consist of a complex mixture the usual components of which are hydrocarbons, waxes, free fatty acids, high-molecular-weight alcohols, aldehydes, terpenes, and flavones.

The lipids of seed coats have been less studied. It has been reported [2] that the lipid layer of seed coats contains the above-mentioned classes of compounds and, in particular, hydrocarbons, waxes, and triterpenes, with more rarely, aldehydes and ketones. At the same time, some of the compounds mentioned have been detected in the lipids of the seed kernels of many plants [3]. Suprunov [4] states that in the seed of *Schizandra chinensis* (family Schizandraceae), the essential oil, waxes, and pigments are present exclusively in the solid coat, and the oil of the kernel consists of triacylglycerols together with sterols and tocopherols. A fatty oil without impurities can therefore be obtained from seeds that have been treated previously with ethanol or acetone.

The question of whether certain classes of lipids belong to the suface lipids or are characteristic of the kernel is of particular interest in those cases where new compounds unusual for neutral lipids have been detected [5].

In order to investigate differences in the lipid compositions of the coats and kernels of seeds, we have investigated the seeds of two species of the family *Labiatae* — *Nepeta pannonica* (Hungarian catmint) and *Lavandula vera* (*Lavandula officinalis*; true lavender). The latter species is cultivated for the production of its essential oil. There is information in the literature only on the composition of the fatty acids of the total lipids of *Nepeta pannonica* [6].

The seeds of *Nepeta pannonica* were collected in August, 1978, in the valley of the R. Chon-Kemin, Kirghiz SSSR, and those of *Lavandula vera* in July, 1977, in the "Dolina Roz" ["Valley of Roses"] collective farm in the Moldavian SSR. They were very small with a smooth surface in the case of the lavender and with a rough surface in the case of the catmint, and

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the weights of 1000 seeds were 1.1 and 0.66 g, respectively. The seeds, which were ripe in appearance, after being freed from impurities, contained no appreciable amounts of broken or defective seeds.

Individual batches of the *Nepeta pannonica* seeds, before comminution, were treated briefly with solvents: diethyl ether or acetone, or a mixture of methanol and acetone. All the extracts apart from the methanol-acetone extract formed homogeneous masses. The inhomogeneous methanol-acetone extract after evaporation was additionally treated with petroleum ether and the resulting extract was analyzed.

The yields of extracts from the surface coatings of the *Nepeta pannonica* seeds were: acetone 1%; ether 1%; methanol-acetone 0.8% of the weight of the seeds.

In spite of the fact that we used solvents of different polarities for the extraction of the surface lipids, the quantitative yields of the total lipids did not well agree with the expected order of extraction and the class compositions of the extract proved to be almost identical in all cases (Table 1).

Thus, quantitatively the main lipid class of the *Nepeta* husks are triacylglycerols (TAGS) while the other classes are represented by small amounts which vary according to the extractant within the limits of error of the experiment. For this reason, it was difficult to give a preference to one of the solvents used. However, in view of the fact that the mixture of methanol and acetone extracted a somewhat larger amount of acylglycerols than the other solvents, we used it for extracting the lipids of the surface coatings of the *Lavandula* seeds. The yield of extract for this species amounted to 0.7% of the weight of the seeds.

The composition of the surface lipids of *Lavandula* differ sharply from those of the *Nepeta* coat lipids. In this case, the total lipids lack acylglycerols and free fatty acids. The main components of the *Lavandula* coating are ursolic acid and its acetate, sterols and their esters, and chlorophyll. When the *Lavandula* seed coatings were treated with diethyl ether the same main compounds were found in the extract as in a methanol—acetone extract.

Characteristic for plants of the family Labiatae is the accumulation of ursolic and oleanolic acids in the surface lipids of the vegetative organs [7]. In our case, the amount of ursolic aicd was 25% of the weight of the extract and its acetate was present in considerably smaller amounts.

After the extraction of the lipids from the external coatings, the seeds were comminuted and the essential oil was extracted with hexane. The yiels of oil from the seed kernels of *Nepeta* and *Lavandula* were 18 and 20%, respectively.

In all the extracts of the seed coats of *Nepeta* a series of saturated C_{25}-C_{33} hydrocarbons was found by IR and mass spectrometry, with a predominance of the C_{25} and C_{26} compounds (mass spectrum: M^+ 352, 366, 380, 394, 408, 422, 436, 450, and 464).

TABLE 1. Class Compositions of the Extracts

Class of compounds	Nepeta pannonica, %				Lavandula vera, %
	coat			kernel	kernel
	diethyl ether	acetone	methanol—acetone	hexane	hexane
Carbohydrates	1,8	0.7	1,2	0,2	0,1
Sterol esters	—	0,3	0,1	Tr.	—
Waxes	Tr.	0.4	Tr.	0,5	—
Triacylglycerols	86.0	84,9	87,9	96.0	97,7
Unidentified	0,9	1,8	1,3	0,3	—
Free fatty acids	4,0	2,8	2,2	1,4	0,6
Diacylglycerols	—	—	—	—	0,9
Ursolic acid acetate	—	—	—	—	0,01
Sterols	0,3	1,5	Tr.	0.4	0,4
Chlorophyll	Tr.	0,1	0,9	Tr.	Tr.
Polar lipids	7,0	7,5	5,6	1.2	0,3

TABLE 2. Acid Compositions of the Seed-Coat and Kernel Lipids of Two Species of the Family Labiatae (%, GLC)

Acid	Nepeta pannonica								Lavandula vera	
	coat						kernel		kernel	
	diethyl ether		acetone		methanol–acetone		TAGs	FFAs	TAGs	FFAs
	TAGs	FFAs	TAGs	FFAs	TAGs	FFAs				
10:0	—	—	—	—	—	—	—	0,1	—	—
12:0	—	—	—	1,0	—	7,8	—	0,1	—	—
14:0	—	—	—	1,4	—	6,5	—	0,2	—	—
16:0	3,9	7,4	1,4	17,2	5,0	26,9	2,6	4,3	3,4	3,2
16:1	Tr.	0,7	Tr.	3,1	Tr.	—	0,7	1,2	0,1	Tr.
18:0	0,9	1,9	Tr.	13,2	1,0	8,7	0,2	2,6	0,1	Tr.
18:1	10,5	9,0	2,4	18,0	7,0	9,8	4,4	10,6	11,3	2,0
18:2	22,5	24,6	10,2	8,7	19,4	11,8	15,7	18,6	8,0	11,1
18:3	62,2	56,2	86,0	37,4	67,6	28,5	76,4	62,3	77,1	83,7
$\Sigma_{sat.}$	4,8	9,5	1,4	32,8	6,0	49,9	2,8	7,3	3,5	3,2
$\Sigma_{unsat.}$	95,2	90,5	98,6	67,2	94,0	50,1	97,2	92,7	96,5	96,8

The seed kernel oils were found to contain the same set of hydrocarbons with traces of higher homologs from C_{35} to C_{41}, while the lipids of the *Lavandula* kernels contained the hydrocarbons from C_{24} to C_{31} with molecular weights M^+ of 338, 352, 366, 380, 394, 408, 422, and 436.

The sum of the waxes of the *Nepeta* seed kernels consisted of esters with molecular weights M^+ of 592, 564, and 536 (mass spectrum).

After the saponification of the waxes, alcohols and acids were obtained with R_f 0.6 and 0.5, respectively, in system 1. The mass spectrum showed the presence of $C_{16:0}$, $C_{18:0}$, $C_{20:0}$, and $C_{22:0}$ alcohols. According to the results of GLC analysis, the acids had the following composition (%): 14:0 — 6.5; 16:0 — 13; 18:0 — 12.5; 20:0 — 68; 22:0 — traces. Thus, arachidic acid makes up more than half of the total acids, on which basis it may be assumed that the maximum peak, with M^+ 564, corresponds to a wax consisting of arachidic acid and octadecyl alcohol.

As already mentioned, the seed coats of *Lavandula* revealed no acyl glycerols, which constitute the bulk of the extracts in the surface lipids of *Nepeta*.

The fatty acid compositions of the lipid classes of *Nepeta* and *Lavandula* are given in Table 2, from which it can be seen that the set of free fatty acids (FFAs) is, in the majority of cases, more diverse than those bound into the corresponding acylglycerols. The total FFAs are more saturated than the acylglycerols, and the saturation of the acids rises with an increase in the polarity of the extractant.

In an analysis of the FFA fractions of the *Nepeta* coats and kernels on chromatograms the lower parts of the acid spots were not revealed with iodine.

A white deposit was removed from the mixture of acids in the cold, and in this deposit saturated acids with chain lengths of from C_{20} to C_{35} were identified by IR and mass spectrometry. Thus, the quantitative set of acids of the kernels of the central Asian species *Nepeta pannonica* was more diverse than indicated in the literature.

In the FFA fraction of the seed kernel oil of *Lavandula*, a compound having a similar chromatograph behavior to the saturated fatty acids was detected. However, on the basis of spectral characteristics it was identified as dimethyladipic acid $HOOC-\underset{\displaystyle CH_3}{\underset{|}{CH}}(CH_2)_2-\underset{\displaystyle CH_3}{\underset{|}{CH}}COOH$. On a GL chromatogram under the conditions given, the dimethyl ester of this acid issued after linolenic acid.

The ursolic acid acetate with the composition $C_{32}H_{50}O_4$ present in the surface lipids of *Lavandula* was isolated from the seed kernel oil in an amount of 0.02 g. No ursolic acid was detected in the kernels.

The unidentified compound present in the lipids of the surface coats and kernels of *Nepeta* has chromatographic mobilities on TLC in systems 1 and 2 almost coinciding with those

TABLE 3. Acid Composition of the Substances Remaining at the Start (%, GLC)

Acid	Nepeta pannonica			
	seed coat			seed kernel
	diethyl ether	acetone	methanol–acetone	hexane
12:0	8,7	—	—	Tr.
14:0	3,8	—	1,3	Tr.
16:0	38,0	37,5	22,3	22,6
16:1	5,4	—	3,7	4,8
18:0	10,6	14,1	6,2	8,3
18:1	26,1	20,8	13,3	21,6
18:2	5,0	14,7	22,9	15,6
18:3	1,8	12,9	30,3	27,1
$\Sigma_{sat.}$	61,7	51,6	29,8	30,9
$\Sigma_{unsat.}$	38,3	48,4	70,2	69,1

of the TAGs. This compound was isolated by steam distillation. The retention time of the substance on GLC without preliminary methylation corresponded to methyl palmitate, although according to its IR spectrum it had the nature not of an acid but of a lactone (1775 cm^{-1}). A considerable content of this compound is characteristic for the seed coats (see Table 1) but the compound is present in only small amounts in the seed kernel oil, which, however, can exaggerate the true palmitic acid content on analysis of the TAGs. After treatment with alcoholic alkali, the compound partially decomposed, giving on GLC a number of peaks with low retention times. The substance was provisionally assigned to the components of the essential oil.

The most saturated fractions of the TAGs of the kernel lipids of *Nepeta* and *Lavandula* had intense absorption in the UV spectrum with maxima at 239, 245, 250, 257, and 267 nm, but in view of their very small amount we were unable to isolate these compounds.

The diacylglycerols of the *Lavandula* seed kernels had the following acid composition (%, GLC): 16:0 — 9.8; 18:1 — 15.0; 18:2 — 25.3; 18:3 — 49.9.

A comparatively large percentage of the total lipids both of the coats and of the kernels of the *Nepeta* seeds is represented by substances remaining at the start including highly polar substances in which we were unable to detect phospholipids by a qualitative reaction. The qualitative reaction for glycolipids was unclear [8].

According to its IR spectrum, this fraction contained a considerable amount of hydroxy and ester groups (3400, 1735, 1110-1270 cm^{-1}). By saponifying the polar products we isolated fatty acids the compositions of which are given in Table 3.

Sterols were present both in the coats and in the kernels of the seeds studied. From the seed kernel oil of *Nepeta* we isolated sterols with mp 134°C, and from *Lavandula* sterols with mp 136°C. According to mass spectrometry, the total steroids of *Lavandula* include campsterol, $C_{28}H_{48}O$, stigmasterol, $C_{29}H_{48}O$, and β-sitosterol, $C_{29}H_{50}O$ with a quantitative predominance of the latter.

The only pigment found in the surface lipids and seed kernel oils of *Nepeta* and *Lavandula* was chlorophyll, mainly concentrated in the seed coats and probably passing into the oil as a result of incomplete extraction from the surface of the seeds.

The results of our investigations have shown that the compositions of the lipids of the seed coats of plants of two species of the family Labiatae differ substantially. The fatty oils of the species studied belong to the linolenic-acid-containing group; in the *Lavandula* oil, the linolenic acid content amounts to 80%.

EXPERIMENTAL

UV spectra were taken on a Hitachi spectrophotometer in hexane, IR spectra on a UR-10 instrument using films and KBr, and mass spectra on a MKh-1303 spectrometer.

The gas–liquid chromatography of the lipids was carried out on a Khrom-4 chromatograph with a flame-ionization detector using a 2.5 × 4 mm column filled with 17% of Reoplex 400 on Chromaton N-AW-DMCS at a column temperature of 198°C.

The column chromatography of the lipids was effected on Chemapol L 100/250 μ silica gel; hexane with increasing concentrations of diethyl ether — 5, 10, 20, 50, and 100% — was used for eluting the fractions.

Analytical TLC was performed on Silufol plates in the following solvent systems: 1) hexane—diethyl ether—glacial acetic acid (7:3:0.1); 2) heptane—methyl ethyl ketone—acetic acid (41:9:0.5); and 3) toluene—ethyl acetate—acetic acid (12:4:0.5) [9].

The extraction of the coat lipids was carried out by means of three or four steepings with the solvent for an hour and a total time extraction of 4-5 h. The methanol:acetone ratio was 2:1.

Waxes were extracted in the cold from the hexane solutions of the initial fractions of acylglycerols and were purified by reprecipitation from petroleum ether. Vigorous alkaline hydrolysis of 0.04-g samples of the waxes was carried out for 6 h [10].

The high-molecular-weight saturated acids were precipitated in the cold from an acetone solution of the FFAs of *Nepeta*. IR spectrum, ν_{max}^{KBr}, cm^{-1}: 940-980, 1710, 2800-3100. Mass spectrum (40 eV, 160°C, 0.5 mA), m/e: M^+, 552, 508, 494, 480, 466, 452, 438, 424, 410, 396, 382, 368, 354, 340, 326, 312, 60.

Dimethyladipic acid was crystallized in the cold from a solution of the total FFAs of *Lavandula* in acetone. The precipitate was methylated with diazomethane and analyzed in the form of the dimethyl ester: IR spectrum, ν_{max}^{KBr}, cm^{-1}: 750, 760, 945, 1060, 1080, 1205, 1310, 1390, 1415, 1480-1520, 1640, 1680-1700, 2855, 2920, 2960.

Mass spectrum (50 V, 60 μA, 130°C), m/e, %: 171 ($M^+ - 31$) (100), 142 ($M^+ - 60$) (20), 88 (10), 69 (10).

The retention time of dimethyl dimethyladipate on GLC relative to the 18:0 acid was 2.9.

Ursolic acid was purified by crystallization from methanol. It was revealed by sulfuric acid: R_f 0.1 (system 1, TLC) and 0.7 (system 3, TLC). In concentrated sulfuric acid it gave a maximum in the UV spectrum at 310 nm.

IR spectrum ν_{max}^{KBr}, cm^{-1}: 1700, 1390, 1380, 1360.

Mass spectrum (50 V, 3 A, 150°C), m/e: M^+ 456, 248.

Ursolic acid acetate was revealed with sulfuric acid, R_f 0.3 (system 1).

IR spectrum ν_{max}^{KBr}, cm^{-1}: 1740, 1700, 1250.

Mass spectrum (50 V, 3 A, 120°C), m/e: M^+ 498, 483 ($M^+ - 15$), 438 ($M^+ - 60$).

The free sterols were revealed with sulfuric acid, R_f 0.2 (system 1). Mass spectrum (3 A, 50 V, 100°C), m/e: M^+ 414, M^+ 412, M^+ 400, 399, 397, 396, 395, 385, 382, 369, 367, 351, 329, 315.

SUMMARY

1. For *Nepeta pannonica* the class compositions of the seed-coat and seed-kernel lipids have been shown to be identical. An extract of the seed coats of *L. vera* is characterized by the presence of ursolic acid and its acetate.

2. The free fatty acids of the seed kernels of *Nepeta pannonica* were found to contain saturated fatty acids with chain lengths of from C_{20} to C_{35}.

3. Dimethyladipic acid has been isolated from the seed oil of *Lavandula*.

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STRUCTURE AND STEREOCHEMISTRY OF GALBANIC ACID

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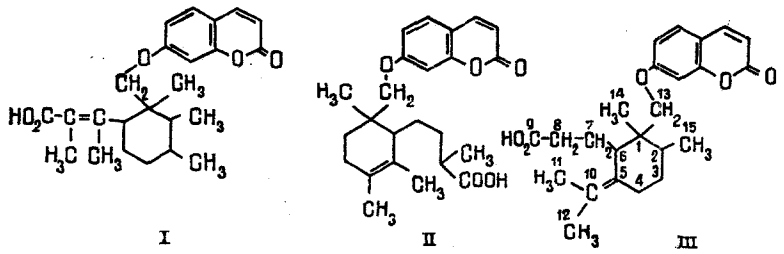
On the basis of the ^{1}H NMR spectrum at 300 MHz and the results of chemical degradation, the structure of 7-[6-(β-carboxyethyl)-5-isopropylidene-1,2-dimethylcyclohexylmethoxy]coumarin has been proposed for galbanic acid.

Galbanic acid, which was first isolated from galbanum resin [1] and then from a number of species of *Ferula* [2-6], was ascribed structure (I) [2, 3] and then, on the basis of PMR and mass spectra, structure (II) [6]. The greater informativeness of the ^{1}H NMR spectrum obtained on a spectrometer with a working frequency of 300 MHz and also the results of the degradation of the substance now permit structure (III) to be suggested for galbanic acid.



I II III

As has been established previously [6], the terpenoid moity of galbanic acid contains a six-membered cyclohexane ring, a carboxy group, two methyl groups at a double bond, a CH_3—CH group, and one methyl group attached to a quarternary carbon atom. However, the oxidative degradation of galbanic acid shows that its structure includes an isopropylidene group. The epoxidation of galbanic acid gives an epoxide $C_{24}H_{30}O_6$ in the ^{1}H NMR spectrum of which the CH_3—C_{11} and CH_3—C_{12} signals have been shifted upfield (1.32 ppm, s 6 H) in comparison with the initial compound (1.43 and 1.63, s, 3 H each); the opening of the epoxide ring in an acid medium and periodic acid oxidation lead to acetone, identified in the form of the 2,4-dinitrophenylhydrazone. Figure 1 shows a fragment of the ^{1}H NMR spectrum of galbanic acid at a working frequency of the spectrometer of 300 MHz ($CDCl_3$: 0 — TMS). The assignment of the signals was made with the aid of double resonance. When the signals of the H_2, H_4a, and $2H_7$ protons, which have the same chemical shift (1.89 ppm), were irradiated, the signals of the H_6 (2.96 ppm), H_4e (2.05 ppm), CH_3-11 (1.45 ppm) and CH_3-15 (0.91 ppm, d, J = 7.0 Hz) protons were converted into singlets and those of the $2H_8$ protons (2.20 ppm) into an AB quartet ($\Delta\nu_{AB}$ = 10 Hz, J = 16.0 Hz). It follows from this that the aliphatic chain has the structure —CH_2—CH_2—COOH and not —CH_2—CH(—CH_3)—COOH, as was previously assumed [6]. The fact that the HOOC—CH_2—CH_2 group is actually present in the α position to the $(CH_3)_2C$= group is shown by the chemical shift and the multiplicity of the signals of the H_6 proton in the spectrum of galbanic acid. The same facts indicate that the other neighboring atom to C_6 is a quaternary

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