

COMPOSITION OF THE WAXES FROM THE PRODUCTION WASTES OF  
LAVENDER AND SAGE OILS

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The chemical compositions of the waxes and the wastes from the production of lavender and sage oils and also the influence of industrial treatment with hydrogen peroxide on their composition have been established.

At the present time, more than 200 thousand tonnes of essential-oil raw material per year is processed in our country [1]. The yield of desired product – essential oil – does not exceed 0.1-1.5%, which reflects the degree of utilization of the plant biomass. The remainder of the raw material is discarded as an industrial waste.

The solution of the problem of the complex utilization of plant raw material presupposes the study of the composition of the wastes from its processing and the search for methods of making practical use of their components. Isolated investigations in this direction have been made only in recent years [2-8].

We have previously reported information on the total volume of lipids in extracts of the raw material and industrial wastes from lavender and sage oils [9]. The aim of the present work was to investigate the composition of lavender and sage waxes which are removed from these extracts individually in the course of the industrial isolation of the essential oils.

We have analysed four samples: the crude waxes of lavender (sample 1) and of sage (3), which have a dark green color, and bleached lavender and sage waxes (2 and 4, respectively). Bleaching with hydrogen peroxide in an acid medium was undertaken to improve the quality of the waxes.

The overall composition of the waxes was first determined by the TLC method. To identify the components, their chromatographic mobilities in systems 1-6 were compared with those given in the literature [10] and with model samples. The definitive assignment of the compounds to particular classes was made on the basis of their UV, IR, and mass spectra and GLC after their isolation by preparative TLC. The amounts of the components were estimated from the results of preparative separation.

The compositions of the lavender and sage waxes are given in Table 1. In each of these plant waxes seven classes of compounds were detected, among which esters predominated (~50% of the weight in all the samples).

A considerable part of all the samples of waxes consisted of hydrocarbons. TLC in system 2 showed the presence of two types of compounds in them, with  $R_f$  0.98 and 0.95. The hydrocarbons from samples 2 and 4 were analyzed in detail.

On treatment with  $I_2$  vapor on Silufol, the hydrocarbons with  $R_f$  0.98 gave a white spot. In the IR spectrum characteristic for this class [9] the bands of the vibrations of olefinic bonds were absent. Homologous series of saturated hydrocarbons from  $C_{17}$  to  $C_{33}$  ( $M^+$  240-464) for lavender and from  $C_{21}$  to  $C_{33}$  ( $M^+$  296-464) for sage were identified from their mass spectra. Their quantitative compositions were determined by the TLC method. The composition of the hydrocarbons of the bleached lavender and sage waxes were as follows (% on the weight of the paraffins):

Sample	$C_{17}-C_{20}$	$C_{21}-C_{24}$	$C_{25}$	$C_{26}$	$C_{27}$	$C_{28}$	$C_{29}$	$C_{30}$	$C_{31}$	$C_{32}$	$C_{33}$
2	Tr.	Tr.	5.4	1.4	5.4	3.1	11.3	6.5	24.7	1.9	27.3
4	—	Tr.	5.7	0.7	12.3	0.6	17.6	0.9	31.1	2.1	29.0

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TABLE 1. Compositions of Lavender and Sage Waxes

Class of compounds	Amount, % on the weight of the sample			
	lavender		sage	
	1	2	3	4
1. Hydrocarbons				
a) saturated	23.9	24.1	22.5	23.2
b) aromatic	5.5	5.2	8.6	8.0
2. Esters				
a) esters of triterpenols	6.2	5.0	4.5	3.0
b) wax esters	—	—	34.7	35.0
c) di(2-ethylhexyl) phthalate	27.1	29.7	Tr.	Tr.
d) esters of aromatic acids	13.4	15.1	11.4	14.7
3. Fatty acids	11.8	10.5	3.9	3.8
4. Unidentified	Tr.	Tr.	—	—
5. Alcohols				
a) fatty alcohols + triterpenols	7.0	7.7	3.0	3.7
b) sterols	Tr.	Tr.	Tr.	Tr.
6. Chlorophylls	1.9	Tr.	2.4	Tr.
7. Sclareol + unidentified	—	—	9.0	8.6
8. Unidentified	3.2	2.7	—	—

Thus, in the set of hydrocarbons of the lavender waxes there were 17, and in those of the sage waxes 13, components of which the main ones in both samples were the  $C_{31}$  and  $C_{33}$  compounds. This composition of the paraffins is typical for plant surface waxes [11].

The second hydrocarbon spot was colored violet when the chromatograms were sprayed with 50%  $H_2SO_4$  followed by heating. It was impossible to isolate these hydrocarbons in the pure form. The presence in the IR spectra of the sum of the hydrocarbons of an intense band of vibrations at  $785\text{ cm}^{-1}$  [12] and intense peaks with  $m/z$  65, 77, 91, and 105 in the mass spectrum of the hydrocarbon fraction [13] enabled them to be assigned to the alkylbenzenes.

The esters of the sage waxes studied consisted of four, and those of the lavender waxes of three types differing in both the acid and the alcohol moieties.

Triterpenol esters ( $R_f$  0.96 in system 1) were identified from the pink-orange coloration on spraying with 50%  $H_2SO_4$  followed by heating that is specific for them. In the products of their saponification, fatty acids and free triterpenols were found ( $R_f$  0.42 and 0.26, respectively, in system 1). The presence of esters of this type was confirmed by the presence in the mass spectrum of the fraction of ions of esters with  $m/z$  189, 203, and 218, which are characteristic for the fragmentation of triterpene alcohols [14].

The main component of the lavender waxes was a phthalic acid ester, which was identified by its TLC behavior and its UV, IR, and mass spectra as di(2-ethylhexyl)phthalate [15].

Di(2-ethylhexyl)phthalate and some other phthalic acid esters are well-known plasticizers used in the production of plastics [16]. When plastic parts and joints are present in an apparatus, phthalates readily pass into hydrocarbon solvents and can then be concentrated in the lipid material [17]. A similar phenomenon is observed when lipids or lipophilic mixtures are stored in vessels containing these plasticizers. Since no phthalic acid ester was detected in an extract of the epigeal part of lavender obtained under laboratory conditions [9], the possibility that this component in the wax may be secondary cannot be excluded.

Phthalic acid esters are biologically active compounds. It has been reported that phthalates participate in the lipid metabolism of mammals but their action on the human organism has not yet been elucidated [18].

The main components of the sage waxes were wax esters ( $R_f$  0.88 in system 1). They were revealed feebly with  $I_2$  vapor. The IR spectrum of the wax esters contained intense bands of the vibrations of ester carbonyl groups at  $\nu_{\max}$   $1730\text{ cm}^{-1}$  and of an aliphatic chain [9]. When they were saponified, fatty acids and fatty alcohols ( $R_f$  0.28 in system 1) were obtained. The mass spectrum of the wax esters showed the presence of peaks of fragments corresponding to residues of saturated  $[RCO_2H_2]^+$  and unsaturated  $[RCO]^+$  and  $[RCO - 1]^+$  acids and of saturated and unsaturated alcohols  $[R' - 1]^+$  [19] and  $[R' - 18]^+$  [20]. According to the results of mass-spectrometric analysis, the wax esters of sage formed a homologous series of components with numbers of carbon atoms from  $C_{20}$  to  $C_{50}$  ( $M^+$  312-732). The strongest peak in the spectrum was that of the molecular ion with  $m/z$  564, corresponding to the  $C_{38}$  ester. The peaks of molecular ions with  $m/z$  536 ( $C_{36}$ ), 508 and 506 ( $C_{34}$ ), 424 ( $C_{28}$ ), and 396 ( $C_{26}$ )

TABLE 2. Composition of the Free Fatty Acids of Lavender and Sage Waxes (% , GLC)

Acid	Lavender		Sage	
	1	2	3	4
13:0	Tr.	Tr.	Tr.	Tr.
14:0	Tr.	Tr.	Tr.	Tr.
15:0	Tr.	Tr.	Tr.	Tr.
16:0	34.8	48.2	43.3	41.8
17:0	Tr.	Tr.	Tr.	Tr.
18:0	8.9	11.6	9.3	20.5
18:1	10.9	23.0	7.2	13.6
18:2	12.5	5.2	7.5	8.0
18:3+20:0	32.9	12.1	33.0	16.1
$\Sigma$ saturated	43.7	57.8	52.3	62.3
$\Sigma$ unsaturated	56.3	40.2	47.7	31.7

had somewhat lower intensities. The peaks with the highest intensities and having  $m/z$  269 and 297 corresponded to the  $C_{18}$  and  $C_{20}$  saturated alcohols, and those with  $m/z$  257, 285, and 313 to the saturated  $C_{16}$ ,  $C_{18}$ , and  $C_{20}$  acids, respectively.

A considerable part of the waxes both of lavender and of sage consisted of compounds with  $R_f$  0.59 in system 1. When they were saponified, no high-molecular-weight aliphatic or cyclic acids and alcohols were obtained. The UV and IR spectra of these compounds were identical with those of esters of phthalic acid. However, when a chromatogram was treated with 50%  $H_2SO_4$  followed by heating they did not give the specific coloration that is characteristic for phthalates. They were assigned to esters of aromatic acids and were not studied in detail.

Free fatty acids as a class were identified from their capacity for being methylated with diazomethane to form methyl esters ( $R_f$  0.77 in system 1) and from their IR spectrum [9]. High-molecular-weight acids deposited from ethereal solutions of the free acids of all the samples at  $-5^\circ C$ , these, according to their mass spectrum, consisting of a mixture of saturated acids of the even series from 22:0 to 36:0 [9]. The compositions of the fatty acids with lower molecular masses were determined on the corresponding methyl esters by the GLC method. The presence of the set of acids listed in Table 2 was shown.

The fact that the compounds with  $R_f$  0.28 and 0.26 in system 1 belonged to fatty alcohols and triterpenols was shown by comparing their chromatographic mobilities with model samples and from the band of the vibrations of free hydroxy groups in the IR spectra at  $3200-3600\text{ cm}^{-1}$ . When a chromatogram was treated with  $I_2$  vapors, the fatty alcohols gave a white spot and when the chromatogram was sprayed with 50%  $H_2SO_4$  followed by heating, the triterpenols gave the pink-orange coloration that is characteristic for this type of compound. The GLC analysis of the total alcohol fraction was carried out under the conditions used for the separation of the fatty alcohols. As a result of the GLC analysis of the total material, the  $C_{22}$  and  $C_{24}$  alcohols were found in this fraction from lavender and the  $C_{22}-C_{28}$  alcohols, with the  $C_{22}$  alcohols predominating, in that from sage.

The free sterols ( $R_f$  0.20 in system 1) corresponded in their chromatographic mobility to the model compound and were revealed as a pink spot after the chromatogram had been sprayed with 50%  $H_2SO_4$  and had been heated.

The chlorophylls ( $R_f$  0.18 in system 1) present in samples 1 and 3 were, according to the UV spectra, chlorophylls "a" and "b" [21]. On treatment with  $H_2O_2/H^+$ , they decomposed almost completely.

Sclareol was detected in fraction 7 ( $R_f$  0.08 in system 1) from the pink-violet coloration after the treatment of the chromatogram with 50%  $H_2SO_4$  followed by heating and also by comparing its chromatographic mobility with that of a model sample in a thin layer and by GLC analysis. Under the GLC conditions used, the retention time of sclareol was 3 min.

To determine the total fatty-acid composition of the waxes, part of the samples was saponified under severe conditions and the acids, in the form of their methyl esters, were analyzed by GLC.

TABLE 3. Fatty-Acid Compositions of Lavender and Sage Waxes (% , GLC)

Acid	Lavender		Sage	
	1	2	3	4
10:0	3,2	0,2	4,2	Tr.
12:0	0,6	0,3	1,3	0,3
14:0-iso	0,5	0,3	—	—
14:0	1,3	0,9	1,0	0,6
16:0-iso	0,6	0,8	Tr.	Tr.
16:0	19,2	13,2	15,8	12,0
16:1	1,6	0,9	0,6	—
17:0	0,8	0,2	0,4	—
18:0	3,1	2,0	3,4	3,6
18:1	10,0	22,4	13,9	5,8
18:2	12,5	28,8	8,7	10,9
18:3	Tr.	Tr.	Tr.	Tr.
20:0	26,4	20,3	13,4	19,7
X <sub>1</sub>	—	—	18,9	24,7
20:1	5,1	1,8	3,2	7,8
X <sub>2</sub>	—	—	Tr.	1,9
22:0	3,4	2,0	3,0	4,1
22:1	1,1	0,8	Tr.	Tr.
23:0	4,1	2,0	6,9	3,9
24:0	2,4	1,4	1,4	0,9
24:1	—	—	Tr.	Tr.
25:0	3,8	1,7	3,9	3,8
26:0	0,3	Tr.	Tr.	Tr.

The compositions of the acids of lavender and sage waxes are given in Table 3. The main acids in both samples were the 16:0, 18:1, 18:2, and 20:0 acids. Iso acids were also detected in trace amounts in the total acids of the samples studied.

An analysis of Tables 1, 2, and 3 shows that under the influence of the treatment of the waxes with hydrogen peroxide in an acid medium their quantitative compositions changed insignificantly, except for the chlorophylls. The chlorophylls, which imparted a dark coloration to the initial waxes, were decomposed almost completely as the result of this treatment.

The treatment of the waxes with  $H_2O_2/H^+$  also decreased the degree of unsaturation of the free fatty acids, but in the total fatty acid composition the amounts of individual unsaturated acids increased.

It is known that unsaturated fatty acids (16:1, 18:1, 18:2, and 18:3) are unstable compounds readily changing and even decomposing under the influence of various factors (light, temperature), and particularly under the action of oxidants. It was to be expected that the treatment of the waxes with  $H_2O_2/H^+$  would lower the amount of unsaturated acids. The fact that the amount of these components increased after the treatment is difficult to explain.

Thus, industrial samples of lavender and sage waxes are characterized by complex chemical compositions.

#### EXPERIMENTAL

The samples of waxes were obtained from the factory of the "Efirnaslo" NPO [Scientific-Production Amalgamation] of the Central Experimental Production Enterprise (Simferopol').

IR spectra were taken on UR-10 instrument in a film, UV spectra on a Hitachi spectrophotometer in diethyl ether, and mass spectra on a MKh-1310 instrument.

Gas-liquid chromatography was performed on a Chrom-4 instrument with a flame-ionization detector in the isothermal regime. The compositions of the hydrocarbons and alcohols were determined by using a steel column, 120 × 0.4 cm, filled with 5% of SE-30 on Chromaton N-AW at 240°C for hydrocarbons and 230°C for alcohols, with a rate of flow of carrier gas (helium) of 130 ml/min. The compositions of the fatty acids in the form of their methyl esters were determined on a 250 × 0.4 cm column filled with 17% of Reoplex-400 on Chromaton N-AWDMCS, at 202°C with a rate of flow of helium of 76 ml/min. The peaks were identified graphically and by the use as a model sample for the hydrocarbons of nonacosane (C<sub>29</sub>), for the alcohols of ceryl (C<sub>26</sub>) and montanyl (C<sub>28</sub>) alcohols, and for the acids of the total methyl esters of the acids of cottonseed oil.

Thin-layer chromatography (TLC) was performed on Silufol and silica gel L 5/40 (Czechoslovakia) with the addition of 10% of CaSO<sub>4</sub> in the following solvent systems: hexane-diethyl ethyl-acetic acid (80:20:1) (1); hexane (2); and hexane-diethyl ether (99:1) (3), (9:1) (4), (8:2) (5), and (6.5:3.5) (6). The plates were developed in parallel with I<sub>2</sub> vapor and with 50% H<sub>2</sub>SO<sub>4</sub> followed by heating to 120°C.

Samples of hydrocarbons, free fatty acids, [9], sterols, and triterpenols [22] isolated previously from various plant oils and lipid extracts, and also sclareol obtained from the "Dolina roz" factory of the Moldavian SSR were used as model samples.

The quantitative compositions of the waxes were determined gravimetrically after preparative TLC on glass plates (20 × 18 cm) coated with silica gel L5/40, to which 10% of gypsum had been added, in the following systems: (2) for the isolation of the hydrocarbons, (3) and (4) for the isolations of the wax esters; (5) for the esters of aromatic acids; and (6) for the other compounds. The edges of the plates were developed with 50% H<sub>2</sub>SO<sub>4</sub> followed by heating to 120°C. The compounds were eluted from the zones of silica gel on a No. 4 nutsch filter, the layer of adsorbent being washed 4-5 times with hot solvents: hexane-benzene (1:1) for the less polar components (from carbohydrates to esters of aromatic acids), followed by diethyl ether, and then with chloroform for all the other compounds. The use of hot solvents permitted desorption losses to be avoided.

The waxes were saponified with a 10% solution of KOH in aqueous methanol (H<sub>2</sub>O<sub>2</sub>-MeOH (1:9 v/v)) with boiling under reflux for 6 h.

#### SUMMARY

1. The compositions of the main components of lavender and sage waxes have been characterized.
2. Lavender and sage waxes each consist of seven classes of compounds among which esters predominate. The main type of esters is di(2-ethylhexyl) phthalate for lavender, and wax esters for sage.
3. The total acids of sage wax include 21 components, and of lavender wax 19.
4. The industrial treatment of the waxes with hydrogen peroxide in an acid medium leads to the decomposition of the chlorophylls and to a change in the degree of unsaturation of the total fatty acids.

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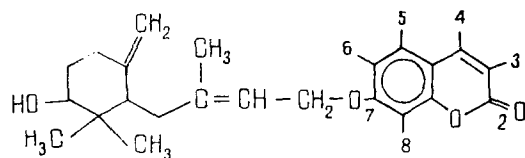
## STEREOCHEMISTRY OF LATILOBINOL

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The results are given of a study of the stereochemistry of a coumarin terpenoid derivative — latilobinol — by nuclear magnetic resonance spectroscopy using lanthanoid shift reagents. On the basis of the results obtained, it has been established that the hydroxy group in the cyclohexane ring occupies the equatorial position.

In a study of the coumarin composition of *Frangos latiloba*, we previously isolated a new terpenoid coumarin — latilobinol (I) [1]. On the basis of chemical and spectral characteristics, the following structure was proposed for it:



However, stereochemical questions remained unsolved. In the present paper we report the results of a study of the stereochemistry of (I) by the methods of nuclear magnetic resonance (NMR) spectroscopy, using lanthanoid shift reagents (LSRs).

To determine the paramagnetic shift caused by the addition of LSRs we used a simplified form of the McConnell-Robertson formula [2]

$$\Delta\delta = K \frac{1}{r^3},$$

here  $\Delta\delta$  is the induced chemical shift in the proton NMR spectra caused by the addition of an LSR; and  $r$  is the radius vector from the center of complex-formation to the observed proton,  $K$  being a constant for a given LSR and given temperature.

The task consisted in selecting the geometric parameter  $r$  that would give the best agreement of the induced chemical shift calculated from it and that found by experiment [3]. The comparison of theoretical and experimental values was made by the graphical method [4]. The method consists in the plotting of lines in the coordinates  $\log \Delta\delta = f(\log r)$ . Such a stereochemistry of the molecule was selected as the most probable in which the NMR parameters  $\Delta\delta$  and the radius vector  $r$  permit the plotting of a straight line in these coordinates with an angle of slope  $\tan \alpha = -3$ .

Figure 1 shows graphically the changes in the chemical shift of the protons as a function of the molar ratios of LSR and substrates  $\delta = f(\rho)$ . From the graphical results the change in the chemical shifts of the protons due to the addition of the LSR was determined.