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COMPOSITION OF THE FLORAL WAXES OF *Rosa gallica, Jasminum grandiflorumm,* AND *Viola odorata*

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The chemical compositions of the waxes of the flowers of Rosa gallica L., Jasminum grandiflorum L., *and* Viola odorata L. *have been characterized. The waxes of these plants mainly contained hydrocarbons and fatty and cyclic alcohols and their esters with fatty acids. The highest level of free fatty acids (12.4%) was found in the* J. grandiflorum *wax. Depending on the plant species, the above-mentioned groups of substances differed in composition and amount, but hydrocarbons predominated.*

The production of essential oils gives rise to wastes — waxes containing a number of biologically active lipidic and lipophilic compounds. The organoleptic, pharmacological, and structure-forming properties of plant waxes are due to the diversity of these components, and their composition depends on the nature of the raw material being processed [1]. The waxes of flowers — for example, chamomiles [2], essential-oil flowers [3], and decorative roses [4] — differ substantially in chemical composition from the waxes of the green biomass of these plants [5]. Particularly diverse are the components of the hydrocarbons and the higher alcohols. There are reports that the concretes and waxes of essential-oil plants exhibit a pronounced selective antimicrobial activity and can be used for the prophylaxis and treatment of stomatological diseases [6].

We have investigated the chemical compositions of the flower waxes remaining as wastes in the production of absolute essential oils from concretes of the flowers of *Rosa gallica* L. (French rose, fam. Rosaceae), *Jasminum grandiflorum* L. (common jasmine, fam. Oleaceae), and *Viola odorata* L. (sweet violet, fam. Violaceae).

In color, the samples of waxes were light brown (*J. grandiflorum*), and brown (*R. gallica*), with a green tinge (*V. odorata*) and had a specific floral odor. According to preliminary TLC results (system 1), the waxes studied included esters of fatty acids (EFAs) with aliphatic alcohols (wax esters) and with cyclic alcohols, methyl esters of fatty acids (MEFAs), free fatty acids (FFAs), and a number of lipophilic components such as hydrocarbons (HCs), fatty alcohols (FAlcs), phytosterols, triterpene compounds, and others. The waxes were fractionated by CC and some multicomponent fractions were separated by preparative TLC in system 2. The assignment of chromatographically individual zones to definite groups of lipids was made on the basis of a comparison of the chromatographic mobilities of the substances under investigation and of model compounds, and also from qualitative reactions and spectral characteristics [7]. The yield of each fraction of waxes was evaluated gravimetrically and the compositions of the fractions were determined (Table 1).

In general, the qualitative compositions of the specimens studied were characteristic for plant waxes [8], but differences were observed in their make-up and in the quantitative levels of individual components. The main components were HCs (29.6—56.1%) and EFAs (15.2—25.5%). A considerable part of the *R. gallica* wax was constituted by alcohols, of the *J. grandiflorum* wax by FFAs, and of the *V. odorata* wax by phytosterols and more polar unidentified substances. Only in the *R. gallica* wax were secondary alcohols and carbonyl and triterpene compounds present, and only in the *J. grandiflorum* wax were there MEFAs. When the *R. gallica* wax was subjected to CC, two fractions were obtained that corresponded, according to TLC in system 3, to primary and secondary alcohols [4]. According to their mass-spectrometric characteristics, the secondary alcohols consisted of a mixture of $C_{25\text{-}33}H_{51\text{-}76}OH$ homologs with the $[M - 18]^+$ 434 ions and fragments with *m/z* 339 and 143

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TABLE 1. Compositions of the *R. gallica*, *J. grandiflorum*, and *V. odorata* Waxes (% by weight)

Component	*R.gallica L.*	*J. grandiflorum*	*V.odorata L.*
Hydrocarbons	42.4	56.1	29.6
Esters of fatty acids with alkanols and cyclic alcohols	21.6	15.2	25.5
Methyl esters of fatty acids	-	Tr.	-
Secondary alkanols, carbonyl compounds	6.3	-	-
Primary alkanols	4.0	4.7	2.3
Free acids	3.3	12.4	4.4
Acetate of a hydroxytriterpene acid	0.1	3.3	-
Triterpenols	2.5	-	-
Sterols	5.5	0.9	9.0
Unidentified components	14.3	7.4	29.2

from hentriacontan-9-ol predominating [3]. According to the results of the Ag^+-TLC of the acetate derivatives in system 3 and the behavior of the initial substances in GLC, the free triterpenols of the *R. gallica* wax corresponded to α- and β-amyrins.

Primary and secondary alcohols and α- and β-amyrins, and also phytosterols, have been detected in *Rosa* species studied previously [3, 4].

The carbonyl compounds of the *R. gallica* wax gave a positive qualitative reaction with dinitrophenylhydrazine and consisted of aldehydes and ketones (TLC in system 3, R_f 0.43 and 0.32, respectively). From the results of mass spectrometry, in this fraction hentriacontan-9-one was identified as the main component of the rose wax ketones [9].

The acetate of a hydroxytriterpene acid was present in the waxes of *T. gallica* and *J. grandiflorum*. Because of its small amount the acetoxytriterpene acid of the *R. gallica* wax was not studied.

The structure of this component in the *J. grandiflorum* wax was determined by comparison with a standard substance and from the products of chemical transformations involving mild alkaline hydrolysis followed by methylation and acetylation. On the basis of the mass spectrum of the methylation product (M^+ 512, *m/z* 262, 249, 203 (main), 189), the compound was identified as oleanolic acid acetate [10].

According to TLC and GLC, the free sterols of each of the three waxes consisted of a mixture of known phytosterols, including cholesterol, campesterol, stigmasterol, and β-sitosterol, with the latter predominating.

Hydrocarbons were analyzed by GLC, IR spectroscopy, and mass spectrometry. Their compositions are given in Table 2. The HCs of the *R. gallica* wax consisted basically of a mixture of 19 alkanes, $C_{17}H_{36}$—$C_{35}H_{72}$ with the odd homologs $C_{19}H_{40}$, $C_{21}H_{44}$, and $C_{27}H_{56}$ and the even homolog $C_{20}H_{42}$ predominating. The HCs also included alkenes, consisting of the four homologs $C_{17}H_{34}$—$C_{31}H_{62}$ and the alkadienes $C_{19}H_{36}$, $C_{21}H_{40}$, and $C_{23}H_{44}$, which were identified by the presence in their mass spectra of the M^+ ions with *m/z* 264, 292, and 320 having a low intensity. The IR spectrum showed the *cis*- configuration of the double bonds in the unsaturated HCs [11]. Earlier, alkanes and alk-*cis*-5-enes were found in the wax of *R. damascena* Mill., while in *R. damascena* Mill., var. *trigintipetala*, there were conjugated alkadienes specific for this species [3]. In all three groups of HCs homologs with C_{19}, C_{21}, C_{27}, and C_{29} chain lengths predominated [3, 4]. Apparently, the presence of more than 50% of medium-molecular-mass alkanes (C_{17}—C_{23}) and the presence of unsaturated hydrocarbons imparts the plasticity that is characteristic for the *R. gallica* wax.

A large part of the HCs of the *J. grandiflorum* wax consisted of the same $C_{26}H_{54}$—$C_{34}H_{66}$ alkanes and isoalkanes (nine components). Among the latter the amount of even homologs was greater than that of odd ones. In the alkanes the C_{29} and C_{31} members predominated, while the proportion of medium-molecular-weight components exceeded 18%. In the isoalkanes the C_{28} homolog predominated. In addition, the GLC chromatograms contained the peaks of three unidentified components with equivalent chain lengths (ECLs) of 30.10, 30.31, and 32.12, which may correspond to other branched C_{31} and C_{33} alkanes [2].

The HCs of the *V. odorata* wax had a more complex composition. They contained almost equal amounts of linear and branched alkanes. A large part of the linear alkanes consisted of the C_{25}—C_{34} high-molecular-mass homologs, with approximately equal amounts of the main ones — C_{27}, C_{29}, C_{31}, C_{32}, and C_{33}. The branched alkanes were a mixture of almost equal amounts of iso- and anteiso- compounds with a predominance of the iso- $C_{34}H_{70}$ and the anteiso-$C_{30}H_{62}$ varieties. A component with an ECL of 32.26 (3.7%, GLC) was not identified. The fatty acid compositions of the waxes and their FFAs and EFAs were determined after severe alkaline hydrolysis. The FAs obtained from the hydrolysis products and also the free fatty acids after CC were analyzed in the form of their methyl esters by GLC and mass spectroscopy.

TABLE 2. Compositions of the Hydrocarbons of the *R.gallica*, *J.grandiflorum*, and *V.odorata* (%, GLC)

Hydrocarbon	*R.gallica*		*J.grandiflorum**		*V. odorata***		
	alkanes	alkenes	alkanes	isoalkanes	alkanes	isoalkanes	anteisoalkanes
$C_{17}H_{36}$	0.5	-	Tr.	-	Tr.	-	-
$C_{18}H_{38}$	0.2	-	Tr.	-	-	-	-
$C_{19}H_{40}$	20.4	Tr.	0.3	-	0.2	-	-
$C_{20}H_{42}$	2.9	-	Tr.	-	-	-	-
$C_{21}H_{44}$	21.5	Tr.	8.5	-	0.2	-	-
$C_{22}H_{46}$	0.5	-	0.5	-	0.8	-	-
$C_{23}H_{48}$	11.5	1.0	9.3	-	1.0	-	-
$C_{24}H_{50}$	1.0	-	0.6	-	0.4	-	-
$C_{25}H_{52}$	9.0	0.3	5.2	Tr.	3.1	1.9	Tr.
$C_{26}H_{54}$	1.1	-	0.9	0.6	0.9	1.4	Tr.
$C_{27}H_{56}$	19.1	0.7	9.5	0.3	6.2	0.7	1.6
$C_{28}H_{58}$	0.7	-	2.8	4.1	1.4	0.8	3.8
$C_{29}H_{60}$	4.3	0.6	17.8	0.7	7.1	0.6	2.6
$C_{30}H_{62}$	0.4	-	8.4	0.5	1.9	1.6	4.9
$C_{31}H_{64}$	4.0	0.3	11.5	1.3	7.2	0.5	3.4
$C_{32}H_{66}$	Tr.	-	5.0	Tr.	6.2	2.0	2.8
$C_{33}H_{68}$	0.5	-	2.8	0.5	8.0	3.9	2.5
$C_{34}H_{70}$	Tr.	-	2.3	-	4.0	9.7	1.0
$C_{35}H_{72}$	Tr.	-	0.6	-	0.8	0.8	0.4
Σ	97.1	2.9	86.0	8.0	49.4	23.9	23.0

*In addition, this contained unidentified components: $C_{31}H_{64}$ — 3.9%; C_{32}—H_{66} — 1,5%; and $C_{33}H_{68}$ — 0.6%.
** Unidentified $C_{33}H_{68}$ — 3.7%.

TABLE 3. Fatty Acid Compositions of the *R. gallica*, *J. grandiflora*, and *V. odorata* Waxes (%, GLC)

Acid	*R.gallica*			*J.grandiflorum*			*V. odorata*		
	Total	EFAs	FFAs	Total	EFAs*	FFAs	Total	EFAs*	FFAs
12:0	1.0	0.4	1.3	0.1	Tr.	Tr.	3.0	6.8	Tr.
13:0	Tr.	0.7	Tr.	Tr.	Tr.	Tr.	0.1	Tr.	Tr.
14:0	1.0	0.7	2.8	0.4	0.3	0.6	1.0	2.5	0.4
15:0	Tr.	Tr.	Tr.	0.4	Tr.	1.7	Tr.	4.6	0.5
16:0	16.3	11.0	11.5	40.6	39.7	36.8	52.9	50.7	43.8
16:1	Tr.	-	Tr.	Tr.	1.4	1.7	Tr.	Tr.	Tr.
17:0	Tr.	0.5	0.2	Tr.	Tr.	Tr.	Tr.	1.7	1.2
18:0	28.2	29.8	25.8	11.0	10.2	13.6	7.3	6.7	9.4
18:1	1.0	1.4	3.5	4.7	5.9	3.0	17.8	12.8	24.6
18:2	5.0	3.3	1.8	7.5	2.3	13.5	7.5	6.2	7.8
19:0	Tr.	Tr.	Tr.	Tr.	2.1	-	Tr.	Tr.	Tr.
20:0	29.1	31.6	31.4	18.7	13.2	22.4	10.4	8.0	12.3
21:0	Tr.	0.5	0.2	Tr.	Tr.	-	-	-	-
22:0	18.4	20.1	17.5	9.8	17.9	6.7	Tr.	Tr.	Tr.
24:0	Tr.	Tr.	4.0	6.8	7.0	Tr.	Tr.	Tr.	-
$\Sigma_{sat.}$	94.0	95.3	4.7	87.8	90.4	81.8	74.7	81.0	67.6
$\Sigma_{unsat.}$	6.0	4.7	5.3	12.2	9.6	18.2	25.3	19.0	32.4

*The 26:0—32:0 acids were also present (mass spectrum).

The FAs of all three waxes were saturated, in the main. Of them, the 16:0 acid predominated in the *J. grandiflorum* and *V. odorata* waxes, and the 18:0, 20:0, and 22:0 acids in the *R. gallica* wax. The *V. odorata* wax proved to be the most unsaturated (25%), mainly through the 18:1 acid, while in the *R. gallica* and *J. grandiflorum* waxes the proportion of the 18:2 acid was greater than that of the 18:1 acid. The compositions of the acids of the EFAs and FFA of the waxes studied were almost identical. According to their mass spectra, the EFAs of the *J. grandiflorum* and *V. odorata* waxes contained, in addition to the acids mentioned above, trace amounts of long-chain acids of the 26:0—30:0 series.

Esters of fatty acids with aliphatic and cyclic alcohols in the three waxes were analyzed by the mass spectrometry of the initial samples on the basis of specific fragmentation [12] and in the light of the composition of the alcohol and acid parts obtained after severe alkaline hydrolysis. To analyze the alcohols we used the TLC of the initial components in system 1 and the Ag^+-TLC of the acetate derivatives in system 3. In the alcohol part of the EFAs of all three waxes we detected (TLC in system 2) alkanols, triterpenols, and sterols.

The alcohols of the EFAs of the *R. gallica* wax were separated by preparative TLC in system 2 into aliphatic (69% of the weight of the alcohols) and cyclic (31%). The compositions of the aliphatic alcohols in the ester form and of the free primary alcohols were studied by GLC and mass spectrometry (Table 4). In the *R. gallica* wax the bound alcohols were represented by a mixture of even and odd saturated and monoenic homologs, $C_{18-26}H_{37-53}OH$ with a predominance of the saturated C_{20} and C_{22} components.

The free primary alcohols differed from the bound ones by a greater diversity of the components and contained a considerably larger amount of the even C_{20}—C_{26} homologs.

The mass spectrum of the cyclic alcohols from the *R. gallica* wax contained peaks of molecular and fragmentary ions confirming the presence of α- and/or β-amyrins (M^+ 426, 12%; *m/z* 411, 393, 218, 203, 189), β-sitosterol (M^+ 414, 100%; *m/z* 396), stigmasterol (M^+ 412, 17%; *m/z* 394), campesterol (M^+ 400, 23%; *m/z* 382), and cholesterol (M^+ 386, 15%; *m/z* 368, 255, 231, 213). The main EFAs of the *R. gallica* wax were the homologous wax esters $C_{38-44}H_{74-88}O_2$ and the esters of the amyrins and of β-sitosterol with the 18:0 and 20:0 acids.

The alcohols of the EFAs of the *J. grandiflorum* wax included mainly the alkanols $C_{20-32}H_{41-65}OH$ ($[M - 18]^+$ with *m/z* 250—448). They were accompanied by β-sitosterol (M^+ 414, 100%), stigmasterol (412, 18%), campesterol (400, 36%), and cholesterol (386, 38%), with a small amount of amyrin (M^+ 426, *m/z* 218, 203, 189). From the combined results it was established that the EFAs of the *J. grandiflorum* wax were represented mainly by wax esters with a set of saturated $C_{38-48}H_{76-96}O_2$ (M^+ 564—704), monoenic $C_{38-48}H_{74-94}O_2$ (M^+ 562—702) and dienic $C_{38-44}H_{72-84}O_2$ (M^+ 560—644) homologs. In the mass spectrum the peaks of the M^+ ions and of fragments characteristic for the breakdown of esters of cyclic alcohols (low intensity) showed the structure of esters of amyrin with the 16:0 acid (M^+ 664, *m/z* 445, 218) and of β-sitosterol with the 18:1 and 18:2 acids (*m/z* 537 and 535, 396, 397).

According to their mass spectrum, the free alcohols of the *J. grandiflorum* wax, unlike the bound alcohols, were identified as a mixture of homologous alkanols with chain lengths of C_{22}—C_{32} ($[M - 18]^+$ with *m/z* 308—448) and isoprenoid alcohols — phytol ($[M - 18]^+$ 278; *m/z* 250, 226, 222, 208, 194, 181, 180, 165, 152, 138, 124, and 123) and dihydrophytol (M^+ 298; *m/z* 280, 252, 228, 224, 210, 196, 183, 182, 167, 154, 140, 126, and 125). In addition, the mass spectrum of the EFAs of the wax contained the peaks of M^+ ions and fragments confirming the presence in the mixture of a small amount of methyl esters of the 16:0, 18:0, 18:1, and 18:2 FAs (M^+ 270, 298, 296, 294; *m/z* 239, 267, 265, 264, 263, 74).

According to the mass spectra of the initial samples and of the hydrolysis products, and also Ag^+-TLC in system 3 of the acetate derivatives in comparison with model specimens of such compounds, the alcohols of the EFAs of the *V. odorata wax* consisted mainly of α- and β-amyrins (M^+ 426, R_f 0.57) accompanying β-sitosterol (M^+ 414, R_f 0.39) and the fatty alcohols $C_{20-32}H_{41-65}OH$ ($[M - 18]^+$ with *m/z* 280—448, R_f 0.90). In the light of the components of the alcohol and acid parts, we identified esters of the amyrins with the 16:0, 17:0, 18:0, 18:1, and 18:2 acids (M^+ 664 (main), 678, 692, 690, 688; $[RH + 207]^+$ with *m/z* 445 (main), 459, 473, 471, 469) and β-sitosterol with the 16:0 acid (M^+ 652 and *m/z* 511) [9]. The FAs of the esters of aliphatic alcohols consisted of the 12:0—24:0, 16:1, 18:1, and 18:2 types. The EFAs included the saturated $C_{36-52}H_{72-104}O_2$ (M^+ 536—760, main peaks with M^+ 620 and 648) and monoenic $C_{40-48}H_{78-94}O_2$ (M^+ 590—702) homologs with predominance of the $C_{42-44}H_{84-88}O_2$ components.

Thus, the chemical composition of the waxes concentrated in the wastes from the processing of flowers of *R. gallica* L., *J. grandiflorum*, and *V. odorata* L. witnesses a high level in them of hydrocarbons and total physiologically active ingredients of lipid nature, such as fatty acids, sterols, triterpenols, and unsaturated FAs and their esters [13]. Not only is it desirable to use these waxes in decorative cosmetics and children's, protective, and special creams [14] simply as structure-forming

TABLE 4. Compositions of the Bound and the Free Alcohols of the *R. gallica* Wax (according to GLC and mass spectrometry)

Component	Bound alcohols		Free alcohols	
	$[M-18]^+$(%)	%, GLC	$[M-18]^+$(%)	%,GLC
$C_{16}H_{33}OH$	-	-	224(41)	2.3
$C_{17}H_{35}OH$	-	-	238(27)	0.4
$C_{18}H_{37}OH$	252(37)	18.4	252(47)	6.1
$C_{18}H_{35}OH$	250(13)		250(26)	
$C_{19}H_{39}OH$	266(14)	Tr.	266(28)	1.7
$C_{19}H_{37}OH$	264(12)		264(28)	
$C_{20}H_{41}OH$	280(100)	40.4	280(79)	17.5
$C_{20}H_{39}OH$	278(8)		278(24)	
$C_{21}H_{43}OH$	294(8)	Tr.	294(15)	2.6
$C_{21}H_{41}OH$	292(4)		292(16)	
$C_{22}H_{45}OH$	308(75)	28.9	308(100)	21.6
$C_{22}H_{43}OH$	306(7)		306(21)	
$C_{23}H_{47}OH$	322(4)	Tr.	322(14)	2.6
$C_{24}H_{49}OH$	336(27)	8.7	336(69)	20.1
$C_{24}H_{47}OH$	334(5)		334(18)	
$C_{25}H_{51}OH$	-	-	350(11)	4.3
$C_{26}H_{53}OH$	364(8)	-	364(63)	17.5
$C_{26}H_{51}OH$	362(3)		362(13)	
$C_{28}H_{57}OH$	392(4)	3.6	392(47)	3.3
$C_{28}H_{55}OH$	-	Tr.	390(16)	
$C_{30}H_{62}OH$	420(2)	Tr.	420(10)	Tr.
$C_{30}H_{60}OH$	418(2)		418(9)	
$C_{32}H_{64}OH$	448(2)	Tr.	448(3)	Tr.
$C_{32}H_{62}OH$	446(2)		446(2)	

components but they may also lead to an enrichment of the formulations with natural physiologically active ingredients of lipid nature. In contrast to the waxes with a dark coloration from the chlorophyll-containing parts of plants, the floral waxes studied are comparatively weakly colored, do not require bleaching, and, consequently, will not appreciably affect the color of cosmetic products.

EXPERIMENTAL

IR spectra were recorded on a UR-20 spectrophotometer in a film. Mass spectra were taken on on a MKh-1321 mass spectrometer at an ionizing voltage of 50—70 eV with a temperature of the ionization chamber of 150—180°C.

GLC analysis of the hydrocarbons was conducted on a Varian Star 3400 CX chromatograph with a capillary column (320 mμ × 4 m) containing the phase DV-1 at a layer thickness of 1.0 μm. The initial temperature was 80°C for 1 min, followed by programming to 250°C at a rate of 10°C/min. The fatty acid methyl esters and the fatty alcohols were analyzed on a Chrom-4 chromatograph in the isothermal regime using packed stainless steel columns, a flame-ionization detector, and helium as the carrier gas. For the fatty acid methyl esters we used 17% of Reoplex 400 at 200°C as the stationary phase, and for the alcohols 5% of SE-30 at 230°C (alkanols) and 260°C (sterols and triterpenols). In both cases the support for the phases

was Chromaton N-AW-DMCS.

TLC was conducted on silica gel 5/40 (Czech Republic) with the addition of 10% of $CaSO_4$ and Silufol with a fixed layer of SiO_2, and Ag^+-TLC containing 20% of $AgNO_3$ in the solvent systems: 1) hexane—diethyl ether—acetic acid (7:3:0.1); 2) hexane—diethyl ether (7:3); and 3) hexane—petroleum ether (2:3).

The wax samples, produced in the Dzintars NPO [Scientific Production Combine], were obtained from the Pyatigorsk Pharmaceutical Institue (Russia).

Hydrocarbons of the *R. gallica* Wax (R_f 1.0, system 1, revealed with I_2). IR spectrum (film, cm^{-1}): 2965, 2930, 2860, 1475, 1385, 905, 735, 670.

Hentriacontan-9-one from the *R. gallica* Wax (R_f 0.32, system 3, orange coloration with 2,4-dinitrophenylhydrazine) [9]. Mass spectrum, *m/z* (*I*, %): 450 (M^+, 1.5) 337 (100), 352 (2.6), 141 (16), 156 (3.6).

Secondary Alcohols of the *R. gallica* Wax. $C_{25-33}H_{51-67}OH$ (R_f 0.20,system 3). Mass spectrum, *m/z* (*I*, %): $[M-18]^+$ 350 (0.9), 364 (0.9), 378 (0.8), 392 (0.6), 406 (5), 420 (0.5), 434 (40), 448 (0.4), 462 (0.4); 143 (72) and 339 (100).

Free Alcohols of the *J. grandiflorum* Wax. Mass spectrum *m/z* (*I*, %): $[M-18]^+$ 278, 250, 226, 222, 208, 194, 181, 180, 165, 152, 138, 124, 123 (phytol); M^+ 298, 280, 252, 228, 224, 210, 196, 183, 182, 167, 154, 140, 126 (main), 125 (dihydrophytol); $[M-18]^+$ 308-448 (alkanols $C_{22-32}H_{45-65}OH$).

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