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WAX COMPOSITION OF THE KAZANLIK AND DAMASK ROSES

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Key Word Index—*Rosa damascena*; Rosaceae; rose flowers; wax composition; taxonomic variation.

Abstract—The Kazanlik and Damask roses differ qualitatively and quantitatively in the composition of the flower waxes. These chemotaxonomic data suggest that these two oil-bearing roses are different from each other.

INTRODUCTION

The Bulgarian oil-bearing rose has been the object of many chemical investigations [1]; a large number of the oil components have been determined [2–4]. Its taxonomy is confusing [5–10], it is known to be closely related to the Damask rose and some authorities believe it to be identical. The Kazanlik rose is usually referred to in the literature as *R. damascena* Mill. var. *trigintipetala* [1].

In 1973 Topalov [8] found for the first time in Bulgaria (in the town of Sozopol) rose bushes which from morphological and anatomical features coincide with those described in the literature for Damask rose, yet they differ from the features of the Kazanlik rose, grown in Bulgaria for industrial purposes. We, therefore, set ourselves the task of settling the taxonomic dispute about the relationship of these two rose cultivars using chemotaxonomy. To achieve this purpose after our detailed investigation of the Kazanlik flower wax [2], it was necessary to study the flower wax from the Damask rose found in Sozopol. This paper reports the results on the composition of the wax from Damask rose, as well as a comparison of the wax components of the two cultivars.

RESULTS AND DISCUSSION

The results on the contents of the concrete, wax and absolute in Damask rose and Kazanlik rose are shown in Table 1. A comparison indicates that both possess almost the same concrete content, but the Kazanlik rose is distinguished by the lower per cent of wax and higher proportion of absolute. The results from group separation of waxes from the two roses obtained by prep. TLC are presented in Table 2. Both roses contain the same classes of compounds, yet with significant quantitative differences in hydrocarbons, carbonyl compounds, secondary alcohols and more polar compounds.

Table 1. Content of concrete, wax and absolute in flowers of Damask rose and Kazanlik rose

Plant	Yield of concrete (% of fresh flower)	% of concrete	
		Wax	Absolute
Damask rose	0.36	53.13	46.87
Kazanlik rose*	0.35	38.44	53.90

* Data from ref. [21].

Table 2. Composition of flower wax from Damask rose and Kazanlik rose

Component	Damask rose		Kazanlik rose	
	%*	R_f†	%*	R_f†
Hydrocarbons	57.1	0.97	48.6	0.96
Esters I	7.1	0.60	9.3	0.58
Carbonyl compounds	2.0	0.39	7.4	0.44
Esters II	3.1	0.31	2.4	0.30
Secondary alcohols	3.4	0.14	8.1	0.16
Primary alcohols and other polar compounds	12.5	0.04	19.0	0.05

*Determined from prep. TLC data.
†TLC on Si gel in C_6H_6–petrol (2:3).

We turned our attenion next to the nature of the carbonyl compounds and of the hydrocarbons, since ketones [11] and conjugated alkadienes [12] were found to be specific for Kazanlik rose in comparison with three decorative roses [13, 14]. The IR spectral data for the carbonyl fraction from Damask rose indicates the presence only of aldehydes. Assuming that in this case the ketones could not be detected because of the small amount of the wax examined, we carried out a separation of the wax by CC but with the same result. We more critically examined our earlier results on the carbonyl compounds in Kazanlik rose [11], which were isolated by CC on aluminium oxide, conditions under which aldehydes in waxes could undergo destruction [15]. The results obtained with Kazanlik rose wax, using prep. TLC as well as CC on Si gel, however, demonstrated the presence of both aldehydes and ketones.

An insignificant amount of hydrocarbons more polar than alkenes was isolated from the total hydrocarbon fraction of Damask rose, but according to IR and UV spectral data they contained no conjugated double bonds [12].

The comparative data concerning the classes of compounds, contained in the two roses and the percentage of members in the homologous series are given in Table 3.

Table 3. Composition (%) of components in flower wax from Damask rose (Rd) and Kazanlik rose (Rk)

Carbon No.	Alkanes Rd	Alkanes Rk [19]	Alkenes Rd	Alkenes Rk [19]	Alkadienes Rd	Alkadienes Rk* [12]	Ketones Rd	Ketones Rk [16]	Aldehydes Rd*	Aldehydes Rk	Secondary alcohols Rd	Secondary alcohols Rk [18]	Primary alcohols Rd	Primary alcohols Rk	Free acids Rd*	Free acids Rk* [25]
12	—	—	—	—	—	3.2	—	—	—	—	—	—	—	—	0.3	2.7
13	—	—	—	—	—	4.5	—	—	—	—	—	—	—	—	—	0.6
14	—	—	—	—	—	4.5	—	—	—	—	—	—	—	—	1.1	0.9
15	—	—	—	—	—	3.1	—	—	—	—	—	0.1	—	—	0.8	—
16	—	—	—	—	—	4.6	—	—	—	—	—	0.4	0.4	—	28.1	23.5
17	—	0.6	—	—	—	3.1	—	0.4	—	—	—	1.0	0.3	—	0.8	0.6
18	—	—	—	—	—	1.1	—	0.2	—	—	—	0.8	0.4	—	10.4	9.8
18:1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.6	1.7
18:2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	21.1	12.2
18:3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	14.1	12.4
19	10.4	18.4	1.3	3.0	—	14.2	—	3.4	—	—	—	1.8	—	0.5	—	0.7
20	1.4	2.2	—	0.3	—	2.9	—	1.0	0.3	—	—	1.2	3.4	3.9	11.5	2.4
21	14.4	17.3	2.4	3.2	—	6.9	—	2.9	1.0	2.7	—	2.2	0.9	2.1	0.2	0.6
22	0.6	0.8	—	0.4	—	0.7	—	1.0	1.1	10.0	—	3.9	12.8	20.9	5.9	2.4
23	11.3	12.6	6.8	6.0	—	4.7	—	2.3	3.6	26.1	—	4.0	1.3	1.4	0.7	0.3
24	0.8	0.6	0.9	1.3	—	1.0	—	1.2	4.5	22.2	—	4.9	18.7	13.1	2.1	1.1
25	8.5	6.8	8.8	9.6	—	6.0	—	2.4	1.5	9.2	—	4.6	1.2	1.1	—	—
26	1.4	0.7	1.8	1.9	—	1.3	—	2.2	4.1	5.7	—	4.9	15.9	12.5	—	0.3
27	29.7	18.9	36.7	24.5	—	8.2	—	6.8	3.1	—	0.5	5.2	1.9	1.5	—	—
28	2.7	1.1	2.7	3.7	—	2.3	—	2.8	28.8	10.2	—	4.7	24.4	17.7	—	—
29	10.2	11.3	24.5	34.8	—	9.3	—	8.3	3.4	—	2.4	5.2	1.5	1.4	—	—
30	—	—	0.9	0.8	—	1.1	—	2.8	30.7	10.0	0.9	3.4	5.5	9.5	—	—
31	7.1	8.8	8.7	8.5	—	2.8	—	55.5	0.7	—	94.3	50.0	—	—	—	—
32	—	—	—	—	—	—	—	2.6	12.1	4.0	0.5	0.3	—	—	—	—
33	1.5	—	4.5	2.1	—	0.8	—	3.2	—	—	1.4	1.8		—	—	—
34	—	—	—	—	—	—	—	1.2	—	—	—	—	—	—	—	—
35	—	—	—	—	—	—	—	0.8	—	—	—	—	—	—	—	—
β-Amyrin	—	—	—	—	—	—	—	—	—	—	—	—	2.3	3.4	—	—
α-Amyrin	—	—	—	—	—	—	—	—	—	—	—	—	9.2	11.1	—	—

*Also contains unidentified constituents not given in this table.

They exhibit certain similarities as well as differences. Thus, conjugated alkadienes [12] and ketones [11, 16] as homologous series are present in Kazanlik rose, whereas the other classes of compounds occur in both roses, but with significant quantitative differences between them. It is worth noting that the main secondary alcohol is C_{31} in both roses. Moreover, data from the mass spectral analysis of the secondary alcohols as the corresponding ketones (M^+ 450, m/z 141, 156, 337, 352) indicate that in Damask rose it is also 9-hentricontanol as is the case in Kazanlik rose [17, 18], but the amount is twice that in Damask rose.

The considerable differences observed in the qualitative and quantitative wax composition between Damask rose and Kazanlik rose are significant, since flowers of the same maturity were used; also previous investigations [19, 20] have shown that the location and season have little effect on the wax composition of the petals. These results prompt us to conclude that Kazanlik rose is not identical to the Damask rose. This inference based on our chemotaxonomic study agrees well with the conclusion of Topalov [8], based on morphological and anatomical comparisons of the two roses.

EXPERIMENTAL

Plant material and isolation of concrete. During spring, Damask rose flowers were collected from domestic gardens near the town of Sozopol. The fresh plant material (786 g) was extracted with petrol and the solvent evaporated under red. pres. to yield the concrete (2.8634 g).

Separation of concrete into wax and absolute. This was carried out as previously described [21]. From the concrete 1.520 g of wax and 1.330 g of absolute were obtained.

Isolation of free acids. Carried out by ion exchange resin Woffatit SBW [20]. After methylation with CH_2N_2 the acids were purified by prep. TLC in C_6H_6–petrol (2:3) (system A).

Isolation of components from the neutral part of wax. (a) *Prep. TLC.* Neutral wax (100 mg) was chromatographed on Si gel in system A. Six fractions were obtained (Table 2). The carbonyl fraction was rechromatographed in hexane–CCl_4 (1:1) (system B) using a triple development. Both the secondary and primary alcohol fractions were purified by prep. TLC in hexane–Et_2O–MeOH (4:1:0.1). The purity of all fractions was monitored by TLC in different solvent systems and by IR spectra. (b) *CC.* Neutral wax (1.0 g) was chromatographed on Si gel. The eluents were hexane and hexane containing increasing amounts of Et_2O. The course of the chromatography was followed by TLC.

Separation of hexane–Et_2O fraction. This was carried out by prep. TLC in C_6H_6–hexane (3:7). Only two bands were observed with R_f-values corresponding to those of long chain ester and long chain aldehyde. With a view to establishing the presence of ketones the area between the two bands was scraped off from the plates and eluted with Et_2O. The product obtained was rechromatographed in system B using a triple development using 15-hentriacontanone as standard.

Isolation of alkanes and alkenes. The total hydrocarbon fraction (0.364 g) was subjected to CC on Si gel–$AgNO_3$[12]. Besides alkanes (0.296 g) and alkenes (0.025 g) a small amount of more polar hydrocarbons was also isolated.

Hydrogenation of alkenes. Effected with PtO_2 [12]. The alkanes and hydrogenated alkenes were treated with urea [19].

Reduction of aldehydes. Carried out using the method of ref. [13]. The alcohols obtained were converted to their acetates.

Oxidation of secondary alcohols. With CrO_3 [22].

GC. The alkanes and hydrogenated alkenes, and free acids (as Me esters) were analysed as described in refs. [19, 23]. The secondary alcohols (as ketones) and primary alcohols (as acetates) were analysed according to ref. [22]. The aldehydes were analysed directly [24] and as the acetates of their corresponding primary alcohol derivatives.

MS. Of the secondary alcohols (as the corresponding ketones) was measured at 70 eV.

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