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COMPOSITION OF NEUTRAL COMPONENTS IN FLOWER WAX OF SOME DECORATIVE ROSES

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Key Word Index—*Rosa americana*; *R. imperial*; *R. virgo*; Rosaceae; rose flower wax; secondary alcohols; primary alcohols; wax esters; α- and β-amyrins; sterols; structure determination.

Abstract—A homologous series of long-chain secondary alcohols in which each member is a mixture of three isomers with a hydroxyl group at 4, 5 and 6 positions has been identified for the first time in a plant wax. The isomer, having an OH-group at C_5 is the prevalent one. Data are also presented on the presence of unsaturated primary alcohols and ester-bound secondary alcohols in the waxes isolated from decorative roses.

INTRODUCTION

In earlier work, a detailed analysis of wax of Bulgarian oil-bearing rose (*Rosa damascena*) has been carried out [1–5]. In order to establish which are the typical components of the oil-bearing rose, we initiated a systematic study of the wax composition from three varieties of decorative roses (*R. americana*, *R. imperial*, *R. virgo*). The composition of hydrocarbons [6], acids [7] and aldehydes [8] in these roses has been studied, and trialkltrioxanes [9], as a polymeric form of the aldehydes, have been found for the first time in a natural product.

Some results from the investigations, carried out on other neutral components in the wax of the decorative roses mentioned above are reported in this paper.

RESULTS

Fractions, which according to TLC data, contained esters, primary and secondary alcohols, sterols and mixtures of the components mentioned, were isolated by Si gel column chromatography of the rose waxes with ether as eluent. They were separated by PLC, and further

Table 1. Composition (%) of primary alcohols in waxes of three decorative roses

Carbon number	*R. americana* free alcohols	*R. imperial* free alcohols	*R. virgo* free alcohols	*R. virgo* ester-bound alcohols
16	—	—	traces	1.4
17	—	—	—	0.3
18	0.1	—	traces	11.0
19	0.5	0.2	—	0.7
20	1.8	0.9	4.2	20.2
21	3.1	1.7	3.5	2.9
22	10.4	2.1	17.5	17.8
23	3.9	2.6	5.4	1.5
24:1	—	—	—	2.5
24	12.8	20.8	12.1	11.7
25	3.6	3.8	2.9	0.9
26	16.9	25.1	8.6	6.8
27	5.7	4.1	3.1	0.7
28:1	—	—	—	0.8
28	32.7	31.7	20.3	7.0
29 + x_1	2.0	1.1	4.9	6.4
x_2	2.5	3.8	14.1	5.0
30	4.0	2.1	2.7	2.5
x_3	—	—	0.5	—
x_4	—	—	0.3	—

x_3 and x_4 unidentified.

purified to chromatographic purity by repetition of the PLC in different solvents.

The free primary alcohols were characterized by the IR-spectra of their acetates (ν_{max} 1743 and 1250 cm^{-1}) and GLC of the acetates showed that the free alcohols in all three waxes were present in an homologous series C_{18}–C_{30} (Table 1). The peaks marked with x_1 and x_2 (Table 1) were further identified by GC–MS of the free alcohols and their acetates from *R. virgo*. The MS of x_1 and x_2 showed M^+ *m/e* 426, while in the acetylated sample M^+ was at 468. These two peaks gave similar MS with the base peak at *m/e* 218. Only several differences in the intensities of the diagnostic ions (207, 205, 203, 189 and 133) were present. The ions observed could be the result of retro-Diels–Alder fragmentation of $\Delta^{12(13)}$ triterpenes of the α- and β-amyrin type [10]. On the basis of both the GLC and MS data on x_1 and x_2, and authentic samples of α- and β-amyrins, it was concluded that x_1 was β-amyrin, and x_2, α-amyrin.

The small amount of chromatographically pure secondary alcohols obtained necessitated the study of their structure by GC–MS and to this end they were oxidized to ketones. The results obtained from *R. virgo* are given in Table 2. GLC showed that a homologous series C_{25}–C_{33} was present, with C_{27} and C_{29} being prevalent. The MS of each ketone with a given chain length contained a base ion at *m/e* 101 and a high intensity of the other ions (see Table 2). All these ions may be explained by assuming that they were fragments of α- and β-splitting [11–13] of a ketone, consisting of three isomers with a carbonyl group at the 4, 5 and 6 positions. The fragmentations of the ketones was confirmed by comparison of their MS with those of synthetic heptacosan-14-one. The amount of each isomer was calculated from the relative intensities of the ions from the α-splitting, according to a formula used by Netting and Macey [13]. This calculation showed that the C_5 isomer was present at *ca* 50%, in each isomeric mixture of ketones of the same length, while the amount of the C-4 and C-6 isomers in each isomer mixture varied (Table 2).

It was established by GLC that the sterols isolated from the ether fraction of the three waxes constituted a mixture of β-sitosterol (91%), cholesterol and campesterol. Since the GLC peak of campesterol was widened, a sample of the sterol fraction from *R. virgo* was subjected to GC–MS. By taking the MS of the shoulders and the apex of that peak, it was shown that campesterol was not mixed with another sterol. At the same time, the MS data confirmed the presence of campesterol (M^+ 400, 385, 382, 367, 315, 289), β-sitosterol (M^+ 414, 399, 396, 381, 329, 303) and cholesterol (M^+ 386, 371, 368, 353, 301, 275).

Only esters of *R. virgo* were subjected to saponification, with a view to studying their neutral components. Primary alcohols, α- and β-amyrin, as well as sterols were established in the manner described above. The individual composition of esterbound primary alcohols is given in Table 1. It is noteworthy that, according to TLC-data, in addition to primary alcohols, also secondary alcohols were present in the neutral components, obtained after the saponification of the esters from *R. virgo*, while according to GC–MS data a minor amount of unsaturated primary alcohols with a main member $C_{24:1}$,

Table 2. Composition and structure of secondary alcohols from *Rosa virgo* wax

Carbon number	%	Structure of secondary alcohols*	% of isomer*	Fragment ions of ketones (*m/e*)
		Pentacosan-4-ol	32	71, 86, 87, 323, 338
25	1.7	Pentacosan-5-ol	39	85, 100, 101, 309, 324 M^+ 366
		Pentacosan-6-ol	29	99, 114, 115, 295, 310
		Hexacosan-4-ol	29	71, 86, 87, 337, 352
26	1.6	Hexacosan-5-ol	60	85, 100, 101, 323, 338 M^+ 380
		Hexacosan-6-ol	11	99, 114, 115, 309, 324
		Heptacosan-4-ol	26	71, 86, 87, 351, 366
27	37.7	Heptacosan-5-ol	50	85, 100, 101, 337, 352 M^+ 394
		Heptacosan-6-ol	24	99, 114, 115, 323, 338
		Octacosan-4-ol	26	71, 86, 87, 365, 380
28	4.3	Octacosan-5-ol	52	85, 100, 101, 351, 366 M^+ 408
		Octacosan-6-ol	22	99, 114, 115, 337, 352
		Nonacosan-4-ol	23	71, 86, 87, 379, 394
29	28.6	Nonacosan-5-ol	54	85, 100, 101, 365, 380 M^+ 422
		Nonacosan-6-ol	23	99, 114, 115, 351, 366
		Triacontan-4-ol	32	71, 86, 87, 393, 408
30	0.9	Triacontan-5-ol	50	85, 100, 101, 379, 394 M^+ 436
		Triacontan-6-ol	18	99, 114, 115, 365, 380
		Hentriacontan-4-ol	36	71, 86, 87, 407, 422
31	16.0	Hentriacontan-5-ol	50	85, 100, 101, 393, 408 M^+ 450
		Hentriacontan-6-ol	14	99, 114, 115, 379, 394
		Dotriacontan-4-ol	37	71, 86, 87, 421, 436
32	1.4	Dotriacontan-5-ol	48	85, 100, 101, 407, 422, M^+ 464
		Dotriacontan-6-ol	15	99, 114, 115, 393, 408
		Tritriacontan-4-ol	31	71, 86, 87, 435, 450
33	7.7	Tritriacontan-5-ol	50	85, 100, 101, 421, 436, M^+ 478
		Tritriacontan-6-ol	20	99, 114, 115, 407, 422

* Determined by GC–MS analysis of corresponding ketone.

giving the diagnostic ions 334 (M^+—18) and 306 (M^+—18–28) were present [14, 15].

DISCUSSION

The analysis of the primary alcohols of decorative roses confirms the fact that the even-numbered homologues are prevalent in plants. From the point of view of the biogenesis of wax components, the correlation observed between the most prevalent primary alcohols and aldehydes in the three decorative roses is very important [8], this confirming the biogenetic relationship between them [16].

So far, plant waxes have been found to contain symmetrical, as well as unsymmetrical secondary alcohols with a OH group located at or near the middle of the chain e.g. nonacosan-15-ol [17], hentriacontan-16-ol [18], nonacosan-10-ol [11, 19]. The occurrence of two or three isomeric alcohols in some waxes has also been established. The C_{29} secondary alcohol in *R. damascena* consists of nonacosan-10-ol and nonacosan-7-ol [11], while the same alcohol in *Brassica oleracea* consists of nonacosan-15-ol and nonacosan-14-ol [13]. The C_{31} alcohol in *Pisum sativum* contains hentriacontan-16-ol, hentriacontan-15-ol and hentriacontan-14-ol [20]. However as it is seen in all these cases the structure of the isomers has been established only in one or two of the prevalent members of the homologous series.

It is for the first time that in a plant wax the structure of each member of the homologous series of secondary alcohols in the C_{25}–C_{33} range has been studied and it is shown that each of them is a mixture of three isomers with a OH group in positions 4, 5 and 6, the C_5 isomer being dominant in all cases. The chain lengths of the secondary alcohols were similar to those of the alkane fraction of the waxes [6]. Up to now no secondary alcohols with such structure have been established in plant waxes. Similar compounds however have been obtained from ester-bound secondary alcohols of insect waxes [21] but with the OH group towards the middle of the chain.

Up to now it has been believed that secondary alcohols are found in the plants only in a free state [11, 22, 23]. It is for this reason that Blomquist *et al* [21] consider that the ester-bound secondary alcohols, found by them in insects, have been formed from free secondary alcohols, ingested with the plant feed. Esterified secondary alcohols, however, of medium chain length with a OH group in position 2 are present in *Eucalyptus* wax [24]. Our results from the study of the neutral components of saponified esters of rose wax confirm also the presence of ester-bound secondary alcohols in plants.

α- and β-Amyrin have been established as components of certain plant waxes [25–28] and β-amyrin has been found in the stem and leaves of *Rosa* Paul's Scarlet [29]. The phytosterols (β-sitosterol, campesterol, cholesterol) isolated from the three roses also occur in other plant waxes [26, 30–32] and in all cases the major sterol is β-sitosterol. It is for the first time that α-amyrin and cholesterol have been found in the genus *Rosa*.

EXPERIMENTAL

Isolation of wax esters. Group separation of the waxes from the 3 roses is as described in ref. [6]. Aldehydes and esters have been isolated from the first Et_2O fractions [8].

Isolation of free secondary and primary alcohols and sterols. Three substances, possessing R_f-values identical to those of sec. and primary alcohols and β-sitosterol were isolated by PLC in Et_2O–hexane (3:7) from the next Et_2O fractions. The primary alcohols and sterols were rechromatographed on Si gel plates, using $CHCl_3$ and hexane–Et_2O–MeOH (4:2:0.1) as solvent systems. The primary alcohols from all three roses showed a spot only for saturated alcohols after TLC on Si gel–$AgNO_3$. The amount of the primary alcohols ranged from 0.8 to 1.7% and that of the sterols, from 0.1 to 0.3% of the total wax in all three roses.

Oxidation of secondary alcohols from R. virgo. Alcohols (4 mg) were oxidized with 50 mg CrO_3 in 3.5 ml of HOAc at 57° for 15 min. After dilution of the reaction mixture with H_2O, the product was extracted with $CHCl_3$. Ketones were purified by PLC in C_6H_6–hexane (1:4).

Saponification of ester fraction from R. virgo. Esters (36.8 mg) were saponified with 2N KOH in EtOH. 21 mg of neutral products and 14.4 mg of acids were obtained. By PLC in hexane–Et_2O (10:3) of the neutral part, 13.4 mg of primary alcohols, 5.4 mg of sterols, a small amount of unsaponified esters and a substance with an R_f-value, equal to that of a secondary alcohol were isolated.

Acetylation of primary alcohols. This was carried out with Ac_2O in C_5H_5N.

GLC. (a) The acetates of the alcohols were chromatographed on a glass column (1 m × 3 mm) packed with 3% OV-17 on Gas Chrom P. The temp was programmed from 150° to 270° at 4°/min, N_2 at 40 ml/min; (b) The sterols and their TMSi derivatives were chromatographed on different phases (3% OV-17 on Gas Chrom Z; 1% SE-30 on Gas Chrom P, and 2.5% OV-1 on Chrom W AWS) at 240° and N_2 at 30 ml/min.

GC–MS analysis of the primary alcohols and their acetates from *R. virgo* and the sterols was done on 2.5% OV-1. The secondary alcohols after transformation in ketones were analyzed on the same phase, temp programmed from 200° to 285° at 4°/min; ionizing energy 30 eV.

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