Characterization of 24 Old Garden Roses from Their Volatile Compositions

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The petals of 24 different rose varieties were extracted with a pentane/dichloromethane mixture, and the extracts obtained were analyzed by gas chromatography/mass spectrometry. Approximately 30 compounds were identified, distributed in different combinations in the various species or cultivars. The main component was phenylethanol, but some roses showed unusually high levels of benzyl alcohol. In some cases nerol and geraniol were also present in considerable concentrations. 4-Vinylphenol was detected in two samples of *Rosa gallica*. A sensorial test was performed by some flavorists to evaluate the most interesting extracts.

Keywords: *Roses; volatiles; GC/MS; essential oil*

INTRODUCTION

Rose oil has drawn the attention of researchers since the 18th century (Kovats, 1987) for its importance in the flavor and fragrance industry. The main objective of this research was *Rosa damascena* (Lawrence, 1991, 1997), although other papers on the oils of other rose cultivars or species (Nishimura et al., 1962; Nakamura, 1987; Tucker and Maciarello, 1988; Ueyama et al., 1990; Brunke et al., 1992; Góra et al., 1995) have been published. However, many of the rose cultivars or species (the so-called old roses or old garden roses) have received less attention. Old roses represent a large population with potential interest both for floriculture and for the fragrance industry. In fact, a large number of these roses have a pleasant odor, a fact that is not well-known.

This paper is an approach to this topic. Its intention is to evaluate the volatile composition of a preliminary group of 24 old roses and to select some of them that might be of potential use in the fragrance industry for further studies.

MATERIALS AND METHODS

Sample Retrieval. The 24 examined species/cultivars were selected from 160 old roses in a collection established in 1993 and sited on a clay-loam soil, pH 7.0 ± 0.1, near Cesena (on the plains of Emilia-Romagna). They were the most fragrant varieties (olfactory test) with a notable flower production per plant.

Grafted plants on *Rosa laxa* rootstock were placed at 2.5 × 2.5 m, and bush cultivars were grown with a natural shape, while climbing varieties were grown against walls. The most important botanical and morphological characteristics of these roses are reported in Table 1.

Sample Preparation and Manipulation. The petals were picked from 9:30 to 10:30 a.m., at maximum flowering time, and stored in polyethylene bags at 10 °C until extraction. After 2 h, solid−liquid extraction was carried out in a 250 mL amber bottle from 20 g of fresh petals with a 40 mL pentane/dichloromethane mixture 7:3 (v/v) (bp 31−32 °C). The bottles were rotated at 6 rpm at room temperature for 24 h. After extraction, the bottles were refrigerated at 0 °C for 30 min to condense the solvent mixture, which was then collected. These operations were repeated five times under the same conditions for each sample. In these conditions the petals were totally extracted and did not reveal any residual odor.

The extracts were concentrated at about 2 mL with a four-ball Snyder column, and 0.5 mL of redistilled hexane was added. The samples were then concentrated at 0.5 mL with a stream of pure N_2 (Gerbi et al., 1992), and submitted to subsequent analyses. The last concentration occurred without any heating device so that evaporation of the solvent rapidly lowered the temperature of the sample below 0 °C.

The dried weight was calculated on a weighed amount of petals dried at 70 °C to a constant weight.

Analysis of Oil Composition by GC and GC/MS. Gas chromatographic analyses were performed by injecting 1 μL of the oil solutions in the split mode (split ratio 1:45) into a Carlo Erba Strumentazione gas chromatograph HRGC 5160 (Milan, Italy) equipped with a 25 m × 0.25 mm i.d., 0.25 mm film thickness, SE 52 capillary column (Mega S.p.A., Legnano, Italy). The column was operated with hydrogen as the carrier gas (1 mL/min) at an initial temperature of 50 °C, which was then raised at a rate of 4 °C/min to 70 °C, held for 8 min, then raised at 5 °C/min to 200 °C, held at this temperature for 6 min, and then raised ballistically to 300 °C. The injector was kept at 250 °C, and the flame ionization detector was set at 300 °C. Chromatograms were displayed and integrated by Chrom-Card data handling (Fisons Instruments, Milan, Italy).

For GC/MS, a QMD 1000 instrument (Fisons Instruments) was used; the same GC conditions described previously were maintained, and mass spectra were recorded from *m*/*z* 33 to 350 at 70 eV.

Chromatographic peaks were identified by retention time and mass spectrometry of authentic compounds, when available.

Each rose extract was injected two times. Analysis of variance was used to compare results.

An informal sensory evaluation of rose scents was performed by the authors and two experts from an Italian firm that produces and commercializes essential oils.

RESULTS AND DISCUSSION

The extraction yields were calculated on the basis of both fresh ($\bar{x}$ = 0.53%; s = 0.12) and dried ($\bar{x}$ = 7.07%; s = 1.56) petals of 22 of the 24 cultivars. Many differences were observed, but no apparent correlation between the two sets of data is evident. The data could be weak because of the small sample size.

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S0021-8561(97)00240-9 CCC: $14.00 © 1997 American Chemical Society

Table 1. Botanical and Morphological Characteristics of Examined Roses (3-Year-Old Plants)

species/cultivar	date of introduction	habitus	color of flowers	height × width (cm)	flowering	flowers/ plant (*n*)	flower diameter (cm)	petals/flower (*n*)
R. × *alba*								
1, Felicité Parmentier	pre-1834	bush-climb.	fresh pink	110 × 160	once	260	6.6	121
2, Maidens Blush	pre-1738	open shrub	fresh pink	230 × 290	once	230	8.3	53
3, Mme Plantier	1835	shrub-climb.	creamy white	160 × 320	once	1500	7.3	147
4, Queen of Denmark	1826	open shrub	pink	130 × 190	once	400	7.7	105
Bourbon R.								
5, Louise Odier	1851	bush	pink-lilac	160 × 130	continuous	130	7.7	76
6, Mme Isaac Pereire	1881	climbing	deep pink	180 × 200	recurrent	200	10.7	58
7, Variegata di Bologna	1909	bush	white, crimson in stripes	200 × 160	once	170	7.5	99
R. × *centifolia*								
8, Blanche Moreau	1880	bush	white	170 × 190	once	80	6.3	106
9, *R. centifolia*	pre-1650	bush	pink	150 × 190	once	120	6.8	102
10, Old Pink Moss	1727	bush	pink	170 × 170	once	150	7.2	65
11, Soupert et Notting	1874	open bush	deep pink	150 × 220	recurrent	550	6.2	28
R. × *damascena*								
12, Kazanlik	pre-1850	bush	pink	140 × 180	once	340	7.1	35
13, Marie Louise	1813	bush	deep pink	140 × 110	once	60	8.0	82
14, Pompon des Princes	pre-1832	bush	pink	150 × 190	once	330	7.1	108
R. × *eglanteria*								
15, Manning's Blush	pre-1797	wide bush	soft pink	140 × 220	once	270	6.3	22
R. × *gallica*								
16, Belle Isis	1845	bush	pink	140 × 210	once	290	5.7	112
17, Charles de Mills	unknown	bush	crimson-purple	160 × 150	once	270	7.0	132
18, Jenny Duval	pre-1821	bush	deep pink	140 × 150	once	250	8.8	68
19, Rose du Maitre d'Ecole	1840	bush	crimson-purple	120 × 130	once	120	9.7	72
20, Tour de Malakoff	1856	bush	magenta-purple	170 × 200	once	280	7.6	78
Noisette R.								
21, Aimée Vibert	1828	climbing	white	400 × 180	once	1600	5.5	60
Perpetual R.								
22, Ferdinand Pichard	1921	bush	pink, crimson in stripes	200 × 160	once	110	7.6	27
Portland R.								
23, Compte de Chambord	1860	bush	deep pink	85 × 70	recurrent	120	7.6	127
R. × *rugosa*								
24, Roseraie de l'Hay	1901	bush	pink-purple	140 × 190	continuous	400	10.2	42

The percent composition (FID trace) of the rose extracts is reported in Table 2. 2-Phenylethanol was almost always the major component; only 5 cultivars showed a phenylethanol concentration <50%, and among these, geraniol exceeded 2-phenylethanol only in roses 15 and 24. Benzyl alcohol, nerol, geraniol, geranyl acetate, and hydrocarbons represented the remaining components of the rose extracts. In fact, the other substances were present in concentrations below 1% each. These data are in sharp contrast with other published data reported in the literature, which described very low concentrations of 2-phenylethanol and high concentrations of geraniol, citronellol, and nerol for *Rosa centifolia* (Góra et al., 1995), corresponding to our samples 8−11. On the other hand, Étienne (1995) found 78% of 2-phenylethanol in *R. centifolia*. The data included the linalool concentration; however, the latter is normally low. Other authors (Tucker and Maciarello, 1986) studied the compositions of a large number of rose cultivars and obtained comparable results. Similar characteristics were also reported for the Bulgarian rose by Kovats (1987). Distillation could cause partial 2-phenylethanol loss, even though it is probably not sufficient to justify such differences.

Phenethyl alcohol and benzyl alcohol were evenly distributed in the different rose groups. These two alcohols were inversely correlated ($y = 70.43 - 1.75x$; $R^2 = 54.76\%$; $p \leq 0.01$) with a good significance, even if some samples were dispersed. The lowest benzyl alcohol concentrations were coupled to the greatest variation of phenylethanol content. Rose 24 had a particular phenylethanol/benzyl alcohol ratio and contained remarkable quantities of aldehydes, particularly neral and geranial and, to a lesser extent, heptanal and benzaldehyde. Methylisoeugenol and benzyl benzoate were present.

The absence of citronellol is remarkable, even in samples 1, 5, 6, 9, 12, 23, and 24, which contained the acetylated derivative. This fact is difficult to explain since geranyl acetate was generally present at lower concentrations than the parent compound. On the other hand, GC/MS analyses excluded the presence of citronellol even in trace amounts. Many authors found citronellol as an important constituent of rose oil (Chen et al., 1985; Li et al., 1988; Baser, 1992). On the contrary, closer to our results, some authors found very small quantities of this compound (Surburg et al., 1993) and some others (Brunke et al., 1992) did not detect this substance in the Lichtkönigin Lucia cultivar.

The hydrocarbon column (Table 2) collected a large number of substances belonging to this class, eluting in the last part of the chromatogram. They do not play an important role in determing the typical rose oil odors but are important for their "fixative" properties.

The *R.* × *alba* group (1−4) was characterized by an abundance of benzyl compounds. In fact, benzaldehyde, benzyl alcohol, and benzyl benzoate were often well represented. Nerol, geraniol, and their corresponding aldehydes contribute significatively to the general richness of these cultivars. 2-Methyl-3-buten-1-ol and 2-methyl-*cis*-2-buten-1-ol were also present. These two substances were never detected in rose extracts. The former is confirmed by its retention time and mass spectrum of the pure standard, while the latter must be considered as tentatively identified by MS. The configuration could be *cis* because of its similarity to angelic acid, a well-represented substance in some plant extracts such as Roman chamomile.

On the other hand, the Bourbon R. group had a remarkably simpler composition almost without alde-

Table 2. Percent Composition of Rose Extracts (FID Trace)

rose[a]	2-methyl-3-buten-1-ol	2-methyl-*cis*-2-buten-1-ol[b]	*cis*-3-hexen-1-ol	2-methyl-2-penten-1-ol[b]	heptanal	α-pinene	benzaldehyde	6-methyl-5-hepten-2-one	octanal	*cis*-3-hexen-1-yl acetate	benzyl alcohol	*trans*-linalool oxide	*p*-cresol	linalool	nonanal + *cis*-rose oxide
1	0.14	0.38	nd[c]	nd	0.02	nd	0.13	nd	0.04	nd	7.96	nd	nd	nd	0.03
2	nd	0.22	0.01	nd	0.04	nd	0.09	nd	0.01	nd	14.56	nd	nd	0.01	0.07
3	0.34	0.99	0.09	0.05	0.10	nd	0.24	0.01	0.01	nd	28.11	0.01	0.01	0.08	0.25
4	nd	nd	nd	nd	nd	nd	0.12	nd	nd	nd	18.24	nd	nd	nd	0.07
5	nd	nd	0.06	nd	0.05	nd	0.01	nd	0.01	nd	4.79	nd	nd	nd	0.01
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	4.54	nd	nd	0.01	0.08
7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.35	nd	nd	nd	nd
8	nd	nd	nd	nd	nd	0.24	nd	nd	nd	nd	0.82	nd	nd	nd	0.09
9	nd	nd	nd	nd	nd	nd	0.01	nd	0.01	nd	4.28	nd	nd	0.01	0.01
10	nd	nd	nd	nd	nd	0.08	nd	nd	0.12	nd	3.40	nd	nd	nd	0.04
11	0.07	0.05	nd	nd	nd	0.08	nd	nd	0.06	nd	4.95	nd	nd	0.01	0.04
12	nd	nd	nd	nd	0.09	nd	nd	nd	nd	nd	2.59	nd	nd	0.01	nd
13	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.37	nd	nd	0.01	nd
14	nd	nd	0.03	nd	nd	nd	0.01	nd	0.01	nd	1.17	nd	nd	nd	nd
15	0.43	0.50	nd	0.10	0.90	nd	0.44	nd	nd	nd	34.24	nd	nd	0.01	0.01
16	nd	nd	nd	nd	nd	nd	nd	nd	0.01	nd	0.94	nd	nd	0.01	0.06
17	nd	nd	0.03	nd	0.04	0.10	nd	nd	0.01	nd	4.32	nd	nd	0.05	0.01
18	nd	nd	nd	nd	0.01	0.32	nd	nd	0.01	nd	2.96	nd	nd	0.01	nd
19	nd	nd	nd	nd	0.01	nd	0.01	nd	nd	nd	2.96	nd	nd	nd	nd
20	nd	nd	0.01	nd	0.01	nd	nd	nd	0.01	nd	0.48	nd	nd	0.01	0.12
21	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.28	nd	nd	nd	0.26
22	nd	nd	2.62	nd	nd	nd	nd	nd	0.01	0.67	0.48	nd	nd	nd	nd
23	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.32	nd	nd	nd	nd
24	nd	nd	0.01	nd	0.29	nd	0.19	nd	0.01	nd	10.67	nd	nd	0.01	nd

rose[a]	2-phenylethanol	methyl salicylate	benzoic acid	4-vinylphenol	nerol	neral	geraniol	geranial	citronellyl acetate	iso-eugenol	geranyl acetate	methyl-isoeugenol	*trans*,*trans*-farnesol	benzyl benzoate	2-phenylethyl benzoate	hydrocarbons
1	69.14	nd	0.01	nd	3.86	0.05	5.96	0.10	0.14	0.49	1.95	nd	0.04	0.50	nd	9.00
2	62.23	nd	nd	nd	2.53	0.01	4.96	0.07	nd	0.67	2.32	nd	0.09	0.78	nd	11.0
3	31.44	0.09	0.01	nd	5.91	0.05	8.63	0.12	nd	nd	0.46	nd	0.37	0.56	1.06	21.00
4	43.40	nd	nd	nd	5.57	0.07	10.88	0.14	nd	nd	2.09	nd	0.10	2.58	nd	17.00
5	64.47	nd	nd	nd	1.43	0.01	4.45	0.01	0.21	0.80	0.33	nd	0.48	0.12	nd	22.50
6	45.66	nd	0.01	nd	6.74	0.01	13.97	0.12	0.46	0.42	1.50	0.22	0.57	1.34	nd	24.50
7	67.88	nd	nd	nd	4.17	0.01	4.85	0.10	nd	nd	0.36	nd	0.08	0.46	nd	21.00
8	56.70	nd	nd	nd	3.32	0.01	7.20	0.07	nd	1.65	5.27	nd	0.14	0.71	nd	23.50
9	76.02	nd	nd	nd	1.44	nd	0.79	nd	0.25	nd	0.35	nd	0.30	0.11	nd	16.50
10	83.75	0.01	nd	nd	1.04	nd	0.70	nd	nd	nd	0.22	nd	0.23	0.12	nd	10.00
11	65.14	nd	0.01	nd	4.93	0.04	13.65	0.13	nd	nd	1.69	nd	nd	0.66	nd	8.50
12	81.92	nd	nd	nd	3.79	0.01	4.04	0.01	0.91	nd	0.71	nd	nd	0.27	nd	6.00
13	72.12	0.01	nd	nd	2.69	0.01	6.97	0.01	nd	0.87	2.13	nd	0.08	0.32	nd	13.50
14	83.33	nd	0.01	nd	1.43	0.03	4.03	0.04	nd	0.62	0.36	nd	0.03	0.15	nd	9.00
15	12.21	nd	nd	nd	9.14	0.01	15.15	0.01	nd	nd	0.49	nd	nd	0.92	nd	25.50
16	85.41	0.01	nd	0.65	0.85	0.01	2.82	0.02	nd	0.46	0.68	nd	0.24	0.35	nd	7.00
17	53.42	nd	nd	nd	2.95	0.01	7.36	0.05	nd	0.17	1.78	nd	0.55	0.80	nd	28.50
18	58.48	0.01	nd	nd	2.56	0.01	5.90	0.01	nd	nd	4.45	nd	0.20	1.08	nd	23.50
19	81.88	nd	nd	nd	1.24	0.01	2.49	0.01	nd	nd	0.56	nd	nd	0.66	nd	10.00
20	80.56	0.01	0.07	0.01	0.85	0.01	0.98	0.01	nd	nd	0.20	0.03	0.26	0.15	nd	16.50
21	89.95	nd	nd	nd	0.01	nd	0.14	nd	nd	nd	nd	nd	0.06	0.14	nd	9.50
22	54.50	nd	nd	nd	7.50	0.10	12.89	0.26	nd	nd	0.26	nd	0.63	0.87	nd	19.50
23	73.21	nd	nd	nd	5.25	0.01	9.75	0.01	0.29	0.92	2.38	nd	nd	0.47	nd	5.50
24	17.17	0.01	nd	nd	18.61	0.10	30.62	0.35	0.91	0.58	0.45	0.46	nd	0.97	nd	18.50

[a] See Table 1 for full description of numbered roses. [b] Tentatively identified by MS library. [c] nd, not detected.

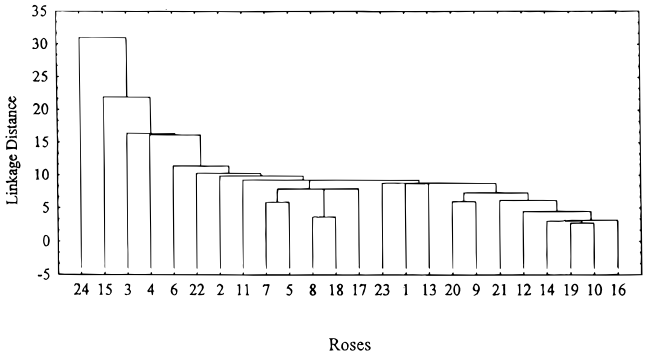

Figure 1. Cluster analysis performed on the whole set of data shown as a tree diagram for 24 roses with single linkage and Euclidean distances.

hydes and other volatile compounds which eluted before phenylethanol. Sample 5, however, did not follow this pattern.

Similar considerations can be extended to *R. damascena* cultivars, which had the lowest content of benzyl alcohol. Rose 12, corresponding to the classical Kazanlik rose used in rose oil production, in our condition is one of the poorest tested. Its oil composition may have been strongly influenced by the existence of different Kazanlik types and by climatic conditions.

The composition of the four samples of *R.* × *centifolia* can be divided into two classes. Compared to roses 8 and 11, roses 9 and 10 were characterized by lower contents of nerol, geraniol, and geranyl acetate. The similarity of roses 9 and 10 is genetical: rose 10 (Old Pink Moss) was derived by a mossed mutation of rose 9 (*R. centifolia*). No other compounds stood out, and the differences among four *R. centifolia* cultivars and all other cultivars were of the same amplitude.

R. × *gallica* had some of the highest phenylethanol concentrations, at least in samples 16, 19, and 20, while roses 17 and 18 reached only 50%, which was partially compensated by the significantly higher nerol, geraniol, and benzyl benzoate concentrations.

The presence of 4-vinylphenol in sample 16 and, to a lesser extent, in sample 20 is the most significant observation for *R. gallica* cultivars. To our knowledge, its presence has never before been detected in rose oils. Some authors (Brunke et al., 1992) reported the presence of a related compound, 4-vinylanisole, in *R. floribunda grandiflora* Queen Elisabeth. These phenolics are widely present in the vegetable kingdom. 4-Vinylphenol is a well-known lignin moiety (Sarkanen and Hergert, 1971) and is also produced by *Saccharomyces* yeasts during alcoholic fermentation (Albagnac, 1975; Steinke and Paulson, 1964).

Apart from these groups, the remaining roses represent different cultivars, each with many differences among them and among other groups. The composition of the oil of rose 21 consisted of almost only phenylethanol and hydrocarbons. Contrary to the simplicity of these samples, roses 15 and 24 exhibited a more complex composition and, at the same time, the lowest phenylethanol content.

All of the results of the rose compositions described here did not clearly differentiate between the groups. In almost every case there was comparable variation both among and between groups. Cluster analyses performed on the whole set of data (Figure 1) clearly show this situation. Only rose 24 was clearly different from the other samples. Then, in decreasing order, roses 15, 3, and 4 formed three groups apart from the other samples, which were distributed regardless of botanical group. In more detail two further clusters were distinguishable: samples 6, 22, 2, 11, 7, 5, 8, 18, and 17 and samples 23, 1, 13, 20, 9, 21, 12, 14, 19, 10, and 16. The cluster contained Bourbon R. and the second *R.* × *damascena*.

Sensory evaluation indicated roses 22 and 24 as the preferred samples. A secondary preference was attributed to roses 1 and 17. All of the selected roses were characterized by low phenylethanol concentration. Nerol and geraniol were positively correlated to the preference, in contrast to benzyl alcohol. In general, more complex sample compositions were preferred to roses characterized by a limited number of substances. *cis*-3-Hexen-1-ol and its high acetate content make rose 22 particularly appreciated for its fresh and grasslike aroma, although it was evident that the bouquet of this sample became worse during the smelling session.

CONCLUSIONS

The study of the 24 rose cultivars showed great differences of composition of the major components, without any apparent influence of botanical classification, problably due to undocumented crossbreeding, which must be added to environmental variability. It cannot be excluded that trace substances, such as damascenone, not detected in our study, would be better markers of both rose quality and different botanical origins.

The phenylethanol content should be confirmed by further studies on the same cultivars, and the influence of hydrodistillation should be carefully evaluated.

ACKNOWLEDGMENT

We thank Dr. M. R. Rizzi and Mr. R. Fiore (Flora srl, Lorenzana, Italy) for their kind contribution to the sensory evaluation of the rose extracts.

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Received for review March 26, 1997. Revised manuscript received August 1, 1997. Accepted August 13, 1997.[⊗]

JF9702407

[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1997.