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Differences in the Fragrances of Pollen, Leaves, and Floral Parts of Garland (*Chrysanthemum coronarium*) and Composition of the Essential Oils from Flowerheads and Leaves

GUIDO FLAMINI,* PIER LUIGI CIONI, AND IVANO MORELLI

Dipartimento di Chimica Bioorganica e Biofarmacia, Via Bonanno 33, 56126 Pisa, Italy

Headspace analyses of pollen, whole flowerheads, ligulate and tubular florets, flower buds, involucral bracts, and leaves have been performed on the food plant *Chrysanthemum coronarium* L. (Asteraceae). The analyses permitted differences in the pattern of volatiles emitted by the different floral parts to be observed and the site and phenological stage of emission of these chemicals to be verified. Camphor and *cis*-chrysanthenyl acetate were emitted mainly by ligulate and tubular florets; the production of myrcene and (*Z*)-ocimene was higher in the flower buds, whereas β-caryophyllene, (*E*,*E*)-α-farnesene, and (*E*)-β-farnesene seemed attributable mainly to the involucral bracts. The leaves showed a quite different volatile profile, with (*Z*)-ocimene as the main constituent. Pollen showed a completely different composition of its volatiles, with perilla aldehyde, *cis*-chrysanthenyl acetate, and camphor among the principal compounds; many carbonylic compounds and linear hydrocarbons have been detected exclusively in pollen. Furthermore, the essential oils obtained from flowerheads and leaves have been studied. These samples showed mainly quantitative differences. Camphor (22.1%) and *cis*-chrysanthenyl acetate (19.9%) were the main constituents of the oil from flowers, whereas the oil from the leaves contained mainly (*Z*)-ocimene (45.4%) and myrcene (28.2%).

KEYWORDS: ***Chrysanthemum coronarium*; garland; Asteraceae; pollen; flower; leaves; volatiles; SPME; essential oil**

INTRODUCTION

Chrysanthemum coronarium L. (Asteraceae), garland, is a very popular food plant in the southern part of China, where it is mainly cultivated in Guangdong Province. It is also appreciated as a food in Japan (*1*). The local names are tong-ho and shungiku, respectively. Garland originated in the Mediterranean region, spreading from there to Europe, Africa, and Asia. It became very popular in Japan, where more than 40 varieties are registered (*2*). This vegetable is rich in β-carotene, minerals, and vitamins (*3*). Furthermore, some antioxidant quinic acid derivatives have been isolated and characterized (*4*−*6*); three of them did not decompose at 100 °C for 30 min, so it can be assumed that garland retains significant amounts of these useful compounds during conventional cooking (*5*). Research aimed at the productive and qualitative characterization of this plant was carried out in northern Sardinia (Italy), to evaluate its introduction and role as pasture species, within forage systems for dairy ewes. It appeared very interesting as forage species (*7*). Previous phytochemical studies showed, besides the cited quinic acid derivatives, the presence of polyacetylenic compounds endowed with insect antijuvenile hormone activity (*8*), sesquiterpene lactones (*9*), coumarins, and steroids (*10*).

Finally, also the essential oil obtained from the capitula have been evaluated for its antifungal activity against 12 agricultural pathogens (*11*). The main constituents were camphor (29.2%), α-pinene (14.8%), lyratyl acetate (9.8%), and β-pinene (9.5%).

Volatiles are the main compounds responsible for the taste of foods, so in the present paper the composition of the essential oils obtained separately from flowerheads and leaves has been analyzed; furthermore, also the solid phase microextraction (SPME) profiles of the volatiles obtained in vivo from whole capitula and isolated ligulate and tubular florets, flower buds, involucral bracts, leaves, and pollen of garland (*C. coronarium*) from Italy have been examined, to evaluate possible flavor changes during plant development. SPME was chosen because of its reproducibility, sensitivity, and high concentration capability; furthermore, most typical sample preparations for compound isolation are more expensive and involve steps that are time- and labor-intensive, are prone to volatile loss, and often use solvents that are toxic or potential carcinogens (*12*−*14*).

MATERIALS AND METHODS

The flowering aerial parts of *C. coronarium* L. (Asteraceae) were collected in the wild in Livorno municipality (Tuscany, Italy) at the end of June 2002, during the morning. Three collections were performed on three different days, within the same week. The samples contained

* Author to whom correspondence should be addressed (telephone +3905044074; fax +3905043321; e-mail flamini@farm.unipi.it).

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also flower buds and were maintained in water. Nine different samples were prepared: 1, whole flowerheads (including involucral bracts); 2, only ligulate florets; 3, only tubular florets; 4, only pollen; 5, only flower buds; 6, only involucral bracts (from open inflorescences); 7, only leaves; 8, essential oil from flowerheads; and 9, essential oil from leaves.

Sample 1 was prepared using five flowerheads collected just after flower opening and cut a few millimeters below the involucre, and the ends were wrapped in aluminum foil to minimize water loss. They were introduced in a 25 mL glass conic flask and allowed to equilibrate for 20 min at 25 °C before sampling.

Samples 2 and 3 were prepared using florets obtained from six to seven freshly opened flowers, avoiding contamination from other flower parts. They were introduced in a 4 mL septum-cap vial and allowed to equilibrate for 20 min at 25 °C before sampling.

Sample 4 consisted of 3−5 mg of pollen obtained by gentle tapping from flowers after anther dehiscence. It was allowed to equilibrate as described above.

Samples 5 and 7 were obtained as described for sample 1, using flower buds or leaves, respectively.

Sample 6 was prepared by introducing in a 4 mL septum-cap vial the involucral bracts randomly collected from six to seven open inflorescences and allowed to equilibrate as described above.

Samples 8 and 9 were obtained by hydrodistillation of fresh flowerheads and leaves (100 g each) for 2 h in a Clevenger-like apparatus.

Samples 1−7 were sampled by means of the SPME technique.

GC analyses of the essential oils were accomplished with an HP-5890 series II instrument equipped with HP-Wax and HP-5 capillary columns (30 m × 0.25 mm, 0.25 μm film thickness), working with the following temperature program: 60 °C for 10 min, ramp of 5 °C/min to 220 °C; injector and detector temperatures, 250 °C; carrier gas nitrogen (2 mL/min); detector dual FID; split ratio, 1:30; injection, 0.5 μL. The identification of the components was performed, for both columns, by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (LRI) relative to the series of *n*-hydrocarbons. All of the reference compounds were obtained from Aldrich Italia (either normal or flavor and fragrances catalogs), except 1-nonene, sabinene, α-copaene, δ-cadinene (Sigma Italia), α-phellandrene, ocimene (mixture of isomers), and (*E*)-β-farnesene (mixture of isomers) (ChromaDex); some compounds, methylthymol (*15*), *trans*- and *cis*-chrysanthenyl acetates, *cis*-3-hexenyl isovalerate, hexyl isovalerate, and (*E*)-3-hexen-1-ol acetate, were prepared by simple synthesis; whereas *cis*-sabinene hydrate, germacrene D, santolina triene, and methyl thymol were confirmed by NMR analyses of other essential oils (*16*, *17*). The only missing reference compounds were yomogi alcohol, artemisia alcohol, artemisyl acetate, β-selinene, bicyclogermacrene, and β-sesquiphellandrene, which were labeled as tentative identification in **Table 1** (however, their retention indices and MS data were in good agreement with the literature).

The relative proportions of the essential oil constituents were percentages obtained by FID peak-area normalization, all relative response factors being taken as one.

GC/EIMS analyses were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (both 30 m × 0.25 mm; coating thickness of 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperatures, 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C/min; carrier gas, helium at 1 mL/min; injection, 0.2 μL (10% hexane solution); split ratio, 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS 95) and homemade library mass spectra built from pure substances and components of known oils and MS literature data (*18*−*23*). Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

SPME analyses were performed with Supelco SPME devices coated with poly(dimethylsiloxane) (PDMS, 100 μm) used to sample the headspace of samples 1−4. After the equilibration time, the fiber was exposed to the headspace for 15 min at 25 °C. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of the GC and GC-MS systems, operating in the same conditions as above both for quantification and for identification of the constituents, except that the splitless injection mode was used and the injector temperature was 250 °C.

All of the quantitative analyses were performed in triplicate. The results were expressed as mean percentages (± SD) obtained by FID peak-area normalization (HP-5 column).

RESULTS AND DISCUSSION

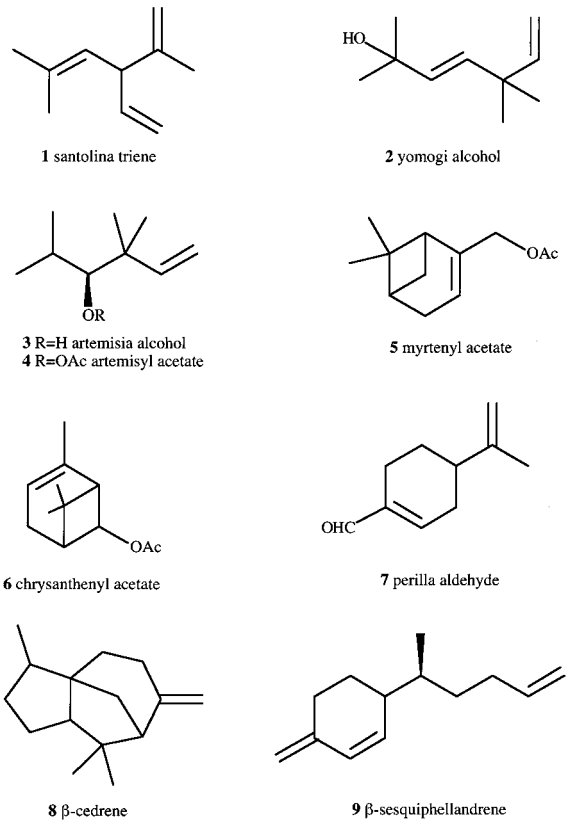
The analyses permitted identification of 62 compounds, accounting for 90.1−99.6% of the whole volatiles. The essential oil yields were 0.04% for the leaves and 0.13% for the flowers. All three collections gave comparable results, from both qualitative and quantitative points of view. The two essential oils showed mainly quantitative differences, but in the essential oil obtained from the leaves fewer compounds were identified (34 vs 28). The principal constituents of the oil of the flowers were camphor (22.1%) and *cis*-chrysanthenyl acetate (**Figure 1**) (19.9%), followed by santolina triene (**Figure 1**) (9.3%), germacrene D (8.5%), (*E*)-β-farnesene (6.4%), and myrcene (5.0%). On the contrary, the oil from the leaves contained mainly (*Z*)-ocimene (45.4%), myrcene (28.2%), and (*E*)-ocimene (8.9%). In a previous investigation (*11*), the major chemicals identified in the essential oil of flowerheads were camphor (29.2%), α-pinene (14.8%), lyratyl acetate (9.8%), and β-pinene (0.5%). Conversely, even if in our flower sample camphor was still the main chemical, pinenes were only minor constituents and lyratyl acetate was not detected at all. Furthermore, santolina triene (**Figure 1**) (9.8%) was not reported by the previous study.

SPME is a fast, solventless technique that permits the establishment of an equilibrium between the sample matrix, the headspace above the sample, and a stationary phase coated on a fused silica fiber. The adsorbed analytes are then thermally desorbed from the fiber in the injector port of a gas chromatograph. This technique permits the sampling of the volatiles emitted by living plants in a fast and easy way. This technique allowed verification that camphor was emitted mainly by ligulate (38.1%) and tubular (34.5%) florets, whereas the outermost parts of the inflorescence gave only a minor contribution (9.1%). Because the flower buds produced only 1.6% of this chemical, it seems that the production of camphor starts only after the opening of the flowerhead. Considering that the production of camphor in the leaves was very low, the biosynthesis of this compound takes place mainly in the full-grown inflorescence. The same is true also for *cis*-chrysanthenyl acetate (**Figure 1**). In fact, it is produced almost exclusively by the ligulate and tubular florets (15.9 and 8.8%, respectively). The production takes place also in this case after the opening of the flowerheads, as demonstrated by the lack of this compound among the volatiles emitted by the flower buds. Perilla aldehyde, santolina triene (**Figure 1**), and camphene showed a similar behavior, even if they are produced chiefly by the ligulate florets. Conversely, the production of myrcene and (*Z*)-ocimene was higher in the flower buds (30.5 and 31.9%, respectively). When the flowerhead was completely developed, these chemicals were still produced, but almost exclusively by the involucral bracts (10.0 and 15.3%, respectively). The synthesis of β-caryophyllene, (*E*,*E*)-α-farnesene, and (*E*)-β-farnesene seemed attributable mainly to the involucral bracts but, in this case, only after the full growth of the inflorescence. The leaves showed a quite different volatile profile, with (*Z*)-ocimene as the main constituent, followed by (*E*,*E*)-α-farnesene (15.9%), germacrene D (7.5%), (*E*)-ocimene (7.0%), and (*E*)-β-farnesene (6.0%). Pollen showed, as already observed in other species (*24*−*27*), a completely different composition of its volatiles. It produced

Table 1. Composition of the Volatiles of Whole Flowerheads, Isolated Floral Parts, Pollen, and Leaves (SPME) and of the Essential Oils from Flowerheads and Leaves of *C. coronarium*

		SPME							essential oils	
constituent[a]	LRI[b]	(1) flower-heads	(2) ligulate florets	(3) tubular florets	(4) pollen	(5) flower buds	(6) involucral bracts	(7) leaves	(8) flower-heads	(9) leaves
3-hexen-1-ol	853	tr[c]	–	tr	–	–	–	0.5 (0.09)	tr	–
(*E*)-2-hexenal	856	–[d]	–	–	–	–	–	–	0.3 (0.04)	tr
1-nonene	892	tr	tr	tr	–	–	–	–	–	–
santolina triene	910	0.6 (0.24)	7.9 (0.60)	2.3 (0.44)	–	0.1 (0.02)	tr	–	9.3 (0.23)	0.2 (0.02)
tricyclene	928	0.5 (0.16)	0.7 (0.18)	0.6 (0.38)	0.8 (0.15)	tr	0.4 (0.23)	–	tr	tr
α-pinene	940	3.6 (0.53)	2.2 (0.11)	1.0 (0.27)	–	4.6 (0.26)	tr	1.1 (0.30)	1.4 (0.35)	1.4 (0.29)
camphene	955	3.3 (0.33)	5.8 (0.96)	3.1 (0.21)	–	0.7 (0.16)	tr	0.1 (0.01)	3.4 (0.32)	1.3 (0.23)
benzaldehyde	962	–	–	–	0.8 (0.03)	–	–	–	–	–
sabinene	978	0.1 (0.10)	tr	tr	–	0.1 (0.09)	–	–	tr	0.1 (0.09)
β-pinene	981	1.3 (0.11)	1.7 (0.31)	1.2 (0.27)	–	0.8 (0.18)	tr	0.2 (0.01)	1.9 (0.25)	0.6 (0.22)
6-methyl-5-hepten-2-one	986	–	–	–	tr	–	–	–	–	–
myrcene	992	15.9 (0.61)	3.4 (0.58)	0.1 (0.02)	1.0 (0.12)	30.5 (2.88)	10.0 (0.56)	5.4 (0.84)	5.0 (0.44)	28.2 (1.58)
yomogi alcohol[e]	999	–	–	–	–	–	–	–	0.3 (0.13)	–
(*E*)-3-hexenyl acetate	1005	tr	–	tr	–	–	–	tr	–	–
α-phellandrene	1007	0.2 (0.18)	–	–	–	0.7 (0.15)	1.2 (0.20)	tr	tr	0.4 (0.07)
p-cymene	1028	tr	–	tr	–	0.2 (0.03)	0.6 (0.22)	–	–	tr
limonene	1033	1.2 (0.39)	1.0 (0.14)	0.9 (0.10)	tr	1.6 (0.18)	0.4 (0.23))	2.6 (0.18)	0.6 (0.25)	2.4 (0.36)
(*Z*)-ocimene	1041	13.0 (0.69)	1.5 (0.57)	0.1 (0.04)	0.7 (0.09)	31.9 (1.62)	15.3 (0.99)	40.8 (1.62)	0.9 (0.26)	45.4 (1.58)
phenylacetaldehyde	1045	–	–	–	8.6 (0.54)	–	–	–	–	–
(*E*)-ocimene	1051	3.5 (0.32)	0.3 (0.10)	tr	–	9.5 (0.45)	6.9 (0.88)	7.0 (0.59)	0.8 (0.20)	8.9 (0.55)
γ-terpinene	1064	tr	–	–	–	tr	–	–	–	–
cis-sabinene hydrate	1070	tr	tr	tr	–	–	–	–	–	–
artemisia alcohol[e]	1085	–	–	–	–	–	–	–	tr	–
terpinolene	1089	tr	–	tr	–	0.2 (0.02)	tr	–	–	0.1 (0.07)
undecane	1100	tr	–	–	tr	–	–	–	–	–
linalool	1101	–	–	–	–	–	–	–	tr	tr
nonanal	1103	–	–	–	0.8 (0.21)	–	–	–	–	–
allo-ocimene	1131	1.9 (0.35)	tr	–	–	4.7 (0.22)	2.1 (0.40)	5.1 (0.31)	–	0.2 (0.11)
camphor	1145	18.6 (0.78)	38.1 (1.66)	34.5 (1.61)	12.3 (0.49)	1.6 (0.38)	9.1 (0.63)	0.4 (0.36)	22.1 (1.27)	1.3 (0.26)
artemisyl acetate[e]	1173	0.1 (0.04)	0.3 (0.13)	0.4 (0.18)	–	–	–	–	–	–
borneol	1175	tr	tr	0.3 (0.13)	–	tr	tr	–	tr	tr
4-terpineol	1182	–	–	–	–	–	–	–	tr	–
naphthalene	1182	–	–	–	–	–	–	0.2 (0.01)	–	–
methyl salicylate	1191	–	–	–	–	–	–	0.1 (0.06)	–	–
α-terpineol	1193	–	–	–	tr	–	–	–	–	–
decanal	1206	–	–	–	4.5 (1.12)	–	–	–	–	–
methyl thymol	1237	–	–	–	–	–	0.3 (0.28)	–	–	–
myrtenyl acetate	1237	–	–	–	–	–	–	–	0.4 (0.04)	–
trans-chrysanthenyl acetate	1238	0.2 (0.11)	tr	0.5 (0.17)	tr	0.2 (0.15)	–	–	4.3 (0.36)	0.3 (0.05)
cis-3-hexenyl isovalerate	1240	tr	–	–	–	–	–	–	tr	–
hexyl isovalerate	1245	–	–	–	–	–	–	–	0.3 (0.03)	–
cis-chrysanthenyl acetate	1264	9.5 (0.58)	8.8 (0.54)	15.9 (0.58)	20.9 (0.90)	–	8.8 (0.74)	–	19.9 (1.19)	1.0 (0.26)
perilla aldehyde	1272	10.6 (0.67)	17.4 (0.65)	24.3 (1.40)	28.9 (1.66)	0.4 (0.12)	14.3 (1.22)	–	–	–
isobornyl acetate	1286	2.2 (0.35)	1.3 (0.02)	1.3 (0.32)	2.1 (0.22)	0.5 (0.13)	1.4 (0.47)	1.3 (0.03)	1.3 (0.26)	1.8 (0.24)
tridecane	1300	–	–	–	0.7 (0.16)	–	–	–	–	–
α-copaene	1377	0.1 (0.04)	tr	–	–	0.3 (0.21)	1.3 (0.04)	0.4 (0.18)	tr	–
tetradecane	1400	tr	tr	–	tr	–	–	–	–	–
β-caryophyllene	1420	0.5 (0.19)	0.5 (0.14)	0.6 (0.04)	0.9 (0.13)	0.2 (0.08)	5.1 (0.65)	1.2 (0.21)	0.8 (0.17)	0.4 (0.17)
β-cedrene	1422	tr	–	–	0.8 (0.16)	–	–	–	–	–
(*E*)-geranylacetone	1454	–	–	–	–	–	–	0.2 (0.08)	–	–
α-humulene	1456	–	–	–	–	–	–	tr	–	–
(*E*)-β-farnesene	1459	6.5 (0.88)	2.3 (0.44)	1.4 (1.36)	6.9 (0.75)	5.7 (0.30)	9.6 (0.51)	6.0 (0.28)	6.4 (0.46)	0.6 (0.02)
germacrene D	1483	1.4 (0.43)	0.7 (0.24)	0.4 (0.09)	3.1 (0.44)	1.8 (0.18)	2.3 (0.33)	7.5 (0.42)	8.5 (0.48)	2.1 (0.41)
(*Z*,*E*)-α-farnesene	1491	–	–	–	–	–	tr	0.8 (0.03)	2.1 (0.52)	0.1 (0.07)
β-selinene[e]	1493	–	–	0.6 (0.11)	–	–	–	–	–	–
bicyclogermacrene[e]	1496	–	–	–	–	–	tr	–	1.0 (0.28)	0.3 (0.03)
pentadecane	1500	–	–	–	0.6 (0.34)	–	–	–	–	–
(*E*,*E*)-α-farnesene	1509	1.5 (0.38)	–	–	1.8 (0.31)	1.5 (0.44)	5.0 (0.36)	15.9 (0.16)	0.6 (0.26)	1.7 (0.24)
δ-cadinene	1525	–	–	–	–	tr	1.5 (0.42)	–	–	–
β-sesquiphellandrene[e]	1527	–	–	–	–	–	–	–	1.1 (0.09)	0.8 (0.21)
hexadecane	1600	–	–	0.6 (0.23)	tr	–	–	–	–	–
α-bisabolool	1684	–	–	–	–	–	–	–	0.8 (0.14)	–
total identified		**96.3**	**93.9**	**90.1**	**96.2**	**97.8**	**95.6**	**96.8**	**93.5**	**99.6**

[a] Percentages obtained by FID peak-area normalization (HP-5 column), mean of three analyses (SD in parentheses). [b] Linear retention indices (HP-5 column). [c] tr < 0.1%. [d] Not detected. [e] Tentative identification.



1 santolina triene
2 yomogi alcohol
3 R=H artemisia alcohol
4 R=OAc artemisyl acetate
5 myrtenyl acetate
6 chrysanthenyl acetate
7 perilla aldehyde
8 β-cedrene
9 β-sesquiphellandrene

Figure 1. Most uncommon compounds identified among the volatiles of *C. coronarium*.

mainly perilla aldehyde (28.9%), *cis*-chrysanthenyl acetate (**Figure 1**) (20.9%), and camphor (12.3%). Many carbonylic compounds and linear hydrocarbons have been detected exclusively in pollen, such as phenylacetaldehyde (8.6%), decanal (4.5%), benzaldehyde (0.8%), nonanal (0.8%), pentadecane (0.6%), and 6-methyl-5-hepten-2-one (trace). The last one is considered to be a defensive chemical against both insects and pathogens (*28*). Probably, pollen odors evolved as defense compounds against pollen-feeding animals, but when plants became dependent on animals for pollination, some attractive compounds were included among pollen volatiles (*24*, *28*). Actually, plants must face two simultaneous contrasting pressures: the need to protect their pollen from nonpollinating insects and the need to advertise it as a reward to pollinators. Other carbonylic compounds could also play a role in these ecological interactions.

Furthermore, knowledge of the exact production dynamic of volatiles during the different phonological stages could be used by aroma chemists either to obtain the compounds of interest by hydrodistillation of the single floral part collected during the correct period or to evaluate the best period for plant harvesting for consumption (*14*).

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