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FLORAL FRAGRANCE COMPOUNDS OF SOME *ANTHURIUM* (ARACEAE) SPECIES AND HYBRIDS

IN HONOUR OF PROFESSOR G. H. NEIL TOWERS 75TH BIRTHDAY

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Key Word Index— *Anthurium*; Araceae; monoterpenes; flower fragrance; headspace analysis; chemotaxonomy.

Abstract—Floral fragrance compounds of five species *Anthurium armeniense*, *A. fragrantissimum*, *A. lindenianum*, *A. ochranthum* and *A. roseospadix*, and three hybrids of *A. armeniense* were studied using headspace adsorption and solvent extraction for sample preparation followed by capillary GC and GC-MS analysis. Twenty eight compounds were identified and their levels (%) assessed at the scent-emitting pistillate stage of the spadix. Variation of compounds in the headspace in response to the time of day were determined in a hybrid, *A. antioquiense* × 'Tatsuta Pink Obake' (UH1299), which emitted strong fragrance all day. Chemical profiles from the headspace and from spadices soaked in CH_2Cl_2 were compared in three species. Combination of these two data sets would provide more complete chemotaxonomic profiles in *Anthurium*. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Anthurium is an important tropical ornamental crop traded in the world market [1]. In Hawaii, it accounts for 64% of the total value of cut flowers [2]. The popularity of *Anthurium* is largely due to the exotic shapes and colors of the spathe and spadix and to the flower's remarkable longevity of up to two months in the vase or even longer on the blooming plant [3]. Varietal development of cut flowers and potted plants relies heavily on intercrossing of species within taxonomic sections.

Plant scents and odors have been recognized as valuable chemotaxonomic markers [4]. However, the study of odorous compounds in the Araceae has focussed on relationships with visiting pollinators [5–7]. A recent example is the presence of dimethyl oligosulphides in the inflorescence headspace of *Amorphophallus* and *Pseudodracontium*, which appears to be a common feature of these sapromyophilous flowers that attract carrion insects [6]. Croat [7] observed that several species of protogynous *Anthurium* emitted odors ranging from pleasant to unpleasant. A more extensive survey of presence or absence of floral scent in 147 *Anthurium* species and hybrids at the University of Hawaii and Missouri Botanical Garden germplasm collections showed that 76% of the plants emitted scents ranging from floral to fishy or foul and from very weak to very strong. A plurality of plants emitted scent only in the morning (45%) and at the pistillate stage (77%) [8]. However, only two fragrance compounds have been identified in one species, *A. ochranthum* [9]. With more than 1,000 species of *Anthurium*, unsettled cases in systematics based on morphological characteristics do exist [3; Croat, personal communication, 1997] and chemical analysis of the fragrance compounds can provide data helpful in correct classification. These results could guide interspecific hybridization programs and assist in development of novel fragrant cultivars. In this study, five species and three hybrids emitting strong, pleasant scent were chosen for chemical analysis. Their emission characteristics and fragrance inheritance in the F1 progeny were compared and discussed based on data from headspace adsorption (HA) and solvent extraction (SE) samples.

RESULTS AND DISCUSSION

Results of chemical characterization of volatile compounds in the headspace and descriptions of fragrance of five *Anthurium* species and three hybrids are summarized in Table 1. Oxygenated monoterpenes

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Table 1. Fragrance description and percentage of chemical components of five *Anthurium* species and three hybrids.[1]

	Species					Hybrids		
	A. fragrantissimum	*A. lindenianum*	*A. ochranthum*	*A. roseospadix*	*A. armeniense*	'Manoa Mist' × *A. armeniense* (Sb_1)	'Manoa Mist' × *A. armeniense* (Sb_2)	'Ellison Onizuka' × *A. armeniense*
Type of fragrance	floral	minty	pine	minty	sweet	sweet, floral	sweet, floral	sweet, floral
Fragrance intensity[a]	strong	strong	strong	medium	strong	strong	strong	strong
Time of emission[b]	midday	day	all day	morning	morning	morning	morning	morning
Compounds								
toluene	3.04	0.20	—	0.18	trace[c]	3.55	2.49	6.43
1-butanol-3-methyl-3-acetate*	—	—	—	—	—	16.53	—	—
α-pinene	—	30.60	3.75	20.90	2.49	—	5.47	1.62
sabinene	—	18.79	—	—	13.17	8.89	8.20	8.79
β-pinene	—	15.66	21.48	32.86	5.99	4.04	3.71	385
myrcene	—	1.63	11.66	—	2.30	trace	1.92	1.71
limonene	2.28	trace	1.25	0.88	0.71	1.46	23.96	1.22
1,8-cineole	—	26.18	55.83	39.89	67.54	59.10	42.20	69.04
benzyl alcohol	0.92	—	—	0.54	—	—	—	2.25
γ-terpinene*	—	—	—	0.24	—	—	—	—
cis-sabinene hydrate*	—	—	0.66	—	—	—	—	—
α-terpinolene*	—	—	0.53	0.35	0.85	—	—	—
methyl benzoate*	—	0.38	—	—	2.49	—	—	—
linalool	93.76	—	—	—	—			
phenylethyl alcohol	—	0.57	—	0.93	0.52	6.43	2.01	3.85
unknown 1	—	5.99	—	1.77	0.21	—	10.10	—
unknown 2	—	—	4.28	—	—	—	—	1.24
α-terpineol	—	—	—	—	trace	—	—	—
dihydrocarvone*	—	—	0.56	0.30	0.19	—	—	—
5-hydroxycineole*	—	—	—	—	trace	—	—	—
unknown 3	—	—	—	0.94	3.54	—	—	—

* Based on GC-MS results compared with published data, [a] Three levels of fragrance intensity, strong, medium and light, [b] Time of collection for midday, 1130–1430 hr; others, 0830–1130 hr., [c] Trace ≤ 0.10%.

and monoterpene olefins such as α- and β-pinene, limonene, 1,8-cineole and linalool are among the major fragrance compounds emitted from the spadix of the inflorescence. Five different fragrance types, floral, minty, pine, sweet and sweet with a note of floral were observed and designated by an expert panel [10]. The fragrance types may be attributed to the differences in the distribution of monoterpenes. For example, *A. fragrantissimum* has a high content (93.76%) of linalool and thus, a strong floral odor. Monoterpenes also contributed to minty and pine-like fragrance. The monoterpene, ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol), previously reported [9] as the major volatile terpene in the headspace of *A. ochranthum*, was not found in the current study. Instead, 1,8-cineole was identified as the major compound (55.83%).

Pollination of *A. armeniense*, a species with a strong sweet fragrance, with two commercial cultivars of either relatively weak floral fragrance (*i.e.*, 'Manoa Mist'), or with no fragrance (*i.e.*, 'Ellison Onizuka'), produced progenies that carried distributions of volatile components similar to that of the parent *A. armeniense*. These shared 1,8-cineole as the major compound. The chemical profiles of the two siblings of *A. armeniense* × 'Manoa Mist', Sb_1 and Sb_2, were similar although differences were noted in 1-butanol-3-methyl acetate, α-pinene, limonene, and an unknownl ($Rt = 19.82$ min; m/z: $M^+ = 152$; base peak = 67). Some differences would be expected for heterozygous lines due to genetic segregation. An apparent inheritance of chemical compositions in headspace is supported indirectly by similarity in fragrance types (*i.e.*, sweet *vs.* sweet with floral note) and emitting characteristics (*i.e.*, strong; morning) between *A. armeniense* and its progenies Table 1.

Anthurium species showed variation in their emission cycle. *A. armeniense* and *A. roseospadix* emitted strong and medium scent, respectively, in the morning (0800–1130 hr) but were relatively scentless after 1200 hr. *A. fragrantissimum* emitted fragrance around noon only, *A. lindenianum* emitted throughout the day time (0800–1600 hr), and *A. ochranthum* emitted its pine-like aroma all day and extending into the night. Such variation in emission time affects accurate data collection. Moreover, studies with other scented flowers also showed variation of headspace chemicals according to the time of the day. In tuberose, *Polyanthes tuberosa*, limonene was found in a greater amount during the night whereas methyl salicylate and α-terpineol were found in the morning sample more than in the night sample. Similarly, in *Stephanotis floribunda* more *n*-hexanol was found in the morning sample whereas more of methyl benzoate and 2-phenyl nitroethane were found in the night sample [11]. In the aroid, *Arum maculatum*, peak odor production and changes of volatile chemical composition during the day were also reported [5]. Thus, in a chemotaxonomic study, it would be important to collect samples from the fragrance emitting inflorescence within an appropriate time frame.

To study specific changes in headspace chemical composition at different times of the day in *Anthurium*, hybrid UH1299 was chosen because of its overall flower quality in addition to emitting a relatively strong sweet and floral fragrance all day. UH1299 is a selection from the F1 progenies of *A. antioquiense* and a popular commercial hybrid, 'Tatsuta Pink Obake' (Plate 1). Table 2 shows that the profiles of fragrance compounds in the morning and noon bear more similarity to each other than to that of the night fragrance, in which α-pinene, β-pinene, sabinene, myrcene and limonene were below detection level in the headspace. This drastic reduction of monoterpene hydrocarbons was accompanied by an increase in benzyl acetate and toluene levels. A subtle difference in the nature of fragrance between day and night time was noticeable due to these chemical changes.

While UH1299 emits strong fragrance all day, neither of its parents, *A. antioquiense* and 'Tatsuta Pink Obake' were noted for being fragrant. The parents were used as breeding materials for other desirable qualities such as disease resistance, shape, color, and size of the flowers (Plate 1). Nevertheless GC-MS analysis of the spadix SE sample of *A. antioquiense* showed the presence of several monoterpenes, limonene, 1,8-cineole and α-terpineol as well as benzyl alcohol and benzyl acetate; which were also found in the HA sample of UH1299. Thus, the SE sample provided relatedness between parent and progeny that was not obvious based on other phenotypes.

Since the anatomy of the inflorescence spadix makes it simple for stripping volatile compounds by soaking in a non-polar solvent such as CH_2Cl_2, and the SE sample could be used directly for GC analysis, SE chemical components were compared with those of HA (Table 3). *A. armeniense* showed similarity in the volatile profile of HA and SE samples. In the corresponding set of *A. lindenianum*, benzyl acetate was the major (64.13%) component in the SE sample, yet its level was below GC detection in the HA sample. In *A. ochranthum*, even greater discrepancies existed; monoterpenes such as α-pinene, β-pinene, sabinene and 1,8-cineole totaled more than 80% of the HA volatiles but accounted for less than 6% in the SE sample. Indole, a compound with relatively long *Rt* (30.93 min) was the major (71.41%) component in the SE sample but was not detected in HA sample. As expected, cuticle waxes such as nonadecane, pentacosane, and tricosane were also identified in the SE samples. Regardless of the similarity or dissimilarity of HA and SE samples in each set of data in Table 3, the combined use of the two sample preparation methods would offer more reliable chemotaxonomic assessment of *Anthurium*.

Table 2. Comparison of fragrance emitted at different times of day from *A. antioquiense* × 'Tatsuta Pink Obake' (*i.e.*, UH1299) using headspace sampling and GC-MS results.[2]

Compounds	% Area		
	morning	afternoon	night[a]
toluene	3.71	9.52	20.68
1-butanol, 3-methyl acetate*	1.85	0.90	—
α-pinene	5.86	7.20	—
benzaldehyde	trace[b]	—	—
sabinene	5.14	6.41	—
β-pinene	3.42	4.28	—
myrcene	—	0.90	—
decane*	1.60	0.77	1.57
limonene	9.19	11.96	—
1,8-cineole	12.49	16.04	2.95
benzyl alcohol	5.54	3.10	6.57
undecane*	12.56	5.76	11.78
phenylethyl alcohol	2.27	1.07	2.24
unknown 1	1.39	0.60	1.34
unknown 2	7.05	4.26	4.48
unknown 3	1.45	0.65	1.42
unknown 4	4.72	2.12	4.49
benzyl acetate	15.87	21.66	33.32
α-terpineol	trace	trace	trace
trans-dihydrocarvone*	1.27	0.64	1.53
cis-dihydrocarvone*	—	—	1.05
nerol	1.73	0.71	1.51
carvone	—	—	trace
unknown 5	2.98	1.45	5.07

* Based on GC-MS results compared with published data.

[a] Collected morning, 0830–1130 hr.; afternoon, 1330–1630 hr.; night, 1900–2200 hr.

[b] Trace ≤ 0.10%.

EXPERIMENTAL

Plant materials

Five species, *Anthurium armeniense*, *A. fragrantissimum*, *A. lindenianum*, *A. ochranthum* and *A. roseospadix* and three hybrids, 'Manoa Mist' × *A. armeniense*, 'Ellison Onizuka' × *A. armeniense*, and *A. antioquiense* × 'Tatsuta Pink Obake' (*i.e.*, UH1299) were maintained in the Horticulture Magoon Greenhouse Facility, University of Hawaii. Two sibling plants, Sb_1 and Sb_2 from the cross 'Manoa Mist' × *A. armeniense*, were included for comparison. These plants varied in size of inflorescences, length of time to flower, and number of flowers from each plant. For *Anthurium* species, it usually takes three years from planting a young seedling to flower, and each plant bears only one to three flowers per year. Sampling of fragrance was carried out during the emitting pistillate stage of the inflorescence.

A. fragrantissimum, *A. ochranthum* and *A. roseospadix* were vouchered at the Missouri Botanical Garden, *Croat* 76249 (MO), *Croat* 69861 (MO) and *Croat* 49091 (MO), respectively. *A. lindenianum* was vouchered at the Harold Lyon Aboretum, Honolulu, *Sheffer* 209 (HLA 8676).

Sample collection and preparation

For headspace adsorption (HA) samples, fragrance of unharvested flowers was trapped on site in the greenhouse from 0830 to 1130 hr at ambient temperature of about 25°. Only in studying fragrance emission in response to time of the day were potted plants of UH1299 moved to a 23° laboratory. Samples were trapped at three different times; morning (0830 to 1130 hr), afternoon (1330 to 1630 hr) and night (1900 to 2200 hr). A closed-loop stripping apparatus [12] courtesy of DRACOGO, Holzminden, Germany was used for trapping headspace volatiles from the spadix. The inflorescence at the pistillate stage was inserted into an Erlenmeyer flask (250 ml) fitted with inlet and outlet connection tubings on the opposite side of the flask. Soft tissue paper enclosing the peduncle minimized external air from entering the flask. Recirculating air was perpetuated by an AC pump at $150 \pm 5\,ml\,min^{-1}$ through the connection tubings.

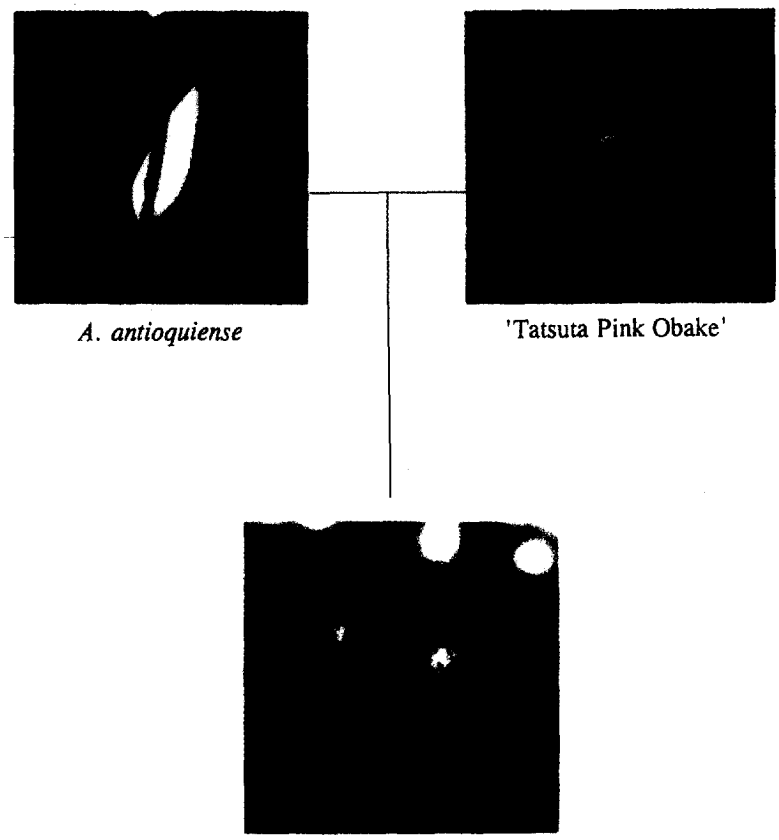

Plate 1. Illustrated parentage of the fragrant hybrid anthurium, UH1299.

Table 3. Comparison of percentage of chemical components present in fragrant *Anthurium* species based on samples prepared by headspace adsorption (HA) or solvent extraction (SE).

Chemical compounds	*A. armeniense*		*A. lindenianum*		*A. ochranthum*	
	HA	SE	HA	SE	HA	SE
toluene	trace[a]	—	0.20	0.42	—	trace
α-pinene	2.49	trace	30.60	0.39	3.75	—
benzaldehyde	—	—	—	0.16	—	—
sabinene	13.17	7.98	18.79	0.25	—	—
β-pinene	5.99	3.62	15.66	0.20	21.48	trace
myrcene	2.30	2.31	1.63	—	11.66	trace
α-terpinene	—	1.02	—	—	—	—
limonene	0.71	1.76	trace	0.11	1.25	trace
1,8-cineole	67.54	54.02	26.18	6.46	55.83	5.06
γ-terpinene*	—	1.19	—	0.12	—	—
cis-sabinene hydrate*	—	—	—	—	0.66	—
α-terpinolene*	0.85	1.55	—	0.16	0.53	—
methyl benzoate*	2.49	26.55	0.57	—	—	—
linalool	—	—	0.38	—	—	1.45
phenylethyl alcohol	0.52	trace		—		
unknown 1	0.21	—	—	1.14		
unknown 2	—	—	5.99	5.54	—	—
unknown 3	—	—	—	—	4.28	16.71
benzyl acetate	—	—	—	43.66	—	—
α-terpineol	trace	trace	—	3.75	—	3.95
cis-dihydrocarvone*	0.19	trace	—	3.16	0.56	trace
nerol	—	—	—	0.15	—	—
5-hydroxycineole*	trace	—	—	2.34	—	1.58
unknown 4	3.54	—	—	31.79	—	trace
indole	—	—	—	0.20	—	71.52

* Based on GC-MS results compared with published data.
[a] Trace ≤ 0.10%.

Headspace volatile compounds were continuously trapped by an XAD-4 resin cartridge (120 mg in 6 × 70 mm tube, Supelco, Bellefonte, PA) connected between the outlet tubing and the pump. The air was recycled back to the flask after passing through an activated charcoal cartridge (20–40 mesh, Supelco) to remove possible contaminants from the pump system. Background of the trapping system was prepared without the flower. After trapping for three hours, fragrance was desorbed by soaking the charged resin in 2 ml of CH_2Cl_2 for one hour in a capped vial on a shaker. Final volume was adjusted under a gentle stream of N_2 to 0.5 ml for GC and GC-MS analysis.

For solvent extraction (SE) samples, flowers were cut between 0830 to 0930 hr and each spadix excised at its base was soaked in CH_2Cl_2 for 15 min. Since spadix size varied, the volume of CH_2Cl_2 was adjusted accordingly, ranging from 5 to 20 ml. Also, care was exercised to avoid contact of the cut surface with the solvent. The extract was concentrated by N_2 to a final volume of 1–1.5 ml for instrumental analysis.

GC analysis

A Hewlett Packard 5890 Series II GC equipped with a 30 m × 0.25 mm id. glass capillary column, DB-5 (J&W Scientific, Folsome, CA) and flame ionization detector was used for the analysis of concentrated essence sample. Results were reported on a HP 3396 Integrator. Conditions were: Injector and detector temperature, 250°; oven temperatures, initial 50° for 2 min, increased to 150° at 2° min^{-1}, subjected to 150° for 1 min followed by a rate of 15° min^{-1} to a final temperature of 280° for 10 min. Flow rates for the gases were: He, 17 mm sec^{-1}; H_2, 35 ml min^{-1}; Air, 350 ml min^{-1}.

GC-MS analysis

A Hewlett Packard 5890 Series II GC equipped with a 30 m × 0.25 mm id. glass capillary column interfaced with a Hewlett Packard 5970 MS was used. Conditions for the GC of the GC-MS were the same as in GC analysis. For MS: Scan mode from 50 to 300 amu; EI ionization voltage, 70 eV; multiplier voltage, 1600 V. Each compound was tentatively identified (> 85% match) by the NBS75K and Wiley138 Libraries. For positive identification, standard compounds were used to match GC retention times and MS spectra. Authentic compounds were obtained from Aldrich Chemical Co. (Milwaukee, WI), Eastman Organic Chemicals (Rochester, NY) and Supelco