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Headspace Compounds from Flowers of *Nicotiana tabacum* and Related Species

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Volatile compounds from flowers of four lines of tobacco, KY 14, TI 1068, TI 1112, and TI 1406, were entrained in air and trapped on Tenax. Each headspace sample was eluted with hexane and separated by capillary GC, and the components were analyzed by GC–MS. Total yields of volatiles ranged from approximately 600 to 900 ppb, which was 30–100 times greater than those from foliage of the corresponding plants. Caryophyllene was the predominant compound in three lines and a major component in the fourth, TI 1068. Studies of volatiles from five other *Nicotiana* species, *Nicotiana alata*, *Nicotiana rustica*, *Nicotiana suaveolens*, *Nicotiana sylvestris*, and *Nicotiana tomentosiformis*, showed that total volatile yields ranged from 88 ppb for *N. rustica* to 2424 ppb for *N. sylvestris*. There was wide diversity in the composition of compounds from the various species studied. *N. tomentosiformis* and *N. sylvestris*, which are putative male and female progenitors of tobacco, respectively, yielded several compounds also identified as tobacco flower headspace components.

Nicotiana is a large predominantly neotropical genus of approximately 57 known species. This genus contains several ornamentals, but tobacco (*Nicotiana tabacum*) is by far its most important member. Tobacco is one of the world's most extensively studied crop plants, not only because of its economic importance but also because of human health concerns. In addition, the genetics of tobacco and other members of the *Nicotiana* fam-

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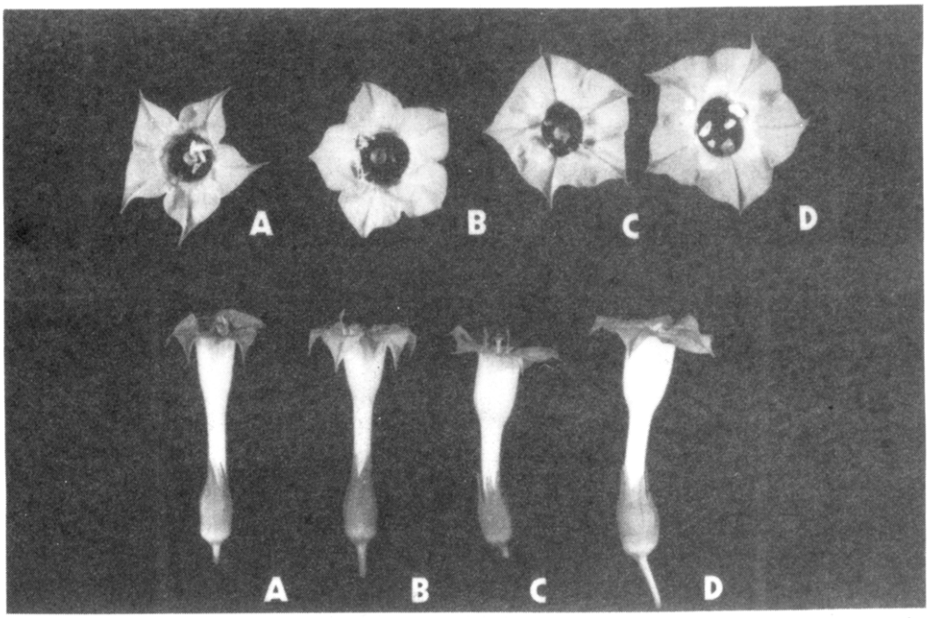

Figure 1. Photograph of flowers from tobacco lines analyzed for headspace volatiles: (A) KY14; (B) TI1068; (C) TI1112; (D) TI1406.

ily are well-known and these plant systems are suitable models for biochemical and molecular biological studies. The chemistry of tobacco leaves has been studied extensively since their composition is important to the genesis of aroma in cured leaves. The majority of the work on volatile compounds in tobacco leaves has been concerned with changes in composition during or after postharvest processing as summarized in reviews (Enzell et al., 1977; Enzell and Wahlberg, 1980). Andersen et al. (1988) recently studied the headspace volatiles of green tobacco leaf in four genetic lines with different trichome morphology. The purpose of the leaf study was to determine whether the emission of volatiles from green leaves was related to functionally secreting trichomes. Four tobacco lines were chosen that included two with secreting trichomes and two with nonsecreting trichomes.

Since flowers are considered to be major producers of volatile compounds, we decided to examine the volatiles from flowers of the four tobacco lines used in studies of leaf volatiles. A comparison was made among the types and quantities of volatile compounds produced by flowers and those from leaves of the same genetic lines. In addition, the studies of flower volatiles were extended to other members of the *Nicotiana* genus including two species that are thought to be progenitors of *N. tabacum* and are therefore considered taxonomically closely related to tobacco.

A headspace sampling method was used to isolate the volatiles from tobacco lines and the various *Nicotiana* species. This procedure, which utilized air entrainment and porous polymer (Tenax) trapping, is relatively mild compared to the steam distillation procedures frequently used to isolate flower volatiles. However, steam distillation does allow the isolation of relatively large quantities of compounds that provide larger amounts of compounds for identification studies.

EXPERIMENTAL SECTION

Plant Material. Four tobacco (*N. tabacum* L.) cultivars, KY 14 burley, Tobacco Introduction (TI) 1068, TI 1112, and TI 1406 (Andersen et al., 1988), were used in the present studies. The following species obtained from the sources listed were also studied: *Nicotiana alata* Link and Otto cv. Nicki Hybrid from Burpee and Co., Warminster, PA; *Nicotiana rustica* L. cv. *brasilia* Schrank, *Nicotiana suaveolens* Lehmann, and *Nicotiana tomentosiformis* Goodspeed from Dr. Verne Sisson of the USDA—ARS Tobacco Research Laboratory, Oxford, NC; *Nicotiana sylvestris* Spegazzini and Cones from Dr. Mark Nielsen of the Department of Agronomy, University of Kentucky. Plants were grown in a greehouse with a soil floor under recommended cultural practices (Andersen et al., 1969). Fully opened flowers that had not begun to senesce were used in this study. All flowers were cut from the plants at the pedicels and were collected within 1 h of 12 noon.

Isolation of Volatiles. The flowers were placed in a 5-L flask that was partially immersed in a water bath maintained at 30 °C. Connected to this flask via Teflon tubing was high-purity compressed air flowing through the headspace apparatus at 500 mL/min. Air exiting the flask passed through a 1-cm-i.d. glass column containing 1.5 g of Tenax. The Tenax trap was conditioned prior to use by washing with 30 mL of hexane and was then heated at 250 °C for 30 min under a stream of high-purity nitrogen (500 mL/min). Headspace analyses were run for 20 h with approximately 50 g of flowers except approximately 25 g for *N. alata*, *N. suaveolens*, *N. sylvestris*, and *N. tomentosiformis*. After a run was completed, the Tenax was removed from the column and eluted with 3 × 10 mL washes of hexane. The combined hexane washings were concentrated to approximately 1 mL on a microstill; cumene was added as an internal standard.

Analysis of Volatiles. Compounds were separated on a Hewlett-Packard 5880A GC equipped with a 60 m × 0.32 mm Supelcowax 10 capillary column (Supelco, Inc.). GC conditions were as follows: inlet temperature, 220 °C; column temperature, isothermal for 1 min at 60 °C and then programmed at 2 °C/min to 220 °C; FID detector temperature, 240 °C; He carrier linear velocity, 31 cm/s. Yields of total volatiles and individual components in the Tenax eluates were estimated from FID peak areas of components and the internal standard, cumene, used in the GC analyses.

GC–MS analyses were performed on a Hewlett-Packard Model 5985A instrument and a Finnigan-MAT ion trap detector both operated at 70 eV. For identification, MS and GC retention data for headspace components were compared to those for authentic compounds unless otherwise noted. Authentic compounds were purchased from commercial sources or were gifts from Bedoukian Research, Inc., Danbury, CT. Dr. H. R. Burton, University of Kentucky, provided a sample of neophytadiene. (*E*)-β-Ocimene and (*E*)-β-farnesene were isolated from a headspace sample of red clover leaves (Buttery et al., 1984).

RESULTS AND DISCUSSION

Top and lateral views of flowers from the various tobacco lines and *Nicotiana* species examined are shown in Fig-

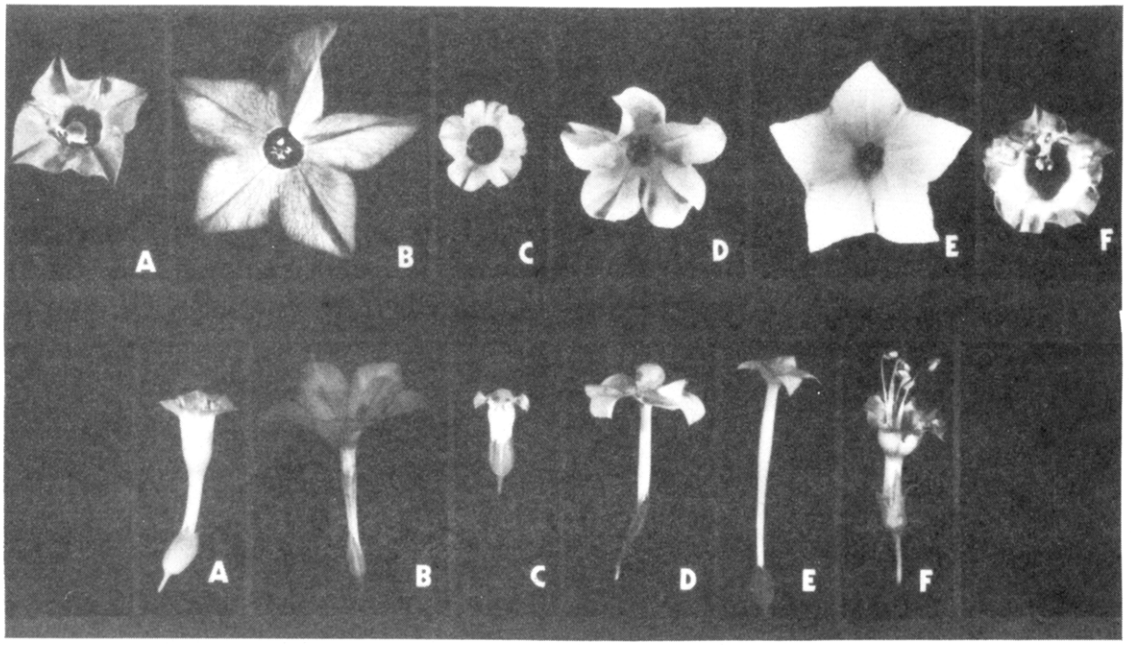

Figure 2. Photograph of flowers from *Nicotiana* species analyzed for headspace volatiles: (A) *N. tabacum* (KY14); (B) *N. alata*; (C) *N. rustica*; (D) *N. suaveolens*; (E) *N. sylvestris*; (F) *N. tomentosiformis*.

ures 1 and 2, respectively. Tobacco lines were similar, but the species yielded a wide diversity of flower shapes and sizes, with *N. rustica* as the smallest and *N. alata* the largest. Tobacco and *N. tomentosiformis* flowers were pink, *N. alata* were red or pink, *N. rustica* were yellow, and *N. suaveolens* and *N. sylvestris* were white.

The compounds identified from the flowers of the *Nicotiana* species including tobacco along with MS and Kovats data for each compound are listed in Table I. The predominant class was terpenes in terms of numbers of compounds identified and their total amounts. Hydrocarbons, alcohols, and ketones were included in this class. Aromatics were a second class of compounds in the flowers and included alcohols, aldehydes, and esters. The third class of compounds identified was short-chain aliphatic compounds (less than 10 carbons) and consisted of alcohols and esters. Several compounds isolated were not identified due to difficulty in interpreting their spectra or the small size of the GC peaks.

The compound yields from tobacco flowers are listed in Table II. The most abundant volatile by far in three lines (KY 14, TI 1112, TI 1406) was caryophyllene, a sesquiterpene hydrocarbon. This compound was also a major volatile in TI 1068, but the relative abundance of a monoterpene alcohol, linalool, exceeded all others in TI 1068 flower headspace isolates. In addition, there were several minor headspace components isolated from the tobacco lines, and those identified are listed in Table II. In comparison to caryophyllene and linalool, the remaining compounds constituted a relatively small portion of the headspace samples.

Among the compounds identified in flowers, caryophyllene, linalool, neophytadiene, and (*E*)-β-ocimene were also found in green leaf headspace samples (Andersen et al., 1988). Linalool, as mentioned, was present in larger amounts in TI 1068 flowers, when compared to other lines; (*E*)-β-ocimene was found only in TI 1112 flowers. These two compounds were also higher in foliage of the corresponding lines. Interestingly, although caryophyllene was a minor and neophytadiene a major component of leaves, these compounds had a reversed order of abundance in flowers. Neophytadiene may be produced from the phytol portion of chlorophyll and therefore be associated closely with leaves. The leaf component (*Z*)-3-hexen-1-ol, known as leaf alcohol, was not detected in tobacco flower volatiles but was present in flowers of other species such as *N. sylvestris*.

Total yields of volatiles from tobacco flowers ranged from 583 to 994 ppb, which was 30–100 times greater than the amounts of volatiles obtained from green leaves (Andersen et al., 1988). The yields from tobacco flowers were in the range of those obtained from headspace entrainments of other flowers, e.g., 500 ppb for red clover (Buttery et al., 1984) and 100 pbb for alfalfa (Buttery et al., 1982).

Table III lists the yields of compounds which were identified in Nicotiana species other than tobacco. It was observed that three of the species, namely *N. alata*, *N. rustica*, and *N. suaveolens*, were quite different in their headspace compositions from *N. tabacum*. One marked difference was that caryophyllene was not detected in these species by our methods except for a trace quantity in *N. rustica*. In addition, linalool was not detected in these three species. However, two of the species, *N. alata* and *N. suaveolens*, did show several similarities to each other. 1,8-Cineole, a monoterpene ether, was a major component in both *N. alata* and *N. suaveolens* as were monoterpene hydrocarbons. These compounds were not detected in *N. rustica*, which produced the lowest quantity of headspace volatiles.

N. alata is sold commercially as an ornamental known as Nicki hybrid, which is not as well-defined genetically as other species studied. Plants used in the present studies bore flowers that were pink or red. Data are presented in Table III for the red flowers. A comparison of volatiles emitted from pink and red flowers showed that they were generally similar except that there were some quantitative differences in the compounds identified (data not shown).

The two remaining species that were analyzed are thought to be closely related to tobacco. *N. sylvestris* and *N. tomentosiformis* are believed to be the female and male progenitors of *N. tabacum*, respectively (Gray et al., 1974).

Flowers from *N. tomentosiformis* yielded relatively low levels of volatiles (Table III). Caryophyllene was present,

Table I. Compounds Identified in Flower Headspace Samples from Tobacco and Other *Nicotiana* Species

compound[a]	mass spectral ions[b]						Kovats index[c]
			Terpenoids				
α-thujene[d]	93	77	*136*	43			<1000
β-pinene	93	69	41	*136*	121		1126
sabinene	93	77	*136*	41	69	121	1137
myrcene	93	69	41	79	*136*	121	1176
limonene	68	93	79	*136*	107	121	1218
1,8-cineole	43	81	93	108	69	*154*	1229
(*E*)-β-ocimene	93	80	43	105	*136*	121	1267
linalool	41	71	93	69	121	136	1564
calarene[e]	91	119	55	105	79	69	1616
caryophyllene	41	93	133	79	69	105	1622
(*E*)-β-farnesene[e]	69	91	77	53	105	133	1663
α-humulene	93	80	121	41	107	147	1696
epoxytagetone[d]	85	56	43	*168*	125	153	1696
α-terpineol	93	136	59	121	81	107	1721
neophytadiene	43	68	82	95	123	109	1931
caryophyllene epoxide	79	93	69	109	121	135	2023
nerolidol	69	93	107	41	81	136	2056
cembrene[d]	93	81	107	119	*272*	69	>2200
			Aromatics				
benzaldehyde	*106*	77	51				1558
methyl benzoate	105	77	*136*	51			1665
benzyl acetate	108	91	*150*	79	65		1759
methyl salicylate	120	*152*	92	65	106	53	1813
benzyl isovalerate[d]	91	108	*192*	57	41	79	1895
benzyl alcohol	79	*108*	51	91	65		1905
benzyl valerate[d]	91	108	*192*	57	41	79	1920
2-phenylethanol	91	105					1942
eugenol	*164*	77	91	149	103	121	>2200
			Aliphatics				
methyl 3-methylpentanoate[d]	74	43	59	99			1145
2-hexanol	45	69	84	56			1232
4-methylpentanol	56	69	41	84			1328
(*Z*)-3-hexenyl acetate	67	43	82				1336
3-methylpentanol	69	56	84	41			1341
hexanol	56	43	69	84			1368
(*Z*)-3-hexen-1-ol	67	82	41	55			1402
4-methylhexanol[d]	70	41	55	83	98		1443
6-methylheptanol[d]	84	69	55	41	97		1524
			Other				
(*Z*)-jasmone	*164*	79	110	149	122	135	1980

[a] Identification based on comparison of mass spectral and GC data from plant components with those from authentic compounds unless otherwise noted. [b] Most intense ion, each 14 mass units above *m/z* 40; in order of decreasing intensity. Molecular ion italicized. [c] Kovats index determined on 60 m × 0.32 mm Supelcowax-10 column. [d] Compound's mass spectrum consistent with published spectrum (EPA/NIH, 1980; Swigar and Silverstein, 1981) but no authentic standard available. [e] Spectrum obtained on a Finnigan-MAT ion trap detector. Most intense ion each 14 mass units above *m/z* 49; in order of decreasing intensity.

Table II. Yields of Flower Headspace Volatiles Emitted by Tobacco Lines

compound	yield,[a] ng compound/g flower			
	KY-14	TI-1068	TI-1112	TI-1406
2-hexanol	6.8 ± 0.8	7.4 ± 2.0	trace[b]	8.4 ± 5.0
(*E*)-β-ocimene			5.5 ± 0.7	
hexanol	9.0 ± 2.4	5.2 ± 1.0	10.3 ± 0.9	14.9 ± 0.2
4-methylhexanol	8.4 ± 2.0	8.3 ± 1.7	10.5 ± 1.7	16.1 ± 1.8
6-methylheptanol	7.4 ± 2.5	6.2 ± 1.1	trace	trace
linalool	7.2 ± 1.9	301.8 ± 61.6		
caryophyllene	541.9 ± 60.4	109.0 ± 22.1	870.5 ± 74.9	551.8 ± 58.0
humulene	15.7 ± 1.9	trace	19.5 ± 8.2	14.6 ± 5.9
benzyl alcohol	trace	trace	trace	trace
neophytadiene	trace	trace	trace	trace
caryophyllene epoxide	trace	12.9 ± 3.0	trace	4.8 ± 0.6
eugenol	trace	7.6 ± 2.6	trace	trace
total volatiles entrained	664 ± 71	583 ± 188	994 ± 52	674 ± 54
% identified	~90	~79	>90	>90

[a] Mean of three determinations ± standard error. [b] Trace = integrated but less than 5 ng of compound/g of flower.

but unlike tobacco it was not a major component. Linalool and several minor compounds found in tobacco were also detected in *N. tomentosiformis*. However, unlike tobacco there was no component predominating in the volatile isolate. Interestingly, *N. tomentosiformis* contained several alcohols including branched-chain compounds not frequently reported in headspace volatiles. Overall, except for a large quantitative difference in caryophyllene, there

Table III. Yields of Flower Headspace Volatiles Emitted by Five *Nicotiana* Species

compound	yield,[a] ng compound/g flower				
	N. sylvestris	*N. rustica*	*N. suaveolens*	*N. alata*	*N. tomentosiformis*
α-thujene				7.6 ± 2.8	
sabinene			60.0 ± 14.0	9.0 ± 1.6	
methyl 3-methylpentanoate		11.1 ± 2.6			
myrcene			42.4 ± 10.4	10.9 ± 1.5	
limonene			58.7 ± 11.0	18.9 ± 2.1	
(*E*)-β-ocimene				trace[b]	12.6 ± 2.5
4-methylpentanol					trace
(*Z*)-3-hexenyl acetate				trace	
3-methylpentanol					38.3 ± 4.6
hexanol					33.5 ± 3.1
(*Z*)-3-hexen-1-ol	27.6 ± 10.6				27.8 ± 5.7
4-methylhexanol	10.5 ± 3.2				31.7 ± 1.1
6-methylheptanol					21.3 ± 1.6
benzaldehyde	211.5 ± 48.5	trace			trace
linalool	trace				11.5 ± 4.1
calarene				trace	
caryophyllene	838.5 ± 169.3	trace			31.8 ± 1.0
(*E*)-β-farnesene				trace	
α-humulene	22.0 ± 5.0				
epoxytagetone		36.0 ± 21.5			
α-terpineol	19.5 ± 8.6				
methyl benzoate	49.1 ± 4.8		473.1 ± 289.8	trace	
benzyl acetate	12.4 ± 3.4				
methyl salicylate			92.1 ± 82.0		
benzyl isovalerate	9.4 ± 2.4				
1,8-cineole			117.2 ± 43.1	50.8 ± 10.9	
benzyl alcohol	403.0 ± 160.5	trace	32.8 ± 11.3		14.0 ● 5.8
benzyl valerate	8.5 ± 3.2				
2-phenylethanol	trace				
(*Z*)-jasmone	6.7 ± 3.6				
nerolidol				26.7 ± 4.2	
cembrene	4.6 ± 1.5				
total volatiles entrained	2424 ± 647	88 ± 29	1303 ± 556	211 ± 44	322 ± 28
% identified	~67	~54	~67	~59	~69

[a] Mean of three determinations ± standard error. [b] Trace = integrated but less than 5 ng of compound/g of flower.

were many similarities between the composition of tobacco and *N. tomentosiformis*.

In contrast to *N. tomentosiformis*, *N. sylvestris* produced large amounts of volatiles (Table III) and the total yield exceeded those of all other species studied. Also, the predominant volatile in *N. sylvestris* flower headspace was caryophyllene, which paralleled the result obtained with tobacco lines. In contrast to tobacco, *N. sylvestris* produced large amounts of benzaldehyde and benzyl alcohol and small quantities of several aromatic esters such as methyl benzoate and benzyl acetate. It is interesting that at least one of these aromatic compounds was present in detectable, although frequently trace, quantities in all the *Nicotiana* species studied. They have also been found frequently as components of headspace volatiles from other flowers such as orchid (Patt et al., 1988), rose (Dobson et al., 1987), and alfalfa (Buttery et al., 1982).

Table III also shows a comparison of the total yields of headspace volatiles from flowers of the additional species. These *Nicotiana* species exhibited a wide range of yields from a low of 88 ppb for *N. rustica* to a high of 2424 ppb for *N. sylvestris* as compared to an average yield of 729 ppb for the tobacco lines.

With regard to the aroma of some of the major compounds, linalool has a characteristic floral aroma and may contribute to the fragrance of TI 1068. Caryophyllene has a weaker aroma that is more reminiscent of conifers whereas 1,8-cineole, which is also called eucalyptol, has an aroma characteristic of eucalyptus. The aromatic compounds encountered have rather weak odors, but some such as the esters are regarded as possessing floral aromas. However, as with other aroma mixtures, the compounds present in the largest quantities do not necessarily make the greatest contribution to the characteristic fragrance. One interesting compound identified in *N. sylvestris* was *cis*-jasmone, which is associated with jasmine aroma. *N. sylvestris* and *N. suaveolens* appear to possess the strongest floral fragrance of the species studied.

Volatile compounds have long been thought to attract insects such as bees and moths to certain flower species for pollination (Pellmyr and Thien, 1986). Certain members of the *Nicotiana* family such as *N. sylvestris* and *N. suaveolens* may be on diurnal cycles of production and/or release of volatile compounds from flowers. We are currently studying the emission of volatiles at different periods during day/night cycles to gain insight into the possible role of diurnal cycles in the release of compounds from *Nicotiana* species. In addition to a role of attracting insects for pollination of certain flower species, volatiles might have a role in pollen germination. French et al. (1979) have shown that volatile compounds stimulated pine pollen germination. There are several examples of relatively low concentrations of volatiles affecting germination of other types of propagules such as fungal spores (French and Gallimore, 1971; Pharis et al., 1982) and seeds (French and Leather, 1979; Bradow and Connick, 1988). The effect of volatiles on germination merits further study, particularly with flowers that are major producers of volatile compounds.

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Registry No. α-Thujene, 3917-48-4; β-pinene, 127-91-3; sabinene, 3387-41-5; myrcene, 123-35-3; limonene, 138-86-3; 1,8-cineole, 470-82-6; (*E*)-β-ocimene, 3779-61-1; linalool, 78-70-6; calarene, 17334-55-3; caryophyllene, 87-44-5; (*E*)-β-farnesene, 18794-84-8; α-humulene, 6753-98-6; epoxytagetone, 124354-88-7; α-terpineol, 98-55-5; neophytadiene, 504-96-1; caryophyllene epoxide, 1139-30-6; nerolidol, 142-50-7; cembrene, 1898-13-1; benzaldehyde, 100-52-7; methyl benzoate, 93-58-3; benzyl acetate, 140-11-4; methyl salicylate, 119-36-8; benzyl isovalerate, 103-38-8; benzyl alcohol, 100-51-6; benzyl valerate, 70340-00-0; 2-phenylethanol, 1321-27-3; eugenol, 97-53-0; methyl 3-methylpentanoate, 2177-78-8; 2-hexanol, 626-93-7; 4-methylpentanol, 626-89-1; (*Z*)-3-hexenyl acetate, 3681-71-8; 3-methylpentanol, 589-35-5; hexanol, 111-27-3; (*Z*)-3-hexen-1-ol, 928-96-1; 4-methylhexanol, 818-49-5; 6-methylheptanol, 1653-40-3; (*Z*)-jasmone, 488-10-8.