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Identification of the Volatile Component(s) Causing the Characteristic Foxy Odor in Various Cultivars of *Fritillaria imperialis* L. (Liliaceae)

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To identify the component(s) causing the foxy odor, characteristic for some *Fritillaria imperialis* cultivars, the headspace of flower bulbs was analyzed using gas chromatography−olfactometry (GC-O) and GC−mass spectrometry (GC-MS). Six *Fritillaria* species and cultivars were selected as follows: *F. imperialis* cv. Premier (very strong foxy odor), *F. imperialis* cv. Lutea (strong foxy odor), *F. imperialis* ssp. Inodora (no odor), *Fritillaria eduardii* (weak mousy odor), *Fritillaria raddeana* (no odor), and an F1 of *F. imperialis* Lutea × Inodora (weak foxy odor). Volatiles from these flower bulbs were accumulated on Tenax and injected into the GC by thermodesorption. The majority of the volatiles consisted of low molecular weight aliphatic compounds. GC-O revealed that the foxy odor was caused by a single component, identified as 3-methyl-2-butene-1-thiol on the basis of smell in GC-O analyses (two GC columns), mass spectra, and retention times. Chemical identification was substantiated by GC-O and GC-MS of an authentic standard of 3-methyl-2-butene-1-thiol, prepared by organic synthesis.

KEYWORDS: Flower bulb; foxy; *Fritillaria imperialis*; gas chromatography/mass spectrometry; gas chromatography/olfactometry; 3-methyl-2-butene-1-thiol; odor

INTRODUCTION

An important way of plants to communicate with the environment is the emission of volatile compounds. Thus, these may attract animal species with which they show positive interactions, e.g., pollinators, or deter animal species that are likely to be harmful, e.g., herbivores. A wide scope of chemical compounds is known to serve this purpose from which the most important belong to three chemical classes: aliphatic, aromatic, and terpenoid compounds (*1*).

For horticultural purposes, these volatiles may serve as a positive quality trait when they result in a pleasant fragrance. In contrast, various plant species, from which many belong to the Liliaceae, produce volatiles that are experienced by humans as "off-odors" in their different fragrance tones, like phenolic, putrid, sulfurous, sweaty, and foxy (*2*, *3*). The most well-known emitters of such offensive odors within the Liliaceae family belong to the genus *Allium*, e.g., onion, garlic, and chive, which emit a wide variety of sulfur-containing compounds (*4*, *5*).

From many of the offensive odor-emitting plant species relevant for horticulture, the causative components have not been identified. From the Liliaceae family, the genus *Fritillaria* was introduced into Europe 400 years ago as one of the first horticultural crops. The genus *Fritillaria* originates from a 3500 km wide zone stretching from southern Turkey to Pakistan (*6*). It is still grown commercially in The Netherlands to a considerable extent to produce bulbs, which are sold locally or exported to the United States, Germany, and Belgium. *Fritillaria* cultivars are mainly grown as ornamental plants for their elegant inflorescences in gardens, but because of the highly pungent, foxy odor (*6*, *7*), which is emitted by bulbs, leaves, stems, and flowers of various cultivars, these are not kept indoors. The chemical structure of the compounds responsible for this odor, which shall be further referred to as "foxy *Fritillaria* odor", has not been identified. Planting *Fritillaria* bulbs is a widely applied strategy to deter moles from penetrating lawns in which they cause mole hills. It is likely that the foxy odor may be the deterring factor. A closely related species, *Fritillaria meleagris*, is the only *Fritillaria* species from which headspace analysis has been described. It emits various small aliphatic compounds and mono- and sesquiterpenoids (*8*), but these cannot be responsible for the pungent, foxy *Fritillaria* odor of *Fritillaria imperialis*. Elucidation of the chemical structure and the availability of an analytical tool for detection may help plant breeders to generate cultivars, which do not emit foxy *Fritillaria*

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odor volatiles but still show the positive horticultural traits, like flower color and plant architecture, of the foxy-smelling *F. imperialis* cultivars.

In the present study, dynamic headspace gas chromatography−olfactometry (GC-O) and GC−mass spectrometry (GC-MS) are used to identify the compound or compounds responsible for the foxy *Fritillaria* odor perception of *F. imperialis* cultivars Premier and Lutea. The foxy smell is not detectable in *F. imperialis*, Inodora, a nonodorous subspecies that can be crossed with the former two cultivars and is used as a control in our analyses. In addition to these three cultivars, we investigated an F1 offspring of a cross between *F. imperialis* Lutea × Inodora, which emits a faint foxy odor, and two other *Fritillaria* species, *Fritillaria eduardii* and *Fritillaria raddeana*, which lack this odor type, as extra controls. Flower bulbs were used as the experimental tool because they are metabolically more stable and more effective in the emission of the foxy *Fritillaria* odor than other intact plant parts, such as leaves, stems, or flowers, which only emit the foxy smell for a few days to 1 week. The foxy tone is very predominant in the overall fragrance perception, which is therefore similar for virtually all plant parts of the foxy-smelling cultivars.

MATERIAL AND METHODS

Chemicals. All chemicals were reagent grade or better. All reference chemicals were purchased from Sigma or Aldrich, now merged to Sigma-Aldrich Chemie BV (Zwijndrecht, The Netherlands). 3-Methyl-2-butene-1-thiol was prepared via organic synthesis from 3-methyl-2-butene-1-ol (Sigma-Aldrich Chemie BV) according to Holscher et al. (*9*) at a 10-fold smaller scale.

Plant Material. Plants of the *F. imperialis* cultivars Premier (very strong foxy odor) and Lutea (strong foxy odor), the *F. imperialis* subspecies Inodora (no odor), a cross between *F. imperialis* Lutea × Inodora (F1 generation, faint foxy odor), *F. eduardii* (mousy odor), and *F. raddeana* (no odor) were grown from bulbs during the spring and early summer in clay soil near Midlum (Province of Friesland, The Netherlands). Bulbs, newly grown from these plants, were harvested in mid-June and stored, after removal of soil, at ambient temperature until analysis, which occurred in October and November.

Headspace Sampling of Volatiles. At least 2 weeks before experiments, bulbs were conditioned to the experimental circumstances by transferring them to a climatic chamber at 20 °C and 50% relative humidity in constant darkness. This prevented or at least strongly reduced stress-related and rhythmicity effects in odor emission during the experimental period, as have been described for many volatiles in higher plants. Single bulbs (between 70 and 160 g each) were placed in glass jars (height 12 cm, diameter 9 cm) closed with a screw cap coated with a Teflon shield at the inside. Ambient air, at a flow rate of 70 mL min^{-1}, was purified at the inlet of the jar by a glass cartridge, containing about 150 mg Tenax TA 20/35 mesh (Alltech, Breda, The Netherlands). Bulb-derived volatiles were accumulated for 24 h at the outlet in glass cartridges, designed for a TDS2 thermodesorption system (Gerstel, Mülheim, Germany), with 125 mg of Tenax TA. Two stainless steel tube connectors were used to protrude the jar cap, and Teflon tubing was applied to connect these to the Tenax cartridges. Tenax cartridges with accumulated volatiles were capped using Teflon tape and stored at −18 °C until GC analysis. The experiments were performed twice with a different set of bulbs. One of the duplicate data sets has been given in the tables.

GC-O and GC-MS. GC-O was performed using two GC systems 1 and 2, where helium was used as a carrier gas at 1 and 8 mL min^{-1}, respectively. Accumulated volatiles were desorbed from Tenax using a TDS2 thermal desorbtion system and a CIS3 cold injection system (both Gerstel), cooled with nitrogen (−150 °C). To start the GC runs, the trap was heated rapidly to 260 °C.

For GC-O in system 1, volatiles were passed onto a BP-5 column (30 m length, 0.25 mm i.d., and 1.0 μm film thickness; Varian, Middelburg, The Netherlands). The oven was programmed at an initial temperature of 40 °C for 2 min with a ramp of 4 °C min^{-1} to 150 °C, then with a ramp of 8 °C min^{-1} to 250 °C, and held for 15 min at this final temperature. Eluting compounds were detected using a self-built sniffing-port system, which was not supplied with humidified air. For GC-O in system 2 (Trace GC 2000, Interscience, Breda, The Netherlands) volatiles were passed onto a BP-Wax column (25 m length, 0.32 mm i.d., and 1.2 μm film thickness; Varian). The oven was programmed at an initial temperature of 33 °C for 4 min with a ramp of 4 °C min^{-1} to 115 °C, then with a ramp of 12 °C min^{-1} to 235 °C, and held for 5 min at this final temperature. Eluting compounds were passed through a three-way splitter (1:1:1 ratio; Gerstel), connected to two sniffing ports to which humidified air was supplied (Interscience) and to a flame ionization detector. The latter was not sensitive enough to show the peak corresponding to the foxy *Friltillaria* odor component and was therefore not used. GC-O detection was performed by two untrained male adults, 44 and 52 years of age, by registering the olfactory perception at a semiquantitative scale for typical *Fritillaria* odor (very strong−strong−weak−absent) and qualitatively for the other odor tones throughout the chromatographic run.

For GC-MS, the conditions and hardware were used as for GC-O system 1. Compounds were analyzed using an Automass II mass spectrometer (Thermo Finnigan, Breda, The Netherlands) operating in the TIC mode (*m*/*z*-range 40−300) at a scan time of 500 ms. Source and transfer line temperatures were 130 and 250 °C, respectively. The filament had a current of 650 mA. Ionization energy and photomultiplier were set at 70 eV and 600 V, respectively.

Because of the vacuum applied for mass spectrometry, components eluted about 3.3 min later under GC-O conditions as compared to GC-MS conditions. This delay was determined using a blend of the following reference chemicals 3-methyl-2-butene-1-thiol (foxy odor), 3-methylthiopropanol (potato odor), and 1-octen-3-ol (mushroom odor) and a mixture of 2-ethyl-3.3-dimethylpyrazine plus 2-ethyl-3.5-dimethylpyrazine (dry sweaty odor).

Table 1. GC/Olfactrometry Pattern of the Volatiles Emitted by Flower Bulbs of *F. imperialis*, Cv. Premier, in Two GC Systems

	R_t (min)	
	GC system	
odor quality	1 (BP-5 column)	2 (BP-Wax column)
foxy	15.7	10.0
mousy	18.8	22.2
citrus	20.8	23.3
mushroom	21.3	22.3
sweaty	28.7	27.1

RESULTS

GC-O Analyses. Table 1 shows the pattern of the major odor tones perceived at the sniffing ports of GC-O systems 1 and 2 after analysis of the headspace, obtained from *F. imperialis* cv. Premier. After GC separation, the foxy *Fritillaria* odor was perceived as "very strong" in both GC systems as a single component in *F. imperialis* cv. Premier, as "strong" in *F. imperialis* cv. Lutea, and as "weak" in the F1 of the hybrid *F. imperialis* Lutea × Inodora. The intensity of the GC-O signals showed a similar ranking order between these cultivars as was observed for whole flower bulbs. Other aroma qualities were noticeable, to different extents, upon GC-O of all six *Fritillaria* cultivars investigated (**Table 1**).

GC-O analyses of an authentic standard of 3-methyl-2-butene-1-thiol, prepared by organic synthesis, revealed the foxy *Fritillaria* odor in both GC systems at the same retention time and with the same odor tone as perceived with *Fritillaria* flower bulbs.

GC-MS Analyses. Table 2 reveals that the flower bulbs from all six *Fritillaria* cultivars investigated emit qualitatively a similar pattern of volatiles, consisting mainly of fatty acid

Table 2. Distribution of Peak Areas in GCMS Profiles of Volatiles from Flower Bulbs of Six *Fritillaria* Species/Cultivars in GC System 1[a]

			peak abundance (% of total peak area)					
			F. imperialis cultivars				other *Fritillaria* species	
compound	RI	R_t (min)	Premier	Lutea	ssp. Inodora	Lutea × Inodora	*F. eduardii*	*F. raddeana*
acetic acid	616	4.9	13.5	12.8	12.0	13.6	19.3	16.5
2-nitroethanol	658	6.7	0.3	61.8	61.0	0.3	0.2	0.2
3-hydroxy-2-butanone*	713	7.9	0.4	0.3	0.5	0.1	0.2	0.5
3-methylpentanol*	738	8.9	0.7	1.1	1.4	0.3	0.6	0.7
2,3-butanediol	793	11.1	2.2	n.d	n.d	1.1	1.2	0.8
n-hexanal	802	11.5	20.4	3.2	3.0	12.1	10.5	9.4
3-methyl-2-butene-1-thiol	823	12.4	2.4	0.1	ND	ND	ND	ND
3-pentene-2-ol	860	14.0	3.6	0.3	0.4	0.1	ND	0.5
1-hexanol	867	14.3	3.5	1.1	0.3	1.5	3.8	2.5
1,2-dimethylbenzene*	872	14.5	1.6	1.1	1.3	3.3	8.6	4.5
cyclohexanone	895	15.5	1.8	1.9	2.5	7.2	7.0	5.9
dihydro-3-methyl 2(3H)-furanone*	948	17.8	1.9	1.5	0.7	1.3	2.8	1.1
benzaldehyde	964	18.5	1.8	1.2	1.2	2.5	3.2	2.9
3-methyl-2(5H)-furanone*	977	19.1	2.9	0.8	0.7	5.1	7.2	18.6
3-hydroxy-4,4,dimethyl 2(3H)-furanone*	1035	21.5	1.7	0.9	0.6	2.7	2.5	2.3
acetophenone	1073	23.0	1.4	0.9	1.0	2.0	2.9	5.4
2-nonene-1-ol*	1105	24.3	2.8	1.2	1.5	2.6	4.5	3.1
octanoic acid	1164	26.6	1.3	0.5	0.8	4.0	4.7	8.9
decanal	1209	28.3	1.9	0.5	0.7	1.0	1.5	0.8
nonanoic acid	1265	30.3	1.9	0.7	1.0	2.3	4.1	2.2
2,4,6-trichlorophenol	1369	33.3	1.5	0.4	2.2	18.2	1.9	1.8
tetradecane	1400	34.2	3.8	0.7	1.1	3.3	2.9	3.7
pentadecane	1500	36.3	6.9	1.5	1.3	4.9	4.1	2.6
3,4-dimethyl-1,5-heptadiene*	1522	36.7	15.2	1.7	0.3	0.5	0.2	0.4
hexadecane	1600	38.1	4.5	3.8	4.4	10.0	6.1	4.6

[a] The *F. imperialis* cultivars Premier, Lutea, and Lutea × Inodora (F1 generation) emit the characteristic foxy *F. imperialis* odor. ND, not detectable (<0.1 % of total peak area). Components marked * are tentatively identified by their mass spectrum from the automated data base; other components are further characterized by mass spectrum plus RI obtained from authentic standards.

metabolites, predominantly C_6 but also C_9 alcohols, longer chain alkanes, and a few aromatic compounds, e.g., benzaldehyde (R_t = 18.5 min) and trichlorophenol (R_t = 33.3 min), but no terpenoids. The origin of the unnatural components 2,4,6-trichlorophenol and 2-nitroethanol is unknown. They may be residues from agrochemicals. 3,4-Dimethyl-1,5-heptadiene is probably a misidentification by the MS library, since this C_9 compound is likely to elute at a lower RI from the BP-5 column. Quantitatively, i.e., expressed as percentage of total peak area for each cultivar, the amounts of these volatiles appeared highly variable, even among replicates of the same cultivar. The major distinction in the GC patterns between cultivars emitting the foxy *Fritillaria* odor and those which lack this characteristic was the presence of a component, identified as 3-methyl-2-butene-1-thiol on the basis of its mass spectrum. GC-MS analyses of an authentic standard, prepared by organic synthesis, confirmed the identity of the component responsible for the foxy *Fritillaria* odor.

The abundance of 3-methyl-2-butene-1-thiol is consistent with the intensity of foxy *Fritillaria* odor in the *F. imperialis* cultivars: Premier > Lutea ≫ Lutea × Inodora, where the latter did not show a detectable peak in GC-MS (**Table 2**). The retention times in GC system 1, after correction for pressure difference in the detection system (see Material and Methods), also indicate that 3-methyl-2-butene-1-thiol causes the typical, foxy *Fritillaria* odor perception. The other odor tones mentioned in **Table 1** could not be matched with any of the volatiles identified by GC-MS (**Table 2**).

DISCUSSION

The results in this paper show that the foxy *Fritillaria* odor, which is characteristic for some *F. imperialis* cultivars (*6*, *7*), is caused by a single component: 3-methyl-2-butene-1-thiol. The chemical identification is based on mass spectrometry and sensory perception after GC separation of the volatiles emitted by flower bulbs of various *Fritillaria* cultivars. It is further substantiated by comparison of retention time and odor quality with an authentic standard, obtained by organic synthesis, in GC-O analyses with two different GC systems and by GC-MS analyses in one of these systems.

Independent from its *Fritillaria* origin, 3-methyl-2-butene-1-thiol has been described in earlier investigations as having a foxy odor, where it occurs in processed plant-derived beverages such as beer, after exposure to light in the 350−500 nm range (*10*, *11*) and in coffee (*2*, *9*). Epple et al. (*12*) tested this compound, also prepared by organic synthesis, on its feeding avoidance activity in mountain beavers where it was shown to be ineffective.

In the above-mentioned reports on occurrence in beer and coffee, the sensory threshold of 3-methyl-2-butene-1-thiol was very low, 20−30 pg/L (*9*). This was also observed in our analyses, where its olfactorally faintly detectable presence in the cross of *F. imperialis* cv. Lutea × Inodora, did not result in a detectable signal on the GC-MS. For this reason, we chose a relatively long sampling time, which will probably interfere with a reliable quantitative determination of this low molecular weight component due to breakthrough in the Tenax cartridges used (*13*−*15*). A rough estimate, based on peak area, with the synthetically prepared 3-methyl-2-butene-1-thiol as a reference, suggests an accumulation in the low picomole range in the most intensely smelling cultivar *F. imperialis* cv. Premier after 24 h of sampling.

A similar compound, 2-butene-1-thiol, has been reported as the major component causing the odor perception of the anal

sac secretion of four different skunk species (*16*−*19*). The foxy tone of 3-methyl-2-butene-1-thiol may be influenced to various extents by other components in the blend of volatiles, as was observed in coffee (*2*, *20*), and also in *Fritillaria* flower bulbs. In the latter source, the foxy tone strongly dominated the overall perception of the blend.

In the closely related plant species *F. meleagris* Hedström (*8*) we observed the emission of some monoterpenes and low molecular weight alkanes but no sulfur-containing compounds. Also, in other Liliaceae, low molecular weight aliphatic compounds predominate the spectrum of emitted volatiles (*21*). Sulfur-containing volatiles, mainly C_3−C_6 aliphatic sulfides and thiosulfinates, are commonly described for the genus *Allium*, where they are responsible for the typical odor of these food sources (*4*, *5*, *22*−*25*). Also, 3-methyl-2-butene-1-thiol may be considered as an aliphatic but at the same time as a terpenoid sulfur-containing volatile. Because of the presence of only five carbons, 3-methyl-2-butene-1-thiol should be further specified as a member of the terpenoid subclass of isoprenoids.

Biosynthesis of low molecular weight aliphatic compounds, which produces the green odor tone in fragrance blends (*3*), probably occurs via degradation of fatty acids (*26*). Terpenoids are generated by either of two different pathways: the mevalonate (*27*) or the pyruvate-dihydroxyacetone phosphate pathway (*28*). Although terpenes were not observed in our study, the C_5-isoprenoid 3-methyl-2-butene-1-ol has been suggested as an immediate precursor for 3-methyl-2-butene-1-thiol (*9*) and is, considering its almost identical chemical structure, a very likely candidate for its biosynthesis.

An objective analytical protocol, together with insight in the biosynthetic pathway and heritability, are very valuable tools for breeding and selection of bulbs lacking emission of the foxy *Fritillaria* odor. The lower abundance in bulbs of the F1 generation of the cross *F. imperialis* Lutea × Inodora indicates an intermediate inheritance. Biosynthesis of higher terpenoids is required for survival due to the crucial role in plant vitality of carotenoids, as photosynthetic and protective pigments, and of steroids, as plant growth regulators. The absence of 3-methyl-2-butene-1-thiol in nonemitting cultivars is probably not due to inactivity of genes or enzymes responsible for biosynthesis of these vital compounds. It is more likely that another biosynthetic step is inhibited, e.g., the introduction of sulfur into the aliphatic or terpenoid backbone. It would be interesting to extend our investigations to the molecular genetic background of 3-methyl-2-butene-1-thiol biosynthesis. This may open the possibility to generate *F. imperialis* cultivars, which lack foxy *Fritillaria* odor but preserve the ornamental value. The primary prerequisite of an objective analytical tool has now become available, together with the identification of the causative foxy *Fritillaria* odor component.

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LITERATURE CITED

(1) Knudsen, J. T.; Tollsten, L.; Bergström, L. G. Floral scents. A checklist of volatile compounds isolated by headspace techniques. *Phytochemistry* **1993**, *33* (2), 253−280.

(2) Bücking, M.; Steinhart, H. Headspace GC and sensory analysis characterization of the influence of different milk additives on the flavor release of coffee beverages. *J. Agric. Food Chem.* **2002**, *50* (6), 1529−1534.

(3) Flament, I.; Sauvegrain, P.; Sauberli, U. De la science à l'art structure et odeur des composants volatiles de la rose. *Quadrinestale Inst. Tetrahedron* **1992**, *3*, 1−13.

(4) Schultz, H.; Krueger, H.; Liebmann, J.; Peterka, H. Distribution of volatile sulphur compounds in an interspecific hybrid between onion (*Allium cepa* L.) and leek (*Allium porrum* L.). *J. Agric. Food Chem.* **1998**, *46* (12), 5220−5224.

(5) Schultz, H.; Krueger, H.; Herchert, N.; Keller, E. R. J. Occurrence of volatile secondary metabolites in selected *Allium* wild types. *J. Appl. Bot.* **2000**, *74* (3−4), 119−121.

(6) Jefferson-Brown, M.; Pratt, K. *The Gardener's Guide to Growing Fritillaries*; David & Charles Publishers: Newton Abbott, United Kingdom, 1997; pp 104−129.

(7) Beck, C. *Fritillaries—A Gardener's Introduction to the Genus Fritillaria*; Faber & Faber Ltd.: London, 1953; pp 64−65.

(8) Hedström, I. Kungsängiljans pollinationsekologi. *Svensk Bot Tidskr* **1983**, *77*, 313−325.

(9) Holscher, W.; Vitzthum, O. G.; Steinhart, H. Prenyl alcohol—Source for odorants in roasted coffee. *J. Agric. Food Chem.* **1992**, *40* (4), 655−658.

(10) Goldstein, H.; Rader, S.; Muramaki, A. A. Determination of 3-methyl-2-butene-1-thiol in beer. *J. Am. Soc. Brew. Chem.* **1993**, *51* (2), 70−74.

(11) Masuda, S.; Kiruch, K.; Harayama, K.; Sakai, K.; Ikeda, M. Determination of lightstruck character in beer by gas chromatography−mass spectrometry. *J. Am. Soc. Brew. Chem.* **2000**, *58* (4), 152−154.

(12) Epple, G.; Russel Mason, J.; Aronov, E.; Nolte, D. L.; Hartz, R. A.; Kaloostian, R.; Campbell, D.; Smith, A. B. Feeding responses to predator-based repellents in the mountain beaver (*Aplodontia rufa*). *Ecol. Appl.* **1995**, *5* (4), 1163−1170.

(13) Bradshaw, N. M.; Ballantine, J. A. Confirming the limitations of diffusive sampling using Tenax TA during long-term monitoring of the environment. *Environ. Technol.* **1995**, *16* (5), 433−444.

(14) Simon, V.; Riba, M.-L.; Waldthart, A.; Torres, L. Breakthrough volume of monoterpenes on Tenax TA: Influence of temperature and concentration for α-pinene. *J. Chromatogr. A* **1995**, *704*, 465−471.

(15) Ventura, K.; Dostàl, M.; Churécek, J. Retention characterization of some volatile compounds on Tenax GR. *J. Chromatogr.* **1993**, *642* (1−2), 379−382.

(16) Wood, W. F. New components in defensive secretion of the striped skunk. *J. Chem. Ecol.* **1990**, *16* (6), 2057−2065.

(17) Wood, W. F.; Morgan, C. F.; Miller, A. J. Volatile compounds in defensive spray of the spotted skunk, *Spilogale putorius*. *J. Chem. Ecol.* **1991**, *17* (7), 1415−1420.

(18) Wood, W. F.; Fischer, C. O.; Graham, G. A. Volatile compounds in defensive spray of the hog-nosed skunk, *Conepatus mesoleucus*. *J. Chem. Ecol.* **1993**, *19* (4), 837−841.

(19) Wood, W. F.; Sollers, B. G.; Dragoo, G. A.; Dragoo, J. W. Volatile components in defensive spray of the hooded skunk, *Mephitis macroura*. *J. Chem. Ecol.* **2002**, *28* (9), 1865−1870.

(20) Hoffmann, T.; Czerny, M.; Calligaris, S.; Schieberle, P. Model studies on the influence of coffee melanoidins on flavor volatiles of coffee beverages. *J. Agric. Food Chem.* **2001**, *49* (5), 2382−2384.

(21) Brunke, E. J.; Schmaus, G.; Baker, K. H. C. Proceedings of the International 13th Congress of Flavours, Fragrances and Essential Oils, Istanbul, Turkey, 15−19 October 1995; Vol 3: Chemistry−Technology, Sensory Biological, 1995; pp 186−215.

(22) Chyau, C. C.; Mau, J. L. Release of volatile compounds from microwave heating of garlic juice with 2,4-decadienals. *Food Chem.* **1999**, *64* (4), 531−535.

(23) Kubota, N.; Yamane, Y.; Toriu, K.; Kawazu, K.; Higushi, T.; Nishimura, S. Identification of active substances in garlic responsible for breaking bud dormancy in grapevines. *J. Jpn. Soc. Hortic. Sci.* **1999**, *68* (6), 1111−1117.

(24) Abbey, L.; Aked, J.; Joyce, D. C. Discrimination amongst Alliums using an electronic nose. *Ann. Appl. Biol.* **2001**, *139* (3), 337−342.

(25) Arnault, I.; Mondy, N.; Cadoux, F.; Auger, J. Possible interest of several sample transfer techniques for fast gas chromatography−mass spectrometric analysis of true onion volatiles. *J. Chromatogr. A* **2000**, *896* (1−2), 117−124.

(26) Hatanaka, A.; Kajiwara, T.; Matsui, K. The biogeneration of green odour by green leaves: its physiological functions—Past, present and future. *Z. Naturforsch.* **1995**, *50c* (7−8), 467−472.

(27) Gershenzon, J.; Croteau, R. B. Terpenoid biosynthesis: The basic pathway and formation of monoterpenes, sesquiterpenes, and diterpenes. In *Lipid Metabolism in Plants*; Moore, Th. S., Jr., Ed.; CRC Press: Boca Raton, FL, 1993; pp 339−388.

(28) Rohmer, M.; Knani, M.; Simonin, P.; Sutter, B.; Sahm, H. Isoprenoid biosynthesis in bacteria: A novel pathway for the early steps leading to isopentenyl diphosphate. *Biochem. J.* **1993**, *295* (9), 517−524.

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