Pergamon

Phytochemistry, Vol. 48, No. 2, pp. 311–315, 1998
© 1998 Elsevier Science Ltd. All rights reserved
Printed in Great Britain
0031-9422/98 $19.00+0.00

PII: S0031–9422(97)01127–8

VOLATILE COMPONENTS IN OKRA SEED COAT

MARIUS CAMCIUC, JEAN MARIE BESSIÈRE,† GÉRARD VILAREM* and ANTOINE GASET

Laboratoire de Chimie agro-industrielle, Unité associée INRA No 31A1010, École Nationale Supérieure de Chimie de Toulouse, 118, route de Narbonne, 31077 Toulouse Cedex 04; † École Nationale Supérieure de Chimie de Montpellier; 4, rue de l'Ecole Normale, 34000 Montpellier, France

(*Received in revised form* 17 *November* 1997)

Key Word Index—*Abelmoschus esculentus*; malvaceae; okra; seed coat; volatiles.

Abstract—The volatile compounds in the coat of okra seeds can be released by simple rubbing of the seeds. They were isolated by hexane extraction and steam distillation to give the essential oil (EO) and by head space (HS) condensation. 72 compounds were identified in the study (40 esters and 24 sesquiterpenes and derivatives).

The most abundant compound was 2-methylbutyl 2-methylbutanoate which represented 49.6% of the HS and 32% of the EO. Among the sesquiterpenes, germacrene D (11.9% of the EO) and the farnesyl derivatives (7.9% of the EO) were the major compounds. Most of these compounds have not been reported previously to be present in okra. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Known for over 2000 years [1], okra (*Abelmoschus esculentus* L.) Moench., Malvaceae, (formerly *Hibiscus esculentus* Linn.) is currently being grown in all the regions of the world with a tropical or Mediterranean climate. It is the seed pods that are used most frequently: they are consumed as a fresh vegetable especially in Africa (gombo) and India (bhindi).

Michotte [2], speaking of the cultivation of okra in Angola, reported that "the aromatic seeds are used as a scent by the local population". This remark is somewhat astonishing in that none of the available literature concerning okra, whether published before or after the works of Michotte, gives the slightest hint of the existence of a perfume or an essential oil in the seeds.

The volatile compounds in okra have already been the object of several studies. Using petroleum ether, diethyl ether and methanol, Osman [3] prepared extracts of the various parts of the plant. A few compounds were identified in the seed: methanol, isopropanol, isobutanol, 3-methyl 1-butanol, cyclohexanol, α-terpenyl acetate and citral. Ames and MacLeod. [1] identified 148 compounds in the fruit, the most abundant being 2-methoxy 4-vinylphenol (9.3%).

Vigorously shaking okra seeds together immediately causes the release of a characteristic odour. It is the origin and the composition of the volatile components of the seed coat that is studied here.

RESULTS AND DISCUSSION

As simple friction is sufficient to release the volatile compounds, it is possible that the specialised cells containing them, occur at the surface of the seeds. A cytological study was carried out which confirmed the existence of the cells storing the aromatic compounds in the seed coat [4].

A plant of the same genus, *Abelmoschus moschatus* (ambrette) has long been used as an essential oil in perfumes. Planchon [5] reports that this oil is produced by cells on the seed coat but the literature on the subject [6–10] does not afford any additional explanations. As for okra, when ambrette seeds are rubbed between the fingers, they give off a characteristic pungent smell. The occurrence of similar structures in okra and ambrette seeds supports the inclusion of these two species in the same genus (*Abelmoschus*).

The seeds used here came from an experimental crop grown near St Perdon, south-west France, in 1994. A total extract of the outer volatile components of the seeds was obtained by vigorously stirring the seeds in hexane for 10 min at 20°C. This procedure avoids extraction of the fats contained within the seed (16–18% weight glycerides and fatty acids). The total extract obtained was steam distilled to give an essential oil (EO) of a greenish yellow colour with a fragrance reminiscent of greenery with overtones of orange, a slight odour of citral and a resinous base. The EO yield represented about 0.06% of the weight of the seeds. In addition, head-space gas (HS) was obtained from seeds shaken energetically for 30 s in a glass vessel. The volatile compounds released from their storage cells were concentrated by means of a

---

* Author to whom correspondence should be addressed.

Tenax trap. Chromatographic analysis was performed after heat desorption.

The compositions of the EO and the HS are reported in Table 1.

Overall 72 compounds were isolated and over 60 identified from their retention index [11–13], their mass spectra [11, 14–16] and data from the literature [17]. In order to confirm the identification of the numerous esters present, several were synthesised from standard alcohols and acids using the method of Lambert [18]. Among these 23 were identified in our extracts. Thus, 88.2% of the HS and 59.6% of the EO were accurately identified. The EO is mainly composed of aliphatic esters (55%) with 2-methylbutyl 2-methylbutanoate alone representing 31%; the other components are sesquiterpenes (including 11% germacrene D) and fatty aldehydes and alcohols. The presence of farnesol derivatives (~ 10%) was noted along with the high proportion of farnesol and farnesyl esters (> 50%) characteristic of ambrette seed essential oil, the latter, however, does not contain aliphatic esters [10]. There are very few common points between our results and those reported by Ames and Macleod [1] for a volatile okra extract obtained directly from the fruit: only a few minor compounds such as nonanal and β-caryophyllene were common to both analyses. These authors do, however, mention [1] the presence of linear terpene esters (non-identified) which seem to correspond to our farnesoyl esters.

The HS gives an overall representation of the compounds detected by smell; by definition, it is composed of the most volatile compounds released on bruising the superficial storage cells of the seeds. Indeed, it contains all the compounds of the EO with an index lower than 1520. In addition, the very light molecules lost during the steam distillation of the seed hexane extract are also present. The major compounds in the HS are the aliphatic esters (96%), the preponderant molecule being 2-methylbutyl 2-methylbutanoate (49.6%).

It is known that certain volatile fractions produced by plants [19] can act as insect repellents. Loughrin [20], for instance, showed that cotton plantlets (*Gossypium*), when attacked by *Spodoptera exigua* Hübner caterpillars, release sesquiterpenes (caryophyllene and humulene) and esters (such as 3-hexenyl 2-methylbutanoate). It can be noted that okra, which is a Malvaceae like cotton, is also attacked by insects of the genus *Spodoptera* (*S. litura* F. and *S. littoralis* Boisd.) [21] which destroy the leaves. It can therefore be hypothesised that these substances could also contribute to the defence of okra seeds.

The major compound of the okra HS and EO, 2-methylbutyl 2-methylbutanoate, is equally found in the glandular secretions of *Omalium rugatum* (Coleopterae) which play a defensive role against predators, the secretions causing intense irritation [22]. The esters present in such secretions act as solvents and also have a synergic action with molecules having irritant properties (studied on *Calliphora* larvae). For instance, 2-methylbutyl 2-methylbutanoate, which is not an irritant on its own, enhances the action of 4-oxo 2-hexenal. This mixture was found to be the most irritant in the study [22]. It cannot be excluded that with okra, where the same ester is present in large proportions, it plays the same role. It is thought to act by increasing the penetration of toxic molecules (e.g. aldehydes) through the predator's tegument.

Sesquiterpenols such as farnesol tend to repel ants and repellents based on farnesols have been tested against the Argentine ant (*Linepithema humile* Mayr.) with good results [23]. Yet, farnesol and dihydrofarnesol are also attractants for pollinating insects [24].

In the present study, we have described the composition of the scent of the okra seed. The data indicate the presence, among other molecules, of farnesol derivatives and aliphatic esters, which had never been described in this plant. As already demonstrated in other plants [19–24], the biological activity of some of these compounds seems to support the hypothesis of their role in defence against seed-eating insects.

EXPERIMENTAL

Plant material

The okra was grown at St Perdon (Landes), France, in an experimental field in 1994 and seeds were obtained from ripe pods in the month of September 1994. The seeds were separated from impurities and dust by sieving.

The plant was identified at the Department of Pharmacognosy, Faculty of Pharmacy, Toulouse and seed specimens were deposited at the Botanical Institute, University of Montpellier I, France.

Isolation of volatile components

Solvent extraction (*EO*). Seed (200 g) were stirred for 10 min at 800 to 1000 rpm with 150 ml HPLC quality hexane in a round-bottomed flask. Continuing the extraction for longer than 10 min gave an extract containing heavy fractions irrelevant for the extraction of the aroma. The final liquor was filtered and the hexane eliminated by evaporation under vacuum at 20–30°. The extract obtained was distilled in an Neo-Clevenger apparatus and the volatile molecules recovered in a minimum volume of hexane (HPLC grade) then analysed by GC and GC-MS.

Dynamic head-space concentration (*HS*). Seed (50 g) were placed in a 250 ml glass cell connected to a dynamic head-space concentrator (Chisa device; SGE). The seeds were shaken for 30 s and the volatile compounds released were drawn off by a flow of helium (30 ml min^{-1}; 25 min; 20°) and concentrated in a Tenax trap. Desorption was performed with a head-space injector (Chisa device; SGE), directly linked up to the analysis column. The desorption tem-

Table 1. Volatile compounds in the okra seeds

Component and ref. MS (lit.)[a]	Kovàts index[b]	RA%[c] (HS)	RA% (EO)	Odour[d]
2-methylbutanol	722	0.3	—	
i-butyl *i*-butanoate [20]	900	0.2	—	sweet-fruity, harsh pineapple-like
n-propyl 2-methylbutanoate [20]	933	0.4	—	
i-amyl propionate [20]	954	0.4	—	very sweet, fruity, apricot-pineapple type
i--butyl *i*-valerate[e]	985	5.0	—	fruity ethereal, apple-raspberry-like
2 methylbutyl *i*-butanoate [20]	1002	0.9	—	
n-butyl 2-methylbutanoate[e]	1020	1.1	—	fruity, cocoa undertones
i-amyl butanoate [20]	1042	0.4	—	powerful fruity, sweet apricot-banana type
nonanal	1084	0.2	—	fatty floral, waxy
2-methylbutyl 2-methylbutanoate[e]	1088	49.6	32.0	fruity, apple-like
n-amyl 2-methylbutanoate[e]	1121	1.8	—	
a valerate	1129	1.6	0.4	
n-hexyl *i*-butanoate [20]	1135	0.1	—	harsh fruity
i-butyl hexanoate [20]	1140	0.2	—	winey-fruity, pineapple-like
i-amyl valerate [20]	1138	2.2	—	
n-propyl heptanoate[e]	1169	1.0	—	green-fruity, winey
n-butyl hexanoate [20]	1174	0.1	—	heavy-vinous, fruity
decanal	1188	0.3	—	sweet-waxy, orange peel-like
n-hexyl 2-methylbutanoate[e]	1218	2.3	0.5	fresh green-fruity
3-hexenyl 2-methylbutanoate [20][h]	1220	0.2	—	warm fruity, unripe apple-pineapple
i-butyl heptanoate[e]	1227	1.6	—	fruity winey, fresh
2-methylbutyl hexanoate[e]	1231	3.9	1.7	
n-heptyl *i*-butanoate [20]	1233	0.3	—	fruity with distinctly, herbaceous undertones
n-propyl octanoate[e]	1270	1.3	0.4	winey, oily-fruity mildly Brandy-like
n-butyl heptanoate [20]	1276	0.2	—	fresh, fruity green, apple-peel, Marigold-like
n-nonyl acetate[e]	1291	0.5	—	powerful, fruity green, leafy
diisopropylbenzene	1295	—	0.4	
n-heptyl 2-methylbutanoate[e]	1317	4.0	1.1	
i-butyl octanoate[e]	1327	0.8	1.3	mild herbaceous, woody, fruity, oily
2-methylbutyl heptanoate[e]	1329	11.0	8.5	
n-octyl *i*-butanoate [20]	1332	0.2	—	refreshing, herbaceous, fruity green
α-copaene	1363	tr	1.7	
β-elemene [25]	1381	0.5	0.3	
n-decyl acetate[e]	1391	0.2	—	sweet fatty-fruity, pineapple, orange-like
1-nonyl propanoate[e]	1392	0.7	—	winey-ethereal, sweet rosy
β-caryophyllene[f]	1405	—	0.8	wood spicy, dry and tenacious
n-octyl 2-methylbutanoate[e]	1418	1.0	1.0	fruity
2-methylbutyl octanoate[e]	1430	1.7	6.9	
α-humulene	1435	tr	1.9	
alloaromadendrene	1446	—	0.6	
phenylethyl *i*-valerate[e]	1459	0.5	3.0	fruity-rosy, rose petal-like
germacrene D	1460	1.1	11.9	
γ-muurolene	1474	0.3	0.3	
$C_{15}H_{24}$	1476	0.6	0.3	
bicyclogermacrene [25]	1479	—	0.2	
γ-cadinene [24]	1496	0.2	1.5	mild, dry, woody, slightly medicinal-tarry
δ-cadinene [24]	1508	0.2	0.1	
cubenene	1511	—	0.1	
1-nonyl 2-methylbutanoate[e]	1518	0.2	—	
i-amyl nonanoate[e]	1527	—	2.5	apricot, floral
2-tridecenal[h]	1545	—	0.8	powerful, citrus, waxy
caryophyllene oxide	1576	—	0.6	
n-undecyl propanoate	1581	—	0.1	
6,7-dihydronerolidol	1587	—	2.9	
unknown	1607	—	0.5	
n-decyl 2-methylbutanoate[e]	1615	—	0.3	
i-amyl decanoate [20]	1627	—	0.2	oily-winey, brandy-like
tetradecanol	1664	—	4.6	fruity-citrusy, Ambre incense note
(*Z*), 6,7-dihydrofarnesol [24]	1668	—	1.5	sweet, mildly floral, woody undertone
$C_{15}H_{26}O$	1677	—	0.3	
(*E*), 6,7-dihydrofarnesol [24]	1681	—	tr	

Table 1. Continued

Component and ref. MS (lit.)[a]	Kováts index[b]	RA%[c] (HS)	RA% (EO)	Odour[d]
pentadecadienal[g]	1704	—	0.5	
phenylethyl heptanoate[e]	1707	—	tr	mild, oily-fruity with herbaceous notes
(*E*, *E*)-farnesal	1714	—	tr	mild, sweet-oily, slightly woody
pentadecanol	1768	—	0.5	
(*Z*), 6,7-dihydrofarnesyl acetate	1782	—	1.9	
(*Z*,*E*)-farnesyl acetate	1787	—	0.3	
(*E*)-dodecenyl 2-methylbutanoate [25]	1804	—	tr	green, fruity, floral
phenylethyl octanoate	1809	—	0.2	
(*E*,*E*)-farnesyl acetate[f]	1814	—	tr	
dihydrofarnesyl propanoate[h]	1865	—	3.6	
(*E*,*E*)-farnesyl propanoate[e]	1902	—	0.4	
dihydrofarnesyl pentanoate[h]	1998	—	0.2	

[a] MS Ref. [13] when not cited.
[b] Kováts index determined on OV 101 column; for conditions, see Experimental, GC-MS, section (b).
[c] RA = relative abundance; < 0.1% quoted as trace (tr).
[d] [25].
[e] identity established with standards synthesised by Lambert method [18].
[f] identity established with purchased standards.
[g] tentative.
[h] undetermined stereochemistry.

perature was 210° and the volatile compounds were cold-concentrated at −20° at the column head before being injected.

GC and GC-MS. Analyses were carried out on a HP-5890 gas chromatograph equipped with a flame ionisation detector and on different columns: (a) a 50 m × 0.22 mm × 1 μm dimethylpolysiloxane BP1 (SGE) fused silica capillary column; carrier gas (He) fixed at 22 psi, splitless injector, 250°, detector 250°; (b) 50 m × 0.22 mm × 1 μm column coated with polysiloxane (OV101); (c) 60 m × 0.25 m × 1 μm coated with Carbowax 20 M; for (b) and (c): carrier gas (He) at 0.9 ml min^{-1}, injector and detector heaters 220°, temp. prog. 100° for 1 min then 1° min^{-1} to 220° injection vol typically 0.2 μl, splitless, no attenuation. The MS is a HP-5791 type with ionisation voltage: 70 eV, scan speed 1.1 s decade^{-1} (repetitive throughout run).

Acknowledgements—We express our gratitude to GIEE Agronove (Greece) for financing this study. We thank Mr Berdoues for the olfactory evaluation of the aroma, Mrs Fouraste for the plant identification and Mr Passera for entomological advice.

REFERENCES

1. Ames, J. M. and MacLeod G., *Phytochemistry*, 1990, **29**(4), 1201.
2. Michotte, F., *Les Hibiscus. Bulletin de la soc. Coloniale Française*, no 3–6, 1928, 64 pp.
3. Osman, A. M., Younes, M. and Ata, M. F., *Ind. J. Chem.*, 1974, **12**(9), 1019.
4. Camciuc, M., Culture de l'espèce *Abelmoschus esculentus*, (L.) Moench en France. Etude du contenu moléculaire et perspectives de valorisation. Thèse INP Toulouse, France. 1997.
5. Planchon, G. and Collin, E., *Les Drogues Simples d' Origine Végétale*, tôme deuxième, Paris, 1896, p. 708–709.
6. Misra, G. and Mitra, C. R., *Res. Ind.*, 1964, **9**, 325.
7. Nee, T. Y., Cart, S. and Pollard, M. R., *Phytochemistry*, 1986, **25**(9), 2157.
8. Singh, B., *Phytomorphology*, 1968, **17**, 282.
9. Hegnauer, R., *Chemotaxonomie der Pflanzen.* IX Bd., Bürkhäuser Verlag, Basel, 1990, p. 22.
10. Buil, J. P., Laurent, R., Fourniol, J. P., Joulain, D. and Hardy, P. Y., *Parfums, Cosmetiques, Arômes*, 1989, **89**, 95.
11. Jennings, W. G. and Shibamoto, T., *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*. Academic Press, New York, 1980.
12. Kondjoyan, N. and Berdagué, J. L., *A Compilation of Relative Retention Indices for the Analysis of Aromatic Compounds. Edition du Laboratoire Flaveur*, INRA de Theix, France, 1996.
13. Davies, N. W., *J. Chromatogr*, 1990, **503**, 1.
14. McLafferty, F. W. and Staufer, D. B., *The Wiley/NBS Registry of Mass Spectral Data*. Wiley Interscience, 1989.
15. Stenhagen, E., Abrahamsson, S. and McLafferty, F. W., *Registry of Mass Spectral Data*. John Wiley Edn., New York, 1974.
16. Banque informatisée de spectres de masse pour

composés aromatiques, INRA Dijon, France, 1997.

17. Kim Ha, J. and Lindsay, R. C., *J. Food Comp. Anal.*, 1989, **2**, 118.
18. Lambert, M. A. and Wayne, C. M., *J. Chromatogr.*, 1972, **74**, 335.
19. Whittaker, R. H., in *The Biochemical Ecology of Higher Plants*, ed. E. Sondheimer and J. B. Simeone. Academic Press, New York, NY, 1970, pp. 43–70.
20. Loughrin, J. H., Manukian, A. Heath, R. R., Turlings, T. C. and Tumlinson, J., *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 11836.
21. Dennis, S. H., *Agricultural Insect Pests of the Tropics and Their Control*. Cambridge University Press, 1987, p. 611.
22. Dettner, K. and Reissenweber, F., *Biochem. Syst. Ecol.*, 1991, **19**(4), 291.
23. Shorey, H. H., Gaston, L. K., Gerber, R. G., Sisk, G. B. and Phillips, P. A., *Environ. Entomol.*, 1996, **25**(1), 114.
24. Borg-Karlson, B. K. A., *Chemica Scripta*, 1987, **27**, 313.
25. Arctander, S., *Perfume and Flavor Chemicals*. Allured Publishing Corporation, U.S.A., 1994.