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Volatile constituents in juice and oil of Australian wild lime (*Microcitrus inodora*)☆

Philip E. Shaw[a,*], Manuel G. Moshonas[a], Kim D. Bowman[b]

[a]*USDA, ARS, SAA Citrus and Subtropical Products Lab, 600 Avenue S., N.W., Winter Haven, FL 33881, USA*
[b]*U.S. Horticultural Research Laboratory, 2120 Camden Road, Orlando, FL 32803, USA*

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Abstract

Fifty-three volatile constituents from the juice and twenty from the peel oil of *Microcitrus inodora* have been identified by gas chromatographic and mass spectral analysis. All except seven had been reported earlier as citrus constituents. Since *M. inodora* is used as a parent for production of new citrus hybrids, this information will be useful to horticulturists, plant breeders and phytochemists. Published by Elsevier Science Ltd.

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1. Introduction

The Australian wild lime (*Microcitrus inodora* [F.M. Bail.] Swing.) is a member of the genus *Microcitrus*, one of six genera comprising the True Citrus Fruit Trees (Reuther, Webber & Batchelor, 1967). This and other species of *Microcitrus* are sexually and graft compatible with the genus *Citrus* and have been used in production of numerous hybrids (Barrett, 1985; Barrett, 1990; Bowman, 1997). *Microcitrus* species are of interest for citrus genetic improvement because of zygotic embryony, very short juvenility, rapid fruit maturation, resistance to *Phytophthora* diseases and nematodes, and a number of morphological traits that are unique or uncommon within *Citrus*. *M. inodora*, so named because the flowers were considered to be odorless, is a small tree that produces large lanceolate leaves and yellow oblong or elliptical fruit about 5 × 3 cm. Knowledge of components in *M. inodora* juice and peel oil would be helpful to plant breeders in screening derived hybrids for fruit quality traits.

A few studies have been reported in citrus in which composition of volatile constituents in juice or peel oil of the parent fruit have been used to help evaluate hybrid progeny. Moshonas, Shaw & Carter (1991) reported analytical data on composition of volatile constituents of juice and peel oil of Ambersweet orange (a hybrid of orange, mandarin and grapefruit) and the parent fruit, and showed that it was indistinguishable from true orange based on the profile of volatile constituents present. Scora (1975) used composition of peel oil in lemon to suggest its origin as a hybrid of citron and lime. One study used gas chromatographic profiles of leaf essential oils in the identification of several species of *Microcitrus* (Katayama, Nito & Machino, 1994), but no identification of specific essential oil components was carried out.

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* Corresponding author. Tel.: +1-941-293-4133; fax: +1-941-299-8678.

E-mail address: pshaw@citrus.usda.gov (P.E. Shaw).

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Table 1
Juice constituents of *M. inodora*

Compound	Identified by	GC area%[a]
Acetaldehyde	Rt	9.4
Methanol	Rt	tr[b]
Ethanol	Rt	14.6
Ethyl Acetate	Rt	0.07
1-Penten-3-ol	Rt	0.07
1-Penten-3-one	Ms-rt	tr
Hexanal	Ms-rt	0.63
2-Hexenal	Ms-rt	tr
cis-3-Hexen-1-ol	Ms-rt	0.38
Hexanol	Ms-rt	0.22
α-Pinene[c]	Ms-rt	0.31
3-Methyl-2,5-furandione[d]	Ms	tr
Myrcene[c]	Ms-rt	1.44
Sabinene[c]	Ms-rt	0.11
Octanal	Rt	0.07
α-Phellandrene[c]	Ms-rt	0.04
Limonene[c]	Ms-rt	68.5
trans-Ocimene[c]	Ms-rt	tr
cis-Ocimene	Ms-rt	0.03
cis-Linalool oxide	Ms-rt	0.03
trans-Linalool oxide	Ms-rt	0.03
Terpinolene[c]	Ms-rt	tr
Linalool[c]	Ms-rt	0.13
Ethyl hexanoic acid	Ms-rt	tr
Alloocimene[c]	Ms-rt	0.01
Benzoic acid	Ms-rt	tr
α-Terpineol	Ms-rt	0.02
Octanoic acid	Ms-rt	tr
Decanal	Rt	0.14
Neral[c]	Ms-rt	tr
Nerol	Ms-rt	tr
Geraniol[c]	Ms-rt	tr
Carvone[c]	Ms-rt	0.01
Geranial	Ms-rt	tr
Perillaldehyde[c]	Ms-rt	tr
Nonanoic acid	Ms-rt	tr
Decanoic acid	Ms-rt	tr
Elemol[d]	Ms	tr
Dodecanol	Ms-rt	tr
1,2,3,-Trimethoxy-5-(2-propenyl)-benzene	Ms	tr
Dodecanoic acid	Ms-rt	tr
Linalyl-3-methylbutanoate[c,d]	Ms	tr
Tetradecanol	Ms-rt	tr
Tetradecanoic acid[c]	Ms-rt	tr
Nootkatone	Ms-rt	tr
Pentadecanoic acid	Ms-rt	tr
Hexadecanol	Ms-rt	tr
7-Hydroxycoumarin	Ms-rt	tr
6-Methyl-8-(2,6,6-trimethyl-1-cyclo)-5-octen-2-one	Ms-rt	tr
7-Hydroxy-6-methoxycoumarin (scopoletin)	Ms-rt	tr
5-Methoxypsoralen[c]	Ms-rt	tr
4,9-Dimethoxy-psoralen[c,d]	Ms	tr
7-[(3,7-dimethyl-2,6-octadienyl) oxy]-coumarin[d]	Ms	tr

[a] Based on headspace GC analysis of juice sacs. The value listed for acetaldehyde includes methanol as well.

[b] tr, trace component too small to quantify by headspace GC.

[c] Also identified in *M. inodora* peel oil were γ-terpenine, β-caryophyllene and camphor.

[d] Tentative identification: no authentic sample was available for comfirmation of retention time.

2. Results and discussion

Fifty-three volatile constituents of *Microcitrus inodora* identified or tentatively identified in the juice by GC and GC–MS analyses are listed in Table 1. In most cases the mass spectrum and the GC retention time were compared to those of authentic samples for identification. Since the trace compounds noted in Table 1 were only identified in the concentrated methylene chloride extract of the juice, they could not be accurately quantified. Five components not normally found in citrus, and for which no authentic sample was available for direct comparison, were considered tentatively identified as indicated by footnote d. The seven constituents in Table 1 for which no mass spectrum was obtained in the juice extract are found in all citrus juices at levels approximating those found here; they all have distinctive retention times as well. Peel oil of *M. inodora* was also analyzed by GC and GC–MS, and fewer constituents were identified. Those juice constituents also identified in peel oil are indicated in Table 1 by footnote c. Three volatile constituents identified only in the peel oil, and not included in this table, were γ-terpinene, β-caryophyllene and camphor. The GC area percent values for the major and some minor constituents are also listed, as determined by HSGC of juice sacs.

Although most of the juice constituents identified are those common to citrus, there were several that had not been identified as citrus volatile constituents. These include ethylhexanoic and benzoic acids, and tetra- and hexa-decanols, which have been reported in other fruit (Maarse & Visscher, 1989) and 3-methyl-2,5-furandione, which is the anhydride of 2-methyl succinic acid reported in cherries (Maarse & Visscher, 1989). Three coumarins and two psoralen derivatives were identified or tentatively identified. Of these, 7-hydroxycoumarin and 5-methoxypsoralen were reported in citrus, and scopoletin was reported in other foods (Maarse & Visscher, 1989). 7-Geranoxycoumarin was found in lime oil, as was 5,8-dimethoxypsoralen, a compound closely related to the 4,9-dimethoxy isomer tentatively identified here (Stanley & Vannier, 1967). The relative amounts of the main constituents found in *M. inodora* are similar to those reported in the major citrus juices and flavor fractions (Shaw, 1986).

This compositional study shows the similarity of *M. inodora* to major citrus cultivars regarding volatile juice constituents present, and also shows several unique constituents that could serve as markers for horticulturists using *M. inodora* in plant breeding.

3. Experimental

3.1. Juice and peel oil samples

Three samples of *M. inodora* fruit were collected from the A. H. Whitmore Foundation Farm at Leesburg, FL in June 1997. Samples A and B each consisted of 16 fruit harvested evenly from all four quadrants of a single tree. Sample C consisted of 80 fruit harvested from two trees and combined. The peel from samples A (43.2 g) and B (42.8 g) was extracted with hexane, concentrated to small volume by a previously described method (Wilson, Shaw, McDonald, Greany & Yokoyama, 1990) and analyzed by gas chromatography–mass spectrometry (GC–MS) as described below. Juice sacs from each sample were collected by cutting the fruit in half and scraping the sacs into a beaker. Samples A, B and C yielded 15.8, 15.3 and 79.4 g of juice sacs, respectively. Juice sacs from sample C were placed in a four-layer piece of cheese cloth which was crushed in a mortar with a pestle to yield 75 ml of *M. inodora* juice.

3.2. Headspace GC analysis of juice and juice sacs

A Hewlett-Packard Model 5890 gas chromatograph equipped with a chrompack Model PTI purge-and-trap headspace sampler (Raritan, NJ) was used for juice analyses. Headspace sampling and cryfocusing were accomplished by placing 5 ml of juice or 5 g of juice sacs in a sample flask equilibrated at 40°C ± 1°C with water circulating through a copper coil surrounding the sample flask. A helium purge at 18 ml/min flowing above the juice for 5 min was used to sweep the headspace gases past a condenser cooled to 0°C (to condense some of the water vapors) and through a glass transfer line at 120°C into an uncoated piece of capillary tubing 20 cm long. A 10 cm portion of this capillary tubing was cooled to −130°C to cryfocus the juice volatiles by condensation. Once the sample was collected, the cooled portion of the capillary tubing was flash heated to 250°C to inject the sample onto the gas chromatographic (GC) column.

The GC column employed was a nonpolar HP-5 capillary column 30 m long and 0.53 mm i.d. with 2.65 μm film thickness (Hewlett-Packard, Wilmington, DE). Both the FID detector and injection port were maintained at 250°C and oven temperature programming was 40°C initially, increasing at 6°C per min to final temperature of 200°C. Column flow rate was 8 ml/min.

3.3. Mass spectral identification of volatile components

Volatile *M. inodora* juice components were separated for identification by GC–MS. Freshly squeezed (65 ml) juice was extracted with 150 ml of CH_2Cl_2 (Burdick and Jackson, capillary GC–MS grade), and the extract was dried (Na_2SO_4) and concentrated to small volume (<0.5 ml) under reduced pressure on a rotary evaporator. Samples (0.1 μl) of the concentrated extracts of juice and of peel oil (described above) were analyzed by GC–MS using previously reported conditions (Moshonas & Shaw, 1994). A Hewlett-Packard Model 5970B, MSD, GC–MS was used with a 0.32 mm × 30 m fused silica column of crossed-linked 5% phenylmethyl siloxane. Column oven temperature programming was 55°C for 9 min, raised at 7.5°C per min to 220°C and held there for 30 min. Injection port and ionizing source were kept at 275°C, and the transfer line was kept at 280°C. Mass spectral matches were made by comparison of mass spectra and retention times with those of authentic compounds, as indicated in Table 1.

References

Barrett, H. C. (1985). *Fruit Var. J*, *39*, 11.

Barrett, H. C. (1990). *Fruit Var. J*, *44*, 113.

Bowman, K. D. (1997). *Hort Science*, *32*, 441.

Katayama, Y., Nito, N., & Machino, J. (1994). *Bull. Fac. Agric. Saga Univ*, *77*, 47.

Maarse, H., & Visscher, C. A. (1989). In *Volatile compounds in food* (6th ed). In *Qualitative and quantitative data*, *Vol. 1*. Zeist: TNO-CIVO Food Analysis Institute.

Moshonas, M. G., & Shaw, P. E. (1994). *J. Agric. Food Chem*, *42*, 1525.

Moshonas, M. G., Shaw, P. E., & Carter, R. D. (1991). *J. Agric. Food Chem*, *39*, 1416.

Reuther, W., Webber, H. J., & Batchelor, L. D. (1967). *The citrus industry*, *Vol. 1* (p. 326). Riverside, CA: University of California Press.

Scora, R. W. (1975). *Int. Flavours Food Addit*, *6*, 342.

Shaw, P. E. (1986). In I. D. Morton, & A. J. McLeod, *Food flavours. Part B: the flavour of beverages* (p. 337). New York: Elsevier.

Stanley, W. H., & Vannier, S. H. (1967). *Phytochemistry*, *6*, 585.

Wilson III, C. W., Shaw, P. E., McDonald, R. E., Greany, P. D., & Yokoyama, H. (1990). *J. Agric. Food Chem*, *38*, 656.