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Essential Oil Constituents of *Ocimum micranthum* Willd.

Denys J. Charles,[†] James E. Simon,*[,†] and Karl V. Wood[‡]

Department of Horticulture and Department of Chemistry and Campus-Wide Mass Spectrometry Center, Purdue University, West Lafayette, Indiana 47907

The essential oil from the leaves, flowers, and stems of *Ocimum micranthum* Willd., a strongly aromatic annual herb used as a local beverage and medicinal plant and native to the lowlands of Central and South America and the West Indies, has for the first time been examined. The essential oil content between plant parts varied significantly with 1.54, 0.63, and 0.08 (percent volume/fresh weight) yield from the leaves, flowers, and stems, respectively. Twenty compounds in the essential oil were identified: 1,8-cineole, eugenol, β-caryophyllene, β-selinene, and elemene isomers were found to be the major constituents. Essential oil composition also varied by plant part. Eugenol, the major constituent in leaves, was present only in trace amounts in flowers and stems. β-Selinene, a minor component in the leaves, was a major constituent in the flowers and stems. Total sesquiterpenes accounted for 48.4, 85.8, and 78.5% of the oil in the leaves, flowers, and stems, respectively. This is the first report of an *Ocimum spp.* to be high in elemenes, 1,8-cineole, β-caryophyllene, and β-selinene.

Ocimum micranthum Willd., a strongly aromatic annual herb native to the lowlands of Central and South America and the West Indies, is used locally to flavor beverages and soups (Morton, 1981). It is also used in domestic medicine for treating colds, fever, stomach disturbances, and dysentery and as a remedy for screwworms parasitizing nasal passages of people in the tropics (Morton, 1981; Standley and Williams, 1978). A decoction of the plant is also used to kill the larvae. The plant is locally used in the treatment of epilepsy, nervous trouble, and earaches, as a remedy for influenza, colic, and convulsion in children, and for painful menstruation (Morton, 1981).

A large part of the aroma and flavor of this plant is due to the presence of essential oils, some constituents of which have also been shown to have biological activity and could be responsible for the plant's use in traditional medicine. While the constituents in the essential oils of *Ocimum basilicum* have been reviewed (Guenther, 1949; Lawrence et al., 1971; Simon et al., 1984), the essential oils of other *Ocimum* species have not been as extensively studied. Many *Ocimum spp.* have been selected and bred for specific essential oil constituents for use in flavoring and perfume products (Sobti et al., 1982a–c). Lesser known yet highly aromatic plants such as *O. micranthum* could serve directly as natural plant sources for specific natural products or used in interspecific hybridizations. Thus, the objective of this study was to determine the essential oil content and composition extracted from fresh leaves, flowers, and stems from *O. micranthum* Willd. in order to evaluate its potential use as a source of aroma chemicals.

MATERIALS AND METHODS

Plant Materials. Seeds of *O. micranthum* Willd. were obtained from Companion Plants (Athens, OH) where it was listed commercially as Peruvian basil. Seeds were sown in the greenhouse and transplanted into the field at the Purdue University Vegetable Research Farm (Oakley silt loam soil) during the summer of 1987. The entire plants were harvested in full-bloom (in October), weighed, and leaves, flowers, and stems immediately separated. Essential oils were then extracted from each of three 50-g samples of fresh leaves, flowers, and stems. Another set of three 50-g samples of leaves, flowers, and stems was dried in an oven at 35 °C and essential oil extracted from the dried plant material. A dried plant specimen was deposited in the Herbarium of the Field Museum of Natural History, Chicago, IL.

Essential Oil Extraction and Isolation. Essential oil was extracted by hydrodistillation for 1 h (fresh samples) and 1 h 15 min (dry samples) with a modified clevenger trap (ASTA, 1968). The essential oil content was determined on a volume to fresh weight or dry weight basis. The values for essential oil content of the three replications were averaged and standard deviations calculated. The essential oil samples were stored in silica vials with Teflon-sealed caps at 2 °C in the absence of light.

Gas Chromatography. Essential oil samples from each of the distillations were analyzed separately and the relative peak areas for individual constituents averaged for each plant part.

[†] Department of Horticulture.
[‡] Department of Chemistry and Campus-Wide Mass Spectrometry Center.

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Table I. Constituents of the Essential Oils of *O. micranthum* Willd.

peak	compound	leaves, %	flowers, %	stem, %	RT
1	α-pinene	0.24 ± 0.05	0.15 ± 0.05	–[a]	5.08
2	*d*-camphene	0.04 ± 0.00	0.03 ± 0.00	–	5.51
3	β-pinene	1.33 ± 0.17	0.81 ± 0.07	0.06 ± 0.00	6.30
4	myrcene	0.13 ± 0.03	0.16 ± 0.00	0.04 ± 0.00	6.48
5	1,8-cineole	20.02 ± 3.10	7.03 ± 0.76	10.89 ± 1.67	8.15
6	*trans*-β-ocimene	0.73 ± 0.02	0.21 ± 0.00	0.07 ± 0.02	8.43
7	linalool	2.71 ± 0.27	3.13 ± 0.56	1.01 ± 0.16	10.79
8	terpinen-4-ol	–	–	0.12 ± 0.00	14.02
9	terpineol	1.10 ± 0.17	0.18 ± 0.02	1.68 ± 0.16	14.74
10	γ-elemene isomer	–	0.17 ± 0.02	0.52 ± 0.08	22.64
11	eugenol	20.50 ± 3.70	tr[b]	tr	23.47
12	γ-copaene	–	–	0.61 ± 0.07	23.56
13	β-elemene isomer	4.18 ± 0.36	8.00 ± 1.16	8.58 ± 1.12	23.66
14	β-caryophyllene	19.26 ± 2.85	18.93 ± 2.86	20.51 ± 3.20	25.17
15	β-selinene	4.73 ± 1.72	14.03 ± 1.67	11.89 ± 2.10	26.50
16	azulene-type sesquiterpene	1.49 ± 0.21	1.50 ± 0.07	3.38 ± 0.76	26.78
17	spiro-type sesquiterpene	2.23 ± 0.75	1.61 ± 0.12	1.01 ± 0.15	27.56
18	γ-elemene isomer	14.44 ± 1.62	36.83 ± 3.99	28.16 ± 3.98	28.58
19	β-elemene isomer	0.51 ± 0.06	0.57 ± 0.05	0.71 ± 0.06	30.23
20	γ-elemene isomer	1.55 ± 0.64	4.32 ± 1.22	4.29 ± 0.86	31.07
		95.19	97.66	94.05	

[a] Absent. [b] Trace (<0.03%).

Essential oil constituents were identified on the basis of retention time and coinjection with authentic compounds and the relative percentages determined using a Varian 3700 gas chromatograph equipped with FID and an electronic 4270 integrator. A fused silica capillary column (12 m × 0.2 mm(i.d.)) with an OV 101 (Varian, polydimethylsiloxane) bonded phase was used. Direct injection of 0.5 μL of essential oil samples with He as a carrier gas (100:1 split vent ratio) and oven temperatures held isothermal at 80 °C for 2 min and then programmed to increase at 3 °C/min to 180 °C gave complete elution of all peaks (sensitivity 10^{-10}). The injector and detector temperatures were 180 and 300 °C, respectively.

GC/Mass Spectrometry Analysis. Pure compounds (α-pinene, *d*-camphene, β-pinene, myrcene, 1,8-cineole, *trans*-β-ocimene, linalool, terpinen-4-ol, terpineol, eugenol, β-caryophyllene) and essential oil constituents were analyzed by a Finnigan 4000 GC/MS using electron impact and hooked online to a Data General Nova/4 data processing system for retention time determination and compound identification in a manner previously described (Simon and Quinn, 1988).

The GC conditions were as follows: direct injection of 1.0 μL of sample diluted 10:1 with MeOH; fused silica column (30 m × 0.25 mm) with DB-1 bonded phase (polydimethylsiloxane) (J&W Scientific, Inc.); He carrier gas with a column pressure of 10.5 psi and split vent of 40 mL/min; oven program, 80 °C at 2 min rising to 180 °C at 2 °C/min; injector temperature, 225 °C.

The MS conditions were as follows: ionization voltage, 70 eV; emission current, 40 μA; scan rate, 1 scan/s, mass range, 40–500 Da; ion source temperature, 160 °C.

RESULTS AND DISCUSSION

We report here, for the first time, the analysis of the essential oil of *O. micranthum* Willd. Yield (percent volume/fresh weight) of essential oil varied by plant part: 1.54 ± 0.12 for leaves, 0.63 ± 0.08 for flowers, and 0.08 ± 0.01 for stems. All essential oil constituents present in concentrations above 0.3% were identified (Table I). Identification of all components except the sesquiterpenes was made by comparison with authentic material. Identification of most sesquiterpenes was accomplished by matching the mass spectra of each compound with three different MS compound libraries for best fit (Finnigan; *EPA/NIH Mass Spectral Data Base*, 1978; *Registry of Mass Spectral Data*, 1974) since pure standards were unavailable. The mass spectrum of β-caryophyllene matched the spectrum reported in the MS libraries (Figure 1). A listing of the mass spectra for the five compounds identified as elemene isomers is presented in Table II. These compounds could be identified as γ- or β-type elemene isomers since their mass spectra were similar to the published reference spectra of β- and γ-elemene (Table II), particularly the diagnostic ions within the reference spectra (γ-elemene, *m/z* 93 and 121; β-elemene, *m/z* 67

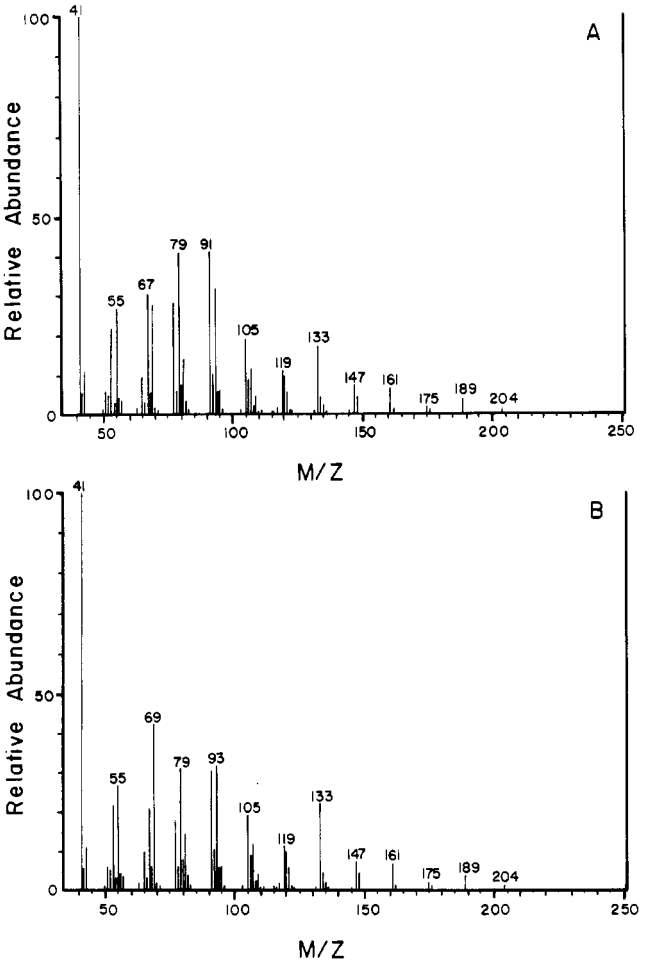

Figure 1. Comparison of the electron impact mass spectrum of β-caryophyllene from MS library (A) and from *O. micranthum* Willd. (B).

Table II. MS of Elemene Isomers

	β-elemene			γ-elemene			
m/z	1 (13)[a]	2 (19)	std[b]	1 (10)	2 (18)	3 (20)	std
41	100	88	95	100	100	74	41
53	50	71	50	32	49	20	20
55	45	41	48	33	41	13	25
67	68	100	56	29	58	13	22
68	65	98	81	2	17	2	16
77				29	23	22	20
79	42	43	41	38	32	24	26
81	69	47	100	21	32	8	27
91	26	17	24	38	35	29	23
93	57	25	66	64	52	85	66
105					38	18	28
107	32	18	42	36	32	14	40
119	14	14	20	20	24	8	21
121	17		30	79	56	100	100
133	11	14	14	6	16	0.8	15
136						57	20
147	17	20	22	3	11		5
161	9	9	21	13	16	24	21
175	2	2	2	0.9	1	0.6	1
189	8	11	16	5	9	2	8
204	0.4	0.5	2	6	6	1	6

[a] Numbers in parentheses represent the peaks listed in Table I. [b] Standard from MS libraries.

and 68). Further conformation was not possible in lieu of either authentic material or more reference spectra. Peaks 16 and 17 labeled azulene-type and spiro-type sesquiterpenes, respectively, had fragmentation patterns representative of these two structural types of sesquiterpenes (as found in the MS libraries), but more complete identification was not possible.

Essential oil composition varied by plant part with the major constituents including 1,8-cineole, eugenol, β-caryophyllene, β-selinene, and elemene isomers. Eugenol, the major constituent in leaves (20.50%), was present only in trace amounts in the flowers and stems. While 1,8-cineole was greatest in leaves (20.02%), its relative content was markedly less in flowers (7.03%) and stems (10.89%). β-Selinene, a minor component in the leaves (4.73%), was a major constituent in the flowers (14.73%) and stems (11.89%). The concentration of sesquiterpenes (Table I, peaks 10 and 12–20) was very high in this plant species, reaching 48.4%, 85.8%, and 78.5% in the leaves, flowers and stems, respectively. Among the sesquiterpenes, β-caryophyllene, β-selinene, and the elemene isomers accounted for 44.7%, 82.7%, and 74.1% in the leaves, flowers, and stems, respectively.

Linalool, a major constituent in the essential oil of European sweet basil, *O. basilicum* L. (Guenther, 1949; Lawrence et al., 1972), occurs in much lower concentrations in this species, with only 3.13% in flowers and 2.71% and 1.01% in leaves and stems, respectively. Methylchavicol, the second major constituent in sweet basil essential oil, was absent in this species. In consideration of the differences in chemical composition which occur in both *O. basilicum* and *Ocimum sanctum*, Hegnauer (Lawrence et al., 1972) subdivided each species into four chemical types of races including for *O. basilicum*, a methylchavicol–linalool, camphor, methyl cinnamate, and a eugenol type, and for *O. sanctum*, a citral, eugenol, methylchavicol, and chavibetol type. The chemical profile of *O. micranthum* does not fit into any of these or other chemical races reported for *Ocimum* and is the only *Ocimum spp.* reported to be very high in elemene, 1,8-cineole, β-caryophyllene, and β-selinene.

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Registry No. α-Pinene, 80-56-8; *d*-camphene, 5794-03-6; β-pinene, 127-91-3; myrcene, 123-35-3; 1,8-cineole, 470-82-6; *trans*-β-ocimene, 3779-61-1; linalool, 78-70-6; terpinen-4-ol, 562-74-3; terpineol, 8000-41-7; eugenol, 97-53-0; γ-copaene, 123750-79-8; β-caryophyllene, 87-44-5; β-selinene, 17066-67-0.

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