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COMPOSITION OF THE ESSENTIAL OIL OF *OCIMUM TRICHODON* GROWN IN RWANDA

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Ocimum trichodon Baker ex Guerke (Lamiaceae) is reported to occur in eastern tropical Africa, in Cameroon and, less frequently, in Rwanda (1). In the latter country the plant, called Ihonoranzobe or Umwenya w'ifumbi, is extensively used in traditional medicine, e.g., to cure cough and headache and as an analgesic after childbirth (2). In a previous study the essential oil of *O. trichodon* showed an antimicrobial activity against some bacteria and a yeast (3). Further studies on this subject are in hand. Because no chemical data on the species in question could be found, and because of the statement of Hegnauer (4) that striking differences in the essential oil composition within species of the genus *Ocimum* may exist, we were interested in analyzing some samples of the volatile oil of *O. trichodon* grown in Rwanda.

MATERIALS AND METHODS

Leaves and flowers of *O. trichodon* growing wild in Butare (Southern Rwanda; samples 213C, 342C, 343C, 395C) and in Kibungo (Eastern Rwanda; samples 268J, 280J, 377J) were collected in January/February 1984. Voucher specimens were deposited in the Herbarium of Curphametra, the National University of Rwanda, at Butare, in that of the National Institute of Scientific Research (INRS) at Butare, and in that of the Botanical Institute, University of Liège, Belgium, under the numbers Ayobangira 1660 and 1697.

The samples of fresh plant material were subjected to hydrodistillation for 2 h using a Clevenger-type apparatus. The yield of oil varied from 0.4% to 0.8% (v/w). Since preliminary gc analyses showed that one sample (268J) differed qualitatively from the other six samples, its was submitted to liquid-solid chromatography (lsc) over silica deactivated by addition to 5% H_2O (5) in order to separate the hydrocarbons from the oxygen-containing compounds (6). The same lsc procedure was carried out with one of the six samples, viz., sample 213C.

Glc was performed on a gas chromatograph Packard 436S equipped with FID and connected with a digital integrator Packard 603 (Packard Instrument BV, Delft, the Netherlands). Gc conditions: column fused silica, 60 m × 0.25 mm i.d., coated with Durabond-DB 1 (J&W Scientific); film thickness 0.25 μm; oven temperature programmed, 60-200° (3°/min); carrier gas N_2; splitting ratio 1:100; gas velocity 16 cm/sec; injector and detector 200°. The seven oil samples and the fractions obtained by lsc were also analyzed by glc on packed columns under conditions comparable to those described previously (7).

The identity of the compounds was assigned by comparing their gc retention times with those of authentic samples. For this purpose also another 50 m capillary, fused silica column coated with CP-Wax 57cb (Chrompack BV, Middelburg, the Netherlands) was used. When necessary, gc/ms was performed as described before (8). The percentage composition of the oil samples was computed from the gc peak areas without correction factors.

RESULTS AND DISCUSSION

The percentage composition of the seven essential oil samples was determined using the 60 m fused silica Durabond-DB 1 column (see Table 1). The components are listed according to the elution on this column. All samples were dominated by eugenol (44-81%). Oct-1-en-3-ol and β-caryophyllene epoxide were

the only other oxygen-containing components which were found in all samples and amounted to more than 1% in some of them. Elemicin was detected in one sample in a relatively high amount (5.8%). To check this result, a pentane-Et_2O (1:1) extract was also analyzed, which confirmed the occurrence of elemicin. In 1981 this compound was reported to be the main component of the essential oil of *Ocimum carnosum* (9).

Among the monoterpene hydrocarbons (MTHC) listed, *cis*-β-ocimene (2-16%) and *trans*-β-ocimene (1-9%) were the most abundant ones, whereas the other MTHC were found in minor quantities. The main sesquiterpene hydrocarbons were β-caryophyllene (2-9%), germacrene-D (1-10%), and α-farnesene (2-5%; tentatively identified). In addition to the components listed in Table 1, traces of *trans*-hex-3-en-1-ol, α-fenchene, α-terpinene, β-phellandrene, 1,8-cineole, γ-terpinene, *trans*-sabinene hydrate, α,*p*-dimethyl styrene, terpinolene, *cis*-sabinene hydrate, terpinen-4-ol, β-bourbonene, β-farnesene isomer, and α-bisabolol were detected.

TABLE 1. Composition of the Essential Oil of *Ocimum trichodon*[a]

Compound	Samples						
	213C	342C	343C	395C	268J	280J	377J
trans-hex-2-enol[b]	0.3[c]	t[d]	0.2	0.1	0.3	0.2	0.4
cis-hex-3-en-1-ol	0.1	—	0.2	0.4	t	t	0.4
α-thujene	t	t	0.1	t	0.1	t	—
α-pinene	t	0.2	0.4	t	0.1	0.1	0.1
camphene	t	0.1	0.2	t	0.1	t	t
oct-1-en-3-ol[b]	0.8	0.5	1.1	0.8	1.3	0.3	1.5
3-octanone	0.1	0.1	0.2	t	0.1	t	0.2
sabinene	t	0.1	0.2	t	0.1	t	t
β-pinene	t	0.1	0.2	t	0.1	0.1	0.1
3-octanol	t	—	—	t	t	t	0.1
myrcene	0.2	0.3	0.6	0.1	0.6	0.3	0.4
p-cymene	t	t	0.2	t	t	t	t
limonene	t	0.1	1.9	0.1	0.1	0.4	0.4
cis-β-ocimene	10.5	6.3	7.8	2.2	14.6	10.3	15.9
trans-β-ocimene	4.4	3.5	2.0	1.4	8.3	7.5	8.8
1-octanol	t	0.1	0.2	0.1	t	t	0.2
allo-ocimene isomer[b]	0.1	0.1	0.1	t	0.3	0.1	0.1
linalool	0.3	0.1	0.8	0.1	0.2	0.2	0.2
α-terpineol	0.1	0.2	0.2	0.1	0.7	0.3	0.3
unknown	0.1	0.2	0.1	0.1	1.3	0.6	0.4
thymol	—	—	0.1	0.1	—	—	t
eugenol[b]	74.7	64.6	54.8	81.2	44.2	60.4	58.8
methyl cinnamate	t	0.1	0.1	t	0.1	0.1	t
methyleugenol	t	t	t	0.2	0.1	t	0.1
α-copaene	0.1	0.6	2.0	0.4	0.7	0.5	0.2
β-elemene	0.2	0.6	1.7	0.5	0.6	0.5	0.5
β-caryophyllene[b]	2.0	6.0	8.6	3.6	3.2	6.5	2.9
bergamotene isomer	0.1	0.1	0.3	0.1	0.2	0.2	0.1
α-humulene	0.2	0.5	0.6	0.3	0.3	0.6	0.2
germacrene-D[b]	1.2	6.2	10.0	4.3	2.9	2.4	3.1
α-farnesene[b,e]	3.5	3.9	1.6	2.2	5.2	4.5	2.9
γ-cadinene	t	0.2	0.1	0.1	0.3	0.1	0.1
δ-cadinene	0.1	0.2	0.4	0.2	—	0.2	0.2
elemicine[b]	—	—	—	—	5.8	—	—
β-caryophyllene epoxide[b]	0.1	1.8	0.6	0.1	1.5	0.8	0.2
apiole	0.1	0.2	0.1	t	0.5	0.2	t

[a]Compounds listed in order of their elution on Durabond-DB 1; for conditions see Materials and Methods.

[b]Identification also based on mass spectrum obtained by gc/ms.

[c]Peak area percentage.

[d]Trace (<0.05%).

[e]Tentatively identified.

In conclusion, it can be stated that the presence of elemicin in one sample was the most striking difference noticed on comparison of the essential oil samples analyzed. Because of the high content of eugenol in some of the samples, the oil of *O. trichodon* might serve as a valuable source of that compound, which is used in dentistry.

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METABOLITES OF *NECTRIA FUCKELIANA*

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Nectria fuckeliana Booth (Pyrenomycetes) was isolated from Douglas fir near Lumby, B.C., by A. Funk, Pacific Forest Research Center, Canadian Forestry Service, Victoria, British Columbia. The fungus is a wound parasite sometimes associated with dieback but most frequently found on damaged, decaying logs (1). We became interested in the metabolites produced by this fungus when Funk observed that on several occasions, orange-red, needle-like crystals appeared on the surface of potato dextrose agar cultures of *N. fuckeliana*. We report herein the isolation and identification of two of these metabolites.

EXPERIMENTAL

A culture of *N. fuckeliana* PFRC 537 was obtained from A. Funk and deposited in the University of Alberta Microfungus Collection (accession number UAMH 5025).

PREPARATION OF FUNGUS.—The fungus was grown on solid media (potato dextrose agar, 20°, 4-6 weeks) or in liquid still culture (malt extract media, 24°, 10 months).

EXTRACTION, ISOLATION, AND IDENTIFICATION.—The aqueous broth (10 liters) was extracted with EtOAc (48 h). Concentration of the extract gave a mixture of products (0.28 g). Flash chromatography on silica gel and elution with Et_2O-Skellysolve B (1:20) afforded 2,5-dimethoxy-3,6-dimethylbenzoquinone (9.4 mg) and (−)-mellein (6.1 mg). The orange-red crystals collected from solid cultures of *N. fuckeliana* were shown to be 2,5-dimethoxy-3,6-dimethylbenzoquinone. The compounds were identified by comparison of their physical (mp) and spectral (ir, nmr, ms, uv) properties with those reported in the literature (2,3).