Essential Oil of Alfavaca, *Ocimum gratissimum*, from Brazilian Amazon

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^{13}C NMR, GC and GC/MS analysis of the steam distillate from leaves and stems of alfavaca, *Ocimum gratissimum,* from the Brazilian Amazon resulted in the identification of methyl eugenol (46.83%) and eugenol (19.26%) as the main components, and another 34 compounds, mainly monoterpene and sesquiterpene hydrocarbons.

Alfavaca, *Ocimum gratissimum* (Labiatae), appears to have been introduced by slaves from Africa by the Portuguese in the 16th or 17th century or it may be a geographical variety native to Brazil, its origin is still doubt. The shrub-like plant is cultivated in the country-sides of the State of Pará and sold in the “Ver-o-peso” market in Belém. It is utilized as a condiment in regional dishes of the cuisine of Brazil. Decoctions of the leaves or of the whole herbs are used in the treatment of fever and coughs, as a diaphoretic and stomachic, and particularly in treating diarrhoea [1] in native medicine in Nigeria. Aqueous solutions of *O. gratissimum* have been shown to have appreciable antibacterial activity [2], antimicrobial efficiency against bacteria was found for the essential oil in ref. [3], the oil of a new strain of *O. gratissimum* showed toxicity against betelvine pathogenic fungi [4].

Oil distilled from experimentally cultivated *Ocimum gratissimum* from Albania consisted of 67–73% eugenol [5], 54–94% eugenol content is reported from Ocimum oil from Eastern and Western Georgia (U.S.S.R.) [6] and Sobti *et al.* reported an eugenol content of 60–80% in selectively cultivated and hybridized strains of *O. gratissimum* [7]. In 1986 a Russian researcher group compared the chemical composition of ten selections of *O. gratissimum* and found altogether 30 components, eugenol (32–91%), germacrene D (2–16%), (*Z*)-β-ocimene (0.3–15%), (*E*)-β-ocimene (trace-12%), Δ^3-carene (0–11%) and β-caryophyllene (0.6–7%) comprising the main constituents [8]. Numerous components were characterized in the leaf and flower oils of *O. gratissimum* grown in Taiwan, eugenol being the main component (85 and 46%, respectively) [9]. In a recent paper the oil composition from plants grown in North America (85,1% eugenol, 3.6% caryophyllene, 2.6% terpinen-4-ol and 2.6% germacrene D) [10] was described. Methyl cinnamate (67%) was reported to be the main constituent of an Ocimum oil from Pakistan [11], the oil from cultivated plants from Nigeria contained thymol and no eugenol [12]. 47.6% Thymol, 16.2% *p*-cymene, 6.2% α-terpinene, 4.2% Δ^3-carene, 4.0% camphene, longifolene, α-pinene, α-terpineol, caryophyllene and limonene were reported as the main constituents of an oil from the leaves and influorescence of *O. gratissimum* [13]. Essential oil from plants wildly growing in Rwanda contained thymol (35%), eugenol (11%) and *p*-cymene (18%) as main constituents, corresponding cultivated plants contained 47% thymol, 0.3% eugenol and 23% γ-terpinene [14].

In a recent paper [15] a first analysis of the oil of *O. gratissimum* from Brazil was reported and the chemical structures of 16 components were clarified, *p*-cymene (30%), (*E*)-β-farnesene (19%), thymol (13%), myrcene (6%) and alloaromadendrene (5%) comprising the major constituents. The discrepancy between the Brazilian *gratissimum* oil most of the others mentioned above regarding their constituents prompted us to reinvestigate the composition of this oil.

Experimental

Plant material

The material was collected in April 1986 near Salvaterra, Marájó Island, Pará (Brazil). The botanical identification was made by comparison

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with authentic samples registered in the herbarium of Emilio Goeldi Museum (Belem).

Steam distillation

Fresh leaves and stems were subjected to steam distillation according to accepted methods [16].

GC

Capillary GC was performed on a) Hewlett-Packard 5890 A gas chromatograph equipped with a Shimadzu Chromatopac CR 3 A data system, and b) Perkin Elmer Sigma 1 chromatograph. Injectors 240°, FIDs 260°, split injection. FSCC SE 54 (25 m × 0.25 mm), temp. progr. 4 min at 60°, 60–260° at 4°/min. Carrier gas N_2, 22 cm/sec linear gas velocity. The retention indices were determined by reference compounds and compared with those from previous publications.

GC/MS

a) Quadrupole mass spectrometer Finnigan 3200 E with Data System 6000, 25 m FSCC SE 54, temp. progr. as above, carrier gas He, 2 sec/scan, 70 eV EI spectra; b) Finnigan MAT 90 GCMS combination, 25 m FSCC SE 30, 4 min at 60 °C, 60–220° at 3°/min, 1 sec/scan. Mass spectra were compared with those from authentic samples and with spectra from the literature.

^{13}C NMR

Spectra were recorded on a JEOL PFT-100 spectrometer in C_6D_6 solutions, using TMS as internal standard.

Results and Discussion

The pentane solution of the alfavaca *(O. gratissimum)* oil obtained by conventional steam distillation (2.1% yield) was analyzed by GC, GC/MS and ^{13}C NMR spectroscopy. The oil consisted of two main constituents (**25** and **28** in Table I) with a total of 66%, the retention times and mass spectra being congruent with those of authentic eugenol (**25**) and methyl eugenol (**28**). A proton-decoupled ^{13}C NMR spectrum of the oil showed signals, the chemical shifts of which also could be assigned with those of the two phenol drivatives **25** and **28** [17].

Comparing the composition of the oil investigated with that of the Brazilian oil reported in ref. [15], no similarity is found. While Maia *et al.* [15] identified *p*-cymene (30%) as the main constituent, methyl eugenol (**28**) and eugenol (**25**), all together 66%, were the main components in our oil sample. Eugenol quite often was found as the main *gratissimum* oil constituent (see above), while none of these two substances were detected in the oil reported in [15].

Among the monoterpene hydrocarbons we found altogether 13 $C_{10}H_{16}$ hydrocarbons by GC, GC/MS and mass chromatography with characteristic fragment ions (Table I), tricyclene (**1,** trace), thujene (**2,** trace), camphene (**3,** 0.14%), α-terpinene (**11,** 0.14%), limonene (**12,** 0.2%, *(Z)*-ocimene (**14,** 3.65%), *(E)*-ocimene (**15,** 0.33%) and terpinolene (**17,** 0.11%) which have not been identified in Brazilian alfavaca before. Out of all monoterpenes, *(Z)*-ocimene (**14,** 3.65%) made the greatest contribution to these compounds, followed by β-pinene (**7,** 0.44%), myrcene (**9,** 0.35%) and *(E)*-ocimene (**15,** 0.33%). The aromatic terpenoid *p*-cymene was only detected in trace amounts. With respect to its monoterpene hydrocarbon pattern the oil investigated resembled well some of the specimen analyzed by Zamureenko *et al.* in ref. [8].

The monoterpene pattern of the sample investigated was also different from that in ref. [15]. Myrcene (**9**), with 5.64% its major $C_{10}H_{16}$ component in [15], was only present in minute quantity (0.35%) in our oil sample, whereas 1,8-cineol (**13**), the main monoterpenoid oxygen compound (3.28%) was not found in ref. [15] at all. No thujone, borneol, thymol and carvacrol, altogether contributing 16.33% to the analysis in [15], and for all of which we have retentional data, mass spectra and authentic material, was found.

Among the oxygenated monoterpenes, 1,8-cineol (**13**) was the main constituent (3.28%), furthermore we identified linalool (**18,** 1.32%), myrcenol (**20,** 0.19%), terpinen-4-ol (**21,** trace) and α-terpineol (**22,** 0.31%), the aliphatic 1-octen-3-ol (**8,** 0.15%) and octan-3-ol (**10,** 0.08%). In addition to the two main components **25** and **28** also small amounts of benzaldehyde (**5**), estragol (**23**) and methyl cinnamate (**26**) were found as aromatic plant constituents.

Nine signals for *m/z* 204, representing the molecular ions of $C_{15}H_{24}$ sesquiterpene hydrocarbons, were detected by mass chromatography, one of the

Table I. Constituents of the essential oil from fresh leaves and stems of Brazilian *O. gratissimum.*

No.	Compound	m.w.	% total (FID)	Ret. index (SE 54)	Method of identification*
1	Tricyclene	136	trace	923	RI MS MC
2	Thujene	136	trace	927	RI MS MC
3	α-Pinene	136	0.27	932	RI MS MC CC
4	Camphene	136	0.14	946	RI MS MC
5	Benzaldehyde	106	trace	963	RI MS
6	Sabinene	136	0.08	972	RI MS MC
7	β-Pinene	136	0.44	975	RI MS MC
8	1-Octen-3-ol	128	0.15	980	RI MS CC
9	Myrcene	136	0.35	991	RI MS MC
10	Octan-3-ol	130	0.08	1005	MS MC
11	α-Terpinene	136	0.14	1017	RI MC CC
12	Limonene	136	0.20	1028	RI MS MC
13	1.8-Cineol	154	3.28	1030	RI MS MC
14	*(Z)*-Ocimene	136	3.65	1038	RI MS MC
15	*(E)*-Ocimene	136	0.33	1048	RI MS MC
16	γ-Terpinene	136	0.07	1059	RI MC
17	Terpinolene	136	0.11	1088	RI MC CC
18	Linalool	154	1.32	1100	RI MS MC
19	$C_{10}H_{18}O$	154	0.07	1130	MC
20	Myrcenol	154	0.19	1168	MS MC
21	Terpinen-4-ol	154	trace	1177	RI MS
22	α-Terpineol	154	0.31	1192	RI MS MC
23	Estragol	148	trace	1200	RI MC
24	δ-Elemene	204	0.17	1338	MS MC
25	Eugenol	164	19.26	1364	RI MS MC NMR
26	Methyl cinnamate	162	trace	1370	MS
27	β-Elemene	204	2.03	1394	MS MC
28	Methyl eugenol	178	46.83	1414	RI MS MC NMR
29	Caryophyllene	204	5.50	1423	RI MS MC CC
30	Humulene	204	1.43	1457	RI MS MC CC
31	Alloaromadendrene	204	0.40	1464	MS MC
32	$C_{15}H_{24}$	204	0.48	1478	MS MC
33	Aromadendrene and Germacrene D	204	2.02	1489	MS MC
34	$C_{15}H_{24}$	204	5.74	1500	MS MC
35	$C_{15}H_{24}$	204	1.66	1508	MS MC
36	$C_{15}H_{26}O$	222	0.41	1559	MS MC
			96.91		

* RI = Kovats retention indices; MS = mass spectrum; MC = mass chromatography; CC = Cochromatography; NMR = ^{13}C NMR spectroscopy.

main components being caryophyllene (**29,** 5.5%), identified by retention and spectra comparison with authentic material. 1.43% of the oil composition was represented by humulene (**30**), another 4.62% identified as β- (**27**) and δ-elemene (**24**), alloaromadendrene (**31**), aromadendrene and germacrene D (**33**) because of conformity of retention and mass spectra.

Compared again with the Brazilian alfavaca oil of ref. [15], also the sesquiterpene composition of the two oils was different. While we found among others caryophyllene (**29,** 5.5%), a common sesquiterpene hydrocarbon, as major constituent in the fraction, a smaller percentage of caryophyllene (2.01%) was reported in the analysis in ref. [15]. Its authors claimed to have identified 18.99% of *(E)*-β-farnesene as main sesquiterpenoid constituent, while we could not detect any appreciable amount of this substance in our sample.

There were four chemotypes of *O. gratissimum* reported, containing thymol [18], eugenol [18], citral [18] or methyl cinnamate [11], as major constituents. Thymol type oils are reported to be obtained from African plants [6, 12, 18], eugenol types among others from cultivated plants in Russia and Brazil [18], the citral type is found in plants growing in India [18], the cinnamate chemotype occurs in Pakistan [11].

According to a study on the biogenesis of the essential oil of *O. gratissimum* from Africa [19], the quantity of phenylpropane derivatives decreased with the development of the plants, while that of the terpenoids increased. In seedlings, methyleugenol represented the main constituent (more than 90%), whereas a small amount of monoterpene hydrocarbons and a greater amount of sesquiterpene hydrocarbons is found [20]. Oxygenated monoterpenes, especially the aromatic thymol is missing. In the leaves of the 1st insertion already a small content of thymol is found, which became the main component from the period of 2nd insertion of leaves. Methyleugenol could not be detected anymore from that point of time and plant development, respectively. The percentage of γ-terpinene and thymol decreased, whereas the amount of *p*-cymene increased, when dried plant material was used for oil production instead of fresh material, and finally became the main component [21].

The essential oil investigated by us belongs to the eugenol/methyleugenol type. Congruently, it contains an appreciable quantity of *(Z)*-ocimene (**14**), linalool (**18**), eugenol (**25**), β-pinene (**7**), α-terpineol (**22**) and myrcene (**9**) [18]. The plants were harvested 1200 km away from the place, lower Amazonas river, where those from ref. [15] originated from. Since it is hard to believe that the essential oil analyzed in [15] was of a new chemotype, we must assume that the difference in the oil composition is due to age and drying of the plants. *p*-Cymene then could represent a final oxidation product, the more since *p*-cymene is the thermodynamically most stable monoterpenoid hydrocarbon [22]. The authors of the first analysis of Brazilian *O. gratissimum* confirmed us to have used dried plant material for the production of the essential oil.

[1] B. Oliver, in: Medicinal Plants in Nigeria, Nigerian College of Arts, Science and Technology, Nigeria, Africa 1960.

[2] F. El-Said, E. A. Sofowora, S. A. Malcolm, and A. Hofer, Planta med. **17,** 195 (1969).

[3] G. S. Grover and Rao J. Tirumala, Parfuem. Kosmet. **58,** 326 (1977).

[4] R. D. Tripathi, R. Banerji, M. L. Sharma, V. R. Balasubrahmanyam, and S. K. Nigam, Agric. Biol. Chem. **49,** 2277 (1985).

[5] Haki Ahmataj, Bul. Univ. Shteteror Tiranes, Ser. Shkencat Natyr. **24,** 21 (1970) [CA 73(24): 123431 f].

[6] A. N. Nisharadze and N. Sh. Bagaturiya, Maslo-Zhir. Prom-st. (4), 33 (1980) [CA 93(7): 66048 y].

[7] S. N. Sobti, P. Pushpangadan, B. L. Bradu, and B. B. Jain, Indian Perfum **23,** 16 (1979) [CA 92(16): 135103 f].

[8] V. A. Zamureenko, N. A. Klyuev, L. B. Dmitriev, S. G. Polyakova, and I. I. Grandberg, Izv. Timiryazevsk. S-kh. Akad. (2), 172 (1986) [CA 105(4): 29769 e].

[9] Yu-shia Cheng and Ming-liang Liu, Paper presented at 9th Intern. Essential Oil Congr. Singapore (1983).

[10] B. M. Lawrence, Perfum. & Flavor **12,** 69 (1987).

[11] M. Erfan Ali and Lohani A. M. Shamsuzzaman, Lohani A. M. Sci. Res. (Dacca) **5,** 91 (1968), [CA 70(14): 6074 c].

[12] E. A. Soforowa, Planta med. **18,** 173 (1970).

[13] M. Sainsbury and E. A. Sofowora, Phytochemistry **10,** 3309 (1971).

[14] L. Ntezurubanza, J. C. C. Scheffer, and A. B. Svendsen, Planta med. **53,** 421 (1987).

[15] J. G. S. Maia, L. S. Ramos, A. I. R. Luz, M. L. da Silva, and M. d. G. B. Zoghbi, in: Flavors and Fragrances: A World Perspective (B. M. Lawrence, B. D. Mookherjee, and B. J. Willis, eds.), Proc. 10th Intern. Congr. Essential Oils, Fragrances and Flavors, Washington D.C., Nov. 1986, p. 177, Elsevier Science Publishers B. V., Amsterdam, Netherlands 1988.

[16] A. A. Craveiro, F. J. A. Matas, and J. W. Alencar, J. Chem. Educ. **53,** 652 (1976).

[17] W. Bremser, L. Ernst, and B. Franke, in: Carbon-13 NMR Spectral Data, Verlag Chemie, Weinheim, New York 1978.

[18] E. Gildemeister and F. Hoffmann, in: Die Ätherischen Öle, **Vol. 7,** p. 502, Ausg., Akademischer Verlag, Berlin 1961.

[19] A. S. Dro and F. W. Hefendehl, Planta med. **21,** 353 (1973).

[20] A. S. Dro and F. W. Hefendehl, Arch. Pharmaz., in press (1973) cited in [19].

[21] A. S. Dro, Dissertation, Univ. Freiburg, F.R.G. (1972).

[22] U. Kobold, O. Vostrowsky, H. J. Bestmann, and K. H. Kubeczka, Liebigs Ann. Chem. **1987,** 557.