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Combined analysis of *Cymbopogon giganteus* Chiov. leaf oil from Ivory Coast by GC/RI, GC/MS and ¹³C-NMR

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Abstract

The composition of the essential oil isolated by hydrodistillation from the leaves of *Cymbopogon giganteus* Chiov. growing wild in Ivory Coast, was determined by GC/RI, GC/SM and ¹³C-NMR after fractionation on silica gel. The oil was characterized by high contents of *trans*- and *cis*-*p*-mentha-2,8-dien-1-ols (18.4% and 8.7%, respectively), *cis*- and *trans*-*p*-mentha-1(7),8-dien-2-ols (16.0% and 15.7% respectively), and limonene (12.5%). Forty-six components were identified, including 25 compounds reported for the first time in the oils of this species. **To cite this article:** J.B. Boti et al., C. R. Chimie (9) 2006.

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Résumé

La composition de l'huile essentielle obtenue par hydrodistillation des feuilles de *C. giganteus* Chiov., poussant à l'état spontané en Côte d'Ivoire, a été déterminée par CPG/IR, (IR = indices de rétention) CPG/SM et RMN-¹³C. L'huile essentielle est caractérisée par une forte proportion de *trans*- et de *cis*-*p*-mentha-2,8-diène-1-ols (18,4 et 8,7 %), des *cis*- et *trans*-*p*-mentha-1(7),8-diène-2-ols (16,0 et 15,7 %) et de limonène (12,5 %). Quarante-six composés ont été identifiés, dont 25 observés pour la première fois dans les huiles essentielles de cette espèce. **Pour citer cet article :** J.B. Boti et al., C. R. Chimie (9) 2006.

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Keywords: *Cymbopogon giganteus* Chiov.; Poaceae; Essential oil composition; *p*-Mentha-1(7),8-dien-2-ols; *p*-Menth-2,8-dien-1-ols; Limonene; ¹³C-NMR

Mots clés : *Cymbopogon giganteus* Chiov. ; Poaceae ; Composition de l'huile essentielle ; *p*-mentha-1(7),8-diène-2-ols ; *p*-menth-2,8-diène-1-ols ; limonène ; RMN-¹³C

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1. Introduction

The *Cymbopogon* genus comprises 56 species [1] among which four species including *Cymbopogon giganteus* Chiov. grow wild in Ivory Coast. *C. giganteus*, an aromatic Poaceae, is a perennial and sweet-smelling grass that grows spontaneously in the savannahs of Asian and African tropical regions. It possesses a rhizome-bearing stem and can grow up to 3 m high [2]. Decoctions of the leaves and flowers are used as an effective treatment against skin disorders [3], conjunctiva and migraine [4] and hepatitis [5]. Solvent extracts of *C. giganteus* have been reported to contain flavonoids [6] and amino acids [7]. The composition of essential oils, obtained from aerial parts of plants growing in different countries, has been investigated: Benin [8], Burkina Faso [9], Mali [10,11], and Cameroon [12]. The composition of two samples of leaf oil from Ivory Coast was briefly investigated; however, only six to eight components were identified [12,13]. The major constituents of *C. giganteus* oils were *p*-menthane derivatives such as *cis*- and *trans-p*-mentha-2,8-dien-1-ols, *cis*- and *trans-p*-mentha-1(7),8-dien-2-ols *cis*- and *trans*-isopiperitenols, limonene and carvone. Conversely, the oils differed from sample to sample by the occurrence of different minor components.

The aim of the present work was to get a better insight on the composition of *C. giganteus* leaf oil from Ivory Coast, using a combination of column chromatography, GC, GC/MS and ^{13}C -NMR following a methodology developed and computerized in our laboratories [14,15].

2. Experimental

2.1. Essential oil

The leaves of *C. giganteus* (1.8 kg) were harvested in August 2002, near Bouaké (Ivory Coast) and water distilled, using a Clevenger-type apparatus (3 h). The leaf oil (9.6 ml) was the cumulative oil of three independent hydrodistillations (yield = 0.20% v/w).

2.2. Oil fractionation

One part of *C. giganteus* leaf oil (2 g) was separated by flash chromatography: silica gel, 63–200 μm , elution using pentane with increasing amount of diethyl ether (from 100/0 to 0/100) as eluent. Nineteen fractions were obtained and analyzed by GC/RI and GC/MS or ^{13}C -NMR.

2.3. Analytical GC

GC analyses were carried out using a PerkinElmer Autosystem GC apparatus equipped with dual FID and fused-silica capillary columns (50 m \times 0.22 mm i.d., film thickness 0.25 μm), BP-1 (polydimethylsiloxane) and BP-20 (polyethyleneglycol). Carrier gas: helium at 1 ml/min (split 1/50); injector temperature, 250 $^{\circ}\text{C}$; oven temperature programmed from 60 to 220 $^{\circ}\text{C}$ at 2 $^{\circ}\text{C}/\text{min}$ and then held isothermal (20 min); detector temperature, 250 $^{\circ}\text{C}$.

2.4. GC/MS analysis

Samples were analyzed with a PerkinElmer TurboMass detector (quadrupole), directly coupled to a PerkinElmer Autosystem XL equipped with fused-silica capillary columns (60 m \times 0.22 mm i.d., film thickness 0.25 μm), Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethyleneglycol). Ion source temperature: 150 $^{\circ}\text{C}$; energy ionization: 70 eV; electron ionization mass spectra were acquired over the mass range 35–350-Da scan rate. Split: 1/80. Other GC conditions were the same as described under GC.

2.5. ^{13}C -NMR analysis

All NMR spectra were recorded on a Bruker AC 200 Fourier transform spectrometer operating at 50.323 MHz for ^{13}C , equipped with a 10 mm (or 5 mm) probe, in deuterated chloroform (CDCl_3), with all shifts referred to internal tetramethylsilane (TMS). ^{13}C -NMR spectra were recorded with the following parameters: pulse width (PW), 5 μs (or 3 μs) (flip angle 45 $^{\circ}$); acquisition time, 1.3 s for 32 K data table with a spectral width (SW) of 12 500 Hz (250 ppm); CPD mode decoupling; digital resolution 0.763 Hz per pt. In a typical procedure, 200 mg (or 70 mg) of the oil were used in 2 ml (or 0.5 ml) of CDCl_3 . The number of accumulated scans ranged between 2000 and 10 000 for each sample, depending on the amount of oil available. Exponential line broadening multiplication (LB = 1 Hz) of the free induction decay (FID) was applied before Fourier transformation.

2.6. Component identification

Identification of the components in each oil and fractions of chromatography was based: (a) on their GC

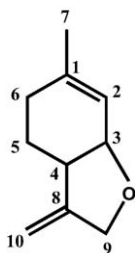


Fig. 1. Structure of 3,9-oxy-*p*-mentha-1,8(10)-diene ((2,3,3a,4,5,7a-hexahydro-6-methyl-3-methylenebenzofurane).

retention indices (RI) on polar and apolar columns, determined relative to the retention times of a series of *n*-alkanes with linear interpolation with those of authentic compounds or literature data [16]; (b) on computer matching with laboratory-made and commercial mass spectral libraries [17,18] and comparison of spectra with those of our own library or literature data [19,20]; (c) and by carbon-13 NMR following the methodology developed and computerized in our laboratories [14,15]. The chemical shift of each carbon in the mixture spectrum is compared with those of the spectra of pure compounds listed in a laboratory made computerized data bank. Each compound is identified taking into account (i) the number of identified carbons, (ii) the number of overlapped signals, (iii) the difference of chemical shift of each resonance in the mixture spectrum and in the reference. The structure of 3,9-oxy-*p*-mentha-1,8(10)-diene (2,3,3a,4,5,7a-hexahydro-6-methyl-3-methylenebenzofurane) (Fig. 1), not present in our MS and ^{13}C -NMR data libraries was determined by extensive NMR studies. Spectral data (^1H , ^{13}C , DEPT, ^1H - ^1H -COSY, HSQC) were measured on a fraction of chromatography (80.0% purity on GC). ^1H -NMR (400 MHz, CDCl_3 , δ = ppm, J = Hertz): 5.51 (1H, br s, H-2), 4.95 (1H, q, 2.4 Hz, H-10), 4.91 (1H, q, 2.0 Hz, H-10), 4.37 (1H, dq, 13.3, 1.9 Hz, H-9), 4.24 (1H, dq, 13.3, 2.1 Hz, H-9), 4.28 (1H, br s, H-3), 2.60 (1H, br s, H-4), 1.90 (2H, m, H-6), 1.71 (3H, s, H-7), 1.63 (2H, m, H-5). ^{13}C -NMR, δ = ppm: 152.30 (C-8), 140.78 (C-1), 119.90 (C-2), 103.46 (C-10), 76.17 (C-3), 70.02 (C-9), 41.15 (C-4), 28.18 (C-6), 24.76 (C-5), 23.84 (C-7).

3. Results and discussion

Fractionation on SiO_2 of the *C. giganteus* leaf oil from Ivory Coast led to 19 fractions that were analyzed by GC/RI. Some fractions, selected on the basis of their chromatographic profile were also analyzed by MS and/or ^{13}C -NMR, depending of the available amount of pro-

duct. The quantitative and qualitative data on the composition of the essential oil, as well as the mode of identification of the individual components, are reported in Table 1. Forty-six constituents, who represented 91.0% of the oil, were identified. The major constituents of the essential oil were: *trans-p*-mentha-2,8-dien-1-ol (18.4%), *cis-p*-mentha-1(7),8-dien-2-ol (16.0%), *trans-p*-mentha-1(7),8-dien-2-ol (15.7%), limonene (12.5%), *cis-p*-mentha-2,8-dien-2-ol (8.7%), while *trans*- and *cis*-isopiperitenols (3.1% and 2.2% respectively), carvone (2.7%) and *trans*-carveol (2.2%) were present at appreciable contents.

Although some quantitative differences could be observed, the major components of the investigated sample are qualitatively and quantitatively similar to those already reported in Ivory Coast [12,13] and other countries [8–11]. However, the detailed analysis carried out after fractionation of the bulk sample, gives a better insight on the composition of the *C. giganteus* leaf oil from Ivory Coast and allows the comparison with oils from other countries. Indeed, 25 compounds were reported for the first time in *C. giganteus* leaf oil, including oxygenated acyclic monoterpenes (citronellal, geraniol, geranial), oxygenated menthane derivatives (*cis*- and *trans*-dihydroperylaldehydes, thymol), phenylpropanoids (safrole), phenylethyl derivatives (hexanoate, octanoate) and oxides (ascaridole, precocene, caryophyllene oxide) as well as sesquiterpene hydrocarbons (β -elemene (E)- β -caryophyllene, β -selinene, δ -cadinene) and linear aldehydes (nonanal, decanal) and alkanes.

4. Conclusion

The present study confirmed that the composition of the leaf oil of *C. giganteus* growing wild in Ivory Coast, is dominated by unsaturated alcohols bearing the menthane skeleton (*p*-menthadienol isomers) as the oils from other countries of West Africa. These compounds have been reported as components of the essential oils of other species of Cymbopogon: *Cymbopogon densiflorus* from Angola, Congo and Zambia [12,21], and *Cymbopogon martinii* var *sofia* from India [22]. However the detailed analysis of a sample from Ivory Coast, by combination of chromatographic (CC, GC) and spectroscopic (MS, ^{13}C -NMR) techniques, allowed the identification of 25 components found for the first time in *C. giganteus* leaf oil (whatever the origin) including oxygenated monoterpenes, sesquiterpene hydrocarbons, aromatic esters, alkanes and linear aldehydes.

Table 1
Composition of the essential oil of *C. giganteus* from Ivory Coast

Compounds	RIa	RIp	%	Identification
Myrcene	978	1157	0.1	RI, MS, ¹³ C-NMR
<i>p</i> -Cymene	1010	1267	0.3	RI, MS, ¹³ C-NMR
Limonene*	1020	1200	12.5	RI, MS, ¹³ C-NMR
1,8-Cineole*	1020	1209	0.1	RI, ¹³ C-NMR
γ -Terpinene	1045	1242	0.1	RI, MS, ¹³ C-NMR
<i>p</i> -Cymenene	1069	1430	0.2	RI, MS, ¹³ C-NMR
Terpinolene	1076	1278	0.1	RI, MS, ¹³ C-NMR
Nonanal	1078	1387	0.1	RI, MS, ¹³ C-NMR
<i>Trans-p</i> -mentha-2,8-dien-1-ol	1102	1619	18.4	RI, MS, ¹³ C-NMR
<i>Cis-p</i> -mentha-2,8-dien-1-ol	1114	1660	8.7	RI, MS, ¹³ C-NMR
<i>Cis</i> -limonene 1,2-oxide	1118	1449	0.5	RI, MS, ¹³ C-NMR
<i>Trans</i> -limonene-1,2-oxide	1121	1470	0.2	RI, ¹³ C-NMR
Citronellal	1127	1472	0.2	RI, MS, ¹³ C-NMR
<i>Trans-p</i> -mentha-1(7),8-dien-2-ol *	1168	1790	15.7	RI, MS, ¹³ C-NMR
3,9-Oxy-mentha-1,8(10)-diene*	1168	1551	2.2	¹ H-NMR, ¹³ C-NMR
<i>Trans</i> -dihydrocarvone *	1171	1600	0.2	RI, ¹³ C-NMR
<i>Cis</i> -dihydroperillaldehyde *	1171	1596	0.6	RI, ¹³ C-NMR
<i>Trans</i> -dihydroperillaldehyde	1174	1606	1.3	RI, ¹³ C-NMR
<i>Trans</i> -isopiperitenol	1178	1744	3.1	RI, MS, ¹³ C-NMR
Decanal	1183	1489	tr	RI, MS, ¹³ C-NMR
<i>Trans</i> -carveol *	1195	1829	2.2	RI, MS, ¹³ C-NMR
<i>Cis</i> -isopiperitenol *	1195	1739	2.2	RI, MS, ¹³ C-NMR
<i>Cis-p</i> -mentha-1(7),8-dien-2-ol	1204	1885	16.0	RI, MS, ¹³ C-NMR
<i>Cis</i> -carveol	1207	1860	0.5	RI, ¹³ C-NMR
Carvone	1213	1725	2.7	RI, ¹³ C-NMR
3-Methylbutyl hexanoate *	1229	1450	0.1	RI, MS, ¹³ C-NMR
Geraniol*	1229	1840	0.4	RI, ¹³ C-NMR
Geranial	1238	1727	0.1	RI, ¹³ C-NMR
Perillaldehyde	1242	1779	0.4	RI, ¹³ C-NMR
Safrole	1260	1872	0.1	RI, MS, ¹³ C-NMR
Thymol	1262	2178	0.9	RI, ¹³ C-NMR
Ascaridole	1273	1860	0.2	RI, ¹³ C-NMR
β -Elemene	1384	1580	tr	RI, MS
Tetradecane	1400	1400	tr	RI, MS
(E)- β -Caryophyllene	1415	1587	0.1	RI, MS
3-Methylbutyl octanoate	1423	1648	0.1	RI, MS, ¹³ C-NMR
Precocene I	1428	2075	0.2	RI, MS, ¹³ C-NMR
β -Selinene	1475	1706	tr	RI, MS
δ -Cadinene	1511	1746	tr	RI, MS
Caryophyllene oxide	1568	1980	0.1	RI, MS, ¹³ C-NMR
Hexadecane	1600	1600	tr	RI, MS
2-Phenylethyl hexanoate	1606	2166	0.1	RI, MS
Benzyl benzoate	1719	2379	tr	RI, MS
Octadecane	1800	1800	tr	RI, MS
2-Phenylethyl octanoate	1814	2232	tr	RI, MS
Eicosane	2000	2000	tr	RI, MS
Total			91.0	

Order of elution and percentages are given on BP-1 column. *: Percentages on BP-20 column. RIa, RIp = RI on apolar (BP-1) and polar (BP-20) columns, respectively. tr < 0.05%.

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