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Chemical composition of the leaf oil of *Cleistopholis glauca* Pierre ex Engler & Diels from Côte d'Ivoire

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The composition of fifteen leaf oil samples from individual plants of *Cleistopholis glauca* Pierre ex Engler & Diels growing wild in Côte d'Ivoire was investigated by gas chromatography (in combination with retention indices) and carbon-13 nuclear magnetic resonance (^{13}C NMR). Thirty-three compounds accounting for 88.8 to 95.4% of the oil have been identified. *Cleistopholis glauca* produces a sesquiterpene-rich oil, germacrene D (16.4–46.5%) and (*E*)-β-caryophyllene (8.0–26.2%) being the main components. In a few samples, monoterpene hydrocarbons were present as appreciable contents: myrcene (up to 39.7%), β-pinene (up to 24.8%) and α-pinene (up to 16.4%).

Keywords: *Cleistopholis glauca* Pierre ex Engler & Diels; essential oil composition; germacrene D; (*E*)-β-caryophyllene; ^{13}C NMR

Introduction

The genus *Cleistopholis* (*Annonaceae*) is a small genus specific to tropical Africa, within the most known species are *C. patens*, *C. glauca* and *C. staudii*. *C. glauca*, native of central Africa, is a rainforest species, common in secondary bushes and along waterways. It is widespread in Cameroon, central African Republic, Gabon, Congo-Kinshasa and Angola. It was introduced in Petit Yapo forest near Adzopé (south-eastern Côte d'Ivoire) thirty years ago. *Cleistopholis glauca* is a tree up to 35 m high, reaching 80 cm in diameter. Its trunk, bole straight, cylindrical, is covered up with a fibrous longitudinally fissured bark. Leaves, 5–15 cm long, 2–5 cm wide; green flowers have outer petals, elliptic-oblong, 10–15 mm long, 5–7 mm wide; fruits are 18–30 mm in length, 10–15 mm wide; seeds, ellipsoid, 15–25 mm long, 10–12 mm wide are slightly smooth to slightly granular. *Cleistopholis Glauca* is easily differentiated from other species by the presence of violets bracts and sessile mericarps.

The bark of *Cleistopholis glauca* is used in the manufacture of African huts and fibers make excellent strings. In decoction, it is used as an emetic (1). The macerated bark of stems and roots is used as a vermifuge in central African Republic (2).

Numerous compounds belonging to various families have been isolated from solvent extracts obtained from several parts of plants of the genus *Clesitopholis* and their structures have been elucidated: alkaloids in *C. patens* (3–8) and in *C. staudtii* (9); oligorhamnosides in *C. patens* (10) and in *C. glauca* (11, 12); lipids in *C. glauca* (13); terpenes in *C. patens* (4, 14) and in *C. glauca* (12, 15); a phenyl propanoïd in *C. glauca* (15); a cinnamic acid ester in *C. staudtii* (16) and a furanic derivative in *C. glauca* (15).

In contrast, only a few studies concerning the chemical composition of the essential oils of *Cleistopholis patens* are reported in the literature. The composition of Nigerian oils have been dominated by monoterpenes (17, 18) while oils from Cameroon contained fair amounts of sesquiterpenes and exhibited antiplasmodial activity (19). To our knowledge, no phytochemical investigations on *C. glauca* essential oil have been undertaken to date. In the present study, we report on the chemical composition of fifteen samples of leaf oil of *C. glauca* investigated by gas chromatography (GC) in combination with retention index (RI) and by carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy.

Experimental

Plant material

Leaves of fifteen individual trees of *Cleistopholis glauca* were collected all around the tree, on lower branches (around 2 m in height), in Petit Yapo forest, near Agboville (south-eastern Côte d'Ivoire), in August 2008 (samples 1–2), May 2009 (samples 3–8) and November 2009 (samples 9–15). Plant material was authenticated by Professor L. Aké Assi, from the

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Centre National Floristique (CNF, Abidjan, Côte d'Ivoire).

Fresh leaves (around 0.8–1.0 kg) were separately subjected to hydrodistillation in a Clevenger-type apparatus for 3 hours. At the end of each distillation the oils were collected, measured, and transferred to glass flasks that were kept at a temperature of +5°C until analysis (5–10 months).

Analysis of the essential oils

GC analysis was carried out with a Clarus 500 apparatus equipped with two flame ionization detectors, and fused capillary columns (50 m × 0.22 mm i.d., film thickness 0.25 μm), BP-1 (polymethylsiloxane) and BP-20 (polyethylene glycol). Carrier gas, helium; linear velocity, 0.8 mL/minute. The oven temperature was programmed from 60°C to 220°C at 2°C/minute and then held isothermal (20 minutes). Injector temperature: 250°C (injection mode: split 1:60). Detector temperature: 250°C. The relative proportions of the essential oil constituents were expressed as percentages obtained by peak area normalization, all relative response factors being taken as one.

All ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 100.63 MHz for ^{13}C, equipped with a 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 μseconds (flip angle 45°); acquisition time, 2.7 seconds for 128 K data table with a spectral width (SW) of 25000 Hz (250 ppm); digital resolution 0.183 Hz/pt. The number of accumulated scans was 3000 for each sample (about 50 mg of essential oil in 0.5 mL of $CDCl_3$).

The fifteen samples were analyzed by GC(RI) and nine samples (samples 1–7, 9 and 13), selected in function of their chromatographic profile, were submitted to

Table 1. Composition of leaf oils of *Cleistopholis glauca.*

Compound	RI^{litt}	RIa	RIp	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
α-thujene	932	926	1029	0.3	0.3	1.1	0.1	0.2	0.2	tr	0.2	0.4	tr	—	tr	tr	—	tr
α-pinene	936	931	1024	0.6	0.4	0.4	16.4	0.3	0.5	0.5	0.5	0.5	0.3	0.3	15.0	0.4	0.3	0.4
camphene	950	944	1072	0.4	tr	0.3	0.1	0.1	0.3	0.3	0.1	0.3	0.1	tr	0.1	0.1	tr	0.1
sabinene	973	965	1125	0.4	0.3	0.3	0.9	0.3	0.3	0.7	0.5	0.5	0.2	0.1	0.8	0.2	0.2	0.2
β-pinene	978	971	1115	0.3	0.2	0.2	24.8	0.1	0.3	0.3	0.4	0.4	0.3	0.6	24.1	0.6	0.5	0.6
myrcene	987	981	1165	6.8	7.8	7.4	1.8	13.3	5.9	39.7	7.3	15.6	12.7	5.1	6.0	5.6	9.7	5.4
α-phellandrene	1002	997	1169	0.7	0.7	0.7	0.1	0.3	0.3	0.1	0.2	0.2	—	0.1	tr	0.1	—	0.1
δ-3-carene	1010	1005	1152	0.6	0.8	0.8	0.2	0.2	0.4	0.1	0.3	tr	tr	0.1	tr	0.1	—	0.1
α-terpinene	1013	1009	1184	1.4	1.9	1.7	0.2	0.5	0.5	0.1	0.3	0.1	—	0.1	0.1	—	—	—
p-cymene	1015	1012	1274	2.1	2.1	2.5	2.3	0.6	3.7	0.2	2.0	0.7	0.4	0.4	0.3	0.6	0.6	0.5
limonene *	1025	1022	1205	9.6	5.2	3.4	3.7	7.0	10.5	2.0	6.4	10.1	1.1	2.2	1.4	3.9	1.2	11.6
1,8-cineole*	1024	1022	1212	0.3	0.8	0.3	0.1	0.2	0.3	0.9	0.2	0.2	0.2	0.1	0.1	0.1	0.3	0.1
β-phellandrene*	1023	1022	1213	0.6	0.4	—	0.4	0.7	0.6	1.1	0.6	0.9	0.4	0.3	0.6	0.5	0.5	0.6
(*E*)-β-ocimene	1041	1036	1253	3.1	4.2	2.3	1.3	1.0	3.0	3.2	3.6	6.1	4.9	3.0	2.0	1.6	1.3	4.6
γ-terpinene	1051	1048	1248	5.6	7.6	7.3	1.1	2.0	2.9	0.2	1.6	0.3	tr	0.1	0.1	0.1	0.1	tr
δ-elemene	1340	1336	1472	1.3	0.7	0.4	0.7	0.7	0.6	0.1	0.3	0.7	0.3	1.9	0.3	1.4	0.7	0.5
α-cubebene	1355	1349	1459	0.7	0.4	0.4	0.3	0.4	0.6	0.2	0.2	0.3	0.1	0.3	0.1	0.3	0.2	0.2
α-copaene	1379	1376	1794	0.6	2.7	2.5	1.5	2.1	0.9	1.1	1.5	2.0	0.5	1.1	0.5	1.5	0.9	2.0
β-elemene	1389	1388	1592	2.5	1.8	1.6	2.1	0.9	2.9	1.4	0.8	3.0	0.8	2.3	0.1	3.7	2.2	0.8
(*E*)-β-caryophyllene	1421	1419	1600	13.0	8.0	17.7	8.1	11.1	16.4	10.6	18.5	17.1	26.2	12.9	8.9	20.5	21.5	20.7
β-copaene	1430	1428	1592	0.5	0.5	0.8	0.7	1.1	0.2	2.2	0.8	2.8	1.3	0.2	0.7	2.3	1.2	1.2
(*E*)-α-bergamotene	1434	1433	1587	0.4	0.3	0.2	0.4	1.2	0.5	0.5	0.5	0.3	0.9	0.9	1.3	1.0	1.0	0.8
(*E*)-β-farnesene	1446	1447	1667	0.5	0.4	0.3	0.4	1.3	0.5	0.7	0.7	0.3	1.2	1.3	1.7	1.2	1.3	1.0
α-humulene	1455	1451	1669	1.4	0.9	1.9	1.1	1.1	3.3	1.2	2.1	1.9	2.7	1.3	0.8	2.2	2.2	2.1
γ-muurolene	1474	1472	1688	0.7	0.7	0.8	0.4	1.1	1.0	0.4	0.5	0.5	0.5	1.3	0.8	1.0	1.0	0.6
germacrene D	1479	1478	1711	35.8	34.5	27.4	18.3	35.4	24.9	16.7	27.5	16.4	27.2	46.5	25.0	30.4	31.5	24.3
bicyclogermacrene	1494	1492	1733	2.4	2.3	1.8	0.9	2.4	1.5	1.2	2.6	1.1	2.8	3.2	1.6	2.1	3.1	2.1
δ-cadinene	1520	1515	1757	1.1	8.2	8.1	4.0	6.1	2.3	3.2	4.7	5.6	1.1	2.8	1.3	3.3	1.7	4.8
β-elemol	1541	1534	2074	0.4	0.1	0.3	0.3	0.1	0.7	1.2	2.2	0.7	2.2	0.9	0.3	0.9	2.9	2.0
germacrene B	1552	1552	1827	0.3	0.1	0.1	0.5	0.2	0.9	3.9	0.7	4.5	0.2	3.0	0.1	3.4	0.3	0.2
spathulenol	1572	1563	2117	0.2	0.4	0.4	0.4	0.1	0.8	0.2	1.1	0.2	0.7	tr	0.2	0.5	0.8	0.8
caryophyllene oxide	1578	1569	1975	0.2	0.1	0.4	0.7	0.1	1.4	0.3	1.1	0.7	1.2	tr	0.2	1.2	1.2	1.5
guaiol	1593	1584	2082	0.6	—	—	0.3	—	0.7	0.4	0.9	0.7	0.9	0.2	0.1	0.2	0.4	0.4
Total:				95.4	94.8	93.8	94.6	92.2	89.8	94.9	90.9	94.6	91.4	92.6	94.6	91.0	88.8	90.3

Notes: Order of elution and percentages are given on apolar column (BP-1), except for compounds with an asterisk (*), percentage on polar column (BP-20); RI^{litt}: retention indices from literature (22); RIa, RIp: retention indices measured on apolar and polar columns, respectively; tr, traces.

NMR analysis, in order to have all the components identified several times by NMR. Identification of the individual components was based: (i) on comparison of their GC(RI) on apolar and polar columns, determined relative to the retention times of a series of *n*-alkanes with linear interpolation ('Target Compounds' software of Perkin–Elmer), with those of authentic compounds, (ii) by ^{13}C NMR spectroscopy, following the methodology developed and computerized in our laboratories, using home-made software, by comparison with spectral data of reference compounds compiled in a laboratory-built library (20, 21). Each component which accounted at least for 0.3 to 0.4% was identified by ^{13}C NMR.

Results and discussion

Hydrodistillation of the fresh leaves collected on fifteen individual trees of *Cleistopholis glauca* produced a clear to pale-yellow essential oil with homogenous yields (0.16–0.19%, *w/w*, calculated on fresh weight basis). Thirty-three compounds accounting for 88.8 to 95.4% of the whole composition of the samples, have been identified (Table 1): fifteen monoterpenes and eighteen sesquiterpenes. Although all the samples were qualitatively similar, a quantitative chemical variability was observed, with a significant variation of the contents of the major components, mostly hydrocarbons: germacrene D (16.4–46.5%), (*E*)-β-caryophyllene (8.0–26.2%), myrcene (1.8–39.7%), β-pinene (0.1–24.8%), α-pinene (0.3–16.4%). Five other components were present as appreciable contents: limonene (up to 11.6%), γ-terpinene (up to 7.6%), (*E*)-β-ocimene (up to 6.1%) as well as δ-cadinene (up to 8.2%) and germacrene B (up to 4.5%). By contrast, oxygenated compounds are poorly represented: β-elemol (0.1–2.9%), caryophyllene oxide (trace–1.5%), spathulenol (trace–1.1%), guaiol (0–0.9%) and 1,8-cineole (0.1–0.9%).

Although germacrene D is the major component of almost all samples, various compositions may be distinguished:

- A main group (samples 1–3, 5, 6, 8, 11, 13 and 14) exhibited a composition largely dominated by germacrene D (24.9–46.5%) followed by (*E*)-β-caryophyllene (8.0–21.5%). Myrcene (5.1–13.3%), limonene (1.2–10.5%) and δ-cadinene (1.1–8.2%) are present at appreciable contents in most samples of that group;
- Samples 10 and 15 contained similar amounts of germacrene D (27.2 and 24.3%, respectively) and (*E*)-β-caryophyllene (26.2 and 20.7%, respectively);
- Samples 4 and 12 contained β-pinene (24.8 and 24.1%, respectively) and α-pinene (16.4 and 15.0%, respectively) among the major components;
- Oil samples 7 and 9 were very high (39.7%) or high (15.6%) in myrcene with moderate amounts of germacrene D (16.7 and 16.4%, respectively).

From these results, it may be concluded that leaves of *Cleistopholis glauca* produces a sesquiterpene-rich essential oil, the composition of almost all investigated samples being dominated by germacrene D and (*E*)-β-caryophyllene.

The composition of the leaf oil of *Cleistopholis glauca* differed from oils isolated from Nigerian *C. patens* (17, 18). Indeed, leaf oil contained (*E*)-β-ocimene (31.0%) and (*E*)-β-caryophyllene (12.8%) as major components; fruit oil contained linalool (23.1%), trans-linalool oxide (furanoid) (17.7%) and cis-linalool oxide (furanoid) (17.0%). The major components of stem bark oil were *p*-cymene (13.4%), germacrene D (12.5%) and myrcene (12.1%) and those of the root bark oil were α-cadinol and bornyl acetate (percentages not specified). Sesquiterpene hydrocarbons were the major components of Cameroonian *C. patens* essential oils (19). Leaf oil contained mainly (*E*)-β-caryophyllene (27.5%), germacrene D (16.1%) and germacrene B (16.0%) while δ-cadinene (28.7%), α-copaene (16.9%) and germacrene B (7.4%) were the major components of the stem bark oil.

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