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Research Paper

Analysis of volatile compounds in *Aucoumea klaineana* oleoresin by static headspace/gas chromatography/mass spectrometry

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ABSTRACT

Static headspace analysis of Gabon Estuary okoume (*Aucoumea klaineana* Pierre) oleoresins indicated that nine products represented the major part (>96%) of the volatile compounds: six monoterpenes (α-pinene, β-pinene, limonene, α-phellandrene, β-phellandrene, 3-carene), one monoterpenic alcohol (α-terpineol) and two other derivatives (p-cymene, eucalyptol). The relative amount of each product appeared to be related to the geographical origin of the trees. Throughout okoume plantations, different compositions of the volatile compounds were observed, probably due to differences in seed origin. Sesquiterpenes were not detected in okoume oleoresins.

Keywords: *Aucoumea klaineana;* headspace/GC/MS; forests; Gabon.

INTRODUCTION

Okoume (*Aucoumea klaineana* Pierre (Burseraceae)) is a commercial forest species grown throughout equatorial forests of Africa (Figure 1). The major use of *A. klaineana* is for the manufacture of plywood (Van Valkenburg, 2008). As the industrial exploitation of natural forests has increased over the last century, the need to avoid overexploitation of natural forests led to the creation of artificial plantations in 1935 (Biraud, et al., 1960). This practice was carried out mainly in the Gabon Estuaire province, but was stopped in 2000. Approximately 30000 ha of plantation forests exist in Gabon, but it is as yet not clear whether wood quality is comparable between plantation grown and natural forests (Medzegue, et al., 2007). The bark of okoume is lightly fibrous and when cut, exudes a strong smelling resin which becomes opaque during coagulation. This oleoresin is used for incense, torches and oil lamps. In traditional medicine, it is also used for the treatment of sores and abscesses, as well as for cosmetic applications in skin and nail care products and for disinfecting water (Van Valkenburg, 2008). The composition of the volatile part of the oleoresin was reported in several documents (Tables 1 and 2). Analysis of the results shows that the chemical nature and/or the amounts of the different identified products are extremely variable.

Within the oleoresin, monoterpenes and some of their derivatives (alcohols) are always present; p-cymene and β-phellandrene are described in all cases. Except in one case (Minkue-M'eny, 2000), the absence of sesquiterpenes is noticeable. Neither the geographical area from which the oleoresin originated, nor the harvesting periods were given. Therefore, the variability of the chemical composition observed may be due to these factors.

We obtained different oleoresin samples from natural and cultivated plantations of okoume in Gabon. We aimed at determining qualitatively the composition of the resin volatile part of okoume. Static headspace seems more suitable for studying volatile compounds (Ioffe, et al., 1984; Kolb, et al., 1997) compared to steam-distillation or solvent extraction, which can also extract non-volatile products present in the material. Headspace also reflects the sample aroma and is easy to use. The aim of this investigation is to analyze for the first time the volatile part of the oleoresins by static headspace/gas chromatography (GC) techniques along with a coupling of headspace/GC/Mass spectrometry (MS) to confirm the attributions of the chromatographic peaks.

MATERIALS AND METHODS

Oleoresin samples: The samples were collected in the Gabon Estuaire province in three different areas (Figure 1). Oleoresins were obtained from the natural forests at N'Toum (three trees) in January 2005 and Cocobeach (six trees) in February 2006 as well as from plantations at Bokoué near Ekouk (six trees) in February 2006. The oleoresins were tainted mainly by barks and earth. To avoid volatile losses, the samples were put into glass vessels, which were tightly closed. The greyish oleoresins were non-homogeneous and more or less viscous in the order: Bokoué < Cocobeach < N'Toum; the latter was almost solid. The vessels were kept at –20°C before analysis of the volatile compounds. Analyses of the N'Toum forest oleoresin were performed in May-June 2005 and again in February 2007. Analyses of the Cocobeach and Bokoué oleoresins were carried out in March 2006 and again in February 2007.

Reference compounds: Formulae are given in Figure 2. α-Pinene, β-pinene, p-cymene, limonene, eucalyptol and α-terpineol were obtained from the Laboratory (LCPO, Bordeaux, France) collection. α-Phellandrene, β-phellandrene and 3-carene were obtained as gifts from Dérivés Résiniques et Terpéniques (DRT, Castets, France).

Static headspace: 10ml vials containing 100mg of cold resin were immediately sealed with silicon rubber Teflon caps and equilibrated in a temperature-controlled oven at 100°C during 20min. Then, a small part of the headspace volume (1ml) was removed with a special syringe and immediately injected into a capillary column.

Gas Chromatography (GC)/Flame Ionization Detector (FID): GC analyses were performed according two different conditions.

(1)- A Hewlett-Packard HP 5890 Series II apparatus was equipped with a fused silica capillary column coated with a DB-5 (5% phenylmethylsiloxane) film (15mx0.25mm i.d.x0.25µm). Helium was the carrier gas. Injections were made in split mode. The injector and detector temperatures were set respectively at 210°C and 230°C. The oven temperature was maintained at 50°C for 5min and programmed first to 120°C at 3°C/min, then to 250°C at 8°C/min.

(2)- A Shimadzu 14B gas chromatograph was equipped with a fused silica capillary column coated with a DB-Wax film (30mx0.25mm i.d.x0.25µm film thickness). Nitrogen was the carrier gas (80kPa). Injections were made in split mode. The injector and detector temperatures were set respectively at 210°C and 230°C. The oven temperature was maintained at 50°C for 5min and programmed to 220°C at 6°C/min. The final temperature was held for 15 min.

Gas Chromatography (GC)/Mass Spectrometry (MS): The analysis was carried out using a Hewlett-Packard G1800 GCD Series II apparatus equipped with a fused silica capillary column coated with a HP-5 (5% phenylmethylsiloxane) film (30mx0.25mm i.d.x0.25µm). Helium (0.8ml/min) was the carrier gas and injections were made in split mode. The injector and transfer line temperatures were set respectively at 210°C and 230°C. The oven temperature was programmed as follows: 40°C for 5min, then programmed to 60°C at 2°C/min, held at 60°C for 5min, further programmed to 250°C at 5°C/min and finally held for 10min. The mass spectrometer operated in the electronic ionisation (EI) mode (70eV) with a scan range of 10 to 450amu.

The volatile compounds were identified by comparison of their retention times with authentic samples, calculation of their Kováts retention indices (Tranchant 1995b) and also by comparison of their mass spectra to those given by the NIST library (version 1.6). Only fully identified products are reported.

RESULTS

Monoterpenes, a terpenic alcohol (α-terpineol) and non-terpenic compounds (p-cymene and eucalyptol) were present in the volatile part of all the resins, but in various amounts (Table 3 and Table 5). Typical chromatograms are represented in Figure 3.

Preliminary work was performed using the oleoresin which originated from the N'Toum natural forest in order to determine the best gas chromatographic analytical conditions before examining all the resins. Two phases (DB-5 and DB-Wax) with largely different polarities were chosen. The separation was better on DB-Wax than on DB-5 (Table 3). On DB-5 (15m), β-phellandrene, limonene and eucalyptol gave only a large peak, which was improved when the column length was increased (30m); β-phellandrene appeared at the beginning of the peak, limonene in the middle and eucalyptol at the end. As limonene is easily identified by its mass spectrum (base peak at m/z 68), a coupling GC/MS allowed unambiguous identification of the three compounds. On DB-Wax all the compounds were suitably separated. Comparison of calculated Kováts indices for DB-Wax with the literature demonstrated good fitting (Table 3). Standard deviations of the retention times were always weak (≤ 0.03min) but standard deviations of the peak areas were greatest (Table 4).

For the Bokoué plantations, two different kinds of resins appeared clearly in the samples, so-called Bokoué 1 and Bokoué 2.

DISCUSSION

As practically the same amount of the resin was introduced in the same type of vials and the same volume of the headspace was analysed, the comparison of relative percentages of the different products is suitable to obtain reliable qualitative results (Tranchant, 1995a; Kolb, et al., 1997). The dispersion of the results can be likely attributed to resin non-homogeneities between trees in the same growing area.

Identified products represented always more than 96% of the total product chromatographic areas. It appears that their relative amounts depend largely on the geographical origin of the resin. These compounds were already described in the literature in essential oils of okoume. Nevertheless a comparison is not adequate. Indeed, the analysis of the volatile compounds by headspace has to be distinguished from the essential oil composition, as completely different physical methods are involved to obtain the analytes : gaseous or vapour-phase in headspace *versus* distillation or suitable mechanical process without heating techniques in essential oil (Rubiolo, et al., 2010).

The observation that two kinds of resins are produced in the Bokoué plantations is not surprising because when the plantation was sown, the okoume seeds used were harvested from all over Gabon even though local ecotypes are the fittest (Koumba Zaou, et al., 1998). The results described here are consistent with a putative preservation of the original oleoresin composition.

The most curious fact is the absence of sesquiterpenes as previously observed. The analysis method is not involved in this problem: sesquiterpenes were already detected in natural substances by headspace methods. For example, longifolene, caryophyllene, α-humulene and δ-cadinene were easily observed in the volatile part of *Pinus pinaster* Ait. sawdust (Gardrat, et al., 1999). A more recent study undertaken by headspace solvent micro-extraction using hexadecane as solvent did not indicate the presence of appreciable amounts of sesquiterpenes in okoume oleoresins (unpublished data). Therefore, it appears that okoume does not produce significant amounts of this type of derivatives.

CONCLUSION

The static headspace analysis of Gabon Estuary okoume oleoresins indicated that only nine products represented the major part of the volatiles: six monoterpenes (α-pinene, β-pinene, limonene, α-phellandrene, β-phellandrene, 3-carene), one monoterpenic alcohol (α-terpineol) and two other derivatives (p-cymene, eucalyptol) often associated with the former compounds in essential oils of plants. The relative amounts appeared to be related to the geographical origin of the trees. In the Bokoué plantations, two different compositions were found, likely depending on seed origin.

Conflict of interest: Dr Medzegue acting as an Engineer of Water and Forests in Gabon was responsible for harvesting the resins.

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Table- 1: Relative proportions of main volatile compounds found in okoume oleoresins according to the literature.

Dupont, et al., 1930, 1932, 1948	Tessier, et al., 1982[a]	Guang-Yi, et al., 1988	Minkue-M'eny, 2000	Gardrat, et al., 2005	Koudou, et al., 2009
		α-pinene	α-pinene	*α-pinene*	α-pinene
			β-pinene	β-pinene	
			sabinene		
α-phellandrene		**α-phellandrene**	**α-phellandrene**	**α-phellandrene**	
		δ-3-carene		δ-3-carene	**δ-3-carene**
p-cymene		**p-cymene**	p-cymene	p-cymene	p-cymene
	β-phellandrene	β-phellandrene	β-phellandrene	*β-phellandrene*	
		limonene	limonene	*limonene*	limonene
			eucalyptol	eucalyptol	
	α-terpineol	**α-terpineol**		α-terpineol	α-terpineol
			α-terpinene		α-terpinene
			terpinolene		*terpinolene*
					p-menth-3-ene
		carane			
		camphor			
		terpinen-4-ol			
		δ-4-carene			

- [a]No quantitative data were given in this paper.
- Only compounds > 0.5 % are reported in the table; in **bold** >10 %; in *italics* between 5 and 10 %

Table- 2: Other minor products.

Product	Reported by
α-thujene; fenchene; 1,2,3,4,-tetramethylbenzene; β-pinene and bicyclo[3.3.1]nonane-2-one; o-cymene; eucalyptol; (E)-β-ocimene; mentha-2,8-dien-1-ol; epoxyterpinolene; mentha-1,5-dien-8-ol; m- and p-cymen-8-ol; verbenone; p-acetylanisole	Koudou, et al., 2009
sabinene	Guang-Yi, et al., 1988; Koudou, et al., 2009
p-menthene; p-cymene; α-terpinene; thymol; carvacrol; cuminyl alcohol	Tessier, et al.,1982
δ-3-carene; α-terpineol; α-copaene	Minkue-M'eny, 2000
β-phellandrene	Dupont, et al., 1932; Koudou, et al., 2009
γ-terpinene	Minkue-M'eny, 1999; Koudou, et al., 2009
p-cymemene; terpinen-4-ol	Tessier, et al. ,1982; Koudou, et al., 2009

Table- 3: Identification of the products.

Compound	Column DB-5 (15m) Retention time (min)	Column DB-Wax (30 m)		
		Retention time (min)[*]	Kováts indexes	
			Calculated	literature
α-pinene	4.86	4.49	1032	1027-1034[a-c]
β-pinene	6.61	6.56	1120	1113-1134[a-c]
α-phellandrene	8.01	8.24	1164	1166-1205[a, c]
δ-3-carene	8.25	7.76	1139	1148[c]
p-cymene	9.01	11.37	1286	1261-1282[a-c]
β-phellandrene	~9.21	9.51	1210	1209-1241[a, c]
limonene		9.23	1201	1198-1234[a-c]
eucalyptol		9.61	1215	1214-1224[a, d, e]
α-terpineol	17.18	22.09	1728	1669-1720[b, c, e-h]

- [*]Mean value (see table 4)
- [a]Hognadottir, et al., 2003; [b]Choi, 2003; [c]Chung, et al., 1993; [d]Nishimura, 1995; [e]Chisholm, et al., 2003; [f]Lee, et al., 2003; [g]Ferreira, et al., 2001; [h] Cullere, et al., 2004.

Table- 4: Analysis of N'Toum oleoresin (Phase: DB-Wax[a])

Compounds	Retention time	Relative area (%)
α-pinene	4.49±0.01	5.9±1.7
β-pinene	6.56±0.01	1.5±0.1
δ-3-carene	7.76±0.03	8.6±1.9
α-phellandrene	8.24±0.03	63.4±5.1
limonene	9.23±0.03	6.1±2.1
β-phellandrene	9.51±0.03	5.9±1.8
eucalyptol	9.61±0.03	0.8±0.3
p-cymene	11.37±0.02	5.4±0.6
α-terpineol	22.09±0.03	1.9±0.1
Not identified	-	0.5±0.1

- Mean of four experiments.
- [a] For conditions, see experimental part.

Table- 5: Analysis of the volatile products of N'Toum, Cocobeach and Bokoué oleoresins (Phase: DB-Wax [a], relative area %).

Compounds	N'Toum	Cocobeach	Bokoué 1	Bokoué 2
α-pinene	5.9	5.8	5.3	5.4
β-pinene	1.5	1.8	1.2	1.1
δ-3-carene	8.6	**40.1**	8.1	12.8
α-phellandrene	**63.4**	13.9	4.1	19.6
limonene	6.1	16.5	24.1	**31.9**
β-phellandrene	5.9	2.1	2.8	2.8
eucalyptol	0.8	1.5	2.1	1.4
p-cymene	5.4	10.3	**42.5**	9.9
α-terpineol	1.9	3.7	7.2	11.9
Not identified	0.5	4.3	2.8	3.2

- Most important compounds in bold.
- Mean of four experiments.
- [a] For conditions, see experimental part.

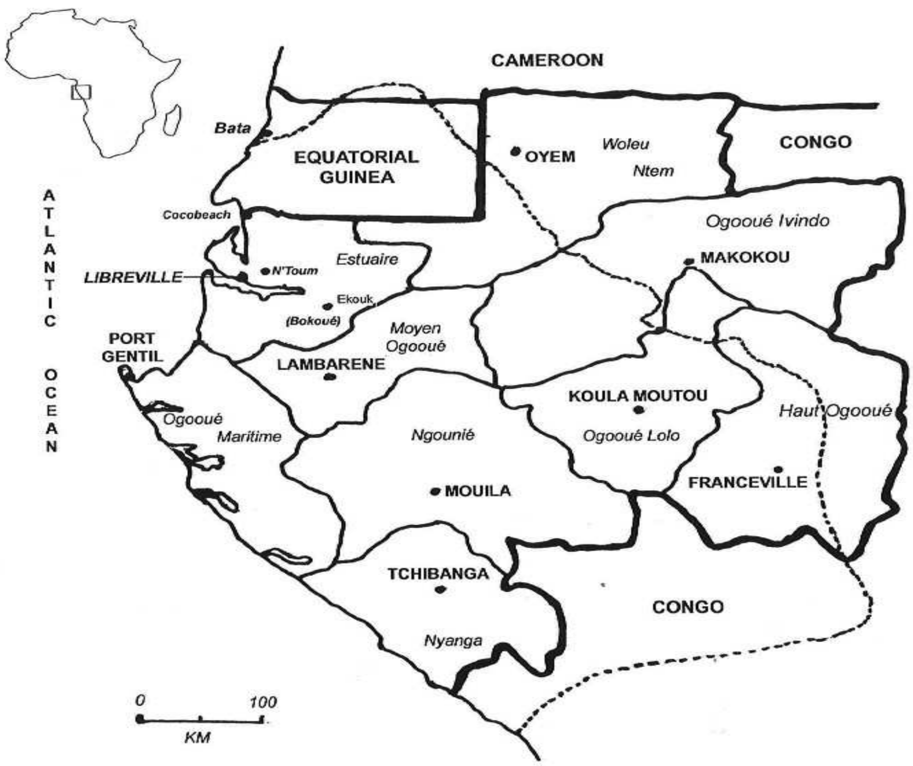
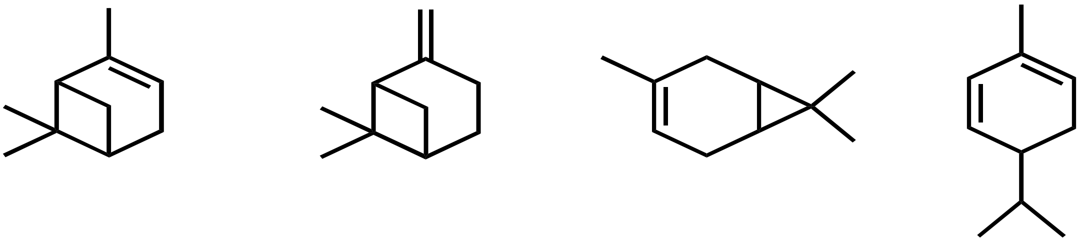
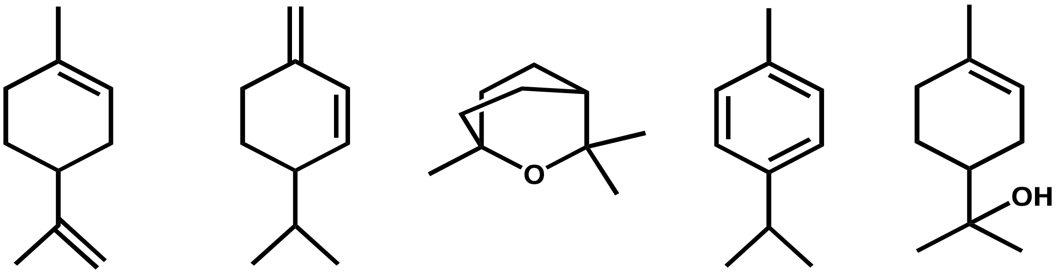


Figure- 1: Gabon map and limit area of natural okoume occurrence.

- *Estuaire*: name of the province; KOULA MOUTOU capital of the province; ----- approximative limit of okoume according to Brunck, et al., (1990).

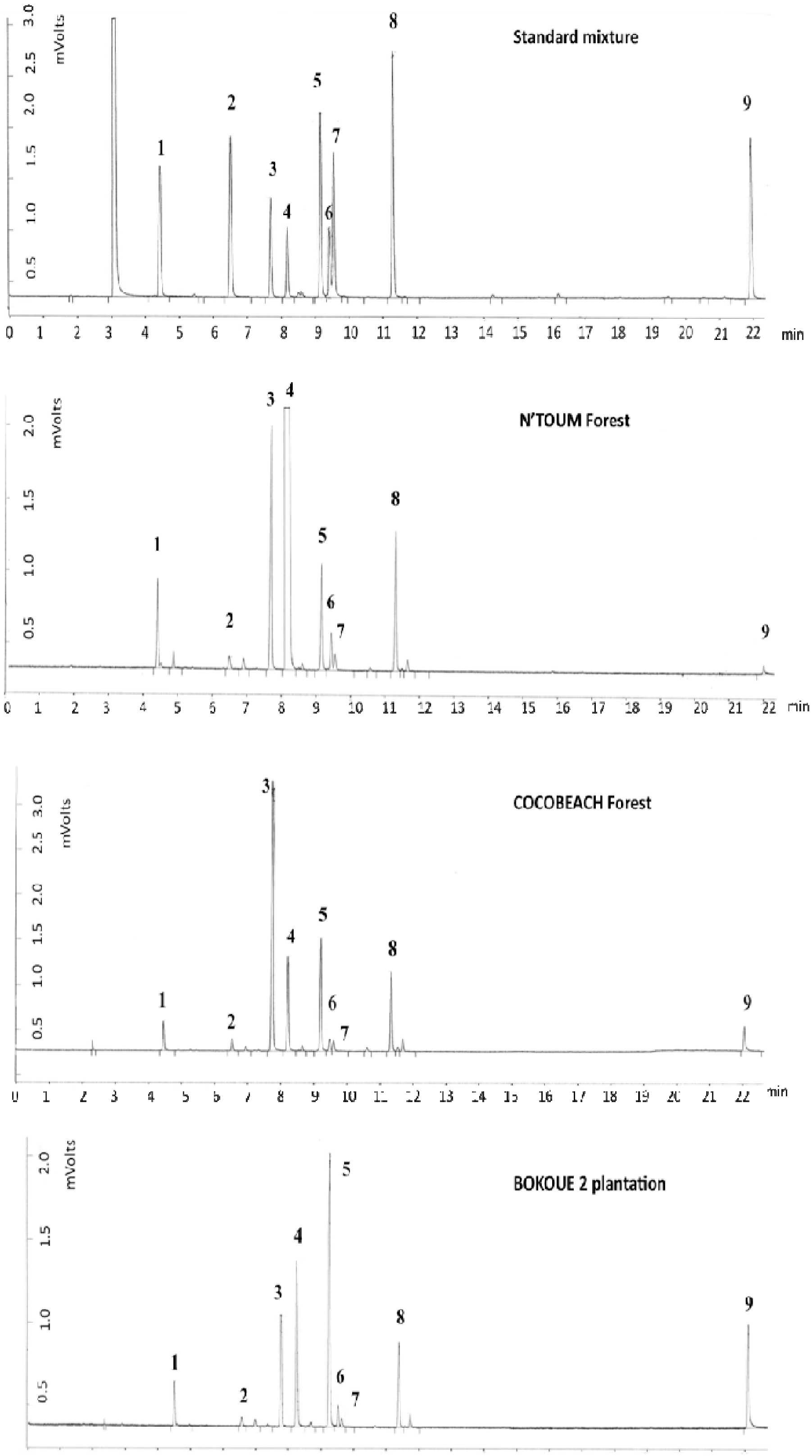


α-pinene 1 **β-pinene 2** **δ-3-carene 3** **α-phellandrene 4**



limonene 5 **β-phellandrene 6** **eucalyptol 7** **p-cymene 8** **α-terpineol 9**

Figure- 2: Volatile products identified in *Aucoumea klaineana* P. oleoresins.


Standard mixture

N'TOUM Forest

COCOBEACH Forest

BOKOUE 2 plantation

Figure- 3: Typical examples of chromatograms of volatile compounds in *Aucoumea klaineana* P. oleoresins (DB-Wax phase; see experimental part for details)