Aromatic Plants of Tropical Central Africa. 23. Chemical Composition of Leaf Essential Oils of *Eucalyptus goniocalyx* F. Muell. and *Eucalyptus patens* Benth. Grown in Rwanda

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The chemical compositions of leaf essential oils of *Eucalyptus goniocalyx* F. Muell. (Syn. *E. elaeophora*) and *Eucalyptus patens* Benth. from Rwanda were determined by GC and GC/MS. About 60 compounds have been identified in both oil samples which are monoterpenoid in character (*E. goniocalyx*, 72.8%; *E. patens*, 91.2%). The major components of the oil of *E. goniocalyx* are α−pinene (29.0%), 1,8-cineole (18.0%), and *p*−cymene (17.2%). In *E. patens* oil, 1,8-cineole is the constituent that is most abundant (39.6%) followed by limonene (32.5%) and α-pinene (11.3%). Among the minor compounds of *E. goniocalyx* and *E. patens* oils, 2,3-dioxabicyclo[4.4.0] decane structures and derivatives (respectively 3.9% and 0.3%) were identified for the first time in the volatile oils of these two species.

INTRODUCTION

Native to Australia, the genus *Eucalyptus* contains more than 700 species (Singh *et al.*, 1988) and belongs to the family Myrtaceae. Owing to the adaptability and fast growth of these trees, extensive plantations were started in many parts of the world, particularly in Africa.

Their wood finds use as firewood, as industrial fuel, and in the paper industry. The essential oils distilled from the leaves and terminal branches are used in perfumes, in medicines, and as chemical raw materials, the nature and amounts of oil components being characteristic of the different *Eucalyptus* species or even of local populations within species.

As part of our study on *Eucalyptus* naturalized in Central Africa, we have already studied several species growing in Congo (Menut *et al.*, 1992) and Burundi (Dethier *et al.*, 1994).

In Rwanda, where *Eucalyptus* is principally used for firewood, 68 species were counted within Ruhande Arboretum, and recently a selection program of individuals suitable for essential oil production was undertaken.

We present here our first results obtained about *Eucalyptus goniocalyx* and *Eucalyptus patens*, the essential oils of which have similar chemical characteristics.

EXPERIMENTAL PROCEDURES

Plant Material and Extraction. Samples of 200–300 g of air-dried leaves of *E. goniocalyx* and *E. patens*, collected in

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January 1993 in Ruhande Arboretum (Rwanda), were subjected to hydrodistillation for 8 h using a Clevenger-type apparatus (Clevenger, 1928). The essential oils were obtained in 0.6% and 0.2% (w/w) yields, respectively.

Gas−Liquid Chromatography. The compounds were first tentatively identified by peak enrichment and by their GC retention indices on two fused-silica capillary columns (25 m × 0.25 mm i.d. coated with OV101 and 25 m × 0.22 mm coated with Carbowax 20M), using a chromatograph (Shimadzu GC−14 A) equipped with a Shimadzu C-R4A Chromatopac integrator. Detector and injector temperatures were set at 250 and 210 °C, respectively; the oven temperature was programmed from 50 to 200 °C at 5 °C min⁻¹, with nitrogen as the carrier gas.

The percentage compositions were obtained from electronic measurements using flame ionization detection without taking relative response factors into account.

Gas−Liquid Chromatography/Mass Spectrometry. All samples were then analyzed by GC/MS, using a Hewlett-Packard capillary GC−quadrupole MS System (Model 5970) fitted with a 25 m × 0.23 mm i.d. fused-silica column coated with DB-1 programmed as follows: 60 °C (1 min), 60−250 °C (5 °C min⁻¹). Helium was used as carrier gas at a flow rate of 0.9 mL min⁻¹; the mass spectrometer was operated at 70 eV.

The identification of the compounds was based on a comparison of retention indices and mass spectra with those of authentic samples and with literature data (Adams, 1989; Jennings and Shibamoto, 1980).

RESULTS AND DISCUSSION

E. goniocalyx and *E. patens* oils are pale yellow fluids with characteristic fresh odors. OV101 capillary gas chromatograms of both samples are given in Figures 1 and 2; the results of the corresponding identifications are presented in Table 1, where the compounds are
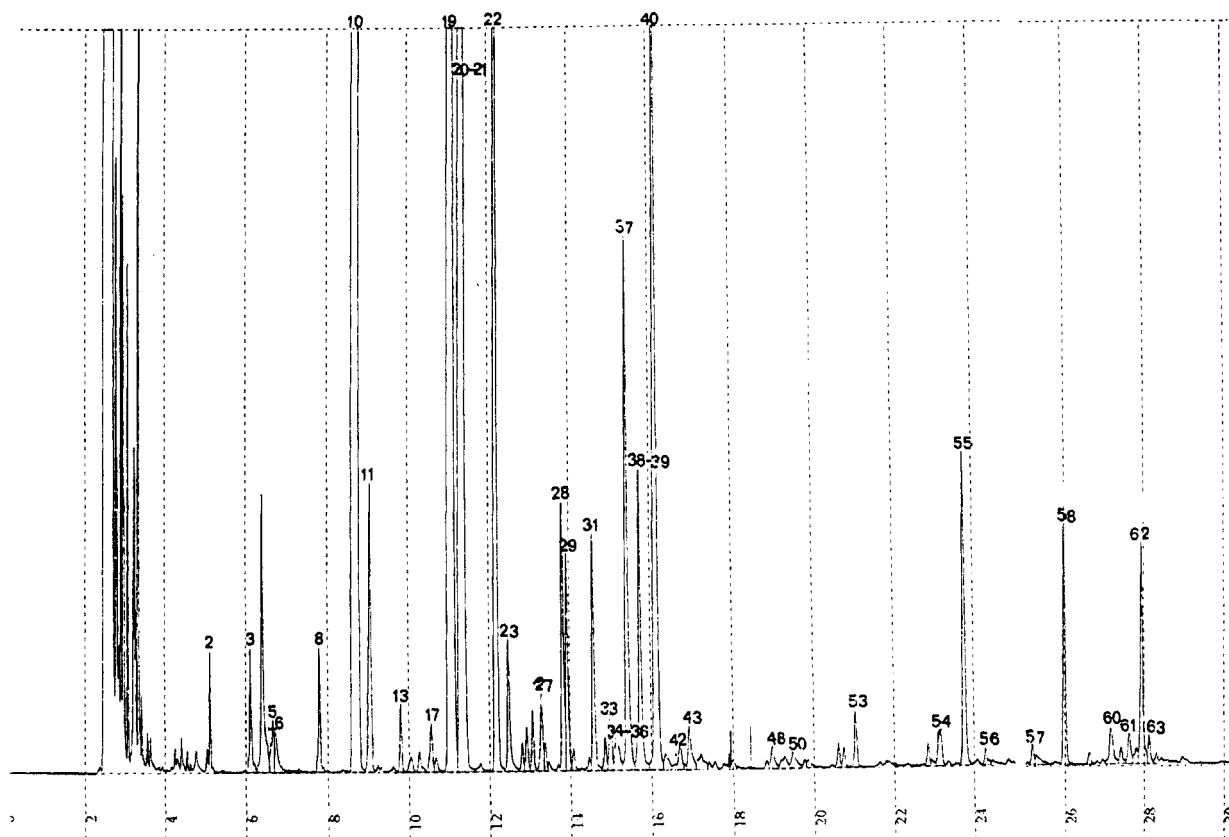
Figure 1. Gas chromatogram of *E. goniocalyx* essential oil from Rwanda (fused-silica column coated with OV101, 25 m, 0.22 mm i.d., 0.25 μm phase thickness; programmed temperature from 50 to 200 °C at 5 °C min⁻¹). See Table 1 for peak identification.
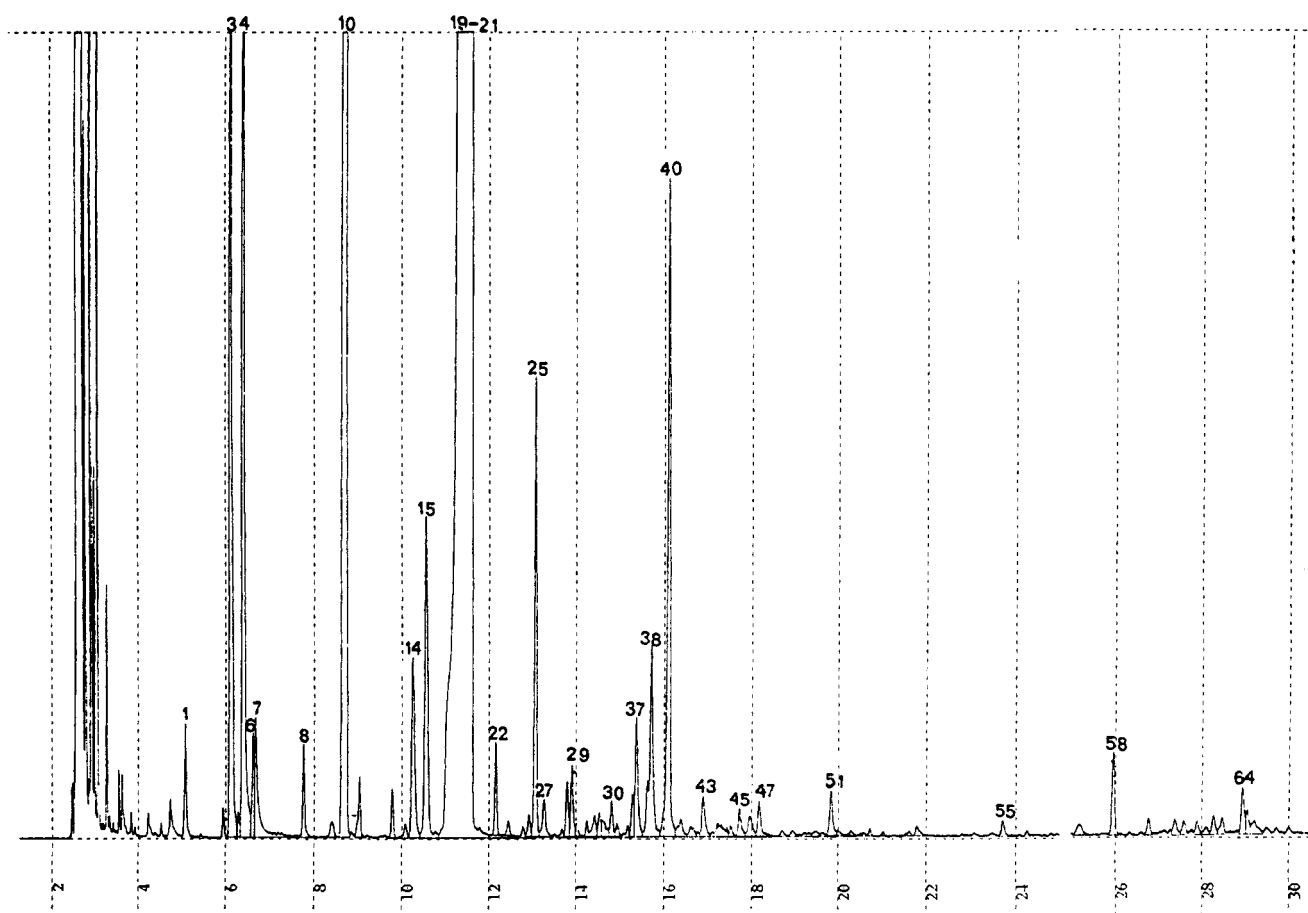

Figure 2. Gas chromatogram of *E. patens* essential oil from Rwanda (fused-silica column coated with OV101, 25 m, 0.22 mm i.d., 0.25 μm phase thickness; programmed temperature from 50 to 200 °C at 5 °C min⁻¹). See Table 1 for peak identification.

Essential Oil Composition of Two *Eucalyptus* Species

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Table 1. Percentage Composition of *Eucalyptus* Essential Oils from Rwanda

peak no.	compd	RT$_{OV101}$	peak area (%) E. goniocalyx	E. patens
1	1-hexanal	778		0.2
2	diisopropylketone	783	0.4	
3	(E)-hex-2-enal	833	0.4	2.5
4	(Z)-hex-3-enol	847	1.2	3.0
5	isobutyl propionate	851	0.3	
6	(E)-hex-2-enol	854		0.2
7	1-hexanol	856	0.3	0.4
8	isobutyl isobutyrate	902	0.5	0.2
9	α-thujene	926		0.1
10	α-pinene	935	29.0	11.3
11	camphene	948	1.2	0.2
12	sabinene	972		0.1
13	β-pinene	974	0.2	<0.1
14	(Z)-hex-3-enyl acetate	982	0.1	0.6
15	myrcene	984		1.0
16	α-phellandrene	998	0.1	
17	n-amyl isobutyrate	999	0.2	
18	isoamyl butyrate	1002	0.1	
19	p-cymene	1016	17.2	1.0
20	limonene	1026	3.3	32.5
21	1,8-cineole	1026	18.0	39.6
22	γ-terpinene	1055	6.0	0.2
23	(Z)-linalool oxide	1063	0.7	
24	(E)-linalool oxide	1074	0.1	
25	terpinolene	1077	0.2	1.0
26	camphen-6-ol	1082	0.2	
27	linalool	1090	0.4	0.1
28	fenchol	1105	1.0	0.1
29	α-camphenal	1109	1.2	0.2
30	camphor	1126		0.1
31	trans-pinocarveol	1131	1.0	
32	camphene hydrate	1140	0.1	
33	MW = 170	1144	0.3	
34	pinocarvone	1154	0.1	
35	isoborneol	1156	0.2	
36	pinocamphone	1157	<0.1	
37	borneol	1159	2.6	0.3
38	p-cymen-8-ol	1169	0.3	0.1
39	terpinen-4-ol	1170	1.2	0.6
40	α-terpineol	1182	5.3	2.0
41	myrtenol	1189	0.1	0.1
42	isopinocarveol	1198	0.2	
43	cis-carveol	1206	0.2	0.1
44	bornyl acetate	1216	0.2	
45	geraniol	1243		0.2
46	piperitone	1250		0.2
47	geranial	1252		0.2
48	cuminic alcohol	1275	0.2	
49	thymol	1280	0.1	
50	carvacrol	1289	0.1	
51	MW = 182	1300		0.2
52	MW = 222	1332	0.1	
53	regulator G$_3$ derivative[a], MW = 224	1347	0.2	
54	β-caryophyllene	1424	0.4	
55	regulator G$_1$ or G$_2$ derivative, MW = 238	1446	1.5	0.1
56	alloaromadendrene	1464	0.1	
57	bicyclogermacrene	1496	0.1	0.1
58	regulator G$_3$ derivative, MW = 252	1529	1.0	0.2
59	nerolidol	1555	0.1	
60	spathulenol	1572	0.3	
61	unidentified	1590	0.2	
62	regulator G$_1$ or G$_2$ derivative,[a] MW = 266	1603	1.1	
63	regulator G$_1$ or G$_2$[b]	1610	0.1	
64	β-eudesmol	1632		0.1

[a] Tentative identification. [b] Indefinite stereochemistry.

arranged according to their order of elution on OV101, and their linear retention indices are indicated.

Altogether, in the two samples, about 60 structures, mainly monoterpenes (*E. goniocalyx*, 72.8%; *E. patens*, 91.2%), were identified; hydrocarbons and oxygenated compounds are equally represented.

The major volatile constituents of *E. goniocalyx* are α-pinene (29.0%), 1,8-cineole (18.0%), and *p*-cymene (17.2%).

E. patens essential oil contains twice as much 1,8-cineole (39.6%) as that of *E. goniocalyx*, followed by limonene (32.5%) and α-pinene (11.3%).

To our knowledge, few chemical data are known about *E. patens*; the only reference found in literature does not concern its essential oil composition (Dell and Wilson, 1985).

On the other hand, studies on oils from *E. goniocalyx* leaves collected in India, Australia, and Spain have been published (Singh *et al.*, 1986, 1989; Lawrence and Reynolds, 1990; Boland *et al.*, 1991); these oils presented different chemical characteristics, with a higher content of 1,8-cineole (50.0−90.0%) than that measured in the present investigation.

Besides the major components previously mentioned, we report the presence, in both samples, of 2,3-dioxabicyclo[4.4.0]decane structures (**63**, regulator G$_1$ or G$_2$) and related compounds (**53, 55, 58**) in low concentrations (*E. goniocalyx*, 3.9%; *E. patens*, 0.3%); the spectral data did not allow us to differentiate G$_1$ from G$_2$, which differ in the stereochemistry of the alkyl substituent attached to the carbon adjacent to the peroxide bridge.

G is a generic term for a family of 2,3-dioxabicyclo-[4.4.0]decane system growth regulators which were originally found, in high amounts, in adult tissues (Nicholls *et al.*, 1970; Paton *et al.*, 1970) and mature leaves (Crow *et al.*, 1971) of *E. grandis*.
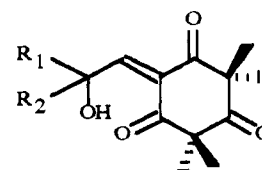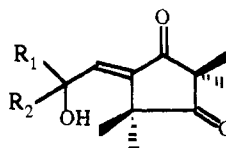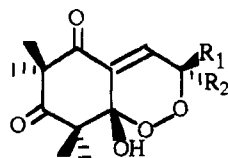
Dhawan *et al.* (1979) observed that juvenile leaves contain 400 times less of these compounds than adult leaves. According to the same authors, the adult leaves of *Eucalyptus delegatensis*, *Eucalyptus melliodora*, and another plant of the Myrtaceae, *Callistemon lanceolata*, contain also small amounts of endogenous growth regulators. They also suggest that growth regulation by G-regulators depends on their concentration: in low concentration (5 × 10^{-6} and 10 × 10^{-5} M), these compounds promote the rooting of cuttings, whereas in high concentration (5 × 10^{-4} M), they have an inhibitor effect, their biological activity being comparable to that of auxin and some other plant hormones. In addition to these properties, G-regulators may be involved in the frost resistance of plants (Bolte *et al.*, 1987; Paton, 1987).

We have previously isolated and characterized the diketo alcohol **55** (2.0−4.4%) from the essential oils of *E. grandis* from Congo (Menut *et al.*, 1992). Its presence (0.7%) was also noticed in leaf oil from the same species grown in Morocco (Zrira, 1991). Here, G-regulators and their derivatives are described for the first time in *E. goniocalyx* and *E. patens* essential oils.

The structures and mass spectra of the different atypical components found in *E. goniocalyx* and *E. patens* are given in Table 2. The structure of compound **53** has been tentatively identified by comparison of its retention index and its mass spectrum with those of its homolog **55**. The structure of compound **58** has been established from the mass spectra data given by Crow *et al.* (1971) and Bolte *et al.* (1982). Compound

Table 2. Chemical Characteristics of Atypical Compounds of *E. goniocalyx* and *E. patens* Essential Oils

compd	IR$_{ov101}$	MW	m/z (rel int, 70 eV)
33	1144	170	170 (33, M$^+$), 127 (100), 113 (71), 95 (25), 85 (30), 71 (31), 57 (41), 44 (43), 44 (67), 41 (34)
51	1300	182	182 (2, M$^+$), 167 (1), 150 (6), 15 (8), 139 (8), 123 (21), 114 (36), 83 (22), 69 (100), 4 (45)
52	1332	222	222 (100, M$^+$), 207 (65), 179 (44), 165 (66), 151 (68), 137 (34), 96 (32), 81 (30), 61 (32), 123 (46), 109 (42), 96 (32), 67 (32), 44 (51), 41 (64)
53	1347	224	224 (8, M$^+$), 209 (45), 181 (34), 167 (21), 154 (34), 139 (100), 137 (34), 123 (14), 111 (34), 109 (46), 96 (22), 81 (12), 67 (25), 59 (34), 43 (84), 41 (52)
55	1446	238	223 (13), 209 (74), 195 (10), 181 (10), 167 (37), 153 (37), 139 (100), 135 (12), 123 (21), 111 (37), 107 (12), 95 (13), 81 (14), 71 (22), 59 (10), 57 (21), 43 (84), 41 (36)
58	1529	252	237 (3), 219 (5), 209 (30), 191 (21), 182 (58), 164 (55), 149 (10), 139 (65), 123 (10), 113 (28), 96 (44), 81 (28), 70 (38), 59 (8), 55 (13), 43 (100), 41 (70)
61	1590	?	223 (23), 209 (26), 195 (66), 181 (15), 167 (43), 153 (28), 139 (70), 111 (78), 135 (24), 107 (42), 95 (22), 81 (20), 73 (34), 67 (23), 55 (34), 43 (100), 41 (71)
62	1603	266	266 (93, M$^+$), 251 (55), 233 (28), 223 (10), 209 (47), 196 (82), 178 (50), 167 (18), 163 (41), 153 (16), 140 (39), 125 (18), 113 (33), 111 (22), 96 (69), 83 (25), 81 (38), 70 (60), 59 (8), 57 (99), 43 (63), 41 (100)
63	1610	282	250 (42), 223 (15), 196 (16), 181 (34), 180 (100), 165 (30), 154 (17), 149 (42), 135 (23), 121 (20), 107 (25), 95 (27), 91 (26), 85 (15), 79 (27), 77 (24), 73 (15), 67 (21), 59 (42), 53 (17), 43 (17), 41 (17)

	R$_1$	R$_2$	G$_i$
63	Et	Me	G$_1$
	Me	Et	G$_2$
	Me	Me	G$_3$

	R$_1$	R$_2$
53 *	Me	Me
55	Me	Et

	R$_1$	R$_2$
58	Me	Me

* tentative identification

63 has been identified as the regulator G$_1$ or G$_2$ on the basis of fragmentations proposed by the same authors (retro-Diels−Alder, loss of O$_2$, and loss of dimethyl ketone).

GC analyses at different injection temperatures (150−250 °C) have shown no modification in the aspect of the chromatogram; products **53, 55, 58,** and **63** are thus components naturally present in the studied essential oils and not artifacts formed during the gas chromatography analysis.

Our current results show a comparable content in G and its derivatives in the volatile oils of *E. goniocalyx* and *E. grandis*, whereas that of *E. patens* contains smaller amounts of these components. However, this observation should be confirmed by studies on alcoholic extracts of the three previous species taking into consideration the physiological leaf age.

Examination of the botanical classification (Pryor and Johnson, 1971) of all *Eucalyptus* species previously cited indicates that they belong to two subgenera: Monocalyptus (*E. delegatensis, E. patens*) and Symphyomyrtus (*E. goniocalyx, E. grandis, E. melliodora*).

With regard to their chemical characteristics, none of the analyzed oils contains any commercially interesting product in large amount. So, the two species *E. goniocalyx* and *E. patens* from Rwanda seem to be unsuitable for eventual exploitation in essential oil production unless further bacteriological tests confirm an interesting biological activity related to the presence of G-inhibitors and derivatives in their volatile oils.

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