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Artigo

ESSENTIAL OILS FROM LEAVES OF *Cryptocarya* spp FROM THE ATLANTIC RAIN FOREST

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The essential oils from leaves of four *Cryptocarya* spp endemic in the Brazilian Atlantic rain forest were obtained by hydrodistillation and shown by GC-MS analysis to contain mono and sesquiterpenes. The major components of the oil of *Cryptocarya moschata* were linalool (34.3%), α-terpinene (17.0%), γ-terpinene (10.4%), 1,8-cineole (5.8%) and *trans*-ocimene (4.8%), whilst those of *C. botelhensis* were α-pinene (22.7%), β-pinene (9.2%), *trans*-verbenol (8.4%), *trans*-pinocarveol (5.5%) and myrtenal (5.4%). The principal compounds of *C. mandioccana* oil were β-caryophyllene (13.8%), spathulenol (10.2%), caryophyllene oxide (7.8%), δ-cadinene (6.9%) and bicyclogermacrene (6.4%), whilst those of *C. saligna* were germacrene D (15.5%), bicyclogermacrene (13.8%), spathulenol (11.8%) and germacrene B (5.7%).

Keywords : essential oils; *Cryptocarya* spp.; Lauraceae.

INTRODUCTION

Cryptocarya (Lauraceae) is a pantropical genus containing around 350 species distributed mainly within Malaysia and Australia.[1] Although 23 species have been described for South America, de Moraes[2] has recognized eight validly published species names. Seven of these species, namely, *Cryptocarya aschersoniana* Mez, *C. citriformis* (Vell.) P.L.R. de Moraes, *C. mandioccana* Meisn., *C. micrantha* Meisn., *C. moschata* Nees & Mart., *C. saligna* Mez and *C. subcorymbosa* Mez. are endemic in Atlantic Rain Forest (Mata Atlântica) of Brazil.

In earlier phytochemical investigations of the essential oils of *Cryptocarya* species, the occurrence of (-)-massoilactone was reported in the bark oil of *C. massoia*[3] and linalool was reported in leaf oil of *C. moschata* and *C. aschersoniana*, although the last of these also contained β-myrcene, 1,8-cineol, and stereo-isomeric linalool oxides.[4] Seventy one components were identified in the essential oil from leaves of *C. alba*, the most important of which were terpin-4-ol, *p*-cymol, cineol, α-pinene, β-pinene and borneol-terpineol.[5] The essential oil from leaves of *C. cunninghamii* contains either bicyclogermacrene (52.4%) or 6-nonyl-5,6-dihydro-5,6-pyrone (78-88%) as its major component, besides 6-heptyl-5,6-dihydro-2H-pyran-2-one, 6-pentyl-5,6-2H-pyran-2-one and 2-phenylethyl benzoate.[6] Analysis of essential oils obtained by hydrodistillation of leaves of *C. mandioccana*, including intraspecific variants, yielded 64 compounds with predominance of β-caryophyllene, spathulenol, caryophyllene oxide, δ-cadinene, germacrene D, benzaldehyde and bicyclogermacrene.[7] Sampling the essential oil from leaves of *C. moschata* Nees (current status *C. mandioccana* Meisner) at different times and seasons revealed no significant variation in oil composition.[8]

In the present study, we have compared the chemical constitutions of the essential oil from leaves of *C. mandioccana, C. moschata* and *C. saligna*, the three most common *Cryptocarya* species from Atlantic Rain Forest of São Paulo State, Brazil, and *C. botelhensis* – a new species described in 2006 by de Moraes.[9]

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EXPERIMENTAL

Plant material

Leaves of *C. mandioccana* Meisn. and *C. botelhensis* P. L. R. Moraes were harvested in November 1999 and December 2000, respectively, from the Parque Estadual Carlos Botelho, São Miguel Arcanjo, São Paulo. Leaves of *C. moschata* Nees & Mart. were collected in February 2002 from the Mata da Mariana, Ibaté, São Paulo, whilst leaves of *C. saligna* Mez were gathered in November 1999 from the Parque Estadual Serra do Mar – Núcleo Picinguaba, Ubatuba, São Paulo. All the species were in fruits at the date of collection except *C. botelhensis*, which was in bud. Plant material was identified by Dr. P. L. R. de Moraes. A voucher specimen of *C. mandioccana* (Cavalheiro CB353) was deposited in the São Paulo State herbarium “Maria Eneyda P. Kaufmann Fidalgo” (SP) and vouchers of *C. botelhensis, C. saligna* and *C. moschata (*Moraes 1243*,* Moraes 2472, and Moraes 2347, respectively)were deposited in Escola Superior de Agricultura Luís de Queiroz herbarium (ESA).

Hydrodistillation

Fresh leaf material (around 500 g) was frozen with liquid nitrogen, triturated using a porcelain mortar and pestle and submitted to hydrodistillation (i. e. plant material in boiling water) in a Clevenger-type apparatus to yield 0.05 to 0.06% of oil. The oil was stored at –18 ºC in a glass vial under nitrogen until required for further analysis.

Essential oil analysis

Analyses were performed using a Shimadzu model QP-5000 gas chromatograph-mass spectrometer equipped with a DB-5 (J&W

Scientific) fused silica capillary column (30 m x 0.25 mm; 0.25 µm). The oven temperature was programmed from 60 to 240 ºC at 3 ºC/min, and the injector and interface temperatures were maintained at 240 and 230 ºC, respectively.[10] The carrier gas was helium at a flow rate of 1.0 mL min^{-1} (constant volume). Mass spectra were obtained at 70 eV. Samples (1 µL) were injected manually in the split mode (20:1). Identifications of essential oil components were made by reference to the NIST 62 library,[11] and their relative percentages were calculated from normalized peak areas (uncorrected for specific responses). Retention indices[12] were determined relative to the retention times of a series of *n*-alkane standards (C-10 to C-30: Sigma, product ref. no. R 8769, Sigma), measured under the same chromatographic conditions described above, and compared with published values.[10]

RESULTS AND DISCUSSION

Ninety compounds present in the essential oils from leaves of four *Cryptocarya* species were identified from chromatographic and spectrometric data (Table 1).The chemical profiles of these oils showed significant differences in composition between the species studied (Figure 1). The main constituents of *C. mandioccana* oil were sesquiterpenes (β-caryophyllene, spathulenol, caryophyllene oxide, δ-cadinene, bicyclogermacrene and germacrene D), whilst those of the oil of *C. moschata* were acyclic and menthane type monoterpenes (linalool and α-terpinene) as previously reported for these species.[4,7] The main constituents of the essential oil of *C. botelhensis* were the pinane monoterpenes, whilst sesquiterpenes were predominant in the essential oil of *C. saligna*.

The major sesquiterpenes identified in the essential oil from leaves of *C. mandioccana* belongs to two main classes (Figure 2), namely, the humulene/caryophyllene type resulting from C1-C11 cyclisation of farnesyl diphosphate (FPP), and those derived from germacrene/bicyclogermacrene produced by C1-C10 cyclisation of the C_{15}-pre-cursor.[7,13,14] The occurrence of distinct sesquiterpene synthases, each associated with a different mode of cyclisation of FPP, is not yet conclusive and it has been suggested that a certain degree of freedom in the process of ring closure might be responsible for the formation of multiple end-products.[15] However, the essential oil from *C. saligna* exhibited a significant predominance of sesquiterpenes deriving from C1-C10 cyclisation and this may be indicative of the occurrence of a specific sesquiterpene synthase within this species.

Table 1. Constituents of the essential oil from leaves of *Cryptocarya* spp. endemic in the Brazilian Atlantic rain forest

			C. mandioccana		*C. moschata*		*C. saligna*		*C. botelhensis*	
No.	Compound	RI_{lit}	RI_{exp}	%	RI_{exp}	%	RI_{exp}	%	RI_{exp}	%
1	α-thujene	931			925	1.4	928	0.2		
2	α-pinene	939	939	1.7	932	2.3	933	4.3	935	22.7
3	α-fenchene	951							947	0.6
4	camphene	953	947	0.2	955	0.4				
5	ni	-					947	6.9		
6	thuja-2.4(10)-diene	957							952	1.2
7	ni	-					955	0.4		
8	benzaldehyde	961	957	3.4						
9	sabinene	976			972	2.6	972	5.6		
10	β-pinene	980	976	1.0	976	3.1	976	2.6	977	9.2
11	β-mircene	991			990	1.9				
12	α-terpinene	1018			1016	17.0			1016	0.3
13	*o*-cymene	1022			1022	3.1	1023	0.3	1023	0.4
14	*p*-menthene	1023							1025	0.3
15	β-felandrene	1031			1026	1.6				
16	limonene	1031					1027	0.6	1027	0.2
17	benzyl alcohol	1032					1029	1.4		
18	1.8-cineol	1033			1028	5.8			1030	3.4
19	*cis*-ocimene	1040			1035	0.5				
20	*trans*-ocimene	1050			1045	4.8				
21	γ-terpinene	1062			1056	10.4	1057	0.2	1057	0.3
22	acetophenone	1065	1069	0.5						
23	*cis*-sabinene hydrate	1068					1065	0.2		
24	*cis*-linalool oxide	1074	1074	1.0	1067	0.7				
25	allyl hexanoate	1080					1069	0.1		
26	*trans*-linalool oxide	1088	1084	0.8						
27	terpinolene	1088			1082	0.8			1082	0.6
28	ni	-							1086	0.3
29	*trans*-sabinene hydrate	1097					1097	0.2		
30	6-camphenone	1093							1088	0.6
31	linalool	1098	1097	0.7	1096	34.3	1091	0.8	1094	1.4
32	ni	1098	1102	0.5						
33	isopenthyl 2-methyl butanoate	1099							1103	0.2
34	isopentyl isovalerate	1103					1102	0.5		
35	*trans*-pinan-2-ol	1119							1120	0.1
36	α-campholenal	1125					1123	0.5	1125	2.6

Table 1. continuation

No.	Compound	RI_{lit}	*C. mandioccana* RI_{exp}	*C. mandioccana* %	*C. moschata* RI_{exp}	*C. moschata* %	*C. saligna* RI_{exp}	*C. saligna* %	*C. botelhensis* RI_{exp}	*C. botelhensis* %
37	*cis*-limonene oxide	1134					1029	0.1		
38	nopinone	1137							1134	0.4
39	*trans*-pinocarveol	1139					1137	0.7	1138	5.5
40	*cis*-verbenol	1140							1140	1.0
41	*trans*-verbenol	1144					1142	1.3	1146	8.4
42	*cis*-dehidro-α-terpineol	1144							1148	0.2
43	sabina ketone	1156					1154	0.3		
44	*trans*-pinocamphone	1160							1158	0.3
45	pinocarvone	1162					1159	0.3	1161	2.5
46	*p*-mentha-1.5-dien-8-ol	1166							1164	0.7
47	terpin-4-ol	1177			1179	0.9	1175	0.7		
48	naphthalene	1179					1183	0.3		
49	*p*-cymen-2-ol	1183							1183	0.3
50	α-terpineol	1189							1184	0.1
51	myrtenal	1193					1194	0.8	1194	5.4
52	verbenone	1204					1207		1207	2.4
53	*trans*-carveol	1217							1217	0.7
54	carvone	1242							1240	0.1
55	bornyl acetate	1285					1285	0.6		
56	δ-elemene	1339	1337	0.3			1337	1.8		
57	α-cubebene	1351	1350	0.8					1349	0.4
58	α-copaene	1376	1376	2.9			1375	1.6	1376	1.2
59	β-bourbonene	1384	1384	0.8			1384	0.5		
60	β-cubebene	1390	1390	0.5			1391	0.5	1389	0.2
61	β-elemene	1391	1392	2.6			1393	2.8		
62	ni	-							1395	0.4
63	β-caryophyllene	1418	1418	13.8	1418	3.3	1419	4.7	1419	0.6
64	β-cedrene	1418	1420	0.3						
65	β-gurjunene	1432	1430	0.4						
66	aromadendrene	1439	1438	0.4						
67	γ-elemene	1433					1433	0.8		
68	α-humulene	1454	1453	2.1	1460	0.5	1453	1.3		
69	*allo*-aromadendrene	1461	1461	2.6			1460	0.3	1461	3.1
70	γ-muurolene	1477	1476	1.4					1477	0.5
71	germacrene D	1480	1481	4.5			1481	15.5		
72	ar-curcumene	1483							1482	1.2
73	β-selinene	1485	1486	1.4						
74	phenyl ethyl 3-methylbutanoate	1489							1486	2.7
75	valencene	1491	1491	0.8						
76	*epi*-cubebol	1493							1495	2.5
77	bicyclogermacrene	1494	1496	6.4			1497	13.8		
78	α-muurolene	1499	1502	1.2						
79	γ-cadinene	1513	1514	1.3					1516	2.3
80	δ-cadinene	1524	1528	6.9			1523	2.2		
81	cadina-1.4-diene	1532	1531	0.3						
82	α-calacorene	1542	1542	0.4						
83	germacrene B	1556			1566	1.0	1556	5.7		
84	spathulenol	1576	1578	10.2			1577	11.8	1582	6.1
85	*trans*-sesquisabinene hydrate	1580							1586	4.9
86	carophyllene oxide	1581	1583	7.8			1582	1.9		
87	globulol	1583					1589	0.6		
88	viridiflorol	1590	1589	2.8						
89	guaiol	1595	1595	1.0						
90	ni	-	1604	2.6						
91	ni	-	1609	0.8						
92	*epi*-cubenol	1627	1628	1.9						
93	ni	-	1638	0.9						
94	cubenol	1642	1642	3.1						
95	α-muurolol	1645	1645	0.8						
96	ni	-	1649	1.1						
97	selin-11-en-α-ol α-cadinol	1653	1653	3.2						
98	14-hydroxy-9-*epi*-caryophyllene	1664	1657	0.7						
99	ni	-	1673	0.8						
100	khusinol	1674	1687	0.5						

RI_{lit} = retention index from literature; RI_{exp} = experimental retention index; % = normalized percentage; ni = not identified

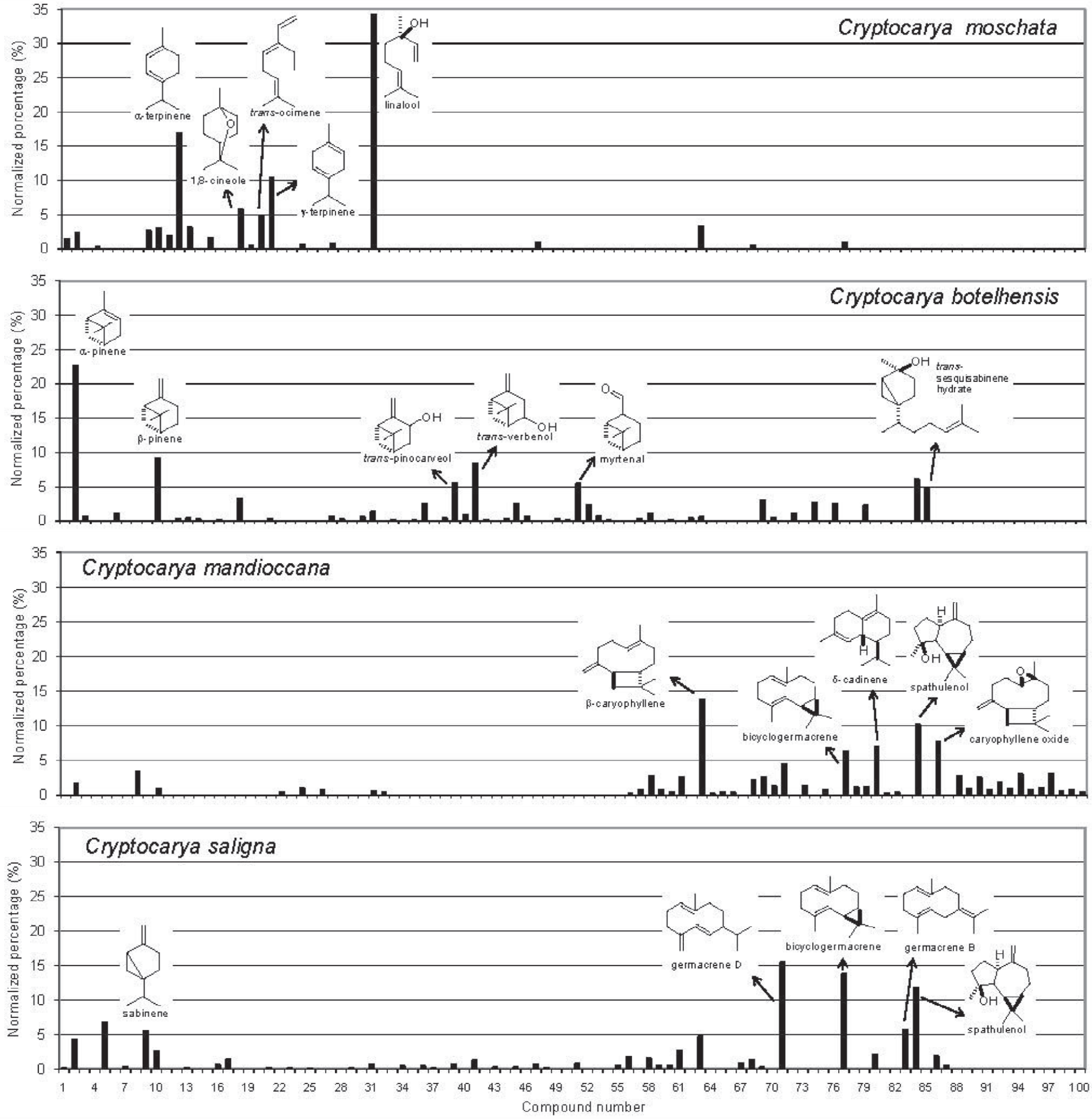

Figure 1*. Chemical profile of essential oils from leaves of* Cryptocarya *spp. For the key to peak identity, see Table 1*

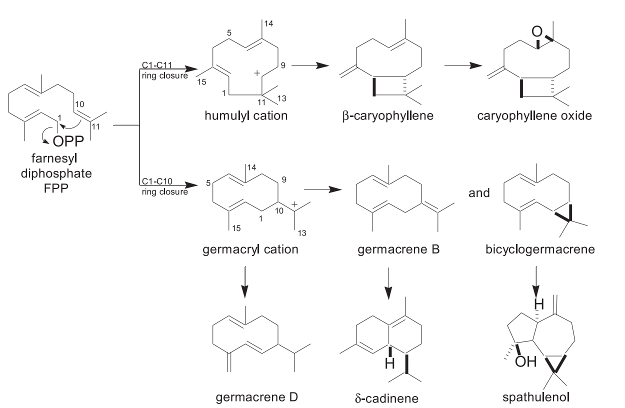

Figure 2. *Biogenetic relationships between the main sesquiterpenes of* Cryptocarya mandioccana *and* C. saligna

CONCLUSION

The chemical profile of essential oils from leaves of four members of the genus *Cryptocarya* was characteristic to each of the species studied. The oil of *C. moschata* presented a predominance of acyclic (linalool, *trans*-ocimene) and menthane (α-terpinene, γ-terpinene, 1,8-cineole) monoterpenes, whilst pinane monoterpenes (α-pinene, β-pinene, *trans*-verbenol, *trans*-pinocarveol, myrtenal) were the main components in the oil of *C. botelhensis.* Sesquiterpenes derived from C1-C11 (β-caryophyllene, caryophyllene oxide) and C1-C10 (spathulenol, δ-cadinene, bicyclogermacrene) cyclization of FPP were the main constituents of *C. mandioccana* oil, although the principal components of the oil of *C. saligna* were sesquiterpenes derived from C1-C10 cyclisation of the FPP precursor (germacrene D, bicyclogermacrene, spathulenol, germacrene B).

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