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Essential Oil Composition of Brazilian *Pourouma* Species

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Abstract

The chemical composition of essential oils isolated by hydrodistillation from ripe fruits and leaves of *Pourouma cecropiifolia* Martius, *Pourouma mollis* Trécul and *Pourouma velutina* Martius ex Miguel was determined by GC and GC/MS. From ripe fruits of *P. cecropiifolia* Martius, 24 compounds were identified, accounting for 73.8% of the oil. Oxygenated monoterpenes were found to be an important group of constituents with linalool (3.1%) predominating. A new monoterpene, 2-methoxy-1,7,7-trimethylbicyclo[2.2.1]hept-2-ene (2-methoxybornylene), was tentatively identified by mass spectrometry. In addition, 29, 37 and 26 constituents were identified in the leaf oils of *P. cecropiifolia*, *P. mollis* and *P. velutina*, respectively. Aliphatic alcohols, aldehydes and esters (C₅-C₉) were quantitatively the most significant components. (Z)-3-Hexenol was clearly the dominant product occurring in contents of 26.7%, 22.3% and 3.2%, respectively. The major sesquiterpene hydrocarbons in the analyzed oils were β -caryophyllene (9.1%) and (E,E)- α -farnesene (4.0%) from *P. cecropiifolia* oil; (E,E)-farnesene (1.7%) from *P. mollis* oil; and (1.5%) from *P. velutina* oil. Linalool (26.1%) was the major compound identified in the volatile fraction of *P. mollis*. It was also identified in the oils of *P. cecropiifolia* (1.2%) and *P. velutina* (1.7%). Phytol was the main constituent of the leaf oil of *P. velutina*. Methyl salicylate was also identified in the leaf oils of *P. cecropiifolia* (1.6%), *P. mollis* (1.4%) and *P. velutina* (2.8%).

Key Word Index

Pourouma cecropiifolia, *Pourouma mollis*, *Pourouma velutina*, Moraceae, essential oil composition, 2-methoxybornylene, hexadecanoic acid, (Z,Z)-octadeca-9,12-dienoic acid, (Z)-3-hexenol, linalool, phytol

Introduction

Pourouma species are small to medium-sized trees of the rain forest areas in South and Central America. These plants are well represented in Amazon Basin and in the Guiana region. *P. mollis* Trécul and *P. velutina* Martius ex Miguel also occur in eastern Brazil. These species have not drawn much attention, except for the fact that the fruits of *P. cecropiifolia* Martius are suitable for human consumption. To the best of our knowledge, only *P. cecropiifolia*—locally called the Amazon grape, uvilla, mapati and imbaúba do vinho—is cultivated as a fruit tree. The taste of its mesocarp strongly resembles that of grapes; they are used to prepare a sweet wine (1). *P.*

cecropiifolia produces fruit as small trees, about three years after planting (2). Other *Pourouma* species may have fruits as tasty as those of *P. cecropiifolia*, but the fleshy mesocarp-like layer is usually thinner. In Brazil, *P. mollis* and *P. velutina* are native trees in the State of Bahia, where they are known as itararanga and tararanga, respectively. Some species of *Pourouma* emit a peculiar odor, described as spearmint, wintergreen, balsam or bengue-like (1). However, no study on the oil composition of *Pourouma* species has been previously reported.

In this study the constituents of the oils from ripe fruits and leaves of *P. cecropiifolia*, *P. mollis* and *P. velutina* were investigated.

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Table I. Percentage composition of the fruit oil of *Pourouma cecropiifolia*

Peak no.	Compound	RI(FFAP)	Peak area (%)	Methods	Ref.
1	limonene	1206	t	Co	20
2	1,8-cineole	1214	t	Co	20
3	hexanol	1364	0.1	MS, RI	21
4	(Z)-3-hexenyl acetate	1381	t	MS, RI	21
5	(Z)-3-hexenol	1396	0.1	MS, RI	21
6	3-octanol	1404	0.1	MS, RI	22
7	cis-linalool oxide(furan isomer)	1447	0.5	MS, RI	22
8	1-octen-3-ol	1463	0.6	MS, RI	22
9	trans-linalool oxide(furan isomer)	1476	0.2	MS	-
10	2-methoxybornylene	1523	0.4	MS	-
11	linalool	1561	3.1	MS, RI, Co	20
12	octanol	1569	t	MS, RI	21
13	p-menth-1-en-9-al (isomer)*	1620	0.2	MS, RI	-
14	p-menth-1-en-9-al (isomer)*	1625	0.3	MS, RI	-
15	α -terpineol	1705	0.9	MS, RI, Co	20
16	methyl salicylate	1786	6.0	MS, Co	-
17	ethyl salicylate	1822	0.2	MS	-
18	geraniol	1863	0.2	MS, RI, Co	22
19	β -ionone	1946	0.1	MS, RI	22
20	(E)-nerolidol	2052	0.1	MS, Co	-
21	tetradecanoic acid	-	1.5	MS	-
22	pentadecanoic acid	-	0.8	MS	-
23	hexadecanoic acid	-	34.5	MS	-
24	(Z,Z)-octadeca-9,12-dienoic acid	-	23.9	MS	-
	Total		73.8		

RI=retention index on polar FFAP column; MS=identification based on the mass spectrum; Co=identity confirmed by co-injection with authentic sample; t=trace (<0.05%); *correct isomer not identified

Experimental

Plant material: Ripe fruits and leaves of *P. cecropiifolia* Martius were collected from cultivated trees growing in the experimental Station of EMBRAPA- Amazônia Ocidental, Manaus District, Amazon, in October 1996 and in January 1997, respectively. Voucher sample is deposited at CEPEC Herbarium, under the number 60767.

Leaves of *P. mollis* and *P. velutina* were collected from trees growing in the Biological Reserve of Mico Leão, Una District, State of Bahia, in March 1997. Voucher samples are deposited at CEPEC Herbarium, under the register number 60768 and 60766, respectively.

Isolation of volatile compounds: Ripe fruits (6.0 kg) of *P. cecropiifolia* were first washed with distilled water and the seeds removed. Pulp and peel of the fruits were blended with distilled water. Leaves of *P. cecropiifolia* (2.0 kg), *P. mollis* (1.0 kg) and *P. velutina* (1.1 kg) were comminuted and placed in a glass vessel with water. These plant materials were submitted to hydrodistillation using a Clevenger-type apparatus for approximately 6 h (3). The oil from ripe fruits of *P. cecropiifolia* yielded 0.003% on dry basis. The leaf oils of all of the *Pourouma* species studied were obtained in smaller quantities. The oils were recovered from aqueous layer by extraction with dichloromethane (spectroscopic grade), dried over anhydrous sodium sulphate and stored at 5°C, for further analysis.

Identification of oil components: Capillary GC was carried out using a Hewlett Packard 5890 Series II Chromato-

graph equipped with flame ionization detector. Hydrogen was used as carrier gas at 1 mL/min; split ratio 100/1; condition 1: HP-1 fused-silica column (25 m x 0.32 mm, film thickness 0.17 μ m); oven temperature was programmed from 40°C (5 min) to 260°C at 3°C/min; injector port temperature 280°C; detector temperature 300°C; condition 2: FFAP fused-silica column (25 m x 0.2 mm, film thickness 0.3 μ m); oven temperature was programmed from 60°C (5 min) to 210°C at 4°C/min; injector port temperature 250°C; detector temperature 280°C. Peak areas were computed by a Hewlett Packard integrator.

GC/MS analyses were carried out on a Hewlett Packard capillary GC-quadrupole MS system (Model 5995C), using the same gas chromatographic parameters as above with helium as carrier gas at 1 mL/min; ionization voltage, 70 eV; ion source temperature, 180°C.

Identification of the oil components was achieved by comparison of retention indices (4-22) and, whenever possible, by co-injection with an authentic sample. Comparison and interpretation of fragmentation patterns in mass spectra with those stored in the NIST computer database and published in reference books (5, 14) were also applied for the identification of the compounds.

Results and Discussion

Twenty four components were identified in the oil isolated by hydrodistillation from ripe fruits of *P. cecropiifolia*, accounting for 73.8% of the mixture. The total content of fatty

Table II. Percentage composition of the leaf oils of *Pourouma cecropiifolia*, *P. mollis* and *P. velutina*

Peak no.	Compound	RI(HP-1)	Peak area (%)			Methods	Ref.
			<i>P. cecropiifolia</i>	<i>P. mollis</i>	<i>P. velutina</i>		
1	1-penten-3-ol	-	3.2	-	-	MS	-
2	2-pentanol	-	0.9	-	-	MS	-
3	3-methylbutanol	719	0.9	-	-	MS, RI	6
4	hexanal	776	3.8	-	-	MS, RI	5
5	(E)-2-hexenal	829	1.4	-	-	MS, RI	6
6	(Z)-3-hexenal	842	26.7	22.3	3.2	MS, RI	5
7	(E)-2-hexenol	849	1.4	0.8	-	MS, RI	5
8	hexanol	855	6.2	3.1	1.1	MS, RI	5
9	heptanal	879	1.4	0.1	0.2	MS, RI	5
10	1-octen-3-ol	965	1.3	1.3	t	MS, RI	16
11	1,8-cineole	1008	-	t	-	MS, RI, Co	5
12	limonene	1012	-	t	-	MS, RI, Co	9
13	cis-linalool oxide(furan isomer)	1055	t	0.3	-	MS, RI, Co	10
14	octanol	1058	-	0.8	-	MS, RI	15
15	trans-linalool oxide(furan isomer)	1073	0.6	0.4	t	MS, RI	10
16	nonanal	1082	1.4	2.6	0.6	MS, RI, Co	15
17	linalool	1084	1.2	26.1	1.7	MS, RI, Co	16
18	trans-p-menth-2-en-1-ol	1117	1.3	-	-	MS, RI	17
19	terpinen-4-ol	1160	0.4	0.2	-	MS, RI	16
20	methyl salicylate	1172	1.6	1.4	2.8	MS, RI, Co	5
21	α -terpineol	1174	1.0	1.5	0.3	MS, RI, Co	11
22	(Z)-3-hexenyl butyrate	1176	1.5	1.2	0.5	MS, RI	5
23	hexyl butyrate	1182	-	0.2	t	MS, RI	5
24	decanal	1184	-	0.2	t	MS, RI	6
25	β -cyclocitral	1187	1.1	0.2	t	MS, RI	4
26	(Z)-3-hexenyl 2-methylbutyrate	1214	-	2.0	t	MS	-
27	(Z)-3-hexenyl isovalerate	1217	-	0.3	-	MS	-
28	hexyl 2-methylbutyrate	1222	-	0.2	-	MS	-
29	2-decenal*	1232	-	1.2	t	MS, RI	6
30	2-undecanone	1272	-	0.2	-	MS, RI	15
31	(Z)-3-hexenyl hexanoate	1361	-	0.3	t	MS	-
32	α -copaene	1365	0.7	-	-	MS, RI, Co	8
33	α -ionone	1397	1.2	0.4	0.6	MS, RI	5
34	β -caryophyllene	1401	9.2	t	-	MS, RI, Co	16
35	α -humulene	1430	1.2	-	-	MS, RI, Co	16
36	β -ionone	1452	1.5	0.4	0.2	MS, RI	5
37	germacrene D	1458	-	0.3	t	MS, RI	16
38	(E,E)- α -farnesene	1492	4.0	1.7	1.5	MS, RI	18
39	δ -cadinene	1506	-	0.2	-	MS, RI	16
40	(Z)-3-hexenyl benzoate	1532	-	1.4	0.4	MS, RI	18
41	(E)-nerolidol	1542	1.5	0.8	0.4	MS, RI, Co	4
42	caryophyllene oxide	1546	1.8	-	-	MS, RI	17
43	pentadecanal	1687	-	0.2	0.5	MS, RI	19
44	hexadecanoic acid	1946	-	4.3	13.7	MS, RI	10
45	phytol	2091	4.7	3.6	38.8	MS, RI	13
46	(Z,Z)-octadeca-9,12-dienoic acid	2107	-	8.3	5.9	MS	-
	Total		83.1	88.5	72.4		

RI=retention index on non-polar HP-1 column; MS=identification based on the mass spectrum; Co= identity confirmed by co-injection with authentic sample; t=trace (<0.05%); *correct isomer not identified

acids in the oil was high approximately 60.7%, comprising mainly of hexadecanoic acid (34.5%) and (Z,Z)-octadeca-9,12-dienoic acid (23.9%). Methyl salicylate (6.0%) was the major constituent detected in the volatile fraction of *P. cecropiifolia* ripe fruits. Oxygenated monoterpenes, aliphatic alcohols and esters (C₆-C₈), in number, contributed significantly to the oil, whereas linalool (3.1%) was in largest amount. (E)-nerolidol (0.1%) was the only identified sesquiterpene

alcohol. Table I lists the composition of the oil obtained from ripe fruits of *P. cecropiifolia*.

A new oxygenated monoterpene was tentatively identified as 2-methoxy-1,7,7-trimethylbicyclo[2.2.1]hept-2-ene (2-methoxybornylene) from its mass spectrum (Figure 1). It revealed a molecular formula C₁₁H₁₈O. Loss of a neutral fragment with 30 mass units indicates the presence of a methyl ether in the structure, directly linked to a double bond.

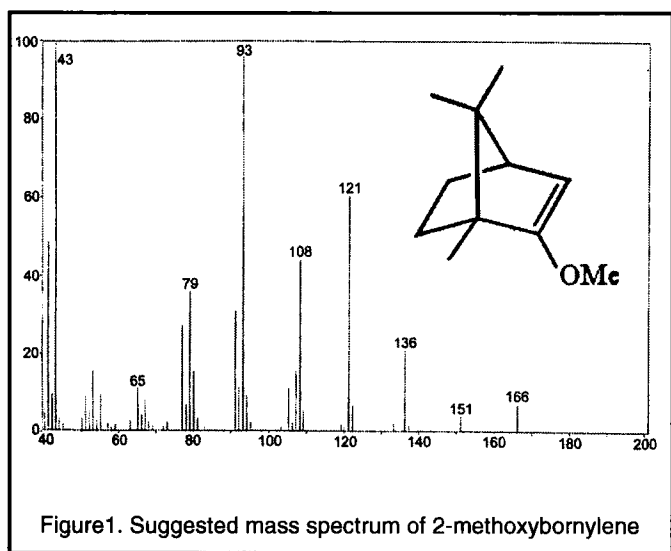


Figure 1. Suggested mass spectrum of 2-methoxybornylene

The loss of this fragment lead to the formation of a fragment at m/z : 136 ($C_{10}H_{16}$), suggesting a monoterpene structure. Subsequent fragmentation of this ion gives rise to a spectrum that is completely superposed by the spectrum of a monoterpene hydrocarbon known as bornylene or 1,7,7-trimethylbicyclo [2.2.1] hept-2-ene. The characteristic peak at m/z 108, due to a fragment formed by a retro-Diels-Alder fragmentation, allows to place the double bond between two and three carbons. The position of a methoxy group is based on biosynthesis considerations. Similar to camphor and borneol, which are the most common examples of this carbon skeleton, the oxygenated function is located at the two position of bornane.

In the oil isolated from leaves of *P. cecropiifolia*, 29 compounds were identified amounting to 83.1%. Aliphatic alcohols, aldehydes and esters (C_5 - C_9) were quantitatively the major components; among them the (Z)-3-hexenol was found in higher percentage. Six oxygenated monoterpenes were also identified. The principal sesquiterpene hydrocarbons were β -caryophyllene (9.1%) and (E,E)- α -farnesene (4.0%). The identified components of the leaf oil of *P. cecropiifolia* and their percentages are given in Table II.

The principal constituent of *P. mollis* leaf oil was linalool (26.1%). Thirty seven constituents were identified, accounting for 88.5% of the oil. Aliphatic alcohols, aldehydes and esters (C_6 - C_{10}), both in number and in quantity, contributed significantly to the oil, (Z)-3-hexenol being the major compound in this group. The most abundant sesquiterpene hydrocarbon was (E,E)-farnesene (1.7%). A complete list of compounds identified in this oil is given in Table II.

In the leaf oil of *P. velutina*, 26 compounds were identified, accounting for 72.4% of the total. In contrast to the other *Pourouma* species leaf oils, a small number of compounds was found, phytol (38.8%) and hexadecanoic acid (13.7%) being the main representatives. Aliphatic alcohols and esters (C_6) accounted for 5.2%, where (Z)-3-hexenol (3.2%) and hexanol (1.1%) predominated. Among the terpenoids linalool (1.7%), (E,E)-farnesene (1.5%) and (E)-nerolidol (0.4%) were found as major products. The compounds identified in leaf oil of *P. velutina* are shown in Table II.

A comparison of these results clearly shows some qualitative and quantitative similarities among the oils obtained from leaves of *Pourouma* species. The aliphatic alcohols, aldehydes and esters (C_5 - C_{10}) fraction was present in these oils whereas (Z)-3-hexenol was always the major component. Methyl salicylate could be found in all oils analyzed: *P. cecropiifolia* (1.6%), *P. mollis* (1.4%) and *P. velutina* (2.8%). (E,E)-Farnesene and (E)-nerolidol were found as major sesquiterpenes in the three leaf oils studied. Linalool and its furanoid derivatives were also present in all oils, but in variable proportions. A new compound identified as 2-methoxybornylene was detected only in ripe fruit oil from *P. cecropiifolia*.

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