



# Characterization of the chemical composition of oleoresins of *Copaifera guianensis* Desf., *Copaifera duckei* Dwyer and *Copaifera multijuga* Hayne

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## Abstract

Oleoresins from different species of *Copaifera* are used in popular medicine and in the cosmetics industry without giving due consideration to the differences between them. Comparison of the compositions of oleoresins from *Copaifera guianensis* Desf. and *Copaifera duckei* Dwyer, species now examined, and of the better known *Copaifera multijuga* Hayne, all of common occurrence in Brazilian Amazônia, shows that significant chemical variation occurs not only between species but also within a given species and in an individual tree source. © 2000 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Copaifera guianensis* Desf.; *Copaifera duckei* Dwyer; *Copaifera multijuga* Hayne; Leguminosae; Copaiba; Sesquiterpenes; Diterpenes; Labdane; Clerodane; Kaurane

## 1. Introduction

The oleoresin obtained by tapping the trunk of trees of the *Copaifera* (Leguminosae- Caesalpinoideae) genus is widely used in Brazilian popular medicine, under the name “óleo de copaíba” (copaiba balsam), mainly as a healing, antiseptic and anti-inflammatory agent (Le Cointe, 1934; Pio Corrêa, 1984). In early Portuguese reports about Brazil, dating from the sixteenth century (Youngken, 1945), its medicinal use by indigenous peoples was mentioned, and its introduction into Europe as a drug for the treatment of blennorrhoea is recorded for the following century (Osol and Farrar, 1955). Still today, copaiba balsam is one of the most used medicines in the Brazilian Amazon, a region where the rural population has little access to industrialised pharmaceutical products and conventional health services.

Neither herbal medicine practitioners nor the cosmetics industry that use copaíba oleoresin always take into account the existence of more than 20 species of *Copaifera* in Brazil (Dwyer, 1951) and the significant

differences of chemical composition that occur among them.

Present Brazilian drug policy requires that medications of natural origin should be standardised chemically and evidence should be presented as to efficacy and safety in use. The analysis and chemical standardisation of the oleoresins of different species of *Copaifera* is clearly essential if one is to relate chemical composition with biological activity and so permit validation as a safe and effective phyto-medicine with adequate quality control (Gilbert et al., 1997).

The oleoresin is a natural solution of diterpene acids in an essential oil composed mainly of sesquiterpenes. Bactericidal (Maruzzella and Sicurella, 1960; Opdyke, 1976), anti-helminthic (Pellegrino, 1967; Gilbert et al., 1972), analgesic (Fernandes and Pereira, 1989), anti-inflammatory (Basile et al., 1988; Fernandes et al., 1992), gastro-protective (Paiva et al., 1998), antitumor (Ohsaki et al., 1994; Pessoa et al., 1994) and trypanocidal (Cascon et al., 1998) activities have been recorded, a variety of substances have been identified, yet little is known of the relationship between the structures and activity of the components.

At the present time, the Amazon region is the main supplier of copaiba oleoresins used and marketed in

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Brazil and in the world in general. Of several species of *Copaifera* that occur in this region most have not been evaluated chemically. The present study has, therefore, been centred on species of this region.

Oleoresin samples were collected from *Copaifera guianensis* Desf. and *Copaifera duckei* Dwyer, species of common occurrence in the Brazilian Amazon but for which chemical data are lacking, and from *Copaifera multijuga* Hayne, a major source of Manaus based balsam, and their chemical composition characterized quantitatively and qualitatively by gas chromatography–mass spectrometry (GC–MS).

## 2. Results and discussion

### 2.1. *Copaifera multijuga*

Of two samples (denoted CM and ICM) examined (Table 1), sesquiterpene hydrocarbons made up about 80% of the oleoresin.

In both oleoresins these are predominantly  $\alpha$ -copaene,  $\beta$ -caryophyllene (the main component comprising 60% of the hydrocarbon content in CM and 43% in ICM),  $\alpha$ -bergamotene,  $\alpha$ -humulene and  $\delta$ -cadinene. ICM had about 16% of oxygenated sesquiterpenes, about a half of these being  $\beta$ -caryophyllene oxide (8.8%), possibly an oxidative artefact produced during storage. CM contained only traces of oxygenated sesquiterpenes but contained over 20% of diterpene acids while ICM

contained less than 3% of these. Copalic, enantiogathic and 3-acetoxy-copalic acids are the main diterpene acids in both balsams; one of them (CM) containing, besides these, eperu-8(17)-en-15,18-dioic acid, all of them bicyclic diterpenoids of the labdane group.

### 2.2. *Copaifera guianensis*

The analysis of this sample (Table 1, CG) showed a much smaller proportion (20%) of sesquiterpene hydrocarbons, but a larger amount (24%) of oxygenated sesquiterpenes, again mainly  $\beta$ -caryophyllene oxide. As this sample was collected and analysed without opportunity for oxidation to have occurred, we can affirm that the caryophyllene oxide is a genuine product of the secondary metabolism of *Copaifera* and not an artefact, resulting from storage as was generally believed up till now. *C. guianensis* contained double the amount (45%) of diterpene acids found in the CM sample of the previous species and, distinctly from the other two species studied, also contained an as yet unidentified diterpene alcohol (11.5%, Table 3). Again  $\alpha$ -copaene,  $\beta$ -caryophyllene,  $\alpha$ -bergamotene and  $\alpha$ -humulene appear among the sesquiterpenes together with  $\beta$ -selinene and  $\beta$ -bisabolene. Of the 45% of diterpene acids, bicyclic diterpene acids of the labdane group, cativic, copalic, eperu-8(17)-en-15,18-dioic and polyalthic acids comprise about 15% (note that polyalthic acid bears a  $\beta$ -furan ring) but 18.5% are tetracyclic acids of the kaurane series (kaur-16-en-19-oic and kauran-19-oic acids) and

Table 1

Terpenoids which occur in all three *Copaifera* species and those which distinguish the three oleoresins<sup>a</sup>

Identified component	Percentages of selected terpenoids in the oleoresins of three <i>Copaifera</i> species							
	<i>C. multijuga</i>		<i>C. guianensis</i>	<i>C. duckei</i> <sup>b</sup>				
	CM	ICM	CG	CJ	PA	AP 04/96	AP 07/96	AP 11/97
<i>Sesquiterpenes</i>								
$\alpha$ -Copaene	2.1	5.2	0.6	nd	nd	nd	nd	nd
<i>trans</i> - $\beta$ -Caryophyllene	60.3	42.9	4.7	6.2	0.7	0.3	7.1	17.7
<i>trans</i> - $\alpha$ -Bergamotene	2.0	7.0	7.2	3.4	7.9	0.4	11.2	19.7
$\beta$ -Selinene	nd	nd	1.5	7.3	5.5	0.5	8.2	14.8
$\alpha$ -Selinene	nd	nd		4.5	2.8	0.2	4.3	8.2
$\beta$ -Bisabolene	nd	nd	2.1	8.9	12.1	1.0	7.5	8.3
$\delta$ -Cadinene	1.6	2.9	nd	nd	nd	nd	nd	nd
$\beta$ -Caryophyllene oxide	traces	8.8	19.1	nd	nd	nd	nd	nd
<i>Diterpene acids</i>								
Kaur-16-en-19-oic	nd	nd	17.5	19.8	24.5	nd	nd	nd
Kauran-19-oic	nd	nd	0.9	nd	nd	nd	nd	nd
Copalic	11.0	1.9	1.4	4.8	traces	0.8	0.6	nd
Polyalthic	nd	nd	10.6	27.7	17.1	16.6	11.8	9.9
Hardwickic	nd	nd	11.0	nd	24.3	11.8	9.3	8.0
3-Acetoxy-copalic	6.2	0.8	nd	nd	nd	nd	nd	nd

<sup>a</sup> Many other compounds were identified. Those listed are suggested as markers for analytical applications. It is noticeable that although percentages may fluctuate the substances present are generally the same within a species.

<sup>b</sup> AP represents a tree whose oleoresin was collected on 11 different occasions (Table 2). The three analyses presented illustrate the range of variation.

nearly 11% of the bicyclic clerodane, hardwickiic acid (also a  $\beta$ -furan).

### 2.3. *Copaifera duckei*

Oleoresins from three specimens, one (AP) from the state of Amapá and two (PA and CJ) from the state of Pará, have been analysed. Here wide differences in proportional content occur within the species. Sesquiterpene hydrocarbons make up 42% of sample CJ, 32% of PA and less than 4% of AP. Although the proportions are so different all three have in common  $\beta$ -caryophyllene,  $\alpha$ -bergamotene,  $\beta$ -bisabolene,  $\beta$ -selinene,  $\alpha$ -selinene and  $\alpha$ -bisabolene. When the hydrocarbon-poor oleoresin was collected for the first time from the Amapá tree (AP, in the month of April 1996) oxygenated sesquiterpenes were found in a significant amount (24%), three quarters of the fraction consisting of 3-methyl-5-(2,6,6-trimethyl-2-hydroxy-1-cyclohexyl)-pentanoic acid, a hydroxy-sesquiterpene acid identified originally in *Copaifera cearensis* Huber (Braga et al., 1998). *C. duckei* contains small amounts of diterpene hydrocarbons, not detected in the other species — 4.5% in CJ, 1.5% in PA and 0.3% in AP, identified in the Pará samples as mainly kaurene. These Pará samples also contain an unidentified diterpene aldehyde but diterpene acids are the predominating component in all three oleoresin specimens 53% of CJ, over 66% of PA and 72% of AP first sample, April 1996. These, however, differ; all three contain copalic and polyalthic acids, both the Pará specimens also contain kaur-16-en-19-oic and enantio-agathic acids, and PA and AP contain hardwickiic acid, absent in CJ. Apart from these, the Amapá oleoresin, AP, also contains eperuic, chrolechinic, eperu-8(17)-en-15,18-dioic, clerodan-15,18-dioic acids and contains no detectable kaur-16-en-19-oic acid. The analysis of the diterpene group distribution in the studied oleoresins showed the presence of bicyclic labdanes in all of them (33% of CJ, 18% of PA and 35% of AP), bicyclic clerodanes in PA (24%) and AP (37%), tetracyclic kauranes in CJ (24%) and PA (26%). All of them contain diterpenes with furane rings (28% of CJ, 41% of PA and 47% of

AP). The samples of Pará State are very similar in their GC–MS chemical profile, apart from the significant presence (24%) in PA of hardwickiic acid (a furanoid clerodane), absent in CJ. The Amapá sample is very different in chemical profile from the others pointing to the existence of chemical types in this species as well as possibly the influence of environmental factors.

In order to investigate the seasonal variation of the oleoresin composition of a single *C. duckei* tree of Amapá State (AP) its oleoresin was collected in April, July, August and December of 1996; March, June, August and November of 1997; February and May of 1998 and May of 1999. Chemical analysis showed insignificant qualitative variation among the components of the samples from these 11 collections, but a great variation in their proportions, with the sesquiterpene content rising and falling in alternation with the diterpene acid content as a general tendency (Table 2). There was little evidence of human interference in this pattern although a collection by the local population did occur shortly before the last revision (May of 1999). The striking quantitative variations may be related to growth phase and to variations in environmental conditions but no consistent pattern has yet been identified. It is significant that two specimens of another *Copaifera* species from the same area and habitat, not yet botanically identified, but distinct from the three described here, showed variations in sesquiterpenoid and diterpenoid content over the same period (Cascon and Gilbert, 1999), which were closely parallel between themselves and similar to the variation observed with *C. duckei*.

The overall picture for the oleoresins of the three studied species is shown in Table 3. *Copaifera multijuga* is the species in which the sesquiterpene fraction predominates while in *C. guianensis* Desf. and *C. duckei* there is usually prevalence of the diterpene acids.

All the samples here studied only have in common the sesquiterpenes  $\beta$ -caryophyllene and  $\alpha$ -bergamotene which are not restricted to the *Copaifera* genus. Copalic acid previously considered a characteristic diterpene of the *Copaifera* genus is, however, not a reliable marker because it occurs only in traces, if at all, in *C. duckei*

Table 2

Variation in the chemical composition of *Copaifera duckei* oleoresin collected at different times from the same trunk borehole<sup>a</sup>

Chemical group	<i>Copaifera duckei</i> — AP oleoresin composition										
	1996				1997				1998		1999
	Apr	Jul	Aug	Dec	Mar	Jun	Aug	Nov	Feb	May	May
Sesquiterpene hydrocarbons	3.8	48.1	8.5	22.8	66.4	46.4	61.2	76.9	73.0	62.1	26.2
Oxygenated sesquiterpenes	23.7	14.6	12.1	9.9	2.7	5.8	4.1	1.8	4.6	4.0	5.6
Total sesquiterpenes	27.5	62.7	20.6	32.7	69.0	52.2	65.3	78.7	77.5	66.1	31.8
Total diterpenes	72.5	37.3	79.4	67.3	31.0	47.8	34.7	21.3	22.5	33.9	68.2

<sup>a</sup> The amount collected varied from 300 to 550 ml, an amount which does not harm the tree. The last collection was, however, very small as the hole had recently been drained. There was no evidence of other extraneous harvests of oil from this tree.

Table 3

An overall picture of the terpenoid distribution in the three *Copaifera* species

Chemical group	Overall terpene distribution in three <i>Copaifera</i> species							
	<i>C. multijuga</i>		<i>C. guianensis</i>	<i>C. duckei</i>				
	CM	ICM	CG	CJ	PA	AP 04/96	AP 07/96	AP 11/97
Sesquiterpene hydrocarbons	79.7	81.4	19.7	42.2	32.0	3.8	48.1	76.9
Oxygenated sesquiterpenes	traces	15.9	23.9	nd	nd	23.7	14.6	1.8
Total sesquiterpenes	79.7	97.3	43.5	42.2	32.0	27.5	62.7	78.7
Neutral diterpenes	nd	nd	11.5 <sup>a</sup>	4.5 <sup>b</sup>	1.5 <sup>c</sup>	0.3 <sup>d</sup>	nd	nd
Diterpene acids	20.3	2.7	45.0	53.3	66.5	72.2	37.3	21.3
Total diterpenes	20.3	2.7	56.5	57.8	68.0	72.5	37.3	21.3

<sup>a</sup> This has the MS breakdown of an alcohol (GC–MS) 70 eV,  $m/z$  (rel. int.): 287 [M–1]<sup>+</sup>, 204 (76), 189 (11), 179 (4), 161 (16), 147 (13), 135 (49), 121 (21), 109 (100), 95 (29), 81 (87), 69 (29), 55 (39); but is not kaurenol.

<sup>b</sup> Of the 4.5%, 0.8% is kaurene, the other 3.7% behave as an unidentified aldehyde, (GC–MS) 70 eV,  $m/z$  (rel. int.): 286 [M]<sup>+</sup> (13), 271 (5), 257 (11), 243 (20), 187 (13), 159 (9), 145 (16), 131 (21), 119 (22), 105 (52), 91 (100), 79 (74), 67 (50), 55 (64), whose MS breakdown pattern is related to that of the methyl ester of kaur-16-en-19-oic acid.

<sup>c</sup> This is identical (MS) to the aldehyde mentioned under b above.

<sup>d</sup> This small peak is very polar, (GC–MS) 70 eV,  $m/z$  (rel. int.): 302 [M]<sup>+</sup>, 287 (5), 259 (2), 205 (5), 166 (4), 137 (34), 123 (20), 109 (12), 98 (100), 69 (20), 55 (18).

(Table 1). From our sampling, *C. multijuga* oleoresin is characterized by the great predominance of  $\beta$ -caryophyllene, the presence of  $\alpha$ -copaene and  $\delta$ -cadinene and the absence of  $\beta$ -selinene and  $\beta$ -bisabolene, and the occurrence of copalic and 3-acetoxycopalic acids and other labdane group diterpene acids without a furanoid ring. In *C. duckei*, the most significant substance is polyalthic acid (a furanoid labdane), the predominant sesquiterpene is  $\beta$ -selinene, with the characteristic presence of  $\alpha$ -selinene and  $\beta$ -bisabolene and the absence of  $\alpha$ -copaene and  $\delta$ -cadinene.  $\beta$ -Caryophyllene oxide distinguishes *C. guianensis* from *C. duckei* while the presence of the diterpenoid kaur-16-en-19-oic, kauran-19-oic, hardwickiic and polyalthic acids and absence of  $\delta$ -cadinene distinguish it from *C. multijuga*.

The differences point to the need for established patterns of control of these oleoresins for use in medicine and cosmetic products since it is clear that the differences of chemical composition will occasion differences in pharmacological action and toxicity (Cascon et al., 1997). With regular analysis and the establishment of standard botanical sources it will be possible to extend and update the present picture.

### 3. Experimental

#### 3.1. General

The crude oleoresins, in an equal volume of dichloromethane, were esterified with diazomethane in ether and analysed directly (no insoluble material was present) by gas chromatography–mass spectrometry (GC–MS) in a Hewlett Packard HP 6890 chromatograph (column 30

m  $\times$  250  $\mu$ m  $\times$  0.25  $\mu$ m) — HP 5 mass spectrometer (70 eV, mass selective detector 5972 A), using PFK as reference. The temperature programme was initiated at 70°C rising 2°C/min to 300°C.

Both sesquiterpenes and methyl esters of diterpene acids were analysed in the same sample and the majority of the compounds were characterized by the Wiley Library/Mass Spectra 275 and by comparison of retention times with literature data (Braga, 1994).

The methyl esters of copalic (Hugel et al., 1966; Sandermann et al., 1967; Mahajan and Ferreira, 1971; Buckwalter et al., 1975; Zinkel and Magee, 1987) and 3-acetoxycopalic (Braun and Breitenbach, 1977; Zinkel and Magee, 1987) acids, isolated by liquid chromatography from *C. multijuga* oleoresin, were identified by comparison of their spectroscopic properties (MS, <sup>1</sup>H and <sup>13</sup>C NMR spectra) with the literature values. All GC–MS injections were identical in volume and sample concentration.

All the samples were stored in the refrigerator at approximately 3°C until used. No chemical changes have been observed in practice over periods of several months.

TLC was used as a preliminary analytical procedure in order to establish correct conditions for the above GC–MS. For this, Merck 60F<sub>254</sub> silica gel plates were used, and substances were detected by irradiation at 254 nm and with the vanillin-sulfuric acid reagent.

#### 3.2. Plant material

The oleoresin of *C. guianensis* Desf. was collected (50 ml) in May 1997, in the region of the Rio Iratapuru, Laranjal do Jari county, Amapá, and a voucher specimen was deposited at the herbarium of the Museu Goeldi (MG), under registration number 158502. The 11 samples

of *Copaifera duckei* Dwyer (AP) were collected (footnote, Table 2) at 00° 02' 46 N, 051° 40' 77 W, in the region of the Rio Preto, Mazagão county, Amapá, and a voucher specimen (No. 158501) was deposited at the MG herbarium. All the collections in Amapá were made with the collaboration of the Instituto de Pesquisas Científicas e Tecnológicas do Estado do Amapá (IEPA) and of the National Health Foundation, FNS-Amapá.

*C. duckei* Dwyer (PA) was collected (70 ml) in August 1998, at the Ferreira Penna Scientific Station, Museu Paraense Emílio Goeldi (MG), Caxiuanã county, Pará, and a voucher specimen (No. 158503) was deposited at the MG herbarium.

The collections of oleoresins and botanical material of the previously cited specimens were made by Vera Cascon and Jonas de Oliveira Cardoso (IEPA). Botanical identification was made by Antônio Sérgio Lima da Silva, Museu Paraense Emílio Goeldi, Belém, Pará.

The oleoresin CJ of *C. duckei* Dwyer was collected (30 ml) with the collaboration of Therezinha Tomassini (Fiocruz), in June 1998, in the Carajás Zoobotanical Park (specimen under registration number 647), Parauapebas, Pará, by kind permission of Fernando Dutra de Moura Lima, environmental manager of the Fundação Zoobotânica de Carajás. Botanical identification was made by Nelson Rosa, MG.

The collections of samples PA and CJ were made with the permission of the environmental authority IBAMA.

All samples were collected by draining from existing boreholes when these trees had been previously tapped. In the case of the specimens CJ and PA of *C. duckei*, which had not been tapped before, new boreholes were made. Care was taken not to overdrain the oleoresins. After collection, the boreholes were stoppered to permit normal reaccumulation of the oleoresins.

The two samples of *C. multijuga* Hayne were collected in the Reserva Ducke Manaus by qualified collectors of the Instituto Nacional de Pesquisas Amazônicas (INPA), Manaus, Amazonas State, and were donated by the Phytochemistry Section of this Institute. This species, whose oleoresin is a large scale commercial product of Manaus, is subject to long studies at this site by the scientific staff (Alencar, 1991).

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