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The mystery of the 'resin-of-canuaru': A medicine used by caboclos river-dwellers of the Amazon, Amazonas, Brazil

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ABSTRACT

Ethnopharmacological relevance: 'Resin-of-canuaru' is a medicine utilized by caboclos living in the Amazon Region, Brazil. There is a mystery regarding its origin because the caboclos maintain that this substance is derived only from animal secretions (from a frog called canuaru), whereas the historic literature claims that 'resin-of-canuaru' is derived solely from a plant exudate (resin). Based on our ethnographic studies, we hypothesized that this substance is a combination of both. Because the past reports on this resiniferous material in the literature are based solely on observations, we aimed to present ethnographic, zoological and chemical data to try to elucidate the origin of the 'resin-of-canuaru'.

Materials and methods: Ethnographic techniques and methods were applied, including participant observation, the use of field diaries and informal and unstructured interviews. The canuaru frog (*Trachycephalus resinifictrix* Goeldi, 1907) and 'resin-of-canuaru' were collected for taxonomic identification and chemical analysis, respectively. The resiniferous 'resin-of-canuaru' was extracted using MeOH and then analyzed by silica gel TLC and NMR.

Results: Canuaru frogs live in tree cavities and secrete a large amount of substances during spawning, resulting in a resiniferous material. NMR analysis of the MeOH extract of this crude material showed peaks assigned to 3,4-secofriedel-4(23)-en-3-oic acid (putranjuvic acid) and its methyl ester derivative (methyl putranjivate) and to biogenetic precursor of these two compounds (a lactone derivative), which is formed by the oxidation of friedelin. Based on evidence that *Protium* species accumulate primarily tetracyclic/pentacyclic triterpenoids and that the co-occurrence of the compounds listed above is rarely described in plant species, we suggest that these compounds could be products of the biotransformation of friedelin by the frog.

Conclusions: According to our data, the 'resin-of-canuaru' seems to have both animal and vegetal origins.

1. Introduction

Amphibians are widely known to secrete various substances through the skin; these substances protect amphibians from microbial infections and are a defense mechanism against predators (Duellman and Trueb, 1994; Daly, 1998). The active metabolites found in amphibian secretions include aliphatic, aromatic and heterocyclic molecules and a variety of steroids, alkaloids, proteins and peptides (Clarke, 1997; Monti and Cardello, 1999). According to Bernarde and Santos (2009), the use of amphibians' secretions by indigenous populations has been documented in the Amazon (Myers et al., 1978; Daly et al., 1992; Caramaschi and Cruz, 2002; Souza et al., 2002; Lima and Labate, 2007).

Few previous ethnopharmacological studies have focused on animal- and plant-derived products. In addition, the administration of these products by inhalation has been poorly recorded during field work or pharmacological trials. Accordingly, in this article we highlight an example of a medicine – the 'resin-of-canuaru', a resiniferous material presumably derived from both animal and vegetal sources – that is administered by inhalation. This medicine was recorded during our ethnographic studies conducted among caboclos river-dwellers living in the Unini (Santos et al., 2012) and Jau Rivers (Rodrigues, 2006), both of which are located in the region of the Middle Negro River. According to reports, the 'resin-of-canuaru' is a pitch produced from the secretion of a frog, known as canuaru (Tupi language: Kunawa'ru), that lives in hollow trees,

Abbreviations: NMR, Nuclear magnetic resonance; TLC, Thin-layer chromatography; TMS, Tetramethylsilane

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approximately 40 to 50 ft high, where they lay eggs. After the frogs leave the egg-laying site, the frog's secretions harden is available to be collected by the caboclos to treat headaches by inhalation and children's diseases by fumigate.[1] The children's diseases are similar to those of culture-bound syndromes described by Hughes (1985).

Caboclos believe that it is very difficult to find this frog because it is very smart and they also expressed some fear, believing that the collection or hunting of this frog could bring bad luck to them or their families. For these reasons 'resin of canuaru' is collected by them from fallen trees, by chance and rarely. According to the interviewees, the substance called 'resin-of-canuaru' is derived only from the frog's secretions and is thus entirely of animal origin. However, according to a recent review conducted by Teixeira and Papavero (2010) on the subject, there are reports in the historic literature from the nineteenth century by Brazilian naturalists and zoologists—Joao Barbosa Rodrigues and Emilio Goeldi-refuting this as a local belief. The authors explain that this substance could be resin produced by trees of the genus *Protium* (Burseraceae) and may not be of animal origin. They explained that the frog collects the resins of these trees and then uses the resin to construct the nursery where the eggs will be laid.

The frog mentioned by the interviewees, *Trachycephalus resinifictrix* Goeldi, 1907 (Anura: Hylidae), is nocturnal and arboreal, and produces two primary types of secretions: spawning and stress secretions. When subjected different types of stress, for example, when threatened by predators or when handled by humans, the frog secretes a white substance that is very sticky to discourage attack by the predator (SMS Pers. obs.).

In view of our field observations and the data from past literature, our aim in this short communication is to present chemical data that were used to determine if the 'resin-of-canuaru' is derived from only animal sources (canuaru frog) or from only plant sources (resin), or if it is derived from a combination of both substances.

2. Materials and methods

2.1. Material

The canuaru frog was collected and deposited at HNIAR under number INPA 26514; its resiniferous material (crystalline, yellowish and odorless) was collected by one of the interviewed in January, 2011.

2.2. Instrumental and chromatography materials

Silica gel PF_{254} (Merck) was used for analytical thin-layer chromatography (TLC). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DPX-300 spectrometer operating at 75 MHz for carbon-13 (^{13}C). Deuterated chloroform ($CDCl_3$) was used as the solvent and tetramethylsilane (TMS) as the internal standard.

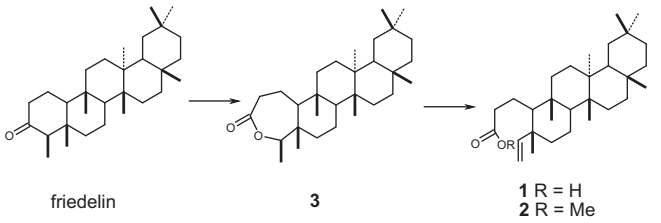

Fig. 1. Biogenetic transformation of friedelin into compounds **1–3**.

2.3. Chemical analysis

The crude resiniferous material (10 g) was extracted using methanol (MeOH) in a Soxhlet system for 2 h. After this extraction, the solvent was evaporated and the obtained MeOH extract (800 mg) was analyzed by TLC and NMR.

3. Results and discussion

TLC chromatographic analysis (CH_2Cl_2:MeOH 8:2) followed by development using ceric sulfate showed three main spots, suggesting the presence of triterpenoids. Thus, based on a method for identification of triterpenes in mixtures (Olea and Roque, 1990), the ^{13}C NMR spectrum of the crude MeOH extract was recorded. This spectrum displayed approximately 30 more intense peaks, being those at δ151.0 (CH) and 110.9 (CH_2) assigned to olefinic carbons C-4 and C-23 while that at δ179.4 was attributed to carboxylic carbon (C-3) of 3,4-secofriedel-4(23)-en-3-oic acid (putranjuvic acid-**1**), confirmed by analysis of remaining intense peaks and comparison of spectroscopic data with those reported in the literature (Leong and Harrison, 1999). However, in the ^{13}C NMR spectrum were also observed additional less intense peaks at δ174.2 (C-3) and δ51.3 (OMe) as well as one signal of a carbinolic carbon at δ62.1 (C-4), indicating the presence of its methyl ester derivative (methyl putranjivate-**2**) and its biogenetic precursor **3** (a lactone derivative) as minor derivatives. The structures of these compounds were confirmed after comparison of spectral data with those reported in the literature (Sengupta and Dey, 1972; Leong and Harrison, 1999). As showed in Fig. 1, compounds **1–3** could be formed by the oxidation of friedelin which is reported to be one of the primary compounds in several *Protium* resins, such as of *P. heptaphyllum* (Aubl.) Marchand (Maia et al., 2000; Bandeira et al., 2002), *P. hebetatum* D.C. Daly (Silva, 1995), and *P. kleinii* Cuatrec. (Lima et al., 2005).

Also, Teixeira and Papavero (2010) explain these resins are employed by canuaru frogs to make their nurseries, where the eggs are deposited. In addition, some studies showed that resins from *Protium* species present some therapeutic indications, such as healing and expectorant as well antiulcer and anti-inflammatory actions (Siani et al., 1999; Maia et al., 2000; Sussunaga et al., 2001; Oliveira et al., 2004a,b), while friedelin and related triterpenes shown analgesic, anti-inflammatory, and antipiretic activities (Antonisamy et al., 2011; Noufou et al., 2012).

4. Conclusion

Based on our field observations and also on the historic literature our hypothesis was that the 'resin of canuaru' was composed of both collected plant resins and frog secretions. Thus, according to the behavior of canuaru frogs, which secrete a large

[1] The biological resources and traditional knowledge associated with biodiversity (TK) referred to in this article are protected under the terms of the United Nations Convention on Biological Diversity (CBD), in force internationally since December 1993. Any individual or public or private entity who wishes to carry out scientific or technological investigations on the biological resources and TK referred shall observe the requirements set forth by Articles 8 (*j*) and 15 of the CBD, as well as, in the case of Brazilian biological resources and TK, the requirements set forth by the Provisional Measure no. 2.186-16/2001, which regulates access to genetic resources, protection and access to TK and the sharing of benefits arising from the use of Brazilian TK and biological resources. The unauthorized use of these resources is an act of misappropriation, and subjects violators to administrative, civil and criminal penalties in Brazil.

amount of substances during spawning, we believe that 'resin-of-canuaru' may correspond to frog secretions because such secretions are likely the most common substance found in the hollow trees where this species spawns. Also, since 'resin of canuaru' is odorless it could not be composed solely of plant-resin since these are rich in essential oils and are therefore aromatics. Moreover, based on evidence that *Protium* species accumulate primarily tetracyclic/pentacyclic triterpenoids (Rüdiger et al., 2007) and that the co-occurrence of **1–3** is rarely described in plant species (Momo et al., 2011), we suggest that compounds **1–3** could be the products of the biotransformation of friedelin by the canuaru frog.

Also, according to our data, one of our questions was answered: the 'resin-of-canuaru' seems to originate from both animal and plant substances. However, these results pose a new question: can 'resin-of-canuaru' be considered a single substance? In our ethnographic studies, caboclos stated that this frog lives in several tree species, such that 'resin-of-canuaru' is most likely not a single substance because the frogs may obtain plant resins from different tree species. Finally, the 'resin-of-canuaru' merit further investigations, since substances derived from animal and plants interaction, as well as the inhalation route, are promising elements in drug discovery, although are poorly investigated by pharmacology.

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