

Chemical Composition of Brazilian Propolis from Sao Paulo State

M. C. Marcucci^a, Jose Rodriguez^b, F. Ferreres^c, V. Bankova^d,
Robson Groto^e and S. Popov^d

^a Engineering and Technological Sciences Department,
Metropolitan University of Santos, Santos, SP, Brazil

^b Chemical Institute of Murcia University, Murcia, Spain

^c Laboratory of Phytochemistry, CEBAS (CSIS), Murcia, Spain

^d Institute of Organic Chemistry with Centre of Phytochemistry,
Bulgarian Academy of Sciences, Sofia, Bulgaria

^e Biological Chemistry Laboratory, Chemical Institute of
Universidade Estadual de Campinas, SP, Brazil

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Two propolis samples from Sao Paulo State were investigated by GC/MS. 39 compounds were identified, 8 being new for propolis. Both samples showed some similarities in their qualitative composition. In one of them, coumaric acid and its prenylated derivatives predominated, while in the other one triterpenic alcohols were the main constituents.

Introduction

Propolis (bee glue) is the material used by bees as a general-purpose glue and a waterproof sealant in their hives. Its 70% ethanolic extract, called “propolis balsam”, is widely used in folk medicine and is known to possess antibacterial, antimycotic, anti-inflammatory and other beneficial properties (Marcucci, 1995). Propolis is now extensively used in foods and beverages intended to maintain human health (Matsuda, 1994).

Bee glue consists primarily of a mixture of beeswax and plant exudates, which are gathered from different plants depending on the geographic location (Crane, 1988). In the temperate zone the exudates gathered by bees are preferentially from poplars and propolis contains mainly typical poplar bud phenolics (Papay *et al.*, 1986; Wollenweber *et al.*, 1987, Greenaway *et al.*, 1987; Bankova *et al.*, 1992). Little is known about the chemical composition of tropical propolis. Investigations in this field started in the last 3–4 years (Tomas-Barberan *et al.*, 1993; Aga *et al.*, 1994; Matsuno *et al.*, 1994; Bankova *et al.*, 1995). In this article we report our investigations on the chemical composition of the balsam from two propolis samples from Sao Paulo State, performed by GC/MS.

Materials and Methods

Propolis

Propolis samples were collected in Brazil, Sao Paulo State, as follows: Sample A near Mogi das Cruzes, sample B near Mairipora.

Extraction and sample preparation

Propolis, cut into small pieces, was extracted twice (x24h) with 70% ethanol at room temperature. The extract was evaporated to dryness. About 5 mg of the residue were mixed with 10 µl dry pyridine and 100 µl BSTFA with 1% trimethylchlorosilane, heated at 100 °C for 30 min and analysed by GC/MS.

GC/MS analysis

For the GC/MS analyses a 30 m x 0.25 mm ID. HP-5 fused silica capillary column was used in a Hewlett-Packard 5890 gas chromatograph with a Hewlett-Packard 5972 series mass selective detector, with He as a carrier gas, split ratio 1:100, temperature program 100–200 °C at 8 deg·min⁻¹, 200–310 °C at 5 deg·min⁻¹ and a 6 min hold at 310 °C; injector temperature 250 °C, detector temperature 280 °C.

Reprint requests to Dr. S. Popov.
Fax: 003592-700-225.

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Identification of compounds

The identification was accomplished using computer searches on commercial libraries. In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed on the basis of its mass-spectral fragmentation. Reference compounds were co-chromatographed where possible to confirm GC retention times.

Results and Discussion

Propolis samples have been collected from two different locations in Sao Paulo State, Brazil, each of them characterized by some type of predominant trees or shrubs. Sample A was harvested from hives in a native forest where pine trees predominated; sample B in a *Eucalyptus* forest (for geographical locations see Experimental). Propolis samples have been extracted at room temperature with 70% ethanol to obtain the balsam, the extracts were silylated and subjected to GC/MS analysis. The results obtained are summarized in Table I.

As it is evident from Table I, the two samples showed some similarities in their qualitative composition. Both of them contained dihydrocinnamic, *p*-coumaric, ferulic and caffeic acid, mono- and diprenyl *p*-coumaric acid as well as carboxyethylbenzopyranes, biogenetically related to the prenylated coumaric acids. However, in sample B the concentration of these compounds was significantly higher. Sample B also contained more flavonoids. In sample A, there were high amounts of triterpenic alcohols, including β -amyrine, some unidentified triterpenic alcohols of the amyrine-type, lanosterol and its isomer with 9(11)-double bond. These triterpenic alcohols are the main components of sample A, comprising more than 30% of the mixture. To the best of our knowledge, triterpenic compounds with oleanan/ursan skeleton have not been found in propolis till now, whereas lanosterol is a known ingredient of poplar propolis (Kardakov *et al.*, 1982).

On the basis of the results obtained, some conclusions could be drawn concerning the plant origin of the investigated samples. One of the plant sources appeared to be common for both samples, and this must be a plant exudate containing preny-

Table I. Composition of alcohol extract of propolis from two locations in Sao Paulo State.

Compound	Sample A % TIC ^b	Sample B % TIC ^b
<i>Aromatic acids</i>		
Dihydrocinnamic acid	0.80	2.19
4-Methoxybenzoic acid	0.08	0.10
Benzoic acid	—	0.38
4-Hydroxybenzoic acid	0.07	—
<i>p</i> -Hydroxydihydrocinnamic acid	0.10	0.38
3-Methoxy-4-hydroxybenzoic acid	0.30	—
3,4-Dihydroxybenzoic acid	—	0.13
Dihydroferulic acid ^a	0.11	—
<i>p</i> -Coumaric acid	0.60	4.13
2,2-Dimethyl-6-carboxyethyl benzopyrane	0.09	0.60
Ferulic acid	0.11	0.22
Caffeic acid	0.19	0.79
Prenyl- <i>p</i> -coumaric acid	1.35	11.78
2,2-Dimethyl-6-carboxyethyl prenylbenzopyrane	0.47	2.07
Diprenyl- <i>p</i> -coumaric acid	12.67	18.35
Methyl dihydrocinnamate	—	0.15
<i>Di- and triterpenes</i>		
Dehydroabietic acid ^a	0.59	—
Abietic acid ^a	0.06	0.14
β -Amyrine ^a	1.82	—
Triterpenic alcohol of amyrine type ^a	8.59	—
Triterpenic alcohol of amyrine type ^a	24.39	—
Lanosterol	1.38	—
Lanosterol isomere with 9(11) double bond ^a	4.54	—
<i>Alcohols, phenols, aldehydes</i>		
Glycerol	0.44	0.19
Vanillyl alcohol	—	0.15
<i>cis-p</i> -coumaric alcohol	0.07	—
<i>trans-p</i> -coumaric alcohol	3.99	0.21
3-Vanillylpropanol ^a	0.11	0.21
Vanillin	1.03	—
Coniferyl aldehyde	1.00	—
<i>Flavonoids</i>		
Trihydroxymethoxy flavanone	0.84	4.43
Tetrahydroxy flavanone	—	0.40
Tetrahydroxyflavone	—	0.23
<i>Others</i>		
Phosphate	—	1.03
Butanedioic acid	0.07	0.11
Inositol	0.14	1.70
D-Glucose	0.09	0.56
Octadecadienoic acid	0.10	0.26
Octadecenoic acid	0.42	0.39

^a For the first time in propolis.

^b The ion current generated depends on the characteristics of the compound concerned and it is not a true quantitation.

lated coumaric acid derivatives and the biogenetically related benzopyranes. Such compounds, known from the literature as Brazilian propolis constituents (Aga *et al.*, 1994; Bankova *et al.*, 1997), have been isolated from different *Baccharis* species (Labbe *et al.*, 1986; Zdero *et al.*, 1986). So leaf exudates of the *Baccharis* species appear to be one of the propolis sources which attracts bees in tropical regions, as it was already suggested (Bankova *et al.*, 1995). On the other hand, the triterpenic alcohols in sample A probably originate from some second plant and it was the main

propolis source for the bees in the region of Mairipora.

The present results confirm the striking variability of the chemical composition of tropical propolis and demonstrate the need for further investigations on its chemistry and biological activity.

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