Phenolics from Brazilian Propolis

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The main phenolic constituents from Brazilian propolis, originating from Sao Paulo State, were isolated and identified: three flavonoids, a prenylated coumaric acid and two new benzopyranes, *E* and *Z* 2,2-dimethyl-6-carboxyethenyl-8-prenyl-2H-benzopyranes.

Introduction

Propolis, or bee glue, is a complex resinous mixture of plant-derived products collected and used by bees as a general purpose sealer and antibiotic in their hives. It is reported to exhibit a broad spectrum of biological activities, including antibacterial, antiviral, antiinflammatory, cytotoxic etc. (Marcucci, 1995). It is widely used in folk medicine and also as an ingredient in "herbal" preparations sold in pharmacies and "health food" stores in Western countries (Matsuda, 1994). The chemical composition of propolis is very complex (Marcucci, 1995) and is dependent upon the source plant. Bud exudates of different poplar species are the main sources of bee glue in the temperate zone including Europe, Asia and North America (Greenaway *et al.*, 1987; Wollenweber *et al.*, 1987; Bankova *et al.*, 1992). Samples originating from these regions are characterized by similar chemical composition; the most important constituents appeared to be phenolics: flavonoids, aromatic acids and their esters (about 50% of the weight of propolis) (Marcucci, 1995). In the last few years, tropical propolis has become a subject of increasing interest, concerning its chemistry, origin and biological activity (Aga *et al.*, 1994; Tomas-Barberan *et al.*, 1993; Matsuno, 1994; Bankova *et al.*, 1995). A brief review of the results published on the chemical composition of tropical bee glue shows a remarkable variability, obviously connected with different plant origin. In this work we report the isolation and characterizatioin of the main phenolic constituents of Brazilian propolis, originating from Sao Paulo State.

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Materials and Methods

Propolis

Propolis was collected in the Beekeeping Section of the School of Veterinary Medicine and Animal Husbandry of Botucatu, UNESP, in the spring of 1996.

Isolation of phenolics

Propolis sample (16.9 g), was extracted twice with 70% ethanol at room temperature for 24 h. The alcoholic extract was concentrated *in vacuo*, diluted with water and extracted successively with *n*-hexane (three times) to give 1.56 g dry extract, and with ethyl acetate (three times) to give 4.46 g dry extract. The ethyl acetate extract was subjected to column chromatography on silica gel and eluted with chloroform – ethyl acetate gradient to produce several fractions. After repeated column chromatography on silica gel with chloroform-methanol and *n*-hexane – methylethylketone; and preparative TLC on silica gel plates with mobile phases chloroform-acetone, *n*-hexane – acetone and *n*-hexane – methylethylketone; compounds **1** – **6** were isolated.

Kaempferid (**1**), 23 mg. UV in MeOH and UV with shift reagents, ^{1}H NMR, ^{13}C NMR and mass

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spectra were identical with literature data for kaempferid (see Results and Discussion).

5,6,7-Trihydroxy-3,4'-dimethoxyflavone (**2**), 32 mg. UV and ^{1}H-NMR spectra identical with literature data. ^{13}C NMR spectrum (DMSO-d_6): δ 55.5 (OMe-4); 60.2 (OMe-3); 94.0 (C-8); 103.6 (C-10); 114.2 (C-3', C-5'); 123.4 (C-1'); 129.5 (C-2', C-6'); 131.0 (C-6); 135.9 (C-3); 151.6 (C-9); 151.9 (C-5); 157.5 (C-7); 160.7 (C-2); 176.4 (C-4).

Aromadendrine-4'-methyl ether (**3**), 13.7 mg. UV, ^{1}H NMR, ^{13}C NMR spectra identical with literature data.

9-E-2,2-dimethyl-6-carboxyethenyl-8-prenyl-2H-1-benzopyrane (**5**, 4.9 mg white solid. UV (MeOH): λ_{max}, nm 231, 269, 317.^{1}H-NMR spectrum ($CDCl_3$): δ 1.44 (6H, s, CH_3-12 and CH_3-13); 1.74 (6H, s, CH_3-17 and CH_3-18); 3.25 (2H, d, *J*=7.3 Hz, H-14); 5.26 (1H, m, H-15); 5.65 (1H, d, *J*=10 Hz, H-3); 6.30 (1H, d, *J*=16 Hz, H-10); 6.33 (1H, d, *J*=10 Hz, H-4); 7.04 (1H, d *J*=2.1 Hz, H-7); 7.18 (1H, d, *J*=2.1 Hz, H-5); 7.67 (1H, d, *J*=16 Hz, H-9). ^{13}C-NMR.-spectrum ($CDCl_3$): δ 17.9 and 25.8 (C-17 and 1–18); 28.0 (C-12 and C-13); 77.1 (C-2); 113.7 (C-10); 121.0; 121.9 (C-3); 124.4 126.2; 129.9 (C-4); 131.1; 140.6; 147.2 (C-9); 153.2 (C-8a); 171.4 (C-11).

9-Z-2,2-Dimethyl-6-carboxyethenyl-8-prenyl-2H-1-benzopyrane (**6**), 1.4 mg. UV.(MeOH) λ_{max}, nm 232, 262, 307.^{1}H-NMR spectrum ($CDCl_3$): δ 1.44 (6H, s, CH_3-12 and CH_3-13); 1.74 (6H, s, CH_3-17 and CH_3-18); 3.25 (2H, d, *J*=7.3 Hz, H-14); 5.26 (1H, m, H-15); 5.65 (1H, d, *J*=10 Hz, H-3); 5.80 (1H, d, *J*=12.8 Hz, H-10); 6.33 (1H, d, *J*=10 Hz, H-4); 6.87 (1H, d, *J*=12.8 Hz, H-9); 3.36 (2H, m, H-5 and H-7).

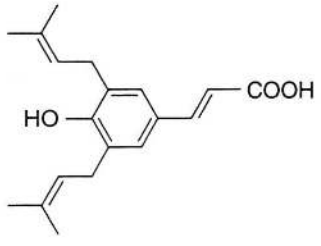
Results and Discussion

From the sample investigated, six individual compounds were isolated.

The first compound isolated was identified as kaempferid (**1**) (4'-O-methyl kaempferol) by com-

1: R=R'=H
2: R=Me. R'=OH

3



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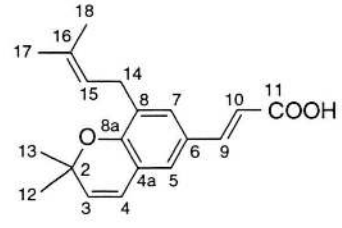

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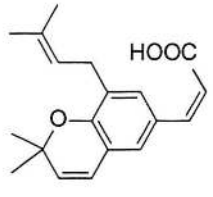

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paring its UV, ^{1}H-NMR and ^{13}C-NMR spectra with literature data (Mabry *et al.*, 1970, Markham *et al.*, 1982, Popravko *et al.*, 1969). This flavonoid was found earlier in propolis from the European part of Russia and was shown to originate from the bud exudate of birch (*Betula* spp.) (Popravko, 1976). It was identified recently in Brazilian propolis, too, its source remaining unknown (Bankova *et al.*, 1996).

The second compound isolated was a flavone, according to its UV spectra (with and without shift reagents). Analysis of the ^{1}H-NMR and ^{13}C-NMR spectra and NOE experiments allowed us to locate three hydroxyl groups in ring A, a methoxyl group at C-3 and a second methoxyl at C-4' position. Reviewing the literature we found that this flavonoid was identical with 5,6,7-trihydroxy-3,4'-dimethoxyflavone (**2**) (UV spectra, including spectra with shift-reagents, and ^{1}H-NMR), which was synthesized by Horie *et al.* (1993). To the best of our knowledge, this is the first isolation of **2** from a natural source.

The third compound isolated was a flavonoid, too. According to its UV-spectra it was a flavanone and was identified as aromadendrine-4'-methy ether (**3**), by comparing its UV, ^{1}H-NMR and ^{13}C-NMR spectra with literature data (Bohm, 1982, and literature cited there). This flavanone has previously been rerported from different plant sources, but this is its first isolation from propolis.

The fourth compound isolated was identified as prenylated *p*-coumaric acid (**4**) (UV, ^{1}H-NMR), isolated from Brazilian propolis by Aga *et al.*, 1994.

Upon analysis of its ^{1}H-NMR spectrum, the fifth compound isolated turned out to be a mixture of two isomeric cinnamic acid derivatives, *E/Z* ratio 2:1. The isomers were separated by TLC and their UV, ^{1}H-NMR and ^{13}C-NMR spectra measured. The structures were determined as 9-*E* and 9-*Z* 2,2-dimethyl-6-carboxyethenyl-8-prenyl-2H-benzopyran (**5**) and (**6**), comparing the spectra with data of similar compounds (3,5-diprenyl-4-hydroxycinnamic acid, Aga *et al.* (1994); 2,2-dimethyl-6-carboxyethenyl-2H-benzopyran, Labbe *et al.* (1986)). To the best of our knowledge, both **5** and **6** are novel compounds.

Identification of new compounds in propolis can give useful hints to its plant sources. Prenylated coumaric acid (**4**), and benzopyranes similar to **5** and **6**, originating from prenylated coumaric acids, have been found in different *Baccharis* species. The genus *Baccharis* is widespread in South America and is known to produce a leaf exudate, containing phenolic compounds, flavonoid aglycones and terpenes (Wollenweber *et al.*, 1986, 1989). According to the literature data and our own results, some *Baccharis* spp. are probably among the plant sources of the investigated sample. Obviously, more investigations are needed, including phytochemical investigations on the probable source plants, in order to clear the origin of tropical propolis and especially Brazilian propolis.

Acknowledgements

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