Constituents of Brazilian Geopropolis

V. Bankova[a], R. Christov[a], C. Marcucci[b] and S. Popov[a]

[a] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria
[b] Engineering and Technological Department, Metropolitan University of Santos, Santos, SP, Brazil

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Three samples of Brazilian geopropolis, collected by three indigenous bee species, were investigated by GC/MS. More than 50 compounds were identified, mainly terpenoids and phenolics. The chemical composition of propolis gathered by different bee species was different.

Introduction

Tropical propolis (bee glue) has recently become a subject of increasing interest for chemists and biologists (Martos *et al.,* 1997; Miyataka *et al.,* 1997; Matsuno, 1995). Significant differences have been found in the chemical composition of tropical propolis samples, collected at different locations (Aga *et al.,* 1994; Bankova *et al.,* 1995; Bankova *et al.,* 1996).Until now, the investigations on tropical propolis concentrated almost only on *Apis mellifera* bee glue. In tropical South America there are indigenous stingless bee species, which collect resinous material from plants and mix it with bees wax and soil to form the so called *geopropolis* (Kerr, 1987). Little is known about its chemical composition. Only one investigation has been published on the phenolic constituents of propolis from 5 species of South American stingless bees in Venezuela (Tomas-Barberan *et al.,* 1993).

In this publication we report the results of a GC-MS investigation of Brazilian geopropolis, collected by three different bee species, widespread in Brazil: *Melipona compressipes, Melipona quadrifasciata anthidioides* and *Tetragona clavipes.*

Experimental

The geopropolis samples were collected in Brazil in June 1995: G-1 in Picas, Piaui State by *Melipona compressipes*, G-2 in Prudentopolis, Parana State, by *Tetragona clavipes* and G-3 at the same location as G-2, but by *Melipona quadrifasciata anthidioides.* They were stored 19 months before analyses.

Extraction of geopropolis

Geopropolis (1 g of each sample) was ground and extracted with 70% ethanol (10 ml) at room temperature. Dry weight of the extracts: G-1 0.05 g, G-2 0.14 g, G-3 0.17 g.

Sample preparation for GC/MS

3 mg of the dry extract was dissolved in 25 μl of dry pyridine, 40 μl N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were added and the mixture heated at 80 °C for 20 min in a screw-cap vial.

Gas-chromatography/mass spectrometry

For the GC/MS analysis a 30 m x 0.2 mm I. D. HP-5 fused silica capillary column; 25 μm film thickens, was used in a Hewlett-Packard 5890 gas chromatograph with a HP 5972 MSD detector. The samples were introduced *via* an all-glass injector working in the split mode, with He as the carrier gas, linear velocity 32 cm/s. Temperature program: 80–240 °C at 8 deg.min^{-1}, 240–300° C at 12 deg.min^{-1} and a 20 min hold at 300 °C.

Identification of compounds

The identification was accomplished using computer searches in commercial libraries. In some cases, when identical spectra were not found, only the type of the corresponding compound was proposed on the basis of its mass spectrum. Reference

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Reprint requests to Dr. Bankova.
Fax: ++35 92-700-225.
E-mail: iochnp@bgcict.acac.bg.

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compounds were co-chromatographed when possible to confirm GC retention times.

Results and Discussion

The preliminary TLC investigation of the alcohol extracts showed significant differences between the three samples. In order to perform a complete analysis of geopropolis and compare the results obtained with Brazilian propolis from *Apis mellifera,* the total alcohol extracts were silylated and subjected to a GC/MS investigation. The results are summarised in Table I. It is evident that all geopropolis samples have a complex chemical composition. Part of the GC/MS peaks remained unidentified, because of lack of authentic samples and library spectra of corresponding compounds. We identified more than 50 compounds, the main group being non-phenolic acids. All three samples contained significant amounts of lactic acid and phosphoric acid**,** as well as long-chain fatty acids (stearic, palmitic, myristic), usually found in propolis. Two odd numbered acids, 15:0 and 17:0 (margarinic acid), were identified in samples G-2 and G-3.

Analogously to all other propolis samples investigated as yet, geopropolis contained aromatic compounds: acids, aldehydes and alcohols, but they were different in different samples and their concentrations were relatively low (Table I.). Only cinnamic acid was common for all the three samples. Surprisingly, dihydrocinnamic acid, which appeared to be characteristic for Brazilian propolis (Bankova *et al.,* 1995), is absent in geopropolis (only traces of it have been identified in G-1).

While prenylated benzophenones were found to be typical for propolis gathered by indigenous bees in Venezuela (incl. *Melipona compressipes*; Tomas-Barberan *et al.,* 1993), no such substances were present in Brazilian geopropolis. We identified only p-hydroxyacetophenone in sample G-1.

In all samples investigated diterpenic acids were found, their amounts being significant in samples G-2 and G-3. Compounds of this type have been identified earlier in Brazilian propolis (Bankova *et al.,* 1996) but not in propolis from temperate zones. The similarity between the spectra of diterpenic acids and lack of library spectra and authentic samples allows their tentative identification by mass spectra only. Pimaric acid and dehydroabietic acid, accompanied by their isomers and analogues were found in the investigated samples but most of them remained unidentified. In G-2 we found also the diterpenic hydrocarbon kaur-16-ene.

In sample G-2, a number of pentacyclic triterpene alcohols were identified. β-Amyrine is among the main components of G-2, accompanied by three other triterpene alcohols, probably isomers of amyrines (they show small differences in retention times and in the mass spectral peaks). In this sample we identified also the pentacyclic triterpenes nor-oleane-12-ene and friedooleanan-3-one. Contrary to G-2, in the other two samples only traces of triterpene alcohols have been found. Triterpene alcohols of amyrine type were recently found in Egyptian and Brazilian propolis (Marcucci *et al.,* 1998; Christov *et al.,* 1998).

Flavonoids are among the main components of propolis from temperate zones (Ghisalberti, 1979). In Brazilian propolis, collected by hive bee (*Apis mellifera)* we also found some flavonoids, although they were different from those of poplar propolis (Boudourova-Krasteva *et al.,* 1997). In samples G-2 and G-3 flavonoids were practically absent. Only in G-1 significant amounts of two flavonoids were present, one of them identified as pinobanksin, while partial structure of the second one is trihydroxymethoxy flavone.

Tomas-Barberan *et al.* (1993) have shown that the composition of propolis from South American stingless bees does not depend on the bee species and that propolis from *Apis mellifera* and indigenous bees has a similar composition. Our results do not support this conclusion. All three samples possess different chemical composition. They also differ from Brazilian hive bee propolis (Bankova *et al.,* 1995, Marcucci *et al.,* 1998). While the specificity of sample G-1 could be explained by the different geographic location (it has been shown earlier that in Brazil the composition of propolis changed very much with the location site: Bankova *et al.,* 1995; Park *et al.,* 1995), samples G-2 and G-3 were collected at the same location and the differences observed might be related to the bee species. Obviously the composition of G-3 is simpler than this of G-2. In the latter, much more terpenic compounds and especially triterpenes were found, while only traces of triterpenes were identified in G-3. This finding indicates that both geopropolis samples have different plant

Table I. Chemical composition assessed by GC-MS of alcohol extracts from Brazilian geopropolis.

Compound		**Samples**	
	G-1	**G-2**	**G-3**
		% TIC[a]	
Acids (aliphatic)			
Lauric acid		0.1	0.2
Myristic acid	0.4	0.7	0.2
Pentadecanoic acid[b]	–	–	0.9
Palmitic acid	2.5	3.2	3.8
Palmitoleic acid[b]	0.8	0.2	1.0
Margarinic acid[b]	–	0.2	0.4
Stearic acid	0.8	0.9	1.4
Oleic acid	1.8	1.3	1.9
Arachidonic acid	–	0.2	–
Lactic acid	0.9	0.7	2.2
Hydracrylic acid[b]	–	0.1	–
Acids (aromatic)			
Benzoic acid	–	0.4	0.2
p-Hydroxybenzoic acid	0.1	0.3	–
Gallic acid	0.1	–	–
Vanillinic acid	–	–	0.4
Cinnamic acid	1.2	0.3	0.5
cis-p-coumaric acid	0.8	–	–
trans-p-coumaric acid	3.0	0.6	–
Dihydroferulic acid[b]	–	–	0.1
Phenols and aromatic alcohols			
Benzyl alcohol	0.1	–	–
p-Vinylphenol[b]	–	0.2	–
Hydroquinone	0.2	–	–
p-Coumaric alcohol	-	–	1.1
3-(2-hydroxyphenyl)-propanol	0.2	–	–
3-(4-hydroxyphenyl)-propanol	0.3	–	–
Aromatic aldehydes and ketones			
p-hydroxybenzaldehyde	0.2	–	–
Vanillin	–	–	1.2
Coniferylaldehyde	–	–	2.0
ð*P*-Hydroxyacetophenone	1.7	–	–
Sugars			
Glucose	0.8	–	–
Pentose	–	0.2	–
C-5 sugar alcohol	–	0.1	–
Flavonoids			
Pinobanksin	5.2	–	–
Dihydroxymethoxyflavanone	5.0	–	–
Diterpenes			
Kaur-16-ene[b]	–	0.6	–
Dehydroabietic acid[b]	1.4	–	–
Diterpenic acid (M=302 RT[c] 21.27)	0.3	1.1	0.7
Diterpenic acid (M=302 RT 21.29)	1.0	2.5	8.1
Diterpenic acid (M=302 RT 21.72)	–	0.6	–
Diterpenic acid (M=302 RT 21.88)	–	0.3	0.3
Diterpenic acid (M=304)	–	–	1.1
Hydroxyditerpenic acid (M=320)	–	1.3	–

Table I. (Continued).

Compound	Samples G-1	G-2 % TIC[a]	G-3
Triterpenes			
β-Amyrine	–	2.5	–
Triterpene alcohol of amyrine type (RT 28.57)	–	11.1	traces
Triterpene alcohol of amyrine type (RT 28.89)	–	4.9	traces
Triterpene alcohol of amyrine type (RT 30.33)	–	8.3	–
Triterpene alcohol (RT 29.98)	–	9.8	–
Friedooleanane-3-one[b]	1.4	7.2	–
Triterpene ketone	–	3.8	–
Others			
Phosphoric acid	0.9	0.5	1.1
Methyl *p*-coumarate	0.2	–	–
Coumaran (pesticide)	0.1	–	0.2
Benzothiazole (pesticide)[b]	0.3	–	–

[a] TIC = total ion current. The ion current generated depends on the characteristics of the compound and is not a true quantitation.
[b] For the first time in propolis.
[c] RT = retention time, minutes.

sources – evidently *Tetragona clavipes* makes use of a specific propolis source, rich of triterpenes. Another difference between G-2 and G-3 is the presence of aromatic aldehydes only in G-3; *Melipona quadrifasciata anthidioides* probably takes these aldehydes from a plant, which is not visited by *Tetragona clavipes.* More investigations are needed to answer the question whether different indigenous bee species have any preferred propolis plant sources, or whether the composition of the local flora is most important for propolis composition.

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