Identification of Amino Acids in Brazilian Propolis

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Amino acids of propolis samples from different origins were analysed and quantified. Seven of them presented almost the same percentage in all samples, indicating that bees may have obtained amino acids from three sources: plants, pollen contamination and from their own metabolism. The regeneration properties of propolis in relation to the presence of some amino acids are discussed.

Introduction

Propolis (bee glue) is the generic name for the resinous substance collected by honey bees from various plant sources. Bees use propolis to fill gaps in the hive, and to prevent the decomposition of creatures which they have killed (such as mice and beetles) after an invasion of the hive (Brumfitt *et al.*, 1990).

Propolis has been used in modern clinics, especially in the treatment of infections, allergic and inflammatory states. It is an effective remedy, exerting antiviral, fungicidal, antibacterial, antiulcer, immunostimulating, cytostatic and hypotensive activities (Brumfitt *et al.*, 1990; Marcucci, 1995). Favourable clinical results were obtained by using propolis on skin inflammations and infections, treatment of mouth and throat infections, ulcers and some stomach problems (Tóth, 1985; Marcucci, 1995).

Its chemical composition is very complex: at least 200 propolis constituents have been identified so far, including: benzoic acid and esters, substituted phenolic acids and esters, flavonoids (flavones, flavanones, flavonols, dihydroflavonols, chalcones), terpenes, β-steroids, enzymes, free and conjugated amino acids (Greenaway *et al.*, 1990, 1991; Bankova *et al.*, 1992, 1995; Marcucci, 1995) suggesting that they may have a metabolic role in mammalian cell development (Gabrys *et al.*, 1986).

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Although many of the constituents of propolis have been identified and their biological properties investigated, only a few reports describe the amino acid content (Greenaway *et al.*, 1990; Gabrys *et al.*, 1986; Moreira, 1986). In order to provide more information on the pharmacological potential of propolis, amino acids present in the propolis resin were identified and quantified.

Experimental

Reagents

Amino acid standard solution was obtained from Sigma Chemical Co., St. Louis, USA.

Propolis

Propolis samples were collected in hives from different places in Brazil by beekeepers: Brunelli (São Paulo, sample no. 1), Chociai (Paraná, no. 2), Wenzel (Ceará, no. 3) and Barbosa (São Paulo, no. 4)(see Table I). The honey bee was *Apis mellifera* (africanized Brazilian bees).

Hydrolysis of samples

100 mg of propolis (samples 1, 2, 3 and 4) were hydrolysed with 6 N HCl at 104°C, for 24 hours. The extracts were then filtered and washed with water. An aliquot was lyophilized and the process repeated until all HCl in excess was eliminated (method previously reported) (Freer *et al.*, 1990).

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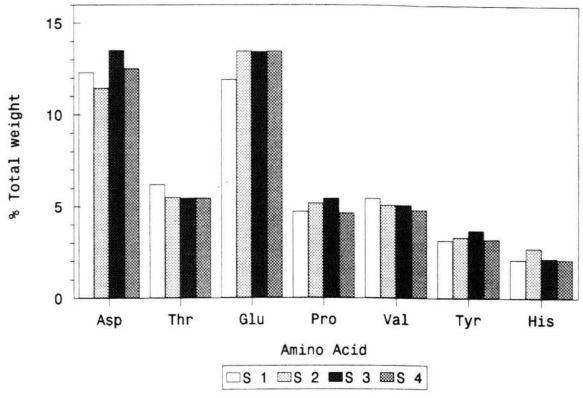
Identification of amino acids

The extracts were suspended in 110 μM citrate buffer pH 2.2, the amino acids were analysed in Amino Chrom II, and quantified with calibration standards of amino acid mixtures.

Statistical analysis

Possible differences among amino acid percentages were tested using a one way ANOVA (Analysis of Variance). The predictor was the origin of the amino acid (pollen, plant and propolis) and the response variable the percentage of the amino acid. The comparisons between values was done using the Ryan-Einot-Gabriel-Welsch multiple comparisons F-test to determine which means were significantly different (SAS Institute, 1986).



S 1: Sample 1; S 2: Sample 2; S 3: Sample 3; S 4: Sample 4.

Fig. 1. Occurrence (%) of seven amino acids in propolis samples (see Table II).

Results and Discussion

The amino acid content from four Brazilian propolis samples was investigated. The plant sources of samples are shown in Table I. The relative weights (μg/g) and percentages (w/w) of eighteen amino acids identified in the propolis resin are presented in Table II. There is a variation between the total weights of samples, because the plant sources are different in the four samples. Although they present these differences, the percentages of aspartic and glutamic acids, threonine, proline, valine, tyrosine and histidine are almost the same in all samples (Fig.1 and Table I). Similar percentage values of these seven amino acids were found by some authors who investigated pollens from woody and herbaceous plants (McLellan, 1977) and sixtynine dicotyledonous plants (Yeoh *et al.*, 1992) indicating that the amino acids from propolis may be collected by bees from plants and

Table I. Plant sources from different propolis samples.

n°	Scientific name[a]	Family[a]	Common Brazilian name	Origin (Place)	Observations
1	*Eucalyptus tereticornis Smith*	Myrtaceae	Eucalipto	near Rio Claro (São Paulo state)	Eucalyptus reforestation
2[b]	*Mimosa scabrella Benth.*	Mimosoideae	Bracatinga	near Prudentópolis (Paraná state)	native forest
2[b]	*Baccharis dracunculifolia DC*	Compositae	Vassourinha	near Prudentópolis (Paraná state)	native forest
2[b]	*Campomanesia maschalantha Berg*	Myrtaceae	Guabiroba	near Prudentópolis (Paraná state)	native forest
2[b]	*Vernonia polyanthes Less*	Compositae	Assa-peixe	near Prudentópolis (Paraná state)	native forest
3	*Anacardium occidentale L.*	Anacardiaceae	Cajú	near Pacajús (Ceará state)	plantation
4[c]	*Citrus aurantium L.*	Rutaceae	Laranja	near Limeira (São Paulo state)	plantation
4[c]	*Citrus aurantium var.pyriformis*	Rutaceae	Laranja	near Limeira (São Paulo state)	plantation
4[c]	*Saccharum officinarum L.*	Graminaceae	Cana-de-açúcar	near Limeira (São Paulo state)	plantation

[a] See reference (Corrêa, 1984). [b] Correspond to the same sample(2). [c] Correspond to the same sample(4).

Table II. Weights (Wt in μg/g) and percentages (%) of free amino acids in propolis samples.

Amino acid	Sample 1		Sample 2		Sample 3		Sample 4	
	Wt [μg/g]	%	Wt [μg/g]	%	Wt [μg/g]	%	Wt [μg/g]	%
Aspartic acid	10.70	12.30	2.16	11.43	1.83	13.49	7,80	12.50
Threonine	5.39	6.19	1.04	5.50	0.74	5.46	3,40	5.45
Serine	4.81	5.53	1.04	5.50	1.16	8.55	4.30	6.89
Glutamic acid	10.37	11.91	2.54	13.45	1.82	13.42	8.40	13.46
Proline	4.13	4.74	0.98	5.19	0.74	5.46	2.90	4.65
Glycine	5.23	6.01	1.08	5.72	0.92	6.78	3.40	5.45
Alanine	5.00	5.74	1.03	5.45	0.84	6.19	3.90	6.25
Cysteine	0.67	0.77	0.45	2.38	0.21	1.55	0.62	0.99
Valine	4.75	5.46	0.96	5.08	0.69	5.09	3.00	4.81
Methionine	0.45	0.52	0.13	0.69	0.06	0.44	0.30	0.48
Isoleucine	4.58	5.26	0.84	4.45	0.48	3.54	2.20	3.43
Leucine	8.40	9.65	1.87	9.90	1.29	9.51	7.50	12.00
Tyrosine	2.74	3.15	0.63	3.33	0.50	3.69	2.00	3.21
Phenylalanine	4.30	4.94	0.87	4.60	0.47	3.47	2.80	4,49
Lysine	7.53	8.65	1.27	6.72	0.58	4.28	3.90	6.25
NH_4	1.12	1.29	0.18	0.95	0.30	2.21	1.15	1.84
Histidine	1.82	2.09	0.51	2.70	0.29	2.14	1.30	2.08
Arginine	5.05	5.80	1.31	6.93	0.64	4.72	3.50	5.61
Total	87.04	100.0	18.89	100.0	13.56	100.0	62.37	100.0

pollen. The presence of pollen in propolis has been reported by Junkunz (1932) who found pollen grains of *Lupinus*, *Robinia* and *Onobrychis sativa* in the residue of propolis extracts. Microphotographs of propolis from different countries in the world confirm the presence of pollen grains, and they are visible in some of them (Vanhaelen and Vanhaelen, 1979). Ricciardelli d'Albore (1979) determined the incidence of pollen from plants in the sediments of propolis samples, and reported that the pollen in propolis samples was characteristic of the flora of particular countries. He also mentioned that the propolis in the sediment derived from the surrounding flora. Parts of pollen pellets or bee bread may also introduced into propolis by bees (Ricciardelli d'Albore, 1979). Zarakomska and Maciejewicz (1992) reported that small quantities of pollen from weeds, meadow and herbaceous plants and anemophilous trees were found in propolis samples. Leaf fragments of dicotyledonous plants were frequently found in the resin.

Considering the previous reports about the origin of pollen and leaf fragments of dicotyledonous plants in propolis samples, and that the amino acids present in the resin originate from these two sources, analysis of variance was performed for each amino acid, in comparison with those from pollen (woody and herbaceous plants), plants and propolis. The predictor was the origin of the amino acid and, the percentage of each amino acid, the response variable. Some of amino acids exhibited significant differences according to the origin, but others did not show differences (level of 5%). The percentage values of alanine, aspartic and glutamic acids, glycine, histidine and lysine are similar for three sources, and represented no statistical differences (S.D., $\alpha = 0.05$). These amino acids probably originated from pollen and dicotyledonous plants in these four propolis samples. Cysteine exhibited no S.D. between propolis and pollen samples. However, arginine, serine, threonine and isoleucine may originate from pollen in propolis samples (no S.D. between pollen and propolis). Leucine showed no S.D. for propolis and leaves. Values for methionine, phenylalanine and tyrosine are significant at 5% for three sources. These amino acids in propolis probably originated from the bee metabolism. The data obtained indicate that the amino acids from propolis may be possibly collected by bees from oil and resinous substances from plants surfaces such as leaves of dicotyledonous plants and (or) pollen. It can also be assumed that the amino acids derived from the metabolism of the bee and are introduced during formation of propolis.

Excess amino acids are converted to common metabolic intermediates for use in biochemical processes. According to Ghisalberti (1979) proline, glutamic acid and arginine increase the insulin level in blood by activating pancreatic enzymes, while its decarboxylation leads to the formation of agmatine which in turn enhances mitosis and protein biosynthesis, thus affecting the ability of propolis to stimulate tissue healing.

The propolis amino acids may be in part responsible for regenerative processes, such as healing and growth cells, attributed to this resin in mammalian tissues (Gabrys *et al.*, 1986).

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