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Article

Comb and Propolis Waxes from Brazil (States of São Paulo and Paraná)

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Amostras de ceras do ninho e da própolis de *Apis mellifera* foram analisadas. Observou-se a predominância de ésteres, seguidos de hidrocarbonetos. Os constituintes foram identificados por cromatografia a gás/espectrometria de massas. Amplas variações foram observadas nos padrões de hidrocarbonetos e dos ácidos e álcoois de ésteres. As cadeias carbônicas dos hidrocarbonetos abrangem a faixa C_{23} - C_{35}, com o predomínio de C_{27} e C_{31}. O principal ácido carboxílico foi $C_{16:0}$, seguido de $C_{18:0}$ e $C_{18:1}$. Os principais álcoois constituintes de ésteres foram homólogos saturados normais, na faixa C_{24} - C_{32}, C_{30} sendo o mais abundante, seguido de C_{24}. Não foram observadas diferenças que permitam distinção, o que sugere uma origem comum para ambas as fontes de cera.

Samples of propolis and comb waxes of *Apis mellifera* were analyzed. Monoesters predominated, followed by hydrocarbons. The constituents were identified by gas chromatography/mass spectrometry. Wide variations in the patterns of hydrocarbons, acids and alcohols of the esters were found. Hydrocarbon chains cover the range C_{23} - C_{35}, C_{27} and C_{31} alkanes predominating. The main carboxylic acid was $C_{16:0}$, followed by $C_{18:0}$ and $C_{18:1}$. The alcohols were predominantly saturated *n*-homologues, ranging from C_{24} to C_{32}, C_{30} being the most abundant, followed by C_{24}. No differences were found to allow a distinction, suggesting a common origin for both wax sources.

Keywords: propolis wax, beeswax, *Apis mellifera*

Introduction

Propolis is a complex mixture of waxes, resins and other organic and inorganic compounds used by bees as a general sealer, draught excluder and antibiotic[1-3]. Bees use propolis to prevent decomposition of creatures such as beetles and mice which they have killed after invasion of the hive[4]. Propolis derived products are widely used in folk medicine and reputedly have antibacterial, antimycotic, anti-inflammatory and other pharmacological properties[1].

The term “waxes” is used to designate mixtures of long-chain non-polar compounds commonly found mainly on the surfaces of plants and animals[5]. Commercially, beeswax is the most important natural wax. It is obtained chiefly from the domesticated European honey bee *Apis mellifera,* although other important taxa exist, such as the Asiatic *A. dorsata, A. florea* and *A. indica* and the African *A. mellifera adansonii*. Aliphatic saturated and monounsaturated compounds are major comb wax constituents[6,7]. The composition of comb wax is dependent on the genetics of the insects[8]. European and African bees produce waxes with different hydrocarbon patterns[9,10] and the process of bee africanization may be detected by analysis of the hydrocarbons encountered in bee products[11]. Recent analysis of comb wax using two-stage resolution of mixtures of heterogenous compounds by supercritical fluid chromatography[12] revealed hydrocarbons, esters of higher alcohols and fatty acids and free higher fatty acids among the wax constituents.

Samples of propolis contain a whitish material which can be extracted by treatment with hot chloroform. This substance has a composition similar to comb wax[13] and is apparently secreted by the bees. In comparison with comb wax, much less is known about the composition of propolis wax. Negri et al.[13] observed that monoesters and hydrocarbons are the predominant constituents of propolis wax. Alkanes, alkenes, alkadienes, diesters, ketones and fatty acids, previously reported as propolis constituents[14,15], are classes of compounds commonly found as natural wax substances.

In Brazil, there has been extensive hybridization between the European bees *A. mellifera mellifera* and *A. mellifera ligustrica* with the African bee *A. mellifera adansonii* (=*A. m. scutellata*) after the introduction of the latter in the 1950’s[16]. Contemporarily, all honey bees found in Brazil are said to be Africanized. The present work presents data of propolis wax from Brazilian localities not included in reference 13. In addition, it includes information about comb wax, for the purpose of comparison between the composition of the waxes from both natural sources.

Results and Discussion

The contents of wax in the collected samples of comb and propolis are presented in Table 1. The values for the samples of propolis range from 4.8% to 19.3%. Apparently, there is no correlation between the percentages and sites of collection. The yields are relatively small compared with those of Bonvehí et al.[17], who found values close to 30% for samples from China. The contents of wax in samples of comb collected in two cities of the state of São Paulo are much lower (1.5% and 3.0%) than the contents found in samples of propolis. Table 1 also presents the percentages of the constituent hydrocarbons and monoesters of the samples of comb and propolis waxes. In both sources, monoesters are clearly the predominant class of constituents, followed by hydrocarbons. Similar results were obtained by Negri et al.[13] for samples of propolis waxes. There is no homogeneity in the results and no correlation with locality. The compositions of comb and propolis waxes are probably more dependent on the genetic characteristics of the bees than on the site of collection. In fact, different degrees of hybridization have been found to occur between European and African bees in Brazil[16,18,19,20].

Table 2 shows the distribution of the hydrocarbon fraction of comb and propolis waxes. A wide variation in the hydrocarbon patterns among the samples is visible. Most samples of comb (1c, 2c and 4c), as well as propolis (2p, 5p, 6p, 7p, 8p and 9p), presented heptacosane as the main component. This alkane has been referred to as the main hydrocarbon of both comb wax[9] and propolis wax[13]. The comb wax sample 3c and that of propolis 1p presented the alkene C_{33} as the main hydrocarbon. Other authors[9,14,21] have reported the predominance of alkenes in comb wax. The main hydrocarbon of propolis samples 3p, 4p and 10p was C_{31}. Occasionally, branched alkanes (*iso*-alkanes) were found in low amounts and exclusively in propolis waxes (samples 1p, 6p and 7p, Table 2). No correlation is apparent between hydrocarbon patterns and localities, contrary to what is known about the composition of the constituents of propolis resin, which is dependent on the local flora[1]. A possible explanation for differences in hydrocarbon patterns between colonies may lie in genetic factors[8, 11], particularly in bee populations from Brazil, which are the result of different levels of hybridization (see Introduction). However, high levels of consistency within and among families of bees has been found by means of correlation analysis[8], indicating structural constancy in comb wax. Analyses of surface hydrocarbons also indicated that a significant proportion of the variation among bees may be attributable to genetic factors[22]. Some insect surface compounds may also be important constituents of comb wax, as are the cases of the hydrocarbons C_{27} and C_{29} (but not C_{31}) and the carboxylic acid C_{14}. It is interesting to note that the latter acid has been found neither in samples of wax of Brazilian propolis analyzed by Negri *et al.*[13] nor in the samples of the present work.

The need to hydrolyze the esters for identification of the constituent acid and alcohol residues is a shortcoming in the analysis of natural waxes, because the outcome is only a partial analysis of the product. Under suitable conditions it is possible to analyze intact high molecular weight esters[9, 23, 24, 25]. Novel techniques involving high temperature gas chromatography have enabled the direct analyses of seed triglycerides[26] and propolis extracts[27,28] without derivatization. In the present work the analysis of the ester fraction followed the conventional procedure of hydrolysis and derivatization prior to GC/MS analysis.

Table 1. Sites of collection of comb (c) and propolis (p) and respective contents (w/w) of wax, hydrocarbons and monoesters. No example of comb and propolis from the same hive is presented.

Sample and site of collection	Wax (%)	Hydrocarbons (%)	Monoesters (%)
1c. Atibaia, state of São Paulo	1.5	23.5	71.8
2c. Jundiaí, state of São Paulo	3.0	25.2	67.4
3c. Jundiaí, state of São Paulo	3.0	15.1	75.8
4c. Jundiaí, state of São Paulo	3.0	16.4	69.4
1p. Atibaia, state of São Paulo	18.0	28.0	67.2
2p. Bragança Paulista, state of São Paulo	14.8	32.5	59.8
3p. Bragança Paulista, state of São Paulo	9.0	15.9	74.3
4p. Bragança Paulista, state of São Paulo	6.6	6.4	73.6
5p. Bragança Paulista, state of São Paulo	11.8	13.8	68.3
6p. Jundiaí, state of São Paulo	4.8	24.9	72.1
7p. Jundiaí, state of São Paulo	14.9	13.3	77.1
8p. Ribeirão Preto, state of São Paulo	19.3	33.3	49.2
9p. Ribeirão Preto, state of São Paulo	17.4	31.0	59.5
10p. Ponta Grossa, state of Paraná	14.5	20.7	73.1

The distribution of the alcohols of monoesters covered the range C_{24}-C_{32} in both sources of waxes (Table 3). Neither branched nor unsaturated homologues were detected. Similar results have been reported for propolis waxes[13]. In general the most abundant homologue in both wax types was C_{30}, but some samples (3c, 4c, 7p-9p, Table 3) presented C_{24} as the main compound. For some samples (1c, 2c, 1p and 6p, Table 3) C_{32} was an important component. In most samples, however, it was a minor constituent of the ester fraction and, in some samples (2p, 7p and 10p), C_{32} was not detected.

Palmitic acid (C_{16}) was the dominant homologue of

Table 2. Percentual distribution of homologues in the hydrocarbon fraction of samples of propolis (p) and comb (c). Unless stated, numbers of carbon atoms correspond to normal chains; *iso* = 2-methyl isomers; colon followed by digit 1 indicates one unsaturation. Digits in sample column correspond to samples listed in Table 1.

Sample	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	iso C_{29}	C_{30}	C_{31}	$C_{31:1}$	iso C_{31}	C_{33}	$C_{33:1}$	$C_{35:1}$
1c	1		3	2	9	3	24	3	16			19	2			15	2
2c	1	1	2	1	13	1	30	1	16		1	17	2		3	3	2
3c					3		12		1							80	4
4c			1		7	2	30	3	16		2	19			5	9	4
1p			2		7		20		12	2		22	3	2		25	3
2p					19		45		14		2	12			1	3	4
3p					4		32		18			40			3		
4p					4		33		20			40			3		
5p					8		36		21			28			3	4	
6p			2	1	6	1	28	1	17	4	1	21	1	2	3	9	2
7p					8		30		13			24		2	2	19	2
8p			7		17		30		7			6			5	28	4
9p			8		18		28		8			6	5			23	3
10p							20		22			53			5		

Table 3. Percentual distribution of *n*-primary alcohols and *n*-carboxylic acids of monoesters from samples of propolis (p) and comb (c) waxes. Colon followed by digit 1 indicates one unsaturation. Digits in sample column correspond to samples listed in Table 1.

Sample/ Fraction	C_{16}	C_{18}	$C_{18:1}$	C_{20}	C_{22}	C_{24}	C_{26}	C_{28}	C_{30}	C_{32}
Alcohols										
1c						15	13	18	40	14
2c						20	17	18	29	16
3c						60	14	12	12	2
4c						43	12	10	29	6
1p						6	9	17	42	26
2p						28	20	21	31	
3p						32	18	11	37	2
4p						6	6	10	73	5
5p						25	7	12	54	2
6p					1	12	13	22	41	11
7p						40	28	14	18	
8p						30	21	19	22	8
9p						39	20	17	21	3
10p						8	3	17	72	
Acids										
1c	100									
2c	56	33	11							
3c	100									
4c	55	14	4	1	2	16	4	3		
1p	80		5			15				
2p	76	4	12							
3p	100									
4p	69	17	8	1	2	3				
5p	100									
6p	58	32	9	1						
7p	29	11	27	2	5	17	4	3		
8p	74	4	18							
9p	72	5	18							
10p	100									

the acyl portion of esters in all samples examined (Table 3), in agreement with previous findings about propolis wax[13]. Although no data were raised about chain lengths of intact esters in the present investigation, the fact that the main alcohol is in general C_{30} and the main acid is C_{16} suggests that triacontil palmitate (C_{46}) predominates among the esters of the samples of propolis and comb wax investigated. Esters ranging from C_{40} to C_{50} were found to occur in comb waxes of *A. mellifera mellifera* and *A. mellifera adansonii*, the most abundant being C_{46}[9]. As in the cases of hydrocarbons and alcohols commented above, a wide variation of patterns of the acid portion of esters is observed in Table 3. For example, samples 1c, 3c, 3p, 5p and 10p presented exclusively palmitic acid. On the other hand, samples 4c and 7p yielded a long series of homologues ranging from C_{16} to C_{28}; oleic acid is an important constituent in some samples (2c, 2p, 7p-9p), but a minor or undetected component in other samples.

In spite of the wide variation observed in the distribution of the constituents of all fractions analyzed, there is a remarkable similarity between the composition of propolis wax and comb wax. The resin and the volatile fractions of propolis are presumably largely derived from plant secretions collected by bees[1]. Since plants produce waxes that coat all aerial cutinized parts[29, 30], the hypothesis could be raised that propolis wax might also be derived from plant secretions. But several differences can be pointed out between the composition of beeswax (as here reported) and plant waxes. For example, the latter rarely present alkenes and oleic acid as important hydrocarbon constituents[29, 30], and esters may predominate in plant waxes, but not always. In contrast, monoesters always appeared consistently as the predominant class of propolis wax (Table 1).

Experimental

Material

Samples of propolis and comb waxes were collected from hives growing in the states of São Paulo and Paraná (Southeast and Southern Brazil, respectively) (Table 1).

Extraction of the waxes

Samples of propolis were extracted with chloroform in a Soxhlet extractor[13]. Amounts of comb ranging from 1.0 to 3.0 g were treated with boiling chloroform and filtered while still hot. The chloroform extracts were evaporated to dryness under reduced pressure and dried in a dessicator to constant weight (Table 1).

Separation and identification of constituent fractions

The fractions of constituents of propolis and comb waxes were separated by CC, using silicagel and a mixture of solvents of increasing polarity[13], and TLC, using silicagel impregnated with sodium fluoresceine and developing with a mixture of hexane: chloroform (73:27)[13]. Functional characterization of the constituent classes was achieved by IR spectroscopy with a Perkin Elmer model FTIR spectrophotometer. The esters were hydrolyzed with methanolic KOH and the resultant acid and alcohol fractions separated by means of extraction with chloroform after neutralization with 10% HCl[13]. The acids were identified as the corresponding methyl esters and alcohols as the corresponding acetyl esters by GC/electron impact mass spectrometry on an HP model 5890 series II GC interfaced with an HP 5989B ChemStation mass spectrometer using conditions identical to those cited in reference 13.

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References

1. Marcucci, M. C. *Apidologie* **1995**, *26*, 83.
2. Marcucci, M. C. *Quim. Nova* **1996**, *19*, 529.
3. Greenaway, W.; May, J.; Scaysbrook, T.; Whatley, F. R. *Z. Naturforsch. C: J. Biosci.* **1991**, *46*, 111.
4. Brumfitt, W.; Hamilton-Miller, J. M. T.; Franklin, I. *Microbios* **1990,** *62*, 19.
5. Hamilton, R. J. In *Waxes: Chemistry, Molecular Biology and Functions*; Hamilton, J. R., Ed.; The Oily Press; Dundee, 1995, p 257.
6. Kolattukudy, P. E. *Chemistry and Biochemistry of Natural Waxes*; Elsevier; Amsterdam, 1976.
7. Brossard, S.; Lafosse, M.; Dreux, M.; Becart, J.; Tranchant, J. F. *Parf. Cosmét. Arômes* **1994**, *117*, 48.
8. Breed, M. D.; Page, R. E.; Hibbard, B. E.; Bjostad, L. B. *J. Chem. Ecol.* **1995**, *21*, 1329.
9. Tulloch, A. P. *Bee World* **1980**, *61*, 47.
10. Hepburn, H. R. *Honey Bees and Wax*; Springer-Verlag; Berlin, 1986.
11. Carlson, D. A. In *Africanized Honey Bees and Bee Mites*; Needham, G. R.; Page, R. E.; Delfinado-Baker, M.; Bowman, C. E., Eds; Ellis Horwood Ltd.; Chichester, 1988, p 264.

12. Takeuchi, M.; Saito, T. *J. Chromatogr.* **1996**, *722*, 317.
13. Negri, G.; Marcucci, M. C.; Salatino, A.; Salatino, M. L. F. *Apidologie* **1998**, *29*, 305.
14. Seifert, M.; Haslinger, E. *Liebigs Ann. Chem.* **1989**, 1123.
15. Bankova, V.; Christov, R.; Stoev, G.; Popov, S. *J. Chromatogr*. **1992**, *607*, 150.
16. Lobo, J. A.; Del Lama, M. A.; Mestriner, M. A. *Evolution* **1989**, *43*, 794.
17. Bonvehí, J. S.; Coll, F. V. V.; Jordá, R. E. *J. Am. Oil Chem. Soc*. **1994**, *71*, 529.
18. Lobo, J. A.; Krieger, H. *Heredity* **1992**, *68*, 441.
19. Diniz-Filho, J. A. F.; Malaspina, O. *Evolution* **1995**, *49*, 1172.
20. Diniz-Filho, J. A. F. *J. Apic. Res*. **1996**, *35*, 104.
21. Seifert, M.; Haslinger, E. *Liebigs Ann. Chem*. **1991**, 93.
22. Page, R. E.; Metcalf, R. A.; Metcalf, R. L.; Erickson, E. H.; Lampman, R. L. *J. Chem. Ecol*. **1991**, *17*, 745.
23. Gülz, P. G.; Müller, E. *Z. Naturforsch*. **1992**, *47*, 800.
24. Summchen, P.; Markstadter, C.; Wienhaus, O. *Phytochemistry* **1995**, *40*, 599.
25. Shepherd, T.; Robertson, G. W.; Griffiths, D. W.; Birch, A. N. E. *Phytochemistry* **1997**, *46*, 83.
26. Reiter, B.; Lechner, M.; Lorbeer, E.; Aichholz, R. *J. High Resolut. Chromatogr*. **1999**, *22*, 514.
27. Pereira, A. S.; Pinto, A. C.; Cardoso, J. N.; Aquino Neto, F. R. ; Ramos, M. F. S.; Dellamora-Ortiz, G. M.; Santos, E. P. *J. High Resolut. Chromatogr*. **1998**, *21*, 396.
28. Pereira, A. S.; Ramos, M. F. S.; Poças, E. S. C.; Dias, P. C. M.; Santos, E. P.; Silva, J. F.; Cardoso, J. M.; Aquino Neto, F. R. *Z. Naturforsch*. **1999**, *54*, 395.
29. Baker, E. A. In *The Plant Cuticle;* Cutler, D. F.; Alvin, K. L.; Price, C. E., Eds; Academic Press; New York, 1982, p 139.
30. Bianchi, G. In *Waxes: Chemistry, Molecular Biology andFunctions*; Hamilton, R. J., Ed.; The Oily Press; Dundee, 1995, p 175.

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