Composition of Propolis from Two Different Spanish Regions

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Propolis and propolis balsam from two different Spanish Regions were analyzed by GC-MS. The majority of the compounds were typical of poplar bud exudate of *P. nigra*. No significant differences were found between the balsam and the entire propolis analyzed, apart from the components of beeswax added to the plant exudate by the bees.

Introduction

Propolis or bee-glue is a resinous bee-hive product, consisting of a mixture of wax, sugars and plant exudate [1], used by bees as a draught excluder and general purpose sealer for their hives. Propolis balsam (a 70% alcohol extract of propolis which contains the bud exudate components) is used in folk medicine in many parts of the world [2]. Propolis can, however, cause allergic reactions in susceptible individuals [3–5]. The composition of propolis from different countries and the similarity between propolis balsam and bud exudate of poplars has been previously reported [6–8]. We here describe the composition of three propolis samples from two different Spanish locations: La Alcarria (Castilla-La Mancha) and Nerpio (Murcia), which as far as we are aware have not previously been studied by GC-MS, and report on its relationship with that of poplar bud exudates previously reported.

Materials and Methods

Two propolis samples from La Alcarria were obtained from the Centro Regional Apicola de Castilla-La Mancha (Guadalajara, Spain). A third sample from Nerpio was obtained from the Laboratorio Agrario Regional de Murcia (Murcia, Spain).

The readgement Bis-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was obtained from Sigma (Poole, U.K.).

About 0.5 mg of propolis was prepared for gas-chromatography by derivatization for 30 min at 100 °C with 50 µl pyridine and 100 µl BSTFA (including 1% TMCS) in a sealed glass tube. About 1 mg propolis was extracted with 70% ethanol to obtain the balsam, which was prepared for gas-chromatography as previously described for entire propolis. The derivatized samples were separated and analyzed in a Finnigan 1020 automated GC-MS as previously reported [7].

Compounds were identified by computer search of user-generated reference libraries incorporating GC retention times and mass spectra. Reference compounds were co-chromatographed with the experimental sample to confirm GC retention times and mass spectra patterns. Peaks were examined by single-ion chromatographic reconstructions to confirm their homogeneity, mixed peaks were resolved by the computer program aimed at resolving the mass spectral data of one compound from overlapping mass spectra of another [6].

Results and Discussion

Composition of propolis

Propolis consists of a mixture of beeswax and plant exudate. The beeswax composition has been previously investigated [9] and will not be further discussed. We here present the major components of three propolis samples and their balsams, from two different regions of Spain.

Table I indicates that all samples were qualitatively similar but they show some variation. The main compounds of all of the propolis samples and their balsams were caffeic acid and isoferulic acid and their esters, flavanones such as pinocembrin (5,7-dihydroxyflavanone) and pinobanksin (3,5,7-trihydroxyflavanone) and their derivatives together with some flavones. Long chain fatty acids and hydrocarbons were present in propolis but are absent from balsam (*i.e.* extracted into 70% ethanol). Variable amounts of sugars such as

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Table I. Composition of propolis assessed by GC-MS of trimethylsilyl derivatives. GC retention times in methylene units (MU)[1] are given to two decimal places to indicate the elution sequence of peaks which chromatograph closely. Factors such as concentration of the compound concerned, together with the characteristics of a particular GC column are liable to affect the chromatography and for general purposes the MU figures are probably reliable to only a single decimal place.

Peak No.	Compound[2]	TMS groups	MU	%TIC[3]		
				A	B	C
1	Benzoic acid	1	12.31	–	0.3	–
2	1,2,3-Propanetriol	3	12.95	0.9	1.2	0.4
3	Butanedioic acid	2	13.10	3.7	–	–
4	Glycerol monoacetate	2	13.20	–	<0.1	–
5	2-Hydroxybutanedioic acid	3	14.92	1.5	0.4	–
6	Guaiol	1	17.01	–	0.3	–
7	3-Hydroxy-4-methoxy benzoic acid	2	17.60	–	0.3	–
8	Fructofuranose (isomer 1)	5	18.50	2.0	0.9	6.6
9	Fructofuranose (isomer 2)	5	18.62	1.2	0.7	6.2
10	Glucofuranose	5	19.01	–	–	0.3
11	*trans*-3(4-Hydroxyphenyl)propenoic acid	2	19.32	0.4	0.9	0.3
12	Sesquiterpene alcohol	1	19.40	–	1.1	–
13	α-Glucopyranose	5	19.51	1.7	–	12.2
14	Unidentified sugar alcohol	–	19.80	0.9	–	2.9
15	*trans*-3(3,4-dimethoxyphenyl)propenoic acid	1	19.90	–	0.4	–
16	Hexadecanoic acid	1	20.30	0.2	1.2	3.2
17	β-D-Glucopyranose	5	20.50	1.9	–	8.5
18	*trans*-3(3-Hydroxy-4-methoxyphenyl)propenoic acid	2	20.68	0.3	0.7	0.5
19	*trans*-3(3-Methoxy-4-hydroxyphenyl)propenoic acid	2	20.80	–	0.8	<0.1
20	Henicosane	–	21.00	–	–	0.3
21	3-Methyl-3-butenyl *trans*-4-coumarate	1	21.28	1.0	–	–
22	*trans*-3(3,4-Dihydroxyphenyl)propenoic acid	2	21.39	6.6	3.4	0.5
23	3-Methyl-2-butenyl *trans*-4-coumarate	1	21.49	0.9	–	–
24	*trans*-9-Octadecenoic acid	1	22.00	0.3	–	–
25	8-Octadecenoic acid	1	22.10	–	1.8	0.7
26	Octadecenoic acid	1	22.20	–	<0.1	–
27	2-Methylpropyl *trans*-caffeate	2	22.30	0.3	–	–
28	2-Methyl-2-propenyl *trans*-caffeate	2	22.50	0.3	–	–
29	3-Methyl-3-butenyl *trans*-ferulate	1	22.81	–	0.2	–
30	Tricosane	–	23.00	0.4	0.4	1.7
31	2-Methyl-2-butenyl *trans*-isoferulate	1	23.10	–	<0.1	–
32	3-Methyl-2-butenyl *trans*-ferulate	1	23.30	–	0.9	–
33	3-Methyl-3-butenyl *trans*-caffeate	2	23.47	10.2	–	1.0
34	Pent-4-enyl caffeate	2	23.60	–	1.1	–
35	2′,6′-dihydroxy-4′-methoxydihydrochalcone	2	23.74	0.1	–	–
36	2-Methyl-2-butenyl *trans*-caffeate	2	23.80	1.2	0.4	0.3
37	3-Methyl-2-butenyl *trans*-caffeate	2	23.98	13.0	1.0	0.9
38	5,7-Dihydroxyflavanone (Pinocembrin)	1	24.20	0.2	0.5	0.6
39	2′,4′,6′-Trihydroxydihydrochalcone	3	24.30	0.2	–	–
40	2′,6′,α-Trihydroxy-4′-methoxychalcone	3	24.39	<0.1	–	–
41	5-Hydroxy-7-methoxyflavanone (Pinostrobin)	1	24.50	2.1	0.9	–
42	2′,6′-Dihydroxy-4′-methoxychalcone	2	24.61	2.2	0.6	–
43	5,7-Dihydroxyflavanone (Pinocembrin)	2	24.90	4.0	5.5	–
44	2′,4′,6′-Trihydroxychalcone	3	24.95	5.5	6.5	–
45	Pentacosane	–	25.00	0.5	1.6	4.1
46	2′,4′-Dihydroxy-6′-methoxychalcone	2	25.26	–	0.9	–
47	Dihydroxymonomethoxyflavanone	2	25.30	0.2	–	–
48	2′,4′,6′,α-Tetrahydroxychalcone	4	25.50	0.1	–	–
49	2′,6′,α-Trihydroxy-4′-methoxychalcone	3	25.60	<0.1	–	–
50	3,5,7-Trihydroxyflavanone (Pinobanksin)	3	25.98	3.7	11.1	1.6
51	Hexacosane	–	26.00	–	–	0.2
52	5,7-Dihydroxyflavone (Chrysin)	1	26.07	0.7	3.3	–
53	5,7-Dihydroxy-3-methoxyflavanone (Pinobanksin-3-methyl ether)	2	26.20	–	3.6	–

Table I. (Continued).

Peak No.	Compound[2]	TMS groups	MU	%TIC[3]		
				A	B	C
54	5,7-Dihydroxy-3-acetyloxyflavanone (Pinobanksin-3-acetate)	2	26.61	9.2	8.7	1.2
55	Benzyl *trans*-caffeate	2	26.81	–	1.8	0.2
56	Heptacosane	–	27.00	2.1	4.4	11.8
57	Glyceryl *trans*-caffeate	4	27.05	0.3	–	–
58	3,5-Dihydroxy-7-methoxyflavone (Galangin-7-methyl ether)	2	27.10	0.7	–	–
59	5,7-Dihydroxyflavone (Chrysin)	2	27.14	4.8	7.0	–
60	5,7-Dihydroxy-3-propanoyloxyflavanone (Pinobanksin-3-propanoate)	2	27.18	–	2.4	–
61	5,7-Dihydroxy-3-methoxyflavone (Galangin-3-methyl ether)	2	27.20	0.8	–	–
62	Sucrose	8	27.42	5.2	–	12.1
63	3,5,7-Trihydroxyflavone (Galangin)	3	27.52	1.9	6.7	–
64	1-Phenylethyl-*trans*-caffeate	2	27.70	0.6	3.1	0.2
65	5,7-Dihydroxy-3-pentanoyloxyflavanone (Pinobanksin-3-pentanoate)	2	28.30	–	0.7	–
66	Tetracosanoic acid	1	28.40	0.8	2.3	8.4
67	Diprenyl-*trans*-caffeate	2	28.71	0.2	–	–
68	Nonacosane	–	29.00	0.7	0.9	5.7
69	*trans*-Cinnamyl-*trans*-caffeate	2	30.01	–	0.7	–
70	Hexacosanoic acid	1	30.41	–	0.2	1.2
71	Henitriacontane	–	31.00	0.7	0.3	3.0
72	Octacosanoic acid	1	32.51	–	–	<0.1

[1] Methylene Units (MU) are defined by Dalgliesh *et al.* [15].
[2] The name given does not include the TMS substituents.
[3] The ion current generated depends on the characteristics of the compound concerned and is not a true quantitation. (A) Nerpio propolis sample, (B) La Alcarria propolis sample 1, (C) La Alcarria propolis sample 2.

glucose, fructose and sucrose were also present, but these are probably introduced by bees accidentally, either during propolis manufacture or during subsequent passage of bees over propolis. Nerpio propolis also contained small amounts of 2′,6′-dihydroxy-4′-methoxydihydrochalcone (0.1%) and 2′,4′,6′-trihydroxydihydrochalcone (0.2%). There are a number of additional compounds present in low amounts which are obscured by other compounds present in larger amounts.

The differences between the two samples from La Alcarria may be due to the fact that they originated from different hives and may have been collected from different poplar trees. It may also happen that wax or sugar content can vary within a single hive, depending on the use to which the propolis is put [7].

Origin of propolis

The high levels of caffeic acid and ferulic acid and their derivatives and the low levels of cinnamic and coumaric acids and their esters, together with the low level of dihydrochalcones (absent from the La Alcarria samples) and absence of terpenoids is typical of bud exudate of *P. nigra,* the European black poplar (Section Aigeiros) [10]. It is from these trees that bees preferentially collect exudate for incorporation into propolis.

P. deltoides (Section Aigeiros) the eastern cottonwood of America, which is widely distributed in Central and Eastern United States, was introduced into Europe after 1700 [10–11]. It has hybridized freely with *P. nigra* yielding many hybrids collectively known as *P.* × *euramericana* [12], which are now widely distributed in Western Europe. The distribution of the phenolics in *P.* × *euramericana* represent a balance (multigenic inheritance) between features of the two parent species, which will be characteristic of each offspring. This is one source of variation in the bud exudate of the poplars used by bees. So characteristic compounds of these last two poplar (*P. deltoides* and *P.* × *euramericana*) bud exudates (previously reported by Greenaway *et al.* [6, 13]) may also be present in Spanish propolis.

While bud exudates of different poplar species are frequently similar in qualitative composition,

they can vary in quantitative composition, as has been described for *P. nigra* over its considerable geographical range [10]. These differences are manifested in the composition of the propolis into which the bud exudate is incorporated [7]. The variation in composition of propolis may be considerable. Caffeic acid and its esters, which play a major part both in the antimicrobial activities of propolis [14] and in its allergic properties [3–5], are present in high amounts in the propolis samples analyzed: the 3-methyl-2-butenyl-*trans*-caffeate in Nerpio propolis represents 13% TIC. The assumption that propolis from apparently similar sources must have similar compositions is incorrect and the use of such a complex and variable product in medicine without first establishing the composition of the particular propolis used is questionable.

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