Composition of Propolis in Oxfordshire, U.K. and its Relation to Poplar Bud Exudate

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Z. Naturforsch. **43c**, 301–305 (1988);
received November 13, 1987

Propolis, Poplar Bud Exudate, Bees

Propolis balsam from four locations in Oxfordshire was analysed. The balsam was qualitatively similar but showed large quantitative differences in composition. These quantitative differences are related to the bud exudate composition of the poplars from which balsam was gathered.

Introduction

Propolis is a mixture of wax, sugars and plant exudates used by bees as a general purpose glue and sealant within hives. There is a long history of medicinal usage of propolis although propolis can cause allergic reactions in susceptible individuals [1, 2]. Various components of propolis have been assessed for their antimicrobial properties [3–7]. Components identified as having antimicrobial properties are all derived from the plant exudate and this exudate may be collected from various trees including *Alnus* sp. [6], *Betula* sp. [8] and *Populus* sp. [9, 10].

We have previously investigated the composition of propolis from England and Scotland and have reported the close similarity between propolis balsam (a 70% alcohol extract of propolis) from Buckland, Oxon., and the exudate of adjacent *Populus* X *euramericana* (Dode) Guinier trees [11]. We here report the variation in composition which occurs in propolis obtained from four locations in Oxfordshire, U.K. and relate this variation to the exudates gathered.

Materials and Methods

Propolis

Propolis was obtained from hives at Buckland, Oxon; the University Museum, Oxford; the Zoology Department, Oxford and Noke, Oxon.

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/88/0003–0301 $ 01.30/0

Sample preparation

Propolis was extracted with 70% ethanol to obtain the balsam. About 1 mg of balsam was prepared for gas chromatography by derivatization for 30 min 100 °C with 50 µl pyridine + 100 µl *bis*-(trimethylsilyl)trifluoroacetamide (BSTFA) including 1% trimethylchlorosilane (TMCS) in a sealed glass tube.

Gas chromatography-mass spectrometry

The derivatized samples were separated and analysed in a Finnigan 1020 automated GC/MS (incorporating a Data General Nova 3 computer); the GC system was fitted with a 50 m, 0.3 mm internal diameter, Thames Chromatography silica column coated with 0.5 micron bonded phase OV1 and a splitless injector with a flush 30 seconds after sample introduction to remove residual gases. The end of the column was introduced directly into the mass spectrometer analyser chamber. The system was operated under the following conditions: helium pressure 138 kN/m^2; injector temperature 310 °C; GC temperature 85–310 °C at 3 °C min^{-1}. The mass spectrometer was set to scan 40–650 AMU per nominal second with an ionizing voltage of 70 eV. The filament was switched on 250 seconds after injection of the sample into the GC.

Identification of compounds

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

Results and Discussion

Composition of propolis

Propolis consists of a mixture of wax, sugars and plant exudates. Various analyses have measured the wax content to be between 14–40% [3] and it seems probable that the percentage of wax in propolis will vary within a single hive depending on the function of the propolis. The beeswax component of propolis

has been analysed in detail by Tulloch [12] and will not be further discussed here.

The aromatic fraction of propolis (essentially that derived from plant exudates collected by bees) may be separated from the wax by extraction into 70% (by volume) alcohol and is termed propolis balsam. The propolis balsam is a complex mixture of compounds (Fig. 1) including substituted benzoic acids and esters, substituted phenolic acids and esters, flavonoid aglycones and terpenoids. Variable amounts (ca. 2–20%) of sugars such as glucose, fructose and sucrose are also usually present in the balsam but these are probably introduced by bees either accidentally during propolis manufacture or during subsequent passage of bees over propolis.

We have already made a detailed analysis of propolis from Buckland [11] and here present analytical data for the major components of four propolis balsam samples from Oxfordshire (Table I). Whereas all four samples are qualitatively similar they show great variation in their quantitative composition. Thus cinnamic acid is high in balsam from the University Museum (18.1%) but much lower in balsam from Buckland (0.2%); Oxford Zoology (0.5%) and Noke (3.5%). Similarly 4-coumaric acid is high in Museum balsam (22.1%) but, whereas Buckland (0.9%) and Oxford Zoology (6.1%) are again low, Noke balsam is high in 4-coumaric acid (20.1%). Schneidewind [5] has identified caffeic acid and its esters as having major activity in the antimicrobial properties of propolis and these compounds form 25.7% of Buckland and 20.2% of Oxford Zoology balsams. However they form only 3.4% of Noke and 1.9% of Oxford Museum balsams.

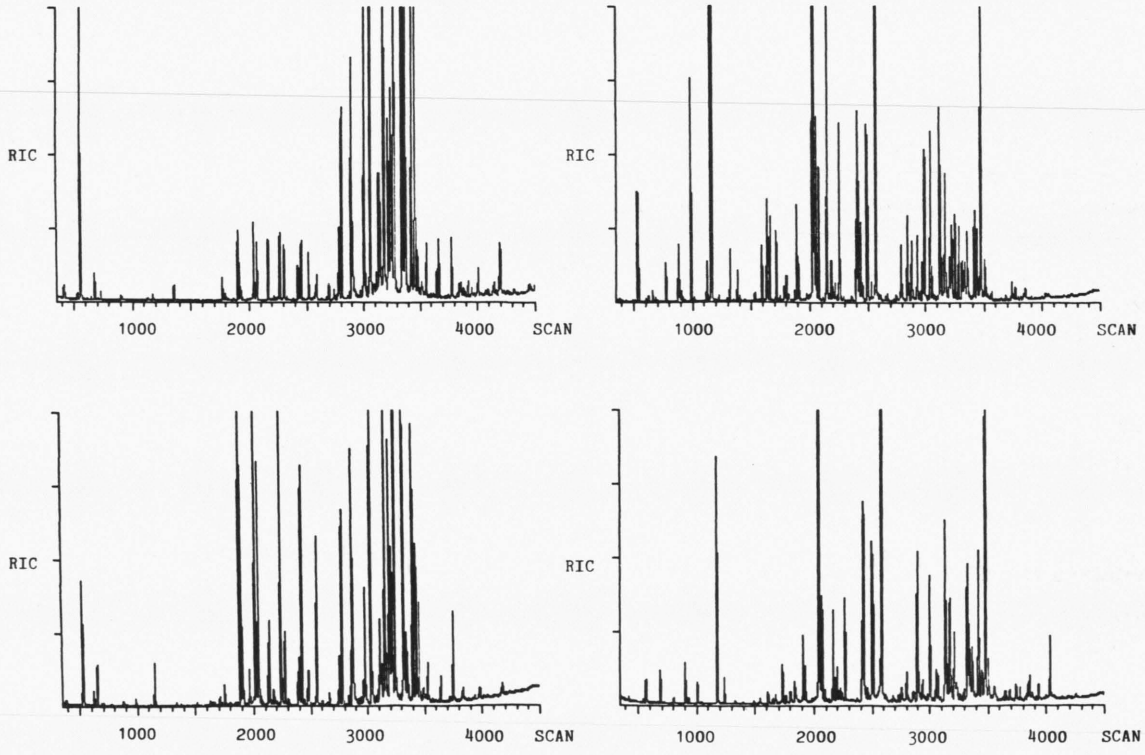

Fig. 1. Reconstructed ion chromatograms (RIC) of propolis balsam from (1) Buckland, Oxon.; (2) University Museum, Oxford; (3) Zoology Department, Oxford; (4) Noke, Oxon. The chromatograms, generated by plotting the total ion signal from the mass spectrometer against the scan number, illustrate the chemical complexity of propolis balsam and demonstrate the differences in composition of the four balsams analysed. The compounds present were identified by computer search as described in the text and the major components are shown in Table I. A detailed analysis of the compounds present in the propolis from Buckland has already been published [11].

Table I. Composition of major components of propolis balsam from Oxfordshire. The percentage figures refer to the ion current generated by the compound in the mass spectrometer.

Compound identified		Source of propolis			
			Oxford		
		Buckland	Museum	Zoology	Noke
	benzoic	11.4	1.7	2.7	0.6
	cinnamic	0.2	18.1	0.5	3.5
Free	4-coumaric	0.9	22.1	6.1	20.1
acids	3,4 dimethoxycoumaric	0.9	–	0.6	–
	isoferulic	0.8	<0.1	0.9	–
	ferulic	0.1	<0.1	0.1	–
	caffeic	0.9	0.7	2.9	0.1
	salicylate	0.1	3.4	0.5	1.6
Benzyl	4-coumarate	3.2	1.6	1.5	2.1
esters	isoferulate	1.7	–	0.4	–
	caffeate	16.3	0.2	6.9	2.8
methylbutenyl	4-coumarate	1.1	3.2	0.2	8.3
esters	caffeate	5.1	0.8	7.1	0.1
Phenylethyl	4-coumarate	0.7	2.1	1.2	3.7
esters	caffeate	3.1	0.1	2.1	0.1
Cinnamyl	4-coumarate	0.2	4.0	1.1	6.5
esters	isoferulate	0.5	–	0.3	–
	caffeate	0.3	0.1	1.2	0.3
Flavonoid[a]	pinocembrin	8.7	1.8	11.8	0.9
aglycones	chrysin	4.8	0.5	4.8	1.4
	galangin	5.1	1.0	5.0	0.9
	fructose	0.9	1.2	7.0	1.8
Sugars	glucose	0.8	3.4	7.7	2.5
	sucrose	1.0	<0.1	0.5	3.4
Terpenoid[b]	1	0.5	8.6	2.1	13.2
alcohols	2	–	1.2	<0.1	0.2
	3	0.1	1.0	0.5	2.9
Ketones	*p*-hydroxyacetophenone	–	2.1	–	0.4

[a] Flavonoid aglycones do not transmit as well through the GC column as do other components of the mixture and the figures given will therefore be an underestimate of their true quantitative occurrence.
[b] We do not know the identity of these terpenoid alcohols.

Although the hives at the University Museum and Zoology Department are only *c* 400 m apart, it is clear from the analyses presented that the balsam from these two hives differs so greatly in the quantitative composition of their common components that the propolis will show different physical and antimicrobial properties.

Relationship of plant exudate and propolis balsam

All samples of U.K. propolis which we have analysed are similar in qualitative composition to bud exudate of various *Populus* species and their hybrids and when only a single clone of poplar is available to the bees the propolis balsam composition is directly related to the bud exudate composition [11]. The composition of poplar bud exudate is characteristic of the species [13–15] and does not change during the period the tree is in leaf [13, 14]. In the U.K. therefore propolis balsam composition is directly related to the composition of the bud exudate collected and this, although consistent within a species, can vary greatly between species. As an example of the differences encountered, bud exudate of *P. balsamifera* L., from Oxford contained, amongst other compounds, 5.5% *p*-hydroxyacetophenone and 6.3% cinnamic acid but less than 0.1% caffeic and caffeic methylbutenyl esters, whereas *P. nigra* L. *'Italica'* contained less than 0.1% of *p*-hydroxyacetophenone and cinnamic acid but 4.7% caffeic acid and 32.4% caffeic methylbutenyl esters.

We conclude that propolis obtained from hives having access to trees of a single species, or even a single clone, which produces a characteristic bud exudate, will contain propolis balsam with the same characteristic composition, although the percentage of wax and sugar in the propolis may vary. However if bud exudate is obtained from different species of poplar the composition of the propolis balsam is likely to show considerable variation, as we have demonstrated for the Oxfordshire propolis.

The production of balsam of a required composition could be controlled by ensuring bees have access only to a single poplar clone which produces a bud exudate of that composition.

Acknowledgements

We thank P. Embden, I. Gourlay and H. P. Powell for providing propolis samples.

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