FLAVOUR AND FRAGRANCE JOURNAL, VOL. 4, 173–175 (1989)

Headspace Volatiles from Propolis

W. Greenaway, T. Scaysbrook and F. R. Whatley

Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RA, UK

Volatile compounds from propolis were trapped on Tenax GC, desorbed and then analysed by capillary GLC and mass spectrometry. All compounds identified appear to originate from popular bud exudates which form a major component of propolis.

KEY WORDS Volatiles Propolis Bee glue *Populus* GC–MS

INTRODUCTION

Propolis (bee-glue) is the material used by bees as a general-purpose glue and waterproof sealant in their hives. It consists primarily of a mixture of beeswax and bud exudates,[1–3] which are gathered preferentially from poplar trees.[3–5] The bud exudates can be extracted from propolis with 70% ethanol to give propolis balsam.

The bud exudate of different poplar species,[6,7] or even clones,[8] have characteristic compositions. The composition of the propolis balsam is directly related to that of the bud exudate[3] and therefore varies according to the source of the bud exudate.[9] We here report the chemical constituents of the headspace volatiles of a sample of propolis and discuss how the volatiles may vary depending on the source of the propolis balsam.

EXPERIMENTAL

Propolis

Propolis was obtained from P. Ross Esq. and collected from a hive at Cleppa Park, near Cardiff, Wales. The composition of the propolis balsam was established by GC–MS and was similar to that of balsam from Buckland, Oxon., which was known to incorporate bud exudate collected by bees from *Populus* × *euramericana* (Dode) Guinier,[3] a widely planted hybrid between *P. deltoides* Marsh and *P. nigra* L.[10] We therefore believe the bud exudate incorporated into the propolis analysed here was gathered from a *P.* × *euramericana* clone.

Trapping of Volatiles

Vapour from 6.0 g of coarsely granular propolis, kept at 40°C in a 10 ml glass tube, was drawn for 24 h at 1.25 ml per min through a Scientific Glass Engineering desorption tube (P/N 093259) packed with Tenax GC, 30–60 mesh ASTM occupying a space 7 cm × 0.7 mm.

Compounds trapped were desorbed by heating the tube at 240°C in a Thames Chromatography Desorb 100 unit. The Desorb 100 unit was installed in the helium carrier gas line prior to the standard injector inlet, which remained operational.

Capillary GLC and MS

Constituents of the desorbed volatiles were separated in a Hewlett-Packard 5890 GLC and analysed in a Hewlett-Packard 5970 series mass selective detector. The GC system was fitted with a 30 m × 0.25 mm i.d. Supelco Inc. fused silica column coated with 0.5 µl bonded phase Supelcowax 10 and was run on the following settings: helium pressure 7.5 lb, GC temperature programme 30–200°C at 3°C per min, with a 10 min hold at 30°C. The mass spectrometer was set to scan 30–250 atomic mass units per nominal 0.5 s with an analysing voltage of 70 eV.

Identification of Compounds

Peaks were examined by single ion chromatographic reconstructions to confirm their homogeneity and were identified by computer searches of commercial and user-generated

0882–5734/89/040173–03$05.00

Received 11 March 1989
Accepted 11 July 1989

Table 1. Volatile components of propolis

Peak no.	Compound identified	Retention time (min)	% total[a] ion current
1	Isobutyl acetate[b]	7.1	1.8
2	Unknown	9.1	0.5
3	Hexanal[b]	10.3	2.5
4	Isopentyl acetate[b]	12.9	9.9
5	Limonene[b]	17.5	0.5
6	1,8-Cineole[b]	18.0	0.4
7	3-Methylbut-3-enyl acetate[b]	18.3	0.7
8	Hex-2-enal[b]	19.3	0.7
9	3-methylbut-3-enol[b]	21.9	5.8
10	Phenylethylene (styrene)[b]	22.0	1.4
11	Unknown branch chain alcohol	22.3	0.7
12	*p*-Cymene[b]	22.8	1.8
13	Isopentyl butyrate[c]	23.3	2.2
14	Unknown aliphatic acid ester	25.2	0.8
15	3-Methylbut-2-enol[b]	26.4	5.2
16	6-Methylhept-5-en-2-one[b]	27.2	16.0
17	Acetic acid[b]	33.8	1.1
18	α-Copaene[c]	35.9	0.1
19	Benzaldehyde[b]	36.1	9.0
20	Isobutyric acid[b]	38.8	0.6
21	Methyl benzoate[b]	40.5	0.1
22	Butyric acid[b]	41.3	0.5
23	Acetophenone[b]	41.6	0.1
24	2-Methylbutyric acid[b]	43.0	4.8
25	4-Hexanolactone[c]	43.7	0.4
26	But-2-enoic acid[c]	43.9	0.9
27	Benzyl acetate[b]	45.0	0.8
28	Napthalene[b]	45.1	0.4
29	Methyl salicylate[b]	46.6	0.4
30	2-Methylbut-2-enoic acid[c]	49.8	5.3
31	Benzyl alcohol[b]	50.6	14.2
32	2-Phenylethyl alcohol[b]	51.8	4.4

[a]The total ion current generated depends on the characteristics of the compound concerned and is not a true quantitation.
[b]Identified by comparison with GC retention time and mass spectral characteristics of authentic reference compound.
[c]Identified by GC and mass spectral characteristics, but without direct comparison with reference compound.

reference libraries. Reference compounds were chromatographed where possible to confirm GC retention times and mass spectral patterns (Table 1).

RESULTS AND DISCUSSION

The total ion chromatogram of volatiles trapped from the propolis is shown in Figure 1 and peaks numbered there are identified in Table 1. The last peaks eluting from the volatile fraction are benzyl alcohol and phenyl ethyl alcohol. When entire propolis is analysed as trimethyl-silyl (TMS) derivatives on a capillary column coated with OV1 these compounds, as their mono-TMS derivatives, are amongst the first peaks to elute.[3] More volatile compounds, which are described here, are obscured by the TMS solvent front. Analysis of volatiles trapped on Tenax therefore complements the analysis of TMS derivatives of the less volatile components.

All the compounds which we identify are either directly related to known chemical constituents of *P.* × *euramericana* bud exudate or are known to be common plant volatiles. We see no compounds which are distinctive products of bee metabolism.

We deduce that the propolis from which these

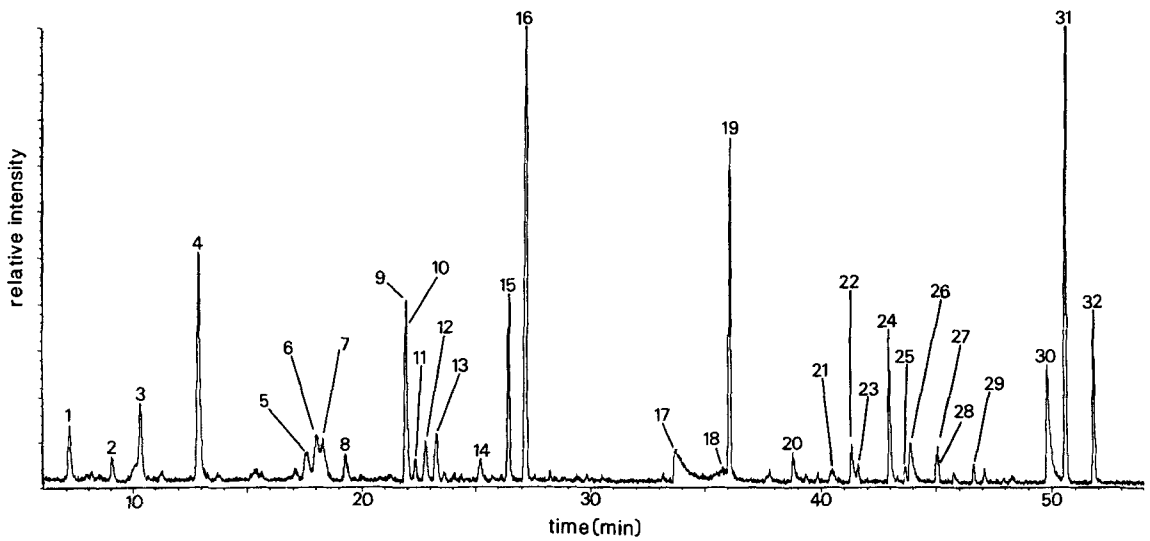

Fig. 1. Total ion mass chromatogram of headspace volatiles from propolis. Identification of numbered peaks is given in Table 1

volatiles were trapped contained bud exudate originating from *P.* × *euramericana* (see above). The composition of bud exudate of different poplar species, whilst often qualitatively similar, can show great quantitative differences. Thus terpenoids account for less than 0.1% of *P. euramericana* bud exudates[3,7] and this is in agreement with the low amounts of terpenoids in the propolis volatiles analysed here. In contrast terpenoids account for over 10% of the bud exudate of the balsam poplar, *P. balsamifera* L. (= *P. tacamahaca* Mill.), and volatiles trapped from these buds consist almost entirely of terpenoids (unpublished). We suggest therefore that propolis incorporating primarily *P. balsamifera* bud exudate would produce a volatile fraction which consists mainly of terpenoids.

Acknowledgement—We thank P. Ross of the Cardiff Bee Research Unit for supplying the propolis used.

REFERENCES

1. E. L. Ghisalberti, *Bee World*, **60**, 59 (1979).
2. M. H. Haydak, *State of Iowa Repts State Apiarist*, 74 (1953).
3. W. Greenaway, T. Scaysbrook and F. R. Whatley, *Proc. R. Soc. Lond. B*, **232**, 249 (1987).
4. V. S. Bankova, S. S. Popov and N. L. Marekov, *J. Nat. Prod.*, **46**, 471 (1983).
5. E. Nagy, V. Papay, G. Litkei and Z. Dinya, *Flavonoids and Bioflavonoids 1985; Proc. 7th Hungarian Bioflavonoid Symp. Szeged, Hungary*, ed. L. Farkas *et al.*, Studies in Organic Chemistry No. 23, p. 223, Elsevier, Amsterdam.
6. E. Wollenweber, *Biochem. Syst. Ecol.*, **3**, 35 (1975).
7. W. Greenaway, J. May and F. R. Whatley, *J. Chromatog.*, **472**, 393 (1989).
8. W. Greenaway, J. Jobling and T. Scaysbrook, *Silvae Genetica*, **38**, 28 (1989).
9. W. Greenaway, T. Scaysbrook and F. R. Whatley, *Z. Naturforsch.*, **43c**, 301 (1988).
10. M. Viart (ed.), *Poplars and Willows in Wood Production and Land Use* 34, Food and Agricultural Organization of the United Nations, Rome (1979).