Identification by Gas Chromatography-Mass Spectrometry of 150 Compounds in Propolis

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

Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, U.K.

Z. Naturforsch. **46c,** 111–121 (1991); received October 11, 1990

Propolis, Volatiles, Phenolic Compounds, Poplar Bud Exudate, GC-MS

Propolis was analyzed by gas chromatography-mass spectrometry for both its headspace volatiles and for the less volatile components of its alcoholic extract (propolis balsam). 181 peaks were located of which 171 representing 150 compounds were identified, including 28 identified in propolis for the first time. The majority of compounds were typical of poplar bud exudate.

Introduction

Propolis is the substance used by bees as a draught excluder and general purpose sealer for their hives. It usually consists of a mixture of poplar bud exudate and beeswax [1]. Propolis balsam (a 70% alcohol extract of propolis which contains the bud exudate components) is a popular herbal medicine [2–4] containing a number of phenolic constituents with antimicrobial activity [4–6].

Bees appear to prefer to collect bud exudate from poplars of the Section Aigeiros, such as *Populus nigra* L., the European black poplar [1, 7, 8]. We have previously identified by gas chromatography-mass spectrometry (GC-MS) 104 components in propolis balsam originating from *P.* × *euramericana* (Dode) Guinier, an intrasectional hybrid between *P. nigra* and *P. deltoides* Marsh, the eastern cottonwood of North America [9]. That sample of propolis contained compounds typical of Section Aigeiros poplars and essentially lacked compounds typical of poplars of other sections. We here describe the detailed analysis by GC-MS of both the headspace volatiles and other less volatile components of a propolis sample which contains, in quantity, series of compounds, such as dihydrochalcones and sesquiterpenols, which are typical of Section Tacamahaca poplars [10, 11], together with pinocembrin (3,5,7-trihydroxyflavanone) and pinocembrin derivatives which are typical of Section Aigeiros poplars [12–14]. The sample of propolis described here (for origin and availability see Methods) is therefore unusually complex, as it contains compounds typical of both Section Aigeiros and Section Tacamahaca poplars.

Reprint requests to W. Greenaway.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0939–5075/91/0100–0111 $ 01.30/0

Materials and Methods

Reagents

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

Propolis

Propolis was collected from a single hive in England at Buckland, Oxfordshire. The propolis (45 g) was 'manufactured' by the bees during a four week period (late May to early June) and used to construct a screen restricting the entrance to the hive. The propolis has been deposited with Professor E. Wollenweber, Institut für Botanik der Technischen Hochschule, Darmstadt, D-6100 Darmstadt, F.R.G., from whom samples may be requested for use as 'standards' in future work.

Analysis of components of propolis

The volatile and non-volatile fractions of propolis were prepared and analyzed using different methods.

The volatile fraction was trapped on Tenax GC (an activated carbon), desorbed by flash heating, and analyzed in a Hewlett-Packard GC-MS system using a polar GC column.

The less volatile components were derivatized with trimethylsilyl reagent, to render them volatile for gas chromatography, and analyzed in a Finnigan 1020 GC-MS system on a non polar GC column.

These different methods are described in detail below.

Trapping and analysis of headspace volatiles

Headspace volatiles (*i.e.* those compounds which are naturally emitted as a vapour) from 45 g of propolis kept at room temperature (*ca.* 25 °C) in a 450 ml glass vessel were drawn for 16 h at 1.25 ml per min through a Scientific Glass Engineering (Victoria, Australia) desorption tube (P/N 093259) packed with Tenax GC, 30–60 mesh ASTM, occupying a space of 7 cm × 0.7 mm. Compounds trapped on Tenax were desorbed by heating the tube at 240 °C in a Thames Chromatography (Maidenhead, U.K.) Desorb 100 unit. The Desorb 100 unit was installed in the helium carrier gas line of a Hewlett-Packard 5890 gas liquid chromatograph (GLC) prior to the standard injector inlet.

Constituents of the desorbed volatiles were separated in the Hewlett-Packard GLC and analyzed in a Hewlett-Packard 5970 series mass selective detector. The GLC system was fitted with a 30 m × 0.25 mm i.d. Supelco Inc. (Bellefonte, PA, U.S.A.) fused silica column coated with 0.25 µm bonded phase Supelco-wax 10 and was run on the following settings: helium pressure 51 kN/m^2, GC temperature programme 30–200 °C at 3 °C per min with a 3 min hold at 30 °C. The mass spectrometer was set to scan 35–250 atomic mass units (AMU) per nominal 0.5 s, with an analyzing voltage of 70 eV.

Derivatization and analysis of propolis balsam

Propolis was extracted with 70% ethanol. The alcohol was evaporated and the residue dried to obtain the balsam. About 1 mg of balsam was prepared for gas chromatography by derivatization for 30 min at 100 °C with 50 µl pyridine + 100 µl BSTFA (including 1% TMCS).

The derivatized samples were separated and analyzed in a Finnigan 1020 automated GC-MS as previously described [15] excepting that a 25 m × 0.32 mm ID Thames Chromatography silica column coated with 0.5 µm of immobilized polydimethylsiloxane was used with a helium pressure of 76 kN/m^2, and the mass spectrometer was set to scan 40–650 AMU per nominal 0.6 s.

Identification of compounds

Compounds were identified by comparison of GC R_ts and mass spectra of reference compounds. Components of mixed peaks were separated either by a computer programme aimed at resolving the mass spectral data of one compound from overlapping mass spectra of another or by manual examination of single ion reconstructions of the data [16] (see also below). Flavonoid standards were either purchased from Apin Chemicals (Abingdon, U.K.), or from Plantech U.K. (Reading, U.K.), or provided as a gift by Professor E. Wollenweber (Darmstadt, F.R.G.). Other reference compounds were synthesized as described previously [9].

The GC R_ts for the headspace volatiles are given in minutes, because we find hydrocarbons not to be satisfactory retention time standards at low temperatures on polar columns when using Tenax desorption tubes. For analysis of TMS derivatives by injection on a non polar column retention times are given in methylene units (MU). These MU retention times are not absolute, but can vary depending on the condition of the GC column [16]. The MU values in Table II were however calculated from a GC-MS run in which a series of straight chain hydrocarbons was added as markers to the derivatized propolis balsam sample.

Results

Headspace volatiles

The total ion chromatogram (TIC) of headspace volatiles is shown in Fig. 1 and the 29 components separated are listed in Table I. Most of the compounds have been previously identified in headspace volatiles from propolis [17] although some, including 2-methylbutyl acetate[2]*, isobutyl isobutyrate[4], 3-methyl-3-buten-1-ol[7] and prenyl acetate[8] (Table I) have not been previously identified from propolis.

The last peak occurring in the headspace volatiles (*i.e.* the least volatile component), benzoic acid[29], is the first compound to elute (as its trimethylsilyl derivative) when entire propolis balsam is analyzed (Table II).

* Superscripts refer throughout to peak numbers in Fig. 1 and 3 and Table I and II.

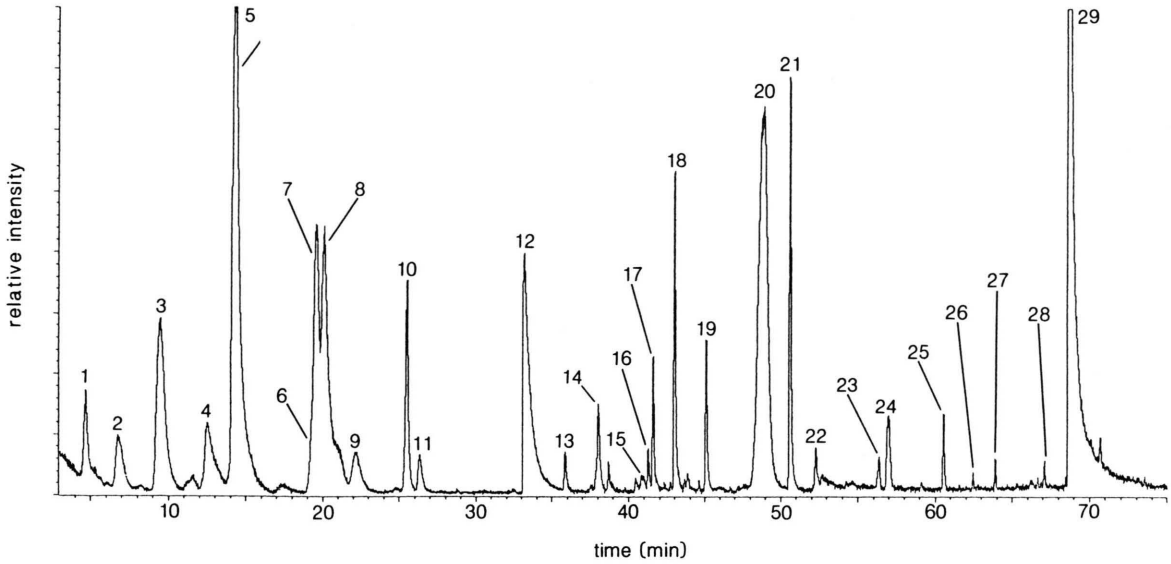

Fig. 1. Total ion chromatogram of headspace volatiles of propolis. Identifications of numbered peaks are given in Table I.

Table I. Headspace volatiles of propolis trapped on Tenax. Peak numbers correspond to those given in the chromatogram shown in Fig. 1.

Peak No.	Compound	Retention time [min]	% TIC[1]
1	2-Methylpropyl acetate (isobutyl acetate)	4.7	1.2
2	2-Methylbutyl acetate[2]	6.8	1.0
3	3-Methylbutyl acetate (isopentyl acetate)	9.5	3.0
4	Isobutyl isobutyrate[2]	12.5	1.1
5	3-Methyl-3-butenyl acetate	14.2	14.7
6	Phenylethylene (styrene)	19.1	0.7
7	3-Methyl-3-buten-1-ol[2]	19.5	4.7
8	3-Methyl-2-butenyl acetate (prenyl acetate)[2]	20.0	5.4
9	3-Methylbutyl butanoate	22.2	0.7
10	3-Methyl-2-buten-1-ol	25.5	3.0
11	6-Methylhept-5-en-2-one	26.3	0.5
12	Acetic acid	33.1	6.1
13	Benzaldehyde	35.8	0.4
14	Linalyl acetate[2]	38.0	1.2
15	Methyl benzoate	40.8	0.1
16	Butanoic acid	41.2	0.4
17	Acetophenone	41.5	1.3
18	2-Methylbutanoic acid	42.9	3.2
19	Benzyl acetate	45.0	1.4
20	Ester of 2-methylpropanoic acid[3]	48.8	17.3
21	Benzyl alcohol	50.6	4.0
22	Methylpentanoic acid	52.2	0.3
23	Geranyl or nerolidyl ester	56.3	0.1
24	Octanoic acid	56.9	0.5
25	Unidentified acid	60.5	0.3
26	Probably elemicin	62.4	0.1
27	Unidentified acid	63.9	0.2
28	Unidentified acid	67.0	0.1
29	Benzoic acid	68.6	21.7

[1] The ion current generated depends on the characteristics of the compound concerned and is not a true quantitation (see 9).

[2] We are not aware of a previous identification of this compound in propolis.

[3] Library searches indicate this to be the 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester of 2-methylpropanoic acid. We are not aware of a common name for this compound nor can we obtain a sample to use as a standard.

Table II. Composition of propolis assessed by GC-MS of trimethylsilyl derivatives. Peak numbers correspond to those given in the chromatogram shown in Fig. 3. GC retention times given 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.[2]	Compound[3]	TMS groups	MU	% TIC[4]
29	Benzoic acid	1	12.31	3.4
30	Phosphate	3	12.86	0.1
31	1,2,3-Propanetriol (glycerol)	3	12.95	0.4
32	Butanedioic acid (succinic acid)	2	13.09	0.2
33	Monoacetyl glycerol[5]	2	13.22	<0.1
34	4-Hydroxybenzaldehyde	1	13.43	<0.1
35	2,3-Dihydroxypropanoic acid (glyceric acid)[5]	3	13.46	<0.1
36	*trans*-1,4-Butenedioic acid (fumaric acid)	2	13.50	<0.1
37	Nonanoic acid (pelargonic acid)[5]	1	13.56	<0.1
38	3-Phenylpropanoic acid (hydrocinnamic acid)	1	13.89	0.3
39	1,4-Benzenediol (hydroquinone)	2	13.95	<0.1
40	*cis*-3-Phenyl-2-propenoic acid[6] (*cis*-cinnamic acid)	1	13.96	<0.1
41	*trans*-3-Phenyl-2-propen-1-ol (*trans*-cinnamyl alcohol)	1	14.00	<0.1
42	4-Hydroxyacetophenone (*p*-acetophenol)	1	14.38	0.9
43	3-Methoxy-4-hydroxybenzaldehyde (vanillin)	1	14.92	<0.1
44	2-Hydroxybutanedioic acid (malic acid)[5]	3	15.02	3.6
45	*trans*-3-Phenyl-2-propenoic acid[6] (*trans*-cinnamic acid)	1	15.13	1.5
46	N-Carboxypyrrolidine-2-carboxylic acid[7] (N-carboxypyroglutamic acid)	2	15.60	<0.1
47	Unidentified, MI m/z = 279	–	15.82	<0.1
48	2,3,4-Trihydroxybutanoic acid (threonic acid)[5]	4	15.87	<0.1
49	3,4-Dihydroxybenzaldehyde (protocatechualdehyde)	2	15.91	0.2
50	Sesquiterpene alcohol[8]	1	16.02	<0.1
51	4-Hydroxybenzoic acid	2	16.12	<0.1
52	3(4-Methoxyphenyl)-propanoic acid[5] (methoxyhydrocinnamic acid)	2	16.20	<0.1
53	Dodecanoic acid (lauric acid)[5]	1	16.44	<0.1
54	*cis*-3(4-Methoxyphenyl)-2-propenoic acid[6] (*cis*-4-methoxycinnamic acid)	1	16.51	<0.1
55	Sesquiterpene alcohol (guaiol?)	1	16.77	0.2
56	*trans*-nerolidol	1	16.80	0.4
57	Sesquiterpene alcohol (isomer of 55)	1	16.98	<0.1
58	Sesquiterpene alcohol	1	17.10	<0.1
59	Sesquiterpene alcohol	1	17.30	<0.1
60	Sesquiterpene alcohol	1	17.36	<0.1
61	Bisabolol	1	17.46	1.8
62	3(4-Hydroxyphenyl)-propanoic acid[5] (hydrocoumaric acid)	2	17.47	0.2
63	Unidentified	–	17.54	0.1
64	Sesquiterpene alcohol	1	17.63	0.1
65	*cis*-3(4-Hydroxyphenyl)-2-propenoic acid[6] (*cis*-4-coumaric acid)	2	17.73	0.1
66	Unidentified	–	17.83	0.2
67	*trans*-3(4-Hydroxyphenyl)-2-propen-1-ol[5] (*trans*-coumaryl alcohol)	2	17.87	<0.1
68	*trans*-3(4-Methoxyphenyl)-2-propenoic acid[6] (*trans*-4-methoxycinnamic acid)	1	17.92	0.5
69	5-Phenylpenta-2,4-dienoic acid (cinnamylideneacetic acid)	1	18.04	0.3
70	Unidentified	–	18.13	0.2
71	Sesquiterpene alcohol	1	18.13	0.2

Table II. Continued.

Peak No.[2]	Compound[3]	TMS groups	MU	% TIC[4]
72	3,4-Dihydroxybenzoic acid (protocatechuic acid)	3	18.25	<0.1
73	Tetradecanoic acid (myristic acid)	1	18.48	<0.1
74	2-Hydroxy-1,2,3-propanetricarboxylic acid[5] (citric acid)	4	18.50	0.2
75	Fructofuranose (isomer 1) (fructose)	4	18.63	2.0
76	Fructofuranose (isomer 2) (fructose)	4	18.71	1.7
77	Glucofuranose (glucose)	5	19.04	<0.1
78	Sesquiterpene alcohol	1	19.12	0.2
79	*trans*-3(4-Hydroxyphenyl)-2-propenoic acid[6] (*trans*-4-coumaric acid)	2	19.32	2.8
80	Benzyl 2-methoxybenzoate[5]	0	19.35	0.1
81	α-D-Glucopyranose (glucose)	5	19.44	4.8
82	Unidentified sugar alcohol	–	19.80	3.7
83	*trans*-3(3,4-Dimethoxyphenyl)-2-propenoic acid (*trans*-3,4-dimethoxycinnamic acid)	1	19.90	0.9
84	Sorbitol	6	19.98	0.1
85	Hexadecenoic acid	1	20.11	<0.1
86	3-Methyl-2-butenyl-*cis*-4-coumarate[6] (prenyl *cis*-4-coumarate)	1	20.11	<0.1
87	Sesquiterpene alcohol	1	20.27	1.0
88	Glucopyranose (glucose)	5	20.48	6.5
89	Hexadecanoic acid (palmitic acid)	1	20.50	2.8
90	*trans*-3(3-Hydroxy-4-methoxyphenyl)-2-propenoic acid (*trans*-isoferulic acid)	2	20.68	2.3
91	*trans*-3(3-Methoxy-4-hydroxyphenyl)-2-propenoic acid (*trans*-ferulic acid)	2	20.78	<0.1
92	4-Hydroxybenzyl benzoate[5]	1	20.96	<0.1
93	3-Methyl-3-butenyl *trans*-4-coumarate	1	21.28	0.6
94	Sesquiterpene alcohol	1	21.34	2.0
95	*trans*-3(3,4-Dihydroxyphenyl)-2-propenoic acid (*trans*-caffeic acid)	3	21.46	1.0
96	Unidentified sugar derivative	–	21.49	0.6
97	Myo-inositol[e]	6	21.51	<0.1
98	2-Methyl-2-butenyl *trans*-4-coumarate[5]	1	21.69	1.0
99	3-Methyl-2-butenyl *trans*-4-coumarate[6] (prenyl *trans*-coumarate)	1	21.76	0.8
100	Octadecadienoic acid (probably linoleic acid)	1	22.00	0.7
101	Octadecenoic acid (probably oleic acid)	1	22.10	1.2
102	Unidentified	–	22.19	2.9
103	Octadecanoic acid (stearic acid)	1	22.41	0.3
104	3-Methyl-3-butenyl *trans*-isoferulate	1	22.63	<0.1
105	Benzyl *cis*-4-coumarate[6]	1	22.68	<0.1
106	3-Methyl-3-butenyl *trans*-ferulate	1	22.78	0.4
107	Unidentified	–	23.03	0.1
108	14-Hydroxyhexadecanoic acid[5] (14-hydroxypalmitic acid)	2	23.13	<0.1
109	15-Hydroxyhexadecanoic acid[5] (15-hydroxypalmitic acid)	2	23.29	0.4
110	3-Methyl-2-butenyl *trans*-ferulate (prenyl *trans*-ferulate)	1	23.30	0.6
111	3-Methyl-3-butenyl *trans*-caffeate	2	23.47	3.0
112	*trans*-Cinnamyl *trans*-cinnamate[5]	0	23.50	0.7
113	2′,6′-Dihydroxy-4′-methoxydihydrochalcone	2	23.74	1.6
114	2-Methyl-2-butenyl *trans*-caffeate	2	23.79	<0.1
115	3-Methyl-2-butenyl *trans*-caffeate (prenyl caffeate)	2	23.93	2.4
116	5,7-Dihydroxyflavanone (pinocembrin)[9]	1	23.98	0.2
117	2′,4′,6′-Trihydroxydihydrochalcone	3	24.23	2.3
118	2′,6′,α-Trihydroxy-4′-methoxychalcone	3	24.38	0.1

Table II. Continued.

Peak No.[2]	Compound[3]	TMS groups	MU	% TIC[4]
119	5-Hydroxy-7-methoxyflavanone (pinostrobin)	1	24.39	0.1
120	2′,6′-Dihydroxy-4′-methoxychalcone (pinostrobin chalcone)	2	24.49	0.4
121	Benzyl *trans*-4-coumarate[6]	1	24.63	1.1
122	5,7-Dihydroxyflavanone (pinocembrin)[9]	2	24.97	4.1
123	2′,4′,6′-Trihydroxychalcone (pinocembrin chalcone)	3	24.99	4.1
124	Pentacosane	0	25.00	1.1
125	Unidentified	–	25.04	<0.1
126	17-Hydroxyoctadecanoic acid[5] (17-hydroxystearic acid)	2	25.15	<0.1
127	2′,4′-Dihydroxy-6′-methoxychalcone (alpinetin chalcone)	2	25.26	<0.1
128	Pinobanksin methyl ether	2	25.27	<0.1
129	Phenylethyl *trans*-4-coumarate	1	25.42	0.1
130	Unidentified	–	25.56	0.1
131	Salicin[e]	–	25.74	0.1
132	3,5,7-Trihydroxyflavanone (pinobanksin)	3	25.78	2.0
133	5,7-Dihydroxy-3-acetyloxyflavanone[9] (pinobanksin-3-acetate)	1	25.81	<0.1
134	Coniferyl benzoate	1	25.83	0.6
135	Benzyl *trans*-isoferulate	1	26.05	<0.1
136	5,7-Dihydroxyflavone (chrysin)[9]	1	26.07	0.4
137	2′,6′-Dihydroxy-4′,4-dimethoxydihydrochalcone[5]	2	26.11	0.2
138	3,7-Dihydroxy-5-methoxyflavanone (pinobanksin-5-methyl ether)	2	26.27	0.5
139	Benzyl *trans*-ferulate	1	26.29	0.2
140	Docosanoic acid (behenic acid)	1	26.42	0.1
141	5,7-Dihydroxy-3-acetyloxyflavanone[9] (pinobanksin-3-acetate)	2	26.45	3.1
142	2′,4′,6′-Trihydroxy-4-methoxydihydrochalcone[5]	3	26.61	1.3
143	Benzyl *trans*-caffeate	2	26.98	2.7
144	3,5,7-Trihydroxyflavone (galangin)[9]	2	26.99	1.8
145	2′,6′,4-Trihydroxy-4′-methoxydihydrochalcone[5]	3	27.10	0.6
146	5,7-Dihydroxyflavone (chrysin)[9]	2	27.11	1.8
147	5,7-Dihydroxy-3-propanoyloxyflavanone (pinobanksin-3-propanoate)	2	27.16	0.1
148	5,7-Dihydroxy-3-methoxyflavone (galangin-3-methyl ether)	2	27.16	0.2
149	Sucrose	8	27.42	0.1
150	3,5,7-Trihydroxyflavone (galangin)[9]	3	27.52	5.2
151	1-Tetracosanol	1	27.58	0.1
152	5,7-Dihydroxy-4′-methoxyflavanone (isosakuranetin)	2	27.59	0.1
153	1-Phenylethyl *trans*-caffeate	2	27.70	1.3
154	2′,4′,6′-Trihydroxy-4-methoxychalcone[5] (isosakuranetin chalcone)	3	27.71	0.1
155	Cinnamyl *trans*-4-coumarate	1	27.82	0.3
156	5,7-Dihydroxy-3-(*iso*)butanoyloxyflavanone[10] (pinobanksin-3-(*iso*)butanoate)	2	27.90	<0.1
157	5,7-Dihydroxy-3-(*iso*)pentanoyloxyflavanone[10] (pinobanksin-3-(*iso*)pentanoate)	2	28.32	<0.1
158	Tetracosanoic acid (lignoceric acid)	1	28.37	0.8
159	5,7,4′-Trihydroxyflavanone (naringenin)	3	28.50	<0.1
160	2′,4′,6′,4-Tetrahydroxychalcone (naringenin chalcone)	4	28.61	<0.1
161	Diprenyl (geranyl) *trans*-caffeate[5]	2	28.62	<0.1
162	5,7-Dihydroxy-3-(*iso*)pentenoyloxyflavanone[10] (pinobanksin-3-(*iso*)pentenoate)	2	28.95	<0.1

Table II. Continued.

Peak No.[2]	Compound[3]	TMS groups	MU	% TIC[4]
163	Cinnamyl *trans*-isoferulate	1	29.17	<0.1
164	Cinnamyl *trans*-caffeate	2	29.94	0.1
165	Kaempferol methyl ether (5-methyl ether?)	3	30.10	<0.1
166	Hexacosanoic acid (cerotic acid)	1	30.33	<0.1
167	5,7,4′-Trihydroxyflavone (apigenin)	2	30.39	<0.1
168	3,5,7,4′-Tetrahydroxyflavone (kaempferol)[9]	3	30.51	<0.1
169	3,5,4′-Trihydroxy-7-methoxyflavone (kaempferol-7-methyl ether)	3	30.61	<0.1
170	3,5,7,4′-Tetrahydroxyflavone (kaempferol)[9]	4	30.95	0.3
171	5,7,4′-Trihydroxy-3-methoxyflavone (kaempferol-3-methyl ether)	3	31.00	<0.1
172	Kaempferol methyl ether (4′-methyl ether?)	3	31.25	<0.1
173	Quercetin methyl ether[9,11]	3	31.49	<0.1
174	Quercetin methyl ether[9,11]	3	31.73	<0.1
175	3,5,7,3′,4′-Pentahydroxyflavone (quercetin)[9]	4	31.90	<0.1
176	Quercetin methyl ether[9,11]	4	31.91	<0.1
177	Quercetin methyl ether[9,11]	3	32.00	<0.1
178	Quercetin methyl ether (7-methyl ether)[9,11] (rhamnetin)	4	32.04	<0.1
179	Quercetin methyl ether (4′-methyl ether?)[9,11]	4	32.14	<0.1
180	3,5,7,3′,4′-Pentahydroxyflavone (quercetin)[9]	5	32.19	<0.1
181	Quercetin methyl ether[9,11]	4	32.51	<0.1

[1] Methylene units (MU) are defined by Dalgliesh *et al.* [27].
[2] Peak numbers are continuous with those given in Table I.
[3] The name given does not include the TMS substituents.
[4] The ion current generated depends on the characteristics of the compound concerned and is not a true quantitation (see 9).
[5] We are not aware of a previous identification of this compound in propolis.
[6] Both *cis* and *trans* isomers of this compound are present.
[7] This compound derives from pyroglutamic acid carboxylated during derivatization with BSTFA [28]. The pyroglutamic acid probably originates from glutamic acid.
[8] The sesquiterpene alcohols are particularly difficult to identify positively, both because their mass spectra are very similar and because pure standards are difficult to obtain.
[9] This compound is present as two TMS derivatives.
[10] We do not know whether the substituent at the 3 position is linear or branched.
[11] These compounds are the different methyl ethers of quercetin which are present as both the tris- and tetra-TMS derivatives.

Entire propolis balsam

The entire propolis balsam was derivatized with trimethylsilyl (TMS) reagent to render its components sufficiently volatile for GC-MS analysis. Except for benzoic acid[29] the volatile headspace components described above are obscured in this analysis by the TMS solvent front.

The total ion chromatogram is shown in Fig. 2, and this chromatogram is expanded and the components identified in Fig. 3 and Table II. We see 153 peaks of which we identify 142, representing 125 different compounds (some compounds occur as two or more TMS derivatives). There will be a number of additional compounds present in low amounts which are obscured by other, more concentrated, compounds and which we therefore failed to recognize.

Of the compounds identified 23 are identified for the first time in propolis (Table II). The majority of these are known components either of poplar bud exudate (malic acid[44] [18], threonic acid[48] [18], coumaryl alcohol[67] [10], citric acid[74] [18], benzyl-2-methoxybenzoate[80] [10], 4-hydroxybenzyl benzoate[92] [10], cinnamyl cinnamate[112] [10, 16], salicin[131] [16], 2′,6′-dihydroxy-4′,4-dimethoxy-dihydrochalcone[137] [10, 11], 2′,4′,6′-trihydroxy-

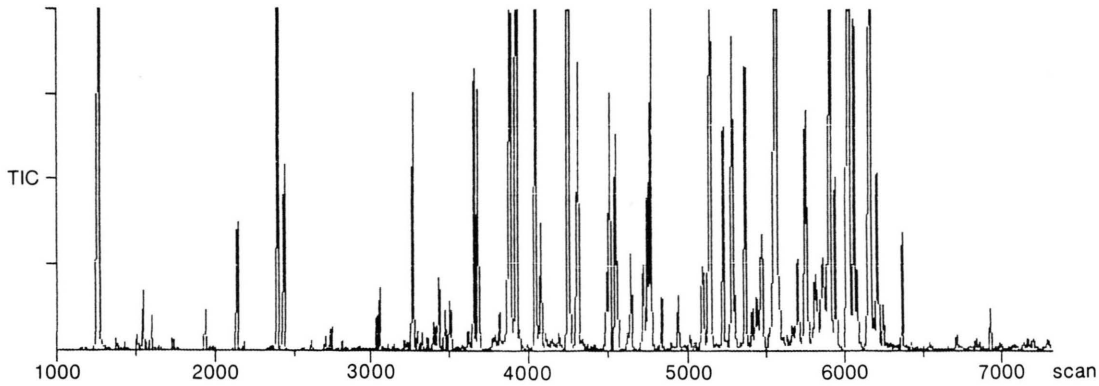

Fig. 2. Total ion chromatogram of entire propolis derivatized with trimethylsilyl reagent, scans 1000–7300. The chromatogram is shown expanded in Fig. 3.

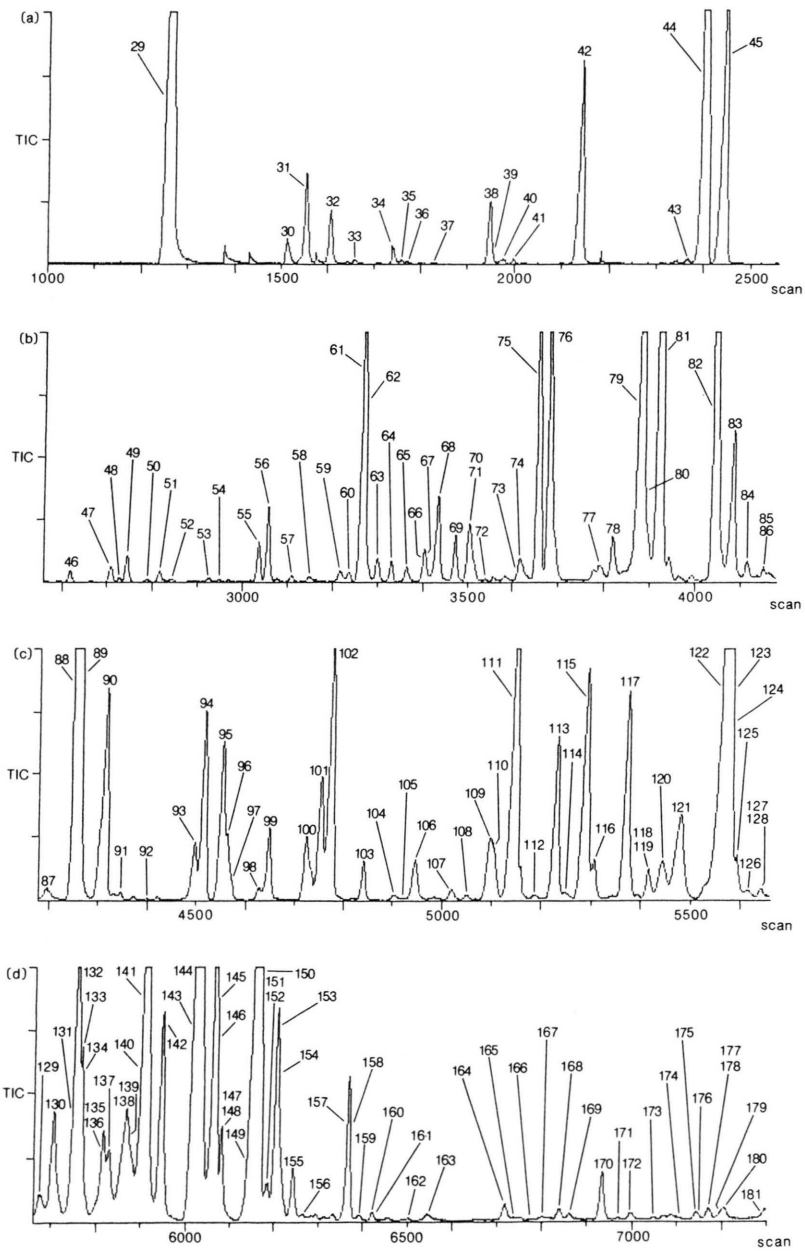

Fig. 3. Expanded total ion chromatogram of entire propolis derivatized with trimethylsilyl reagent. (a) Scans 1000–2560 (MU 11 to 15.5); (b) scans 2560–4180 (MU 15.5 to 20.2); (c) scans 4180–5660 (MU 20.2 to 25.3); (d) scans 5560–7300 (MU 25.3 to 32.5). Identifications of numbered peaks are given in Table II.

4-methoxydihydrochalcone[142] [10, 11], 2′,6′,4-trihydroxy-4′-methoxydihydrochalcone[145] [10, 11], and geranyl *trans*-caffeate[161] [19] or of beeswax (14-hydroxypalmitic acid[108] [20], 15-hydroxypalmitic acid[109] [20] and 17-hydroxystearic acid[126] [20]), although the organic acids could equally be the product of bee metabolism.

We have already demonstrated that many lower molecular weight flavonoids can be successfully located from the mass spectrometry data by single ion reconstructions (SIR) of their characteristic $[M-15]^+$ ions [16]. The higher molecular weight tetrahydroxy-[168,170] and pentahydroxyflavones[175,180] and their methyl ethers[171–174,176–179,181] can similarly be located by SIR of their $[M-15]^+$ ions (Fig. 4) although they are only present as minor peaks. In most cases the mass spectra of such small peaks are not sufficiently detailed in themselves to accurately identify the compounds present, and these must then be identified by their GC retention times. We do not currently have retention time data for the full range of methyl ethers of kaempferol and quercetin and can only therefore positively identify some of these compounds (Table II). These higher molecular weight flavones do not transmit well through a GC column and their percentage occurrence may be seriously underestimated by GC-MS analysis [9].

Origin of propolis balsam

Bud exudate of Section Aigeiros poplars has a characteristic composition, which is different from that of Section Tacamahaca poplars [10–14, 21]. Of the compounds identified in this propolis sample some, such as pinobanksin[132] and pinobanksin esters[137,141,147,156,157,162] are characteristic of bud exudate of Section Aigeiros poplars, whereas others, such as the dihydrochalcones[113,117,137,142,145] and sesquiterpenols[56–61] are characteristic of bud exudate of Section Tacamahaca poplars. The very complex chromatogram obtained from the propolis sample analyzed here is typical of the intersectional hybrids between Section Aigeiros and Section Tacamahaca poplars.

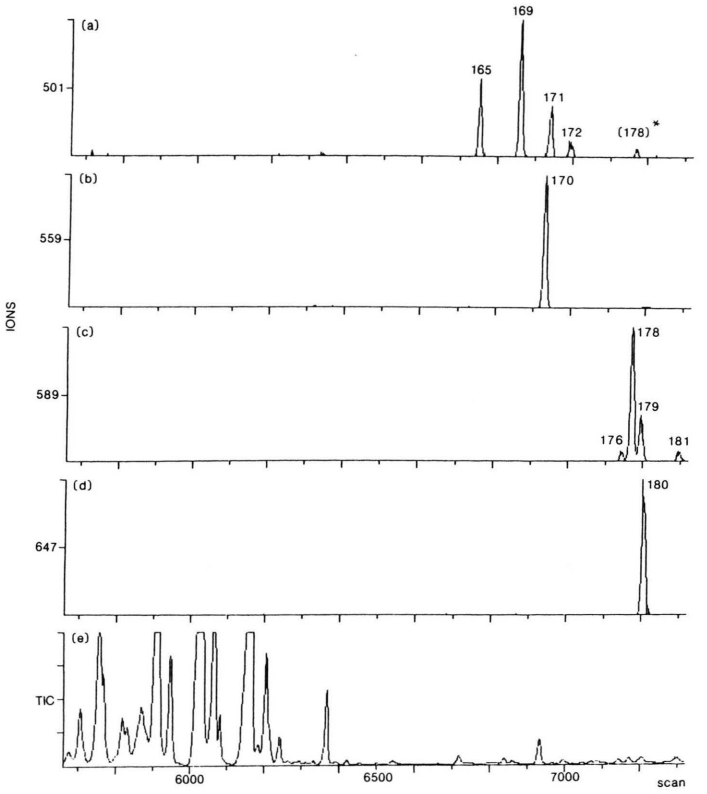

Fig. 4. Single ion reconstructions of $[M-15]^+$ ions locating specific groups of high molecular weight flavones. (a) m/z = 501, locating tris-TMS trihydroxymonomethoxyflavones[165,169,171,172] (kaempferol methyl ethers); (b) m/z = 559, locating a tetra-TMS tetrahydroxyflavone[170] (kaempferol); (c) m/z = 589, locating tetra-TMS tetrahydroxymonomethoxyflavones[176,178,179,181] (quercetin methyl ethers); (d) m/z = 647, locating a penta-TMS pentahydroxyflavone[180] (quercetin); (e) total ion current of scans 5560–7300 (MU 25.3 to 32.5). Numbered peaks are located in Fig. 3 and identifications given in Table II. The lower TMS derivatives of these compounds can be similarly located, when present, by single ion searches of the appropriate $[M-15]^+$ ions: for kaempferol tris-TMS[168], m/z = 487; for quercetin methyl ethers tris-TMS[173,174,177], m/z = 517 and for quercetin tetra-TMS[175], m/z = 575.

* Rhamnetin[178] (3,5,3′,4′-tetrahydroxy-7-methoxyflavone tetra-TMS) has, as a minor ion, in its mass spectrum m/z = 501, and is therefore also located in this single ion reconstruction.

Discussion

In 1979, in a major review of propolis and its antimicrobial properties [5], Ghisalberti concluded "Many of the claims made about the pharmacological activity of propolis have not been well confirmed, but it nevertheless seems likely that propolis will be used increasingly for commercial purposes. It is abundantly clear that it will have to be studied much more before it can be properly considered for therapeutic purposes; because its constitution is largely unknown, propolis should not be recommended in medicines". Whereas Ghisalberti's prediction concerning the increased use of propolis for commercial purposes has been correct, and more is now known of the allergenic properties of propolis [24–26], there have been few attempts to study the variability of propolis or to define its constituents in detail.

The primary source of the plant exudate incorporated into propolis in the Northern Hemisphere is bud exudate of poplar trees [1, 7, 8] and the composition of propolis is therefore directly related to the composition of the poplar bud exudate collected by the bees. Each species or clone of poplar has its own characteristic mixture of compounds in its bud exudate [22, 23] and there can be considerable differences in bud exudate composition between different poplar species [10–14]. Propolis is potentially, therefore, a very variable product, both in its content of compounds with desirable antimicrobial activity, and in its content of undesirable allergens, such as prenyl caffeate[115] [1].

Propolis from the Northern Hemisphere has previously incorporated bud exudates collected primarily from *P. nigra* and its widespread hybrids with *P. deltoides (i.e. P. × euramericana)*, and has contained compounds typical of these trees [1, 7, 8]. However the propolis analyzed here also contains, in quantity, additional compounds (such as dihydrochalcones) which occur in bud exudate of Section Tacamahaca poplars. It probably originates from one of the recently introduced intersectional hybrids, such as the *P. × interamericana* Van Brockhuizen (*P. deltoides* × *P. trichocarpa* Torr. and Gray) clones.

The selection of poplars for forestry and aboricultural uses will inevitably alter in response to the increasing introduction of new and improved clones of intersectional hybrid poplars. Many such improved clones are now becoming available and each will have a distinctive, and different, bud exudate composition [23]. Propolis manufactured by bees from bud exudate of these new clones will reflect these differences in composition. The use of such a potentially variable and complex product in commercial pharmaceutical preparations without establishing the composition of the particular propolis used is perhaps questionable.

[1] W. Greenaway, T. Scaysbrook, and F. R. Whatley, Bee World **71,** 107–118 (1990).
[2] Pliny the Elder (AD 23–79). Natural History II, **Vol. 18,** p. 6, original text and translation: see Loeb Classical Library, 1940 (reprint 1947), **Vol. 3** (transl. H. Rackham). London: William Heinemann Ltd; Cambridge, Massachusetts: Harvard University Press.
[3] R. Hill, Propolis the natural antibiotic, Thorsons Publishers, Wellingborough 1977.
[4] A. P. Walker, International Bee Research Association Bibliography No. 16, IBRA, Cardiff 1976.
[5] E. L. Ghisalberti, Bee World **60,** 59–84 (1979).
[6] E. M. Schneidewind, A. Büge, H. Kala, T. Metzner, and A. Zscunke, Pharmazie **34,** 103–106 (1979).
[7] V. Papay, L. Toth, M. Soltesz, E. Nagy, and G. Litkei, Flavonoids and Bioflavonoids 1985. Proc. 7th Hungarian Bioflavonoid Symposium, Szeged, Hungary. Studies in Organic Chemistry **23** (L. Farkas *et al.*, ed.), 233–240, Elsevier, Amsterdam 1986.
[8] V. S. Bankova, S. S. Popov, and N. L. Marekov, Phytochemistry **28,** 871–873 (1989).
[9] W. Greenaway, T. Scaysbrook, and F. R. Whatley, Proc. R. Soc. Lond. B **232,** 249–272 (1987).
[10] S. English, W. Greenaway, and F. R. Whatley, Phytochemistry **90,** 521–533 (1991).
[11] W. Greenaway, J. May, and F. R. Whatley, J. Chromatography **472,** 393–400 (1989).
[12] W. Greenaway, S. English, and F. R. Whatley, Z. Naturforsch. **45c,** 587–593 (1990).
[13] W. Greenaway, C. G. Davidson, T. Scaysbrook, J. May, and F. R. Whatley, Z. Naturforsch. **45c,** 594–598 (1990).
[14] W. Greenaway, S. English, and F. R. Whatley, Z. Naturforsch. **45c,** 931–936 (1990).
[15] W. Greenaway, T. Scaysbrook, and F. R. Whatley, Z. Naturforsch. **43c,** 301–305 (1988).
[16] W. Greenaway and F. R. Whatley, J. Chromatography **519,** 145–158 (1990).
[17] W. Greenaway, T. Scaysbrook, and F. R. Whatley, Flavour Frag. J. **4,** 173–175 (1989).
[18] W. Greenaway, T. Scaysbrook, and F. R. Whatley, Phytochemistry **27,** 3513–3515 (1988).
[19] W. Greenaway, E. Wollenweber, T. Scaysbrook, and F. R. Whatley, Z. Naturforsch. **43c,** 795–798 (1988).
[20] A. P. Tulloch, Chem. Phys. Lipids **6,** 235–265 (1971).
[21] W. Greenaway, S. English, and F. R. Whatley, Biochem. Syst. Ecol. **18,** 439–445 (1990).
[22] E. Wollenweber, Biochem. Syst. Ecol. **3,** 35–45 (1975).
[23] W. Greenaway, J. Jobling, and T. Scaysbrook, Silvae Genetica **38,** 28–32 (1989).
[24] B. M. Hausen, E. Wollenweber, H. Senff, and B. Post, Contact Dermatitis **17,** 163–170 (1987).
[25] B. M. Hausen, E. Wollenweber, H. Senff, and B. Post, Contact Dermatitis **17,** 171–177 (1987).
[26] B. M. Hausen and E. Wollenweber, Contact Dermatitis **19,** 296–303 (1988).
[27] C. E. Dalgliesh, E. C. Horning, M. G. Horning, K. L. Knox, and K. Yarger, Biochem. J. **101,** 792–810 (1966).
[28] W. Greenaway and F. R. Whatley, J. Chromatography **409,** 383–389 (1987).