Comparison of Organic Extractives Found in Leatherwood (*Eucryphia lucida*) Honey and Leatherwood Flowers and Leaves

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Hotrienol (3,7-dimethyl-1,5,7-octatrien-3-ol, **II**) is the principal aroma component detected by headspace analysis in leatherwood (*Eucryphia lucida*) honey. It arises from the dehydration of 2,6-dimethyl-3,7-octadiene-2,6-diol (**I**), the principal terpene in methylated and unmethylated ether extracts of leatherwood honey. The diol **I** was detected in leatherwood plant extracts. Methylated ether extracts of the honey also contained methyl 2-hydroxy-2-(4-methoxyphenyl)acetate (**III**), which has not been previously reported in honey, in addition to many other aromatic substances. Compound **III** was a major component of the acidic fraction of the plant extract. A methylated extract of unripe leatherwood honey collected after 1 week in the hive was found to contain methyl 2-hydroxy-3-phenylpropionate (**IV**) and an unidentified compound [m/z 91 (100), 180] as the principal components. The unidentified compound found in immature honey was not detected in the plant extracts.

Keywords: *Eucryphia lucida; leatherwood; honey; flavor constituents; volatile components*

INTRODUCTION

Honeys produced from different floral sources may often have distinctly different aromas and tastes. It has been proposed that the volatile compounds present in honey arise from the plant nectar or via some modification of plant constituents by the bee (Bonaga and Giumanni, 1986). The usual practice has been to identify the floral source of honey by pollen analysis; however, a chemical approach to the characterization of the floral sources of honey might prove to be more accurate and more readily available (Tan *et al.*, 1989b). The use of flavonoid analysis in the identification of honeys has been suggested (Amiot *et al.*, 1989), and this technique has been used as a tool for studying the geographical origins of honey (Ferreres *et al.*, 1991).

There is mounting evidence that the extractives of some unifloral honeys are chemically different from one another (Tan *et al.*, 1988, 1989a,b; Wilkins *et al.*, 1993). For example, orange flower honey is known to contain methyl anthranilate which is not found in other honeys. Linden honey contains 8-*p*-menthene-1,2-diol as the major volatile component (Tsuyena *et al.*, 1974), and 3-aminoacetophenone has been reported as the principal volatile component of chestnut honey (Bonaga and Giumanini, 1986). Graddon *et al.* (1979) investigated some Australian unifloral honeys and found a range of hydrocarbons and other compounds, some of which were suggested to be unique to the floral source. Degraded carotenoid type compounds have been isolated from some honeys and may be specific to different types of honey (Ede *et al.*, 1993; Tan *et al.*, 1989a; Broom *et al.*, 1992). Linalool derivatives occur in nodding thistle honey (Wilkins *et al.*, 1993). Steeg and Montag (1987, 1988a,b) have reported a wide range of aromatic acids in unifloral honeys. Manuka and kanuka honeys were found to contain higher amounts of aromatic acids than clover honey which has a low level of extractable components (Tan *et al.*, 1988). More recently, Bouseta *et al.* (1992) have studied 14 types of unifloral honeys from 10 countries by dynamic headspace GC/MS with a view to characterizing and differentiating the honey aromas.

Little work has been done to correlate the chemical constituents of the plant to those of the corresponding honey. Tan *et al.* (1989b) suggest that most of the compounds on which assessment of floral origin is based seem to originate from the nectar. Some of the chemical components of nectar have been investigated by Cremer and Riedmann (1965, 1964). HPLC profiles of nectar have been used as an aid in plant breeding, but the nectar was not chemically characterized (Erickson *et al.*, 1979). In this study, qualitative and quantitative differences were shown to exist between nectars secreted from freshly opened flowers and those from flowers a few days old. It is known that some plant toxins are transferred to honey such as atropine from *Datura stramonium* and scopolomine from Egyptian henbane (*Datura metel*) (White, 1981). Pyrrolizidine alkaloids found in *Echium plantagineum* (Paterson's curse) have been detected in samples of echium honey at levels of 0.27–0.95 ppm (Culvenor *et al.*, 1981), and alkaloids are present in honey produced from ragwort (*Senecio jacobaea*) (Deinzer *et al.*, 1977). Tan *et al.* (1988) extracted whole manuka flowers but did not detect any compounds that could be related to those found in the honey extract. Yet the odorant compounds found in linden (*Tilia cordata*) honey, *cis*-rose oxide and 3,9-epoxy-1,4(8)-*p*-menthadiene, have been detected by GC/MS in extracts of linden blossoms (Blank *et al.*, 1989) and presumably are transferred to the honey unchanged by the bee.

Honey is prepared by the worker bees from nectar and pollen collected from flowers. The nectar is ingested in a honey stomach where the enzymatic conversion of sucrose into fructose and glucose commences. This unripe honey is then deposited into the cells of the hive

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where it is further modified by the reduction of the water content. During processing, the honey may be heated to temperatures of about 55–60 °C (Ayton, 1991; Hunt, 1991). Flavor components in the honey may arise from (a) the direct transferral of plant constituents, (b) the conversion of plant constituents by the bee, (c) the production of compounds by the bee, (d) the transformation of compounds present in unripe honey, or (e) postharvest processing.

Leatherwood honey is renowned in Australia for its pungent flavor. It is produced from the flowers of *Eucryphia lucida* (Eucryphiaceae) a temperate rainforest species endemic to the island of Tasmania (Curtis and Morris, 1975). The flowers have reliable and copious nectar flows ensuring its importance as a nectar source. An aromatic resin which is reputed to have antiseptic qualities covers the leaves and buds (Lassak and McCarthy, 1983). The distribution of another Tasmanian species, *Eucryphia milliganii*, overlaps with *E. lucida*, but it is more restricted and replaces *E. lucida* in exposed positions at elevations above 800 m. *E. milliganii* has smaller leaves and flowers than *E. lucida* and a later flowering season (Curtis and Morris, 1975). It has also been used as a nectar source in the production of leatherwood honey.

Leatherwood honey is of economic importance to Tasmania. Of a total annual honey production of about 1000 tons, between 69% and 74% of the honey produced comes from leatherwood nectar (Gifford, 1990). Almost one-half of the leatherwood honey produced in Tasmania is exported. A Chilean species of *Eucryphia*, *Eucryphia cordifolia,* also produces a honey with a distinctive flavor that is much sought after (Zeigler, 1993). Levels of benzoic acid and phenylacetic acid in Tasmanian leatherwood honey and other varieties of honey have been quantified by Speer and Montag (1984, 1985, 1987) as 0.9–3.0 and 2.4–10.0 mg/kg, respectively.

In this study we have identified the principal volatile flavor components of leatherwood honey and attempted to correlate them to the chemical constituents present in the extracts of the flowers, particularly those in the nectar and the stamens. A sample of unripe leatherwood honey was also studied to investigate the effect of maturation on the volatile component profile.

METHODS AND MATERIALS

Reagents. The solvents diethyl ether and ethyl acetate were analytical grade and redistilled and checked by gas chromatography before use.

Apparatus. Combined gas chromatography/mass spectrometry was performed on a Hewlett-Packard 5890/5970 GC-MSD system with helium as a carrier gas. Two columns were used for analysis: HP-1 and FFAP. Analyses of methylated samples and headspace extracts were performed on the HP-1 column (0.25 mm i.d. × 25 m, 25 μm film thickness). The oven was temperature programmed for on-column injection from 30 °C (4 min initial hold) to 200 °C at 6 °C/min and then to 250 °C at 15 °C/min (20 min final hold). The detector temperature was 270 °C, and the injector temperature was 260 °C. Splitless injections of methylated samples were also carried out on this column. The temperature program differed from the above in that it commenced at 50 °C (4 min hold). The temperature gradient of the FFAP column (0.25 mm i.d. × 30 m) was programmed as follows: 50 °C (4 min hold) and then increased at 6 °C/min to a final temperature of 190 °C (20 min hold). The injector was held at 270 °C and the detector at 220 °C.

The nectar sample was analyzed on a Kratos Concept ISQ GC-MS system equipped with a DB-1 column (J&W) (0.25 mm i.d. × 25 m, 25 μm film thickness). The oven was programmed with an initial temperature of 40 °C with a temperature rise of 6 °C/min to 250 °C. Both the injector and detector were set at 250 °C.

Source of Honey Samples. Samples of leatherwood honeys were obtained from commercial suppliers and beekeepers as follows.

Mature Honey. Sample code (supplier): A1, creamed honey (Four Roses, Launceston, Tasmania), heated to a maximum temperature of 25 °C during processing; A2 (Four Roses), heated to 72 °C prior to bottling; A3 (Golden Nectar, Mole Creek, Tasmania), heated to temperatures between 40 and 45 °C during centrifugal extraction, flash heating to a maximum temperature of 65 °C was performed prior to bottling of the honey; A4 (Picton River region, C. Klapp, Hobart, Tasmania), centrifugal extraction of the honey was performed without additional heat, warmed to a temperature not exceeding 45 °C to aid transfer.

Unripe Honey. The honey was collected 1 week after the hive had been placed on site in the South West National Park in Tasmania. The supplier was H. Hoskinson of Golden Pearl, Woodbridge, Tasmania.

Plant Material. Samples of leatherwood (*E. lucida*) flowers and leaves were collected from the Picton River area in Tasmania in the months of January and February 1994. The material was kept at −20 °C until required.

Honey Extraction. *Mature Honey: Methylated Extracts.* Honey (100 g) was placed into a flask with deionized (DI) water (150 mL) and sodium chloride (20 g) and dissolved by stirring at room temperature. The samples were then exhaustively extracted with ether. The emulsion that formed was separated by centrifugation at 2000 rpm, and then the organic layer was reduced under vacuum to 5 mL. Each sample was then methylated with diazomethane (Lombardi, 1990) and the volume made up to 10 mL. An internal standard of 143 μg of methyl undecanoate in ethanol/water (3%) was added to each sample prior to extraction.

Mature Honey: Unmethylated Extract. In a typical extraction, honey (25 g) was placed in a beaker and extracted by stirring rapidly with ethyl acetate (5 × 20 mL) at room temperature for 2 min by means of a mechanical stirrer. The combined decanted extract was dried over $MgSO_4$ and then concentrated under reduced pressure in an all-glass rotary evaporator at 30 °C. When the volume was suitably reduced (1–2 mL), the extract was quantitatively transferred (using dichloromethane) to a 2 mL vial. After the extract was further concentrated to 0.1 mL with a stream of dry nitrogen, the vial was sealed and stored at −18 °C until required.

Unripe Honey. A 1 week old sample of comb honey (180 mL) was separated from the beeswax by centrifugation at 2000 rpm. The honey (30 g) was mixed with NaCl (10 g) and DI water (100 mL). This solution was extracted with three 30 mL portions of ether. The solvent was reduced to 2 mL under vacuum at room temperature. The sample was methylated with diazomethane and made to a final volume of 5 mL.

Steam Distillation. Honey (50 g) was dissolved in 300 mL of DI water and subjected to continuous distillation for 6 h. The distillate was collected into dichloromethane and dried over $MgSO_4$. The solvent was reduced under vacuum to 3 mL. An internal standard of hexanol (81 μg) was added. Leatherwood leaves were steam distilled in a similar manner.

Plant Extraction. *Flowers.* Leatherwood flowers (220 g) were examined whole and also partitioned into (a) stamens (12 g), (b) petals (60 g), and (c) flower parts without petals (22 g). Leaves from the plant were also investigated. These samples were soaked in ether for several hours at room temperature. The solvent was decanted from the residue, dried over $MgSO_4$, and reduced to 5 mL under vacuum at room temperature. The extracts were methylated with diazomethane and made to a final volume of 5 mL. Methyl undecanoate (120 mg) was added as an internal standard.

Acids Fraction. An ether extract of whole flowers (240 g) was extracted with 5% sodium hydrogen carbonate. This aqueous layer was then acidified with HCl and extracted with ether. The organic layer was dried over $MgSO_4$ and the solvent removed using a rotary evaporator. The extract was

methylated and made to a volume of 5 mL. An internal standard of methyl undecanoate (120 μg) was added.

Nectar. Nectar (50 μL), obtained from the flowers with a capillary tube, was extracted with 3 drops of ether and the extract injected into a GC/MS, equipped with the DB-1 column. The sample was analyzed on a Kratos ISQ GC/MS system.

Headspace Analysis. *Static Headspace Analysis.* Honey (5 g), NaCl (2 g), and 5 mL of DI water were placed in a 20 mL vial and sealed with a crimp top. The vial was incubated at 50 °C for 45 min. One milliliter of the headspace was analyzed by GC/MS equipped with a FFAP column.

Purge and Trap: Honey. The honey sample (12.3 g) was mixed with 50 mL of DI water. Nitrogen was passed through the solution at 200 mL/min for 2 h at room temperature. The headspace components were trapped on an activated charcoal tube (Orbo-32 small absorption tube). The charcoal was desorbed with 1.5 mL of carbon disulfide.

Purge and Trap: Flowers. Nitrogen was passed through a flask of leatherwood flowers (170 g) at 200 mL/min at room temperature for 4 h, and the headspace vapors were trapped on an activated charcoal tube. The sample was eluted with ether (2 mL).

RESULTS

Tables 1 and 2 give data from splitless and on-column injections of methylated extracts of both mature and unripe leatherwood honeys. Initially, the methylated samples were introduced on to the GC column via splitless injection. However, it was suspected that the passage of the sample through a hot injection port led to the thermal dehydration of one of the major volatile components, 2,6-dimethyl-3,7-octadiene-2,6-diol (**I**), to the monoalcohol hotrienol (3,7-dimethyl-1,5,7-octatrien-3-ol, **II**). An authentic sample of the diol when analyzed by GC/MS using splitless injection showed hotrienol (10%) in the GC profile. Thermal degradation of the diol was not observed when on-column injections were performed on the same sample. Hence, all honey and plant samples were analyzed using on-column injection.

Honey. *Methylated Extracts.* Table 1 lists the levels of components in methylated diethyl ether extracts of four leatherwood honey samples. Over 100 peaks have been identified and quantified using methyl undecanoate as the internal standard. The components were identified on the basis of published mass spectral data and GC carbon number. All leatherwood honey samples gave very similar GC profiles.

The principal volatile components of interest in the GC/MS profile are 2,6-dimethyl-3,7-octadiene-2,6-diol (**I**) and 3,7-dimethyl-1,5,7-octatrien-3-ol (**II**), known as hotrienol. The mass spectrum and the retention time of the diol were verified by comparison with an authentic sample. The major components of the honey extracts appear to be long chain hydrocarbons and fatty acids and aromatic derivatives. The extract contained a large number of aromatic substances, of which the methyl ester of 2-hydroxy-2-(4-methoxyphenyl)acetic acid (4-methoxymandelic acid, **III**) predominated. This compound may be present in the honey as 4-hydroxymandelic acid, the phenolic group being methylated during derivatization. However, both 4-methoxymandelic acid and 4-hydroxymandelic acid may be too polar to be observed by gas chromatography. Evidence for these compounds was not found when an underivatized sample was chromatographed on the FFAP and HP-1 columns.

Unmethylated Extract. Table 3 lists the constituents of unmethylated ethyl acetate extracts of samples of leatherwood honey. The diol **I** and hotrienol (**II**) were the major volatile components. Compounds present as methyl esters in these extracts were methyl syringate and methyl furoate. Compounds detected in the unmethylated extract that were not found in the methylated extract were butane-1,3- and -2,3-diols, benzyl alcohol, phenylacetaldehyde, 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one, lilac aldehydes, lilac alcohol isomer 1, an isomer of 2,6-dimethyl-3,7-octadiene-2,6-diol (**I**), (*Z*)- and (*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol, and (*Z*)-2,6-dimethyl-6-hydroxy-2,7-octadienal.

Headspace Analysis. The headspace volatiles of leatherwood honey were purged with nitrogen and captured on an activated carbon tube. GC/MS analysis showed hotrienol to be the only compound detected. However when static headspace analysis was performed, trace amounts of dimethyl sulfide and an unidentified compound (m/z 39, 67, 82) were also observed.

One Week Old Honey. *Methylated Extract.* A sample of honey was removed from the hive 1 week after the hive was placed on site. Constituents of the methylated extract of the unripe honey are listed in Table 2. The most striking difference between the mature honey and the unripe honey was that hotrienol was not detected in the unripe honey although the diol **I** was found at a level of 21 μg/g. Another difference between the two types of leatherwood honey was the increased concentrations of the methyl ester of 2-hydroxy-3-phenylpropionic acid (**IV**) and an unidentified compound [m/z 91 (100), 180)], both of which were detected in trace amounts in the mature honey.

On the other hand, methyl 2-hydroxy-2-(4-methoxyphenyl)acetate (**III**) was present at a similar concentration in both the mature and unripe honeys. Other prominent components of the sample included the aromatic compounds methyl 3,4,5-trimethoxybenzoate and 3,4,5-trimethoxybenzyl methyl ether and the diacid derivatives dimethyl decanedioate and dimethyl 2-decenedioate. The ratio of the major hydrocarbons appeared constant as did some of the higher molecular weight neutral carbonyl compounds. Some compounds were present in the immature honey in higher concentrations than found in the mature honey such as methyl mandelate, methyl 2-phenylacetate, methyl 2-methoxybenzoate, and methyl 2-(4-methoxyphenyl)acetate.

Plant Extracts. *Flowers and Leaves.* Compounds that were found in both plant and honey extracts are given in Table 4. The diol **I** was found in all plant extracts analyzed with the exception of the leaves and appeared to be concentrated in the stamen extract. On-column injections of the whole flowers, stamens, and petal extracts showed no evidence of hotrienol. Other compounds detected in the plant extracts that occur in leatherwood honey include dimethyl butanedioate, methyl 2-phenylacetate, *p*-aminoacetophenone, and a number of long chain hydrocarbons.

As might be expected, the acid fraction of the flower extract contained several of the aromatic acids found in the honey. The major acid derivative detected in this fraction was methyl 2-(4-methoxyphenyl)acetate (**III**). Hotrienol was found in trace amounts in extracts of the steam-distilled flowers and the acid fraction of the flowers and in splitless injections of the stamens and petals.

Nectar. Splitless injections of an ether extract of leatherwood nectar were performed. At low injection port temperatures (140 °C), 2,6-dimethyl-2,7-octadiene-2,6-diol (**I**) was prominent in the GC profile and only trace amounts of hotrienol were observed. At elevated injection port temperatures (250 °C), significant amounts

Table 1. Concentration (Micrograms per Gram) of Methylated Components in Leatherwood Honey

peak	carbon no.	t_R (min)	compound	leatherwood honey samples[a]							
				splitless injection				on-column			
				A1	A2	A3	A4	A1	A2	A3	A4
1		4.59	methyl 3-furancarboxylate[c]	t	t	1.6	t	-	t	0.1	–
2		5.3	phenol[b,d]	–	t	–	–	–	–	–	–
3	100.9	6.5	dimethyl butanedioate[c,d]	0.4	1.2	1.5	0.4	t	0.9	0.1	t
4	1034	7.96	methyl furoate[b,c]	t	t	t	–	–	–	t	–
5	1052	8.8	methyl benzoate[c,d]	0.3	0.5	0.8	0.6	5.4	1.9	0.4	1.1
6	1074	9.6	isophorone[b,f]	–	–	–	0.5	–	–	t	0.6
7	1079	9.8	3,7-dimethyl-1,5,7-octatrien-3-ol (hotrienol) (**II**)[c]	2.1	3.2	6.8	1.5	4.3	4.6	2.7	0.2
8	1090	10.4	3,5,5-trimethylcyclohex-2-ene-1,4-dione[b,d,f]	1.7	2.1	4.2	1.9	t	t	0.2	t
9	1132	12.1	unknown (168, 83, 92, 69, 39)	–	–	–	0.2	t	–	–	t
10	1150	12.3	methyl 2-phenylacetate[c,d]	0.6	0.4	1.9	1.6	t	1.0	1.3	2.6
11	1152	13.0	methyl 2-hydroxybenzoate[d]	0.6	0.3	t	0.2	t	0.1	–	–
12	1185	13.8	2,6-dimethyl-3,7-octadiene-2,6-diol (**I**)[c]	16.8	52.2	31.3	19.2	62.6	58.9	33.30	28.0
13	1187	14.5	unknown (54, 82, 151, 166, 110, 123)	0.4	–	–	–	–	–	–	–
14	1188	14.6	HMF[b,c,d]	1.8	1.7	–	–	–	–	–	–
15	1195	14.9	unknown (82, 71, 89, 67, 43, 99) lilac alcohol	–	–	t	–	–	–	–	–
16	1198	15.0	4-methoxybenzaldehyde[c,d]	0.6	1.1	1.5	0.3	8.5	0.6	0.2	t
17	1207	15.4	unknown (74, 55, 43)	–	–	t	t	–	–	–	–
18	1211	15.6	unknown (59, 129, 101, 42)	–	t	t	–	–	–	–	–
19	1230	16.4	unknown (71, 43, 55, 103, 111, 93)	0.2	–	t	0.3	–	–	–	–
20	1234	16.6	unknown (43, 71, 42, 39, 55, 82)	t	0.2	0.6	t	–	–	–	–
21	1239	16.8	unknown (71, 43, 59, 55)	0.3	0.3	1.2	0.3	–	–	–	–
22	1244	17.0	methyl 2-hydroxy-2-phenylacetate[d] (methyl mandelate)	–	–	t	t	–	t	–	–
23	1247	17.2	unknown (135, 77, 120, 150, 92)	t	t	t	t	t	2.6	2.2	–
24	1252	17.4	unknown (98, 70, 69)	0.5	–	–	t	–	–	–	–
25	1265	18.0	unknown (139, 93)	0.6	1.5	t	–	0.8	–	–	
26	1289	19.0	3-hydroxy-4-phenyl-2-butanone[b,h]	–	–	0.2	0.1	–	–	0.2	2.3
27	1290	19.1	methyl 2-aminobenzoate	t	–	–	t	–	–	–	–
28	1295	19.3	methyl 2-methoxybenzoate[d]	1.8	t	2.6	1.4	–	–	2.2	1.9
29	1297	19.4	unknown (129, 97, 156, 69, 41)	–	t	1.4	t	–	–	t	–
30	1302	19.6	unknown (43, 71, 55, 87)	1.9	2.1	2.8	3.1	–	–	–	3.4
31	1311	20.0	unknown (71, 43, 55, 96, 121)	2.2	1.9	3.5	2.9	–	–	t	3.8
32	1320	20.4	methyl 2-hydroxy-3-phenylpropionoate (**IV**)[c,d]	7.4	6.5	3.5	5.6	18.5	12.0	10.8	9.6
33	1325	20.6	methyl 3-methoxybenzoate[d]	t	–	1.9	3.6	–	16.7	–	–
34	1327	20.7	4-methoxybenzaldehyde	4.2	2.7	11.2	3.7	22.2	10.1	10.8	9.1
35	1331	20.9	unknown (43, 71, 67, 70, 81, 119, 134)	1.4	1.8	5.5	2.0	–	–	3.4	3.3
36	1336	21.1	methyl 3-phenyl-2-propenoate[c,d]	t	t	7.5	0.5	–	–	t	–
37	1337	21.1	unknown (101, 103, 162, 77)	0.2	–		0.5	–	–	–	–
38	1358	22.1	unknown (74)	5.2	5.4	1.4	3.6	–	–	–	–
39	1385	23.2	methyl 2-(4-methoxyphenyl)acetate[d]	t	t	0.4	t	–	–	–	–
40	1412	24.4	dimethyl octanedioate[c,d]	0.5	0.5	0.7	0.8	–	–	–	–
41	1419	24.7	3,4-dimethoxybenzaldehyde[d]	2.3	2.2	t	4.0	0.6	2.7	3.9	10.7
42	1429	25.1	methyl 3-hydroxybenzoate[d]	0.8	0.8	4.2	0.3	–	–	0.6	0.8
43	1430	25.3	methyl 3-hydroxydecanoate[d,e]	0.2	–	–	0.2	–	–	–	–
44	1436	25.5	3-oxo-α-ionone[c,d,f]	–	–	–	0.2	–	–	–	–
45	1440	25.7	*p*-aminoacetophenone	t	t	0.7	t	–	–	–	–
46	1449	25.9	methyl 3-hydroxyphenylacetate[i]	0.2	0.3	0.1	t	–	–	–	–
47	1450	26.1	methyl dihydroxybenzoate	0.4	0.3	1.1	0.4	–	0.7	t	–
48	1458	26.5	methyl 4-hydroxy-3-methoxybenzoate	t	t	0.1	0.4	–	–	–	–
49	1471	27.1	unknown (88, 101, 69)	0.2	t	–	t	–	–	–	–
50	1481	27.5	methyl *N*-formylanthranilate	0.2	t	0.7	0.4	–	–	0.3	–
51	1494	28.0	methyl 3,4-dimethoxyhydrocinnamate	1.1	t	2.4	0.3	–	–	–	–
52	1496	27.8	methyl 2-hydroxy-2-(4-methoxyphenyl)acetate (**III**)	15.6	153	168	12.1	105.9	25.6	20.8	22.9
53	1513	28.5	dimethyl nonanedioate[c,d]	0.3	0.6	0.7	0.4	–	–	t	–
54	1521	28.8	carbaldehyde furyl butyl ketone[b]	t	–	–	–	–	–	–	–
55	1522	28.9	unknown (196, 137, 77, 124, 165)	t	–	t	–	–	–	–	–
56	1537	29.6	methyl 3,4-dimethoxybenzoate[d]	3.4	4.4	12.8	5.6	14.3	13.8	10.5	15.4
57	1576	30.7	methyl 2-hydroxy-3-(4-methoxyphenyl)propionate[d]	3.9	3.7	9.3	0.8	24.3	12.6	8.1	1.1
58	1585	31.4	4-isopropylacetophenone	4.2	3.7	9.1	4.4	19.9	15.0	8.5	12.7
59	1588	31.5	3-methylphenylacetate	1.1	1.1	1.5	0.8	–	1.6	0.6	1.6
60	1609	32.3	unknown (147, 162, 106, 119, 133, 210)[d]	t	t	t	t	–	–	7.8	–
61	1615	32.5	dimethyl decanedioate[c,d]	4.8	3.9	4.0	5.0	8.3	10.4	6.1	11.1
62	1623	32.8	unknown (137, 210, 150)[d]	0.4	0.5	0.6	0.7	–	0.2	0.1	1.1
63	1653	33.9	unknown (165, 238)	–	–	–	0.6	–	–	–	1.0
64	1659	34.1	dimethyl 2-decenedioate[c,d]	5.1	7.7	11.8	9.7	18.1	28.6	16.8	29.3
65	1669	34.5	methyl 3,4,5-trimethoxybenzoate[c,d]	2.3	3.2	2.5	2.9	15.5	13.0	7.8	8.2
66	1684	35.0	unknown (55, 41, 167, 151)	0.2	1.8	3.0	0.4	–	–	–	–
67	1686	35.1	methyl 2-hydroxy-2-(3,4-dimethoxyphenyl)acetate	t	1.6	2.9	t	–	5.8	–	–
68	1700	35.6	unknown (43, 85, 95, 124, 141, 165, 208, 252, 282, 71, 55)	1.6	–	t	t	–	–	0.4	–
69	1700	35.6	unknown (85, 43, 109)	t	1.9	2.5	t	–	–	–	–
70	1703	35.7	unknown (43, 109, 208)	1.2	1.5	–	1.6	–	–	–	–
71	1708	35.9	3,4,5-trimethoxybenzyl methyl ether[c]	4.9	2.9	7.3	2.1	25.1	7.8	8.3	5.0
72	1714	36.1	dehydrovomifoliol isomer[d]	4.3	t	8.3	5.1	36.1	28.7	15.0	12.8
73	1728	36.6	dehydrovomifoliol[d,g]	10.4	7.2	15.3	7.8	10.7	14.5	8.6	10.1
74	1750	37.4	unknown (186, 217, 128, 156, 115, 101)	0.3	t	0.5	0.3	–	–	–	– 1

Table 1 (Continued)

peak	carbon no.	t_R (min)	compound	leatherwood honey samples[a] splitless injection A1	A2	A3	A4	on-column A1	A2	A3	A4
75	1908	42.8	methyl palmitate[b-e]	1.7	1.8	2.6	1.2	3.7	5.6	1.4	0.8
76	2011	46.3	carbonyl (190, 162, 134, 207)	3.4	3.0	4.9	4.1	20.8	14.6	5.3	7.9
77	2047	47.8	$C_{21:1}$ alkene	0.8	1.2	0.5	0.5	–	5.5	8.6	–
78	2054	48.1	methyl octadecenoate[c-e]	2.4	3.0	4.6	3.3	1.1	15.0	3.6	2.4
79	2103	48.4	*n*-heneicosane (C_{21})[c-e]	2.3	–	2.6	0.6	–	–	8.8	2.5
80	2109	48.6	carbonyl (190, 134, 162, 91, 222, 245, 260, 147, 205)	0.9	0.5	1.2	1.0	2.4	1.8	1.4	1.
81	2121	48.9	unknown (57, 43, 71, 85, 99, 113, 147)	0.3	–	–	–	–	–	–	–
82	2127	49.1	methyl stearate ($C_{18:0}$)[c-e]	0.4	0.2	0.4	0.3	–	–	0.1	–
83	2312	53.6	*n*-triacosane (C_{23})[d,e]	2.3	2.6	4.4	2.5	9.0	12.6	2.0	–
84	2392	54.9	$C_{21:4}$ alkene	0.6	0.5	0.8	0.2	–	–	–	–
85	2402	55.8	$C_{25:1}$ alkene[e]	0.2	0.4	1.1	t	–	0.6	0.5	–
86	2502	56.1	C_{25} alkane[e]	6.0	6.0	12.7	6.3	40.2	–	8.3	13.6
87	2511	56.2	methyl ester $C_{22:0}$ acid[d]	1.7	1.8	5.1	2.6	9.7	11.7	3.3	–
88	2593	57.1	$C_{27:1}$ alkene	0.9	1.2	2.3	1.1	2.0	3.4	0.8	–
89	2635	57.6	$C_{27:0}$ alkane[e]	0.2	–	0.7	–	–	–	–	–
90	2692	58.3	$C_{27:0}$ alkane[d,e]	15.3	45.6	40.4	16.1	15.6	13.5	17.1	31.1
91	2692	58.3	methyl ester $C_{24:0}$ acid[d,e]	15.3	45.6	40.4	14.8	16.8	15.2	18.2	32.8
92	2708	58.5	methyl ester $C_{22:1}$ acid	15.0	46.2	43.4	5.4	1.8	–	–	–
93	2900	61.3	$C_{29:0}$ alkane[d,e]	7.5	8.8	18.3	7.1	76.2	6.2	8.1	16.6
94	2903	61.5	methyl ester $C_{26:0}$ acid[d,e]	4.0	4.5	11.1	3.6	45.8	46.4	4.6	10.6
95	3045	64.9	$C_{31:1}$ alkene isomer[d,e]	2.0	2.3	3.3	0.7	–	–	–	–
96	3060	65.1	$C_{31:1}$ alkene isomer[d,e]	1.8	2.0	3.3	4.8	15.4	–	4.0	–
97	3068	65.8	unknown (57, 71, 85, 99, 225, 267, 295, 310)	4.8	5.2	10.3	3.4	18.7	–	3.0	–
98	3106	66.3	$C_{31:0}$ alkane[d,e]	4.7	5.2	5.8	8.5	47.3	–	5.1	–
99	3108	66.3	methyl ester C_{28} acid[d,e]	2.7	2.9	8.5	2.8	37.4	–	4.1	–

[a] A1, creamed leatherwood honey (Four Roses, Tasmania); A2, liquid leatherwood honey (Four Roses); A3, liquid leatherwood honey (Golden Nectar, Tasmania); A4, liquid leatherwood honey (C. Klapp, Tasmania). t = trace amount. – = not detected. The prominent ions in the MS are given in decreasing order in parentheses for each unknown compound. [b] Graddon *et al.* (1979). [c] Wilkins *et al.* (1993). [d] Tan *et al.* (1988, 1989a,b). [d] Bonaga *et al.* (1986). [f] Enzell and Waklberg (1986). [g] Hausler and Montag (1989, 1991). [h] Watanabe *et al.* (1986). [i] Steeg and Montag (1987).

of hotrienol were present, and it is likely that it is an artifact. Several other components were observed in the GC profile (injector, 250 °C) of the nectar extract that are of interest as they also occur in the honey extracts. These results may not be reliable as these components may also be thermally derived artifacts.

An ether extract of nectar obtained from leatherwood flowers contained the diol **I** (20.9%) and 4-methoxybenzaldehyde (19.6%) as the major volatile components. Other volatile components that were present in the extract were hotrienol (7.4%), 3,4-dimethoxybenzaldehyde (14.2%), and three related terpenoid compounds, (a) 17.0%, *m/z* 55 (100), 43 (95), 82 (79), 67 (76), 71 (67), 119 (27); (b) 11.0%, *m/z* 71 (100), 43 (73), 40 (65), 55 (53), 67 (34), 83 (22); and (c) 9.6%, *m/z* 71 (100), 55 (63), 67 (52), 41 (47), 79 (25), 82 (25). Relative areas of the GC profile are quoted as percentages and given in parentheses. The components were not quantified due to the difficulty of collecting a sufficiently large sample of nectar from the flowers.

Headspace. The diol and hotrienol were not detected in the headspace of leatherwood flowers. The compounds limonene, pinene, and safrole were found.

DISCUSSION

The compounds 2,6-dimethyl-3,7-octadiene-2,6-diol (**I**) and 3,7-dimethyl-1,5,7-octatrien-3-ol (hotrienol, **II**) are the major terpenes found in both methylated and unmethylated extracts of leatherwood honey. The diol **I** has also been detected in the nectar of leatherwood flowers and the solvent extracts of the flowers. Hotrienol is the major volatile component detected by headspace analysis in leatherwood honey, and it probably makes an important contribution to the aroma of the honey, as the flavor of this compound has been described as sweet and flowery (Nakatani *et al.*, 1969). The increased polarity of the diol **I** is likely to render it too involatile to contribute significantly to the headspace vapors; furthermore, it has been reported to be odorless (Wintoch *et al.*, 1993). Hotrienol can be made from the thermal dehydration of 2,6-dimethyl-3,7-octadiene-2,6-diol (**I**) (Williams *et al.*, 1980b; Etoh *et al.*, 1980; Wintoch *et al.*, 1993), the principal terpene in ether extracts of leatherwood honey. Hotrienol has been detected in the GC/MS profile of an authentic sample of the diol when injected in splitless mode. Increased concentrations of hotrienol were also found in an extract of steam-distilled honey and flowers in which the diol was not detected. As hotrienol has not been detected in leatherwood flower extracts or in a sample of 1 week old unripe honey, it appears that this compound may be formed either in the hive or during postharvesting processing of the honey. Warm, acidic conditions in the hive or the heat (about 60 °C) applied during the postharvest processing might promote the dehydration of the diol to hotrienol. Williams *et al.* (1980b) have prepared hotrienol by heating of the diol at 70 °C at pH 3.2. More recently, it has been shown that a glycoside of the diol isolated from the fruit peelings of *Solanum vestissimum* (lulo fruit) can be thermally degraded under acidic conditions to yield hotrienol (Wintoch *et al.*, 1993).

Dehydration of the diol appears to occur only at the C-2 position. No evidence could be found in the GC/MS of the honey extract for the presence of the C-6 dehydrated compounds 2,6-dimethyl-3,5,7-octatrien-2-ol or 2-methyl-6-methylene-3,7-octadien-2-ol. The latter is produced by *Ips confusus* from Ponderosa pine and has a base peak *m/z* 79 (Silverstein *et al.*, 1966). Likewise, it might be expected that hotrienol would further dehydrate to the 3,7-dimethyl-1,3,5,7-octatetraene (cosmene) or (*E*)-2-methyl-6-methylene-1,3,7-octatriene. The octatriene is stable at room temperature

Table 2. Constituent Levels (Micrograms per Gram) in a Methylated Extract of Unripe Honey

carbon no.	t_R (min)	compound[a]	splitless	on-column
	9.7	unknown (69, 43, 87)	0.5	1.5
100.9	11.4	dimethyl butanedioate	1.4	0.5
1052	13.9	methyl benzoate	3.0	1.9
1079	15.2	3,7-dimethyl-1,5,7-octatrien-3-ol (hotrienol) (**II**)	1.1	–
1090	15.7	3,5,5-trimethylcyclohex-2-ene-1,4-dione	0.6	–
1132	17.8	unknown (68, 83, 92, 69, 39)	0.4	–
1150	17.9	methyl 2-phenylacetate	19.9	10.4
1152	18.6	methyl 2-hydroxybenzoate	0.5	t
1185	20.2	2,6-dimethyl-3,7-octadiene-2,6-diol (**I**)	20.7	21.0
1198	20.9	4-methoxybenzaldehyde	4.1	2.5
1244	23.3	methyl mandelate (methyl 2-hydroxy-2-phenylacetate)	18.4	19.0
1289	24.5	3-hydroxy-4-phenyl-2-butanone	8.8	4.2
1295	25.4	methyl 2-methoxybenzoate	24.3	15.1
1309	26.1	methyl nonanoate	t	–
1320	27.4	methyl 2-hydroxy-3-phenylpropionate (**IV**)	480	430
1325	27.5	methyl 3-methoxybenzoate	10.1	3.3
1327	28.6	4-methoxybenzaldehyde	t	–
1331	28.9	unknown (43, 71, 67, 70, 81, 119, 137)	t	–
1385	29.4	methyl 2-(4-methoxyphenyl)acetate	2.6	2.0
1412	30.2	dimethyl octanedioate	4.0	1.6
1419	30.9	3,4-dimethoxybenzaldehyde	5.7	3.3
1429	32.2	methyl 3-hydroxybenzoate	1.0	1.9
1436	31.5	3-oxo-α-ionone	t	t
	32.0	unknown (112, 55, 92, 140)	4.1	–
1440	32.7	*p*-aminoacetophenone	0.6	–
	33.2	unknown (107, 77, 180, 131)	2.1	–
1481	33.9	methyl *N*-formylanthranilate	2.0	–
1496	34.2	methyl 2-hydroxy-2-(4-methoxyphenyl)acetate (**III**)	30.9	44
1513	34.5	dimethyl nonanedioate	3.3	1.3
1537	35.5	methyl 3,4-dimethoxybenzoate	2.5	t
	36.3	unknown (91, 180)	293	165.9
1576	37.2	methyl 2-hydroxy-3-(4-methoxyphenyl)propionate	57.2	29.2
1585	37.5	4-isopropylacetophenone	8.2	6.6
1588	37.8	3-methylphenylacetate	4.6	2.7
1615	38.5	dimethyl decanedioate	11.7	8.0
1623	39.4	*p*-menthan-1-one	6.5	2.9
1659	40.2	dimethyl 2-decenedioate	37.4	31.9
1669	40.7	methyl 3,4,5-trimethoxybenzoate	148.6	78.6
1708	42.1	3,4,5-trimethoxybenzyl methyl ether	17.6	13.8
1714	42.4	dehydrovomifoliol	21.8	8.2
1719	42.6	dehydrovomifoliol isomer	20.5	8.4
1908	48.7	methyl palmitate ($C_{16:0}$)	5.5	4.9
2011	52.6	carbonyl (190, 162, 134, 207)	2.1	1.3
2047	53.7	$C_{21:1}$ alkene	2.8	1.7
2054	54.1	methyl octadecenoate	6.1	4.2
2103	54.2	*n*-heneicosane (C_{21})	1.9	1.4
2109	54.8	carbonyl (190, 134, 162, 91, 222, 245, 260, 147, 205)	0.8	1.0
2121	55.0	unknown (57, 43, 71, 85, 99, 113, 147)	0.8	–
2127	55.1	methyl stearate ($C_{18:0}$)	1.1	1.1
2312	59.8	*n*-triacosane (C_{23})	12.4	6.8
2402	62.1	$C_{25:1}$ alkene	2.8	1.5
2502	62.4	C_{25} alkane	44.9	18.7
2511	62.6	methyl ester $C_{22:0}$ acid	20.8	10.8
2593	63.6	$C_{27:1}$ alkene	3.4	1.8
2692	64.6	$C_{27:0}$ alkane	132.6	54.5
2692	64.8	methyl ester $C_{24:0}$ acid	145.8	80.4
2900	67.4	$C_{29:0}$ alkane	70.2	29.2
2903	67.6	methyl ester $C_{26:0}$ acid	48.5	22.2
3045	70.8	$C_{31:1}$ alkene isomer	26.9	14.7
3060	70.9	$C_{31:1}$ alkene isomer	22.7	13.6
3106	71.6	$C_{31:0}$ alkane	41.4	20.8
3106	72.1	methyl ester C_{28} acid	38.4	21.0

[a] The prominent ions in the MS are given in decreasing order in parentheses for each unknown compound. t = trace amount. – = not detected.

and gives a base peak of m/z 79 in the mass spectrum (Mignani *et al.*, 1986). However, cosmene has been reported to be highly unstable (Christensen *et al.*, 1990), and at present, neither cosmene nor isomers have been detected in the extracts of leatherwood honey.

The diol **I** that was present in honey was transferred to the hive without modification by the bee. The diol was first prepared by the photosensitized oxidation of linalool (Matsura and Butsugan, 1968) and then isolated from the oil of Japanese ho-leaf tea (Takaoka *et al.*, 1976). It has been identified as a constituent of grapes, wines (Williams *et al.*, 1980a,b; Strauss *et al.*, 1987; Rapp and Knipser, 1979), and tea (Etoh *et al.*, 1980) and is a constituent of *Boronia megastigma* (Davies and Menary, 1984). The diol has been isolated as a major glycosidically bound constituent of lulo fruit (Wintoch *et al.*, 1993) which has been suggested as a natural precursor to hotrienol which has also been detected in this fruit. Williams *et al.* (1980b) have made hotrienol from the acid-catalyzed thermal dehydration of 2,6-

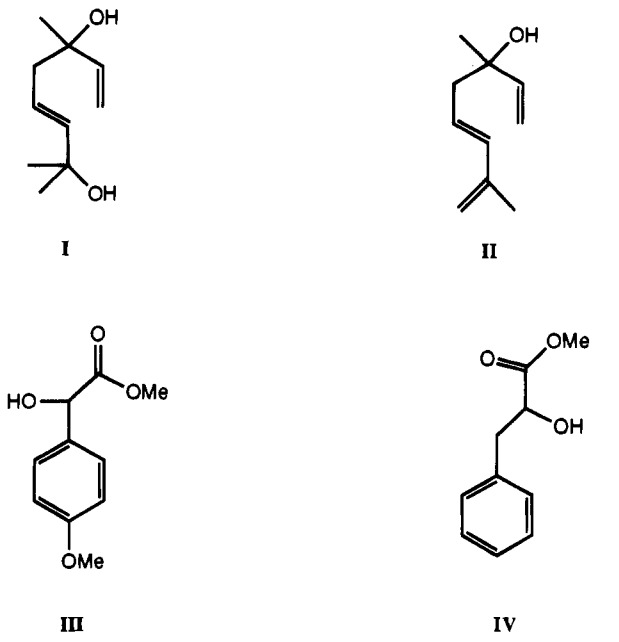

Figure 1. (**I**) 2,6-Dimethyl-3,7-octadiene-2,6-diol, (**II**) 3,7-dimethyl-1,5,7-octatrien-3-ol, (**III**) methyl 2-hydroxy-2-(4-methoxyphenyl)acetate, and (**IV**) methyl 2-hydroxy-3-phenylpropionate.

Table 3. Volatile Compounds Found in Unmethylated Extracts of Leatherwood Honey

compound	identification
butane-1,3-diol	MS[g]
butane-2,3-diol	GC, MS[a]
phenol	GC, MS[a]
benzyl alcohol	GC, MS[a]
phenylacetaldehyde	MS[g]
hotrienol (**II**)	MS[b,g]
oxoisophorone	GC, MS[a]
2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	MS[a,g]
lilac aldehydes (four isomers)	MS[b,c,g]
2,6-dimethyl-3,7-octadiene-2,6-diol (**I**)	MS[b,g]
hydroxymethylfurfural	GC, MS
isomer of 2,6-dimethyl-3,7-octadiene-2,6-diol	MS[b,g]
lilac alcohol (isomer 1)	MS[b,d,g]
4-methoxybenzaldehyde (*p*-anisaldehyde)	MS[a,b]
(*Z*)-2,6-dimethyl-2,7-octadiene-1,6-diol	MS[b,g]
(*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol	MS[b,g]
(*Z*)-2,6-dimethyl-6-hydroxy-2,7-octadienal	MS[b,g]
4-methoxybenzoic acid (*p*-anisic acid)	MS[g]
3-methoxybenzoic acid (*o*-anisic acid)	MS[g]
3,4-dimethoxybenzaldehyde (veratraldehyde)	GC, MS
2-hydroxy-3-phenylpropionic acid (phenyllactic acid)	MS[g]
(5′-carbaldehyde) 2-furyl butyl ketone (three isomers)	MS[a,g]
methyl syringate	GC, MS[a,e]
dehydrovomifoliol	GC, MS[f,g]
isomer of dehydrovomifoliol	MS[g]

[a] Graddon *et al.* (1979). [b] Wilkins *et al.* (1993). [c] Wakayama and Namba (1974). [d] Wakayama and Namba (1970). [e] Tan *et al.* (1989). [f] Hausler and Montag (1989). [g] Tentative identification since only by comparison of mass spectral data.

dimethyl-3,7-octadiene-2,6-diol. Hotrienol is also a constituent of ho-leaf tea (Nakatani *et al.*, 1969), *Cinnamomum camphora* (Yoshida *et al.*, 1969), grapes (Williams *et al.*, 1980a,b; Schreier *et al.*, 1974; Ribereau-Gayon *et al.*, 1975), papaya (Winterhalter *et al.*, 1986), passion fruit (Engel and Tressl, 1983), elderberry flowers (Eberhardt and Pfannhauser, 1985), and *S. vestissimum* (Suarez and Duque, 1991; Suarez *et al.*, 1991; Wintoch *et al.*, 1993) and has been isolated from the distillate of beeswax (Ferber and Nursten, 1977). Both of these compounds have been isolated from nodding thistle honey together with many other linalool derivatives (Wilkins *et al.*, 1993).

The methylated extract of leatherwood honey afforded a GC/MS profile containing many aromatic acid derivatives, many of which have been previously described in honey by other workers (Speer and Montag, 1984, 1985, 1987). The principal aromatic acid derivative in the extract was methyl 2-hydroxy-2-(4-methoxyphenyl)-acetate (**III**). This compound is probably present in the honey as 2-hydroxy-2-(4-hydroxyphenyl)acetic acid, the phenol and acid groups being methylated during derivatization. Compound **III** was a major component of the acidic fraction of leatherwood flower extracts. The compound 2-hydroxy-2-(4-hydroxyphenyl)acetic acid has been found in tobacco smoke (Snook *et al.*, 1985) and human urine (Armstrong *et al.*, 1956; Higa and Kishimoto, 1986; Spiteller and Spiteller, 1979). It has antiamebic activity (La Manna *et al.*, 1964) and is known to be a chemoattractant for the microorganism *Pseudomonas putida* (Harwood *et al.*, 1984). The *R* isomer has also been isolated from cultures of the ectomycorrhizal Basidiomycete fungus *Pisolithus tinctorius* and has been found to have antifungal activity (Tsantrizos *et al.*, 1991a,b). Neither 2-hydroxy-2-(4-hydroxyphenyl)acetic acid nor its methyl derivatives have been reported to occur in honey, yet Tan *et al.* (1988) have reported that a structurally similar compound, 2-hydroxy-3-phenylpropionic acid (which has also been detected in leatherwood honey), is the major component in kanuka and manuka honeys. Although Tan *et al.* (1988) were not able to find any constituents in the manuka flower extracts that corresponded to those in the honey, Steeg and Montag (1988b) have suggested that many aromatic acids found in honey are secondary plant metabolites and correspond to the floral source.

In addition to hotrienol and the diol, the nectar extract contained the aromatic components 4-methoxybenzaldehyde and 3,4-dimethoxybenzaldehyde. These two components have also been found in unmethylated extracts of leatherwood honey. Other components that are present as methylated derivatives in the unmethylated extracts include 4-methoxybenzoic acid (*p*-anisic acid), 3-methoxybenzoic acid (*o*-anisic acid), and methyl syringate. Methoxybenzaldehyde, 3,5-dimethoxybenzaldehyde, and methyl syringate have been previously identified in unmethylated extracts of a range of Australian honeys (Graddon *et al.*, 1979).

The principal aromatic acid derivatives of the sample of unripe honey were methyl 2-hydroxy-3-phenylpropionate (**IV**) and an unidentified compound [*m*/*z* 91 (100), 180 (51)]. As methyl 2-hydroxy-3-phenylpropionate is a minor component and the unidentified compound is a trace component of the matured leatherwood honey, it is likely that these compounds are degraded into other components during the course of maturation. The unidentified compound may be produced by the bee or arise from other flower species as it has not been detected in the flower extracts. In the unripe honey, methyl 2-hydroxy-2-(4-methoxyphenyl)acetate (**III**) was present at a similar concentration to that found in mature honey. Other major aromatic components of the immature honey extract were methyl 2-hydroxy-2-phenylacetate, methyl 3,4,5-trimethoxybenzoate, methyl 2-hydroxy-3-(4-methoxyphenyl)propionate, and methyl 2-methoxybenzoate. Concentrations of these compounds in the unripe honey were higher than those in the matured honey, and it is likely that they undergo

Table 4. Components (Micrograms per Gram) of Leatherwood Honey Found in Plant Extracts

I_W	t_R (min)	compound[a]	stamens	whole flowers	acidic fraction	petals	flower: steam distilled	flower: without petals	leaves
100.9	6.5	dimethyl butanedioate	7.4	1.7	0.4	0.1	–	13.5	5.9
1079	9.8	3,7-dimethyl-1,5,7-octatrien-3-ol (**II**)	t	–	1.3	t	2.3	–	–
1090	10.4	3,5,5-trimethylcyclohex-2-ene-1,4-dione	–	–	–	–	t	–	–
1150	12.3	methyl 2-phenylacetate	–	–	0.9	t	t	t	–
1152	13.0	methyl 2-hydroxybenzoate	–	–	7.1	0.4	0.2	1.2	–
1185	13.8	2,6-dimethyl-3,7-octadiene-2,6-diol (**I**)	9.4	2.3	2.9	t	–	6.4	–
1295	19.3	methyl 2-methoxybenzoate	–	–	–	–	–	–	2.5
1320	20.4	methyl 2-hydroxy-3-phenylpropionoate (**IV**)	–	–	4.6	–	–	t	–
1336	21.1	methyl 3-phenyl-2-propenoate	–	–	–	–	–	–	4.3
1429	25.1	methyl 3-hydroxybenzoate	5.6	–	4.6	–	–	1.8	–
1440	25.7	*p*-aminoacetophenone	2.2	–	–	0.2	t	3.1	–
1458	26.5	methyl vanillate (methyl 4-hydroxy-3-methoxybenzoate)	–	–	9.4	t	–	–	–
1471	27.1	unknown (88, 101, 69)	–	12.0	–	–	–	26.8	–
1496	27.8	methyl 2-hydroxy-(4-methoxyphenyl)acetate (**III**)	t	t	25.0	t	–	t	–
1537	31.3	methyl 3,4-dimethoxybenzoate	–	–	t	–	–	–	–
1609	32.3	unknown (147, 162, 106, 119, 133, 210)	–	–	1.9	t	–	–	–
1669	34.5	methyl 3,4,5-trimethoxybenzoate	t	–	1.2	–	–	1.2	–
	35.0	unknown (137, 210, 150)	t						
1702	35.7	methyl 3-(3-hydroxyphenyl)-2-propenoate	–	–	4.2	t	–	t	–
1703	35.7	3,4,5-trimethoxybenzyl methyl ether	t	–	t	t	–	–	–
1708	35.9	methyl syringate	3.6	–	2.5	t	–	1.9	–
1908	42.8	methyl palmitate ($C_{16:0}$)	10.8	4.4	7.6	3.2	0.3	17.5	52.7
2011	46.3	carotenoid	–	–	3.8	–	–	t	–
2047	47.8	$C_{21:1}$ alkene	7.2	–	0.5	1.6	–	22.7	–
2054	48.1	methyl octadecenoate	5.2	6.4	1.6	t	0.6	19.4	40.7
2127	49.1	methyl stearate ($C_{18:0}$)	4.1	–	2.2	–	–	16.2	15.1
2312	53.6	*n*-triacosane (C_{23})	107	–	3.8	18.4	3.3	274	–
2502	56.1	C_{25} alkane	124	75.6	5.2	16.3	1.4	10.6	512.4
2511	56.2	methyl ester $C_{22:0}$ acid	17.6	6.8	354	2.5	–	9.7	318.2
2692	58.3	$C_{27:0}$ alkane	–	35.6	–	8.3	0.1	115	318
2692	58.3	methyl ester $C_{24:0}$ acid	–	10.0	–	2.4	–	59.5	574
2900	61.3	$C_{29:0}$ alkane	27.5	20.2	–	t	–	79.4	546
2903	61.5	methyl ester $C_{26:0}$ acid	16.7	5.8	–	t	–	37.9	149.1

[a] The prominent ions in the MS are given in decreasing order in parentheses for each unknown compound. t = trace. – = not detected.

some modification while in the hive. All of these compounds were detected in extracts of the flowers. The diacid derivatives dimethyl octanedioate, dimethyl nonanedioate, dimethyl decanedioate, and dimethyl 2-decenedioate were also present in the unripe honey. These compounds were not detected in the plant extracts and thus may be manufactured by the bee. The diacids are thought to be part of the pheromone system of the bee and have been reported to occur in the extracts of royal jelly (Lercker *et al.*, 1981). It must be noted that only one sample of unripe honey could be obtained for analysis. Due to unusual weather patterns in 1994, the flowering season of leatherwood trees was delayed, and it is possible that other pollen sources may have contributed to this sample of honey, thereby affecting the chemical profile. Further work is required to confirm these results.

Leatherwood honey contains a number of neutral carbonyl compounds, many of which are 3,5,5-trimethylcyclohex-2-ene derivatives. Those identified in this study are isophorone, 4-oxoisophorone, 3-oxo-α-ionone, and dehydrovomifoliol [4-hydroxy-4-(3-oxo-1-butenyl)-3,5,5-trimethylcyclohex-2-en-1-one]. Isophorone, 4-oxoisophorone, and dehydrovomifoliol were among the 3,5,5-trimethylcyclohex-2-ene derivatives detected in New Zealand heather honey (Tan *et al.*, 1989a). Our results support the assertion by Tan *et al.* (1989a) that the published mass spectra of compounds B, C, and H of uncertain structure from several Australian honeys (Graddon *et al.*, 1979) correspond to the mass spectra of isophorone, 4-oxoisophorone, and 3-oxo-α-ionone, respectively. Dehydrovomifoliol has been shown to be present in French and Spanish heather honeys in much higher levels than in a range of other honeys surveyed (Hausler and Montag, 1989). Dehydrovomifoliol has been proposed as a marker compound for heather honey and described as a precursor of flavor compounds (Hausler and Montag, 1991). Other studies have put forward 3,5,5-trimethylcyclohex-2-ene derivatives as specific for different varieties of honey (Ede *et al.*, 1993; Broom *et al.*, 1992).

The neutral carbonyl compounds were not detected in unripe leatherwood honey nor in the plant, except for dehydrovomifoliol as a flavor precursor. Degradation of abscisic acid or a carotene-like compound might lead to the formation of these compounds. Since degraded carotenoids are implicated in contributing to flavor and aroma (Tan *et al.*, 1989a; Hausler and Montag, 1991), the above result may be relevant to the development of the ripe honey. The mass spectrum of another neutral carbonyl compound, 3-hydroxy-4-phenyl-2-butanone, that was found in some samples of mature leatherwood honey (but not in unripe honey) corresponds to the published spectrum of a compound of uncertain structure (E) by Graddon *et al.* (1979). This compound has been found previously in wisteria flowers (Watanabe *et al.*, 1986) and wine (Brock *et al.*, 1984).

Splitless injection appeared to favor the formation of thermally induced artifacts. Levels of some compounds found in GC/MS profiles performed with splitless injection were reduced or not detected when an on-column injection was performed. Methyl furoate, 3,5,5-trimethylcyclohex-2-ene-1,4-dione, several linalool-related compounds, and some of the aromatic acid derivatives such as methyl 2-(4-methoxyphenyl)acetate, methyl 3-hydroxyphenylacetate, and methyl 4-hydroxy-3-methoxybenzoate appear to be thermally induced artifacts. In contrast, the levels of methyl 2-hydroxy-3-phenyl-

propionate, methyl 2-hydroxy-2-(4-methoxyphenyl)-acetate, 4-methoxybenzaldehyde, and the diacid derivatives dimethyl decanedioate and dimethyl 2-decenedioate increase when an on-column injection is performed and presumably are partly degraded on heating. The presence of hotrienol in the nectar extract may also be heat derived. Other compounds found in the nectar were 4-methoxybenzaldehyde and 3,4-dimethoxybenzaldehyde. Hotrienol, the diol **I**, 4-methoxybenzaldehyde, and 3,4-dimethoxybenzaldehyde have been found in honey. Trace amounts of dimethyl sulfide and an unidentified compound (m/z 39, 67, 82) were detected in the headspace of leatherwood honey. These compounds may be thermally induced artifacts formed during the incubation of the honey at 50 °C. Maillard reactions with sulfur-containing amino acids are known to produce many volatile sulfur compounds including dimethyl sulfide (Tressl *et al.*, 1983). Bouseta *et al.* (1992) have detected dimethyl sulfide in the headspace of several different types of honey. Dimethyl sulfide is present in a wide variety of foods, and small amounts of it are thought to contribute to the characteristic flavor of the foods (Peer, 1971).

In addition to hotrienol (**II**) and the diol **I**, an isomer of the diol **I**, the linalool derivatives (*Z*)- and (*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol, (*Z*)-2,6-dimethyl-6-hydroxy-2,7-octadienal lilac aldehydes (four isomers), and a lilac alcohol isomer (assigned as isomer 1 on comparison with nodding thistle retention data) were characterized by comparison with the mass spectral data published by Wakayama and Namba (1970, 1974). The levels and ratios of hydrocarbons and fatty acids found in all the honey samples are similar to those reported elsewhere (Bonaga *et al.*, 1986; Graddon *et al.*, 1979) and thought to arise from the residual beeswax in the honey. Some of the hydrocarbon components were detected in the plant extracts although the concentration and ratios of these components varied among the samples.

CONCLUSIONS

Our results support the assertions by other researchers (Bonaga and Giumanini, 1986) that many of the differences in honey flavor and aroma arise from components in the nectar of the floral source. The major terpene found in leatherwood honey, 2,6-dimethyl-3,7-octadiene-2,6-diol (**I**), originates in the nectar. Some of the diol **I** is thermally dehydrated in the hive or during postharvest processing to form hotrienol (**II**), the principal aroma component in the honey. These two compounds contribute to the characteristic flavor and aroma of leatherwood honey.

Many of the aromatic substances detected in methylated extracts of leatherwood honey have also been detected in the flower extracts. The major aromatic acid derivative methyl 2-hydroxy-2-(4-methoxyphenyl)acetate (**III**) in honey was also present as an important component of the acidic fraction of the leatherwood flower extract. This compound has not been previously reported in honey.

In addition to the degradation of 2,6-dimethyl-3,7-octadiene-2,6-diol to hotrienol, it appears likely that considerable modification of plant components takes place in the hive or during postharvest processing. A methylated extract of an unripe sample of honey was found to contain methyl 2-hydroxy-2-phenylpropionate (**IV**) and an unidentified compound with prominent peaks in the MS of m/z 91 (100) and 180 (51) as the major components. These compounds were present as minor components in matured samples of leatherwood honey, and it is possible that they undergo some chemical transformation while in the hive. The source of the unidentified compound is uncertain as it was not detected in the plant extracts. It may be produced by the bee or arise from another plant source. Although leatherwood honey is a predominantly unifloral honey, other plant species are in flower at the same time in the areas where it is collected and may contribute to the volatile component spectrum. As only one sample of unripe honey was analyzed, further investigation is needed to confirm these results.

Some naturally methylated components have been detected in unmethylated extracts of leatherwood honey. They include 4-methoxybenzaldehyde, 3,4-dimethoxybenzaldenyde, 4-methoxybenzoic acid, and 3-methoxybenzoic acid. Other classes of compounds found in the honey include hydrocarbons (C_{21}–C_{31}), fatty acids (C_{16}–C_{28}), diacids, and neutral carbonyl compounds.

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