Extractives from New Zealand Unifloral Honeys. 2. Degraded Carotenoids and Other Substances from Heather Honey

Seng To Tan,* Alistair L. Wilkins,* Patrick T. Holland, and Tony K. McGhie

Extractives from New Zealand ling heather (*Calluna vulgaris*) honey samples have been found to contain compounds with degraded carotenoid-like structures (3,5,5-trimethylcyclohex-2-ene skeletons) possessing fifteen, thirteen, nine, or eight carbon atoms. Two of the substances were present at 30–180 μg/g levels. Their structures were established by a combination of high-resolution mass spectroscopy and one- and two-dimensional NMR spectroscopy. A further 10 related compounds, including abscisic acid, present at 1–60 μg/g levels were identified by GC/MS. Other prominent extractives include benzyl alcohol, benzoic acid, phenylacetic acid, and 2-hydroxy-3-phenylpropionic acid. Degraded carotenoids have not been hitherto identified from honey. The GC profiles of heather honeys readily distinguish them from other New Zealand unifloral honeys.

Studies of extractable substances present in New Zealand honeys have revealed a range of compounds that appear characteristic of the floral source. We have reported levels of a range of aliphatic and aromatic acids plus diacids in clover, manuka, and kanuka honeys (Tan et al., 1988). The levels of aromatic acids, both free and bound, have been reported previously in a variety of unifloral honeys (Steeg and Montag, 1987). There are also reports of more detailed analyses of minor components, especially honey volatiles (Bonaga and Giumanini, 1986; Bicchi et al., 1983; Graddon et al., 1979; Tsuneya et al., 1974). Our investigation of heather honeys revealed some similarities in the composition of the extractables, but a variety of unidentified components were also present at relatively high levels. We now report the characterization in heather honey of a family of 3,5,5-trimethylcyclohex-2-ene derivatives, often described as degraded carotenoids. Concentrations of these substances and other extractives in heather honeys are presented.

MATERIALS AND METHODS

Source of Honey Samples. Heather (*Calluna valgaris*) honey samples (unheated, 1986–1987 summer season) were obtained from beekeepers (H2, H3, H4) with hives kept in the cental North Island of New Zealand (National Park region). A commerical heather honey sample (H1) from the same region was obtained from Wilson and Neill-Hororata Honey Exports Ltd. Honey purity was verified by pollen analysis (Moar, 1985).

Methods and procedures used in the analysis of the heather honey samples were as described in Tan et al. (1988), other than the use of a 250-mL extractor with a 12-h extraction time and of a Shimadzu CR-3A reporting integrator. Gas chromatographic/flame ionization detector (GC/FID) analyses of the methylated extracts were performed on a 0.22 mm (i.d.) × 16 m column, coated with methylsilicone (BP-1; SGE Ltd., Melbourne), combined gas chromatographic/mass spectroscopic (GC/MS) analyses were carried out on a Hewlett-Packard 5890/5970 GC/MSD system interfaced to a 12-m HP-1 methylsilicone column or a Varian 3700 GC instrument coupled to a Finnigan Mat 700 ion trap detector system interfaced to a 12-m BP-1 column, and high-resolution GC/MS was performed on a Kratos MS80RFA instrument with a Carlo Erba Mega GC. Concentrations of degraded carotenoid substances were determined from GC/FID area responses calibrated against methyl 3-phenylprop-2-enoate. Other compounds were quantified as described previously (Tan et al., 1988). ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were determined on Bruker AM-400 (peak 37) or Varian XL-300 (peak 45) instruments.

A bulk extraction of samples H2, H3, and H4 (100 g combined weight) afforded a mixture of extractives separated by multiple preparative layer chromatography (PLC) on silica gel (Merck $PF_{254+366}$) with toluene–dioxane–acetic acid (90:25:3) (first and second developments) and hexane–ether (4:1) (third and fourth developments). Twenty bands were recovered from the PLC plate (ether as eluent); the two major degraded carotenoid bands (peaks 37 and 45, 1 and 2 mg, respectively) were deep purple when viewed under UV illumination at 366 nm as did the band for peak 25 (1.5 mg). Methylation of the latter band with diazomethane afforded methyl 2-hydroxy-3-phenylpropionate, identical with an authentic specimen.

RESULTS

Figure 1 is the GC/FID trace of the derivatized extractable organic substances recovered from heather honey sample H1. Peaks 61–73 were shown to be hydrocarbons or fatty acids; a similar collection of fatty acids (detected as the corresponding methyl esters since the extracts were methylated with diazomethane prior to analysis) and hydrocarbons were found in other unifloral honeys (Tan et al., 1988). Since the concentrations and distribution of high molecular weight hydrocarbons (C_{21}–C_{33} or higher) and fatty acids (C_{18}–C_{28} or higher) have been well documented (Bonaga et al., 1986; Graddon et al., 1979; Tulloch and Hoffman, 1972), details of their characterization are not repeated here.

An array of methylated aromatic substances (see Table I) including methyl benzoate, methyl phenylacetate, methyl 2-hydroxy-3-phenylpropionate, methyl 3-hydroxybenzoate, methyl 3-methoxybenzoate, methyl 3,5-dimethoxybenzoate, and methyl 3,4-dimethoxybenzoate were detected in each of the heather honey samples. While it is reasonable to assume that the parent acids occur in the honey samples, since aromatic acids are readily methylated by diazomethane, the origin of the aryl methoxy groups in some of the foregoing esters is less obvious. For example, the detection of both of methyl 3-hydroxybenzoate and methyl 3-methoxybenzoate suggests the latter compound may be derived from the former compound by partial derivation of the phenolic hydroxyl group since phenolic hydroxyl groups react more slowly with diazomethane than is the case for acid groups.

A variety of unknown compounds were also detected. Extraction of 100 g of heather honey afforded a mixture of extractives, which when separated by PLC on silica gel (see Materials and Methods) yielded two major components (peaks 37 and 45) in quantities sufficient for their structures to be elucidated, principally by a combination of high-resolution mass spectroscopy and high-field one- and two-dimensional NMR spectroscopy. Thereafter, a

Chemistry Department, University of Waikato, Hamilton, New Zealand (S.T.T., A.L.W.), and Ruakura Research Centre, Ministry of Agriculture and Fisheries, Private Bag, Hamilton, New Zealand (P.T.H., T.K.M.).

0021-8561/89/1437-1217$01.50/0

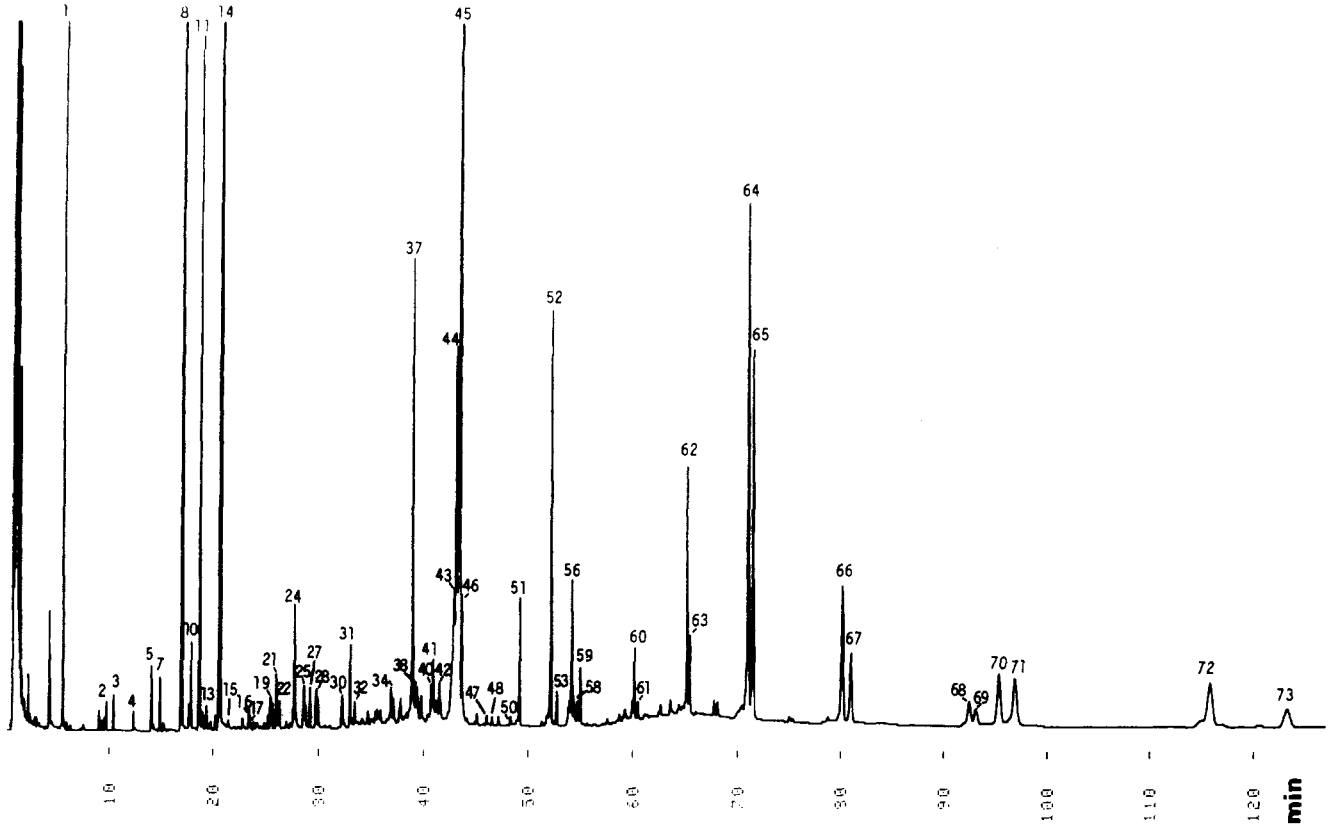


Figure 1. FID GC profile of heather honey extractives. For peak identifications, see Table I. GC conditions: 12-m BP-1 column; H_2 as carrier gas; 40 °C (2-min hold), up 4 °C/min to 240 °C (75-min hold).

consideration of the mass spectral fragmentation patterns of these substances, and of those reported in the literature for related compounds, enabled the structures of related compounds to be determined (structures 1–12).

Peak 45. ^{13}C NMR spectroscopy revealed the presence 13 carbon signals (see Table II), assignable to four methyl carbons, two conjugated carbonyl groups, and the carbons of two olefinic double bonds (one trisubstituted, the other disubstituted), while high-resolution mass spectroscopy established the molecular formula $C_{13}H_{18}O_3$ (m/z 222, M^+). Since this molecular formula requires a total of five rings and/or double bonds, it follows that peak 45, which the ^{13}C NMR data require to be a diketo diene, must be monocyclic. High-resolution mass spectroscopy established that the ion of m/z 205 arises by loss of a hydroxyl radical. This suggested the presence of a tertiary hydroxyl group, an observation that is in keeping with the occurrence at 79.3 ppm in the ^{13}C NMR spectrum of a signal assignable to an oxygenated quaternary carbon. ^1H NMR spectroscopy indicated the presence of two aliphatic tertiary methyl groups, an olefinic methyl group, a methyl ketone, an isolated two-proton multiplet (AB q, ^2J = 17.5 Hz), and three conjugated olefinic protons, two of which were mutually trans coupled (AB q, ^3J = 15.8 Hz). While these data establish the functional groups present and demonstrate that peak 45 possesses a six-membered ring, it does not define the stereochemical disposition of substituent groups.

Even at 90 MHz, the peak half-width, and therefore height of the two tertiary methyl group signals, differed by ca. 15%, this despite the observation that the methyl group protons possess similar T_1 values and therefore by implication T_2 values. Hence, it can be concluded that one of the tertiary methyl group signals is more extensively long range coupled than the other. This observation prompted the determination of the two-dimensional double-quantum-filtered COSY (DQFCOSY) spectrum of peak 45 at 300 MHz under conditions designed to detect ^4J couplings. While ^4J couplings are usually not resolved in conventional ^1H NMR spectroscopy, they can be detected as cross peaks in two-dimensional COSY and DQFCOSY spectra. Thus, it emerged (Figure 2) that the tertiary methyl groups were mutually ^4J coupled and that one of the methyl groups (that with the lesser peak height) was

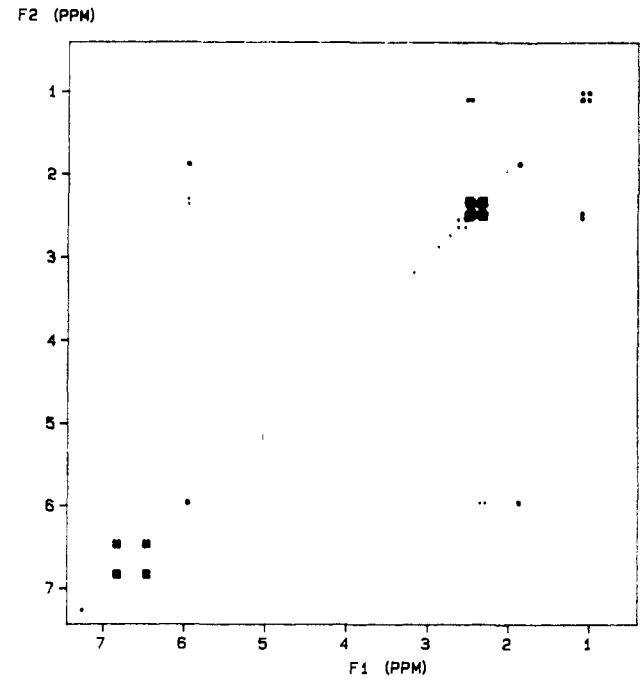


Figure 2. 300-MHz phase-sensitive double-quantum-filtered COSY NMR spectrum of 4-hydroxy-4-(3-oxo-1-butenyl)-3,5,5-trimethylcyclohex-2-en-1-one (1).

Table I. Concentrations (μg/g) of Methylated Components in Heather Honey

peak	carbon no.	compound[a]	sample no. H1	H2	H3	H4	mean
1	8.11	unknown (44, 58, 74, 89)	45	4.4	5.6	4.2	14.8
2	9.09	methyl caproate (6:0)	1.1	1.6	1.5	1.3	1.4
3	9.23	benzaldehyde	1.0	0.3	0.3	0.3	0.5
4	9.66	phenol	0.5	0.7	0.7	0.5	0.6
5	10.02	benzyl alcohol	2.0	1.7	1.8	1.2	1.7
6	10.03	phenylacetaldehyde	0.2	0.4	0.3	0.2	0.3
7	10.20	unknown (43, 69, 73, 101, 126 M^+)	3.0	1.7	2.2	1.4	2.1
8	10.65	methyl benzoate	114	68	83	63	82
9	10.78	2-phenylethanol	0.9	0.8	0.9	0.6	0.8
10	10.82	3,5,5-trimethylcyclohex-2-en-1-one (**10**)	5.0	2.5	2.9	2.1	3.1
11a	10.99	3,5,5-trimethylcyclohex-2-ene-1,4-dione (**11**)	*[c]	*	*	*	*
11	11.00	*n*-undecane (C_{11})	internal standard				
12	11.06	methyl caprylate (8:0)	0.9	2.5	2.7	1.6	1.9
13	11.18	unknown (56, 70, 98, 112, 139, 154 M^+)	1.5	1.3	1.1	0.8	1.2
14	11.48	methyl phenylethanoate	213	173	214	153	189
15	11.62	methyl 2-hydroxybenzoate	0.3	0.3	0.4	0.2	0.3
16	12.07	methoxybenzaldehyde	0.9	2.9	2.6	2.0	2.1
17	12.09	ethyl phenylethanoate	0.2	0.2	0.2	0.2	0.2
18	12.39	methyl 3-phenylpropionate	0.2	0.2	0.3	0.1	0.2
19	12.52	2-methoxy-3,5,5-trimethylcyclohex-2-ene-1,4-dione (**12**)	1.6	1.4	1.6	1.2	1.5
20	12.56	unknown (69, 83, 100, 125, 128, 152)	1.1	0.8	1.0	0.7	0.9
21	12.65	3-phenylprop-2-en-1-ol	2.4	3.2	3.7	2.5	3.0
22	12.73	unknown (43, 85, 109, 127, 141, 169)	1.1	1.3	1.5	1.0	1.2
23	12.84	trimethylphenol	0.3	0.2	0.3	0.2	0.3
24	13.07	unknown (43, 71, 96, 109, 121, 139)	7.1	5.4	5.8	4.3	5.7
25	13.28	methyl 2-hydroxy-3-phenylpropionate	2.2	5.1	6.8	3.2	4.3
26	13.31	methyl 3-methoxybenzoate	0.7	0.6	0.7	0.7	0.7
27	13.43	methyl 3-phenylprop-2-enoate	1.8	5.2	5.2	4.2	4.1
28	13.59	unknown (43, 97, 139, 181, 196 M^+)	3.1	0.9	0.6	0.8	1.4
29	13.63	unknown (41, 83, 123, 151, 180 M^+)	1.5	1.4	1.8	1.2	1.5
30	14.15	methyl 3-hydroxybenzoate	2.1	2.1	2.7	2.4	2.3
31	14.40	unknown (55, 70, 95, 127, 196 M^+)	3.8	3.6	4.2	3.3	3.7
32	14.52	4-(3-oxo-1-butynyl)-3,5,5-trimethylcyclohex-2-en-1-one (**8**)	1.2	1.2	1.1	1.4	1.2
33	15.07	methyl laurate (12:0)	0.9	0.7	0.7	0.7	0.8
34	15.41	methyl 3,5-dimethoxybenzoate	1.8	1.7	1.7	1.2	1.6
35	15.47	methyl 3,4-dimethoxybenzoate	1.1	1.5	1.3	0.6	1.1
36	15.62	fatty acid? (55, 74, 87, 111, 129, 152, 172)	1.3	0.3	0.5	0.5	0.7
37	16.00	4-(3-oxobut-1-enylidene)-3,5,5-trimethylcyclohex-2-en-1-one (**2**)	27	35	36	32	33
37b	16.01	4-(3-hydroxybut-1-enyl)-3,5,5-trimethylcyclohex-2-en-1-one (**9**)	*	*	*	*	*
38	16.08	unknown (55, 67, 81, 95, 127, 168, 198)	4.1	3.4	4.3	3.8	3.9
39	16.23	unknown (45, 77, 93, 119, 147, 162 M^+)	1.5	2.4	1.9	2.2	2.0
40	16.49	4-hydroxy-4-(3-oxo-1-butynyl)-3,5,5-trimethylcyclohex-2-en-1-one (**7**)	1.6	1.7	1.4	1.5	1.6
41	16.53	4-hydroxy-4-(3ξ-hydroxy-1-butenyl)-3,5,5-trimethylcyclohex-2-en-1-one (isomer 1[b]) (**3**)	4.4	0.5	0.3	0.3	1.4
42	16.71	methyl 3,4,5-trimethylbenzoate	1.9	1.6	1.9	1.8	1.8
43	17.07	methyl myristate (14:0)	16.3	3.5	4.2	3.8	7.0
44	77.18	4-hydroxy-4-(3ξ-hydroxy-1-butenyl)-3,5,5-trimethylcyclohex-2-en-1-one (isomer 2[b]) (**4**)	30	36	60	39	41
45	17.29	4-hydroxy-4-(3-oxo-1-butenyl)-3,5,5-trimethylcyclohex-2-en-1-one (**1**)	107	158	148	185	149
46	17.35	isomer of peak 45	9.0	7.0	8.1	8.0	8.0
47	18.08	methyl pentadecanoate (15:0)	0.5	0.7	0.3	0.6	0.5
48	18.25	methyl 3-(3,4-dimethoxyphenyl)-prop-2-enoate	0.5	0.5	0.4	0.8	0.6
49	18.44	unknown (43, 91, 134, 162, 278 M^+)	0.4	0.6	0.8	0.9	0.7
50	18.81	methyl palmitioleate (16:1)	0.6	1.6	1.1	0.8	1.0
51	19.09	methyl palmitate (16:0)	6.0	6.9	4.5	5.2	5.7
52	20.10	methyl margarate (17:0)	internal standard				
53	20.30	methyl abscisate (**5**)	1.6	4.2	5.0	5.7	4.1
54	20.70	methyl linoleate (18:2)	1.1	1.1	0.8	1.5	1.1
55	20.73	methyl α-linolenate (18:3)	1.1	6.4	2.6	3.3	3.4
56	20.79	methyl oleate (18:1)	6.9	16.5	0.8	7.3	7.9
57	20.86	methyl oleate isomer (18:1)	1.7	1.0	–	–	1.4
58	21.00	*n*-heneicosane (C_{21})	0.9	1.6	0.4	1.2	1.0
59	21.09	methyl stearate (18:0)	2.7	5.5	4.7	5.5	4.6
59b	21.12	methyl abscisate (**6**)	#	#	#	#	#
60	23.00	*n*-triacosane (C_{23})	3.1	3.0	1.2	2.1	2.4

[a] Prominent ions are given in parentheses for unknown compounds; alternatively, structure numbers appear in bold type while fatty acid designations or hydrocarbon numbers are given in plain type. [b] Absolute configuration at C3 not determined. [c] *, unresolved peak, the presence of which was verified by GC/MS analysis; #, trace component (<0.1 μg/g honey).

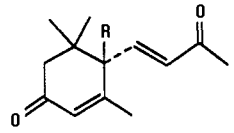
also ^4J coupled with one of the adjacent methylene protons. Such a ^4J coupling between an axial methylene proton and the protons of 1,2-trans-oriented methyl group is typical of that occurring in steroids (Marat et al., 1987) and triterpenes (Wilkins et al., 1989). These protons can be aligned such that they exhibit a planar *W*-type interaction. The other (equatorial) methylene proton exhibited a ^4J coupling (0.9 Hz) with the conjugated olefinic proton that resonated at 5.95 ppm. Such a coupling is typical of that which occurs between protons flanking a carbonyl group. The olefinic proton also displayed a resolvable ^4J coupling (1.4 Hz) with the olefinic methyl group. The foregoing four-bond connectivities require that peak 45 is 4-hydroxy-4-(3-oxobut-1-enyl)-3,5,5-trimethylcyclohex-2-

Table II. ^{13}C and ^{1}H NMR Chemical Shifts in $CDCl_3$ of Peaks 45 and 37

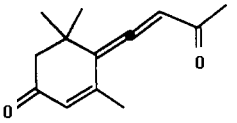
site	δ(45)		δ(37)	
	^{13}C	^{1}H	^{13}C	^{1}H
1	197.5[a]		197.4	
2	127.8	5.96	127.3	6.20
3	160.5		148.4	
4	79.3		117.1	
5	41.5		37.1	
6	49.6	2.48, 2.35[b]	50.9	2.45
7	24.4	1.02	28.8	1.24
8	18.7	1.10	21.5	1.28
9	23.0	1.88	27.2	2.02
1′	145.1	6.82[c]	214.2	
2′	130.5	6.45[c]	102.5	6.00
3′	197.1[a]		196.9	
4′	28.4	2.30	28.8	2.27

[a] Assignments are interchangeable. [b] AB q, J = 17.5 Hz. [c] AB q, J = 15.8 Hz.

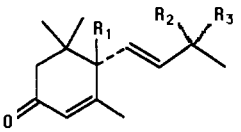
en-1-one (1). In accord with this conclusion, there appeared in the mass spectrum of this compound strong losses of ketene (from the oxobutenyl side chain) and butene (from the six-membered ring). Levels of this component in honeys ranged from 100 to 180 μg/g. A minor component (peak 46) exhibited an identical mass spectrum; since ^{1}H NMR establishes that peak 45 possesses a trans bond, it is possible that peak 46 is the cis analogue.



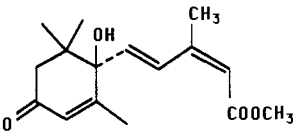
(1) R = OH
(13) R = H



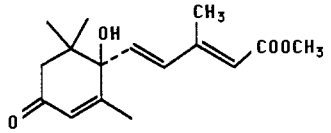
(2)



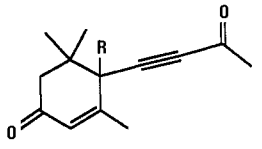
(3) $R_1 = R_2$ = OH, R_3 = H
(4) $R_1 = R_3$ = OH, R_2 = H
(9) $R_1 = R_2$ = H, R_3 = OH



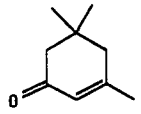
(5)



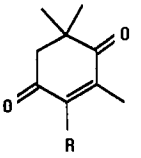
(6)



(7) R = OH
(8) R = H



(10)



(11) R = H
(12) R = OCH_3

Peak 37. High-resolution mass spectroscopy established the molecular formula $C_{13}H_{16}O_2$ (m/e 204, M_+), while ^{13}C and ^{1}H NMR spectroscopies established the presence of an array of functional groups similar to that found for 1 save that the tertiary hydroxyl group and the disubstituted double bond had been replaced by an allenic system. Structure 2 was thus assigned to peak 37. Consistent with this conclusion, a DQFCOSY NMR experiment established that within the six-membered ring system there existed a network of ^{4}J couplings identical with those established for 1, while the allenic proton proved to be long range coupled with the olefinic proton, the olefinic methyl group, and the methyl ketone protons. The mass spectrum of this compound exhibited a strong loss of ketone but lacked a butene loss. Levels of this component in honeys ranged from 27 to 36 μg/g.

Peaks 41 and 44. The mass spectra of peaks 41 and 44 were indistinguishable; both displayed molecular ions at m/z 224 ($C_{13}H_{20}O_3$ by accurate mass measurement). Reduction of one of the carbonyl groups in structure 1 ($C_{13}H_{18}O_3$) was indicated. Since the strong ketene loss of 1 was in both peaks 41 and 44, replaced by a 44-amu loss (C_2H_4O by accurate mass measurement), it can be concluded that these compounds possess structures 3 and 4. These alcohols are a pair of diastereoisomers (C3 is asymmetric); hence, they are resolved on the GC column. We have not determined however, the absolute congifuration of the alcohols. Compound 4 (the later eluting isomer) predominated in the honey with levels of 30–60 μg/g.

Abscisic Acid Isomers. The presence of 1–4, all of which are related to abscisic acid, a well-known plant growth hormone, prompted a search for this substance. Methylation of authentic specimens of *trans,cis-* and *trans,trans*-abscisic acid afforded methyl ester 5 and 6, respectively. GC/MS analysis on two capillary columns of differing polarity, together with high-resolution mass measurements and selected ion chromatograms, revealed the presence in the extracts of low levels of the trans,cis and trans,trans esters (peaks 53 and 59b, respectively). Since GC analysis of an unmethylated honey extract was devoid of these peaks, abscisic acid must be present in heather honey predominantly as the free-acid isomers.

Other Extractives. High-resolution mass spectroscopy established the presence of two other compounds possessing 13 carbon atoms. These substances exhibited molecular ions at m/z 204 ($C_{13}H_{16}O_2$, peak 32) and m/z 220 ($C_{13}H_{16}O_3$, peak 40). Since peak 40 possessed two fewer hydrogens than 1, another ring or double bond must be present. The mass spectra of both compounds included a distinctive loss of a C_4H_8 (butene) fragment. Mass spectral studies (Enzell and Wahlberg, 1986) have demonstrated that a butene loss is characteristic of degraded carotenoids possessing a 3,5,5-trimethylcyclohex-2-en-1-one ring system. Thus, a six-membered ring system similar to that established for 1–4 was present, and it follows that the additional double bond must be located in the side chain. This proposition lead to the conclusion that peak 40 was the acetylenic analogue of 1 and possessed structure 7.

The presence of an acetylenic bond in 7 appears to inhibit the loss of the side chain methyl ketone as ketene. A weak CH_3CO^+ ion loss and a strong CO loss also occur in the mass spectrum of 7. Peak 32 also lacked a ketene loss but displayed strong butene and CO losses and a weak CH_3CO^+ ion loss. Since this compound possesses two oxygen atoms, it follows that is has structure 8. Selected ion GC/MS analysis demonstrated the presence of a shoulder peak (not resolved in GC/FID traces) substance of molecular weight 208 with a mass spectrum identical with a library mass spectrum of 4-(3-hydroxybut-1-

enyl)-3,5,5-trimethylcyclohex-2-en-1-one (**9**).

Several of the early-eluting compounds also gave mass spectra showing strong losses of butene and CO. Two of these compounds were characterized. Peak 10 (M^+ 138, $C_9H_{14}O$) proved to be identical with an authentic specimen (Sigma, St. Louis, MO) of 3,5,5-trimethylcyclohex-2-en-1-one (**10**) (isophorone), while peak 11a (M^+ 152, $C_9H_{12}O_2$) was considered to be the diketo analogue (**11**). Another substance, peak 19 (M^+ 180), gave a mass spectrum identical with a library spectrum of 2-methoxy-3,5,5-trimethylcyclohex-2-ene-1,4-dione (**12**).

The presence of the foregoing extractives prompted a search for carotenoids in the honey samples; however, none were detected by an established nonaqueous reversed-phase/high-pressure liquid chromatography procedure (Lauren et al., 1986). Detection limits were below 1 $\mu g/g$ for β-carotene and a range of hydroxy- or carboxycarotenes.

DISCUSSION

The heather extractives included a number of compounds previously detected in other types of unifloral honeys: among others, a range of aliphatic and aromatic acids. The diacids that were prominent components of clover and manuka honey extractives were not detected in the heather honeys. In a recent report, Steeg and Montag (1987) described the composition of both free and bound acids from a variety of European honeys including heather. The levels of benzoic acid and phenylacetic acid found in their study were similar to those found in this study; however, the level of 2-hydroxy-3-phenylpropionic acid reported here is much less. We have observed levels of 2-hydroxy-3-phenylpropionic acid akin to those reported by Steeg and Montag in some honey samples that apiarists described as heather honey.

The four samples utilized in our study all possessed similar levels of 3,5,5-trimethylcyclohex-2-en-1-one derivatives (see Table I). Substances possessing structures of this type are frequently referred to as degraded carotenoids and have been reported from a number of plant sources. For example, an array of closely related compounds possessing allenic, olefinic, or acetylenic side chains were isolated from tobacco by Enzell and Wahlberg (1986). Compounds of uncertain structure were detected by Graddon et al. (1979) in several Australian honeys. The published mass spectra of compounds suggested to be 4-butylcyclopenten-3-one and 4-butylcyclohexen-3-one corresponded exactly with those we determined for compounds **10** (isophorone) and **11a**, respectively. Additionally, we note that the mass spectrum from Graddon et al. (1979) of a compound of uncertain structure and molecular weight 206 corresponds exactly with that presented by Enzell and Wahlberg (1986) for 3-oxo-α-ionone (**13**).

Since degraded carotenoids are absent in New Zealand white clover, manuka, kanuka, and most other unifloral honeys, the implication of the present findings is that they originate specifically from the heather plant source. The degraded carotenoids are probably of specific biosynthetic origin. However, no intact carotenoids were detected in the honeys. Two commonly encountered C_{13} plant substances of related structure are α- and β-ionone: both are described as possessing sweet floral fragrances reminiscent of violets (Bauer and Garbe, 1985). There compounds were not detected in the heather honey. New Zealand heather honey is described by Walsh (1967) as being "reddish in color and of mild but pronounced flavor". Possibly the degraded carotenoids detected in this study contribute to the heather honey flavor. The apparently characteristic distribution of these compounds points to their utility in distinguishing heather honey from less valuable honeys derived from other floral sources.

ACKNOWLEDGMENT

We acknowledge with appreciation the assistance from the following people: G. M. Reid, National Apicultural Advisory Officer, Ministry of Agriculture and Fisheries, Hamilton, New Zealand; Wilson and Neill-Hororata Honey Exports Ltd., Hororata, New Zealand (for honey samples); N. T. Moar, Botany Division, DSIR, Christchurch, New Zealand (for pollen analyses); D. McNaughton and D. R. Lauren, Ministry of Agriculture and Fisheries, Hamilton, New Zealand (for carotenoid analyses); and J. Coddington, Chemistry Department, University of Auckland, New Zealand (for NMR spectra).

Registry No. **1**, 14398-38-0; *cis*-**1**, 120925-93-1; **2**, 120853-11-4; **3**, 24427-77-8; **5**, 21293-29-8; **6**, 6755-41-5; **7**, 120853-12-5; **8**, 120853-10-3; **9**, 34318-21-3; **10**, 78-59-1; **11**, 1125-21-9; **12**, 41654-27-7; C6:0, 142-62-1; C8:0, 124-07-2; C12:0, 143-07-7; C14:0, 544-63-8; C15:0, 1002-84-2; C16:0, 57-10-3; C17:0, 506-12-7; C18:2, 60-33-3; C18:3, 463-40-1; C18:1, 27104-13-8; C18:0, 57-11-4; benzaldehyde, 100-52-7; phenol, 108-95-2; benzyl alcohol, 100-51-6; phenylacetaldehyde, 122-78-1; benzoic acid, 65-85-0; phenylethanol, 60-12-8; *n*-undecane, 1120-21-4; phenylacetic acid, 103-82-2; 2-hydroxybenzoic acid, 69-72-7; ethyl phenylacetate, 101-97-3; 3-phenylpropanoic acid, 501-52-0; 3-phenylprop-2-en-1-ol, 104-54-1; trimethylphenol, 26998-80-1; 2-hydroxy-3-phenylpropionic acid, 156-05-8; 3-methoxybenzoic acid, 586-38-9; 3-phenyl-2-propenoic acid, 621-82-9; 3-hydroxybenzoic acid, 99-06-9; 3,5-dihydroxybenzoic acid, 99-10-5; 3,4-dihydroxybenzoic acid, 99-50-3; 3,4,5-trimethylbenzoic acid, 1076-88-6; (3,4-dihydroxyphenyl)prop-2-enoic acid, 331-39-5; palmitoleic acid, 373-49-9; oleic acid, 112-80-1; *n*-heneicosane, 629-94-7; *n*-triacosane, 638-67-5; hydroxybenzaldehyde, 28777-87-9.

LITERATURE CITED

Bauer, K.; Garbe, D. *Common Fragrance and Flavor Materials*; VCH Verlagsgesellschaft: Weinheim, West Germany, 1985.

Bicchi, D.; Belliardo, F.; Frattini, C. Identification of the Volatile Components of Some Piedmontese Honeys. *J. Apic. Res.* **1983**, *22*(2), 130–136.

Bonaga, G.; Giumanini, G. A. The Volatile Fraction of Chestnut Honey. *J. Apic. Res.* **1986**, *25*(2), 113–120.

Bonaga, G.; Giumanini, G. A.; Grazia, G. Chemical Composition of the Chestnut Honey: Analysis of the Hydrocarbon Fraction. *J. Agric. Food Chem.* **1986**, *34*, 319–326.

Enzell, C. R.; Wahlberg, I. Mass Spectra of Degraded Tobacco Isoprenoids. *Mass Spectrom. Rev.* **1986**, *5*, 39–72.

Graddon, D.; Morrison, J. D.; Smith, J. F. Volatile Constituents of Some Unifloral Australian Honey. *J. Agric. Food Chem.* **1979**, *27*(4), 832–837.

Lauren, D. R.; Agnew, M. P.; McNaughton, D. E. *J. Liq. Chromatogr.* **1986**, *9*(a), 1997–2012.

Marat, K.; Templeton, J. F.; Sashi Kumar, V. P. *Magn. Reson. Chem.* **1987**, *25*, 25–29.

Moar, N. T. Pollen Analysis of New Zealand Honey. *N.Z. J. Agric. Res.* **1985**, *28*, 39–70.

Steeg, E.; Montag, A. Aromatic Carbonic Acids of Honey. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 17–19.

Tan, S. T.; Holland, P. T.; Wilkins, A. L.; Molan, P. C. Extractives from New Zealand Honeys. 1. White Clover, Manuka, and Kanuka Honeys. *J. Agric. Food Chem.* **1988**, *36*, 453.

Tsuneya, T.; Shibai, T.; Yoshioka, A.; Shiga, M. The Study of Shina (Linden, *Tilia japonica Simk.*) Honey Flavour. *Koryo* **1974**, *109*, 29–34.

Tulloch, A. P.; Hoffman, L. L. Canadian Beeswax: Analytical Values and Composition of Hydrocarbons, Free Acids and Long Chain Esters. *J. Am. Oil Chem. Soc.* **1972**, *49*, 696–699.

Walsh, R. S. *Nectar and Pollen Sources*; National Beekeepers Association of New Zealand: Upper Hutt, N.Z. 1967.

Wilkins, A. L.; Bremer, J.; Ralph, J.; Holland, P. T.; Ronaldson, K. J.; Jager, P. M.; Bird, P. W. *Aust. J. Chem.* **1989**, in press.

Received for review May 27, 1988. Accepted January 4, 1989.