# Extractives from New Zealand Honeys. 3. Unifloral Thyme and Willow Honey Constituents

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The levels of extractable organic substances in some New Zealand thyme and willow honeys have been determined. Substances detected include aromatic acids and phenols, aliphatic acids and diacids, and degraded carotenoids. Among the degraded carotenoids 1-(3-oxo-1-butenyl)-2,6,6-trimethyl-1,2-epoxycyclohexan-4-ol (two isomers) and 1-(3-oxo-*trans*-1-butenyl)-2,6,6-trimethylcyclohexane-*trans*,*cis*-1,2,4-triol were detected in thyme honey extracts while *trans*,*cis*-abscisic acid and *trans*,*trans*-abscisic acid were detected in willow honey extracts. The GC profiles of thyme and willow honeys readily distinguish them from other New Zealand unifloral honeys.

Our previous investigations (Tan et al., 1988, 1989a) of extractable organic substances present in New Zealand unifloral honeys have revealed a range of compounds that appear characteristic of the floral source. We have reported levels of a range of aliphatic, aromatic, and/or degraded carotenoid-like substances in clover, manuka, kanuka, and heather honeys (Tan et al., 1988, 1989a). Other workers have reported the levels of aromatic acids, both free and bound, 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). We now report the presence in some New Zealand thyme and willow honey samples of a range of aliphatic and aromatic substances together with some degraded carotenoids that appear to be unique to these honeys.

## MATERIALS AND METHODS

**Source of Honey Samples.** A commercial willow honey sample (W1) and five commercial thyme honey samples (T1–5) (unheated, 1986–1987 summer season) were obtained from Wilson Neill-Hororata Honey Exports Ltd. A further six thyme honey samples (T6–11) were obtained directly from beekeepers. All samples originated from the Central Otago district, South Island, New Zealand.

The floral authenticity of the commercial honeys was verified by pollen analysis (see Table I). Thyme honey is known to be underrepresented in terms of pollen analysis (Moar, 1985; Maurizio, 1975), a minimum of 20% thyme pollen being required (Moar, 1985) for classification as a unifloral honey. Chemical analysis (see Table II) subsequently verified that although not formally classifiable as unifloral, sample T4 (18% thyme pollen) included a dominant thyme contribution. Pollen analysis also established that samples T1–5 included some contributions from willow and white clover type plants.

**Methods and procedures** used in the analysis of the heather honey samples were as described in part 1 (Tan et al., 1988), other than the use of a 250-mL extractor with a 12-h extraction time. 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); peak area integration was carried out by using a Shimadzu CR-3A reporting integrator. 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. High-resolution GC–MS was performed on a Kratos MS80RFA instrument coupled to 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).

**Table I. Pollen Composition of Thyme and Willow Honeys Expressed as Percent Total Pollen of Nectar Plants Counted**

| pollen type | W1 | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|---|
| thyme (*Thymus*) | | 26 | 28 | 28 | 18 | 27 |
| willow (*Salix*) | 90 | 41 | 33 | 24 | 28 | 5 |
| white clover type (*Trifolium*) | | 13 | 6 | 22 | 26 | 7 |
| matagouri (*Discaria*) | | | 13 | 12 | 10 | 27 |
| rosaceae | | 5 | | 7 | 13 | 8 |
| gorse type (*Ulex* type) | | | | | | 20 |
| remainder | 10 | 15 | 10 | 7 | 5 | 6 |

## RESULTS

Figures 1 and 2 are the GC–FID profiles of the derivatized extractable organic substances recovered from the thyme and willow honey samples T5 and W1, respectively. Peaks eluting after peak 93 (*n*-tricosane, $C_{23}$) were shown to be higher chain length hydrocarbons [$C_{25}$ (peak 96), $C_{27}$ (peak 98), $C_{29}$ (peak 10), $C_{31}$ (peak 104), and $C_{33}$ (peak 106)] or fatty acids [22:0 (peak 97), 24:0 (peak 99), 26:0 (peak 101), and 28:0 (peak 105)]. A similar collection of hydrocarbons and fatty acids (detected as the corresponding methyl esters since the extracts were methylated with diazomethane prior to analysis) were found in other unifloral honeys (Tan et al., 1988). Since the concentrations and distribution of high molecular weight hydrocarbons and fatty acids have been well documented (Tan et al., 1988; 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 II) including methyl 3-(3,4-dimethoxyphenyl)-*trans*-prop-2-enoate (**1**) (peak 86), methyl 4-hydroxy-3,5-dimethoxybenzoate (**2a**) (peak 76), methyl 3,4,5-trimethoxybenzoate (**2b**) (peak 72), methyl 4-hydroxy-3-methoxybenzoate (**3a**) (peak 59), and methyl 3,4-dimethoxybenzoate (**3b**) (peak 64) (see Chart I for structures) were detected in the methylated thyme honey extracts. While it is reasonable to assume that the parent acids occur in the honey samples (carboxyl groups are readily methylated by

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**Table II. Concentration (Micrograms per Gram) of Methylated Components in Willow and Thyme Honey Extracts**

| peak | compound[a] | sample | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | W1 | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 | T11 |
| 1 | unknown[b] | 7.4 | 7.5 | 11 | 8.0 | 7.0 | 4.7 | 4.5 | 7.6 | 7.2 | 2.1 | 0.9 | 3.9 |
| 2 | unknown (43, 58, 59, 101) | 1.1 | 1.4 | 0.5 | 2.0 | 1.5 | 0.5 | | 0.3 | 1.5 | | | |
| 3 | methyl 3-hydroxybutanoate | | 0.7 | | | | | 0.1 | 0.1 | 0.6 | 0.7 | 0.5 | 0.5 |
| 4 | methyl 3-hydroxy-3-methylbutanoate | | 0.5 | 0.2 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.4 | 0.4 | 0.4 | 0.3 |
| 5 | 2,5-cyclohexadiene-1,4-dione | | 0.3 | | | | | 0.2 | 0.4 | | | | |
| 6 | methyl caproate (6:0) | | 0.8 | 0.8 | 1.0 | 0.9 | 1.1 | 1.6 | 1.8 | 0.7 | 0.8 | 0.9 | 0.7 |
| 7 | methyl 3-hexenoate | | 4.6 | 3.8 | 4.7 | 4.3 | 5.7 | 6.5 | 8.1 | 3.9 | 3.7 | 4.3 | 3.5 |
| 8 | methyl 3-furancarboxylate | | 1.5 | 1.6 | 0.6 | 0.4 | 0.1 | 0.2 | 0.3 | 1.1 | 1.0 | 1.1 | 0.8 |
| 9 | unknown (41, 69, 84, 96, 128) | | 1.0 | | | | | | | 0.6 | 0.7 | 0.6 | 0.5 |
| 10 | dimethyl butanedioate | 0.1 | 7.0 | 0.3 | 0.4 | 0.4 | 0.1 | 0.3 | 0.3 | 8.0 | 9.1 | 8.4 | 2.3 |
| 11 | benzyl alcohol | 0.1 | | | | | | | | | | | |
| 12 | phenylacetaldehyde | | | | | | | trace[c] | 0.1 | trace | trace | trace | 0.4 |
| 13 | methyl 2-methylsuccinaldehydate | | 0.2 | | | | | | | 0.2 | 0.2 | 0.2 | 0.1 |
| 14 | methyl benzoate | 0.2 | 1.5 | 1.3 | 1.5 | 1.4 | 0.6 | 0.8 | 1.1 | 2.6 | 2.8 | 3.0 | 2.4 |
| 15 | 2-phenylethanol | 0.1 | 0.1 | | 0.1 | 0.1 | | | | | | | |
| 16 | unknown (43, 67, 71, 82, 125) | 0.1 | 3.3 | 2.0 | 1.7 | 1.3 | 0.5 | 0.8 | 1.4 | 2.7 | 3.1 | 3.6 | 3.3 |
| 17 | unknown (51, 53, 80, 91, 108, 109) | 2.5 | | | | | | | | | | | |
| 18 | methyl pyridinecarboxylate | | 2.3 | 0.8 | trace | 0.1 | 0.1 | 0.2 | 0.2 | *[d] | * | * | * |
| 19 | *n*-undecane ($C_{11}$) | internal standard | | | | | | | | | | | |
| 20 | dimethyl pentanedioate | | | | | | | | | 0.4* | 0.5* | 0.6* | 0.4* |
| 21 | methyl caprylate (8:0) | | 0.3 | | | | | trace | trace | 0.9 | 1.1 | 0.8 | 0.9 |
| 22 | methyl 2-phenylethanoate | 0.5 | 1.4 | 2.7 | 1.9 | 1.5 | 0.3 | 0.8 | 0.6 | 1.8 | 2.7 | 1.9 | 1.7 |
| 23 | unknown (43, 59, 83, 101, 141, 159) | 0.4 | 0.4 | 0.7 | 0.8 | 0.8 | 0.3 | 0.3 | 0.1 | 2.2 | 0.9 | 1.1 | 1.2 |
| 24 | unknown (43, 55, 67, 71, 82, 109) | 0.5 | 17 | 13 | 14 | 12 | 5.8 | 8.0 | 8.3 | 21 | 22 | 22 | 20 |
| 25 | 5-(hydroxymethyl)-2-furfural | | 0.2 | | | | | | 0.1 | | | | |
| 26 | unknown (67, 79, 94, 108, 136, 168 $M^+$) | | 0.6 | 0.6 | 0.8 | 0.7 | 0.4 | 0.7 | 0.6 | 1.0 | 1.0 | 1.1 | 0.9 |
| 27 | methyl nonanoate (9:0) | 0.2* | | | | | | | | | | | |
| 28 | methoxybenzaldehyde | * | | | | | | | | | | | |
| 29 | dimethyl hexanedioate | | 0.5 | | | | | 0.1 | 0.1 | | | | |
| 30 | unknown (43, 55, 61, 71, 94, 103, 154) | | 2.0 | 0.9 | 0.9 | 0.8 | 0.4 | 0.5 | 0.6 | 2.0 | 2.3 | 2.5 | 1.9 |
| 31 | unknown (55, 85, 98, 111, 115, 151, 183) | | 0.2 | 0.7 | 0.7 | 0.6 | 0.2 | 0.3 | 0.2 | 1.3 | 2.1 | 1.6 | 1.9 |
| 32 | methyl 3-phenylpropionate | | | | 0.1 | 0.1 | trace | 0.1 | 0.3 | 0.3 | 0.2 | 0.2 | 0.8 |
| 33 | 1,4-dihydroxybenzene | | 5.5 | | | | | | | | | | |
| 34 | 2′-methoxyacetophenone | 0.2 | | | | | | | | | | | |
| 35 | unknown (43, 71, 95, 109, 123, 151, 169) | | 2.9 | 0.1 | * | * | 0.5 | 0.3 | 0.3 | 1.9 | 2.5 | 1.3 | 2.7 |
| 36 | 2-methoxy-3,5,5-dimethylcyclohex-2-ene-1,4-dione | 1.4 | | | | | | | | | | | |
| 37 | 3′-aminoacetophenone | | 2.6 | 2.5 | 2.8 | 2.0 | 0.7 | 1.4 | 0.9 | 4.8 | 5.1 | 4.8 | 4.9 |
| 38 | methyl *cis*-3-phenylprop-2-enoate | | | | | | | trace | 0.1 | | | | |
| 39 | unknown (43, 55, 85, 127, 140, 169) | 0.1 | 1.1 | 0.4 | 0.4 | 0.2 | 0.2 | 0.3 | 0.2 | 1.5 | 2.3 | 1.2 | 1.5 |
| 40 | unknown (43, 59, 87, 118, 130) | 0.1 | 2.9 | 0.7 | 0.7 | 0.7 | 0.3 | 0.2 | 0.5 | 0.7 | 0.6 | 1.1 | 0.6 |
| 41 | unknown (43, 71, 102, 109, 135, 139, 170) | 0.6 | | | | | | | | | | | |
| 42 | unknown (43, 67, 71, 119, 137, 152, 180) | 0.6 | 5.6 | 7.7 | 6.5 | 5.1 | 2.4 | 3.7 | 3.0 | 10.2 | 10.2 | 12 | 10.7 |
| 43 | methyl 2-hydroxy-3-phenylpropionate | | | | 0.4 | 0.6 | trace | | | 1.7* | 1.7* | 1.8* | 1.7* |
| 44 | methyl 4-methoxybenzoate | 0.1 | | 0.7 | 0.4 | 0.5 | 0.3 | 0.3 | 0.3 | * | * | * | * |
| 45 | unknown (69, 81, 85, 117, 121, 181, 199) | | 0.5 | 0.4 | 0.3 | 0.2 | 0.1 | 0.1 | | 0.9 | 0.9 | 1.1 | 0.9 |
| 46 | methyl *trans*-3-phenylprop-2-enoate | | 0.6 | 0.4 | * | * | * | 0.1 | 0.7 | * | * | * | * |
| 47 | unknown (43, 59, 71, 94, 111, 155) | 0.3 | 2.2 | 1.7 | 1.6* | 1.0* | 0.5* | 0.3 | 2.0 | 0.8* | 0.8* | 1.0* | 0.9* |
| 48 | unknown (43, 55, 69, 85, 117, 121, 181, 199) | | 5.4 | 6.7 | 7.0 | 6.9 | 2.8 | 2.9 | | 0.6 | 0.8 | 0.8 | 0.6 |
| 49 | unknown (51, 77, 91, 107, 138) | 5.2 | | | | | | | | | | | |
| 50 | dimethyl octanedioate | trace | 0.9 | 0.2 | 0.3 | 0.3 | 0.1 | 0.1 | 0.2 | 0.7 | 0.7 | 0.7 | 0.8 |
| 51 | 1-(3-oxo-1-butenyl)-2,6,6-trimethyl-1,2-epoxycyclohexan-4-ol (isomer 1) | | | trace | | | | * | 0.3 | | | | |
| 52 | methyl 4-hydroxybenzoate | * | * | | | | | * | * | | | | |
| 53 | unknown (43, 71, 95, 117, 138, 166) | 1.6* | 4.7* | 3.9 | 3.1 | 2.9 | 1.1 | 1.3* | 1.3* | 4.8 | 6.1 | 5.1 | 5.0 |
| 54 | unknown (43, 59, 79, 109, 121, 152) | 4.6 | | | | | | | | | | | |
| 55 | unknown (55, 83, 111, 126, 143, 158) | | | 0.2 | 0.8 | 0.8 | 0.1 | 0.1 | 0.2 | 1.6 | 1.8 | 1.9 | 1.7 |
| 56 | unknown (41, 55, 107, 121, 151, 166) | 7.6 | | | | | | | | | | | |
| 57 | unknown (59, 81, 108, 136, 140, 168) | | 1.5 | 0.8 | | 0.4 | 0.1 | 0.5 | 0.2 | 1.1 | 1.5 | 1.5 | 1.8 |
| 58 | unknown (43, 65, 92, 120, 135, 163) | | 1.4 | 5.0 | 5.6 | 4.1 | 2.6 | 4.4 | 2.7 | 4.4 | 4.6 | 4.1 | 3.1 |
| 59 | methyl 4-hydroxy-3-methoxybenzoate | | 1.0 | 2.1 | 1.7 | 1.2 | 1.3 | 1.1 | 0.9 | 1.1 | 1.4 | 1.3 | 1.2 |
| 60 | 1-(3-oxo-1-butenyl)-2,6,6-trimethyl-1,2-epoxycyclohexan-4-ol (isomer 2) | | 0.6 | 0.8 | 1.1 | 0.8 | 0.4 | 1.7 | 0.6 | 3.5 | 1.4 | 4.8 | 4.1 |
| 61 | methyl laurate (12:0) | | 0.1 | 0.7 | | | | trace | 0.8 | 0.2 | 0.2 | 0.2 | 0.1 |
| 62 | dimethyl nonanedioate | | 0.3 | 0.1 | 0.1 | 0.2 | trace | 0.1 | 0.1 | 0.3 | 0.3 | 0.3 | 0.2 |
| 63 | unknown (55, 81, 95, 113, 138, 166, 192) | 3.0 | 0.9 | | | | | 0.2 | | | | | |
| 64 | methyl 3,4-dimethoxybenzoate | | 0.4 | 0.9 | 1.6 | 1.5 | 0.6 | 0.7 | 0.3 | 0.5 | 0.9 | 0.6 | 0.7 |
| 65 | unknown (41, 69, 121, 175, 193, 208) | 0.4 | | | | | | | | | | | |
| 66 | methyl 2-hydroxy-3-(4-methoxyphenyl)propionate | | | | | | | | | 0.5 | 0.6 | 0.2 | 0.2 |
| 67 | 4-(3-oxo-1-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one | 0.9 | | | | | | | | | | | |
| 68 | dimethyl decanedioate | 0.4 | 4.4 | 1.3 | 1.8 | 1.5 | 0.4 | 0.6 | 1.1 | 2.0 | 2.3 | 2.9 | 2.5 |
| 69 | methyl 3-(4-methoxyphenyl)-*trans*-prop-2-enoate | 0.7 | 0.2 | 0.1 | 0.2 | 0.2 | trace | 0.1 | 0.1 | 0.3 | 0.3 | 0.2 | 0.3 |
| 70 | methyl 3-hydroxy-3-(methoxyphenyl)propionate | 0.1 | 0.3 | trace | | 0.1 | trace | 0.1 | 0.2 | 0.6 | 0.6 | 0.6 | 0.2 |
| 71 | dimethyl *trans*-2-decenedioate | 0.7 | 14 | 3.3 | 3.5 | 2.5 | 0.7 | 1.1 | 2.3 | 8.2 | 7.6 | 9.8 | 8.5 |
| 72 | methyl 3,4,5-trimethoxybenzoate | 0.5 | 0.3 | 4.1 | 4.4 | 3.6 | 2.4 | 3.2 | 0.5 | 0.4 | 0.3 | 0.5 | 0.5 |

**Table II. (Continued)**

| peak | compound[a] | sample W1 | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 | T11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 73 | unknown (43, 107, 125, 167, 208) | 1.8 | | | | | | | | | | | |
| 74 | methyl 3-(4-hydroxyphenyl)-*trans*-prop-2-enoate | 1.5 | 0.7 | 0.2 | | 0.4 | 0.1 | 0.1 | 0.2 | 0.7 | | 0.3 | 0.4 |
| 75 | methyl myristate (14:0) | 0.2 | 0.4 | 0.5 | 0.1 | 0.3 | 0.2 | 0.2 | 0.2 | 0.5 | 0.4 | | 0.4 |
| 76 | methyl 4-hydroxy-3,5-dimethoxybenzoate | | 4.2 | 0.7 | 2.4 | 3.1 | 0.7 | 0.4 | 2.8 | 11 | 14 | 12 | 8.4 |
| 77 | methyl 3-(3,4-dimethoxyphenyl)-*cis*-prop-2-enoate | | 0.9 | | 0.2 | 0.3 | 0.2 | 0.3 | 0.4 | 0.6 | 0.3 | 0.3 | 0.5 |
| 78 | unknown (43, 77, 95, 124, 163, 209) | 5.2 | | | | | | | | | | | |
| 79 | 4-hydroxy-4-(3-oxo-1-butenyl)-3,5,5-trimethylcyclohex-2-en-1-one | 0.6 | 1.8 | 1.3 | | | | | | | | | |
| 80 | methyl pentadecanoate (15:0) | 0.2 | trace | 0.2 | 0.1 | 0.2 | trace | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| 81 | methyl 3-(3,4-dimethoxyphenyl)-*trans*-prop-2-enoate | 0.1 | | | | | | 0.3 | 0.2 | | | | |
| 82 | isomer of peak 83 | | 4.4 | 15 | 17 | 10.2 | 9.4 | 7.4 | 2.0 | 1.2 | 43 | 1.8 | 23 |
| 83 | 1-(3-oxo-*trans*-1-butenyl)-2,6,6-trimethylcyclohexane-*trans*,*cis*-1,2,4-triol | | 82 | 72 | 61 | 52 | 40 | 25 | 27 | 72 | 82 | 110 | 87 |
| 84 | methyl palmitate (16:0) | 1.5 | 1.9 | 4.3 | 4.5 | 4.9 | 1.7 | 2.5 | 2.7 | 7.6 | 10.5 | 9.5 | 8.9 |
| 85 | methyl margarate (17:0) | internal standard | | | | | | | | | | | |
| 86 | methyl *trans*,*cis*-abscisate | 106 | 10.1 | 8.1 | 7.7 | 10.6 | 0.5 | 1.6 | 5.3 | 1.3 | 0.8 | 0.9 | 1.5 |
| 87 | methyl linoleate (18:2) | * | * | * | * | * | * | 1.0 | * | * | * | * | * |
| 88 | methyl $\alpha$-linolenate (18:3) | 2.3* | 2.4* | 4.7* | 7.8* | 6.9* | 1.2* | 1.9 | 3.5* | 9.9* | 15* | 13* | 14* |
| 89 | methyl oleate (18:1) | 1.0 | 2.1 | 3.5 | 2.7 | 3.4 | 1.8 | 1.8 | 1.7 | 3.0 | 4.5 | 5.2 | 3.9 |
| 90 | *n*-heneicosane ($C_{21}$) | 0.2 | 0.1 | 0.7 | 0.5 | 0.5 | 0.2 | 0.3 | 0.5 | 0.2 | 0.8 | 0.5 | 0.4 |
| 91 | methyl stearate (18:0) | trace | * | 5.3 | 4.0* | 5.6* | 0.6 | 1.2 | * | 1.0 | 0.5 | 0.5 | 0.4 |
| 92 | methyl *trans*,*trans*-abscisate | 42 | 7.4 | * | * | * | | | 3.1* | | | | |
| 93 | *n*-triacosane ($C_{23}$) | 0.4 | 0.9 | 1.3 | 1.1 | 1.3 | 1.0 | 1.4 | 1.4 | 1.7 | 2.6 | 3.4 | 1.5 |

[a] Prominent ions observed in the mass spectra of unknown compounds, or fatty acid designations and hydrocarbon numbers are given in parentheses. [b] Detected in GC–FID but not in GC–MS. [c] Trace designates a component present at a level <0.1 $\mu$g/g honey. [d] An asterisk designates an unresolved GC–FID peak.

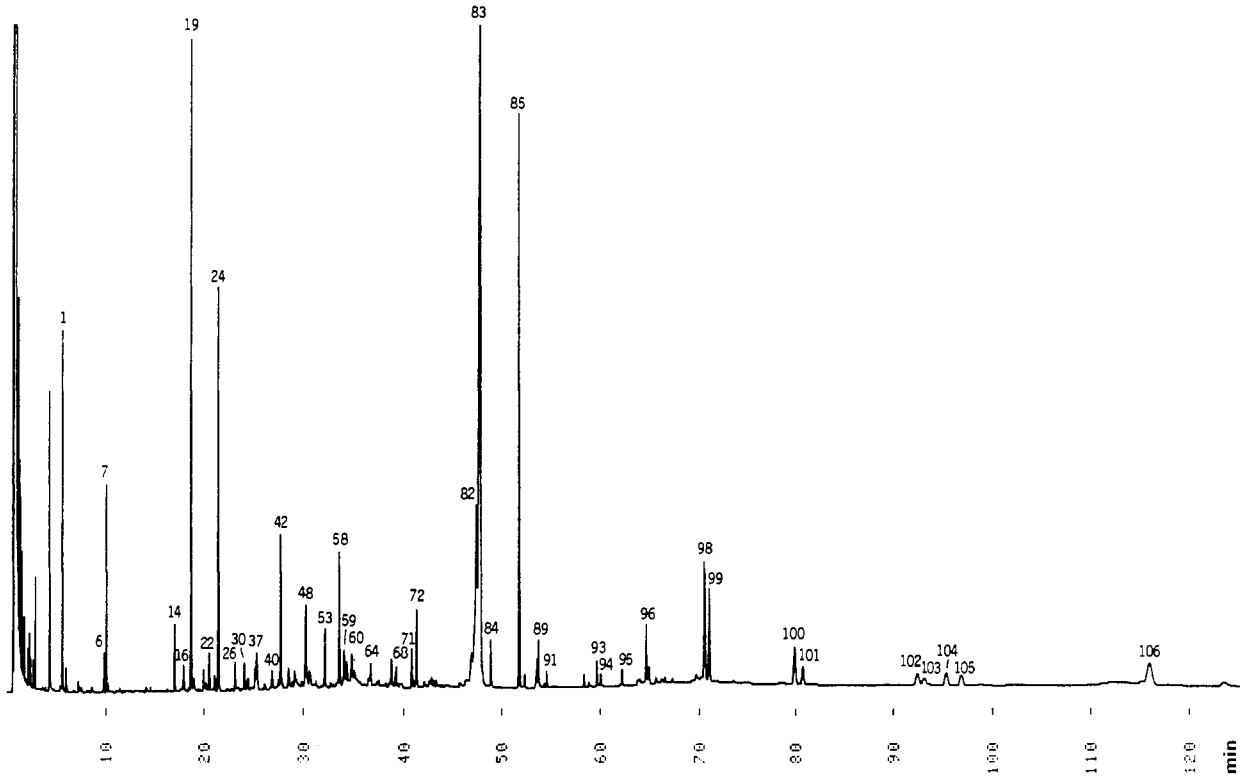



**Figure 1.** FID–GC profile of methylated thyme honey extractives (sample T5). For peak identifications see Table II. GC conditions: 16-m BP-1 column, $H_2$ as carrier gas ($\mu$ 46 cm/s); 40 °C (3-min hold) up 4 °C/min to 250 °C (65-min hold).

diazomethane), the origin of the aryl methoxy groups in some of the foregoing esters is less obvious. The detection of methyl 4-hydroxy-3-methoxybenzoate (**3a**) and methyl 3,4-dimethoxybenzoate (**3b**), and likewise methyl 4-hydroxy-3,5-dimethoxybenzoate (**2a**) and methyl 3,4,5-trimethoxybenzoate (**2b**), suggests that the fully methylated compounds may be derived from the 4-hydroxy analogues since phenolic hydroxyl groups react more slowly with diazomethane than is the case for carboxyl groups.

The most striking characteristic of the 11 thyme honey samples was the dominance of peak 83. Since the highest observed ion in the mass spectrum of peak 83 occurred at $m/z$ 224 ($C_{13}H_{22}O_3$ by high-resolution measurement), it appeared that this substance might be a degraded carotenoid similar to those which we recently detected in heather honey samples (Tan et al., 1989a). Extraction of a bulk thyme honey sample, followed by separation of the extractives by preparative layer chromatography on silica gel, afforded a quantity of peak 83 sufficient for its structure to be established by X-ray crystallographic analysis. By this means peak 83 was shown to be 1-(3-oxo-*trans*-1-butenyl)-2,6,6-trimethylcyclohexane-*trans*,*cis*-

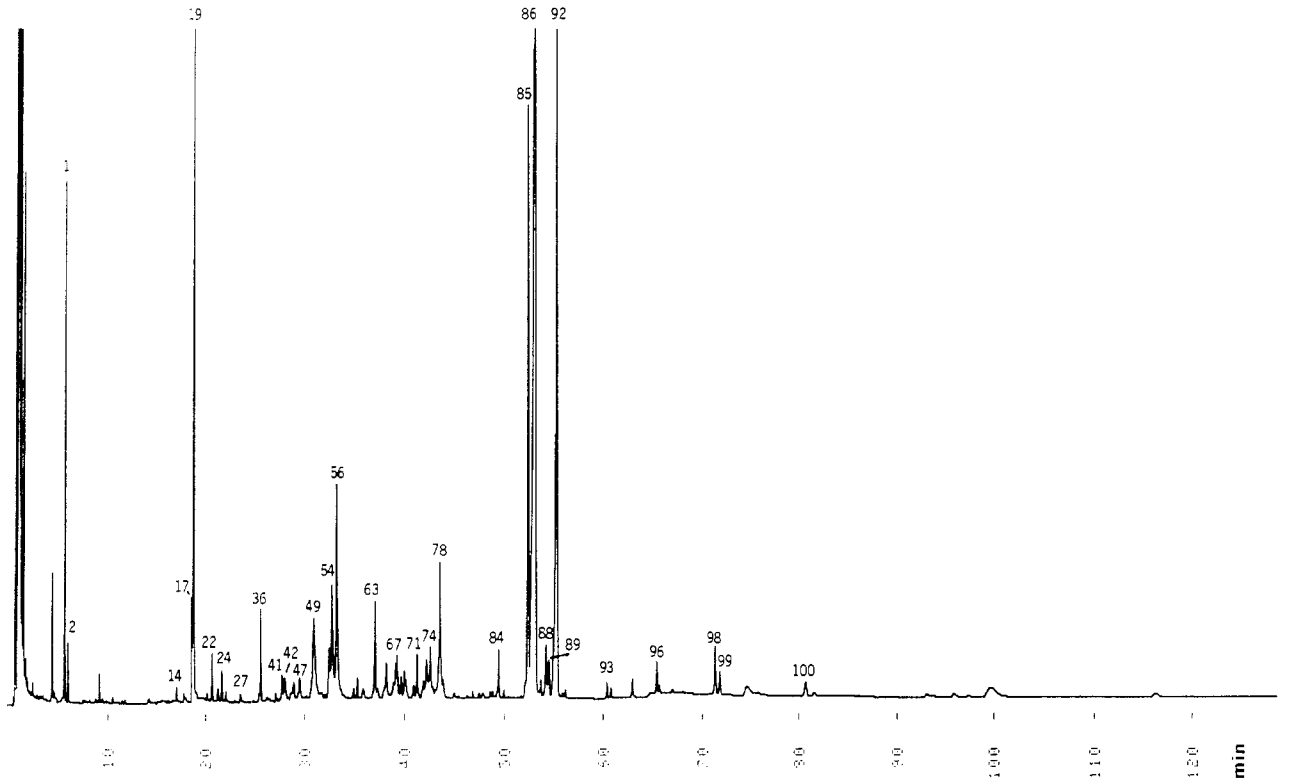



**Figure 2.** FID–GC profile of methylated willow honey extractives (sample W1). For peak identifications see Table II. GC conditions: 16-m BP-1 column, $H_2$ as carrier gas ($\mu$ 46 cm/s); 40 °C (3-min hold) up 4 °C/min to 250 °C (65-min hold).

1,2,4-triol (**4**) (Tan et al., 1989b), and it follows that the ion of $m/z$ 224 arises from an absent molecular ion of $m/z$ 242 ($C_{13}H_{24}O_4$) by loss of a water molecule. A lesser quantity of an earlier eluting shoulder peak exhibited a similar mass spectrum (peak 82) and is presumably a stereoisomer of **4**.

Enzell and Wahlberg (1986) have reported the mass spectral fragmentation pathways of a variety of degraded carotenoid-like substances, including the pair of isomeric epoxides **5a** and **5b**, $C_{13}H_{22}O_3$. Strong ions of $m/z$ 123 appeared in the mass spectra of these epoxides, while ions of $m/e$ 125 were observed in the mass spectra of the related dihydroxyepoxides **6a** and **6b**. Selected ion GC–MS analysis ($m/z$ 224 and 123 ion profiles) of the thyme honey extractives established the location of two substances, peaks 54 and 60 (peak 54 in trace quantities only), the mass spectral characteristics of which corresponded with those reported for the epoxides **5a** and **5b**. We were not, however, able to assign the absolute configuration of the pair of thyme honey epoxides.

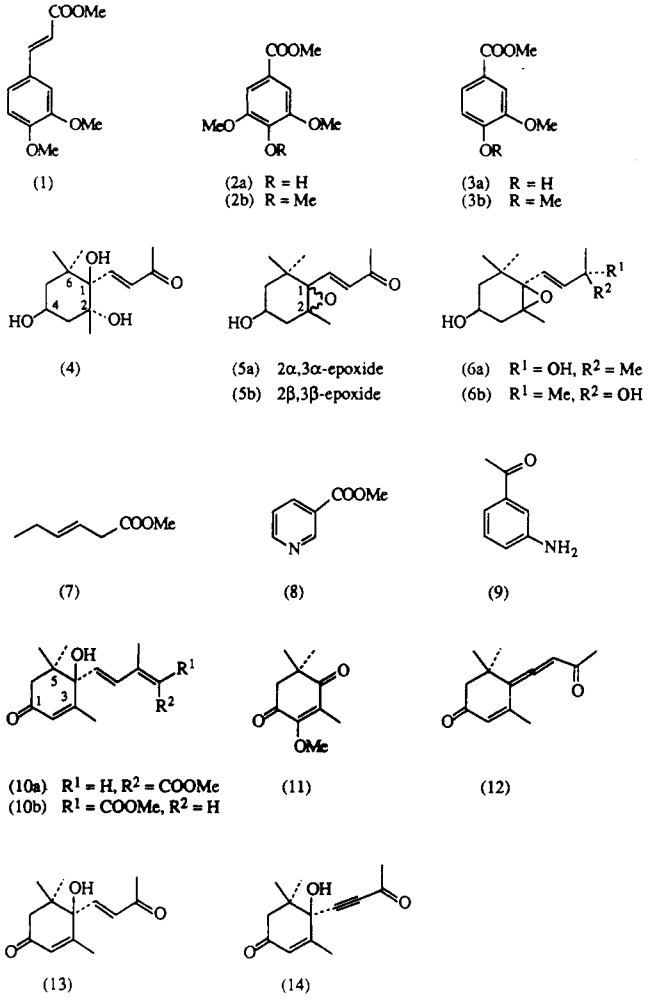
The thyme honey samples are also characterized by the presence of some peaks not found in other unifloral New Zealand honeys, for example, methyl 3-hexenoate (**7**) (peak 7), methyl pyridinecarboxylate (peak 18) [possibly methyl nicotinoate (**8**); found $m/z$ 137.0449 ($M^+$); $C_7H_7NO_2$ requires 137.0477], 3′-aminoacetophenone (**9**) (peak 37) [found $m/z$ 135.0718 ($M^+$); $C_8H_9NO$ requires 135.0684], and an unknown compound (peak 58) [found $m/z$ 163.0672 ($M^+$); $C_9H_9NO_2$ requires 163.0633]. The concentration of these compounds, among which only 3′-aminoacetophenone has been previously reported from honey (Bonaga and Giumanini, 1986), ranged from 3.5 to 8.1 (peak 7), from 0.1 to 2.3 (peak 18), from 0.7 to 5.1 (peak 37), and from 1.4 to 5.6 $\mu$g/g (peak 58), respectively. Other components detected in all of the methylated thyme honey extracts include the methyl esters of an array of aliphatic fatty acids (12:0, 14:0, 16:0, 18:0, 18:1, 18:2, etc.) and some diacids including butanedioic acid (succinic acid), octanedioic acid, nonanedioic acid, decanedioic acid, and *trans*-2-decenedioic acid. A similar array of acids and diacids occurs in other New Zealand unifloral honeys (Tan et al., 1988, 1989a).

A number of components could not be identified. For example, peak 24 exhibited a base peak at $m/z$ 82; high-resolution GC–MS established the molecular formula $C_{10}H_{16}O$ [found $m/z$ 152.1188 ($M^+$) and 134.1076 ($M^+ - H_2O$); $C_{10}H_{16}O$ and $C_{10}H_{14}$ require $m/z$ 152.1201 and 134.1045, respectively]. These observations suggest peak 24 might be a hydroxylated monoterpene. Peak 42 also appears to be a monoterpene of formula $C_{10}H_{16}O$. The majority of the other unknown peaks were trace or coeluting components.

The GC profile of the willow honey sample W1 (90% willow pollen frequency) was dominated by peaks arising from two 16-carbon substances, the mass spectral fragmentation patterns of which were reminiscent of those reported for methyl abscisate isomers. Co-injection using authentic specimens of the methylated acids (Sigma, St. Louis, MO) established peaks 86 and 92 to be methyl *trans*,*cis*-abscisate (**10a**) and methyl *trans*,*trans*-abscisate (**10b**), respectively. Other degraded carotenoid-like substances detected in the extracts of willow honey sample were 2-methoxy-3,5,5-trimethylcyclohex-2-ene-1,4-dione (**11**) (peak 36) and 4-(3-oxo-1-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one (**12**) (peak 67).

The level of methyl *trans*,*cis*-abscisate (peak 86) in the willow honey sample (106 $\mu$g/g) can be compared with the much lower levels of this compound found in most of the thyme honey samples, e.g., 10.1, 8.1, 7.7, and 6.9 $\mu$g/g in samples T1, T2, T3, and T4, respectively. We associate the levels of methyl *trans*,*cis*-abscisate (peak 86) in these honeys with minor willow contributions of between 10% and 7% by peak area ratios. By contrast, the willow pollen frequencies of these samples are between 41% and 24%. The discrepancy between the two results is a consequence of the underrepresentative frequency of thyme honey pol-

**Chart I**




lens; this results in a percent pollen frequency that greatly overestimates the percent by weight (or volume) willow contribution to the thyme honeys. The much reduced level of methyl *trans,cis*-abscisate (peak 86) found in sample T5 (0.5 μg/g) is consistent with a diminished willow contribution, 5% by pollen frequency (see Table I) or less than 0.5% by peak area ratio compared to the level of peak 86 observed in sample W1 (see Table II).

## CONCLUSIONS

Examination of the extractives of more than 200 New Zealand honey samples has revealed the occurrence of 1-(3-oxo-*trans*-1-butenyl)-2,6,6-trimethylcyclohexane-*trans,cis*-1,2,4-triol (**4**) to be confined to samples that include a thyme component, while the occurrence of abscisic acid isomers is indicative of a willow component. Hitherto degraded carotenoid-like substances have been reported from ling-heather honey; however, a different array of substances including 4-hydroxy-4-(3-oxo-1-butenyl)-3,5,5-trimethylcyclohex-2-en-1-one (**13**) and 4-hydroxy-4-(3-oxo-1-butynyl)-3,5,5-trimethylcyclohex-2-en-1-one (**14**) occur in ling-heather honey extracts. Our studies indicate that in unifloral grade thyme honey 1-(3-oxo-*trans*-1-butenyl)-2,6,6-trimethylcyclohexane-*trans,cis*-1,2,4-triol (peak 83) should be present at a level greater than 40 μg/g of honey. The presence of degraded carotenoid-like substances in the thyme and willow honey samples prompted a search for carotenoids; however, none were detected by using an established nonaqueous reverse-phase high-pressure liquid chromatography procedure (Lauren et al., 1986) with a detection limit below 1 μg/g for β-carotene and a range of hydroxy- or carboxycarotenes.

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**Registry No.** 3-Hydroxybutanoic acid, 300-85-6; 3-hydroxy-3-methylbutanoic acid, 625-08-1; 2,5-cyclohexadiene-1,4-dione, 106-51-4; caproic acid, 142-62-1; 3-hexenoic acid, 4219-24-3; 3-furancarboxylic acid, 488-93-7; butanedioic acid, 110-15-6; benzyl alcohol, 100-51-6; phenylacetaldehyde, 122-78-1; 2-methylsuccinaldehydate, 35643-98-2; benzoic acid, 65-85-0; 2-phenylethanol, 60-12-8; pyridinecarboxylic acid, 32075-31-3; *n*-undecane, 1120-21-4; pentanedioic acid, 110-94-1; caprylic acid, 124-07-2;

2-phenylacetic acid, 103-82-2; 5-(hydroxymethyl)-2-furfural, 67-47-0; nonanoic acid, 112-05-0; methoxybenzaldehyde, 50984-52-6; hexanedioic acid, 124-04-9; 3-phenylpropionic acid, 501-52-0; 1,4-dihydroxybenzene, 123-31-9; 2′-methoxyacetophenone, 579-74-8; 2-methoxy-3,5,5-trimethylcyclohex-2-ene-1,4-dione, 41654-27-7; 3′-aminoacetophenone, 99-03-6; *cis*-3-phenylprop-2-enoic acid, 102-94-3; 2-hydroxy-3-phenylpropionic acid, 156-05-8; 4-methoxybenzoic acid, 100-09-4; *trans*-3-phenylprop-2-enoic acid, 140-10-3; octanedioic acid, 505-48-6; 1-(3-oxo-1-butenyl)-2,6,6-trimethyl-1,2-epoxycyclohexan-4-ol, 38274-01-0; 4-hydroxybenzoic acid, 99-96-7; 4-hydroxy-3-methoxybenzoic acid, 121-34-6; lauric acid, 143-07-7; nonanedioic acid, 123-99-9; 3,4-dimethoxybenzoic acid, 93-07-2; 2-hydroxy-3-(4-methoxyphenyl)-propionic acid, 28030-15-1; 4-(3-oxo-1-butenylidene)-3,5,5-trimethyl-cyclohex-2-en-1-one, 127619-37-8; decanedioic acid, 111-20-6; 3-(4-methoxyphenyl)-*trans*-prop-2-enoic acid, 943-89-5; 3-hydroxy-3-(methoxyphenyl)propionic acid, 127619-38-9; *trans*-2-decenedioic acid, 37443-67-7; 3,4,5-trimethoxybenzoic acid, 118-41-2; 3-(4-hydroxyphenyl)-*trans*-prop-2-enoate, 501-98-4; myristic acid, 544-63-8; 4-hydroxy-3,5-dimethoxybenzoic acid, 530-57-4; 3-(3,4-dimethoxyphenyl)-*cis*-prop-2-enoic acid, 14737-88-3; 4-hydroxy-4-(3-oxo-1-butenyl)-3,5,5-trimethylcyclohex-2-en-1-one, 7070-24-8; pentadecanoic acid, 1002-84-2; 3-(3,4-dimethoxyphenyl)-*trans*-prop-2-enoic acid, 14737-89-4; 1-(3-oxo-*trans*-1-butenyl)-2,6,6-trimethylcyclohexane-*trans*,*cis*-1,2,4-triol, 127643-61-2; palmitic acid, 57-10-3; margaric acid, 506-12-7; *trans*,*cis*-abscisic acid, 21293-29-8; linoleic acid, 60-33-3; $\alpha$-linolenic acid, 463-40-1; oleic acid, 112-80-1; *n*-heneicosane, 629-94-7; stearic acid, 57-11-4; *trans*,*trans*-abscisic acid, 6755-41-5; *n*-triacosane, 638-67-5; 3,4-dihydroxybenzoic acid, 99-50-3; 3,4,5-trihydroxybenzoic acid, 149-91-7.