Extractives from New Zealand Honeys. 5. Aliphatic Dicarboxylic Acids in New Zealand Rewarewa *(Knightea excelsa)* Honey

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Thirty-two aliphatic dicarboxylic acids were identified as methyl esters in the methylated diethyl ether extracts of four unifloral grade New Zealand rewarewa *(Knightea excelsa)* honeys using combined gas chromatography–mass spectrometry (GC–MS). 2-Methoxybutanedioic acid (*O*-methylmalic acid) and 4-hydroxy-3-methyl-*trans*-2-pentenedioic acid are proposed as floral marker substances for New Zealand rewarewa honey. The total level of aliphatic dicarboxylic acids identified in the rewarewa honey samples ranged from 64 to 111 mg/kg, with an average level of 88 mg/kg.

Keywords: *Rewarewa (Knightea excelsa) honey; aliphatic dicarboxylic acids; 2-methoxybutanedioic acid; 4-hydroxy-3-methyl-trans-2-pentenedioic acid*

INTRODUCTION

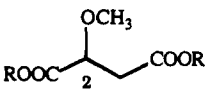
We have previously reported the levels of extractable organic substances (mainly aliphatic acids, aromatic acids, and degraded carotenoid-like substances) in a number of unifloral New Zealand honeys (Tan *et al.*, 1988, 1989a,b, 1990; Wilkins *et al.*, 1993a,b). Some of the substances detected in these studies have been proposed as floral marker compounds. Other authors (Bicchi *et al.*, 1983; Bonaga and Giumanini, 1986; Graddon *et al.*, 1979; Bouseta *et al.*, 1992) have investigated the volatile substances contributing to honey flavor.

Acidicity is a well-recognized characteristic of honeys. Gluconic acid (Stinson *et al.*, 1960) and numerous aliphatic acids including formic, acetic, propionic, butyric, valeric, caproic, and palmitic acids have been identified in honey (Cremer and Riedmann, 1965). Aliphatic dicarboxylic acids including oxalic, malonic, succinic, fumaric, malic, α-ketoglutaric, tartaric, *cis*-aconitic, and citric acids have also been identified in honey by Echigo and Takenaka (1974), while we have identified glutaric, adipic, suberic, azelaic, sebacic, and *trans*-2-decenedioic acids in our investigations of New Zealand unifloral honeys (Tan *et al.*, 1988, 1989a,b, 1990; Wilkins *et al.*, 1993a,b). Steeg and Montag (1988a,b) have reported the presence in some European honeys of an array of aromatic carboxylic acids, most of which are postulated to arise from phenylpropanoid metabolism. Elevated levels of other aromatic acids, *e.g.*, β-phenyllactic acid and syringic acid, have been identified in New Zealand manuka *(Leptospermum scoparium)* honeys (Tan *et al.*, 1988; Wilkins *et al.* 1993a).

In the course of our ongoing chemical investigations of New Zealand honeys we observed that the diethyl ether extracts of rewarewa *(Knightea excelsa)* honey were dominated by acidic substances (mainly aliphatic diacids), some of which were of uncertain structure. Two of the aliphatic diacids, 2-methoxybutanedioic acid (**1**) and 4-hydroxy-3-methyl-*trans*-2-pentenedioic acid (**3**), the structures of which were verified by isolation, spectroscopic analyses, and synthesis, are proposed as floral marker compounds for rewarewa honey.

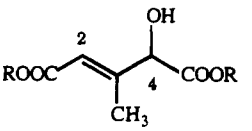
MATERIALS AND METHODS

Honey Samples. Four rewarewa *(K. excelsa)* honey samples (RW1, RW2, RW3, and RW4) (1985–1992 flowering season) were obtained directly from beekeepers. Honey samples were stored at 5 °C prior to chemical analysis (in 1992). The floral integrity of samples RW1 and RW3 (11.2 and 12.8% rewarewa pollen respectively) was verified by pollen analysis. Since rewarewa pollen is known to be under-represented, a minimum of 10% rewarewa pollen is required for classification as a unifloral honey (Moar, 1985). Bulk extraction was performed using the RW1 sample.



(1) R = H

(2) R = Me



(3) R = H

(4) R = Me

General Procedures. Methods and procedures used in the extraction, derivatization, and gas chromatographic (GC) analysis of the rewarewa honey samples were as described previously (Tan *et al.*, 1988), modified by the use of a 250 mL extractor with a 12 h extraction time and with diethyl octanedioate as the internal standard. GC conditions are given in the caption to Figure 1. Supporting GC–MS analyses were carried out on a Hewlett-Packard 5890/5970B GC–MS system fitted with a 20 m × 0.22 mm (i.d.) HP-1 methylsilicone column. The concentrations of aliphatic diacids detected in the four rewarewa honey samples are listed in Table 1. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were determined as $CDCl_3$ solutions using a Bruker AC-300 instrument.

Bulk Extraction. A bulk extraction of sample RW1 (200 g) using diethyl ether afforded a mixture of extractives (60 mg) which, after methylation with diazomethane, were separated by multiple (×3) preparative layer chromatography (PLC) on silica gel (Merck$_{254+366}$) (1.5 mm layer thickness) with *n*-hexane/diethyl ether (9:1) as the developing solvent. Twelve fractions were recovered and examined by GC–MS. Repeated PLC of fractions 6 and 8 afforded dimethyl 2-methoxybutanedioate (peak 7) (**2**) (0.5 mg) and dimethyl 4-hydroxy-3-methyl-*trans*-2-pentenedioate (peak 12) (**4**) (0.7 mg), respectively.

Dimethyl 2-methoxybutanedioate (**2**): MS (see Table 2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.70 (**ABX** system, J_{AB} = 12.3, J_{AX} = 7.6, J_{BX} = 5.0 Hz, CH_AH_B), 3.37 (s, OCH_3), 3.62 (s, $COOCH_3$), 3.70 (s, $COOCH_3$), 4.11 (dd, J_{AX} = 7.6, 5.0 Hz, $CHOCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 37.5 (CH_2), 51.9 ($COOCH_3$), 52.1 ($COOCH_3$), 58.7 (OCH_3), 76.1 ($CHOCH_3$), 170.4 ($COOCH_3$), 171.7 ($COOCH_3$).

Dimethyl 4-hydroxy-3-methyl-*trans*-2-pentenedioate (**4**): MS (see Table 2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.15 (d, J = 1.4 Hz, C=CCH_3), 3.29 (d, J = 5.4 Hz, OH), 3.72 (s, OCH_3), 3.82

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Table 1. Concentrations (Milligrams per Kilogram) of Aliphatic Dicarboxylic Acids, Identified as the Corresponding Methyl Esters, in the Methylated Extracts of Four New Zealand Rewarewa Honey Samples

peak	retention time, min	compound	sample RW1	RW2	RW3	RW4
1	12.4	butanedioic acid (succinic acid)	32	68	72	58
2	13.5	2-methylbutanedioic acid	0.6	1.1	0.9	0.4
3	14.2	2,2-dimethylbutanedioic acid	0.2	tr[a]	0.1	tr
4	14.5	butanedioic acid monoethyl ester	tr	0.1	0.2	0.2
5	15.0	2-hydroxybutanedioic acid (malic acid)	0.3	0.7	0.1	0.6
6	15.2	pentanedioic acid	0.6	0.5	0.7	1.3
7	15.5	2-methoxybutanedioic acid	2.3	2.5	3.3	3.0
8	17.1	2-hydroxy-2-ethylbutanedioic acid	0.1	0.1	tr	tr
9	17.5	3-hydroxy-3-methylpentanedioic acid	tr	0.2	0.1	tr
10	18.1	hexanedioic acid	0.2	0.3	0.6	0.2
11	18.9	2-hydroxy-2-isopropylbutanedioic acid	0.3	0.2	0.4	0.4
12	20.3	4-hydroxy-3-methyl-*trans*-2-pentenedioic acid	3.9	1.6	3.2	0.2
13	20.5	heptanedioic acid	0.3	0.4	0.7	0.1
14	22.9	octanedioic acid	1.1	1.1	1.4	0.5
15	23.9	2-octenedioic acid	0.3	0.9	0.8	0.3
16	25.1	nonanedioic acid	0.2	0.4	0.6	0.2
17	27.3	decanedioic acid	5.5	5.4	6.5	2.0
18	28.4	2-decenedioic acid	16	17	19	7.6
		total aliphatic dicarboxylic acids	64	101	111	75

[a] tr, trace, which indicates the compound is present at a level of less than 0.1 mg/kg of honey.

(s, OCH_3), 4.58 (dd, J = 5.4, 1.1 Hz, C*H*OH), 6.03 (dq, J = 1.1, 1.4 Hz, H-2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.6 (CH_3), 51.3 (OCH_3), 53.4 (OCH_3), 75.5 (C-4), 118.9 (C-2), 153.2 (C-3), 166.5 (C-1), 172.9 (C-5).

Preparation of Dimethyl 2-Methoxybutanedioate [after the Method of Haeberle and Eberle (1982)]. Dimethyl fumarate (4.3 g) was added to the sodium methanolate (2.3 g of sodium dissolved in 30 mL of methanol) and the mixture stirred under reflux for 2 h. Workup gave dimethyl 2-methoxybutanedioate (**1**) (2.2 g) (spectroscopic data identical to that given above).

Preparation of Dimethyl 4-Hydroxy-3-methyl-*trans*-2-pentenedioate [after the Method of Gilchrist and Rees (1968)]. Dry HCl gas was passed into ethyl acetoacetate (25 g) cooled with ice/salt for 8 h. The reaction mixture was then allowed to stand for 7 days at room temperature. Distillation under vacuum gave ethyl 2,4-dimethyl-6-oxopyran-3-carboxylate (13.3 g): bp (2 mmHg) 130–140 °C; MS (70 eV) *m/z* (%) 196 (M^+, 50), 168 (77), 151 (68), 140 (63), 138 (49), 125 (29), 123 (35), 122 (67), 109 (26), 98 (18), 94 (14), 53 (39), 43 (100). The pyrone (5.0 g) was dissolved in dry chloroform and bromine (4.5 g) added in one portion with shaking. The reaction mixture was then allowed to stand overnight at room temperature. Workup gave ethyl 5-bromo-2,4-dimethyl-6-oxopyran-3-carboxylate (5.5 g): mp 82–83 °C; MS (70 eV) *m/z* (%) 276 (16), 274 (17) (M^+), 248 (8), 246 (9), 231 (18), 229 (19), 195 (97), 167 (100). The bromopyrone (1.0 g) was added to a solution of potassium hydroxide (1.5 g of KOH in 8 mL of H_2O) at room temperature and stirred overnight. The mixture was then acidified with 50% hydrochloric acid and extracted with diethyl ether. The solvent was evaporated and the residue methylated with an ethereal solution of diazomethane. Separation by multiple PLC (×4) on silica gel with *n*-hexane/diethyl ether (9:1) afforded dimethyl 4-hydroxy-3-methyl-*trans*-2-pentenedioate (**4**) (130 mg) (spectroscopic data identical to that given above for **4** isolated from the methylated honey extracts) and dimethyl 2-oxo-3-methylpentanedioate (25 mg): MS (see Table 2); ^1H NMR (300 MHz, $CDCl_3$) δ 1.20 (d, J = 7.2 Hz, CH_3), 2.51 (dd, J = 16.9, 5.4 Hz, 4-C*H*$_A$H$_B$), 2.82 (dd, J = 16.9, 9.0 Hz, 4-CH$_A$C*H*$_B$), 3.66 (s, OCH_3), 3.70 (m, H-3), 3.89 (s, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.9 (CH_3), 36.8 (CH_2), 38.3 (CH), 52.0 (OCH_3), 53.0 (OCH_3), 161.3 ($COOCH_3$), 172.2 ($COOCH_3$), 196.3 (CO).

Preparation of Dimethyl 3-Methyl-2-pentenedioate. Ethyl 2,4-dimethyl-6-oxopyran-3-carboxylate (1.0 g) was treated with potassium hydroxide solution (1.5 g of KOH in 8 mL of H_2O) as described above for ethyl 5-bromo-2,4-dimethyl-6-oxopyran-3-carboxylate. Workup gave dimethyl 3-methyl-2-pentenedioate (0.8 g), (*cis:trans* 1:2). Dimethyl 3-methyl-*cis*-2- pentenedioate: MS (see Table 2); ^1H NMR (300 MHz, $CDCl_3$) δ 1.97 (d, J = 1.4 Hz, CH_3), 3.66 (s, OCH_3), 3.74 (d, J = 2.3 Hz, CH_2), 5.85 (d, J = 0.7 Hz, H-2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.0 (CH_3), 38.3 (CH_2), 51.1 (OCH_3), 52.0 (OCH_3), 118.9 (C-2), 151.1 (C-3), 166.6 ($COOCH_3$), 170.8 ($COOCH_3$). Dimethyl 3-methyl-*trans*-2-pentenedioate: MS (see Table 2); ^1H NMR (300 MHz, $CDCl_3$) δ 2.22 (d, J = 1.3 Hz, CH_3), 3.14 (d, J = 0.9 Hz, CH_2), 3.68 (s, OCH_3), 3.70 (s, OCH_3), 5.77 (d, J = 1.2 Hz, H-2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.8 (CH_3), 45.7 (CH_2), 51.1 (OCH_3), 52.2 (OCH_3), 119.3 (C-2), 151.2 (C-3), 166.6 ($COOCH_3$), 170.4 ($COOCH_3$).

RESULTS AND DISCUSSION

Eighteen aliphatic dicarboxylic acids were identified in the methylated extracts by GC–MS analyses. Since the extracts were methylated with diazomethane, prior to GC–FID and GC–MS analyses, the detection of a methyl ester was considered indicative of the parent acid. This proposal was verified by ethylating a subsample of the extractives with diazoethane. Without exception, GC–MS analysis demonstrated the presence of the corresponding ethyl esters. Separation of a portion of the methylated extracts by PLC afforded a diester fraction which, in addition to the methylated diacids reported in Table 1, was found to possess low levels (less than 0.1 mg/kg per component) of a further 14 methylated aliphatic dicarboxylic acids (see Table 2).

Figure 1 is a typical GC–FID profile of the extractable organic substances in the methylated extract of a predominantly rewarewa honey sample. The numbered peaks (1–18) arise from the methylated aliphatic dicarboxylic acids, while other substances (not diacids) are designated by the letters a–q. Moar (1985) has demonstrated that New Zealand rewarewa honeys usually include contributions from other plant sources, including dandelion, kamahi, lotus, white clover-type, and willow. Chemical data and pollen analysis indicated that one of the rewarewa samples examined in this investigation (sample RW1) possessed a significant kamahi contribution. We have shown that the broad peak at 32.7 min (peak n, Figure 1) arises from three new degraded carotenoid-like substances designated kamahines A, B, and C, characteristic of kamahi honeys (Broom *et al.*, 1992).

Saturated Aliphatic Dicarboxylic Acids. The straight-chain (nonbranched) dicarboxylic acids from

Table 2. Mass Spectral Fragment Ions Observed for Methylated Aliphatic Dicarboxylic Acids Detected in Some New Zealand Rewarewa Honey Samples

peak[a]	retention time, min	compound	EIMS at 70 eV, *m/z* (%)
*	9.2	dimethyl propanedioate	42 (49), 57 (30), 59 (100), 69 (11), 74 (45), 101 (58)
1	12.4	dimethyl butanedioate	45 (14), 55 (100), 59 (79), 87 (23), 114 (30), 115 (88)
2	13.5	dimethyl 2-methylbutanedioate	41 (23), 59 (100), 69 (13), 87 (9), 100 (13), 101 (13), 128 (13), 129 (19)
3	14.2	dimethyl 2,2-dimethylbutanedioate	59 (36), 73 (100), 83 (14), 101 (8), 114 (14), 115 (17), 142 (7), 143 (7)
4	14.5	ethyl methyl butanedioate	55 (79), 59 (39), 87 (20), 101 (46), 114 (16), 115 (100), 128 (9), 129 (12)
5	15.0	dimethyl malate	43 (100), 59 (30), 61 (48), 71 (100), 103 (70)
6	15.2	dimethyl pentanedioate	55 (46), 59 (100), 74 (9), 87 (14), 100 (47), 101 (33), 128 (18), 129 (26)
7	15.5	dimethyl 2-methoxybutanedioate	47 (12), 55 (8), 59 (22), 75 (100), 85 (3), 117 (26), 146 (2)
*	16.3	dimethyl 3-methylpentanedioate	55 (44), 59 (100), 69 (62), 101 (45), 114 (50), 115 (23), 142 (14), 143 (32)
*	16.4	dimethyl 2-methylpentanedioate	55 (100), 59 (69), 73 (48), 99 (29), 114 (62), 115 (30), 142 (14), 143 (24)
*	16.9	dimethyl 3-methyl-2-*cis*-pentenedioate	53 (57), 55 (64), 59 (70), 81 (43), 97 (33), 112 (100), 140 (33), 141 (26)
8	17.1	dimethyl 2-hydroxy-2-ethylbutanedioate	43 (30), 57 (100), 59 (14), 99 (18), 101 (10), 131 (30)
9	17.5	dimethyl 3-hydroxy-3-methylpentanedioate	43 (100), 59 (12), 69 (8), 74 (8), 85 (33), 101 (13), 117 (37), 143 (9)
*	17.8	dimethyl 3-methyl-2-*trans*-pentenedioate	53 (69), 55 (87), 59 (88), 69 (50), 73 (59), 97 (61), 112 (100), 140 (75)
*	18.0	dimethyl 3-methyl-2-oxopentanedioate	41 (19), 59 (100), 69 (6), 101 (6), 129 (23), 157 (0.5)
10	18.1	dimethyl hexanedioate	55 (77), 59 (100), 74 (34), 101 (39), 111 (35), 114 (47), 142 (7), 143 (22)
11	18.9	dimethyl 2-hydroxy-2-isopropylbutanedioate	43 (100), 59 (19), 71 (84), 74 (11), 101 (21), 113 (13), 145 (30), 161 (4)
12	20.3	dimethyl 4-hydroxy-3-methyl-2-pentenedioate	59 (50), 69 (55), 97 (77), 101 (39), 129 (100), 156 (22), 157 (10), 170 (1)
13	20.5	dimethyl heptanedioate	55 (100), 59 (77), 69 (64), 74 (86), 115 (75), 125 (27), 128 (22), 157 (19)
14	22.9	dimethyl octanedioate	55 (97), 59 (74), 69 (98), 74 (100), 97 (79), 129 (82), 138 (74), 171 (39)
15	23.9	dimethyl 2-octenedioate	55 (67), 59 (79), 81 (100), 108 (49), 136 (87), 140 (25), 168 (12), 169 (6)
16	25.1	dimethyl nonanedioate	55 (100), 59 (100), 74 (78), 83 (57), 111 (38), 143 (20), 152 (44), 185 (19)
*	26.1	dimethyl nonenedioate	55 (100), 59 (52), 81 (66), 95 (43), 150 (40), 154 (13), 155 (14), 182 (10)
17	27.3	dimethyl decanedioate	55 (100), 59 (56), 69 (38), 74 (81), 98 (47), 138 (22), 166 (14), 199 (19)
18	28.4	dimethyl 2-decenedioate	55 (100), 59 (78), 81 (75), 95 (68), 136 (83), 164 (49), 168 (45), 196 (17)
*	28.6	ethyl methyl decanedioate	55 (100), 74 (38), 88 (36), 98 (51), 125 (40), 138 (24), 199 (20), 213 (9)
*	29.5	dimethyl methyldecenedioate	55 (100), 69 (66), 81 (71), 94 (50), 136 (62), 164 (36), 182 (15), 210 (5)
*	29.8	dimethyl 3-hydroxydecanedioate	43 (100), 55 (43), 59 (38), 74 (36), 87 (65), 103 (43), 144 (17), 196 (4)
*	30.1	dimethyl methyldecenedioate	55 (100), 59 (55), 69 (44), 81 (55), 95 (33), 150 (36), 182 (14), 210 (4)
*	31.1	dimethyl dodecanedioate	55 (97), 59 (51), 69 (65), 74 (93), 98 (100), 153 (28), 185 (19), 227 (29)
*	31.9	dimethyl 2-dodecenedioate	55 (100), 59 (43), 81 (63), 164 (21), 192 (17), 196 (16), 224 (3), 225 (4)
*	33.4	dimethyl tridecanedioate	55 (100), 69 (69), 83 (46), 88 (54), 98 (93), 101 (29), 199 (28), 241 (47)

[a] Numbered peaks (1–18) were identified and quantified in the methylated extracts (see Figure 1). Peaks designated with an asterisk were identified (but not quantified) in a diester fraction obtained by PLC.

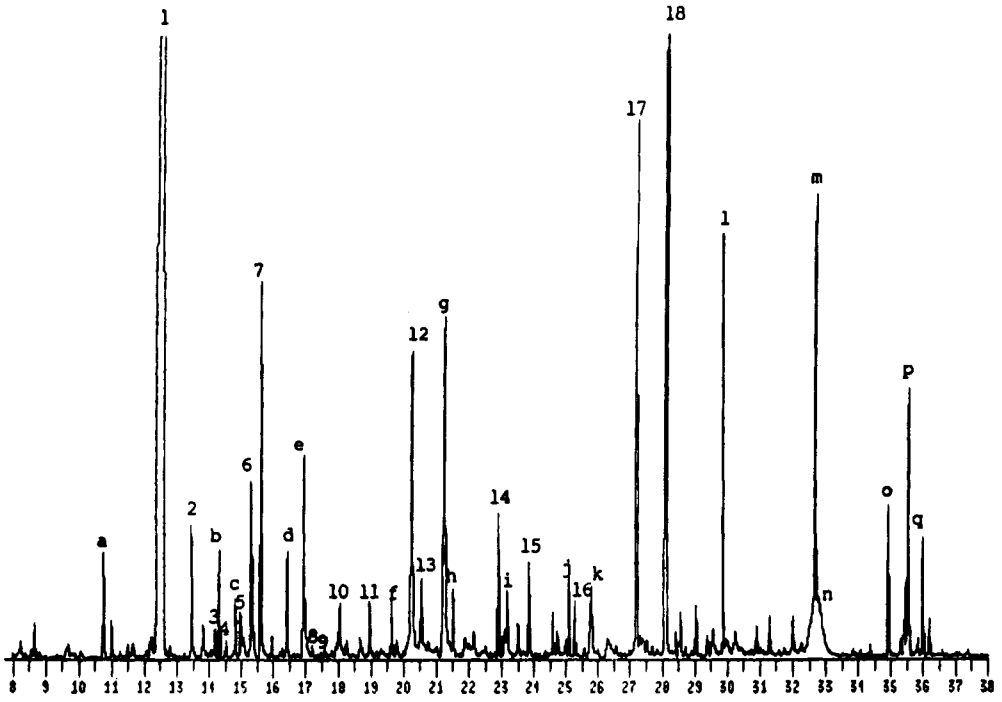

Figure 1. GC profile of the methylated extracts of rewarewa honey sample RW3. GC conditions: 20-m HP-1 column, He as carrier gas (μ 46 cm/s), 40 °C (3 min hold) raised at 6 °C/min to 280 °C. Peak identifications: numbered peak identifications (peaks 1–18) are given in Table 1; (a) methyl 2-furancarboxylate; (b) methyl benzoate; (c) 3,5,5-trimethylcyclohex-2-ene-1,4-dione; (d) methyl phenylacetate; (e) 2,6-dimethyl-3,7-octadiene-2,6-diol; (f) methyl *cis*-cinnamate; (g) methyl β-phenyllactate; (h) methyl *trans*-cinnamate; (i) methyl salicylate; (j) methyl vanillate; (k) methyl veratrate; (l) diethyl octanedioate (internal standard); (m) methyl palmitate; (n) kamahines A, B, and C; (o) methyl abscisate; (p) methyl oleate; (q) methyl stearate.

malonic acid to tridecanedioic acid were, with the exception of only undecanedioic acid, identified by comparisons of GC and GC–MS retention times and mass spectra with authentic samples. Peak designations refer to the elution order on a HP-1 methylsilicone capillary GC column. Butanedioic acid (peak 1) (average level 58 mg/kg) and decanedioic acid (peak 17) (average level 5 mg/kg) dominated the GC–FID profiles

of each of the rewarewa honey samples. Pentanedioic acid (peak 6), hexanedioic acid (peak 10), heptanedioic acid (peak 13), octanedioic acid (peak 14), and nonanedioic acid (peak 16) were found to be present in concentrations averaging between 0.1 and 1.4 mg/kg. Traces (less than 0.1 mg/kg) of malonic acid, dodecanedioic acid, and tridecanedioic acid were also detected in the methylated extractive mixtures after separation using PLC.

2-Methylbutanedioic acid (peak 2), 2,2-dimethylbutanedioic acid (peak 3), 2-methylpentanedioic acid, and 3-methylpentanedioic acid were determined on the basis of their correspondence with the NBS library mass spectra of these compounds.

Unsaturated Dicarboxylic Acids. 2-Decenedioic acid (peak 18), the second most dominant constituent of the rewarewa honey samples (average level 15 mg/kg), has been reported to be a significant constituent of royal jelly (Lercker *et al.*, 1981) and clover honeys (Tan *et al.*, 1988). Modest levels of 2-octenedioic acid (peak 15) (see Table 1) and 2-dodecenedioic acid (traces) (see Table 2) were also detected. The *cis:trans* dimethyl 3-methyl-2-pentenedioates (traces) were identified by comparisons with synthetic samples (see Materials and Methods).

Oxygenated Aliphatic Dicarboxylic Acids. A bulk extraction of the RW1 sample, followed by methylation, afforded a mixture of extractives which were separated by multiple PLC on silica gel. Two fractions, corresponding to peaks 7 and 12, were recovered in sufficient quantity and purity for structural elucidation using one- and two-dimensional NMR spectroscopy.

Peak 7. ^{13}C NMR analysis demonstrated the presence of seven carbon signals, assignable to three methoxy, two carbonyl, one methylene, and one oxygenated carbon. ^{1}H NMR analysis confirmed the presence of three methoxy groups (3.37, 3.62, and 3.70 ppm) and a mutually coupled set of methylene and methine protons (2.70 and 4.11 ppm, ABX system, $J_{AB} = 12.3$, $J_{AX} = 7.6$, $J_{BX} = 5.0$ Hz, $CH_AH_BCH_X$). These spectral data identified peak 7 as dimethyl 2-methoxybutanedioate (**1**), the ^{1}H NMR spectrum of which has been reported by Finnegan and Mueller (1965). The mass spectral ion of m/z 117 exhibited by this compound (see Table 2) can be generated from the molecular ion by loss of an ester group, while the base peak at m/z 75 can be envisaged as arising from the migration of a methoxy group and subsequent loss of a ketene (Howe and Williams, 1968). The identity of peak 7 was also substantiated by a comparison of the NMR and GC–MS characteristics of this compound with those of a synthetic specimen of dimethyl 2-methoxybutanedioate (**2**) prepared by treatment of dimethyl fumarate in methanol with sodium methanolate (Haeberle and Eberle, 1982).

The occurrence of the parent acid, 2-methoxybutanedioic acid (**1**), in rewarewa honey was proven by the esterification of the honey extract with ethanol in the presence of acid or by reaction with an ethereal solution of diazoethane. In each case this gave diethyl 2-methoxybutanedioate [m/z 174 (13%, $M^+ - H_2C{=}O$), 158 (9%, $M^+ - C_2H_5OH$), 131 (58%, $M^+ - COOC_2H_5$), 89 (100%)]. Methylation of 2-hydroxybutanedioic acid (malic acid) using diazomethane did not give dimethyl 2-methoxybutanedioate, except in the presence of boron trifluoride.

Peak 12. ^{1}H and ^{13}C NMR data identified peak 12 as dimethyl 4-hydroxy-3-methyl-*trans*-2-pentenedioate (**4**). ^{1}H NMR resonances at 2.15 and 6.03 ppm indicated the presence of an olefinic methyl group and an olefinic proton, respectively. ^{13}C NMR spectroscopy demonstrated the presence of a conjugated ester group (118.8, 153.2, and 166.5 ppm) and an oxygenated carbon (75.5 ppm). The position of the olefinic methyl group at C-3 was deduced from an analysis of the ^{13}C NMR data. C-2 (a methine carbon) exhibited a chemical shift (118.8 ppm) typical of an olefinic carbon α to a carbonyl group, whereas C-3 (a quaternary carbon) exhibited a chemical shift (153.2 ppm) typical of a β carbon (Breitmaier and Voelter, 1987). These observations, in combination with two-dimensional COSY NMR spectral data (which indicated H-4 was coupled to the 4-hydroxyl proton, but not H-2), indicated peak 12 to be dimethyl 4-hydroxy-3-methyl-*trans*-2-pentenedioate (**4**).

This identification was confirmed by synthesis (Gilchrist and Rees, 1968). Ethyl acetoacetate (as starting material) was converted to α-pyrone, which was brominated and treated with a strong base to form the required acid. The synthetic specimen of dimethyl 4-hydroxy-3-methyl-*trans*-2-pentenedioate (**4**) exhibited GC–MS and NMR data identical to that determined for peak 12.

Other oxygenated dicarboxylic acids, including 2-hydroxy-2-ethylbutanedioic acid (peak 8), 2-hydroxy-2-isopropylbutanedioic acid (peak 11), 3-hydroxy-3-methylpentanedioic acid (peak 9), and 3-hydroxydecanedioic acid (trace), were also detected in the honey extracts and identified as methyl esters by comparison with published mass spectra (Spiteller and Spiteller, 1979a,b; Jellum *et al.*, 1988). This appears to be the first occasion these compounds have been identified in honeys.

Other Substances. In addition to the diacids reported in Tables 1 and 2, modest levels of a number of other substances (peaks a–q) (see Figure 1) were also detected in the extracts. The more dominant of these were 2-furancarboxylic acid (peak a), benzoic acid (peak b), phenylacetic acid (peak d), 2,6-dimethyl-3,7-octadiene-2,6-diol (peak e), β-phenyllactic acid (peak g), palmitic acid (peak m), oleic acid (peak p) and stearic acid (peak q). Abscisic acid (peak o) was detected in sample RW1 but not the other samples. With the exception only of an elevated level of β-phenyllactic acid (14 mg/kg) in sample RW1, suggestive of a minor manuka input (Tan *et al.*, 1988; Wilkins *et al.*, 1993a), the levels of these compounds were typically in the range 0.5–4 mg/kg.

Conclusions. Our investigations of the extractable organic substances present in New Zealand unifloral honeys have shown that the floral source of many New Zealand honeys can be reliably determined by GC analysis, provided suitable marker substances can be identified. While butanedioic acid, decanedioic acid, and 2-decenedioic acid are dominant rewarewa constituents, they also occur in many other unifloral New Zealand honeys; hence, their detection does not assist in floral source discrimination. However, 2-methoxybutanedioic acid and 4-hydroxy-3-methyl-*trans*-2-pentenedioic acid are of importance in the characterization of the floral source since in our examinations of more than 200 New Zealand honey samples we have only detected these compounds in samples possessing a significant rewarewa contribution.

Registry No. Supplied by the Author: Propanedioic acid (malonic acid), 141-82-2; dimethyl propanedioate, 108-59-8; butanedioic acid (succinic acid), 110-15-6; dimethyl butanedioate, 106-65-0; 2-methylbutanedioic acid, 498-21-5; dimethyl 2-methylbutanedioate, 22644-27-5; 2,2-dimethylbutanedioic acid, 597-43-3; dimethyl 2,2-dimethylbutanedioate, 49827-44-3; 2-hydroxybutanedioic acid (malic acid), 6915-15-7; dimethyl

malate 38115-87-6; pentanedioic acid (glutaric acid), 110-94-1; dimethyl pentanedioate, 1119-40-0; 2-methoxybutanedioic acid, 1726-80-3; dimethyl 2-methoxybutanedioate, 4148-97-4; 3-methylpentanedioic acid, 626-51-7; dimethyl 3-methylpentaedioate, 19013-37-7; 2-methylpentanedioic acid, 617-62-9; dimethyl 2-methylpentanedioate, 14035-94-0; 3-methyl-2-pentenedioic acid, 5746-90-7; dimethyl 3-methyl-2-pentenedioate, 52313-87-8; 2-hydroxy-2-ethylbutanedioic acid, 1944-62-3; dimethyl 2-hydroxy-2-ethylbutanedioate, 72718-97-9; 3-hydroxy-3-methylpentanedioic acid 503-49-1; dimethyl 3-hydroxy-3-methylpentanedioate, 56652-39-2; hexanedioic acid (adipic acid), 124-04-9; dimethyl hexanedioate, 627-93-0; 2-hydroxy-2-isopropylbutanedioic acid, 43119-99-9; dimethyl 2-hydroxy-2-isopropylbutanedioate, 43064-52-4; 4-hydroxy-3-methyl-2-pentenedioic acid, 18152-89-1; heptanedioic acid (pimelic acid), 111-16-0; dimethyl heptanedioate, 1732-08-7; octanedioic acid (suberic acid), 505-48-6; dimethyl octanedioate, 1732-09-8, 2-octenedioic acid, 58447-36-2; dimethyl 2-octenedioate, 54526-85-1; nonanedioic acid (azelaic acid), 123-99-9; dimethyl nonanedioate, 1732-10-1; 2-nonenedioic acid, 72461-80-4; decanedioic acid (sebacic acid), 111-20-6; dimethyl decanedioate 106-79-6; 2-decenedioic acid, 6048-93-7; dimethyl 2-decenedioate, 28598-91-6; 3-hydroxydecanedioic acid, 73141-46-5; dodecanedioic acid, 693-23-2; dimethyl dodecanedioate, 1731-79-9; 2-dodecenedioic acid, 124-00-5; dimethyl 2-dodecenedioate, 70086-90-7.

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