

# Oligosaccharides in New Zealand Honeydew Honey

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A series of oligosaccharides based upon successive addition of glucose (1→4) to the glucopyranosyl residue of sucrose and another series similar to the first but with the final residue linked (1→6) have been isolated from New Zealand honeydew honey and fully characterized. Because the trisaccharide in this series is erlose, it is inferred that the honeydew of the indigenous scale insect, *Ultracoelostoma assimile*, which lives upon the Southern beech, *Nothofagus* spp., is of the erlose type. These oligosaccharides and others have been quantified by GC and LC in six New Zealand honeydew honeys.

**Keywords:** Honeydew; honey; *Ultracoelostoma assimile*; scale insect; *Nothofagus* spp.; Southern beech; oligosaccharides; erlose; New Zealand

## INTRODUCTION

The scale insect, *Ultracoelostoma assimile*, lives under the bark of all but one of the species of the Southern beech (*Nothofagus* spp.) that grow on the South Island of New Zealand. This insect draws nourishment directly from the phloem of the tree and secretes a waste product known as honeydew (Crozier, 1981; Gaze and Clout, 1983). The solids content of honeydew is 90–95% carbohydrate, predominantly sucrose, glucose, and fructose but also including higher sugars produced by the action of glycosyl transferases or glycosidases in the insect gut (Maurizio, 1975). Honeydew is collected by honey bees in the absence of floral sources of nectar and is processed, with the addition of further enzymes, into a dark colored and strongly flavored specialty honey.

Honeydew honey is also produced in areas of the United States and in central Europe, where its importance has increased as modern agricultural practice reduces available floral sources of nectar (Kloft, 1963; Siddiqui, 1970). In central Europe it is highly prized (Siddiqui, 1970); indeed, the major part of the New Zealand honeydew honey crop is exported to Europe. The honeydew from which American and European honeys are produced would have a source similar to that found in New Zealand but would differ in the genus of insect and tree since *U. assimile* and *Nothofagus* spp. are endemic to New Zealand (Crozier, 1981).

Maurizio (1975) broadly divides honeydews into two types according to the predominance of one or the other of two trisaccharides, erlose and melezitose. Analysis of erlose-containing honeydews has indicated the presence of higher sugars formed by the stepwise addition of glucose to erlose (Duspiva, 1954; Wolf and Ewart, 1955).

No detailed study exists of the honeydew produced by *U. assimile*, but seasonal fluctuations in total sugar content have been recorded (Gaze and Clout, 1983).

The literature on the carbohydrate composition of honeydew honeys is not as comprehensive as that for floral honeys, but an average composition based upon

14 U.S. honeydew honeys gave moisture (16.3%), fructose (31.8%), glucose (26.1%), sucrose (0.8%), maltose (8.8%), higher sugars (4.7%), and undetermined (10.1%) (White, 1962).

Liquid chromatography (LC) of a New Zealand honeydew honey revealed relatively large amounts of oligosaccharides, and this study aimed to isolate and characterize the oligosaccharides present and to quantify them in six samples from different sources within the South Island with the additional aim of using these data as a possible means of fingerprinting this type of honey.

## EXPERIMENTAL PROCEDURES

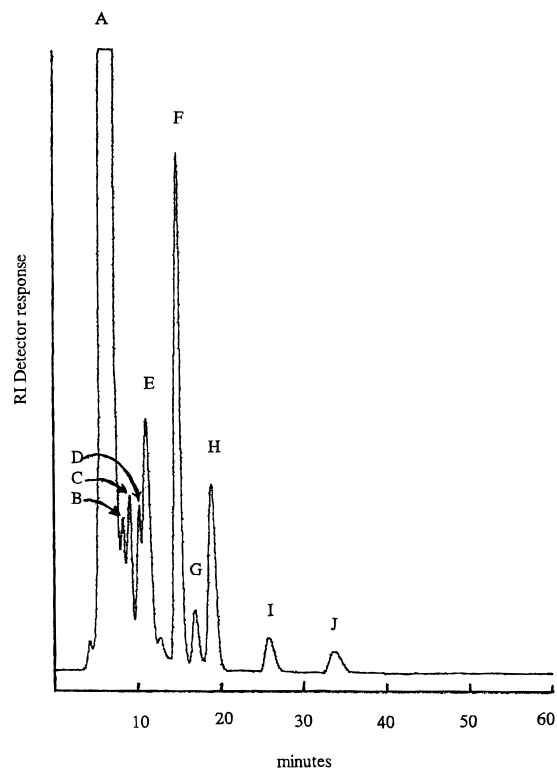
**Materials.** Standards of sucrose, maltose,  $\beta$ -cellobiose, gentiobiose, isomaltose, laminaribiose, melibiose, nigerose, palatinose,  $\alpha,\alpha$ -trehalose, turanose, maltotriose, melezitose, and panose were obtained from Sigma. 1-Kestose was obtained by preparative LC of Nutraflora-FOS obtained from Golden Technologies. Six samples of unpasteurized centrifugally extracted honey from honeydew sources were obtained from different commercial apiarists on the South Island of New Zealand. The source of the honeys was identified by the suppliers on the basis of color, aroma, texture, and taste of the honey and the location of the hive at the time of production. Samples were stored at ambient temperature before extraction.

**Preparation of Honey Samples for LC and GC.** Honey samples were extracted with diethyl ether to remove fatty acids and organics, which might otherwise interfere with chromatography, freeze-dried, and stored in airtight containers at  $-18\text{ }^{\circ}\text{C}$ . Before GC, honey samples and standards were reduced with borohydride and per-*O*-trimethylsilylated.

**Liquid Chromatography.** Oligosaccharides were isolated by repeated chromatography using three Waters Delta-Pak C<sub>18</sub> 25  $\times$  100 mm Radial-Pak columns in series eluted with water at 10 mL min<sup>-1</sup>; detection was by refractive index (RI). Analytical LC was carried out using a Waters Resolve C<sub>18</sub> 8  $\times$  10 mm Radial-Pak column eluted with water at 0.5 mL min<sup>-1</sup> and RI detection. For analytical LC 20  $\mu$ L injections of solutions of honey in water (20 mg mL<sup>-1</sup>) were made; triplicate injections of two samples were used for quantitation. Size exclusion chromatography (SEC) was carried out using a 95  $\times$  2.6 cm column of Bio-Gel P2 eluted with water at 0.5 mL min<sup>-1</sup> and RI detection; for preparative SEC 100 mg samples were injected.

**Gas Chromatography and Mass Spectrometry.** Gas chromatography with flame ionization detection (GC-FID) of

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**Figure 1.** Analytical LC trace of a typical New Zealand honeydew honey; conditions used and identification of peaks are discussed in the text.

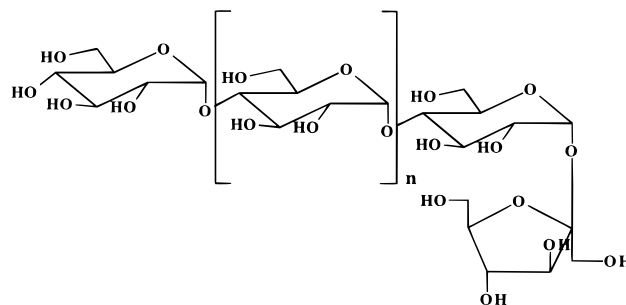
trimethylsilyl derivatives was carried out using a Hewlett-Packard Ultra 2 (25 m  $\times$  0.33 mm) cross-linked phenyl methyl silicone fused silica capillary column. Conditions used were 55 °C (1 min), 30 °C/min to 170 °C, and 3 °C/min to 320 °C for both disaccharides and trisaccharides. For purposes of quantitation, duplicate samples of each honey were prepared and xylitol was used as an internal standard. For partially methylated alditol acetates conditions used were 55 °C (1 min), 30 °C/min to 180 °C, and 4 °C/min to 320 °C. Gas chromatography with mass spectrometry (GC/MS) of partially methylated alditol acetates and trimethylsilyl ethers was carried out using a Hewlett-Packard HP1 cross-linked methyl silicone fused silica capillary column interfaced to a Hewlett-Packard 5970 mass spectrometer (70 eV) and a temperature program similar to that outlined above.

**Electrospray Mass Spectrometry (ES-MS).** Electrospray MS was carried out using a Fisons Instrument VG Platform II mass spectrometer with 50:50 MeOH/water as the mobile phase eluted at 0.02 mL min<sup>-1</sup>. The spectrometer probe temperature was 60 °C and the cone voltage +180V. Samples were spiked with NaCl to assist ionization.

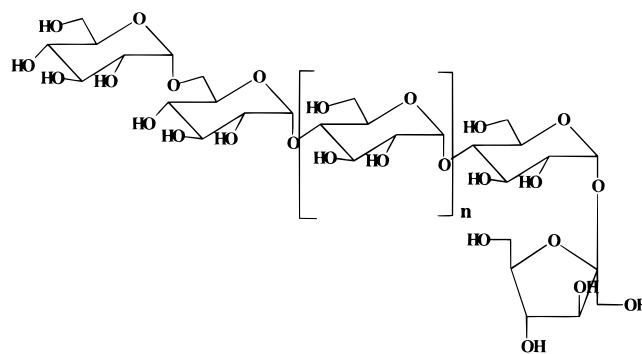
**Nuclear Magnetic Resonance (NMR) Spectrometry.** NMR spectra were obtained, using a Bruker ADV DRX400 400 MHz spectrometer, in D<sub>2</sub>O referenced to internal *t*-BuOH at 1.2 and 31.6 ppm. <sup>1</sup>H, <sup>13</sup>C, DEPT, H,H-COSY, TOCSY, HSQC, and HMBC experiments were carried out. For HSQC a *J* value of 145 Hz was used, for HMBC a delay value of 80 ms was used for evolution of long-range couplings, and for TOCSY a mixing time of 0.3 s was used.

**Methylation Analyses.** Samples were methylated with NaOH and MeI in Me<sub>2</sub>SO after the method of Ciucanu and Kerek (1984) with modifications to the procedure as described by Needs and Selvendran (1993). Methylated samples were hydrolyzed by a two-step hydrolysis procedure, at each stage being reduced (NaBD<sub>4</sub>) and acetylated after the method of Blakeney et al. (1983). The hydrolysis procedure was necessitated by the presence of both fructose and glucose in the oligosaccharides. The glycosidic linkage of a fructofuranosyl residue is more readily cleaved by acid than the glucosidic linkage, and at the same time the fructose residue is more

susceptible to degradation. Acid conditions required to cleave glucosidic linkages would destroy the major part of the fructose released. Therefore, an initial mild hydrolysis (15% HOAc, 100 °C, 60 min) was followed by reduction and then a further strong hydrolysis (15% TFA, 120 °C, 60 min) and subsequent reduction and acetylation (Manley-Harris and Richards, 1993). This double-hydrolysis technique does not unfortunately give reliable quantitation especially for the fructose moiety. A combination of methylation analysis, ES-MS, and NMR was therefore used to characterize compounds 1–6.



Oligosaccharides in New Zealand Honeydew Honey : 1 (n=0); 2 (n=1); 4 (n=2)



Oligosaccharides in New Zealand Honeydew Honey : 3 (n=0); 5 (n=1); 6 (n=2)

## RESULTS AND DISCUSSION

Figure 1 shows the LC trace of a typical New Zealand honeydew honey; peaks labeled A–J are discussed below. Preliminary preparative LC was used to remove the bulk of the monosaccharides (peak A) from the honey samples. The residual material was rechromatographed, and fractions were collected for analysis and characterization.

Peaks B and C were a mixture of di- and trisaccharides that were identified by GC-FID and GC/MS of the *per-O*-trimethylsilyl ethers in the latter case of the unreduced fractions. The electron impact mass spectrum of *per-O*-trimethylsilylated di- and trisaccharides did not reveal a molecular ion, the mass of which lies outside the range of the instrument used for this experiment. One of the larger ions observed in most cases was *m/z* 451, corresponding to [G]<sup>+</sup>. However, certain fragment ions are diagnostic of particular residues. The observation of an ion *m/z* 217 is diagnostic of the presence of a furanose residue; pyranose rings show a characteristic ion at *m/z* 204 (Karady and Pines, 1970). The ion *m/z* 437 is characteristic of 1→1 and 1→2-linked aldohexosylfructoses (Kamerling et al., 1972), and this ion together with *m/z* 217 was prominent in the spectra of sucrose and trisaccharides derived from sucrose such as 1-kestose and erlose. The identities of di- and trisaccharides were confirmed by co-injection of the reduced, *per-O*-trimethylsilylated fraction with the appropriate standard. By this means cellobiose, mal-

**Table 1. Oligosaccharide Constituents of New Zealand Honeydew Honey**

constituent <sup>a</sup>	mean <sup>b</sup> percentage of honey solids	SD	range
sucrose	0.55	0.12	0.40–0.77
trehalose	0.044	0.0036	0.042–0.051
cellobiose	0.33	0.046	0.25–0.37
turanose	1.5	0.26	1.2–1.8
nigerose	1.1	0.13	0.94–1.3
maltose	2.2	0.20	1.9–2.6
gentiobiose	0.85	0.26	0.45–1.3
palatinose	1.3	0.34	0.66–1.8
isomaltose	0.32	0.072	0.24–0.42
erlose, 1	1.2	0.33	0.87–1.8
melezitose	0.085	0.0080	0.069–0.093
maltotriose	0.54	0.19	0.32–0.84
panose	0.51	0.16	0.27–0.73
maltotetraose	0.51	0.23	0.18–0.85
<b>2</b>	3.9	1.2	1.8–5.5
<b>3</b>	0.46	0.13	0.21–0.63
<b>4</b>	1.1	0.89	0.11–2.9
<b>5</b>	0.33	0.15	0.08–0.60
<b>6</b>	0.28	0.13	0.07–0.53
higher sugars except maltose and sucrose	14.31	3.5	8.71–20.57

<sup>a</sup> Compounds **2–6** are discussed and named in the text. <sup>b</sup> Mean of duplicate (GC) or triplicate (LC) samples of six different honeydew honey samples.

tose, gentiobiose, nigerose, turanose, trehalose, palatinose, isomaltose, sucrose, maltotriose, melezitose, and panose were identified. The aforementioned di- and trisaccharides have all been previously identified in honey samples from various origins, and elution orders have been published (Siddiqui, 1970; Siddiqui and Furgala, 1967, 1968; Doner, 1977; Low and Sporns, 1988). 1-Kestose, laminaribiose, and melibiose, which have been found in floral honeys (Siddiqui, 1970; Doner, 1977; Low et al., 1988), were not found. We did not have standards of theandrose and kojibiose, which were also identified by Low and Sporns (1988) in floral honey, or maltulose (Siddiqui, 1970; Doner, 1977). A peak eluting at the position predicted for kojibiose was, however, observed in all samples, and some other minor unidentified peaks in the disaccharide and trisaccharide regions may also be attributable to theandrose and maltulose. The constituents of peaks B and C are all readily amenable to GC as the trimethylsilyl ethers of the reduced sugars and were therefore quantified in six honey samples by this method using response factors obtained from appropriate standards. Mean percentages and ranges are recorded in Table 1.

Peak D was shown to be maltotetraose by comparison of the <sup>13</sup>C NMR spectra with the literature (Bock et al., 1984). Quantitation was achieved by LC, although the results should be viewed with reservations due to the overlap with the following peak.

Peaks E–J proved to be single oligosaccharides, respectively, **1–6**. These oligosaccharides were isolated and characterized by methylation analysis and NMR spectroscopy (Tables 2 and 3). **1**, **2**, and **4** gave, upon methylation analysis, 2,5-di-*O*-acetyl-(2-*O*-deuterio)-1,3,4,6-tetra-*O*-methylhexitol (terminal fructofuranosyl residue 2-linked), 1,5-di-*O*-acetyl-(1-*O*-deuterio)-2,3,4,6-tetra-*O*-methylhexitol (terminal glucopyranosyl residue 1-linked), and 1,4,5-tri-*O*-acetyl-(1-*O*-deuterio)-2,3,6-tri-*O*-methylhexitol (glucopyranosyl residue 1,4-linked). Compounds **3**, **5**, and **6** gave these three partially methylated alditol acetates and in addition 1,5,6-tri-*O*-

acetyl-(1-*O*-deuterio)-2,3,4-tri-*O*-methylhexitol (glucopyranosyl residue 1,6-linked).

**1** was consistent with the known compound erlose [ $\alpha$ -D-Glcp-(1 $\rightarrow$ 4)- $\alpha$ -D-Glcp-(1 $\leftrightarrow$ 2)- $\beta$ -D-Fruf] by methylation analysis, by ES-MS (527.1 [Gly<sub>3</sub> + Na]<sup>+</sup>), and by comparison of the <sup>13</sup>C NMR spectrum with the literature (Bock et al., 1984). **1**, named fructomaltose, was first isolated from the honeydew of the citrus mealy bug, *Pseudococcus citri* (Risso), by Gray and Fraenkel (1953), who also demonstrated its presence in the honeydews of cottony maple scale, *Pulvinaria vitis* (L.), and the spirea aphid, *Aphis spiraeicola* (Patch). White and Maher (1953) demonstrated that erlose was a transient product of the action of honey invertase upon sucrose, and erlose has also been isolated from the reaction of sucrose with honey  $\alpha$ -glucosidase (Ono et al., 1981, Low et al., 1988).

Compounds **2** and **4** proved to be part of a series formed by successive addition of glucopyranosyl residues to erlose being, respectively,  $\alpha$ -D-Glcp-(1 $\rightarrow$ 4)- $\alpha$ -D-Glcp-(1 $\rightarrow$ 4)- $\alpha$ -D-Glcp-(1 $\leftrightarrow$ 2)- $\beta$ -D-Fruf (ES-MS, 689.2 [Gly<sub>4</sub> + Na]<sup>+</sup>) and  $\alpha$ -D-Glcp-(1 $\rightarrow$ 4)- $\alpha$ -D-Glcp-(1 $\rightarrow$ 4)- $\alpha$ -D-Glcp-(1 $\rightarrow$ 4)- $\alpha$ -D-Glcp-(1 $\leftrightarrow$ 2)- $\beta$ -D-Fruf (ES-MS, 851.2 [Gly<sub>5</sub> + Na]<sup>+</sup>). This series has previously been shown to be present in the honeydews produced by the soft scale *Coccus hesperidum* (L.) (Wolf and Ewart, 1955).

Erlose and melezitose are trisaccharides produced by the action of transferases in the gut of the plant-sucking insect and are characteristic of honeydews (Maurizio, 1975; Bacon and Dickinson, 1957).

Maurizio (1975) indicates that, although melezitose and erlose are not mutually exclusive in insect honeydews, one or the other normally predominates. Because the erlose series is dominant in New Zealand honeydew honey, and in the absence of any reported analysis of the honeydew of *U. assimile*, it can be inferred that the honeydew of the scale insect *U. assimile* is of the erlose type.

Compounds **3**, **5**, and **6** were part of a separate series of oligosaccharides differing from the erlose series only in that the terminal glucopyranose was linked (1 $\rightarrow$ 6) instead of (1 $\rightarrow$ 4). **3**, **5**, and **6** are, respectively,  $\alpha$ -D-Glcp-(1 $\rightarrow$ 6)- $\alpha$ -D-Glcp-(1 $\rightarrow$ 4)- $\alpha$ -D-Glcp-(1 $\leftrightarrow$ 2)- $\beta$ -D-Fruf (ES-MS, 689.2 [Gly<sub>4</sub> + Na]<sup>+</sup>),  $\alpha$ -D-Glcp-(1 $\rightarrow$ 6)- $\alpha$ -D-Glcp-(1 $\rightarrow$ 4)- $\alpha$ -D-Glcp-(1 $\rightarrow$ 4)- $\alpha$ -D-Glcp-(1 $\leftrightarrow$ 2)- $\beta$ -D-Fruf (ES-MS, 851.3 [Gly<sub>5</sub> + Na]<sup>+</sup>), and  $\alpha$ -D-Glcp-(1 $\rightarrow$ 6)- $\alpha$ -D-Glcp-(1 $\rightarrow$ 4)- $\alpha$ -D-Glcp-(1 $\rightarrow$ 4)- $\alpha$ -D-Glcp-(1 $\leftrightarrow$ 2)- $\beta$ -D-Fruf (ES-MS, 1013.8 [Gly<sub>6</sub> + Na]<sup>+</sup>). **3**, described as  $\alpha$ -panosyl- $\beta$ -D-fructofuranoside, has previously been isolated from Danish lynghonning (heather honey), and the <sup>13</sup>C spectrum was identical with the reported spectrum (Munksgaard, 1981; Bock et al., 1984). We infer that the transfer of the terminal (1 $\rightarrow$ 6)-linked glucopyranose residue is brought about by transferase enzymes originating from the bee rather than from *U. assimile*.

Tetrasaccharides and higher oligosaccharides are not as readily amenable to GC as their trimethylsilyl ethers. Compounds **2–6** were therefore quantified by LC using prepared concentration/response curves (Table 1).

If significant oligosaccharides having degrees of polymerization (dp) >6 are present, these would not be amenable to LC. To determine if this was so, a honeydew honey sample was subjected to size exclusion chromatography (SEC) (Figure 2) and fractionated, and the dp values of the fractions were determined by ES-MS. This showed that only a trace of material had a dp >6.

**Table 2.**  $^1\text{H}$  NMR Spectra of Higher Oligosaccharides 1–6 Found in New Zealand Honeydew Honey

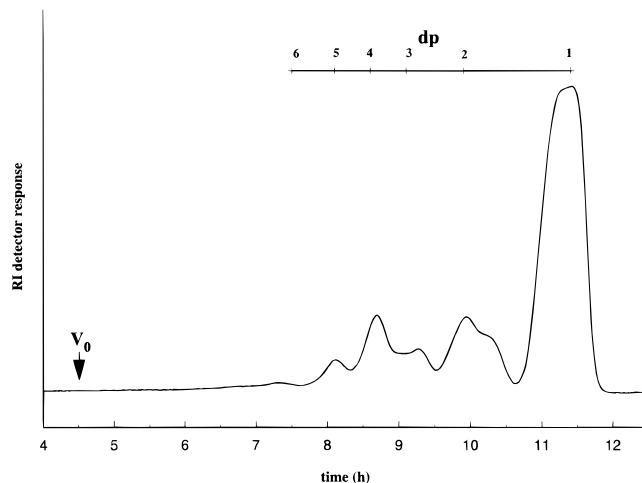
residue	H-1	H-2	H-3	H-4	H-5	H-6
<b>1 (erlose)</b>						
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	5.35 ( $J_{1,2} \sim 4.0$ Hz)	3.53	3.63	3.39	3.68	3.72, 3.80
$\alpha\text{-D-Glcp}(1\leftrightarrow 2)$	5.36 ( $J_{1,2} \sim 4.0$ Hz)	3.54	3.97	3.65	3.92	3.78
$\beta\text{-D-Fruf}$	3.62		4.15 ( $J_{3,4} = 8.7$ Hz)	3.98	3.84	3.75
<b>2</b>						
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	5.340	3.53	3.68	3.37	3.88	3.72, 3.80
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	5.343	3.58	3.91	3.59	3.80	3.75
$\alpha\text{-D-Glcp}(1\leftrightarrow 2)$	5.355	3.54	3.96	3.64	3.92	3.78
$\beta\text{-D-Fruf}$	3.62		4.157 ( $J_{3,4} = 8.7$ Hz)	3.97	3.83	3.75
<b>4</b>						
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	5.33	3.50	3.60	3.33	3.62	3.68, 3.76
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	5.34	3.54	3.87	3.57	3.75	3.68
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	5.34	3.54	3.87	3.57	3.75	3.68
$\alpha\text{-D-Glcp}(1\leftrightarrow 2)$	5.35	3.51	3.93	3.61	3.87	3.75
$\beta\text{-D-Fruf}$	3.62		4.154 ( $J_{3,4} = 8.7$ Hz)	3.95	3.80	3.75
<b>3</b>						
$\alpha\text{-D-Glcp}(1\rightarrow 6)$	4.91 ( $J_{1,2} = 4.0$ Hz)	3.52	3.70	3.39	3.68	3.73, 3.80
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	5.353 ( $J_{1,2} = 4.5$ Hz)	3.60	3.4	3.45	3.88	3.71, 3.92
$\alpha\text{-D-Glcp}(1\leftrightarrow 2)$	5.365 ( $J_{1,2} = 4.0$ Hz)	3.55	3.93	3.66	3.93	3.81
$\beta\text{-D-Fruf}$	3.62		4.160 ( $J_{3,4} = 8.7$ Hz)	4.00	3.85	3.76
<b>5</b>						
$\alpha\text{-D-Glcp}(1\rightarrow 6)$	4.912 ( $J_{1,2} = 3.7$ Hz)	3.52	3.73	3.39	3.68	3.72, 3.82
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	5.353 ( $J_{1,2} = 5.4$ Hz)	3.56	3.67	3.46	3.88	3.69, 3.92
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	5.342 ( $J_{1,2} = 4.0$ Hz)	3.59	3.92	3.6	3.82	3.8
$\alpha\text{-D-Glcp}(1\leftrightarrow 2)$	5.365 ( $J_{1,2} = 4.0$ Hz)	3.55	3.99	3.66	3.93	3.78
$\beta\text{-D-Fruf}$	3.63		4.170 ( $J_{3,4} = 8.3$ Hz)	4.00 ( $J_{4,5} = 8.5$ Hz)	3.85	3.76
<b>6</b>						
$\alpha\text{-D-Glcp}(1\rightarrow 6)$	4.915 ( $J_{1,2} = 3.7$ Hz)	3.52	3.71	3.40	3.68	3.74, 3.81
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	5.35	3.57	3.65	3.46	3.87	3.70, 3.94
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	5.36	3.6	3.93	3.63	3.82	3.8
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	5.36	3.6	3.93	3.63	3.82	3.8
$\alpha\text{-D-Glcp}(1\leftrightarrow 2)$	5.37	3.55	3.99	3.66	3.93	3.80
$\beta\text{-D-Fruf}$	3.63		4.167 ( $J_{3,4} \sim 8.6$ Hz)	4.01 ( $J_{4,5} \sim 8.5$ Hz)	3.86	3.76

**Table 3.**  $^{13}\text{C}$  NMR Spectra of Higher Oligosaccharides 1–6 Found in New Zealand Honeydew Honey

residue	C-1	C-2	C-3	C-4	C-5	C-6
<b>1 (erlose)</b>						
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	101.7	73.7	74.9	71.3	74.7	62.5
$\alpha\text{-D-Glcp}(1\leftrightarrow 2)$	93.9	72.8	75.0	78.8	73.0	62.1
$\beta\text{-D-Fruf}$	63.4	105.6	78.5	76.0	83.3	64.3
<b>2</b>						
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	101.7	73.7	74.9	71.3	74.7	62.5
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	101.6	73.5	75.3	78.8	73.2	62.5
$\alpha\text{-D-Glcp}(1\leftrightarrow 2)$	93.9	72.8	74.9	78.9	72.9	62.1
$\beta\text{-D-Fruf}$	63.4	105.6	78.5	76.0	83.3	64.3
<b>4</b>						
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	101.7	73.7	74.9	71.3	74.7	62.5
$\alpha\text{-D-Glcp}(1\rightarrow 4)^a$	101.5	73.5	75.3	79.0	73.1	62.4
$\alpha\text{-D-Glcp}(1\rightarrow 4)^a$	101.6	73.5	75.3	78.8	73.2	62.5
$\alpha\text{-D-Glcp}(1\leftrightarrow 2)$	93.9	72.8	75.1	78.8	72.9	62.1
$\beta\text{-D-Fruf}$	63.4	105.6	78.5	76.0	83.3	64.3
<b>3</b>						
$\alpha\text{-D-Glcp}(1\rightarrow 6)$	100.0	73.4	75.1	71.5	73.8	62.5
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	101.9	73.7	75.1	71.4	73.3	68.0
$\alpha\text{-D-Glcp}(1\leftrightarrow 2)$	93.9	72.8	74.9	79.3	72.9	62.3
$\beta\text{-D-Fruf}$	63.4	105.6	78.5	76.0	83.3	64.3
<b>5</b>						
$\alpha\text{-D-Glcp}(1\rightarrow 6)$	101.1	73.4	75.1	71.5	73.8	62.5
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	101.8	73.7	75.1	71.4	73.3	67.9
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	101.6	73.5	75.3	79.2	73.2	62.5
$\alpha\text{-D-Glcp}(1\leftrightarrow 2)$	93.9	72.8	74.9	78.9	72.9	61.0
$\beta\text{-D-Fruf}$	63.4	105.6	78.5	76.0	83.3	64.3
<b>6</b>						
$\alpha\text{-D-Glcp}(1\rightarrow 6)$	100.1	73.4	75.1	71.5	73.8	62.5
$\alpha\text{-D-Glcp}(1\rightarrow 4)$	101.9	73.7	75.1	71.4	73.3	68.0
$\alpha\text{-D-Glcp}(1\rightarrow 4)^b$	101.6	73.5	75.3	79.2	73.2	62.5
$\alpha\text{-D-Glcp}(1\rightarrow 4)^b$	101.5	73.5	75.3	79.0	73.2	62.4
$\alpha\text{-D-Glcp}(1\leftrightarrow 2)$	93.9	72.8	74.9	78.8	72.9	62.1
$\beta\text{-D-Fruf}$	63.4	105.6	78.5	76.0	83.3	64.3

<sup>a,b</sup> The assignment for these peaks may be interchanged.

The results in Table 1 may be compared to those obtained by White (1962) for 14 American honeydew



**Figure 2.** SEC of a typical New Zealand honeydew honey on Bio-Gel P-2. The dp was ascertained by ES-MS of the fractions collected.  $V_0$  indicates the exclusion limit of the gel. Conditions for the chromatography are discussed under Experimental Procedures.

honeydew honeys. Note, however, that the figures in White (1962) are percentages of total honey including the moisture, whereas those in Table 1 are percentages of solid components. The sucrose component of New Zealand honeydew honey is smaller than that obtained by White, and the maltose component is significantly smaller. The total percentage of higher sugars, excepting maltose and sucrose, is much greater in the New Zealand samples than in the American samples. These effects may arise from the enzymic activity of the scale insect or of the bee, and this may also possibly be related to seasonal changes. Detailed information relating to these factors in a New Zealand setting is not yet available, although

Gaze and Clout (1983) showed seasonal variations in total sugar content of beech honeydew droplets of 16–81%; these variations were thought to be caused by changes in the translocation of solutes within the tree, although some effect was attributed to atmospheric conditions.

#### ABBREVIATIONS USED

LC, liquid chromatography; RI, refractive index; SEC, size exclusion chromatography; dp, degree of polymerization; GC, gas chromatography; MS, mass spectrometry; FID, flame ionization detection; ES-MS, electrospray mass spectrometry; NMR, nuclear magnetic resonance spectrometry; DEPT, distortionless enhancement by polarization transfer; COSY, correlation spectroscopy; TOCSY, total correlation spectroscopy; HSQC, homonuclear single quantum coherence; HMBC, heteronuclear multiple bond coherence; SD, standard deviation.

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