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The oligosaccharide composition of some New Zealand honeys

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Abstract

The oligosaccharide fraction of samples of manuka *(Leptospermum*), heather (*Calluna*), clover (*Trifolium*) and beech honeydew (*Nothofagus*) honeys from New Zealand was separated from the monosaccharides and then analysed by high performance anion-exchange chromatography with pulsed amperometric detection (hpaec-pad). Significant oligosaccharide components of manuka honey were isomaltose (or maltulose), kojibiose, turanose (or gentiobiose), nigerose and maltose which was the major component. The composition of clover honey was identical to that of manuka, while heather honey differed from these two only because isomaltose was the major component. Beech honeydew honey was characterised by the complexity of the oligosaccharide composition. The trisaccharides melezitose and panose were the most abundant components. No differences were observed between the oligosaccharide compositions of manuka honeys which did or did not exhibit non-peroxide residual antibacterial activity. Manuka honey was shown to be derived from nectar and not honeydew as has been suggested.

1. Introduction

An earlier paper from this laboratory (Weston et al., 1998) described work which identified some phenolic substances in honey produced from nectar of the New Zealand manuka tree (*Leptospermum scoparium*; Myrtaceae). That work was part of a programme that is attempting to identify substances which are responsible for the non-peroxide residual antibacterial activity of this honey. *In-vitro* studies have demonstrated a bacteriocidal effect for manuka honey against *Helicobacter pylori* the bacterium which causes human stomach ulcers (Somal et al., 1994). Adhesion of this bacterium to the lining of the stomach wall is mediated by the tetrasaccharide sialyl Lewis x antigenic determinant, which forms the terminus of several glycoproteins and glycolipids on gastric mucosa (Boren et al., 1993). Since that discovery, an intense effort was begun to prepare mimics of this tetrasaccharide (Shih-Hsiung Wu et al., 1996) which will bind to receptors on the surface of the bacterium and thereby prevent its adhesion to the stomach lining.

While honey is composed primarily of the sugars, glucose, fructose, sucrose and maltose (White, 1978) many oligosaccharides have been identified in honey (Siddiqui and Furgala, 1967, 1968). The oligosaccharide composition of honey derived from honeydew is especially complex and manuka honey is believed to be a honeydew honey (Tan et al., 1988). We surmised that it was possible for a unique oligosaccharide to be present in manuka honey similar in structure to the Lewis x tetrasaccharide that might be responsible for the observed bacteriocidal effect of manuka honey *in vitro* against *H. pylori* and other bacteria. This hypothesis prompted us to examine the oligosaccharide composition of antibacterially active *and* inactive New Zealand manuka honeys to determine whether any differences existed between these two variants of the same honey and also to confirm the previous deduction that this honey was derived from honeydew.

2. Materials and methods

2.1. Materials

Honey samples were obtained from the Honey Research Unit, Department of Biological Sciences, Waikato University, Hamilton.

2.2. Methods

2.2.1. Antibiotic activity

All antibiotic activity discussed in this paper refers to *in vitro* non-peroxide antibacterial activity, determined

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by well diffusion bioassays on agar plates inoculated with *Staphylococcus aureus*. The procedures for these bioassays are described by Allen et al. (1991). Results of the bioassays are reported as the diameter of the area of inhibition of growth of the bacteria. The concentration is that used for the bioassay. For the active honey, used as a standard, the minimum inhibitory concentration (MIC) was 125 g litre^{-1}.

2.2.2. Sample preparation

The oligosaccharide fraction of several honeys was obtained by a variation of the method described by Swallow and Low (1990). Honey (1.0 g) was dissolved in water (20 ml) and activated carbon (4.0 g) was then added. The mixture was stirred at room temperature for 18 hr and then added to a glass column, which was packed with activated carbon and Celite (1:1; 4.0 g). Monosaccharides were eluted from the column with a solution of ethanol in water (1:999; 1.0 litre) and the oligosaccharides with ethanol/water (1:1; 500 ml). Solutions were concentrated at 40°C on an evaporator and then freeze-dried (Table 1).

2.2.3. High pressure anion-exchange chromatography

Analyses of the oligosaccharides were carried out according to the method described by Swallow and Low (1990). The chromatography was performed with a Dionex system incorporating a Carbopac PA1 (4×250 mm) column, a PA1 (4×50 mm) guard column and pulsed amperometric detection. The flow rate was 0.7 ml min^{-1} at a pressure of 1500 psi. A gradient solvent system was used as follows:

Solvents; A: 1M NaOH, B: 1M NaOAc, C: water.
Gradient A/B/C was

T0	10/0/90	T4	10/0/90	T20	10/3/87
T50	10/10/80	T60	10/10/80	T61	10/0/90

Table 1
Oligosaccharide and monosaccharide content of various honeys

Honey Sample	Monosaccharides (%)	Oligosaccharides (%)	Total
Manuka (active M61)	80	8	88
Manuka (inactive)	89	12	101
Active fraction F (from M61 honey)	81	5	86
Heather (MV)	68	8	76
Honeydew (MV)	62	17	79
Manuka (inactive no. 9)	73	5	78
	Ave. 76	Ave. 9	Ave. 85

Chromatograms of the oligosaccharides from various honeys are shown in Figs. 1–4 and the compositions of the oligosaccharide fractions are given in Table 2.

3. Results and discussion

3.1. Oligosaccharide composition of the honeys

The purpose of this work was to determine qualitative differences between the various honeys and absolute concentrations of the sugars in the extracts or honeys were not determined. The mass of glucose and fructose, which was separated from the honeys, might have differed slightly from one sample to another and therefore the compositional data in Table 2 is useful only to determine the abundance of the sugars *relative* to one another (except for fructose and glucose). No account of relative response factors of the sugars was taken for the compilation of this data. These factors were recorded by Swallow and Low (1990). Approximate absolute concentrations of individual sugars in the honeys can be calculated from the fact that oligosacharides constitute

Table 2
Oligosaccharide composition of extracts of some New Zealand honeys

R_t (min)	Sugar	%(w/w) in sample			
		Manuka honey		Heather	Beech honeydew honey
		Antibacterially active	Antibacterially inactive		
8.2	Glucose	11.6	10.2	10.2	5.9
9.3	Fructose	4.3	9.2	5.1	1.9
14.7	Isomaltose[a]	15.5	13.7	16.4	18.4
16.9	Sucrose	15.4	5.0	5.0	8.7
17.5	Kojibiose	15.4	10.5	3.7	8.7
19.1	Unknown	—	—	2.8	—
19.8	Turanose[b]	10.2	6.0	6.5	3.0
20.1	Unknown	—	—	3.1	—
21.7	Melezitose	—	1.7	1.4	4.3
23.7	Nigerose	7.1	6.1	8.7	5.6
24.7	Maltose[c]	19.6	23.5	9.2	5.5
25.5	Theanderose	2.3	1.3	3.4	0.9
27.4	Erlose	3.2	3.9	—	3.6
30.7	Unknown	2.3	2.3	1.8	—
31.8	Panose	1.5	—	3.0	6.6
32.8	Unknown	—	—	—	2.0
35.9	Maltotriose	—	—	—	6.1
36.5	Unknown	—	—	—	2.3
40.7	Unknown	—	—	1.6	3.7
43.8	Unknown	—	—	—	2.5
44.6	Unknown	—	—	—	3.0
49.9	Unknown	—	—	—	1.8

[a] and/or maltulose.
[b] and/or gentiobiose.
[c] and/or 1-kestose.

10% of the mass of honey (see data under *sample preparation* above. White (1978) provides an average oligosaccharide content of 10%.

3.1.1. Manuka honey (Figs. 1 and 2)

Significant sugars in the oligosaccharide fraction of this honey were isomaltose (or maltulose), kojibiose, turanose (or gentiobiose), nigerose, maltose and minor components were sucrose, erlose and panose. Maltose was the major oligosaccharide.

3.1.2. Clover honey

The chromatogram and compositon of the oligosaccharides of this honey were identical to those of manuka honey.

3.1.3. Heather honey (Fig. 3)

The major difference between the oligosaccharide composition of heather honey and manuka honey was the higher ratio of isomaltose to maltose and the insignificance of erlose.

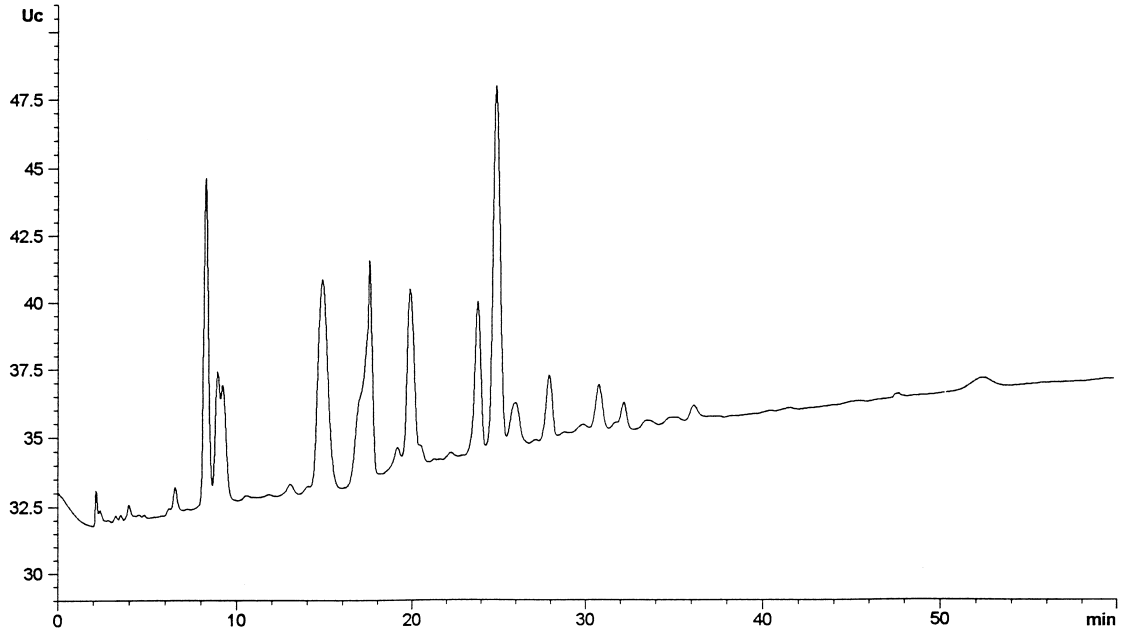

Fig. 1. Oligosaccharides of manuka honey with residual non-peroxide antibacterial activity.

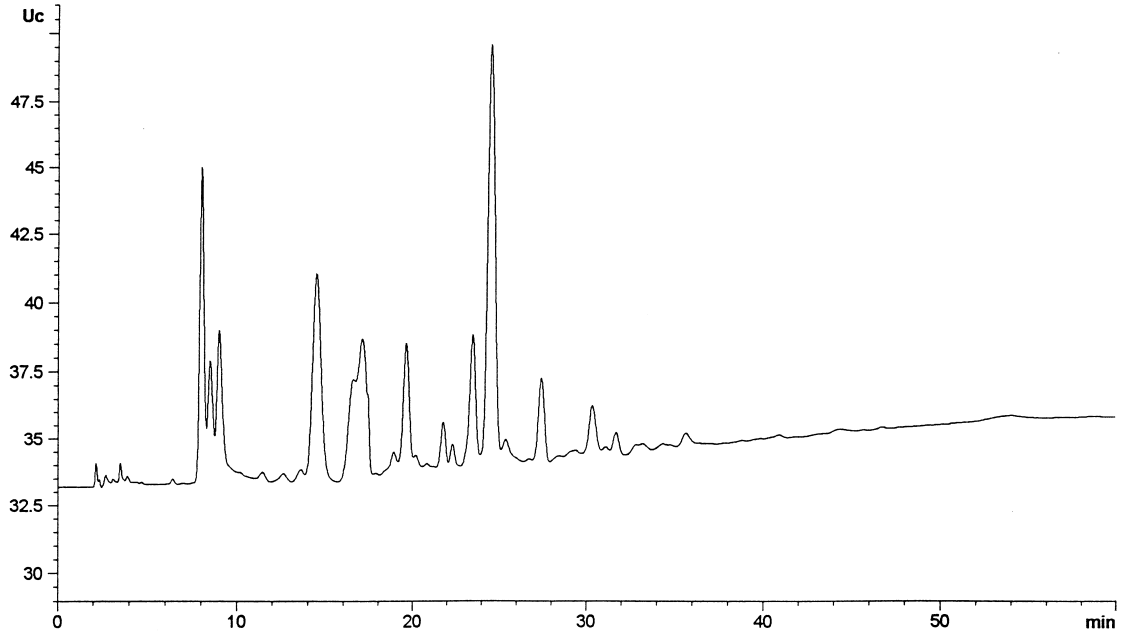

Fig. 2. Oligosaccharides of manuka honey with no residual non-peroxide antibacterial activity.

3.1.4. Honeydew honey (Fig. 4)

The oligosaccharide fraction of this honey was characterised by its large number of components, the origin of which is discussed below. Isomaltose was the most abundant disaccharide while melezitose, panose and maltotriose were significant trisaccharides. In particular, the prominance of melezitose is regarded as a characteristic feature of honeydew honey (Doner, 1977).

3.2. Differences between Manuka honeys

There were no differences whatsoever between the oligosaccharide composition of antibacterially active and inactive manuka honeys. Excluding carbohydrate acids, which were not detected with this solvent system, the oligosaccharides clearly do not contribute to the antibacterial activity of this honey as was surmised in the introduction.

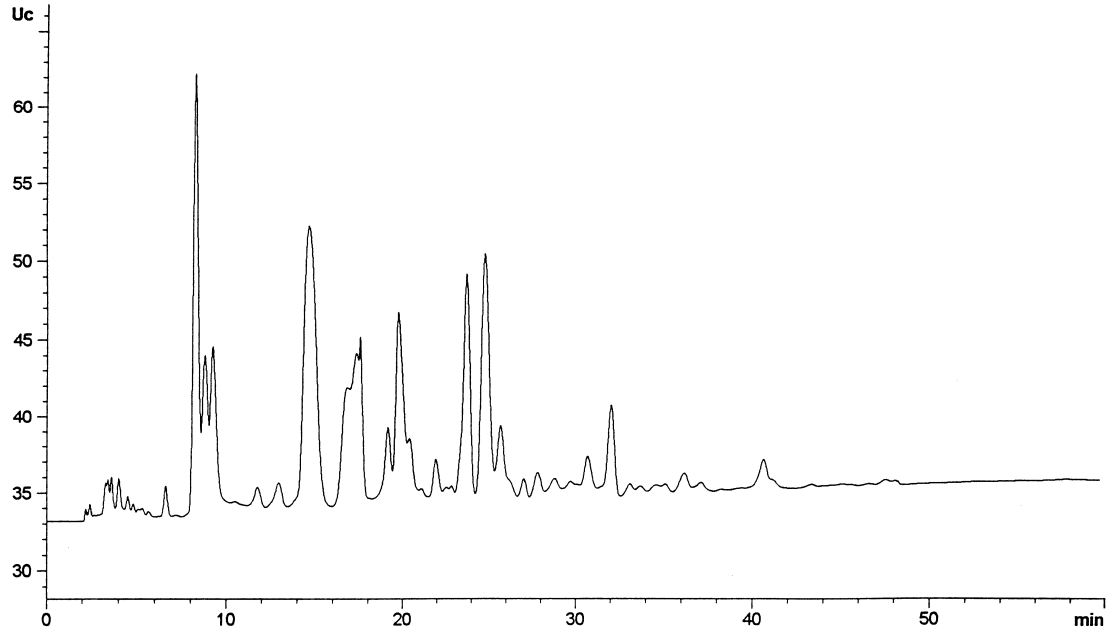

Fig. 3. Oligosaccharides of heather honey.

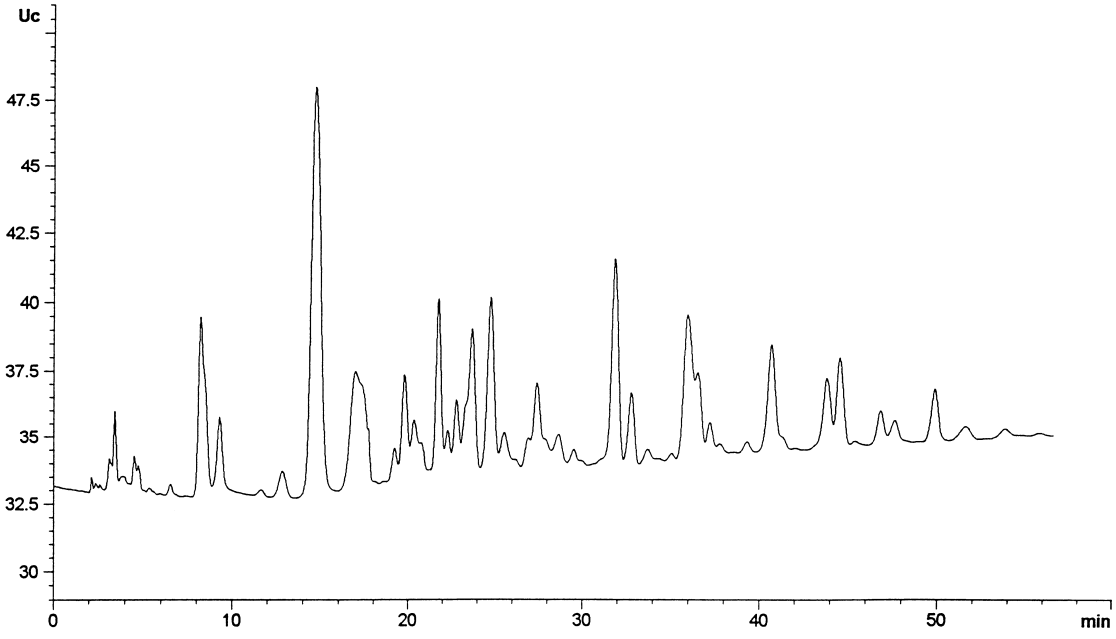

Fig. 4. Oligosaccharides of beech honeydew honey.

3.3. Nectar and honeydew honeys

Manuka, clover and heather honeys are all derived from the flower nectar of the respective plants, whereas the honeydew honey (used in this work) was derived from the sap of New Zealand beech trees (*Nothofagus* spp). The sap is ingested by the native scale insects *Ultracoelostoma assimile* and *U. brittini* (Margarodidae: Hemiptera) and then excreted as droplets of honeydew onto the trunk of the trees. In beech forests which are not infested with wasps, the bees are able to collect the honeydew for conversion to a honey which is darker in colour and more strongly aromatic than most other honeys (Crozier, 1981).

The complexity of the oligosaccharide composition of honeydew honeys arises from the action of transglucosylation enzymes in the gut of scale insects, on the sucrose of tree sap (Bacon and Dickinson, 1957), which results in the formation of a large number of oligosaccharides. The permutations of the stereochemical linkages and the ultimate oligosaccharide composition has been found recently to depend on the species of both the insect and plant or tree (Hendrix et al., 1992).

Manuka honeydew is produced by the native scale insect *Coelostomidia wairoensis* (Margarodidae: Hemiptera) which infests manuka trees (Morales, 1991). In view of the work described by Hendrix et al. (1992), a honey made from manuka honeydew might be expected to have a slightly different composition from that of a beech honeydew honey, but the two honeys would nevertheless exhibit the complexity of oligosaccharide composition characteristic of a honeydew honey.

Comparison of the oligosaccharide composition of the samples of manuka honeys used in this work, illustrates clearly that manuka honey is a nectar honey and is not derived from manuka honeydew as was surmised by Tan et al. (1988).

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