

The Sugars of Honey—A Review

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Surveys of floral honey composition have established that the three major components are fructose, glucose, and water, averaging 38.2, 31.3 and 17.2%, respectively. Glucose and fructose are the only monosaccharides in honey and it is these sugars, combined in various forms, that comprise the di- and trisaccharide fractions of floral honey. Several laboratories, utilising various chemical and physical methods, have been responsible for the isolation and characterisation of ten disaccharides, ten trisaccharides, and two higher sugars from floral honey. Several of these occur only rarely in nature, and the trisaccharide erlose, produced by the action of honeybee invertase on sucrose, was first discovered as a component of honey. Honeydew honey is produced by the honeybee from honeydew deposits left by various hemipterous insects on their host plant. Honeydew contains a more complex mixture of sugars than does nectar, and honeydew honey is appreciably higher in reducing disaccharides and higher sugars than is floral honey. The trisaccharide melizitose, not found in floral honey, is often present in levels exceeding 10% in honeydew honey. The precipitation of glucose from honey, termed granulation, is often technologically undesirable as it is sometimes followed by fermentation. Indices such as the glucose/water ratio have been used to predict granulation tendency. Small amounts of hydroxymethylfurfural (HMF) occur naturally in honey, resulting from the acid catalysed dehydration of the hexoses, particularly fructose. High levels of HMF suggest adulteration of honey with acid inverted invert syrup and several methods are available for its determination. The conversion of nectar and honeydew to the complex array of honey sugars by the honeybee involves a variety of chemical and biochemical processes, some of which are now understood, while others remain to be elucidated.

1. Honey precursors

Before discussing the sugar composition of honey and honeydew honey, the composition of the precursors to these commodities will be covered in some detail.

1.1. Nectar and honeydew

1.1.1. *Origins*

The raw materials for the production of honey by the honeybee, nectar and honeydew, both originate in the sap of vascular plants, the circulating fluid that distributes nutrients throughout the plant. The floral nectaries are supplied through phloem (tissue conducting organic materials) and xylem (tissue conducting water and dissolved minerals), with the ratio of phloem to xylem determining the sugar concentration of the nectar.¹ These nectaries may contain up to 80% sugar,² but most often 20–40%, and are the bees' source of raw material from which to produce floral or nectar honey. Honeydew most often comes to the bee more indirectly; hemipterous insects (aphids, leaf hoppers, scale insects) feed on phloem sap of various trees and then excrete from their alimentary canal a sweet liquid that is collected by the bee. Less often, honeydew is collected in the form of plant secreted phloem sap, or manna, without other insects acting as intermediaries.

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Honeybees generally show a preference for floral nectar over honeydew, gathering the latter during periods of low nectar availability such as drought. Both nectar and honeydew are processed by the bee in the same manner, with the compositional differences in the two being reflected in the final products, floral honey and honeydew honey.

1.1.2. Sugar composition of nectar

In 1886 Planta³ reported that nectar contains glucose, fructose, and sucrose. Much later (1952), small amounts of raffinose and melibiose were reported by Wykes.⁴ Furgala⁵ examined several varieties of clover nectar and found maltose to account for as much as 26% of the total solids, but suggested that the nectar may have been contaminated by honeydew of aphids. Maurizio⁶ reported finding maltose in small amounts in the nectar of some plants, but more often found only glucose, fructose, and sucrose. These studies used paper chromatographic separation of nectar sugars and comparison of R_F values with appropriate standards as their method of analysis. Complete nectar and honeydew analysis requires the use of more sophisticated methods, such as have been applied with success in the identification of minor sugars in honey. The difficulty in obtaining sufficient quantities of nectar has been the handicap to this point. Kleinschmidt *et al.*² and Echigo,⁷ however, applied gas chromatography in analyses of nectar, and found only glucose, fructose, and sucrose to be present. More recently, Battaglini and Bossi⁸ used this technique and found, in addition, traces of raffinose and melizitose.

Paper chromatographic examination of the glucose, fructose, and sucrose content of nectar from a large number of floral species has suggested that the ratio of these sugars is related to the anatomy of the sugar conducting system of the plant and may be quite constant for a species. Percival⁹ surveyed 889 species and found three patterns of sugar composition: (a) high sucrose nectar, (b) about equal amounts of glucose, fructose, and sucrose, and (c) high glucose and fructose. Sucrose⁹ dominant nectar was associated with long-tubed flowers in which the nectar was protected (clovers), while open flowers usually contained only glucose and fructose. Her findings confirmed earlier reports¹⁰⁻¹² suggesting a relationship between the ratio of the three sugars and the species of a flower. Quantitative paper chromatography of the hexoses in nectar from a variety of species has shown the fructose/glucose ratio to range normally from 2 to 28; only rarely does glucose predominate (dandelion, rape, pear).⁶ As will be elaborated upon later, the fructose/glucose ratio is much more variable in nectar than in honey. Wykes⁴ observed that honeybees preferred a test solution containing equal amounts of glucose, fructose, and sucrose to other ratios, and Furgala⁵ found that bees prefer sweet clover (balanced composition) to alfalfa (high sucrose) nectar.

The total sugar concentration in nectar varies over a wide range, depending on plant species and environmental conditions, but typically the range is 20-40%.¹⁰ In the gas chromatographic analysis of Wyoming alfalfa nectar by Kleinschmidt *et al.*² in 1968, 81% sugar was found. The very low relative humidity under which the alfalfa was grown could explain this extraordinarily high value.

1.1.3. Sugar composition of honeydew

Honeydew deposits left on their host plant by aphids have been analysed by various groups and the evidence suggests that it is a more complex mixture of sugars than is nectar. Glucose, fructose, sucrose, and melizitose have long been known to be constituents of honeydew. Melizitose has been reported to represent as much as 30% of honeydew,^{13,14} though typical values are less than 5%.¹⁵ Only trace amounts of this trisaccharide have been found in floral honey, so it is obvious that the honeybee does not produce this sugar. A divergence of opinion existed for some time regarding the origin of melizitose. Some, including Hudson,¹⁶ believed that melizitose was present in the sap of the plants concerned, while others concluded that the insect is responsible for its formation. Those holding the latter view argued that melizitose was absent from the sap of trees (spruce, oak) on which melizitose was found. That the insects produce the sugar was confirmed in 1957 by Bacon and Dickinson¹⁷ who demonstrated in aphids a transglucosylase activity capable of converting sucrose to melizitose. Honey invertase was shown to possess transglucosylase activity by White and Maher^{18,19} in 1953, producing from sucrose six sugars in addition to glucose and fructose. The

major product was shown to be erlose [O- α -D-glucopyranosyl-(1 \rightarrow 4)- α -D-glucopyranosyl- β -D-fructofuranoside], previously unknown. This sugar was then found by Gray and Fraenkel²⁰ in several samples of honeydew and in 1954 Duspiva²¹ reported a series of sugars in honeydew related to erlose by the stepwise addition of glucose molecules. This was confirmed the next year by Wolf and Ewart,²² who established that erlose, maltotriosylfructoside, maltotetraosylfructoside and possibly maltopentaosylfructoside are present in honeydew. It has been suggested²³ that there are two types of honeydew, the melizitose type and the erlose type. In her 1962 review on the sugars of nectar and honeydew, Maurizio⁶ states that different species of insects feeding on the same host plant can produce honeydews with quite different sugar compositions and with variable attractiveness to bees.

2. Honey

2.1. Production by the honeybee

A comprehensive discussion of the anatomy and physiology of the honeybee, as well as the processes of gathering nectar and subsequently converting it to honey are included in two recently published volumes entitled *The Hive and the Honeybee*²⁴ and *Honey*.²⁵ Briefly, the sugar-containing raw material is drawn in through the proboscis and transported to the honey sac, which is sealed off from the digestive tract by the proventricular valve. Enzyme secretions of the hypopharyngeal gland are mixed with the contents of the honey sac. The foraging bee then returns to the hive and passes its load (estimated at 50 mg)²⁶ of material to the house bees, who undertake the task of "ripening" the honey. Ripening is accomplished by the house bees alternately expelling and ingesting the honey sac fluid repeatedly for 15–20 min, mixing it with additional enzyme secretions and reducing its water content. When the honey is properly ripe, with nectar sucrose inverted and concentrated to about 82% solids,²⁷ the comb cells are capped over by the bees and the honey stored as food.

2.2. Definition and early surveys of honey

The US Food and Drug Act of 1906 defined honey as "the nectar and saccharine exudation of plants, gathered, modified, and stored in the comb of honeybees (*Apis mellifera* and *Apis dorsata*); is levorotatory; contains not more than 25% water, not more than 0.25% ash, and not more than 8% sucrose." The limits established in this definition were largely based on a survey of commercial honey samples by Wiley,²⁸ which was published in 1892. Much of the honey on the market at that time was contaminated with other carbohydrate materials.¹⁵ The analytical methods used by Wiley were the basis of a 1908 survey by Browne²⁹ of 100 samples of honey and honeydew honey. Determinations were made of glucose, fructose, dextrin (not well defined), ash and free acidity. Other surveys were conducted over the next 30 years,^{30–32} using the methods of Wiley and Browne with slight modification. These methods, utilising polarimetry and reducing sugar analyses, appeared in the Official Methods of the Association of Agricultural (now Analytical) Chemists³³ until after the development of an entirely new procedure by White and Maher in 1954.³⁴

2.3. Methods of sugar analysis of honey

The need for an alternative method of sugar analysis was demonstrated in a paper chromatographic study by Tafel and Reiss³⁵ and in a critical study of previously used methods by White *et al.* in 1952.³⁶ The methods in general use until that time^{37–41} were shown³⁶ to give values that did not reflect the true composition of the sample, and variance due to methods was as great as that due to differences among various types of floral honey. The new method, termed "selective adsorption",³⁴ provided for more accuracy in the determination of glucose and fructose and revealed the presence of new categories of honey sugars, the reducing disaccharides and higher oligosaccharides. Before this time, honey had been considered to be primarily a mixture of glucose, fructose, and sucrose, and methods using polarimetry and reducing sugar analyses, though themselves precise, were unknowingly interfered with by a number of other sugars, both reducing and non-reducing. The selective adsorption procedure utilises the charcoal-celite column (described by Whistler and

Durso⁴² in 1950) and effects the fractionation of honey into monosaccharides, disaccharides, and higher oligosaccharides before analysis. Results were shown to be superior to traditional methods of honey analysis by collaborative testing⁴³⁻⁴⁵ and accepted as first action by the Association of Official Agricultural Chemists in 1959.⁴⁶ The method has also been used in Canada,⁴⁷ Chile,⁴⁸ South Africa,⁴⁹ Japan,^{50, 51} and the Philippines.⁵⁴

Details of the selective adsorption method can be found in the references to White,^{34, 53} and they will be briefly described here. The honey sample (0.8-1.0 g) is subjected to adsorption on a charcoal-celite (equal amounts) column under controlled conditions, followed by elution under pressure with aqueous solutions of increasing ethanol content. Monosaccharides are eluted with 1% ethanol, disaccharides with 7% and the higher oligosaccharide fraction with 50% aqueous ethanol. This preliminary class fractionation of the honey sugars, when used prior to other chromatographic methods, has made possible the isolation of many new honey sugars, which will be discussed later.

2.4. Post-1950 surveys of honey composition

In 1954 White and Maher⁵³ conducted a survey of 21 honey samples representing 19 floral sources, using selective adsorption chromatography and analytical methods described in their earlier paper.³⁴ Following class fractionation of the sugars, the monosaccharide fraction was shown by paper chromatography to consist of only glucose and fructose. Glucose was determined by a slight modification of the method of Marshall and Norman.⁴⁰ In this procedure, glucose oxidation by hypiodite is determined and residual fructose then determined by copper reduction. A correction is included to account for hypiodite oxidation of fructose.

The reducing disaccharides (termed "maltose") present in the 7% ethanol eluate were determined by copper reduction calibrated against maltose, and sucrose was estimated by the increase in reducing power of this fraction following mild acid hydrolysis. The 50% ethanol eluate contained all other sugars in the sample (trisaccharides and higher sugars) and was analysed collectively as glucose after acid hydrolysis.

The average values found in this survey were: moisture, 16.72%; glucose, 32.29%; fructose, 39.28%; sucrose, 1.62%; "maltose", 7.11%; and higher sugars, 1.03%. This represents a considerably lower glucose content, somewhat lower fructose and sucrose levels than previous surveys, and significant amounts of reducing disaccharide ("maltose") that had not been previously reported. From these results, it is apparent that honey is essentially a carbohydrate material, with 95-99% of the total solids being sugars.

The results obtained in this limited survey of 21 honey samples differed significantly from those of previous surveys and suggested the need for a more comprehensive survey. Such a survey was conducted by White *et al.*¹⁵ who felt that a fuller knowledge of the variation of honey with floral source, age, production area and crop year would be of great value to honey producers. A survey of 504 samples of honey and honeydew honey included samples from 47 of the 50 states and represented 82 single flora types and 93 blends of known composition.

Several parameters were included that had not been considered in their previous honey survey.⁵³ These were granulating tendency, pH, free acidity, lactone content, total acidity, ash, nitrogen, and diastase (amylase) value. The sugar, moisture, and undetermined content of the floral and honeydew honeys analysed in the survey are compiled in Table 1. Honeydew honey differs from floral honey in several respects: lower in glucose and, as a result, usually non-granulating; it is lower in fructose; higher in oligosaccharides, ash, pH value, free and total acidity and the amount of material undetermined by methods used. This undetermined category is taken as the difference between 100 and the total sugars plus the moisture content. It can include nonreducing disaccharides other than sucrose, such as trehalose, and weakly reducing disaccharides such as kojibiose, along with the small amounts of protein, lipid, polysaccharide and organic acids.

Several tables are included in the survey bulletin¹⁵ and among them are: effects of storage on honey composition; average composition classified by state of origin; average composition of single source samples classified by plant family; and a comparison of 1956 and 1957 samples from the same floral source and location.

Table 1. Average composition of 490 US floral honey samples and 14 US honeydew honey samples and range of values¹⁵

	Average (%)	Standard deviation	Range (%)
<i>Floral Honey</i>			
Composition:			
Moisture	17.20	1.46	13.4 -22.9
Fructose	38.19	2.07	27.25-44.26
Glucose	31.28	3.03	22.03-40.75
Sucrose	1.31	0.95	0.25- 7.57
"Maltose"	7.31	2.09	2.74-15.98
Higher sugars	1.50	1.03	0.13- 8.49
Undetermined	3.10	1.97	0.0 -13.2
<i>Honeydew Honey</i>			
Composition:			
Moisture	16.30	1.74	12.2 - 8.2
Fructose	31.80	4.16	23.91-38.12
Glucose	26.08	3.04	19.23-31.86
Sucrose	0.80	0.22	0.44- 1.14
"Maltose"	8.80	2.51	5.11-12.48
Higher Sugars	4.70	1.01	1.28-11.50
Undetermined	10.10	4.91	2.70-22.4

Aso *et al.*⁵⁰ and Watanabe *et al.*⁵¹ examined 25 samples of Japanese honey, some from floral sources peculiar to Japan, but in only one case was fractionation employed prior to analysis. Glucose, fructose, sucrose, and moisture were reported, with the values having a wide range and, in most cases, glucose was found to predominate over fructose. The high moisture content reported, 21.65%, was said by the authors to result from the high humidity in Japan. In the one sample that was fractionated by selective adsorption chromatography, then analysed by paper chromatography, 22 sugars were shown to be present, 15 of them classified as ketoses from their reaction to spray reagents.⁵⁰

In his review on the sugars of honey, Siddiqui⁵⁴ briefly discussed his survey of 95 Canadian honey samples (Table 2), but details of analytical methods were not described. Classes of sugars were separated by paper chromatography, eluted, then determined spectrophotometrically. Di- and higher saccharides were reported collectively as oligosaccharides. The average composition of the samples in this survey is similar to that found by White *et al.*¹⁵ with glucose being somewhat higher, and the fructose and oligosaccharide content lower.

Table 2. Average composition of 95 Canadian honey samples and range of values⁵⁴

Components	Average (%)	Range (%)
<i>Honey</i>		
Moisture	17.9	15.0-21.8
Fructose	37.1	31.1-41.4
Glucose	33.7	28.5-40.7
Oligosaccharides	7.4	2.2-15.2
Undetermined	3.9	0.0-10.8

2.5. Granulation and glucose analysis

Some attention will now be given to the technologically important subject of honey granulation, which is the precipitation of glucose from honey. Methods will be presented for predicting this tendency in honey using convenient analytical methods.

The major problem resulting from glucose granulation is that the resulting increased moisture content of the liquid phase allows yeast cells, which occur naturally in honey, to multiply and fermentation to proceed. It is well known that properly ripened honey is not susceptible to spoilage by microorganisms, with the exception of osmophilic yeasts, and then only at moisture levels above 17%.^{55, 56} Considerable effort has been directed toward preventing granulation in honey which is to be sold in the liquid form and in controlling granulation in honey which is to be sold in finely granulated form.

Attempts to understand honey granulation have been made with model systems of sugars. A thorough discussion of this topic and further references can be found in the chapters by White on the composition and physical properties of honey in the recently published volume entitled *Honey*.⁵⁷

Honey is more complex than the model systems examined to date, but valuable information has been gained by examining the roles of the major components of honey (glucose, fructose, and water) in the crystallisation of glucose. The results of Lothrop⁵⁸ on the solubility of glucose in solutions of fructose approximating those found in honey suggest that glucose solubility increases with increasing fructose concentration. The explanation given for this observation was in terms of an equilibrium between anhydrous glucose and glucose monohydrate (the form found in granulated honey). It was stated that at high fructose levels, the equilibrium favors the anhydrous form, which shows a greater solubility in water. The importance of this equilibrium to granulation was supported by Kelly,⁵⁹ who observed that in solutions saturated with fructose, the transition temperature from glucose monohydrate to anhydrous glucose is below 30°C.

In the survey of White *et al.*¹⁵ it was shown statistically that granulating tendency can be estimated either from the D/W ratio (first applied by Austin⁴⁷ in 1956), or from the D-W/L ratio (D, glucose; L, fructose; W, water). The D/W ratio was shown¹⁵ to give the most highly significant relationship to granulating tendency and requires only glucose and moisture determinations. D/W ratios of 1.70 and lower are associated with nongranulating honey and values of 2.10 and higher predict rapid granulation.

A recent survey of 54 honey samples from throughout the world by Hadorn and Zürcher⁶⁰ suggests, however, that only a loose correlation exists between the D/W or L/D ratio and granulation tendency. They found that in only 40–50% of the samples can granulation be predicted from these ratios.

A method for glucose determination by which the D/W ratio can be readily determined without prior class separation of the sugars was developed by White in 1964.⁶¹ Commercial glucose oxidase (with contaminating α -glucosidase activity inhibited by tris buffer) catalyses the specific oxidation of glucose to gluconic acid. The hydrogen peroxide produced as a byproduct reflects the glucose content and is determined photometrically in an assay procedure utilising peroxidase. The method was found to be reproducible and the accuracy compared favourably with previous methods of honey glucose analysis and was more convenient.

Recently Wood *et al.*⁶² described a facile method for honey glucose and fructose determination using gas chromatography. Dried aliquots of honey were treated with n-butaneboronic acid in pyridine, which converts the sugars to their cyclic n-butaneboronic derivatives. Subsequent trimethylsilylation produces volatile derivatives of glucose separable by gas chromatography. Results were found to be reproducible, but the accuracy of the method remains to be determined. In three of four honey samples analysed, fructose content was reported to be greater than 40%, and in one case the glucose content was 40.6%. These values are appreciably higher than those compiled in Tables 1 and 2. Should the accuracy of this method be established it will be preferable to other gas chromatographic analyses applied to nectar and honey as glucose and fructose each give a single peak, rather than the multiple peaks^{2, 7, 8, 63, 64} representing both anomers in either of two ring forms when trimethylsilylation is used.

2.6. Hydroxymethylfurfural (HMF) in honey

Hydroxymethylfurfural (HMF) formation results from the acid catalysed dehydration of hexose sugars with fructose being particularly susceptible to this reaction. Small amounts (0.06–0.20 mg/

100 g) are present in fresh honey and White *et al.*⁶⁵ have shown that both heat treatment and storage result in the formation of increased amounts of HMF. They showed that in natural honey stored for one year at 25°C, the HMF level often reaches 3.0 mg/100 g honey and if heated to 60°C this level can be reached in less than 3 days. Although heat treatment of honey is essential at various stages of honey processing to prevent granulation and fermentation, it is apparent that the temperature and period of heating must be controlled, as an excessive amount of HMF is considered in many countries as evidence of overheating and a loss of freshness of the honey.

High levels of HMF also suggest the possibility that natural honey has been adulterated with invert syrup, prepared from sucrose by acid hydrolysis. Acid-inverted invert syrup invariably contains high amounts of HMF. Prolonged storage or overheating of honey can result in an HMF level exceeding 3.0 mg/100 g and may rise to 10.0 mg/100 g or more.⁶⁶ A content of 15.0 mg/100 g or more is taken to indicate adulteration with acid-inverted invert sugar.⁶⁶

Several methods are available for the determination of HMF, the two most commonly used being the Fiehe Test⁶⁷ and the method described by Winkler.⁶⁸ The Fiehe Test has been adapted by Schade⁶⁹ to give a quantitative procedure, but this is sensitive only at a level of 3.0 mg/100 g honey. The colorimetric Winkler method is sensitive to 0.2–3.0 mg/100 g honey and is a simpler procedure.

2.7. Sugar composition of floral honey

2.7.1. Introduction

Particular emphasis will be given to the composition of floral honey, as its sugar composition has been more closely examined than that of honeydew honey. This is probably because floral honey is the type preferred in most parts of the world. In extensive regions of central Europe, however, honeydew is the main source and some forest honeys are more highly prized than floral honeys. Honeydew honey sugar composition will be discussed in a later section.

Floral honey was long thought to be primarily a mixture of glucose and fructose, with lesser amounts of sucrose, maltose and an ill defined material termed "dextrin". This simple concept could be readily explained by honeybee invertase acting on nectar sucrose. In the past 25 years the application of modern methods to honey analysis has established that the "dextrin" material includes a mixture of at least 22 di-, tri- and higher oligosaccharides. Early paper chromatographic studies indicating that honey is indeed a complex mixture of sugars were done by several groups, including those of Malyoth in 1951,⁷⁰ Vavruch⁷¹ and Taufel and Reiss³⁵ in 1952, and Keup⁷² in 1957, and paper chromatography has continued to play a key role in honey sugar analysis. Sugars that have been definitely established as being components of honey are listed in Table 3, along with reference to the group responsible for their positive identification.

The presence of a sugar in honey has frequently been reported on the basis of its having an R_f value and behaving similarly to spray reagents as a standard sugar. These criteria are considered here to be insufficient, and Table 3 does not include sugars reported only on the basis of such observations. An example showing this evidence to be inadequate is the often reported presence of raffinose in honey. It was shown by Siddiqui and Furgala⁷⁹ that raffinose and theandrose behave identically in paper chromatography and in colour reactions to ketose spray reagents. Sugars listed in Table 3 have been isolated from the honey mixture and characterised by sound physical or chemical methods of analysis.

2.7.2. Monosaccharides

Glucose and fructose, the major constituents of honey, account for about 85% of the honey solids. Many reports have suggested^{72, 81–83} that raffinose is a minor sugar in honey, but if so, galactose would also be expected. Galactose has never been observed by paper chromatography or by gas chromatography of honey sugar hydrolysates by Battaglini and Bossi.⁶⁴ It should be mentioned that gluconic acid (in equilibrium with its lactone) was found in honey by Stinson *et al.*⁸⁴ in 1960. The subsequent finding by White *et al.*⁸⁵ of the enzyme glucose oxidase in honey explained the origin of this acid.

Table 3. Sugars established as honey constituents and group responsible for positive identification

Trivial name	Systematic name	Reference
Glucose		
Fructose		
Sucrose	α -D-glucopyranosyl- β -D-fructofuranoside	Elser (73), Liggett (74), Van Voorst (75)
Maltose	O- α -D-glucopyranosyl-(1 \rightarrow 4)-D-glucopyranose	White and Hoban (76)
Isomaltose	O- α -D-glucopyranosyl-(1 \rightarrow 6)-D-glucopyranose	White and Hoban (76)
Maltulose	O- α -D-glucopyranosyl-(1 \rightarrow 4)-D-fructose	White and Hoban (76)
Nigerose	O- α -D-glucopyranosyl-(1 \rightarrow 3)-D-glucopyranose	White and Hoban (76)
Turanose	O- α -D-glucopyranosyl-(1 \rightarrow 3)-D-fructose	White and Hoban (76)
Kojibiose	O- α -D-glucopyranosyl-(1 \rightarrow 2)-D-glucopyranose	Watanabe and Aso (77)
Laminaribiose	O- β -D-glucopyranosyl-(1 \rightarrow 3)-D-glucopyranose	Siddiqui and Furgala (78)
α , β -Trehalose	α -D-glucopyranosyl- β -D-glucopyranoside	Siddiqui and Furgala (78)
Gentiobiose	O- β -D-glucopyranosyl-(1 \rightarrow 6)-D-glucopyranose	Siddiqui and Furgala (78)
Melizitose	O- α -D-glucopyranosyl-(1 \rightarrow 3)-O- β -D-fructofuranosyl-(2 \rightarrow 1)- α -D-glucopyranoside	Siddiqui and Furgala (79)
3- α -Isomaltosylglucose	O- α -D-glucopyranosyl-(1 \rightarrow 6)-O- α -D-glucopyranosyl-(1 \rightarrow 3)-D-glucopyranose	Siddiqui and Furgala (79)
Maltotriose	O- α -D-glucopyranosyl-(1 \rightarrow 4)-O- α -D-glucopyranosyl-(1 \rightarrow 4)-D-glucopyranose	Siddiqui and Furgala (79)
1-Kestose	O- α -D-glucopyranosyl-(1 \rightarrow 2)- β -D-fructofuranosyl-(1 \rightarrow 2)- β -D-fructofuranoside	Siddiqui and Furgala (79)
Panose	O- α -D-glucopyranosyl-(1 \rightarrow 6)-O- α -D-glucopyranosyl-(1 \rightarrow 4)-D-glucopyranose	Siddiqui and Furgala (79)
Isomaltotriose	O- α -D-glucopyranosyl-(1 \rightarrow 6)-O- α -D-glucopyranosyl-(1 \rightarrow 6)-D-glucopyranose	Siddiqui and Furgala (79)
Erllose	O- α -D-glucopyranosyl-(1 \rightarrow 4)- α -D-glucopyranosyl- β -D-fructofuranoside	Siddiqui and Furgala (79)
Theanderose	O- α -D-glucopyranosyl-(1 \rightarrow 6)- α -D-glucopyranosyl- β -D-fructofuranoside	Siddiqui and Furgala (79)
Centose	O- α -D-glucopyranosyl-(1 \rightarrow 4)-O- α -D-glucopyranosyl-(1 \rightarrow 2)-D-glucopyranose	Siddiqui and Furgala (80)
Isopanose	O- α -D-glucopyranosyl-(1 \rightarrow 4)-O- α -D-glucopyranosyl-(1 \rightarrow 6)-D-glucopyranose	Siddiqui and Furgala (79)
Isomaltotetraose	O- α -D-glucopyranosyl-(1 \rightarrow 6)-[O- α -D-glucopyranosyl-(1 \rightarrow 6)] ₂ -D-glucopyranose	Siddiqui and Furgala (79)
Isomaltopentaose	O- α -D-glucopyranosyl-(1 \rightarrow 6)-[O- α -D-glucopyranosyl-(1 \rightarrow 6)] ₃ -D-glucopyranose	Siddiqui and Furgala (79)

2.7.3. Disaccharides

The presence of maltose in honey was suggested in 1924 by Elser⁷³ on the basis of its characteristic osazone formation. In the separation of honey sugars by distillation of propionate esters Liggett,⁷⁴ in 1941, obtained a crystalline material from the disaccharide fraction with a melting point close to that of authentic maltose octapropionate. Van Voorst⁷⁵ fermented several honey samples with a maltase-free yeast and found all sugars except maltose were removed.

In 1959 White and Hoban⁷⁶ confirmed the presence of sucrose and maltose and identified four additional disaccharides. The disaccharide fraction was obtained by the selective adsorption chromatographic method⁸⁴ described earlier, and further resolved using preparative paper chromatography and stearic acid-treated charcoal column chromatography. The latter method had been shown by Hoban and White⁸⁶ to resolve disaccharide pairs (turanose-sucrose, isomaltose-gentiobiose, maltulose-nigerose and melibiose-lactose) not separable by paper chromatography. From 2.6 g in the original disaccharide fraction were obtained 50-100 mg each of five purified sugars.

Portions were converted to their β -octaacetates and the infrared spectrum of each sugar and its acetate then compared with the spectra of known disaccharides and their acetates. Characteristic differences, even among closely related disaccharides, are present between 650 and 1500 cm^{-1} .⁸⁷ Isomaltose, maltulose, turanose, maltose and nigerose were identified in this manner and confirmed by paper electrophoresis with standards. Unresolved column fractions were shown by zone electrophoresis to contain an additional 12 compounds. One of the unresolved fractions contained sucrose, so an alternate method was used to confirm its presence in honey. A procedure (described by Adcock⁸⁸ in 1957) was applied to the mixed disaccharide fraction. Reducing sugars in the fraction were removed by ion exchange treatment after oxidation to acids. The nonreducing sugars that remained gave five bands on preparative paper chromatography. The band migrating with sucrose was isolated and its infrared spectrum and that of its octaacetate corresponded to authentic samples.

In 1960 Watanabe and Aso^{77, 89, 90} confirmed the presence in honey of nigerose, maltose, and isomaltose and also identified kojibiose for the first time. Successive elutions of a charcoal-celite column with seven solvents of increasing ethanol content (2.5–30%) resulted in a partial fractionation of the disaccharides. The first fraction was suspected by paper chromatography to contain kojibiose and isomaltose so it was rechromatographed on a charcoal-celite column containing pH 10 borate buffer. This effected the separation of the two sugars, which were then acetylated. The octaacetates did not crystallise until further purification by magnesol-celite column chromatography (developed by McNeely *et al.*⁹¹ in 1945). By the same methods, nigerose and maltose were obtained as crystalline octaacetates. The melting points and specific rotations of the octaacetates compared well with literature values. Both α - and β -kojibiose octaacetate were obtained crystalline, thus establishing kojibiose as a new honey sugar. The presence of leucrose [(O- α -D-glucopyranosyl-(1 \rightarrow 5)-D-fructopyranose] was also reported in this sample of honey, the only evidence, however, was that when mixed with an authentic sample, one spot alone appeared on a paper chromatogram.

Siddiqui and Furgala⁷⁸ added to the list of honey disaccharides in 1967 by isolating α, β -trehalose, gentiobiose, and laminaribiose and characterising them as their crystalline octaacetates. Sucrose and turanose were isolated and crystallised as free sugars, while maltose, isomaltose, maltulose, nigerose, and kojibiose were obtained as crystalline derivatives, thus confirming that these sugars are not peculiar to the honey type from which they were originally isolated. Following the removal of monosaccharides from 2 kg by adsorbing them on a charcoal-celite mixture, steps were taken to remove ethanol insoluble material, organic acids, and lipids. The oligosaccharide fraction remaining (67 g) was then fractionated on a charcoal-celite column using aqueous ethanol (0–30%) in a stepwise elution. Fifteen fractions were collected, concentrated and then resolved by paper chromatography and paper electrophoresis. The approximate yields of the principal components in the oligosac-

Table 4. Yields of the principal sugars in the oligosaccharide fraction (3.65%) of honey^{78, 79}

Disaccharide	%	Trisaccharide	%	Higher oligosaccharide	%
Maltose	29.4	Erllose	4.5	Isomaltotetraose	0.33
Kojibiose	8.2	Theanderose	2.7	Isomaltopentaose	0.16
Turanose	4.7	Panose	2.5		
Isomaltose	4.4	Maltotriose	1.9		
Sucrose	3.9	1-Kestose	0.9		
Maltulose (and 2 unidentified ketoses)	3.1	Isomaltotriose	0.6		
Nigerose	1.7	Melzitose	0.6		
α, β -Trehalose	1.1	Isopanose	0.24		
Gentiobiose	0.4	Centose	0.05		
		3- α -Isomaltosyl-glucose	trace		
Laminaribiose	0.09				
Total	56.99		13.69		0.49

charide fraction accounted for about 71% of the fraction and are compiled in Table 4. Among the disaccharides, maltose predominated, with gentiobiose, α,β -trehalose and laminaribiose accounting for only 1.6% of the fraction. In this paper, evidence for the presence of at least 22 oligosaccharides in honey was obtained. On the basis of finding two components with electrophoretic mobilities close to the literature values for isomaltulose and 1-O- α -D-glucopyranosyl-D-fructose, the authors have tentatively identified these as honey sugars. More solid evidence to establish these as sugars common to honey has not yet been presented.

In 1957 Keup⁷² reported finding melibiose in honey, the evidence being a paper chromatographic spot with an R_F value identical to that of authentic melibiose. No subsequent analyses have reported this sugar, and the gas chromatographic study of Battaglini and Bossi⁶⁴ revealed neither melibiose nor galactose in honey or its acid hydrolysate.

2.7.4. Tri- and higher oligosaccharides

In 1968 Siddiqui and Furgala⁷⁹ reported the first positive identification of ten honey trisaccharides, one tetrasaccharide and one pentasaccharide. In addition, the possible presence of two other trisaccharides was indicated.

The general fractionation procedures and experimental methods were as described in their previous study⁷⁸ of disaccharides. Melizitose, 3- α -isomaltosylglucose, maltotriose and isomaltotriose were isolated and characterised as crystalline β -undecaacetates. Melting points (undepressed) and specific rotations corresponded with those of authentic samples. 1-Kestose and panose were obtained as crystalline free sugars with the correct physical constants.

Erlöse was characterised by methods involving partial chemical and enzymatic hydrolysis after its undecaacetate failed to crystallise. Partial hydrolysis with weak acid gave quantitative cleavage to maltose and fructose and salivary α -glucosidase produced sucrose.

The first report of erlöse was published by White and Maher in 1953.¹⁸ They demonstrated that it is produced (11% yield) during a short incubation of sucrose with honey invertase and characterised the sugar by showing that maltose and sucrose were produced by treatment with yeast invertase and honey invertase, respectively. The implications of honey invertase possessing glucosyl-invertase activity are discussed in section 2.9.

Theanderose was identified by showing that the substance from honey and authentic theanderose behaved identically on partial acid hydrolysis (giving isomaltose and fructose) and on digestion with amyloglucosidase (an α -glucosidase). Peracetylated derivatives had the same R_F 's by thin-layer chromatography and the free sugar showed a specific rotation corresponding well to the literature value.

Authentic isopanose coincided by paper chromatography and paper electrophoresis with one honey sugar and the specific rotations of the sugar and its acetate corresponded well also. Partial hydrolysis by acid and incubation with enzymes gave the expected fragments to support further the presence of isopanose in honey.

Centose was isolated and characterised⁸⁰ on the basis of several observations. Total hydrolysis yielded only glucose, and partial hydrolysis yielded the unhydrolysed trisaccharide, maltose, kojibiose and glucose. This and the ease of hydrolysis with emulsin established the glucosidic linkages as alpha. The linkages between the sugars were established by characterising the methylated sugars after methylation and hydrolysis of the trisaccharide.

The evidence for isomaltotetraose and isomaltopentaose appears adequate, as the former on partial acid hydrolysis produced isomaltotriose, isomaltose, and glucose with the latter giving these sugars and isomaltotetraose. The sugars were resistant to emulsin (a β -glucosidase) and incubation with amyloglucosidase gave large amounts of glucose. Further evidence for their presence was their having specific rotations corresponding to the literature values.

Two additional trisaccharides were suggested as possible components of honey on the basis of partial acid hydrolysis but further evidence is needed. These were 4- α -kojibiosylglucose and 4- α -gentiobiosylglucose.

2.8. Sugar composition of honeydew honey

The minor sugar components in the higher saccharide fractions of honeydew honey have not been examined with the detail that has been applied to those of floral honey. From the survey¹⁵ described in Table 1, however, it can be seen that on the average, honeydew honey is lower in glucose by 5.2%, lower in fructose by 6.4% but appreciably higher in reducing disaccharides and higher sugars. A distinctive feature of honeydew honey when compared with floral honey is its optical rotation. Honeydew honeys are dextrorotatory, while floral honeys are invariably levorotatory. It was shown by Battaglini and Bossi⁶⁴ that honeys containing high levels of fructose (levulose) and glucose (dextrose) along with low di- and trisaccharide levels are levorotatory. Conversely, low fructose, glucose levels with large quantities of di- and higher saccharides (as honeydew honey) are dextrorotatory. Reference was made earlier to the large amounts of melizitose sometimes produced by aphids acting upon sucrose.^{13-15, 17} Honeybees, in their conversion of honeydew to honeydew honey, are capable of digesting melizitose to lower sugars,⁶ but its level (as high as 10-20%)¹⁶ often remains high enough to granulate in the comb. In their 1974 gas chromatographic examination of honeydew honey, Hadorn *et al.*⁶³ provided evidence for the presence of maltose and raffinose.

2.9. Formation and metabolism of the honey sugars

A definitive explanation of the mode of formation of each of the honey sugars requires a knowledge of the sugar and enzyme content of nectar and honey, the enzymes added by the honeybee, and an understanding of the nonenzymatic interactions of sugars over extended periods of time. The complexity of this task is obvious, but evidence has accumulated in recent years that might explain the formation of at least a few of the sugars. Siddiqui⁵⁴ treated this topic in some detail in his 1970 review.

The most important enzyme in honey, invertase, has long been known to be responsible for the hydrolysis of nectar sucrose with the formation of fructose and glucose. It is now known that an additional activity resides in this enzyme, the capability of transferring α -D-glucosyl units from sucrose to suitable acceptors. There has been a question as to whether this enzyme originates in the nectar of the plant or is added by the honeybee. Maurizio⁶ suggested that invertase from both the plant nectary and the honeybee are present in honey, with that from the honeybee being more active and having the dominant effect on the sugar spectrum in honey.

In the 1967 report of White and Kushnir,⁹² an invertase fraction was prepared from several bulk honeys, comb honey, and from honey produced by caged bees (no contribution from nectar). Invertase activity was fractionated from other enzyme activities (amylase, glucose oxidase) and the purified invertases were examined by various methods. Evidence was presented indicating that honey invertase differs from honeybee invertase (added by caged bees) in that the former is stabilised by nectar components, probably proteins.

In 1973 Rinaudo *et al.*⁹³ presented evidence confirming that honey invertase originates in the honeybee. The catalytic properties of invertase preparations from honey were compared with preparations from the hypopharyngeal gland of the honeybee and found notably similar. A preparation of invertase from the nectar from which the honey was made differed significantly with regard to pH optimum, temperature stability, and competitive inhibition by fructose.

More conclusive evidence that the honeybee is the source of the invertase present in honey was recently provided by Huber and Matheson,⁹⁴ using enzyme preparations purified to homogeneity. They established that the enzymes (shown to be glycoprotein) from both sources displayed very similar kinetics.

Honey invertase was shown by White and Maher¹⁹ to catalyse, in addition to the hydrolysis of sucrose, the production of six oligosaccharides, the major one being erlose. This sugar can be envisioned as resulting from the transfer of a glucose moiety from sucrose to the 4-hydroxyl of glucose in an intact sucrose molecule. This evidence for honey invertase transferring glucose, together with an earlier report by Gorbach and Schneiter⁹⁵ establish the enzyme as an α -glucosidase, or a gluco-invertase (transferring glucose from sucrose). This enzyme differs from yeast invertase in that the yeast enzyme is a fructo-invertase, transferring fructose to an acceptor molecule. The enzymes'

relative inactivity toward raffinose, and the production of higher glucans from maltose support its characterisation as a gluco-invertase. Should this enzyme be shown to transfer glucose to positions other than the 4-hydroxyl of an acceptor, many of the sugars in Table 3 may be formed by its action, with theandrose the most likely. The transference of glucose to disaccharide acceptors helps explain the observation that the fructose: glucose ratio is greater than one.

In a recent paper by Echigo and Takenaka,⁹⁶ honeybees confined in a cage were fed exclusively on sucrose. Invertase was extracted from the honey produced and was shown to produce erlose from its reaction with sucrose. This report confirms that α -glucosidase activity resides in the bee and is responsible for the transference of glucose from sucrose.

Consideration of the role of nonenzymatic reactions in the formation of honey sugars was made¹⁵ in an analysis of honey samples before and after a two year storage at room temperature. Extensive changes in sugar composition had occurred, and the change was in the direction of increased complexity. Monosaccharide content decreased 18.5%, while the disaccharide and trisaccharide content increased by 68 and 13%, respectively, over their original levels. Residual transglucosylase activity would account for some accumulation of higher sugars, but it was suggested that the acidic pH of honey could promote reversion (acid catalysed condensation of monosaccharides) and the formation of compounds such as difructose anhydrides.

Additional information regarding the role of reversion in the formation of di- and possibly higher saccharides, is needed. In floral honey (average pH 3.91) and honeydew honey (average pH 4.45)¹⁵ it is very possible that reversion is responsible for the formation of some of the rare honey sugars not ordinarily found in nature. The acidic pH, the high sugar concentration of honey, and the long equilibration times all favour reversion occurring, but this area has remained largely unexplored.

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