CHEM 191

Module 4

Structures and reactions of biological molecules

Lecture 4

Chemistry of Carbohydrates: Oligosaccharides and Polysaccharides, case study: honey

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Module 4 Lecture 4

Learning objectives

Learning Objectives:

- Understand the mechanism how hemiacetals react to form acetals (covered in lecture 2)
- Understand that acetals are much more stable than hemiacetals
- Recognise that oligosaccharides and polysaccharides are assembled via acetals, identify the glycosidic bond(s) and name the type of glycosidic linkage(s)
- Identify a reducing versus non-reducing sugar (also in lecture 3)
- Recognise that different glycosidic bond connectivity can result in different carbohydrate structures/shapes, and that this is important for physical properties and molecular recognition
- Apply previous knowledge (naming glycosidic linkages, formation of imines) to the case study of honey

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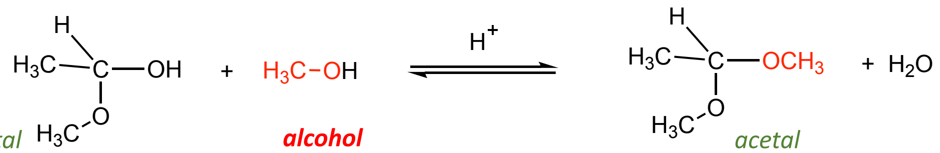
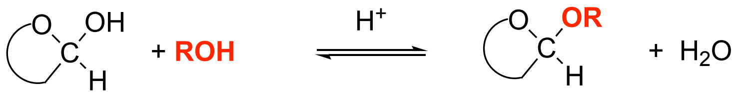
Hemiacetal to acetal

- Recap from Module 4 Lecture 2, we covered the mechanism for



hemiacetal **alcohol** *acetal*

- Cyclic hemiacetals react in the same way……



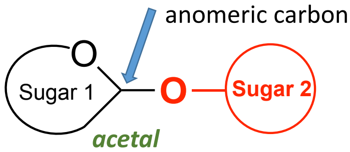
*Cyclic **hemiacetal*** **alcohol** *Cyclic **acetal***

e.g. a sugar

[28.33] on page 1322

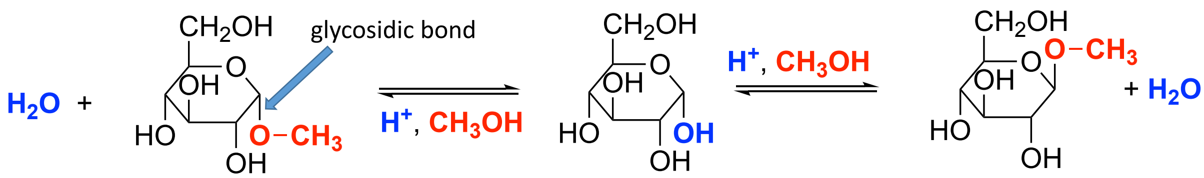
Today's lecture

If "**R-OH**" is an **alcohol** from another sugar….. then…..



acetal

Hemiacetal to acetal example - D-glucose and methanol



α-**glycoside**
an acetal

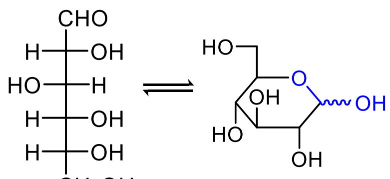
α-D-glucopyranose
a hemiacetal

β-**glycoside**
an acetal

- Acetal to hemiacetal equilibrium is reversible in the presence of a strong acid
- As the alcohol nucleophile can attack either side of the carbocation intermediate (lecture 2), both α and β glycosides (acetals) are formed from a single hemiacetal anomer, without ring opening of the hemiacetal occurring (*more info on naming α and β glycosides in a few slides time*)
- The new C-O bond formed is called a glycosidic bond (or glycosidic linkage)
- The acetal is called a "glycoside", the term glycoside includes anything linked via a glycosidic bond

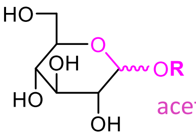
Acetals are <u>much</u> more stable than hemiacetals

- In Module 4 lecture 3 we learnt that hemiacetals exist in equilibrium with the open chain monosaccharide form, even in neutral aqueous conditions

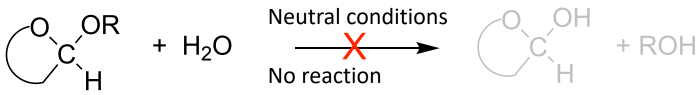


hemiacetal

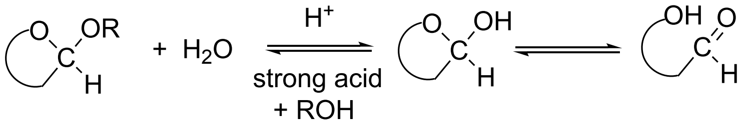
- In contrast to this, **acetals** are **stable in neutral conditions**.



acetal



A **strong acid** is required to chemically convert between an acetal and hemiacetal



Repeating sugar units linked by acetals are <u>chemically quite stable</u> in the <u>absence of strong acids</u>

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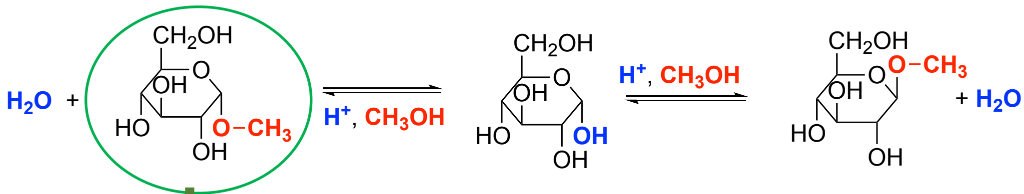
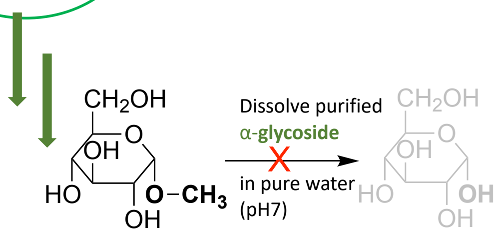
Acetal stability example - D-glucose and methanol

- Once the acetal is synthesised



Purifying and removing it from the reaction conditions, e.g. crystalize into pure form



α-glycoside
an acetal

- Dissolving in neutral aqueous conditions – nothing happens
- The α-**glycoside** is very stable
- There is no equilibrium

- This **glycoside** is a **non-reducing** sugar (there is no hemiacetal hence no aldehyde in equilibrium)

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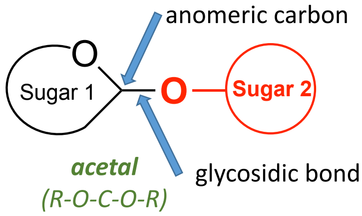
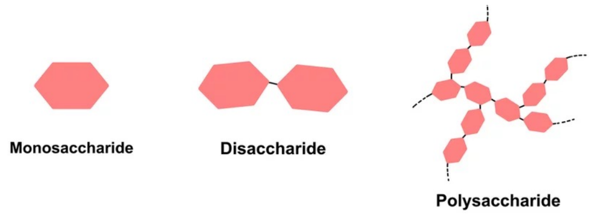
Higher saccharides

- Repeating units of sugars are joined by acetals, which contain a glycosidic bond
- Very stable chemically (unless strong acid present)
- In a biological setting, acetal hydrolysis is achieved using **enzymes**



Term	Number of sugar units
monosaccharide	1
disaccharide	2
trisaccharide	3
oligosaccharide	anything between 2-10
polysaccharide	>10, often hundreds

Higher saccharides



Can be branched
Varied linkage stereochemistry

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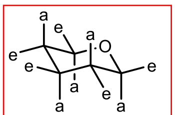
Glycosidic linkage – stereochemistry and connectivity

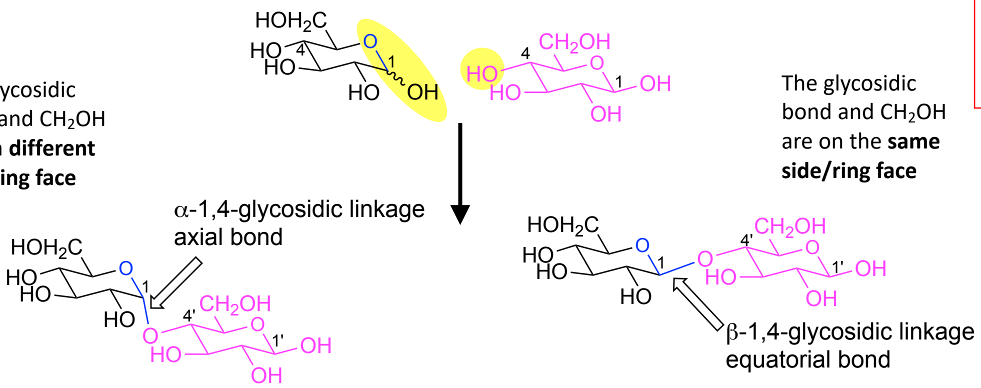
Pages 1322-1323

- As with the reaction of D-glucose and methanol, two acetal anomers are chemically possible, so we must name the glycosidic linkage as α or β
- Depending which sugar alcohol reacts, different bond connectivity is possible



Lecture 3 'Chairs'

The glycosidic bond and CH_2OH are on **different side/ring face**



The glycosidic bond and CH_2OH are on the **same side/ring face**

- The **biosynthesis** of oligosaccharides is done using **enzymes** that selectively make only one isomer

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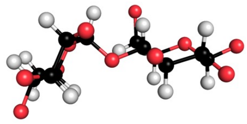
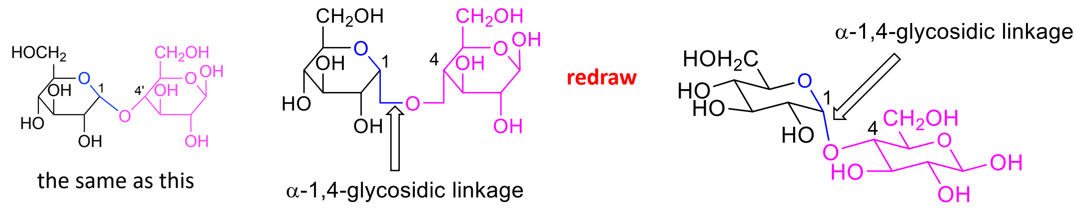
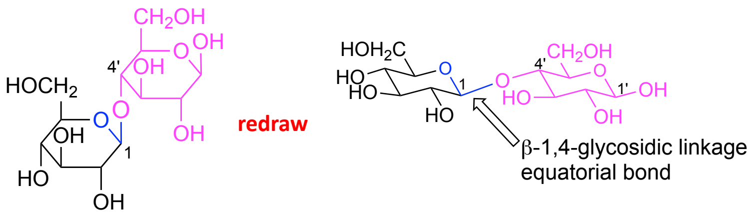
Glucose disaccharides

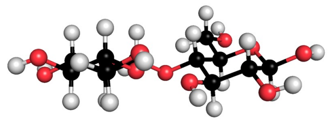


- Maltose - two D-glucopyranose units linked by an α-1,4-glycosidic bond



- Cellobiose - two D-glucopyranose units linked by an β -1,4-glycosidic bond





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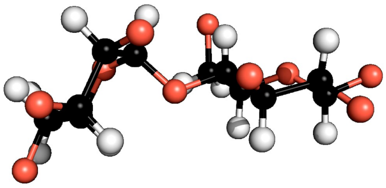
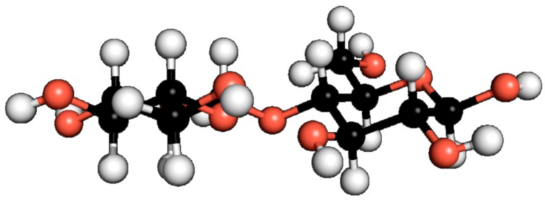
Maltose



Cellobiose



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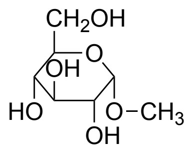
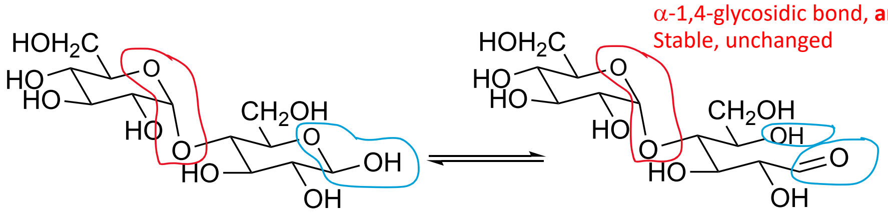
Identifying reducing saccharides

- We previously identified this as a **non-reducing** sugar as it does **not** contain a hemiacetal



- Is maltose a reducing sugar?



- **Hemiacetal** will exist to some extent (even if small) in equilibrium with the ring opened form (aldehyde + alcohol)
- A 'silver mirror' test will still come up positive since the one-way redox reaction (oxidation of aldehyde to carboxylic acid in the 'silver mirror test reaction') will still occur.

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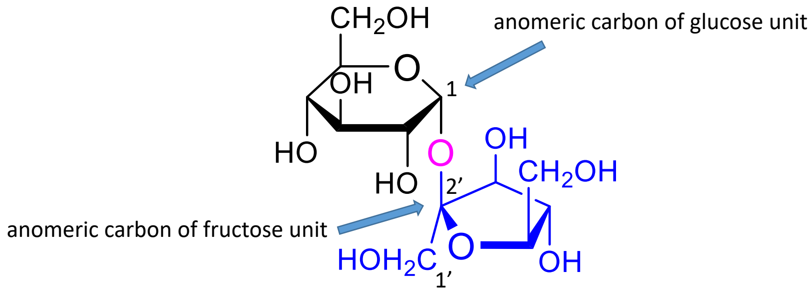
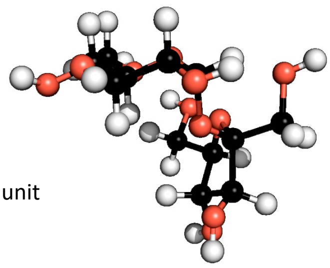
Sucrose, a disaccharide

- D-gluco<u>pyranose</u> and a D-fructo<u>furanose</u> linked by an α-1,2-glycosidic bond





How many acetals? How many hemiacetals?
Is sucrose a reducing or non-reducing sugar?

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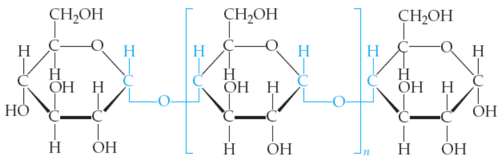
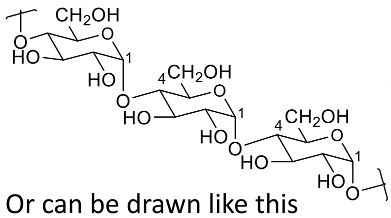
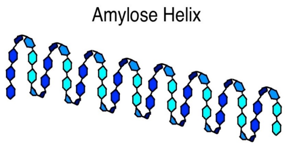
Amylose, a polysaccharide

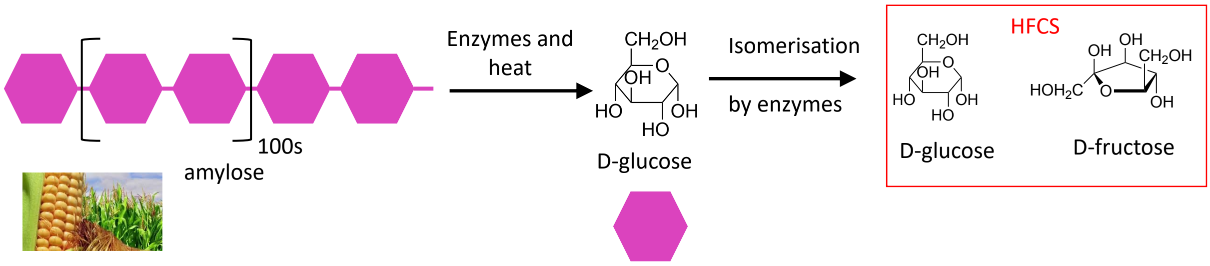
- D-glucose only linked by **α-1,4-glycosidic bonds**, up to 4,000 glucose units
- One of the polysaccharides in starch





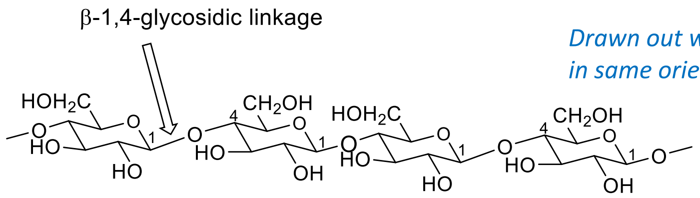


- High fructose corn syrup (HFCS) is made from amylose from corn starch -



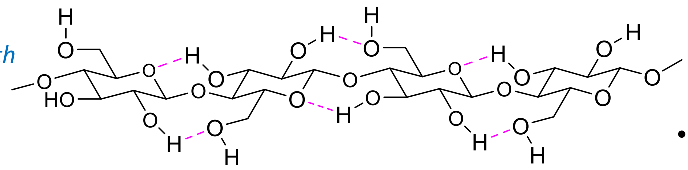
Cellulose, a polysaccharide

- Still only D-glucose, but linked by **β-1,4-glycosidic bonds**, up to 4,000 glucose units
- A fibrous carbohydrate found in all plants, is the structural component of plant cell walls



Drawn out with glucopyranose oxygen in same orientation on the page

The stable 3D orientation is with alternate glucopyranose's 'rotated'



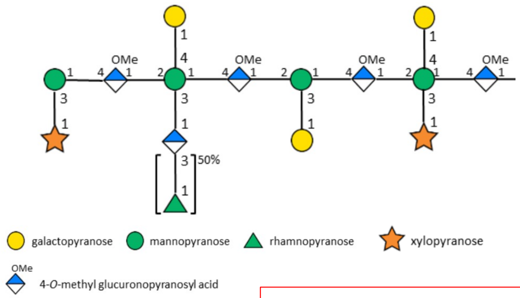
- Flat 3D shape, straight 'molecular rods' stabilised by a network of **hydrogen bonds**
- Good for structural material, strong (cotton is mostly cellulose)

Mammals without a rumen cannot digest cellulose

The human digestive enzyme α-amylase **cannot** break the **β**-1,4-glycosidic bonds

Natures makes complex carbohydrates

- Gel extracts from Aotearoa's native Mamaku (black tree fern) are a rongoā rākau (plant remedy) used topically as a healing extract to soothe and revive skin as part of rongoā Māori.
- The gel extract contains mostly carbohydrates, one found in larger amounts is -



Adapted from Fig 1, doi: 10.3390/plants8060163

The **repeating β-1,4-linkages and α-1,2- glycosidic linkages** give this carbohydrate unique shape and contribute to the gel-like consistency of the extract

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Carbohydrates and molecular recognition

- Carbohydrates (via attachment to a protein or lipid) also form a cell surface **molecular recognition code** based on 3D shape
- There is a HUGE variety in 3D shape
    - Each shape and colour = a different monosaccharide
    - Each glycosidic bond can vary in **linkage position** (e.g. **1,4**) and **linkage stereochemistry** (**α or β**) = *many* 3D shapes possible

- Many of the initial interactions between human cells and an invading microorganisms are governed by cell surface carbohydrates

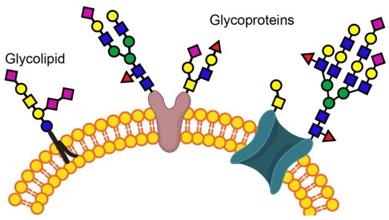
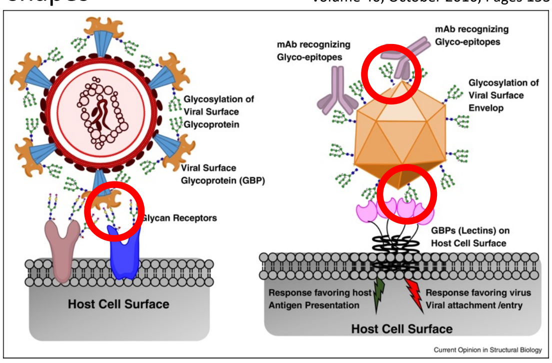


Image adapted from, https://communities.springernature.com/posts/cell-based-glycan-arrays-for-probing-glycan-glycan-binding-protein-interactions

Current Opinion in Structural Biology
Volume 40, October 2016, Pages 153-162



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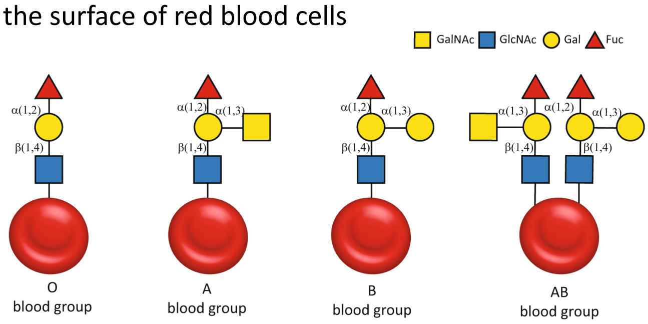
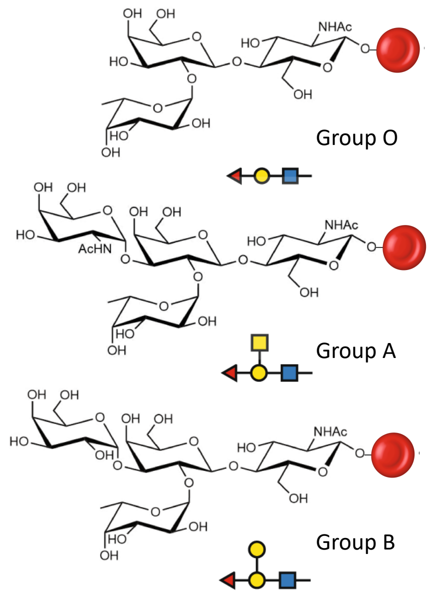
Molecular recognition - blood group example

- The blood groups A, B and O have ***different carbohydrates*** on the surface of red blood cells



This seemingly minor different in carbohydrate 'code' has huge implications for antibodies recognising foreign red blood cells

You don't need to memorize blood types, sugar names
But by looking at the structures you can name the glycosidic linkages (e.g. α-1,3 etc)
HOMEWORK – self check you can name these linkages



Graphics adapted from
https://www.nature.com/articles/s41467-023-37324-z

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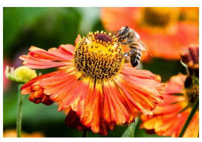
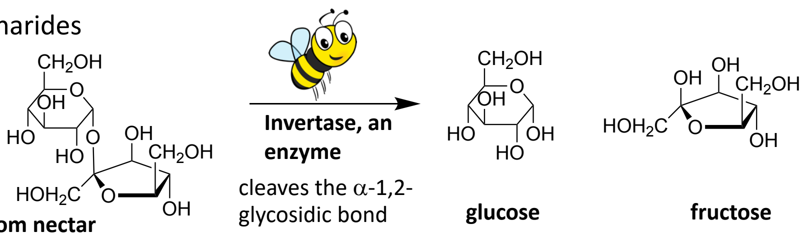
Case study - honey

- What is in honey?
  - Mostly monosaccharides (glucose and fructose), smaller amounts of disaccharides and other compounds

- Honey bees collect nectar that contains hundreds of compounds including sucrose, that bees break down into monosaccharides





Sucrose from nectar

- Honey contains a unique 'chemical signature' based on the plant's nectar (including carbohydrates, natural products) and pollen, and is even season and region dependent

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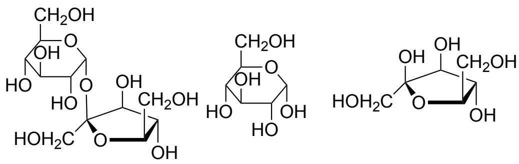
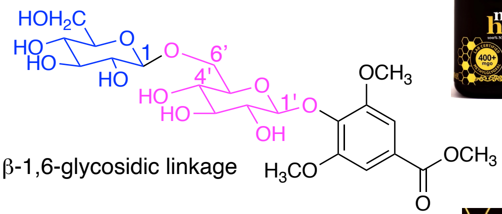
Mānuka honey

- Mānuka is taonga (treasure) and has significant cultural heritage and connection to Māori, who are the kaitiaki of Mānuka
- Long before honey production, Mānuka extracts were used as a rongoā rākau
- Many 'hive to shelf' Aotearoa honey businesses incorporate mātauranga Māori (including plant knowledge, environmental stewardship, sustainability) and chemical analysis to produce the end honey product. ***Extra reading on Blackboard if you are interested***

- Chemical analysis includes -



measuring the sucrose, glucose, fructose levels



unique to Mānuka honey, the natural product leptosperin, a "gentiobiose glycoside"
*Gentiobiose is made up of 2 x D-*glucose

- Another important component of Mānuka honey is methylglyoxal….

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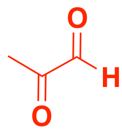
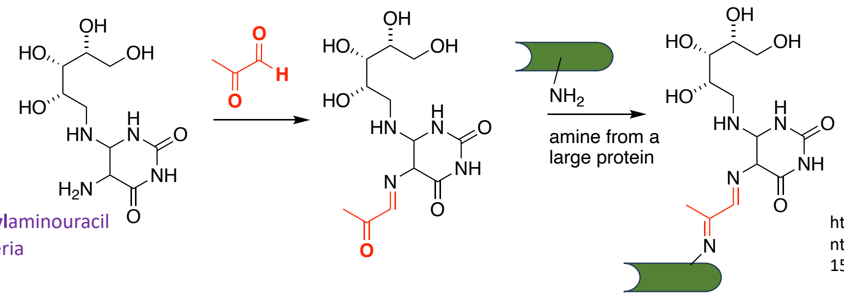
Methylglyoxal in Mānuka honey



- The level of methylglyoxal (MGO) is thought to *contribute to* the antimicrobial activity of the honey
- MGO is very reactive compound, *recap from lecture 2 – aldehydes and ketones can react with an amine to produce an imine, this is a common covalent reversible bond in the body*



5-amino-6-D-**ribityl**aminouracil produced by bacteria

https://pubs.rsc.org/en/content/articlepdf/2020/fo/d0fo01153c

- MGO can react twice**, each time forming an imine**, to join small 'foreign' compounds to larger proteins, which are recognised by and stimulate an immune response to an infection

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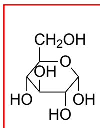
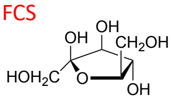
Adulterated honey

- Fake or adulterated honey is when colorants, sweeteners, or other substances that were not present when extracted from the beehive are added.
- Adding ‘synthetic’ methylglyoxal is a concern for the Mānuka honey industry in Aotearoa.
- Adding high fructose corn syrup (HFCS) is one of the common ’fake’ honey additives.

HFCS



D-glucose



D-fructose

- For interest only, not examined - the glucose:fructose ratio is actually very similar in honey and HFCS, so how do we tell a fake…?

Analyse the carbon isotope ratio in the sugars
- Glucose from corn starch has a different ^{12}C:^{13}C ratio then glucose from plant nectar

DNA testing
- DNA from the plant’s pollen can be identified
- HFCS contains small fragments of corn DNA that can be detected

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* Homework *

Page 1329, exercise 28.36
Page 1330, exercise 28.54

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