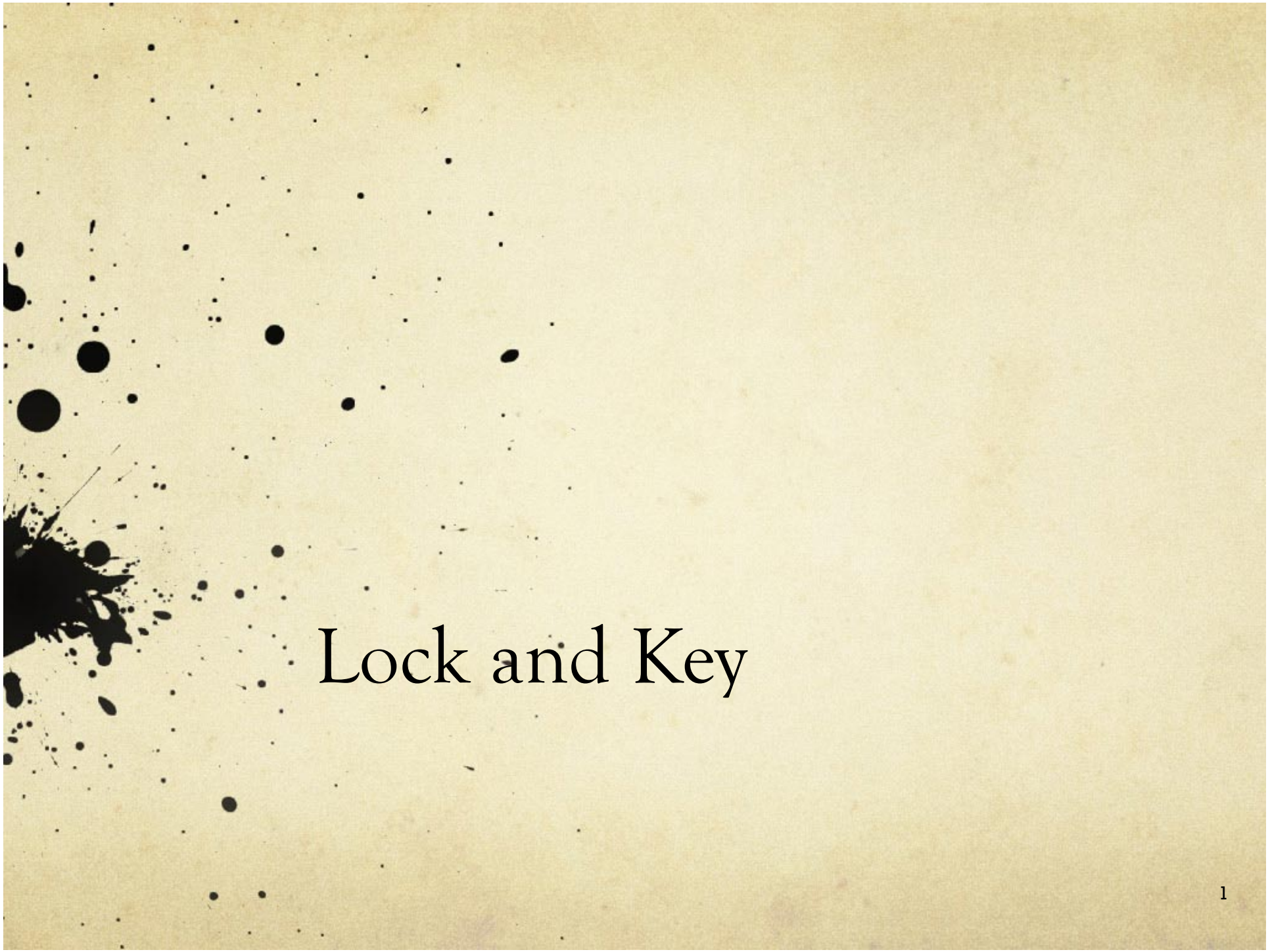
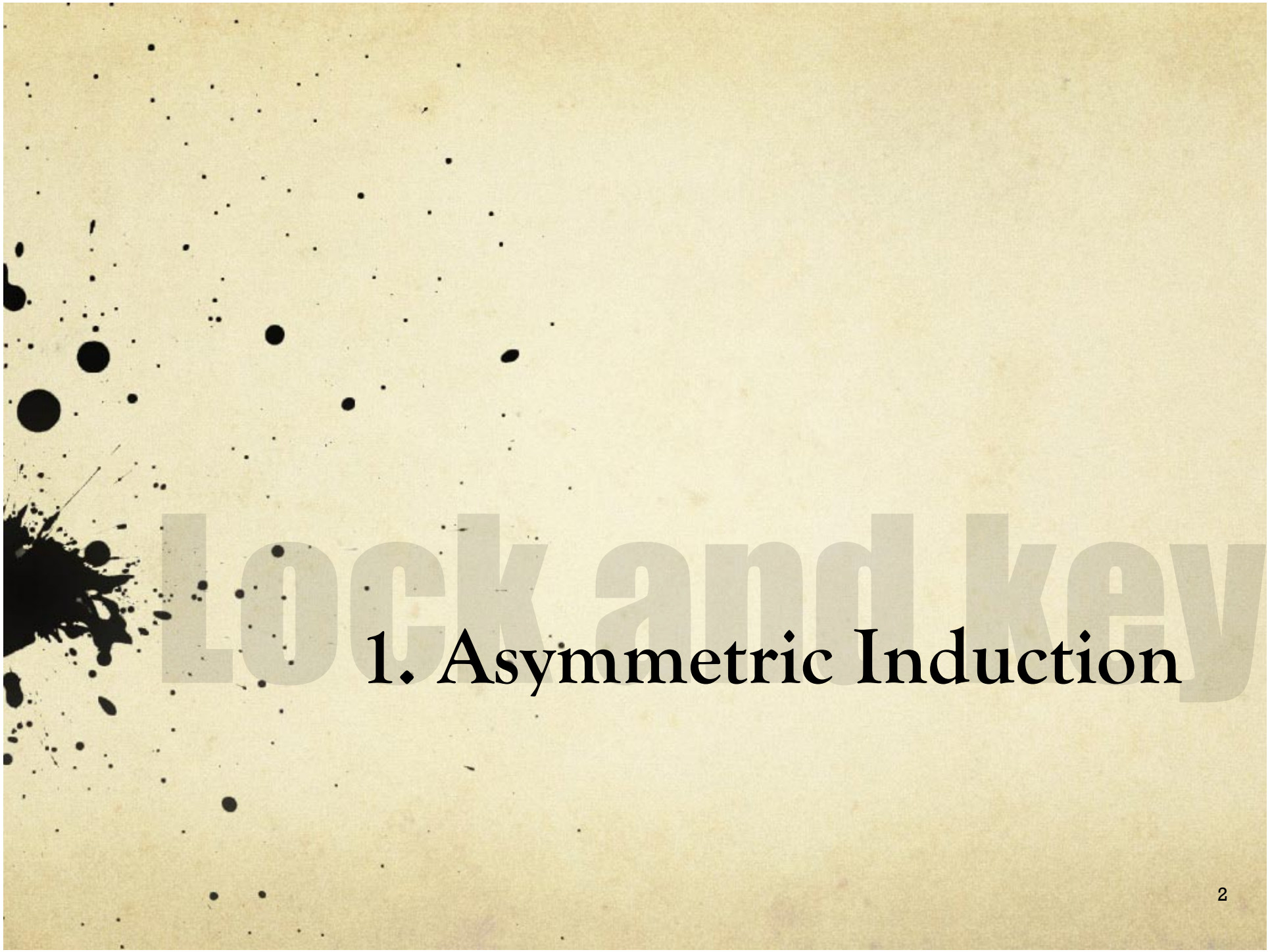
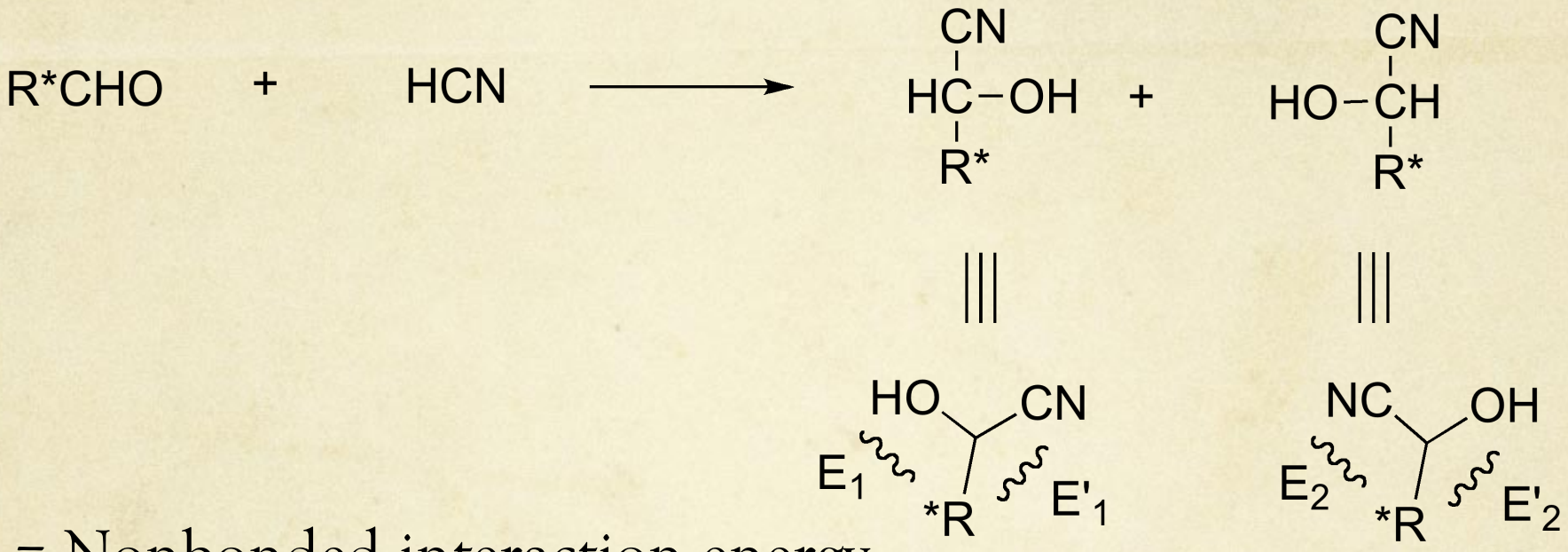

Lock and Key



1. Asymmetric Induction

Modern concept of assymmetric induction



E = Nonbonded interaction energy

When R* is dissymmetric,

$$E_1 + E'_1 \neq E_2 + E'_2$$

The preferential metabolism of the *dextro*-enantiomer of tartaric acid is 15 years prior to Fischer's doctoral studies

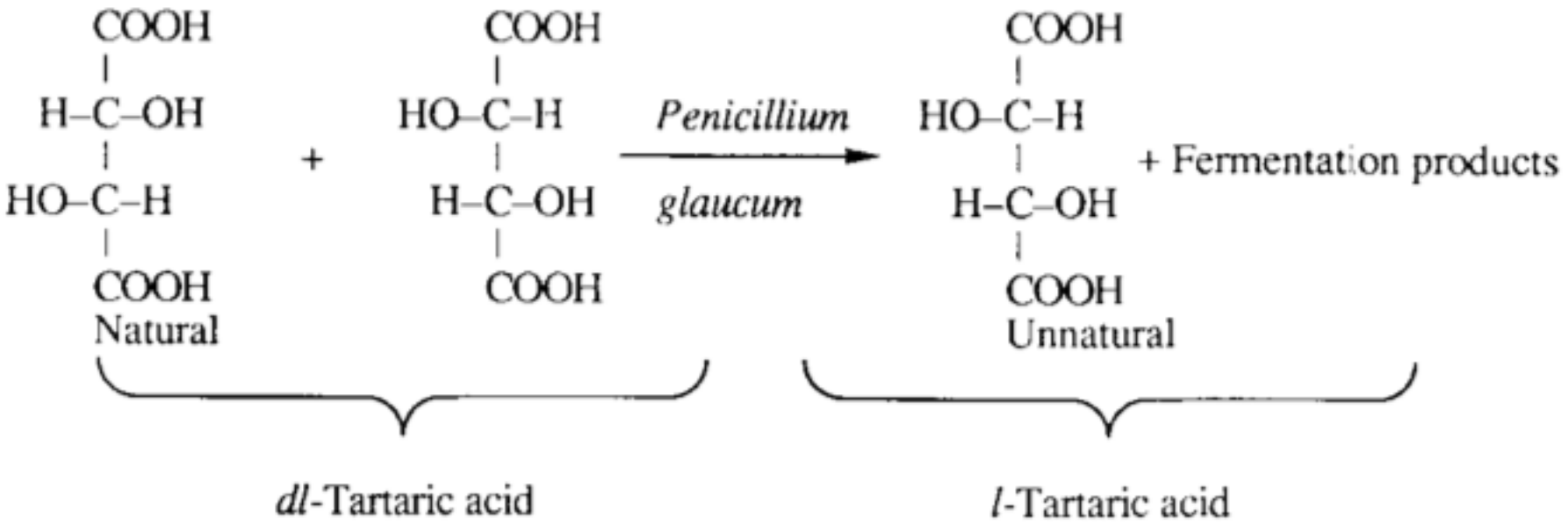


FIG. 2.—Louis Pasteur's preparation of D-*(levo)*-tartaric acid.

An understanding of optical isomerism had been provided in 1874 by van't Hoff – Le Bel theory of asymmetric carbon

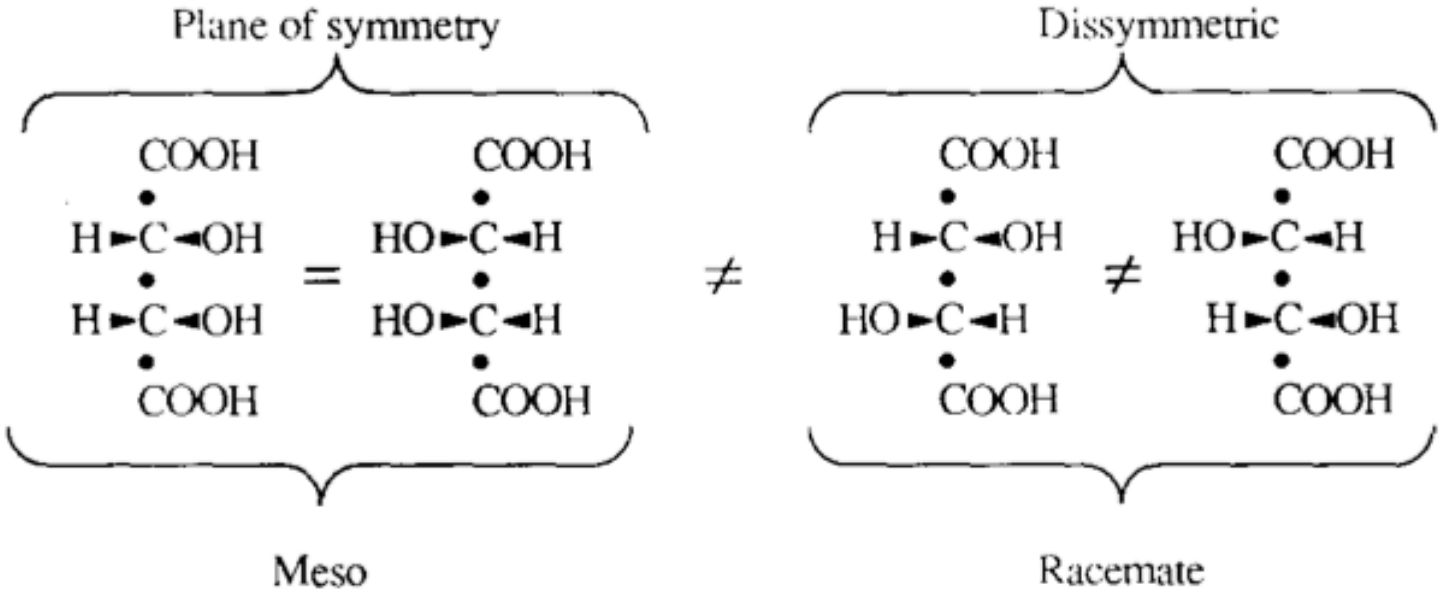
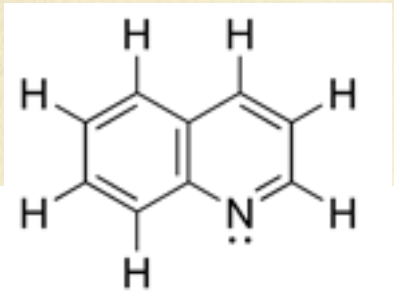


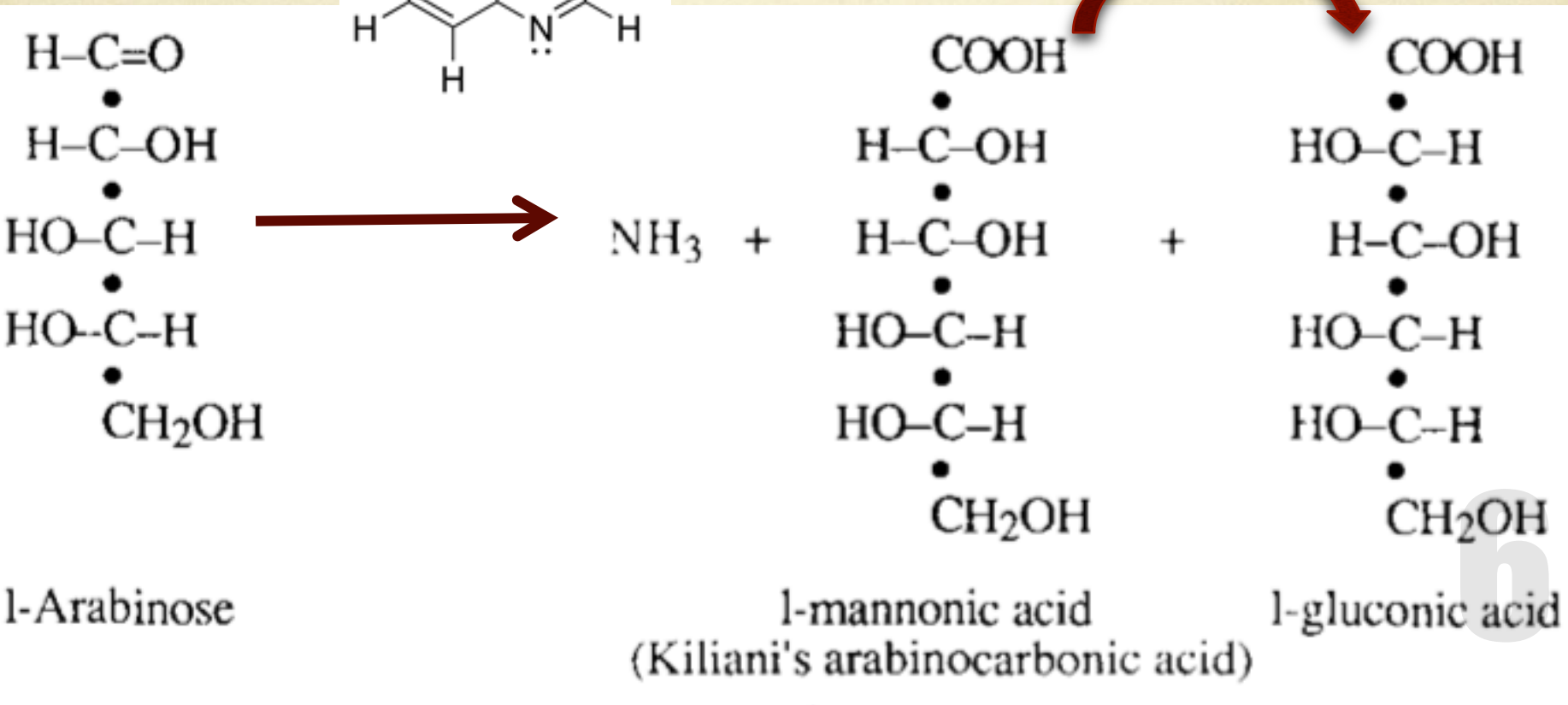
FIG. 3.—The basic assumption for Fischer's research of the optical isomerism of sugars.

All previous observations in the sugar group are in such complete agreement with the theory of asymmetric carbon that the use of this theory seems justifiable

On the way to explicit the configuration of glucose……



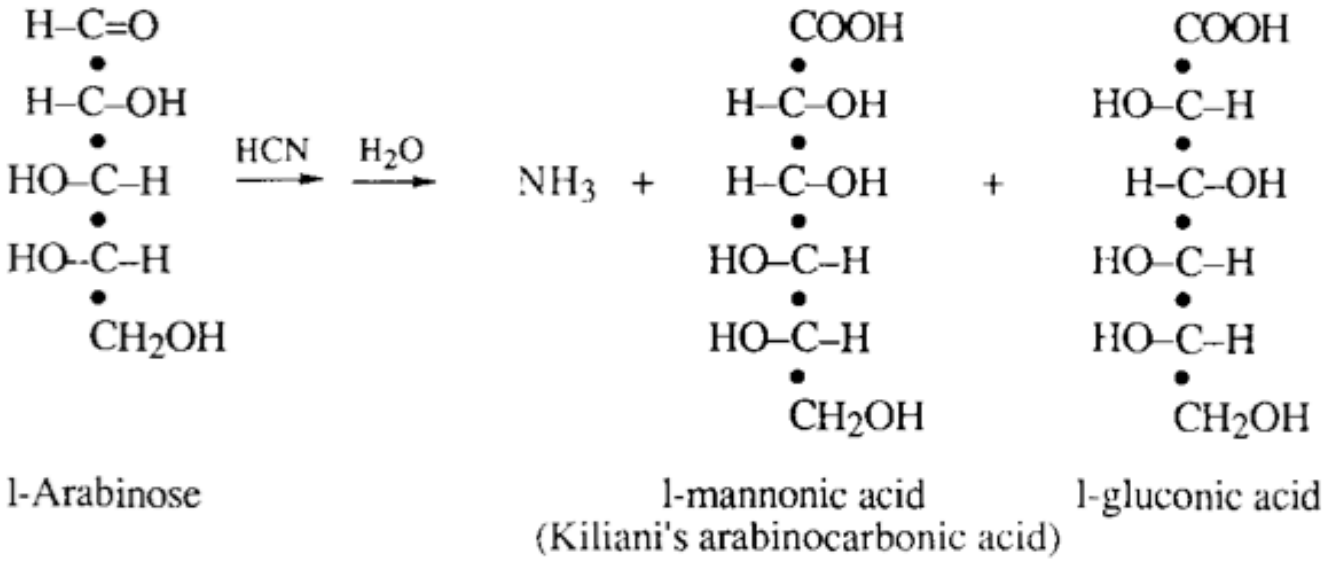
Heating with quinoline



l-Arabinose

l-mannonic acid
(Kiliani's arabinocarbonic acid)

l-gluconic acid



l-Arabinose

l-mannonic acid
(Kiliani's arabinocarbonic acid)

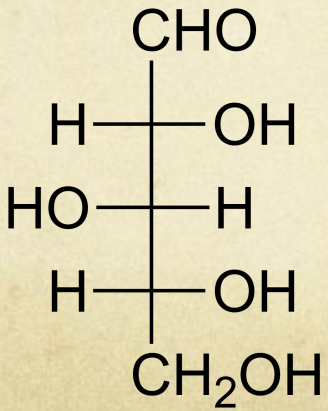
l-gluconic acid

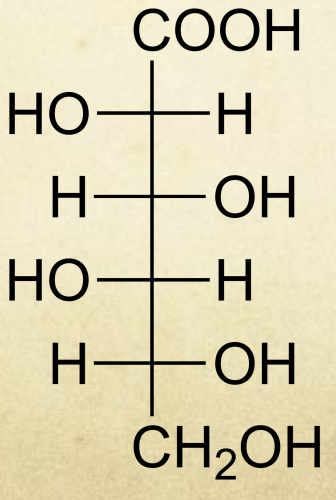
l-mannonic acid 3 times greater than gluco isomer

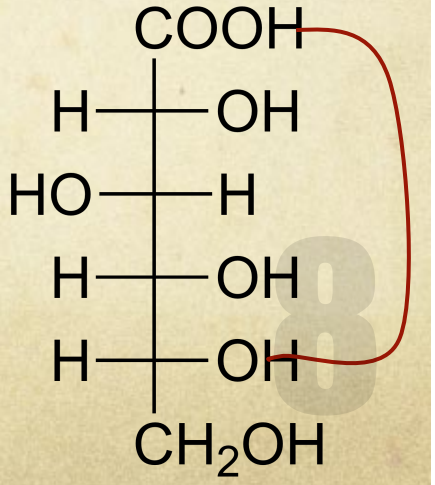
The simultaneous formation of the two stereoisomeric products on addition of hydrogen cyanide to aldehydes, which was observed here for the first time, is quite remarkable in theoretical as well as in practical terms.

He accumulated pure substances with relative figurations. And observed more asymmetric induction

			Yield (%)	Reference
l-Arabinose (50 g)	⟶	Mannonic acid	34	20
		Ca-gluconate	11	
d-Mannose (2 kg)	⟶	α-Mannoheptonic acid	87	21
d-Xylose (40 g)	⟶	Gluconic acid lactone	51	22
		Idonic acid	35	





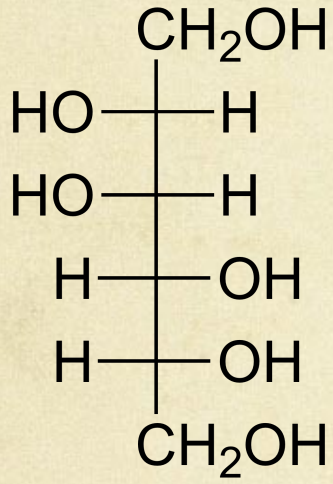


Chirality

In 1894, "in the case of asymmetric systems, the further synthesis occurs in an asymmetric sense."

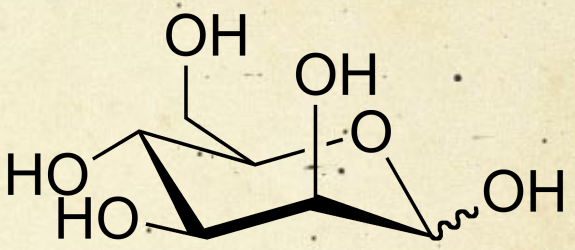
2. Yeast Fermentations and Enzymes

1889 Fischer reported the fermentation of d-Man



Mannitol

HNO_3



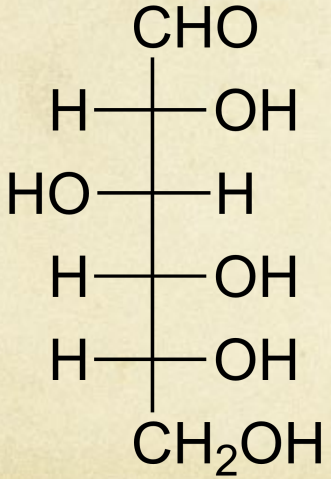
fermentation

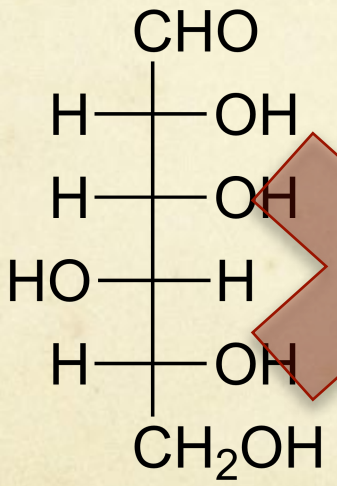
Experiments That Demonstrated the Chemical Basis of Biology

Racemic mannose $\xrightarrow{\text{yeast}}$ *l*-mannose + CO_2 + ethanol

Similarly, *d*-glucose, *l*-fructose, or *d*-galactose $\xrightarrow{\text{yeast}}$ CO_2 + ethanol

However, *l*-glucose, *d*-fructose, or *l*-galactose $\xrightarrow{\text{yeast}}$ no fermentation





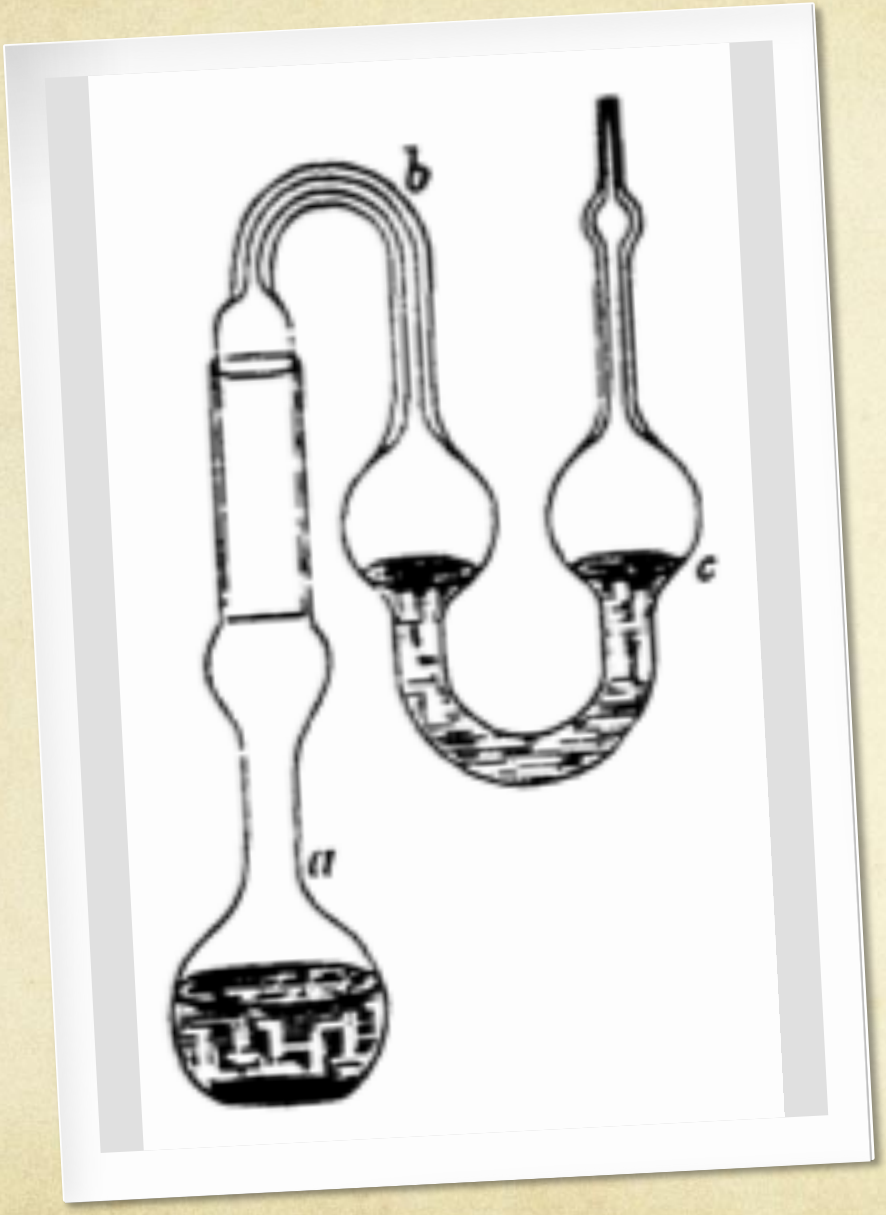
D-Gulcose

L-(dextro)-fructose

Optically active

Heptoses

Octoses



Actual size

Semi-micro scale rxn

(a) 70 mg sugar

0.35 ml water

0.35ml sterilized yeast extract

13 mg yeast species

(b) S-trap for evolved CO_2

(c) $Ba(OH)_2$ (aq)

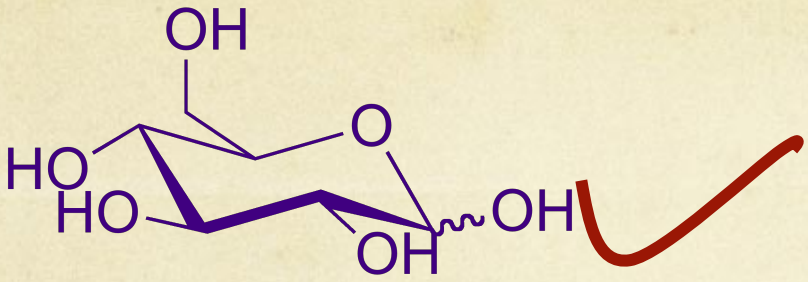
The Selective Fermentation of Natural Sugars by Pure Yeasts

Yeast	Sugar							
	Glucose		Mannose		Galactose		*l*-Fructose[a]	*d*-Sorbose[b]
	d	*l*	*d*	*l*	*d*	*l*		
S. pastorianus I	+++	−	+++	−	+++	−	+++	−
S. pastorianus II	+++	−	+++	−	++	−	+++	−
S. pastorianus III	+++	−	+++	−	+++	−	+++	−
Brauereihefe	+++	−	+++	−	+++	−	+++	−
Brennereihefe	+++	−	+++	−	+	−	+++	−
Milchzuckerhefe	+++	−	+++	−	+	−	+++	−

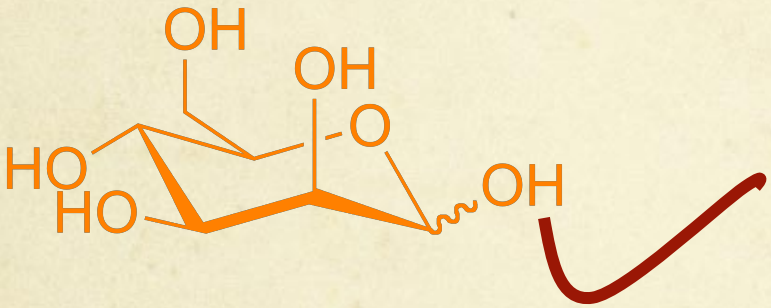
[a] Used by Fischer to designate natural D-fructose.

[b] Also negative were *d*-talose, *l*-gulose, *l*-arabinose, rhamnose, α-glucoheptose, and α-glucooctose.

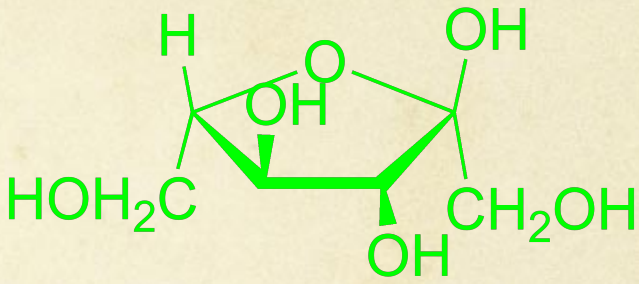
The same observation is likely to be found for other microorganisms as well as for other groups of organic compounds and perhaps a very great number of chemical processes occurring within an organism are influenced by the geometry of the cell.



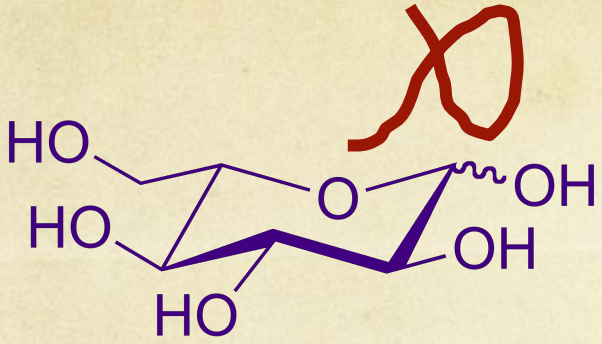
D-Glucose



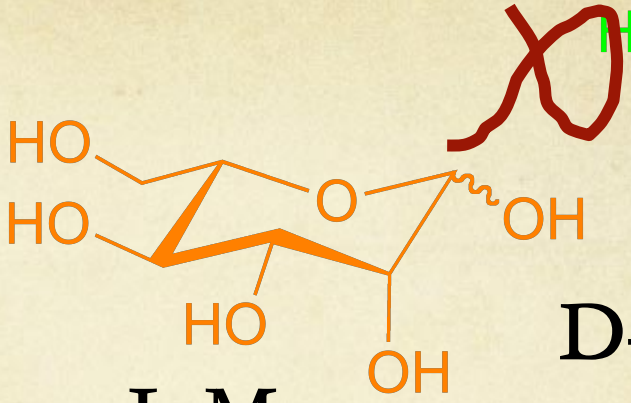
D-Mannose



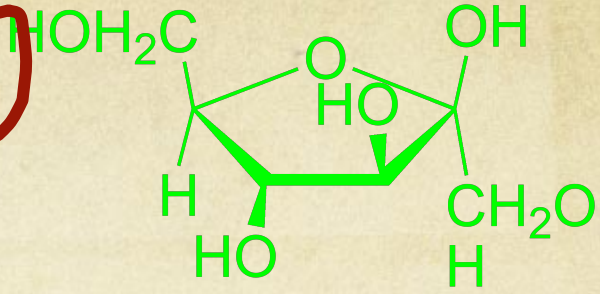
L-Fructose



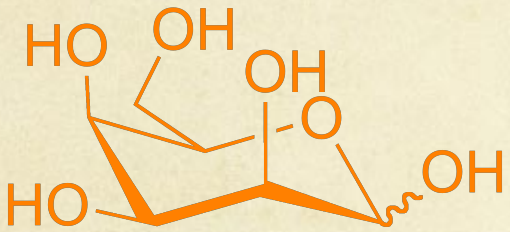
L-Glucose



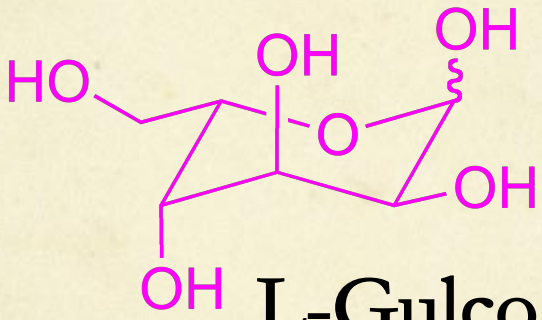
L-Mannose



D-Fructose



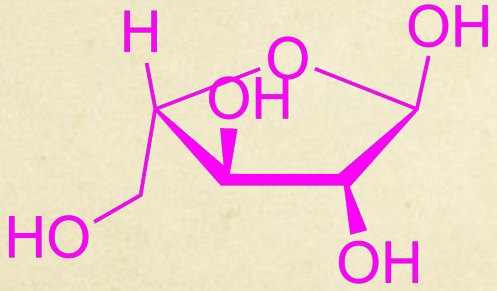
D-Talose



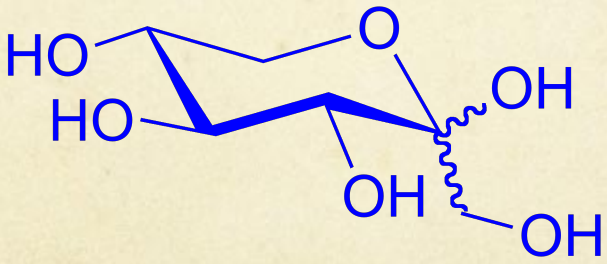
L-Gulcose

α-glucoheptose

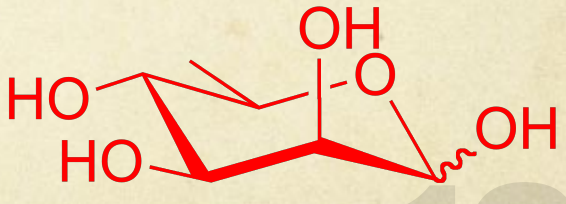
α-glucooctose



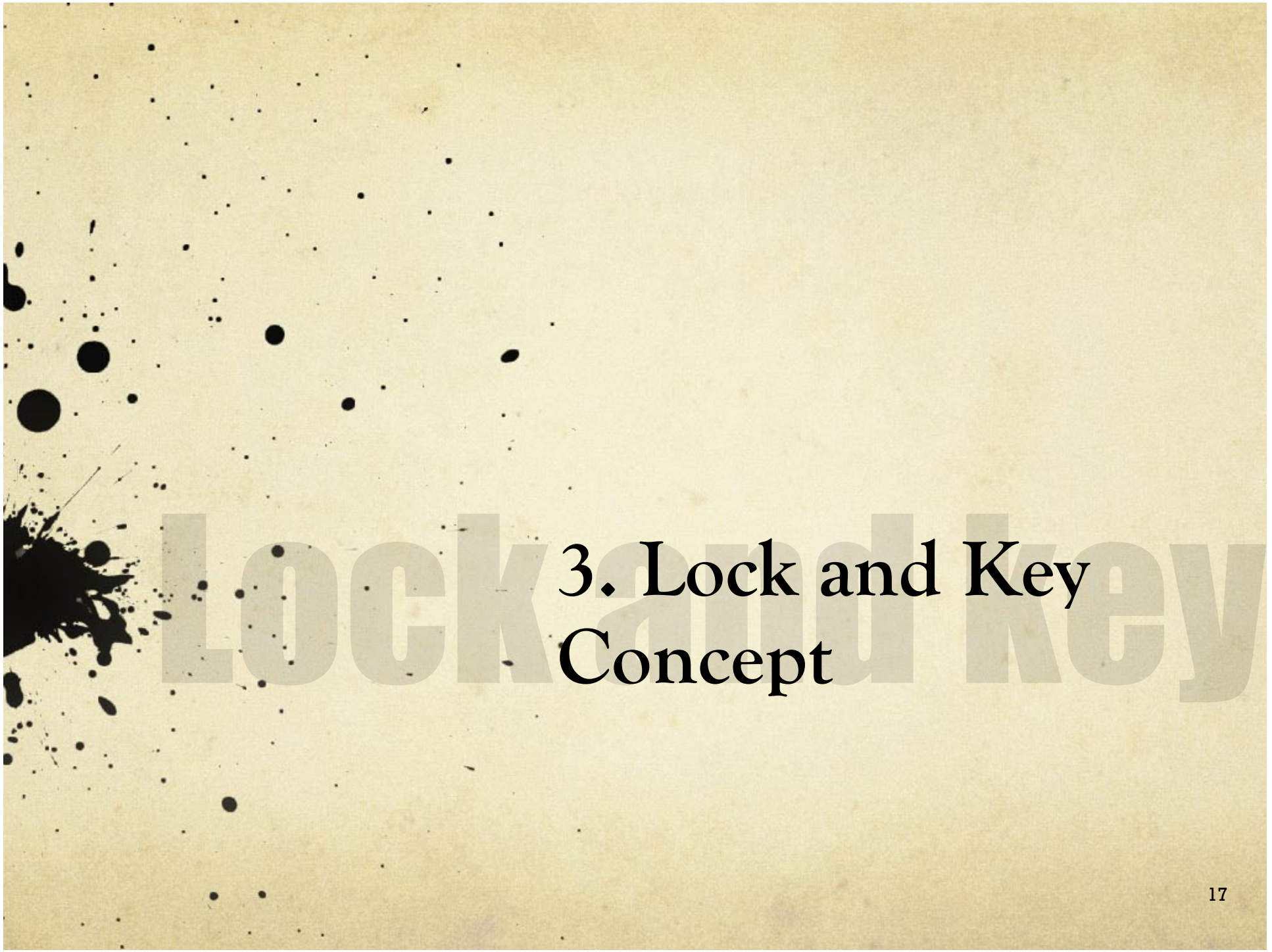
L-arabinose



D-sorbose



D-Rhamnose



3. Lock and Key Concept

The Fermentation of Glycosides by Different Pure Yeasts

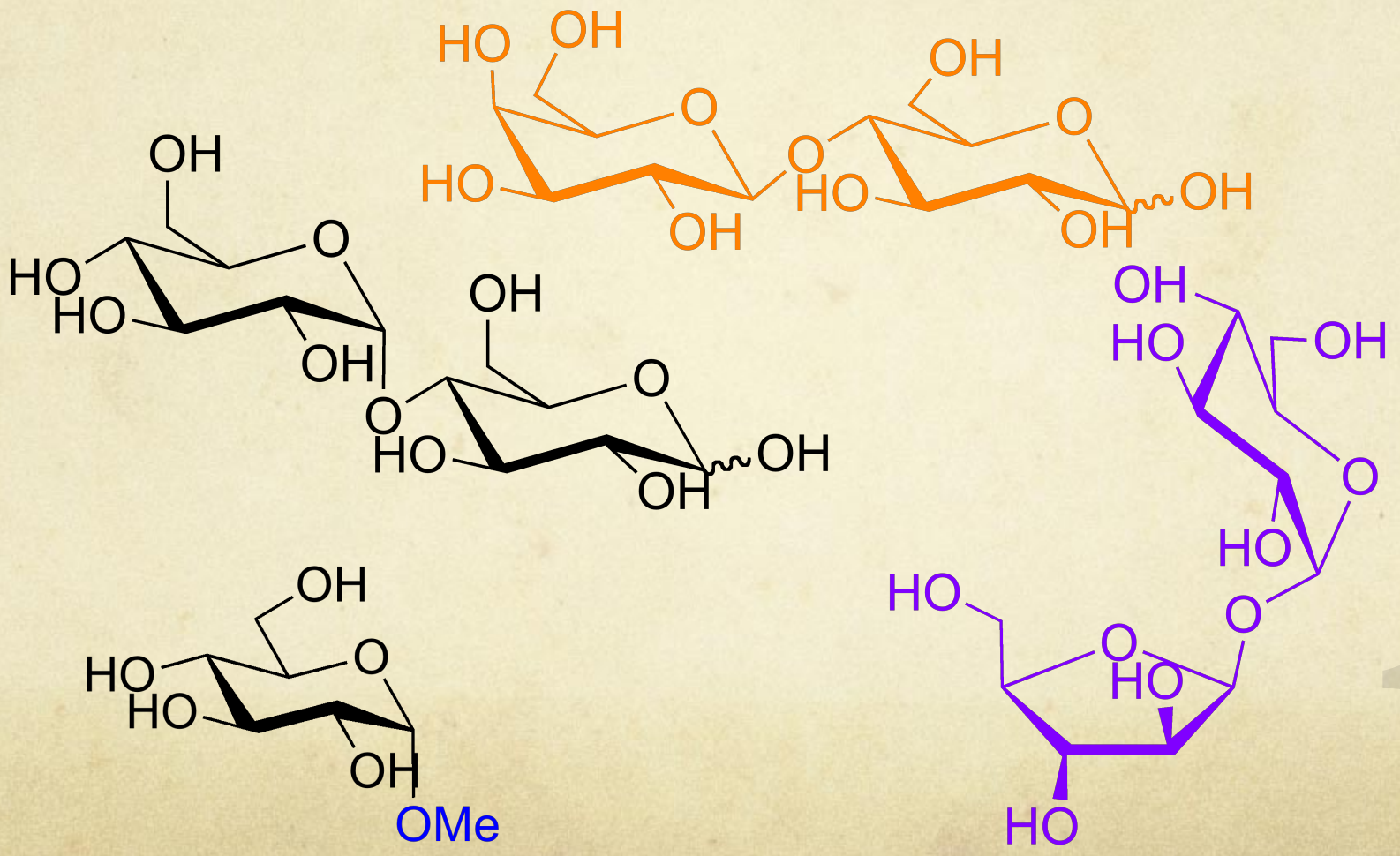
	Sucrose	Maltose	Lactose	Methyl α-glucoside[a]	Glucosyl resorcinol[b]
S. pastorianus I	+++	+++	−	+	−
Brauereihefe	+++	+++	−	+	−
Brennereihefe	+++	+++	−	+	−
S. productivus	+	+++	−	+	−
Milchzuckerhefe	+++	−	+++	−	Not tested

[a] From Fischer synthesis.
[b] From Koenigs–Knorr synthesis.

Different yeasts possessed different enzymes?

Maltose, lactose

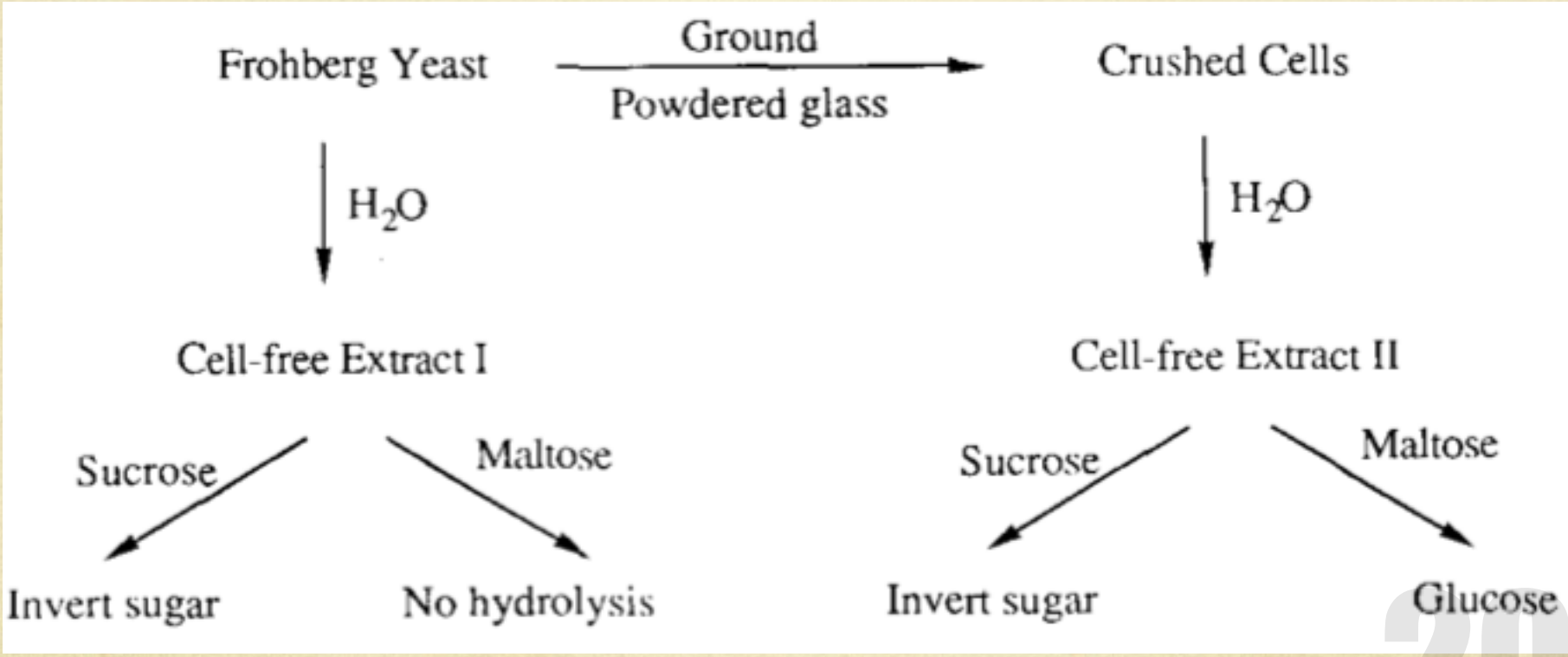
sucrose



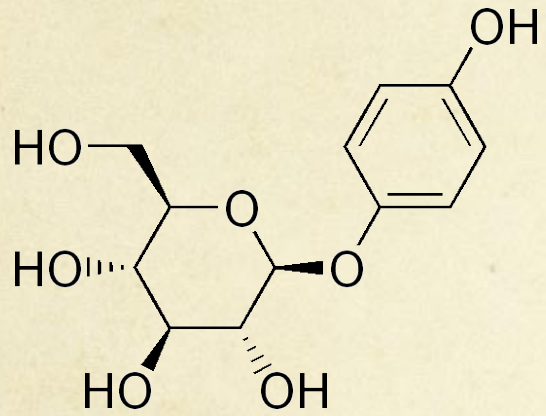
yeasts contains at least two different enzymes

Extracellular enzyme

Cells contained enzyme

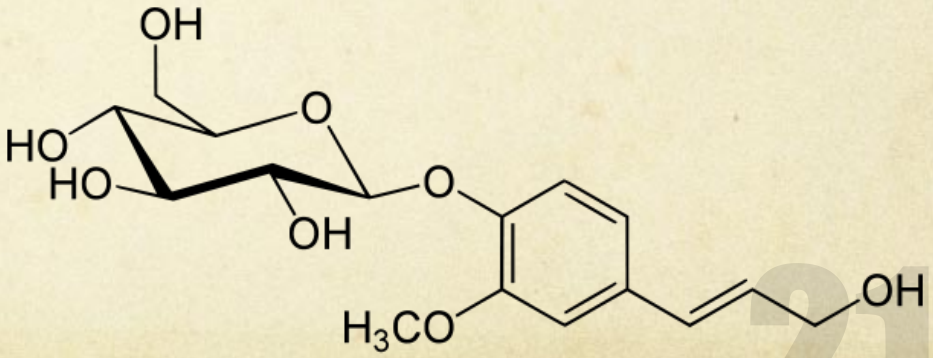


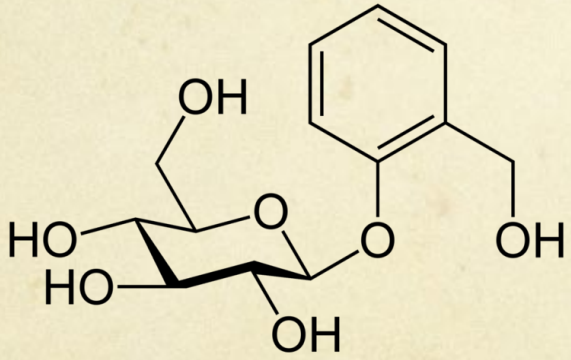
Invertin ✗ emulsin ✓



熊果素(Arbutin)

松果苷Coniferin





水杨苷Salicin

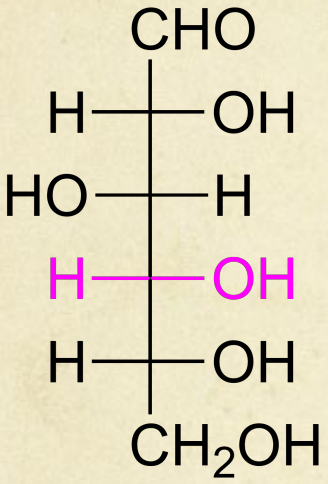
Enough artificial glucosides

Note: Fischer did not know the configurations of anomeric carbon

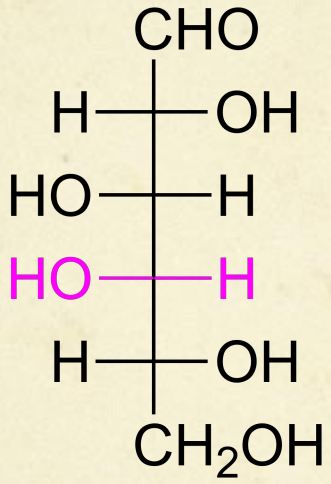
Effects of Structure and Configuration on Enzymatic Hydrolysis

Glycoside	Crude enzyme preparation: Invertin[a]	Crude enzyme preparation: Emulsin[b]
α-Glucosides		
Methyl	+	−
Ethyl	+	−
Sucrose	+	−
Maltose	+	−
β-Glucosides		
Methyl	−	+
Phenyl	−	+
Salicyl	−	+
α-Galactoside[c]		
Methyl	−	−
β-Galactosides		
Methyl	−	+
Lactose	−	+

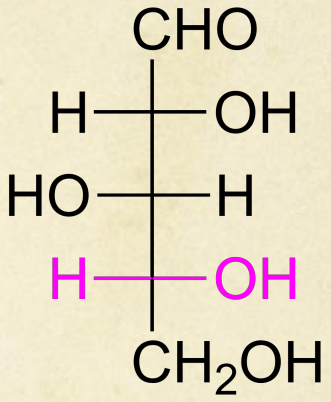
Fischer was intrigued by the hydrolysis of β-glucosides and β-galactosides, but had no effect on either α- or β-xyloside



Glucose

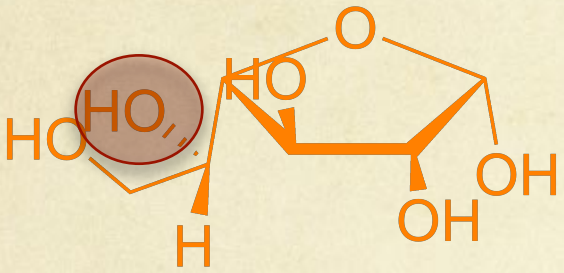


Galctose

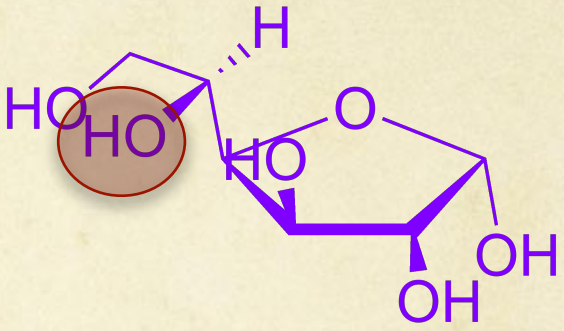


Xylose

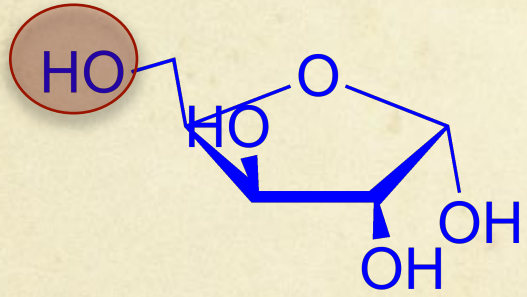
Since then, he expected glycosides to be furanosides



Galctose



Glucose



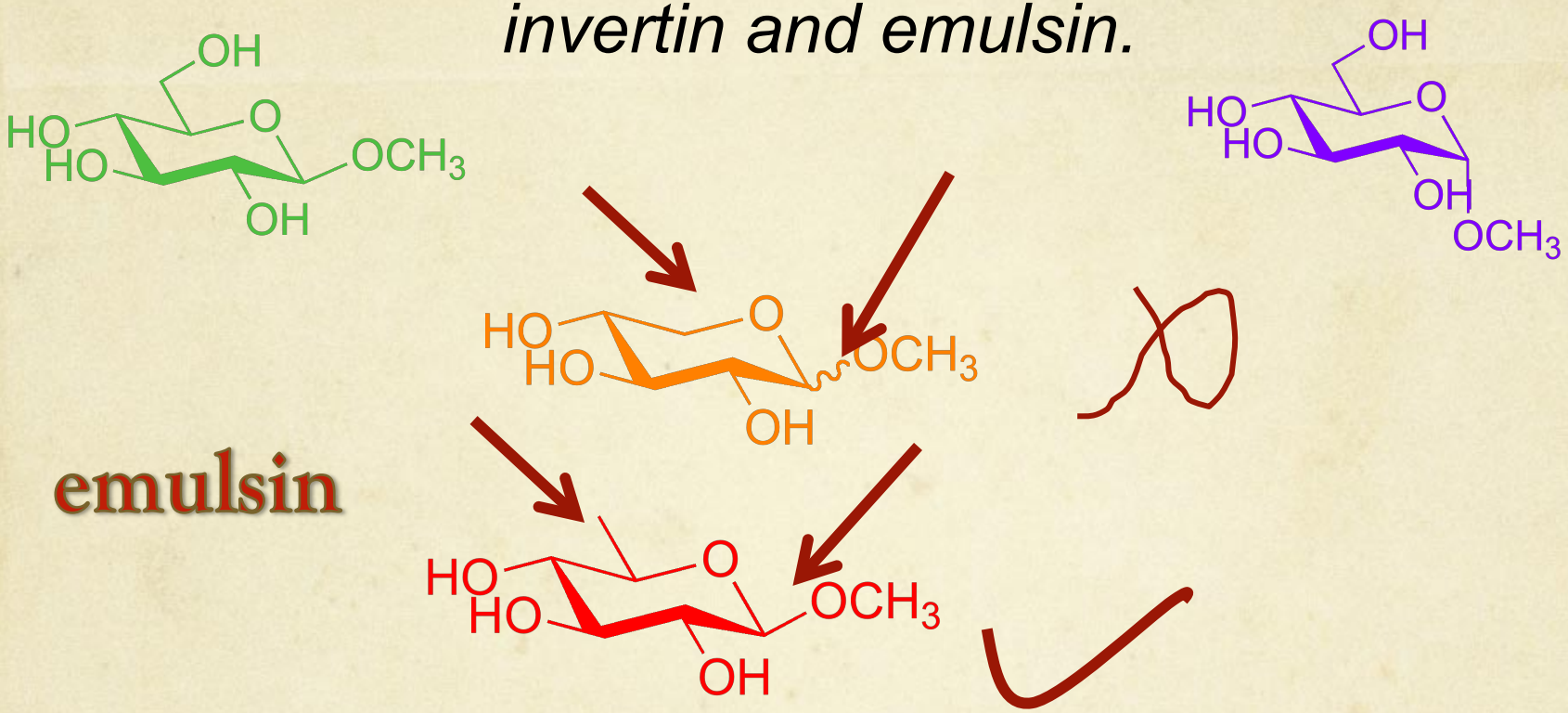
Xylose

A free hydroxyl group at position 5 of a hexoside is required by the enzyme

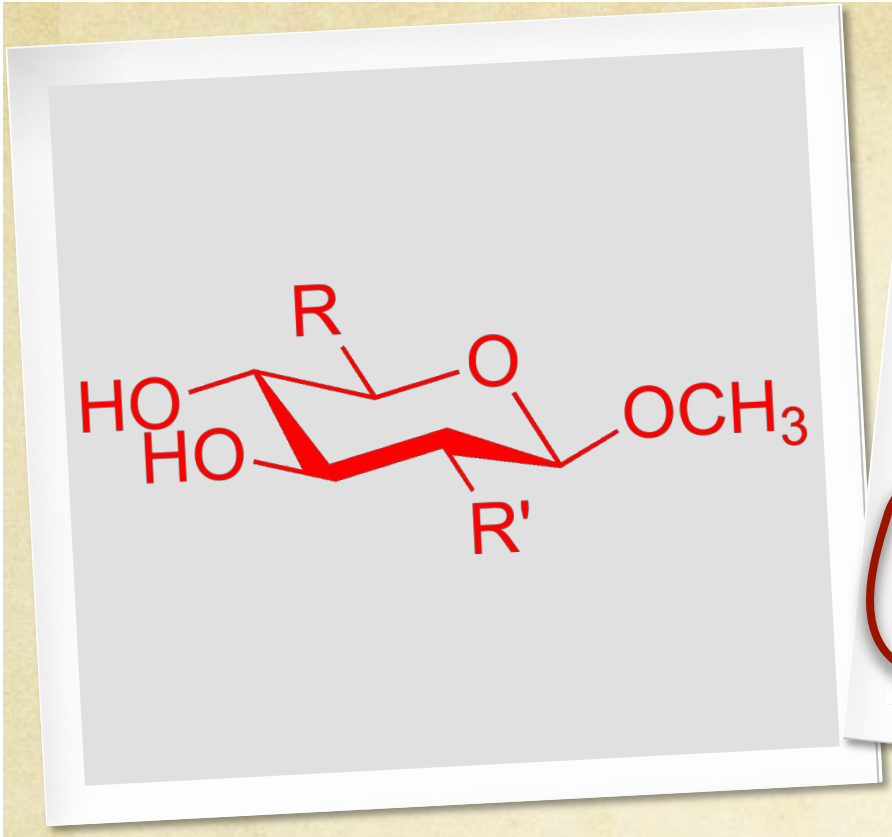
Enzymes are as fastidious as yeast in terms of the cofigurations of substrates

4. Insights on enzyme specificity

Effects of changes of α- and β- glucosides on their properties as substrates for the enzymes invertin and emulsin.



It appears to us very strange that the effect of the enzyme on the methoxyl group at the other end of the carbon chain depends on the sixth carbon atom.



Substituent		Hydrolysis by emulsin
R	*R′*	
CH_2OH	OH	+
CH_2OH	H	−
H	OH	−
CH_3	OH	+
CH_2Br	OH	−

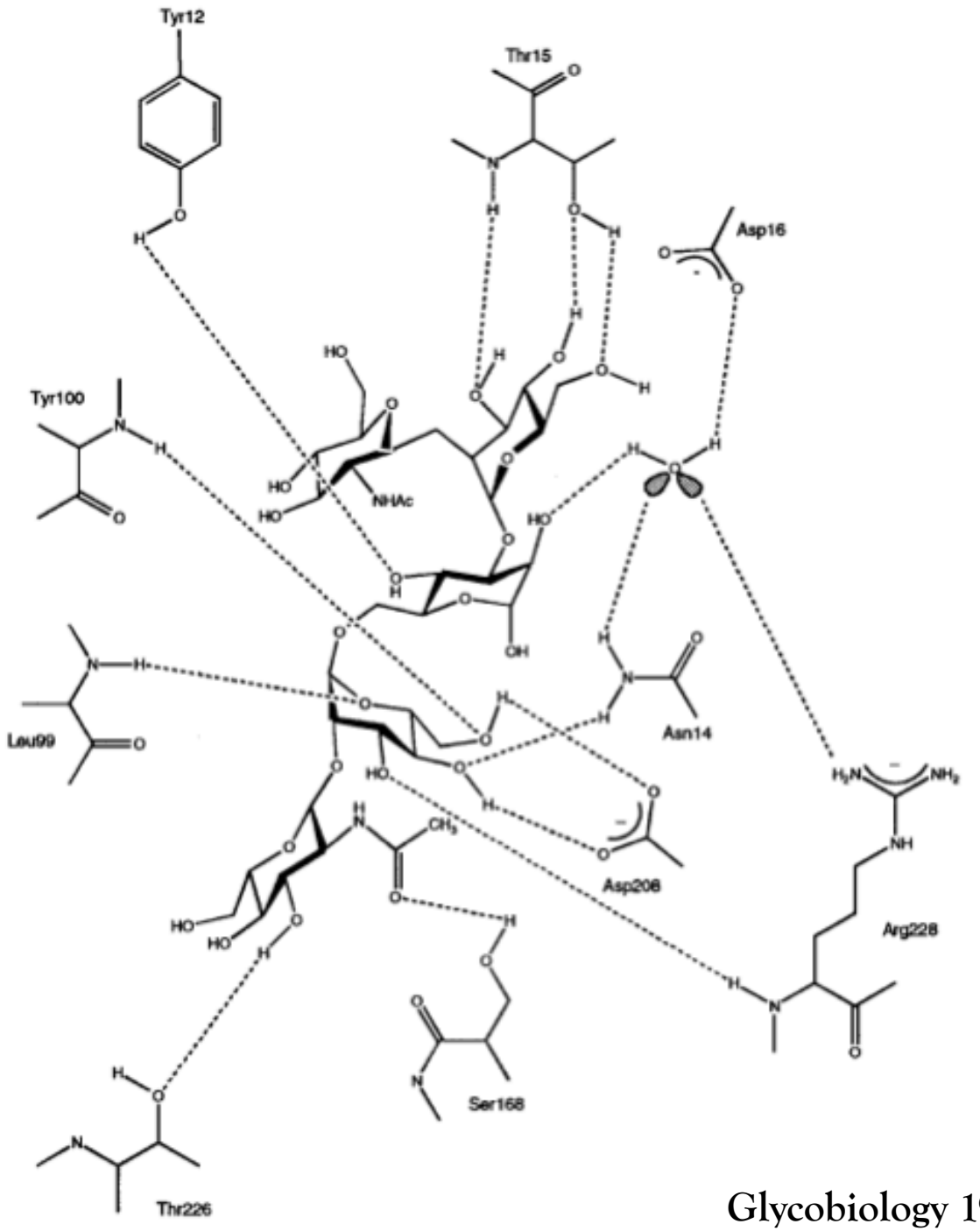
How will the enzyme behave if there is a carbon richer alkyl at the end of the chain?

After his death, his colleagues published that…….

Fischer: Cell level to Molecular level

Concanavalin A

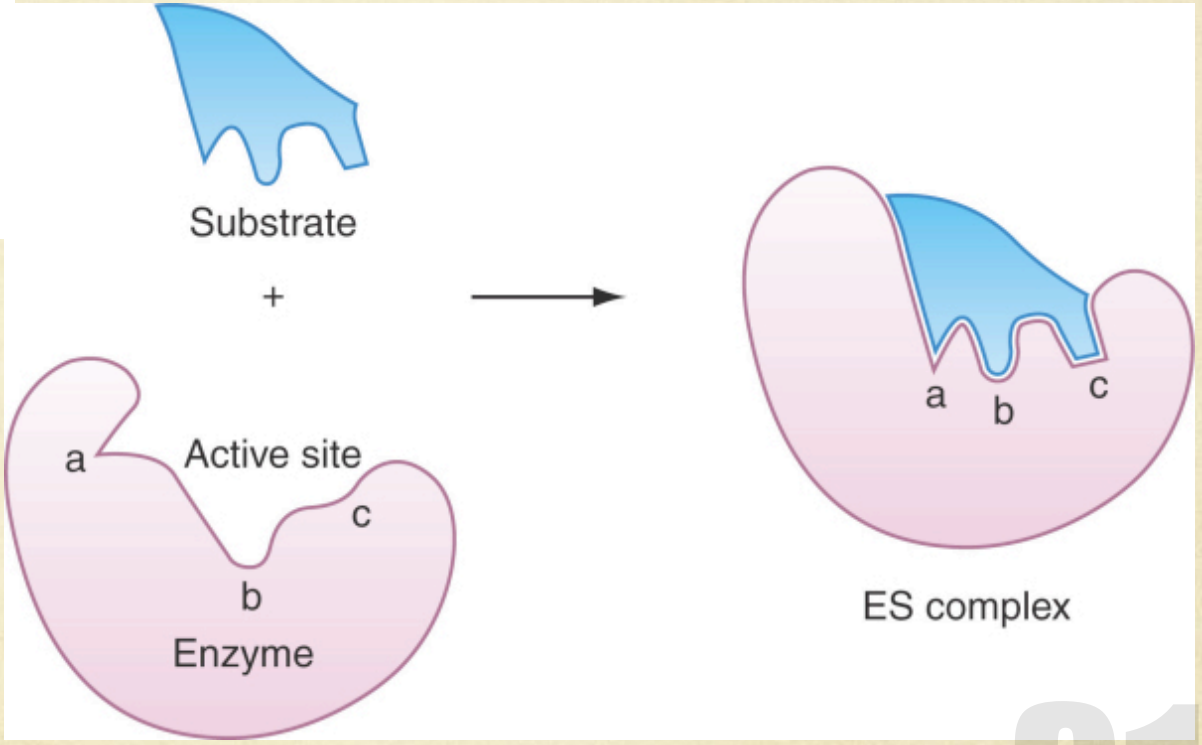
Picture from http://biochem.szote.u-szeged.hu/astrojan/prot1t.htm



Glycobiology 1998, 8, 173–181

5. Impact of Lock and key

Specific structural features of the substrate act somewhat like the ward of a key

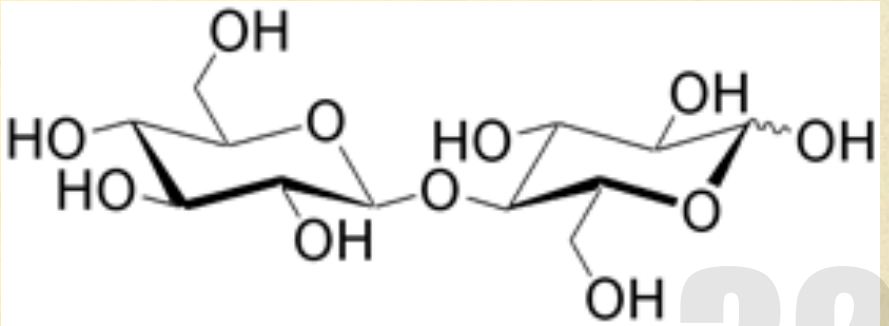


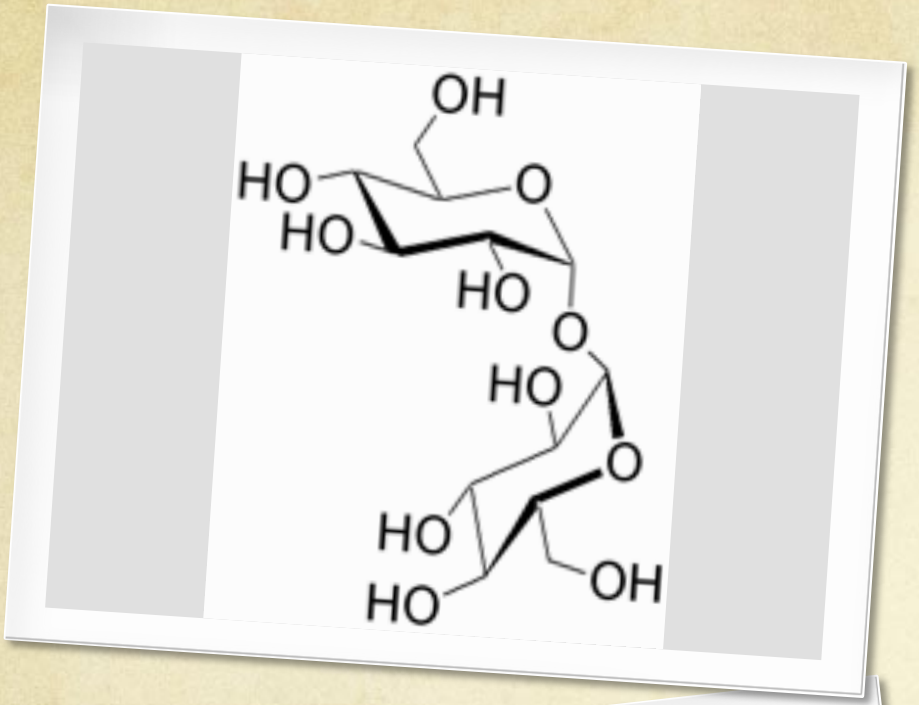
Complementarity is strongly demanding

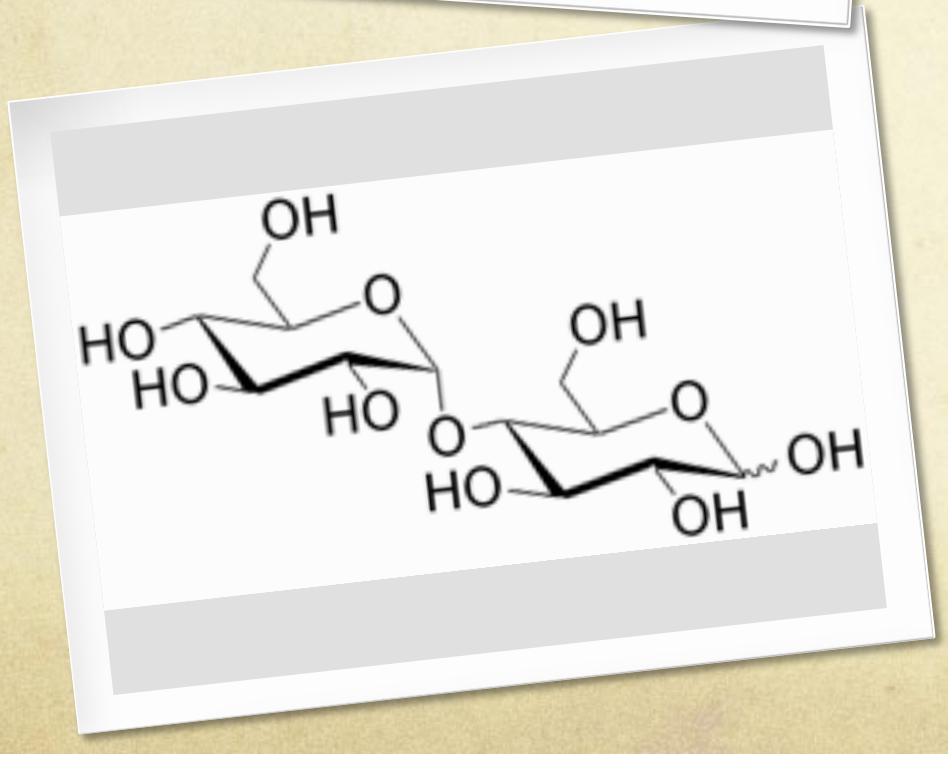
Close proximity and proper orientation

Glucoamylase (amyloglucosidase, AMG)

- Enzyme effect on cellobiose
- Extracted from *Aspergillus niger*
- No catalysis to hydrolysis of cellobiose
- Whereas emulsin did
- Cellobiose must have β-linkage
- (now) hydrolysis of maltose and other α-linked glucosides





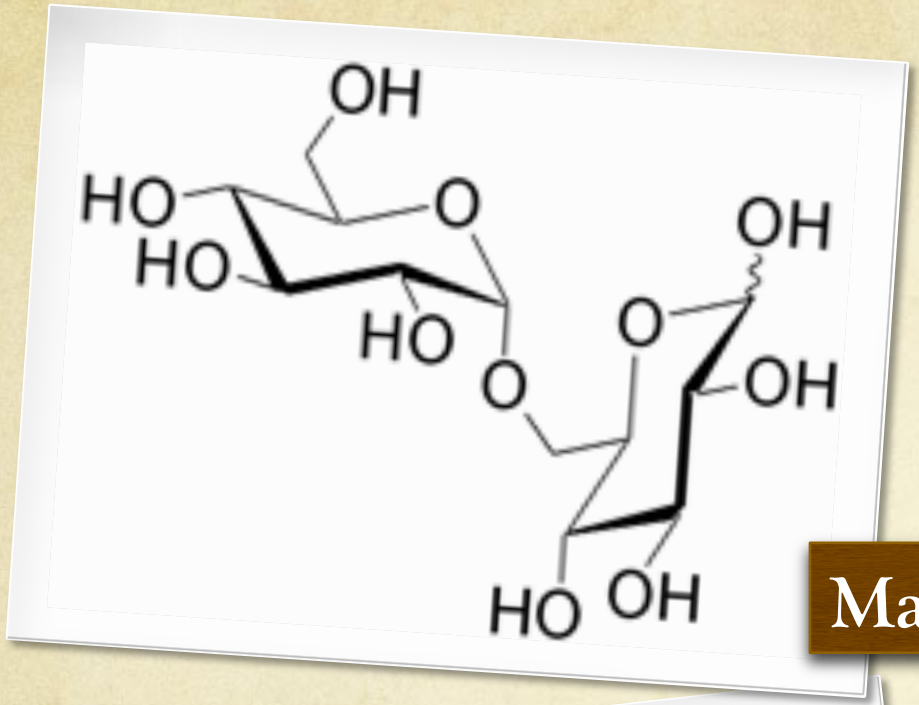


1883, Bourquelot reported that an extract from *A. niger* hydrolyzed maltose

The solution also caused hydrolysis of trehalose

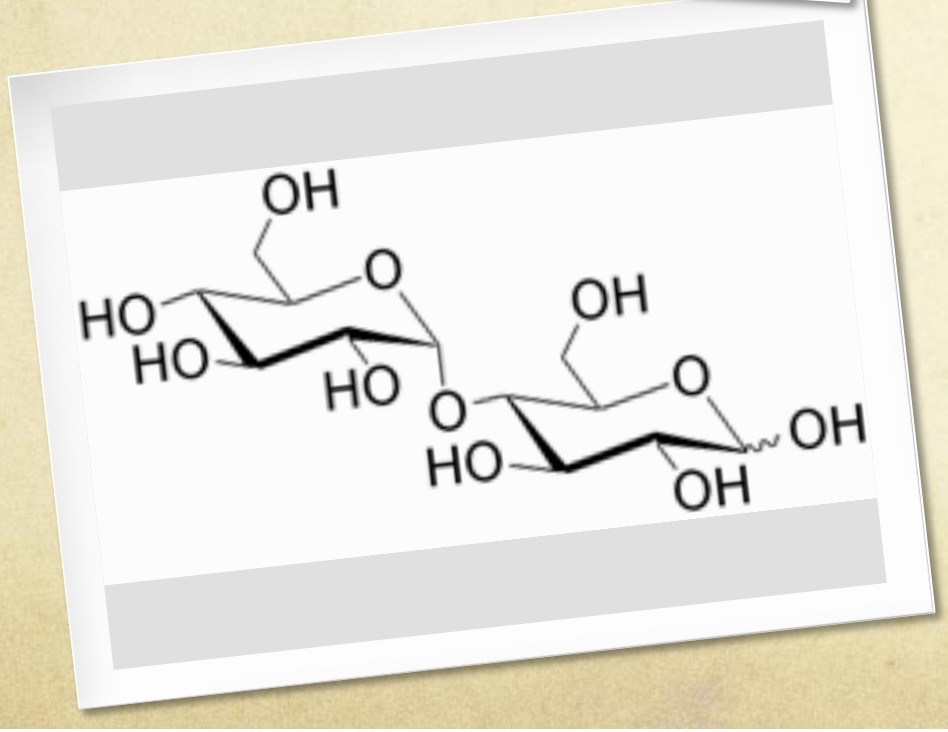
On heating to 63°C the trehalose ability lost but maltose maintained till 75°C

Two enzymes inside, maltase and trehalase

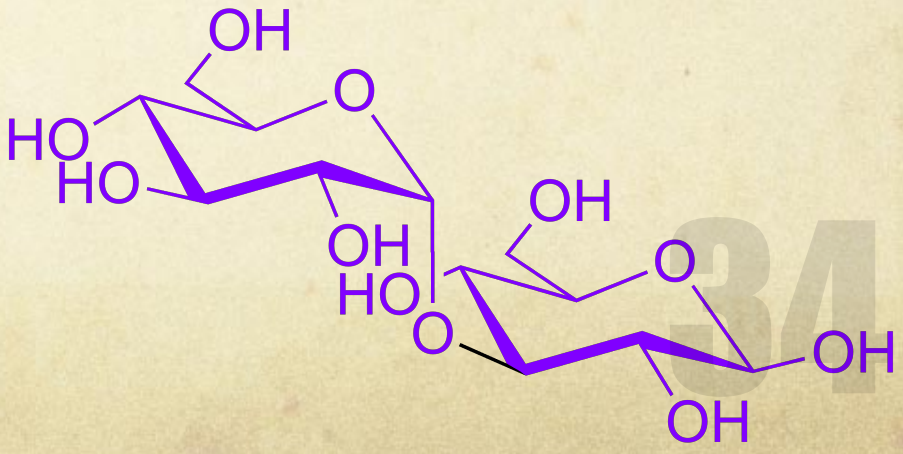


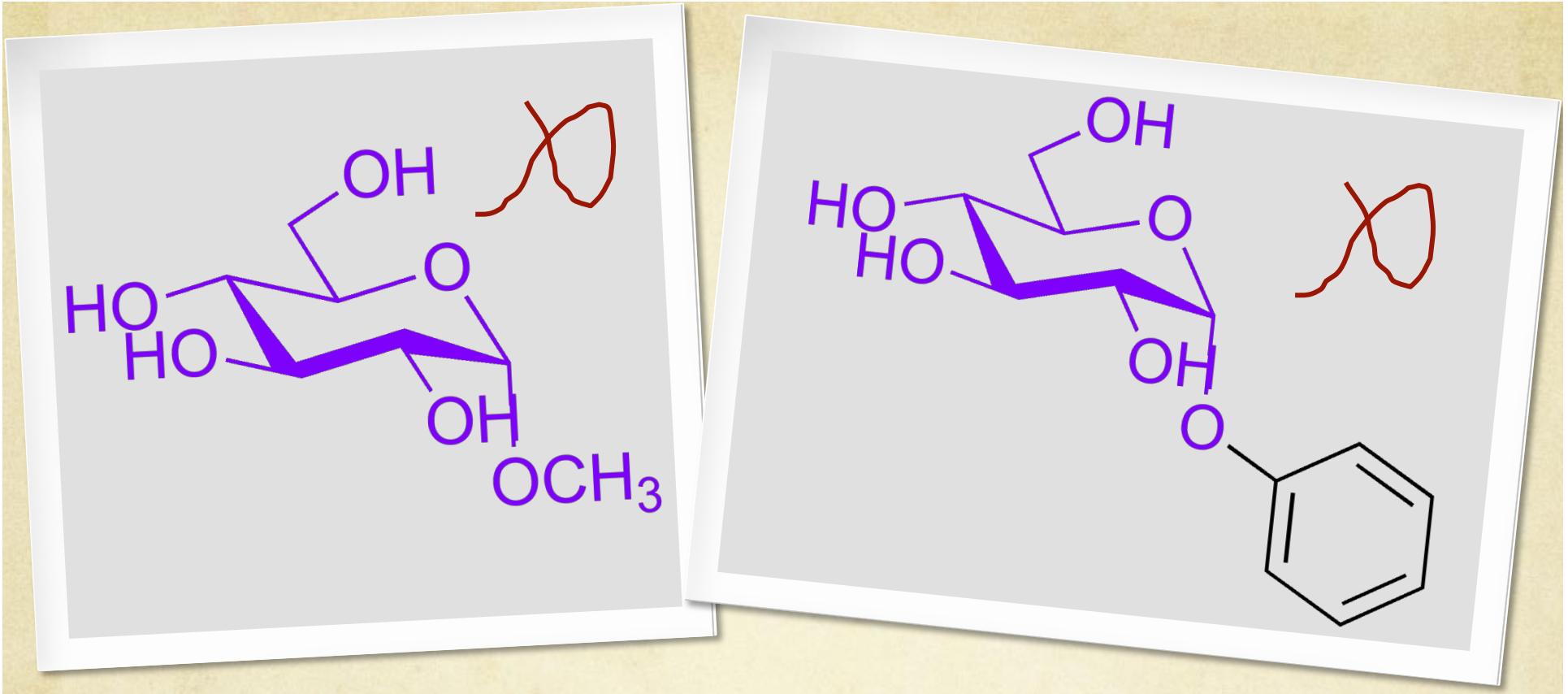
Isomaltose, much lower hydrolysis rate than maltose

Maltose > nigerose > isomaltose

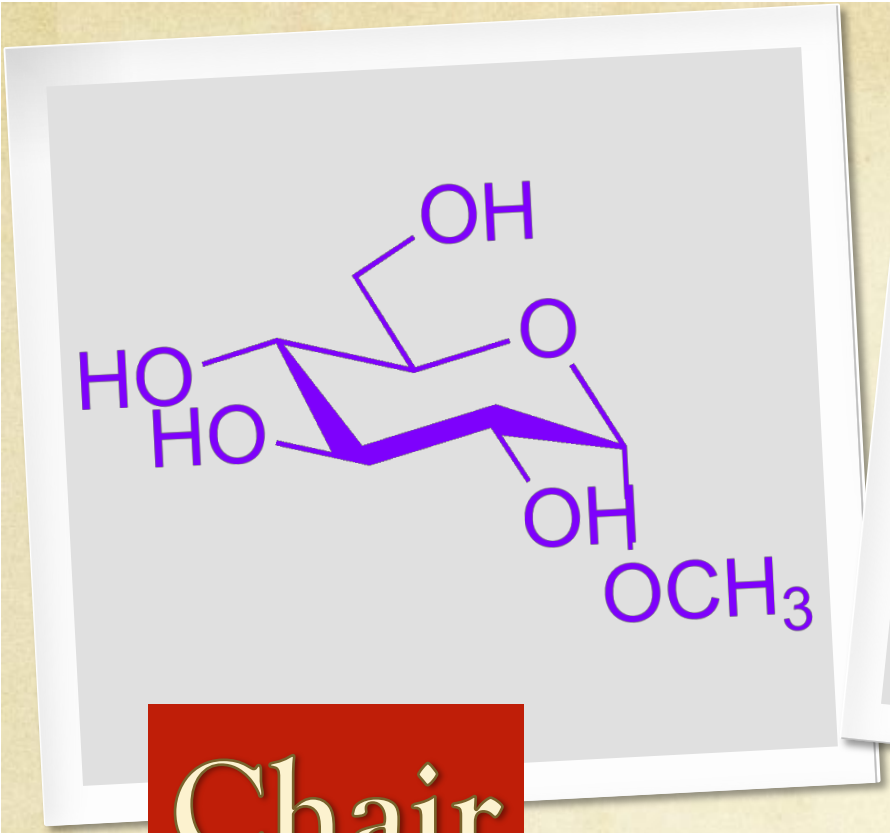


then hydrolysis to nigerose:

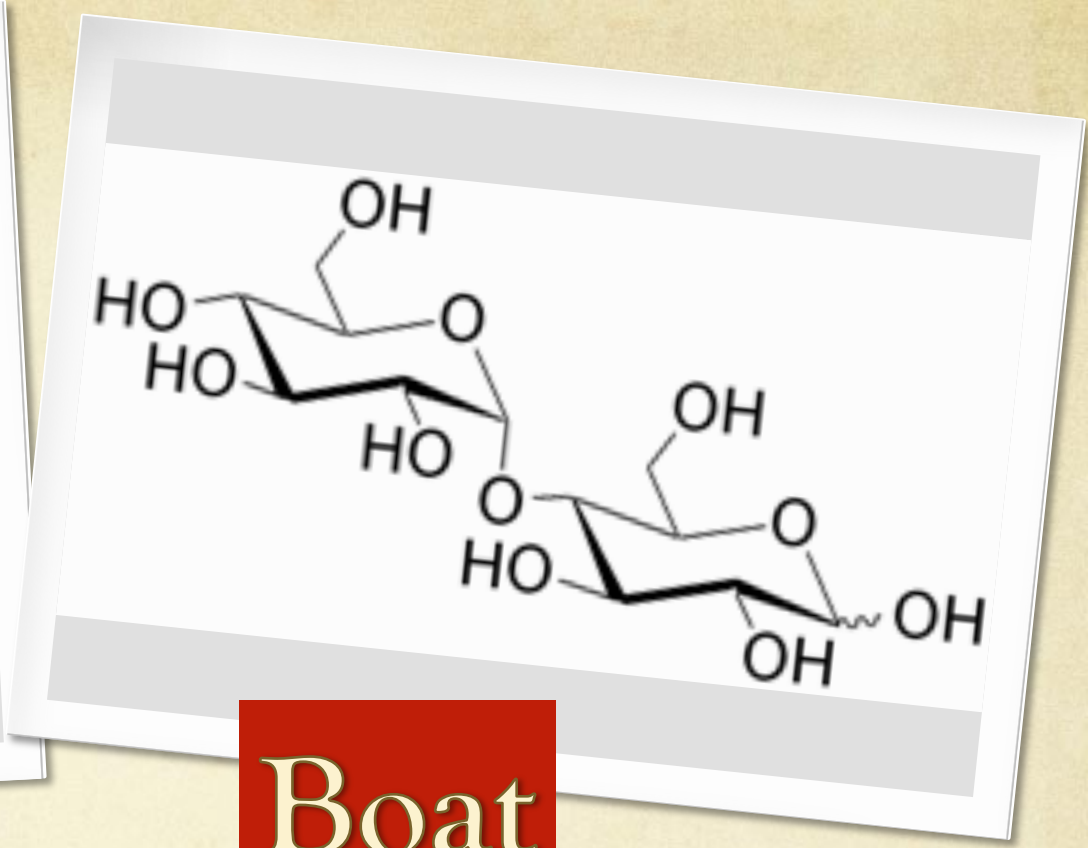




Although the enzyme has a high specificity for an α-D-glucopyranosyl disaccharide, the structure of aglycon can be varied without total loss of activity



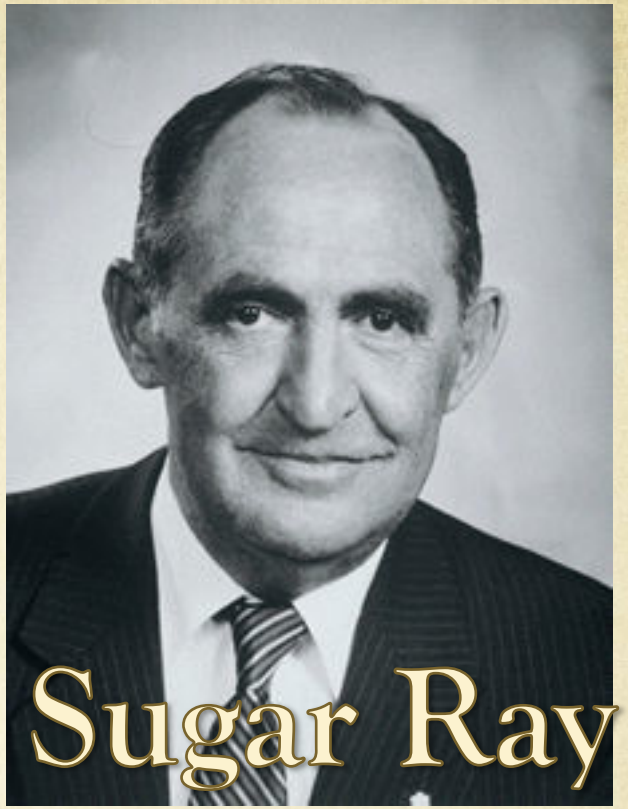
Chair



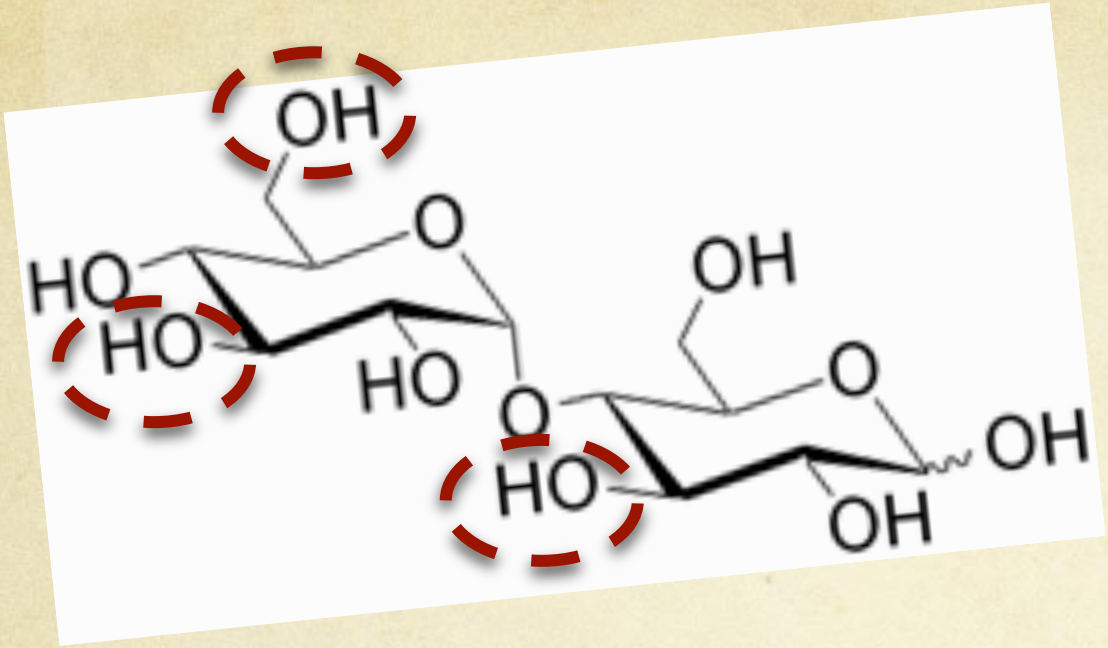
Boat

Which glucosyl units can transform faster into the preferred conformation by the enzyme

Later, by NMR, the same chair conformation!



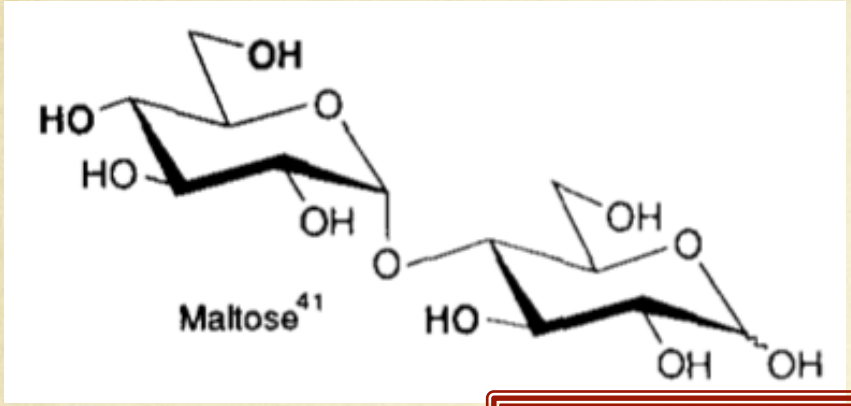
Lemieux : Hydroxyl groups are involved, but not all.
Key hydroxyl groups

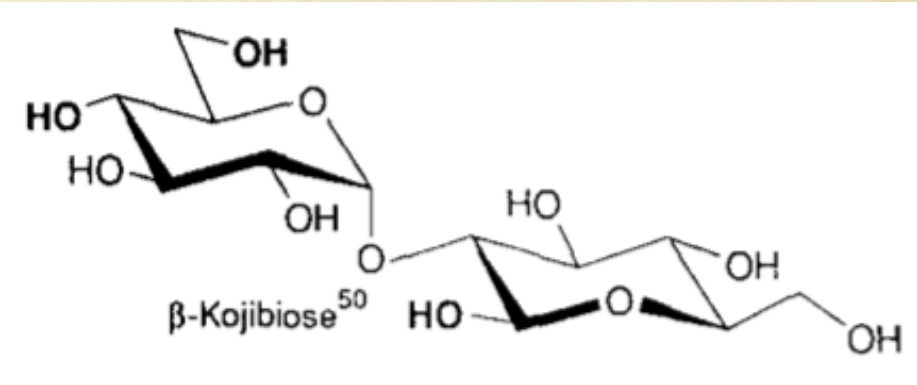


The real answer was given by Bock and Pedersen, who studied the effect of deoxygenation at various hydroxyl-group positions on rate of hydrolysis

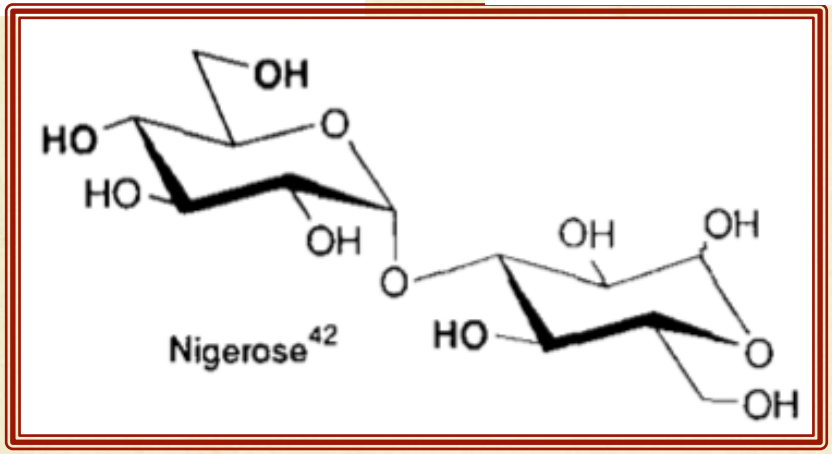
The two key OH of the non-reducing unit must establish polar interactions that tend to anchor this glucose unit in the enzyme's active site.

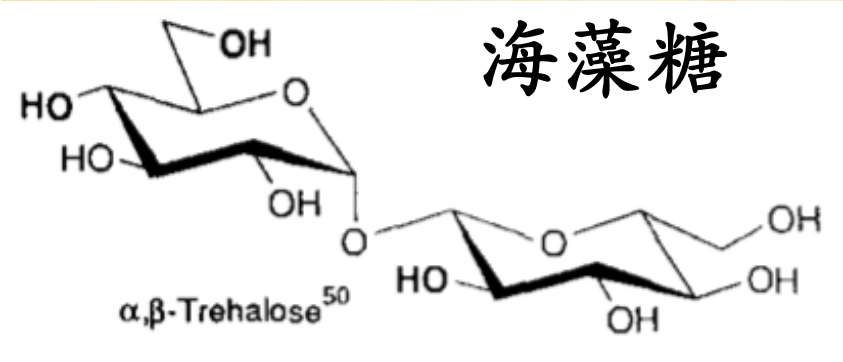
Key hydroxyl groups



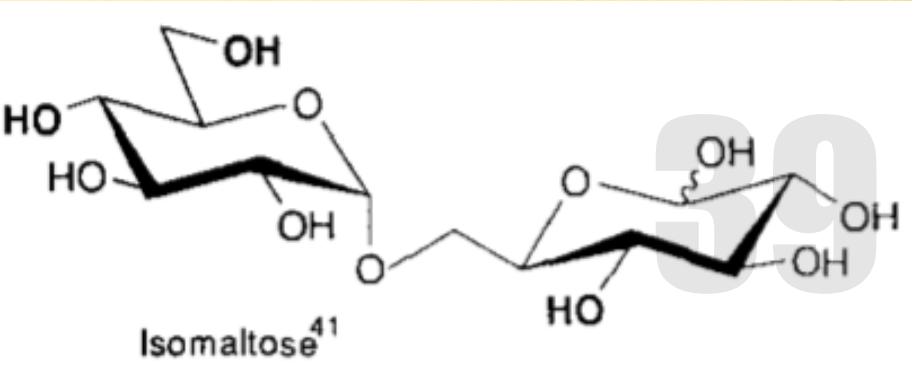


曲二糖



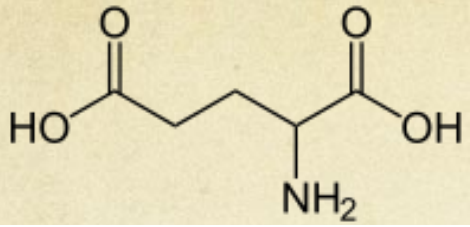


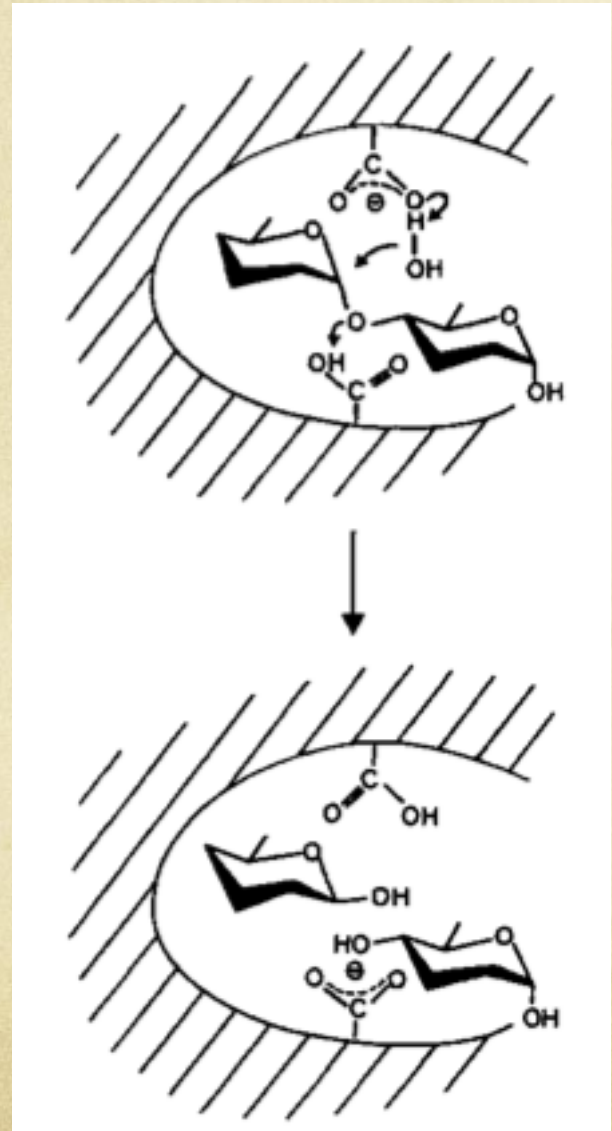
海藻糖



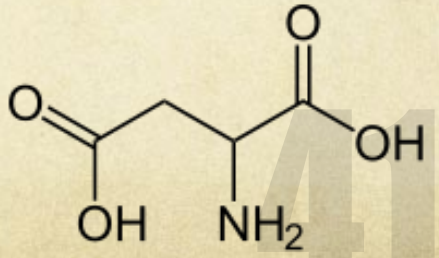
Studies followed up

- Commercial A. niger glucoamylase was separated into two catlytic componets, G1 and G2
- G2 is a proteolytic fragment derived from G1
- They behave similarly, which is common to all major variants of amloglucosidase
- Amino acid sequences of G1 and G2 had been established
- Using glucoamylase-specific synthetic oligonucleotides and molecular cloning of the complementary DNA synthesized from A. niger,
- the primary structure of the mRNA for G1 was established
- In vitro translations of the mRNA followed by immunoprecipitations with glucoamylase-specific antisera showed the presence of G1 and G2





- Crystal structures of G2
- Chemical approach
- Asp-176, Glu-179 and Glu-180 form an acid cluster crucial to the functioning of the enzyme
- Tested by site-specific mutagenesis
- Substitution at Glu-179 provided an inactive protein
- Other two only affected the kinetic parameters
- Asp-176, Glu-179



Eur. J. Biochem. 188,29-38 (1990)

The end of Fischer's story