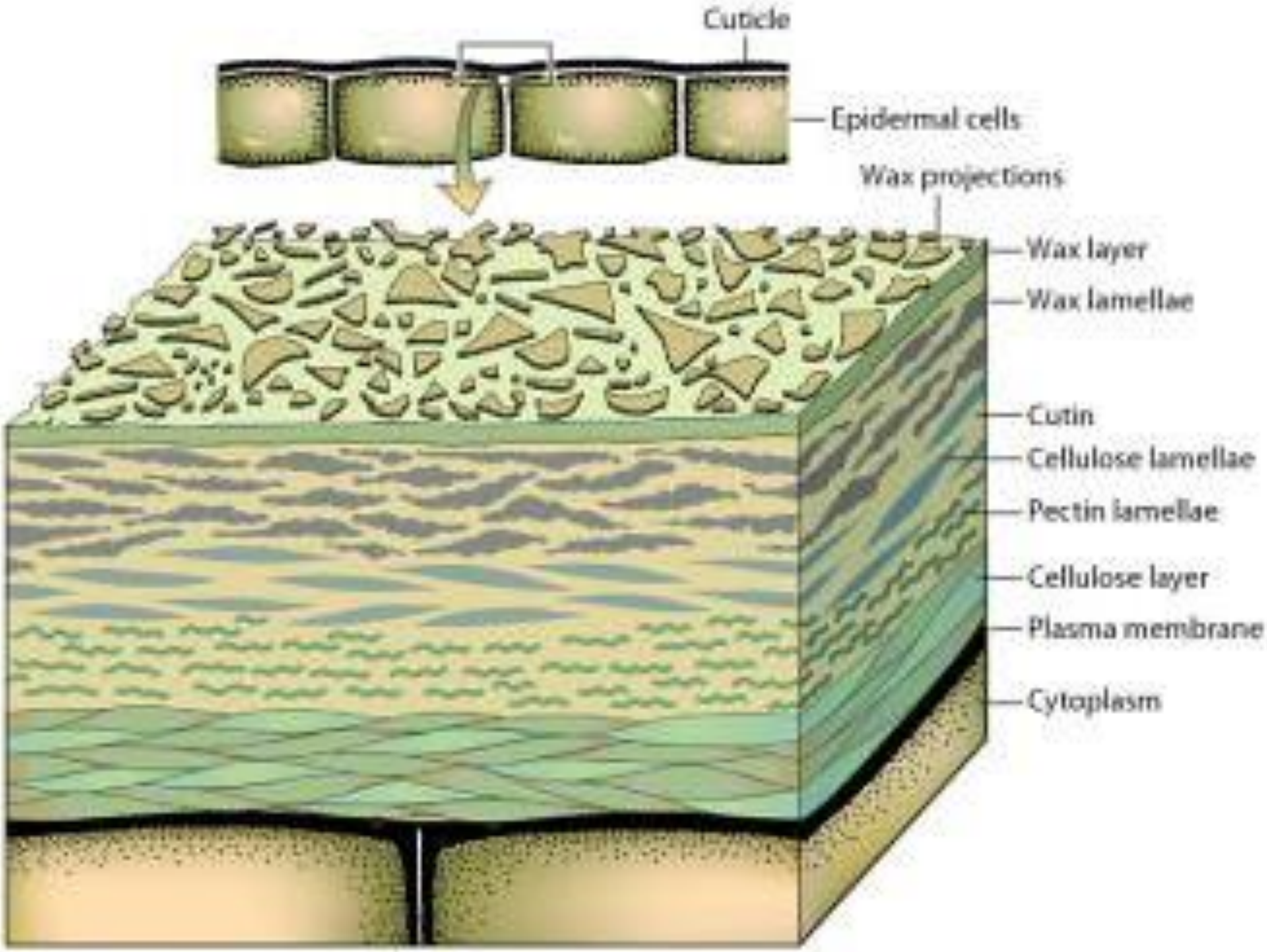
Unit 4

Host Pathogen Interaction

Virulence factors of pathogens: Enzymes


Cuticle
Epidermal cells
Wax projections
Wax layer
Wax lamellae
Cutin
Cellulose lamellae
Pectin lamellae
Cellulose layer
Plasma membrane
Cytoplasm

α cellulose (23%),

hemicellulose (only xyloglucan 21%),

lignin

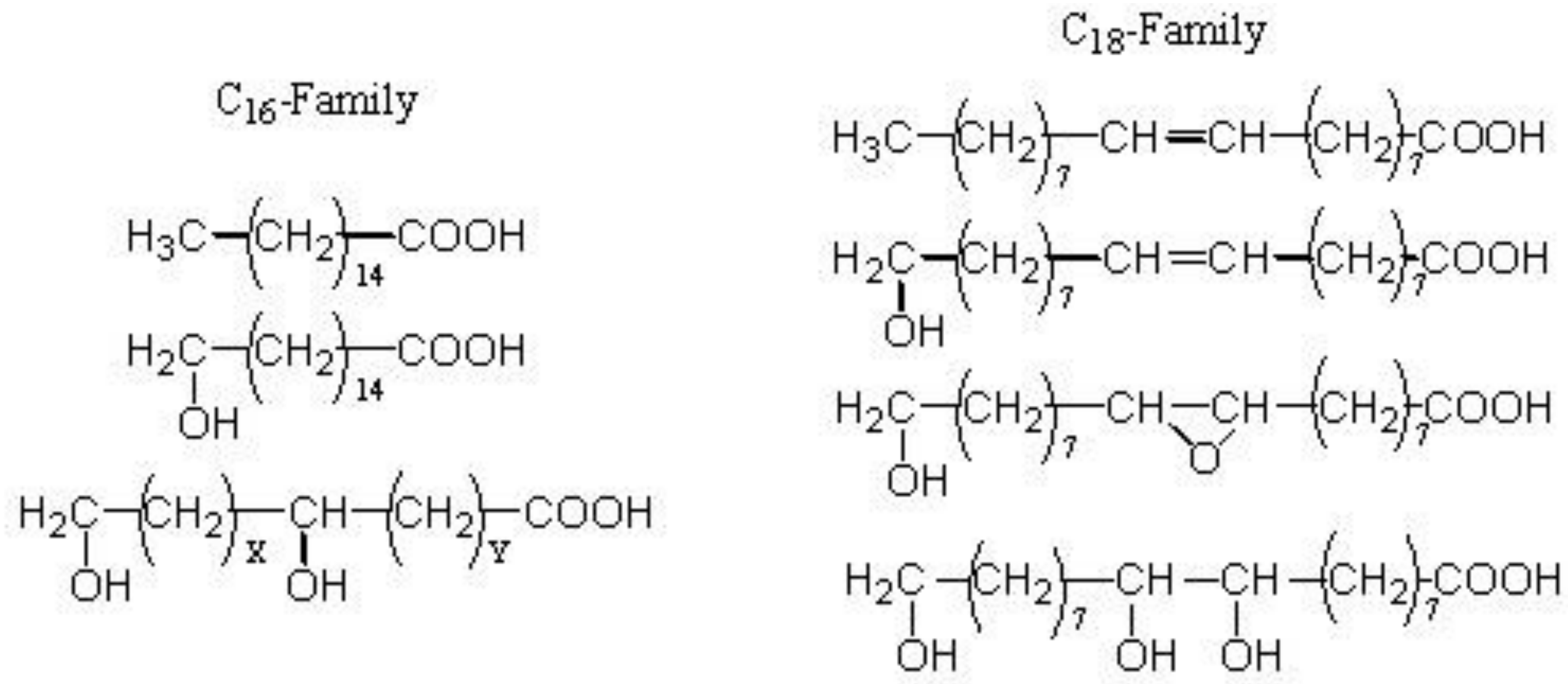
pectic substances (rhamnogalacturonan- 16%, arabinan- 10%, galactan- 8%, arabinogalactan- 2%, together- 36%),

glycoprotein (protein-9%, hydroxyproline arabinoside- 9%, together 19%)

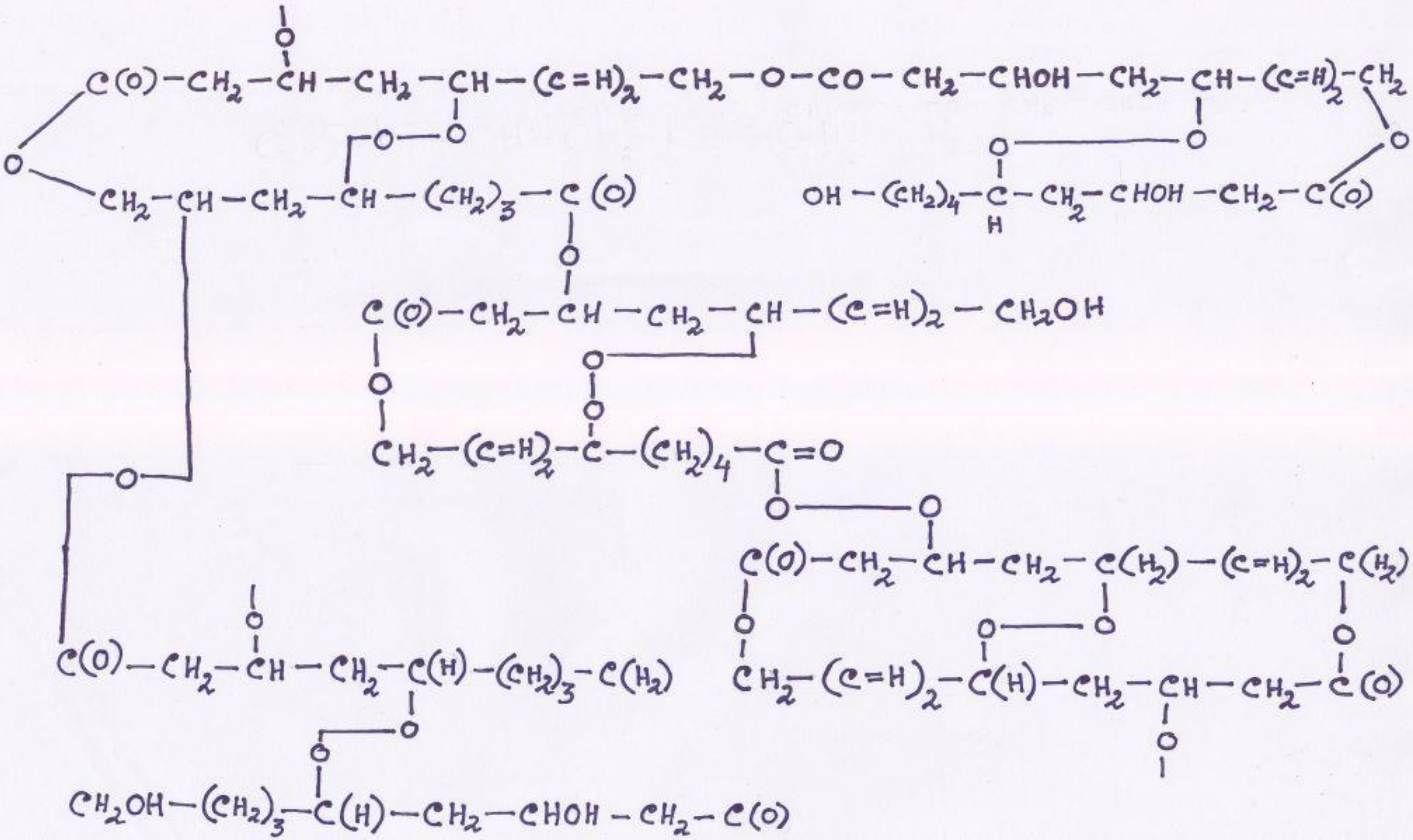
accounting for 99%of the cell wall.

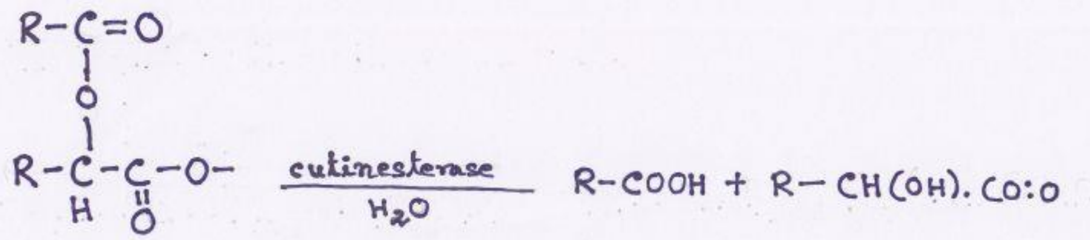
Cutin is a chemical polymer of hydroxylated octa- and hexadecanoic acids, mostly phloinolic acid, a trihydroxy monocarboxylic acid

Cutin is a lipid-like polyester found in the cuticle of higher plants that serves as an armor protecting plant cells from microbial attack

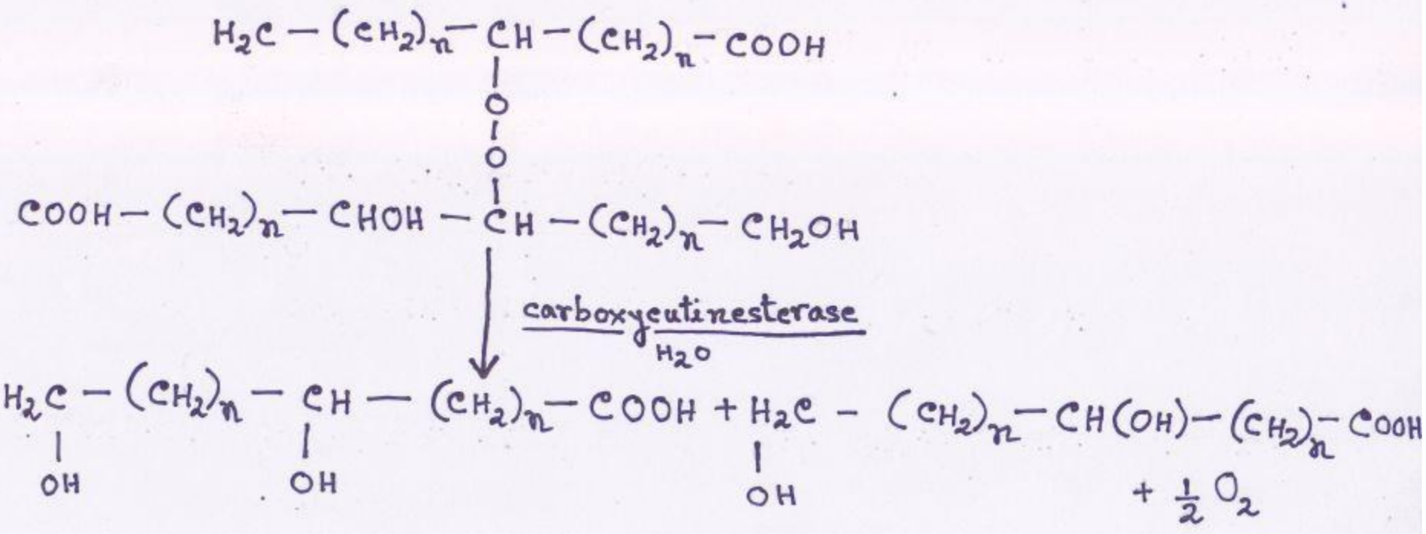


Major cutin monomer structures. X= 5, 6, 7, 8; Y= 5, 6, 7, 8, X+Y= 13. Minor monomers such as fatty acids, fatty alcohols, aldehydes, ketones, diacids as well as hydroxycinnamic acids can also be liberated.

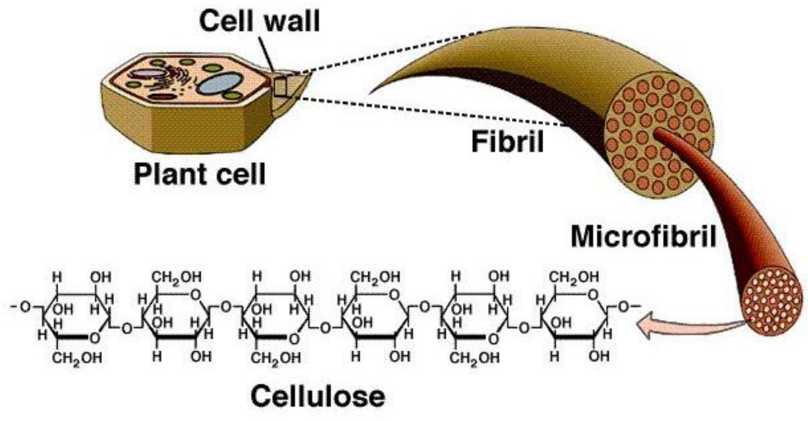


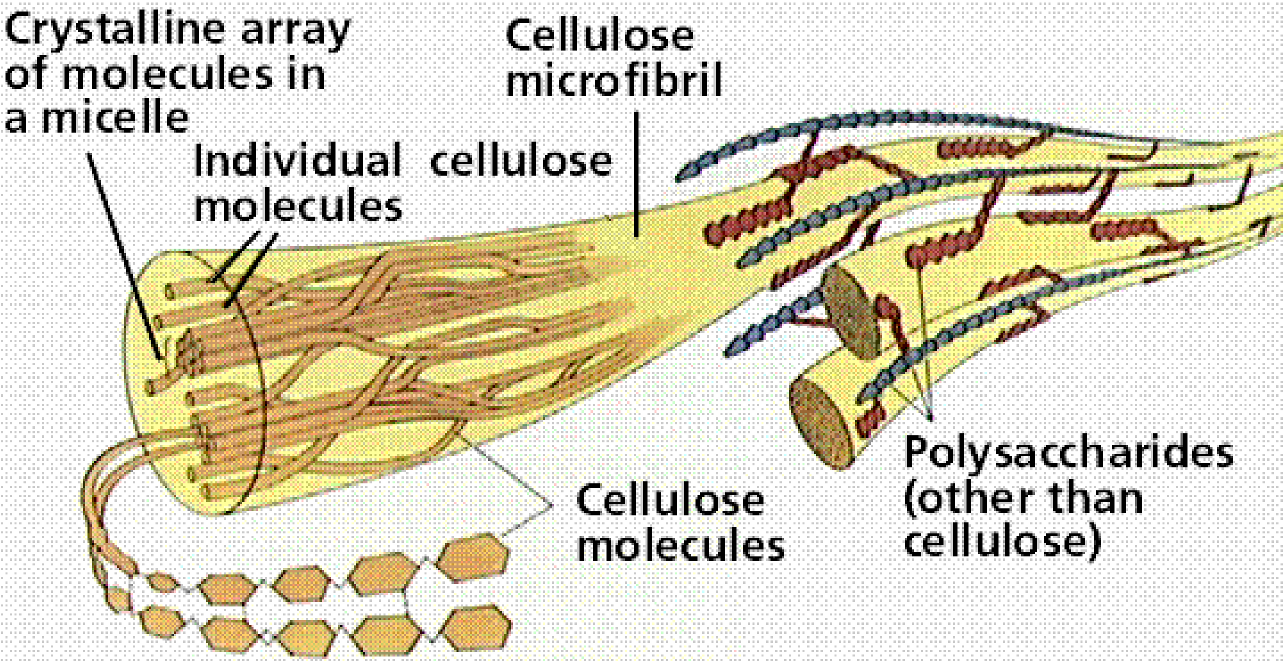


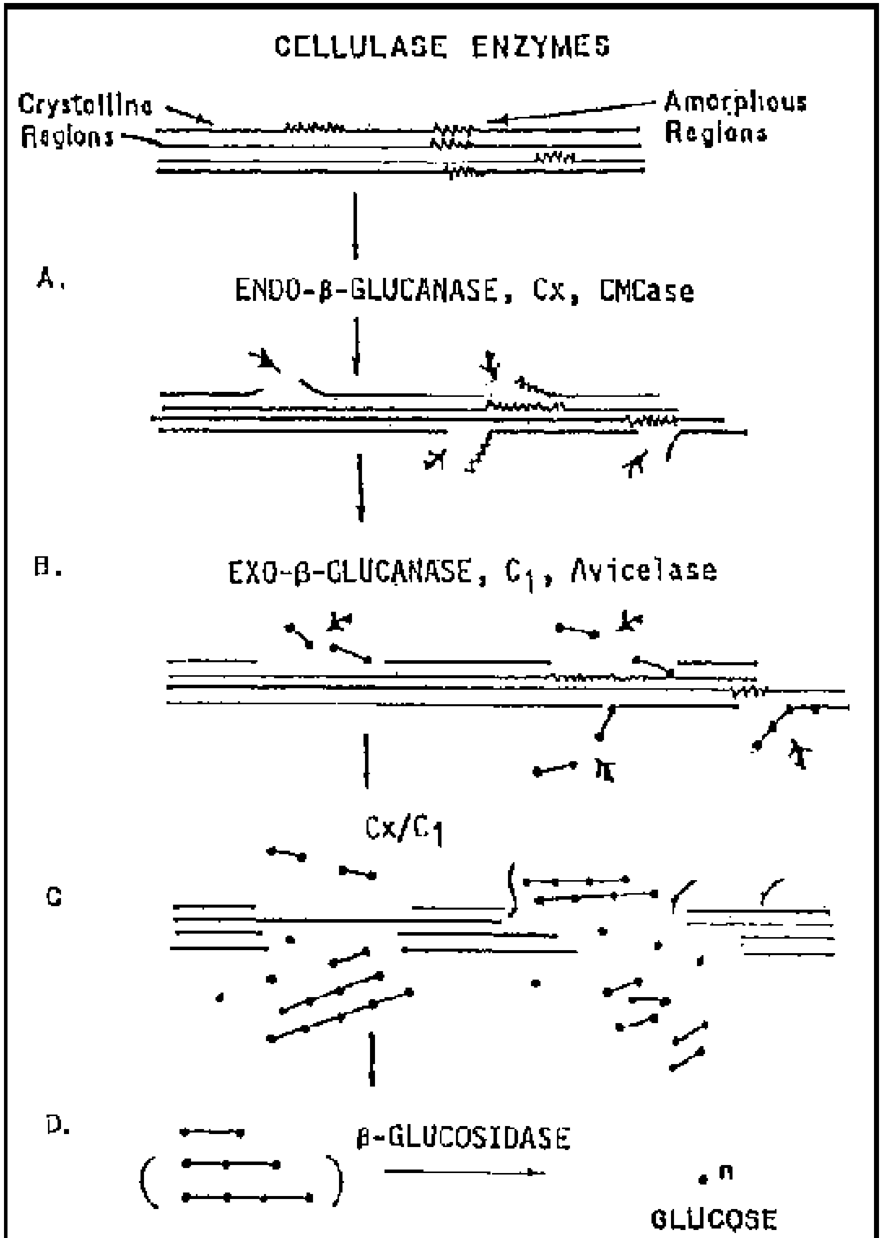
The cutinesterase catalyses the hydrolyses of ester bonds occurring between free hydroxyl and carboxyl groups of cutin acid



Arrangement of Fibrils, Microfibrils, and Cellulose in Cell Walls







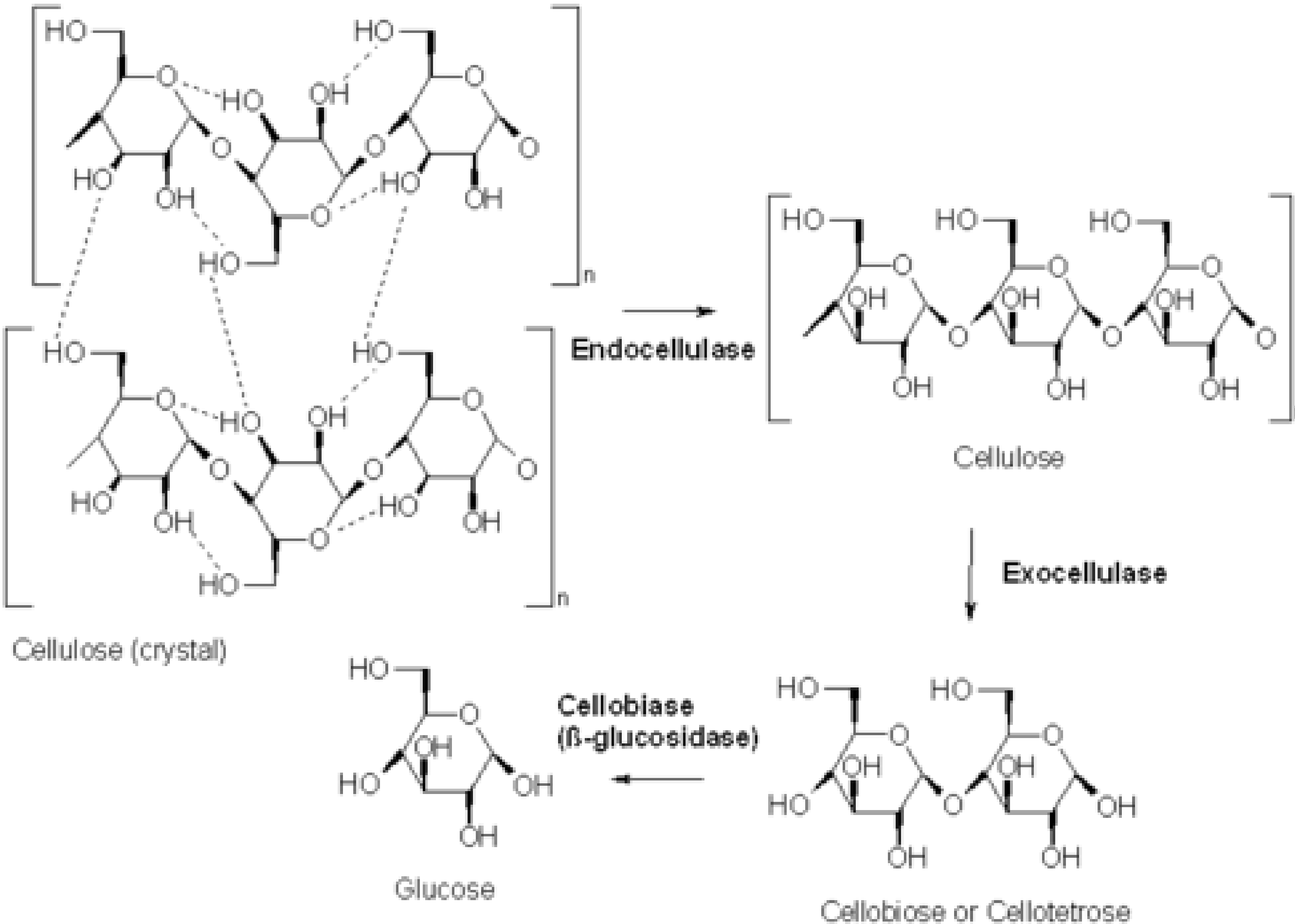
1. C1
2. β-(1 ⟶ 4) glucanase or Cx
3. β-glucosidase

Four general types of cellulases based on the type of reaction catalyzed:

Endocellulase (EC 3.2.1.4) randomly cleaves internal bonds at amorphous sites that create new chain ends.

Exocellulase (EC 3.2.1.91) cleaves two to four units from the ends of the exposed chains produced by endocellulase, resulting in the tetrasaccharides or disaccharides, such as cellobiose. There are two main types of exocellulases [or cellobiohydrolases (CBH)] - CBHI works processively from the reducing end, and CBHII works processively from the nonreducing end of cellulose.

Cellobiase (EC 3.2.1.21) or beta-glucosidase hydrolyses the exocellulase product into individual monosaccharides.


Cellulose (crystal)
Endocellulase
Cellulose
Exocellulase
Cellobiose or Cellotetrose
Cellobiase
(ß-glucosidase)
Glucose

Introduction…

Hemicellulose

- Hemicellulose makes up 25-30 % of total wood dry weight
- Present along with cellulose in almost all plant cell walls

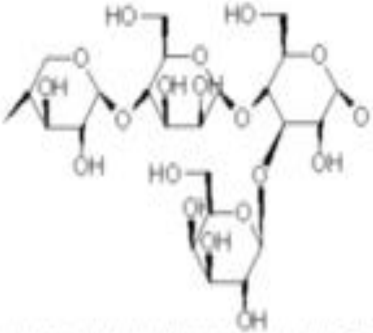
Hemicelluloses are of two types-

(a) **Homoglycans**- These contain only a single monosaccharide type. Typical homoglycans are-xylan,mannan or galactan

(b) **Heteroglycans**- These contain more than one kind of monosaccharide or uronic acid.

Hemicellulose and Role of Microorganisms in its Degradation

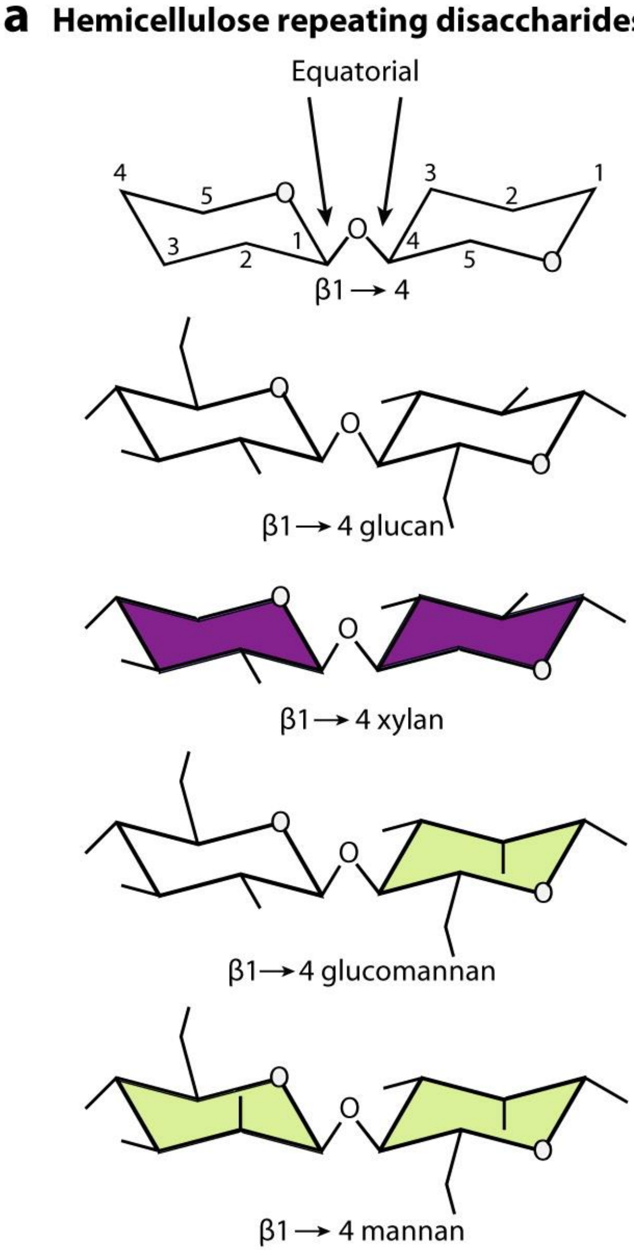
- Monomeric units of hemicellulose are D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, 4-O-methyl-glucuronic, D-galacturonic and D-glucuronic acid
- Predominate linkage > β-1,4 glycosidic bond. Also, β-1,3 glycosidic bonds are found at some places
- Polysaccharides > glucose, xylose, mannose, galactose, rhamnose, and arabinose
- Present in all plant cell walls with cellulose
- Less resistant against biodegradation
- Easily hydrolyzed by dilute acid or base as well as a large group of hemicellulase enzymes



- Xylose - ß(1,4) - Mannose - ß(1,4) - Glucose - - alpha(1,3) - Galactose

Hemicellulose

Hemicelluloses are polysaccharides in plant cell walls that have β-(1→4)-linked backbones with an equatorial configuration. Hemicelluloses include xyloglucans, xylans, mannans and glucomannans, and β-(1→3,1→4)-glucans.



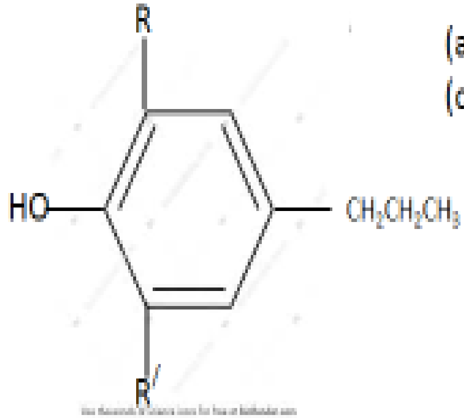
Enzymes Concerned in Hemicellulose Breakdown

Endo enzyme Xylanase- yields xylobiose (the disaccharide) or oligosaccharides
Exo enzymes Xylanase- yields xylose
Glycosidases Xylosidase produce xylose, Mannosidase generates mannose

Galactans may have β-(1 → 4) or β-(1 → 3) linkages between the galactose units and rupture of these linkages requires different enzymes both of which are termed Galactanases. The enzymes produce galactose, galactobiose and/or galactotriose, the product depending on how the substrate is cleaved.

LIGNIN DECOMPOSITION

The lignin molecule contains only three elements- carbon, hydrogen and oxygen but the structure is aromatic rather than being Carbohydrate. The basic unit in lignin is a phenyl-propane type of structure which may exist in three types



(a) R and R/ are H, (b) R is H and R/ is a methoxyl and (c) both R and R/ are methoxyl groups.

- Lignin is also an important component in plant cell wall
- A group of complex organic polymers of plant cell wall
- Held with cellulose, hemicellulose, and pectin
- Imparts mechanical strength to plant cell wall

Lignin is found in the secondary layers of the cell wall and also to some extent in the middle lamella.

Lignin Degrading Microorganisms

Fomes officinalis
Pleurotus ostreatus
Polyporus fumosus
Poria taxicola

Chemical Properties of Lignin

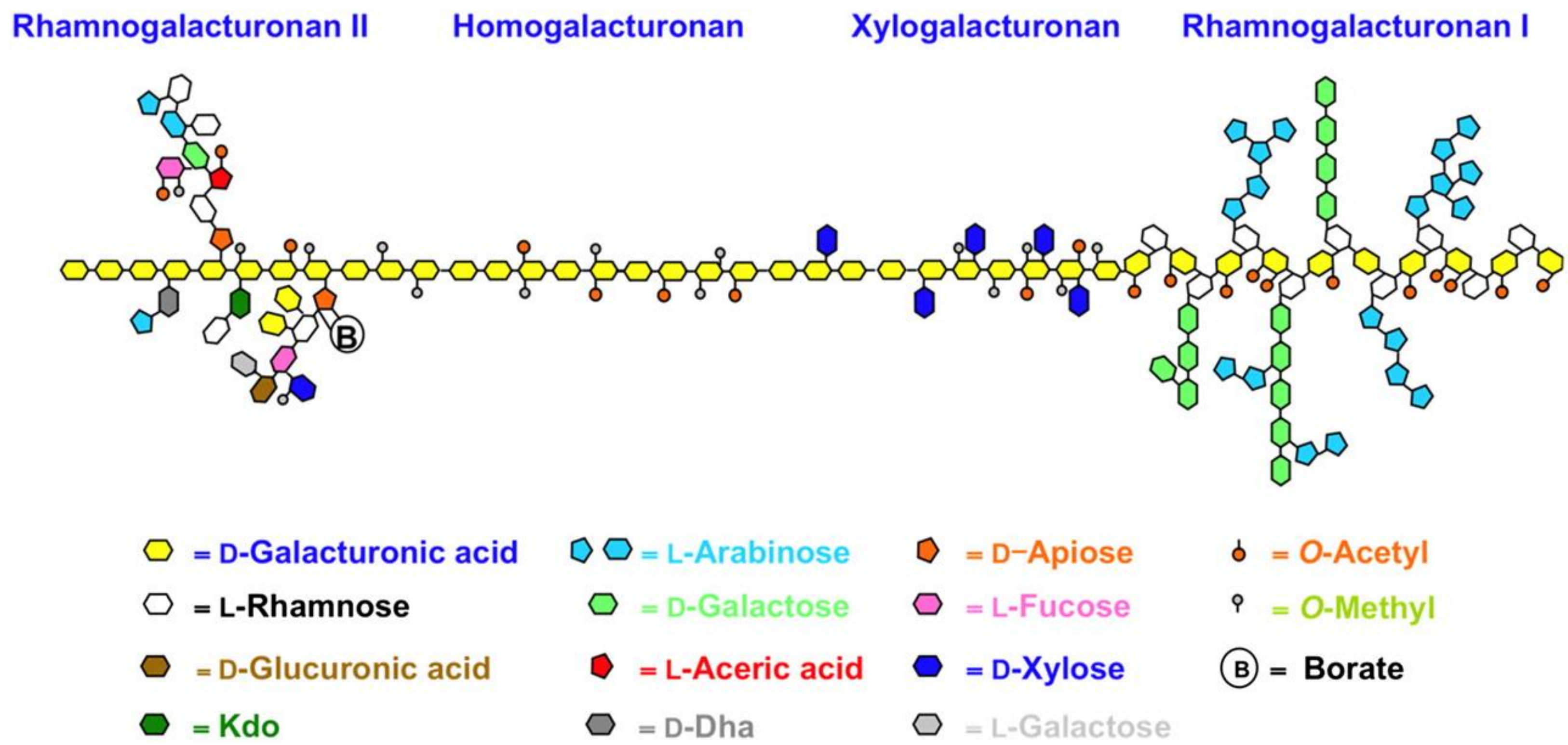
- It is resistant to acid hydrolysis
- Insoluble in hot water and neutral organic solvents
- Solubilized by alkali
- Solutions containing lignin give characteristic bands in the ultraviolet region of the light spectrum with an absorption maximum in the vicinity of 280 nm. This result show that lignin is a modified benzene derivative.

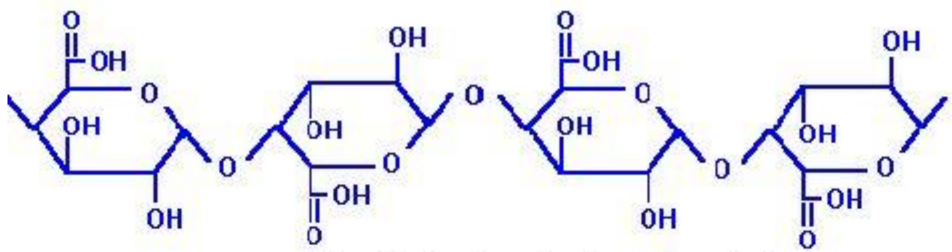
ENZYMES DEGRADING LIGNIN

- A single lignin degrading enzyme, a so called **LIGNASE** has yet to be described.
- Enzymes known as **Phenol Oxidases, Laccases and Peroxidases** have been postulated as being the responsible catalysts.
- **Phenol oxidases** oxidize aromatic compounds containing one or two hydroxyls.
- **Laccases** oxidize only those aromatic compounds with more than one hydroxyl group.
- **Peroxidases** can oxidize aromatic molecules but only in presence of hydrogen peroxide.
- These enzymes are said to be able to cleave ether linkages, remove side chains and cause loss of methoxyl groups.

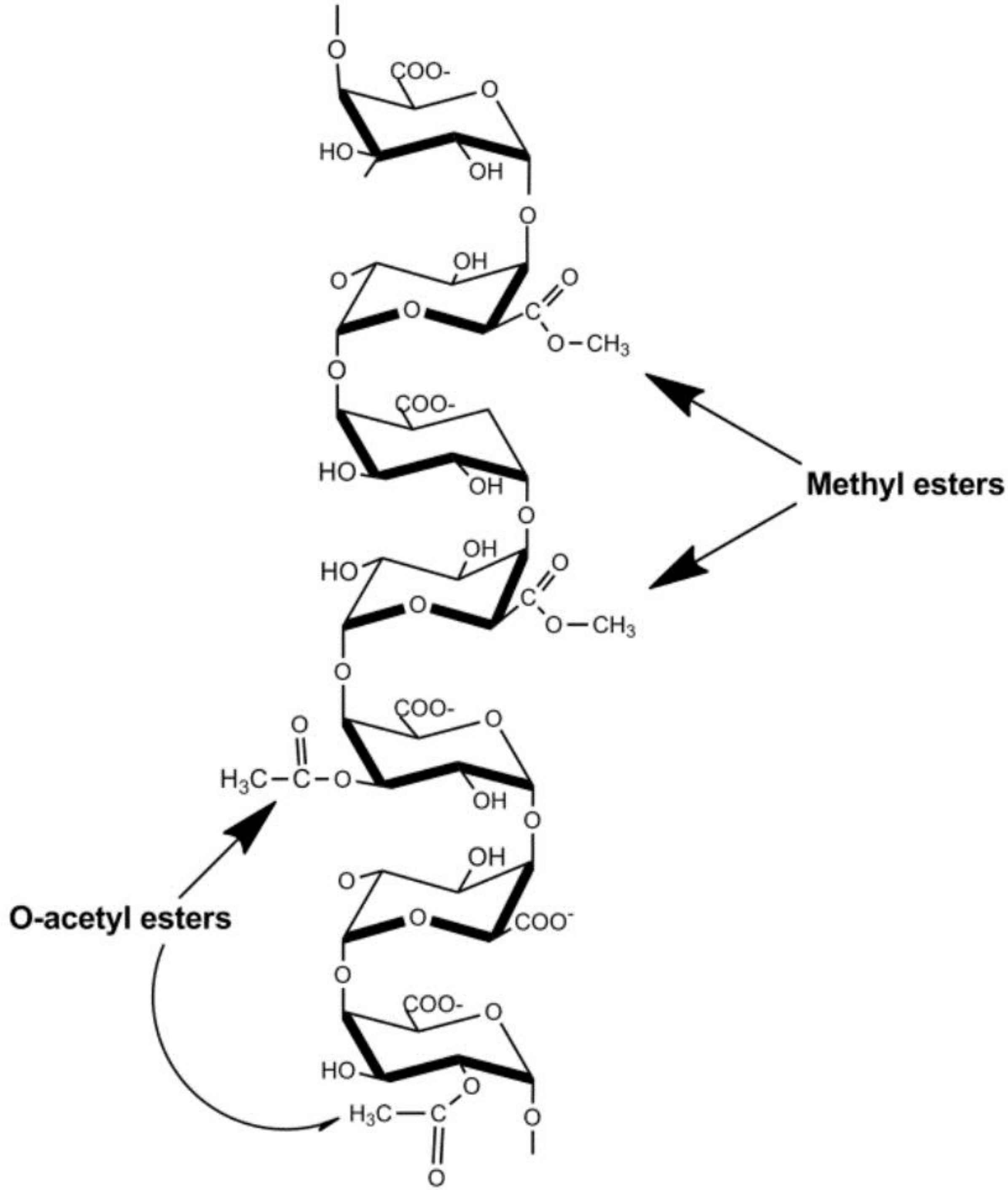
Pectic substances

a) **Protopectin**- a water insoluble constituent of the cell wall
b) **Pectin**- water soluble polymer of galacturonic acid containing many methyl ester linkages
c) **Pectinic acid-** colloidal pectic substances that are also galacturonic acid polymers with few methyl ester linkages
d) **Pectic acid-** water soluble polymer of galacturonic acid devoid of methyl ester linkages





Pectin (polygalacturonic acid)



Methyl esters
O-acetyl esters

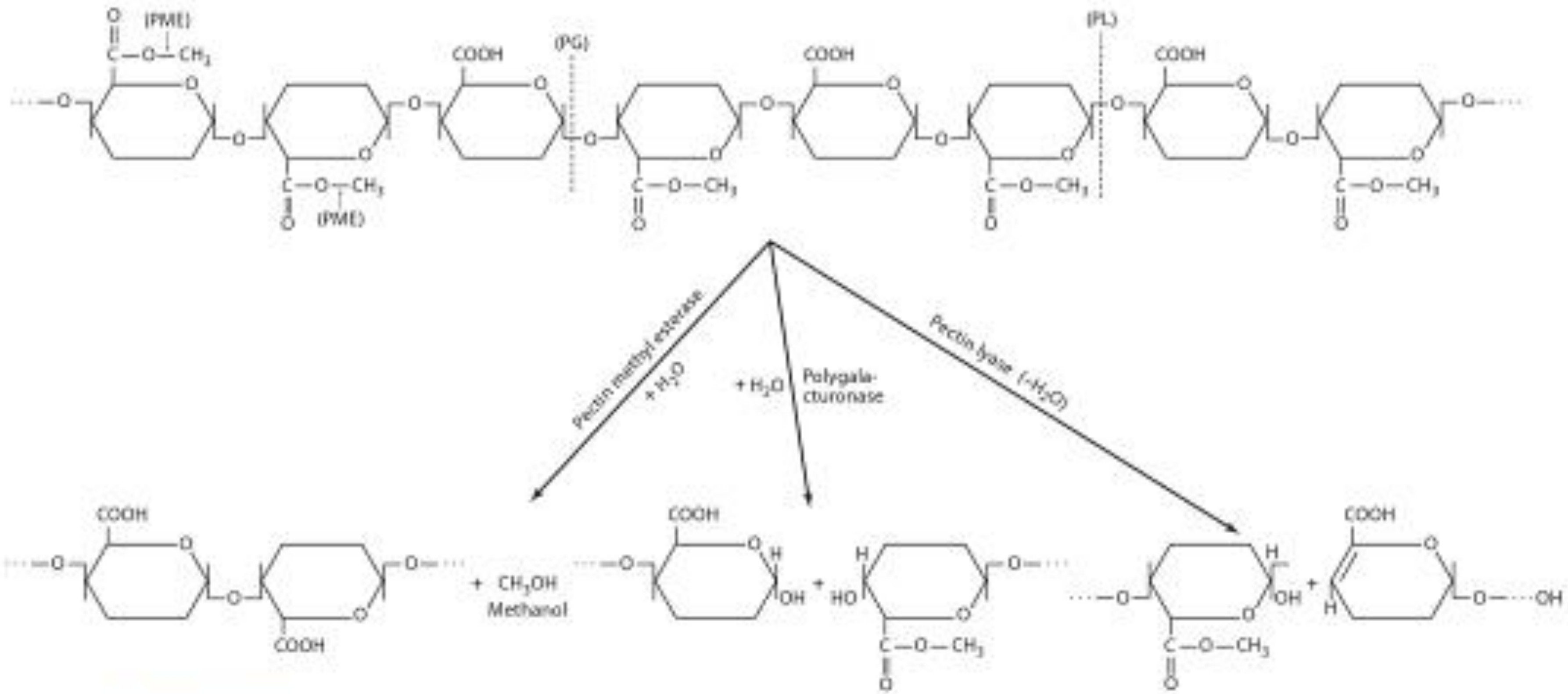


FIGURE 5-8 Degradation of a pectin chain by the three types of pectinases into modified and smaller molecules.

Pectinolytic enzymes

Demethylases (hydrolyse methylesters)

PME (pectinmethyl esterase)- this saponifies methyl ester groups of pectins and pectinic acids to give methyl alcohol and pectinic acids of lower methoxy content.

They remove the methyl groups from the methyl esters, thereby converting pectin or pectinic acids to pectic acid.

Hydrolysases (hydrolytically cleaves α-1,4 glycosidic bonds)

- **Polymethylgalacturonase (PMG)-** if pectin and less methylated pectinic acids.
- **Polygalacturonase (PG) or polygalacturonic acid transeliminase (PGTE)-** for pectic acids.

Transeliminases or lyases (cleave -1,4 glycosidic bonds by lytic mechanism)

- **pectinmethyl transeliminase (PTE)-** for pectin and less methylated pectinic acids.
- **Pectic acid transeliminase (PATE)-** for pectic acids.