Aalto University
School of Chemical Technology

Hemicellulose: structure, characterization, dissolution, modification

CHEM-E2140
Cellulose-based fibres

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22nd September 2020

Learning objectives

After this lecture you will be able to:

- Identify a hemicellulose
- Distinguish the main hemicellulose types from each other
- Tell which hemicellulose is typical to a certain plant or plant species
- Gain knowledge on the main properties of hemicellulose
- Identify the most common ways to chemically modify hemicellulose

Outline

(1) Definition of hemicellulose

(2) Types and structures of different hemicelluloses:
- Xylan
- Mannan
- Xyloglucan

(3) Occurrence and function of hemicelluloses

(4) Characterization of hemicelluloses

(5) Dissolution of different hemicelluloses

(6) Solution properties

(7) Chemical modification of hemicelluloses

Definition of hemicellulose

- A broad, inexplicit definition of hemicellulose (half-cellulose) refers to plant polysaccharides that are not cellulose

- They are all short chain polysaccharides (DP of ~50-200)

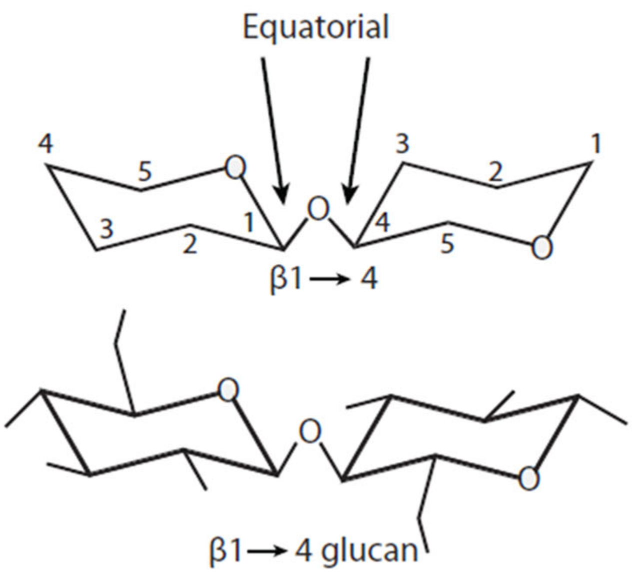
- The term hemicellulose was introduced long before the structures were properly understood

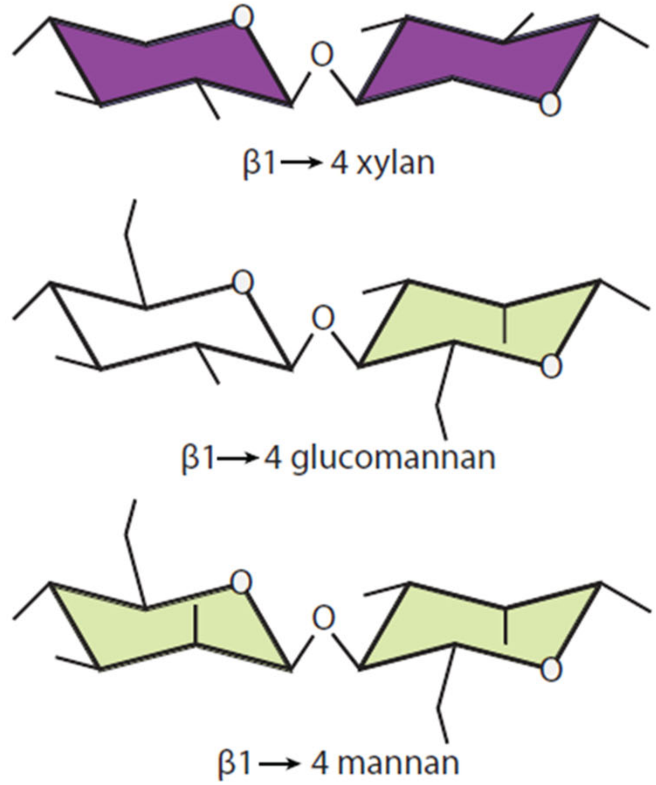
$\rightarrow$ A lot of controversy on the real meaning of the term

Most widely accepted explicit definition:
Hemicelluloses are characterized as a group of cell wall polysaccharides that are neither cellulose nor pectin and have a β-(1$\rightarrow$4)-linked backbones of glucose, mannose or xylose linked in equatorial configuration.

Definition of hemicellulose

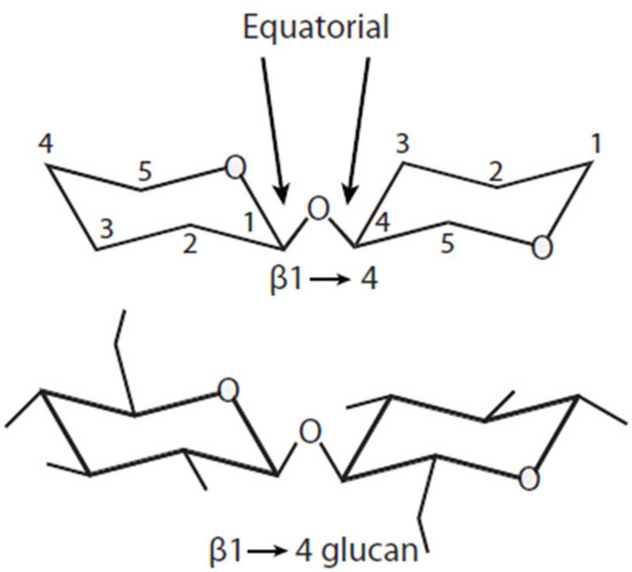
Repeating disaccharide
units of hemicellulose



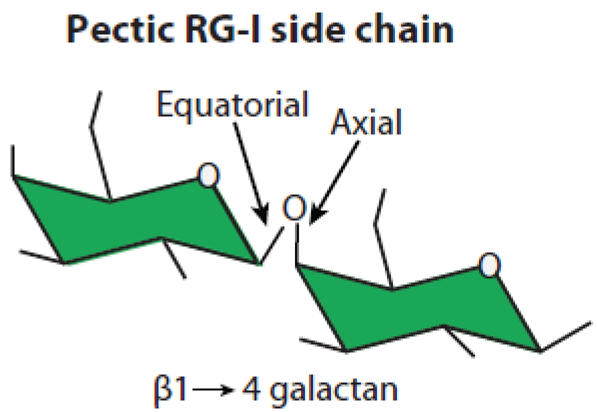


Definition of hemicellulose

Repeating disaccharide units of hemicellulose



NOTE: Pectin with β-(1→4)-linked galactan with axial configuration at C4 is not included with hemicelluloses



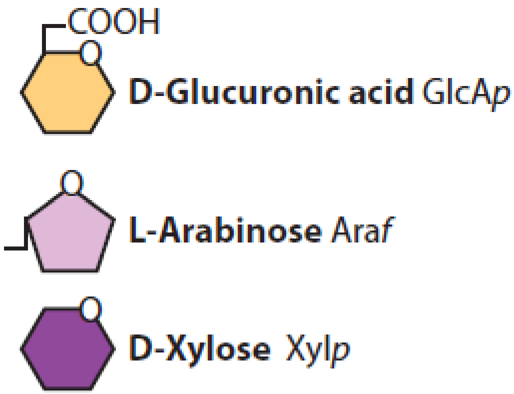
- Also glucans with β-(1→3)-linked backbone (e.g., callose) are not included

Xylan

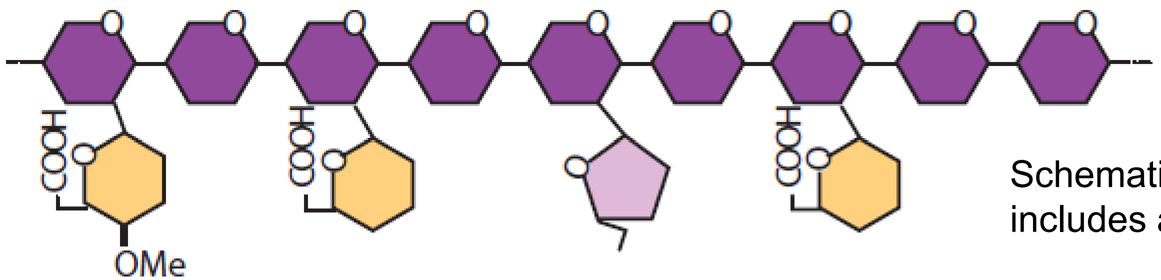
General issues on xylans

- Found in most plant cell walls (both terrestrial and algae): sometimes termed the second most abundant biopolymer
- Backbone consists exclusively of xylose
- Often partially acetylated

Xylan structures: glucoronoxylan



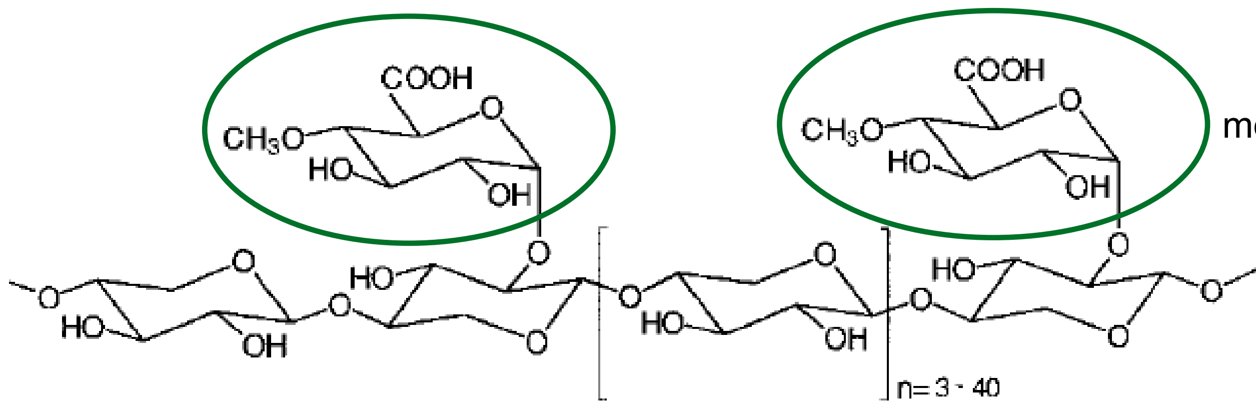
- The most common hemicellulose in the secondary walls of hardwoods
- Backbone consists exclusively of xylose



Schematic structure that includes also arabinose

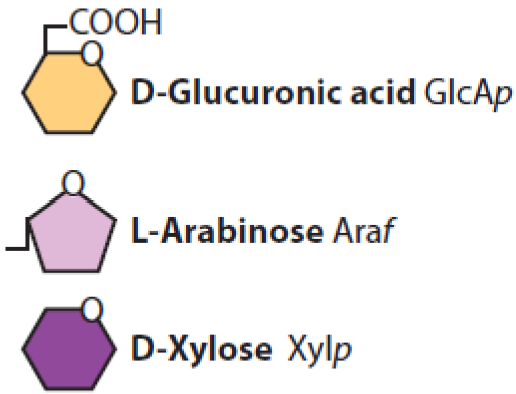
Xylan structures: glucoronoxylan

- Distinguishing side group: **2-linked 4-methyl glucuronic acid unit**
- Xylose : glucuronic acid ratios range from 4-16:1 in hardwoods

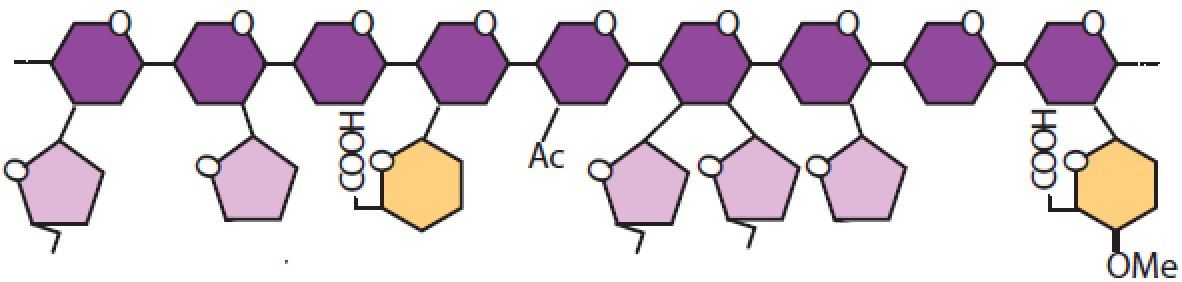


molecular structure

Xylan structures: arabinoglucoronoxylan



- Second most common hemicellulose in the secondary walls of softwoods
- Typical for the lignified tissues of grasses and annual plants

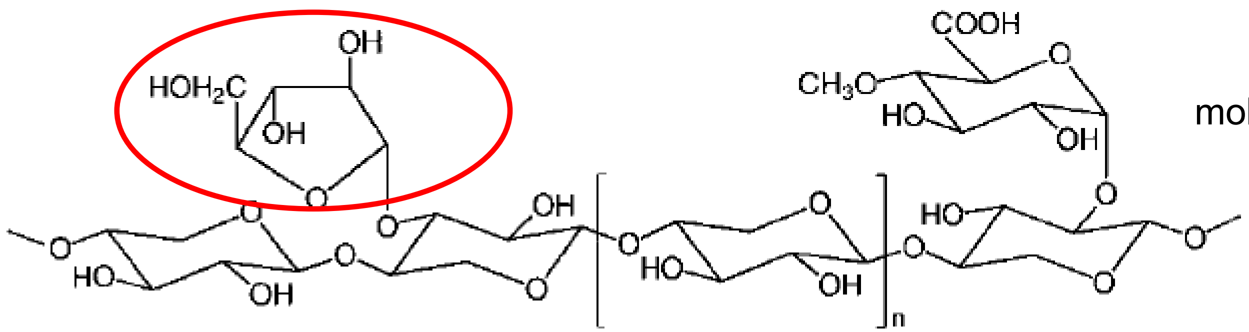


Schematic structure

Xylan structures: arabinoglucoronoxylan

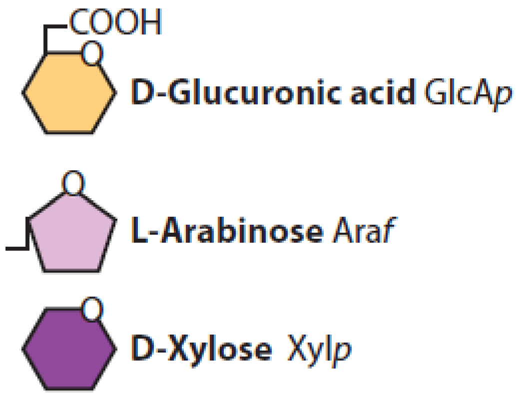
Distinguishing side groups:

- 2-linked 4-methyl α-D-glucuronic acid unit
- 3-linked α-L-arabinofuranosyl (arabinose)

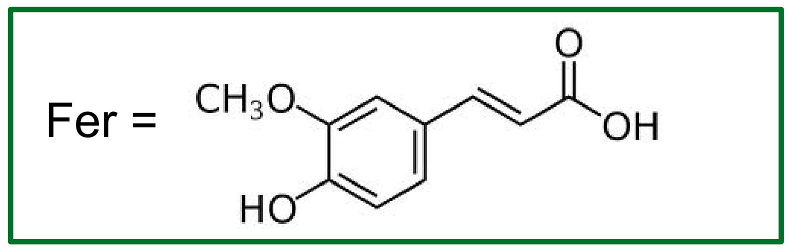


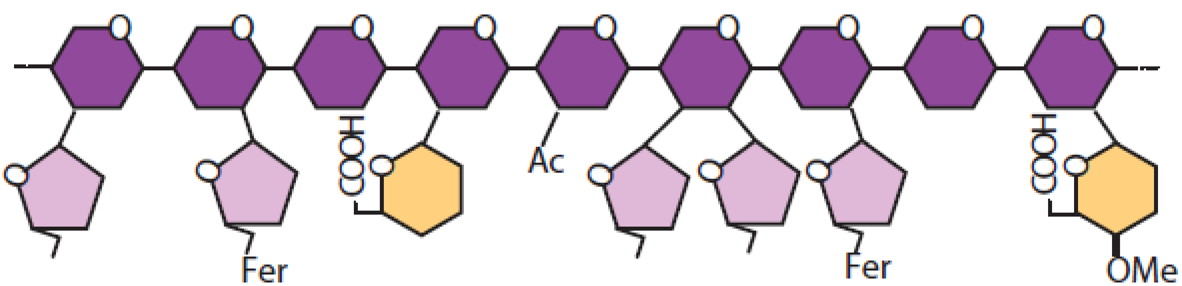
molecular structure

Xylan structures: arabinoglucoronoxylan



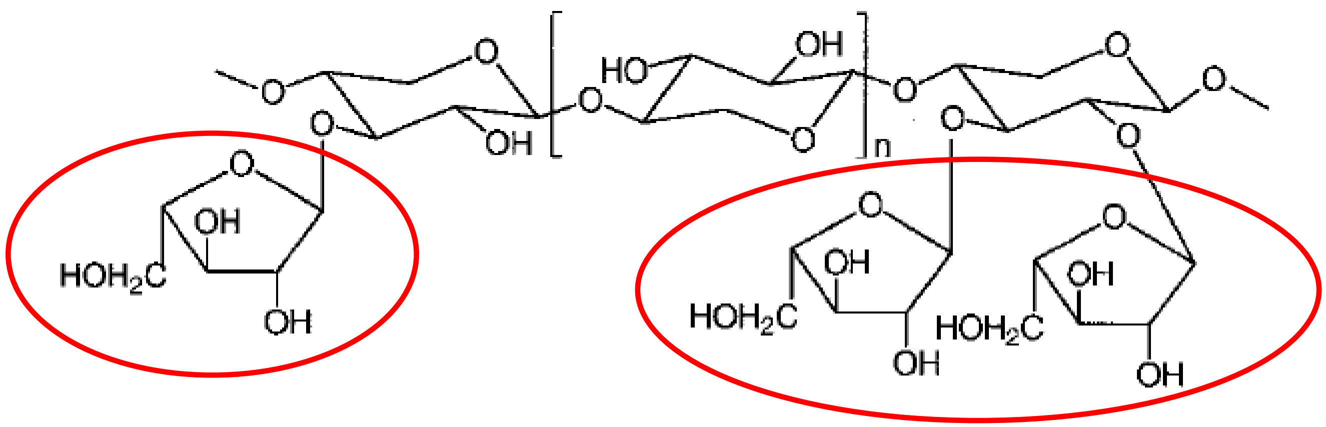
- In *grass xylan*, ferulic acid esters are linked to O5 position of some of the arabinose residues
- Ferulate esters enable cross-linking and enhance cell wall recalcitrance against enzymatic attack



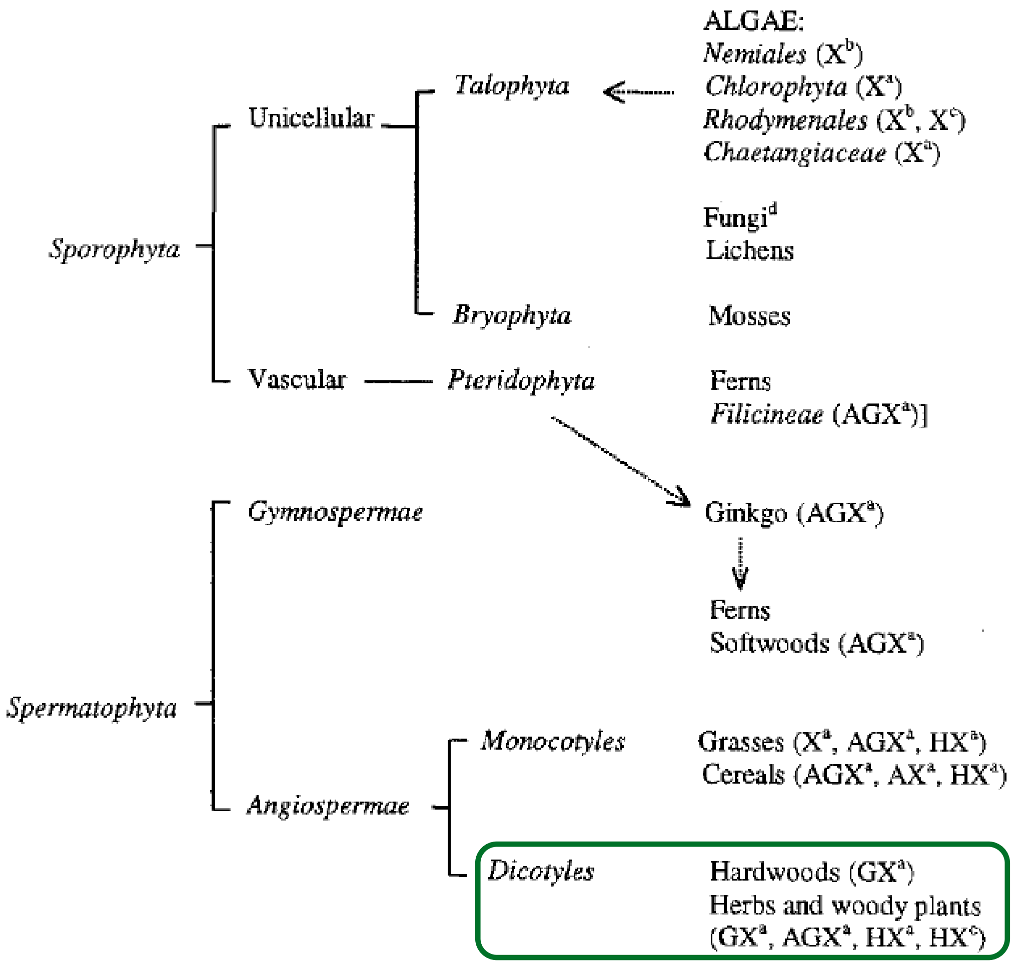


Xylan structures: arabinoxylan

- Uncharged xylan (without the methyl glucuronic acid side group)
- Common in cereal grains
- Distinguishing side group: **2- and/or 3-linked arabinose**



Evolutionary path of xylan



- Arrows indicate the direction of evolution

X – straight chain xylan
GX – glucuronoxylan
AGX – arabinoglucuronoxylan
HX – heteroxylan

The most developed species

Mannan

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General issues on mannans

- Found in nearly all plant cell walls
- Backbone may consist exclusively of mannan or of mannose and glucose in a nonrepeating pattern
- Often partially acetylated

General issues on mannans

- Mannans have been highly abundant in early land plants
- They are still abundant in mosses and lycophytes

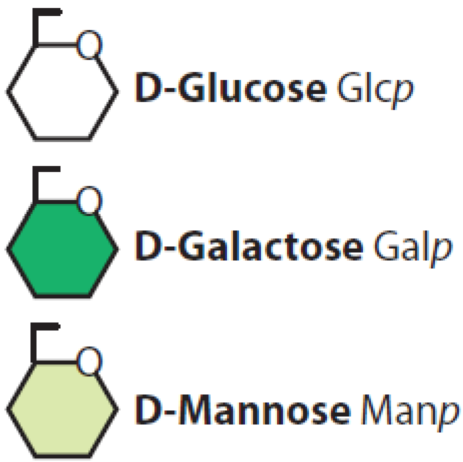
Lycophytes

Mosses

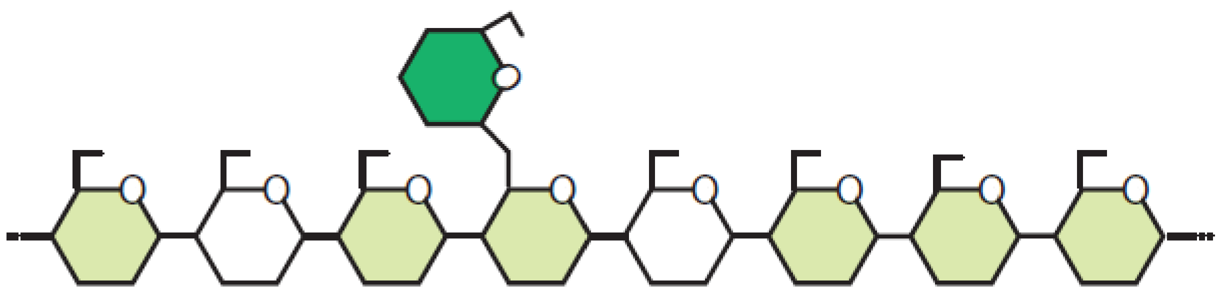
General issues on mannans

- In conifers, galactoglucomannan is the major hemicellulose
- In hardwood, glucomannans are much less abundant


Structure of galactoglucomannan

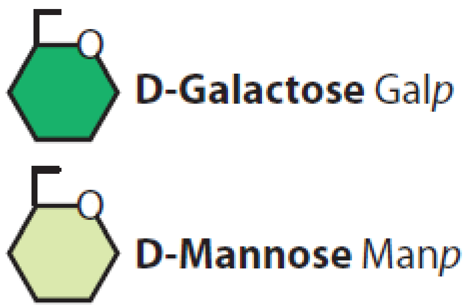


- The most common hemicellulose in the secondary walls of softwoods
- Mannose : glucose : galactose ratio in softwoods is 3.5-4.5 : 1 : 0.5-1.1

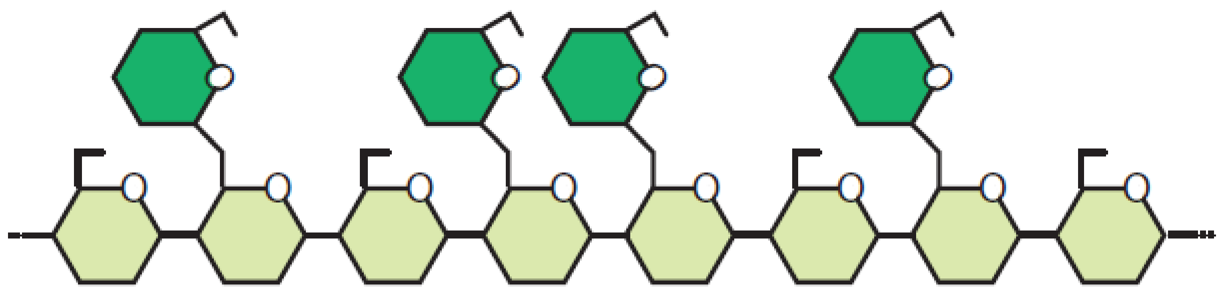


Schematic structure

Structure of galactomannan



- Present in, e.g., Fabacae seeds and some gums
- Exclusive mannose backbone



Schematic structure

Various mannan structures (I)

(a) Ivory nut mannan Pure mannan

→4)-β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-β-D-Man*p*-(1→

(b) Tara gum (galactomannan) Without glucose

→4)-β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-β-D-Man*p*-(1→

α-D-Gal*p*-(1→6)-

α-D-Gal*p*-(1→6)-

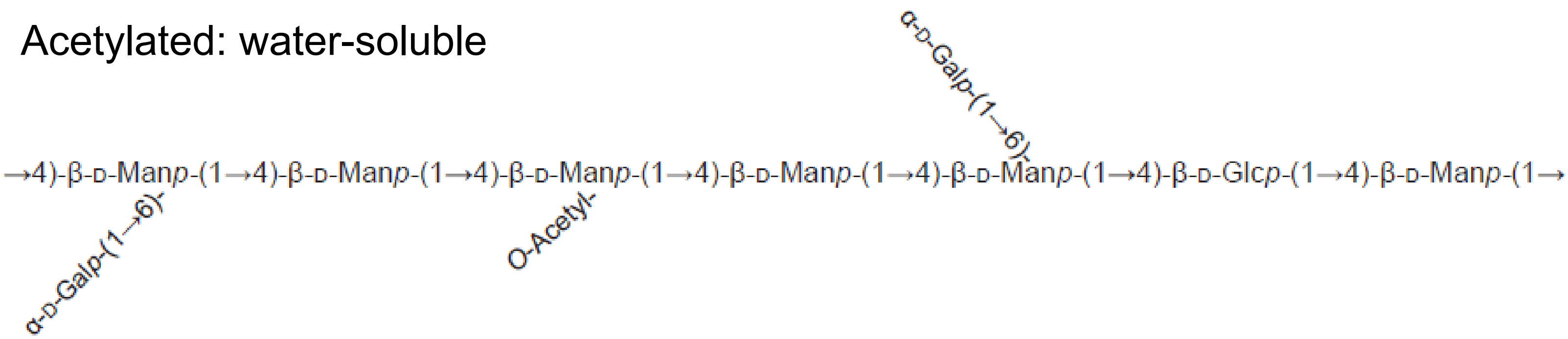
(c) Konjac gum (glucomannan) Without galactose, occurs in secondary walls of hardwoods

→4)-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→

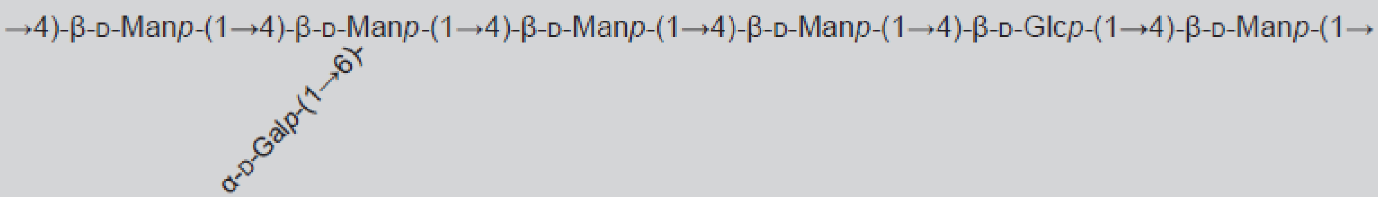
Various mannan structures (II)

Typical softwood galactoglucomannan structures

Acetylated: water-soluble



Non-acetylated: insoluble in water



Xyloglucan

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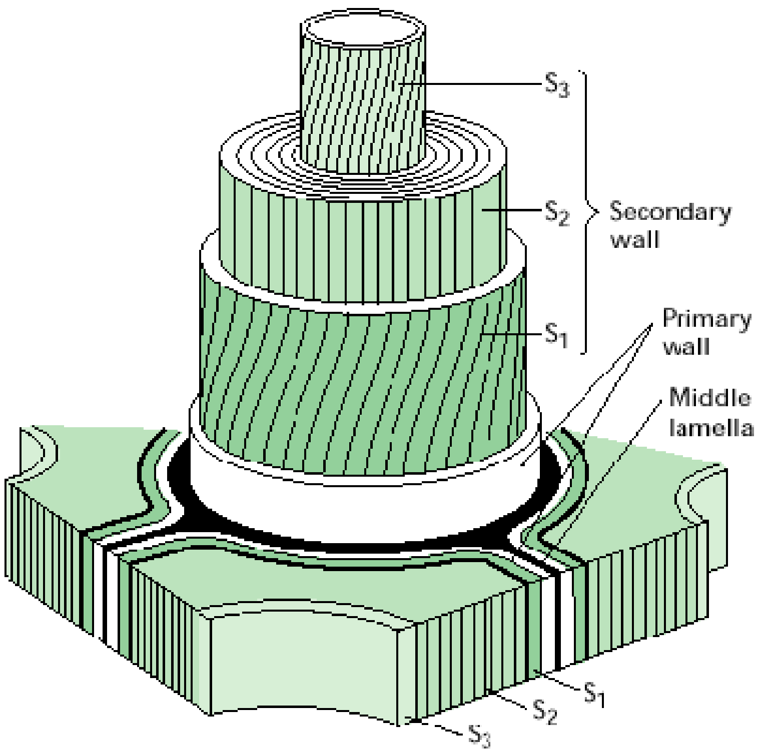
General issues on xyloglucans



- Xyloglucans are present in the *primary walls* of all land plants
- Xyloglucans are scarcely present in secondary walls

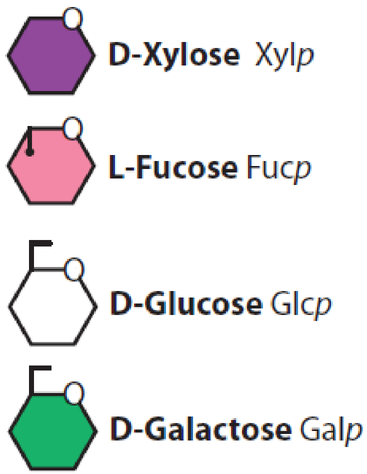
NOTE: Many plants do not possess a secondary wall.

Wood cells possess a very thick secondary wall.

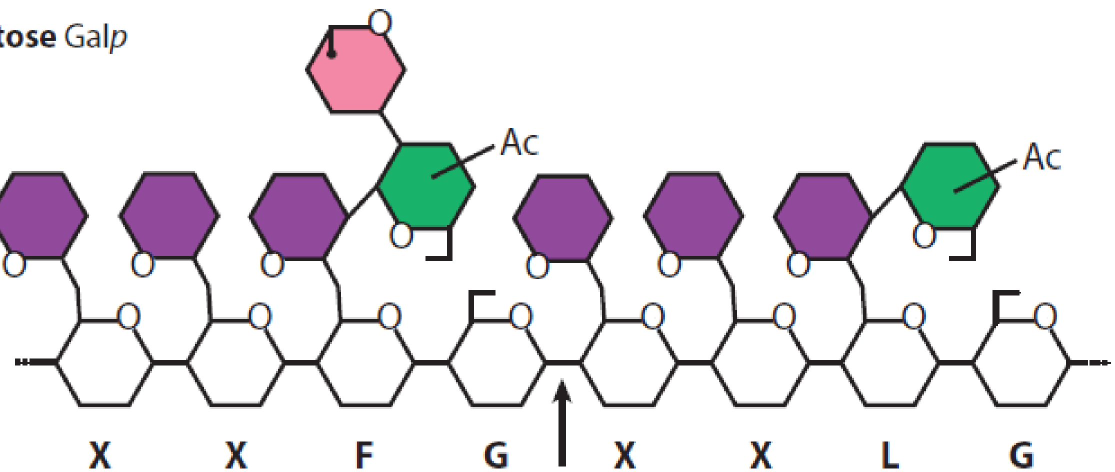
General issues on xyloglucans

- Backbone consists of glucose with xylose linked to most glucose groups by a 6→1 bond
- Other branching saccharides are galactose and fucose

Structure of xyloglucan



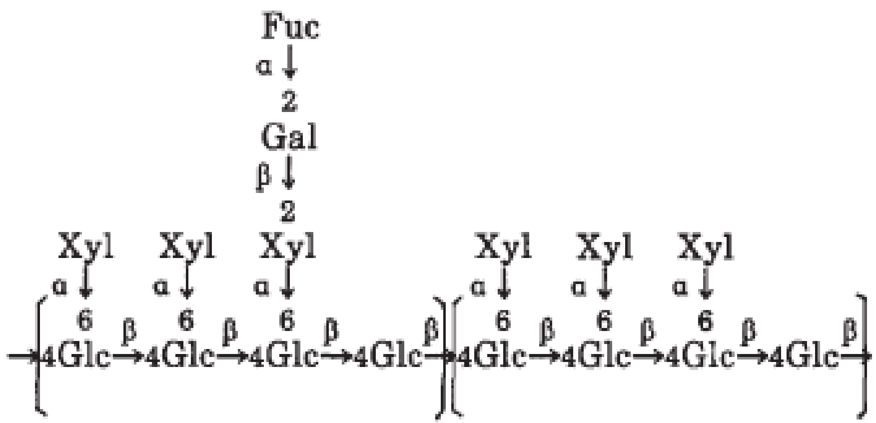
- This structure is typical for the primary wall of eudicotyledons
- It is sometimes referred to as fucogalactoxyloglucan



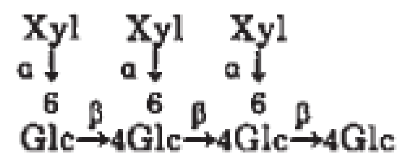
Schematic structure

Xyloglucan nomenclature

- Unambiguous nomenclature exists for xyloglucan oligosaccharides



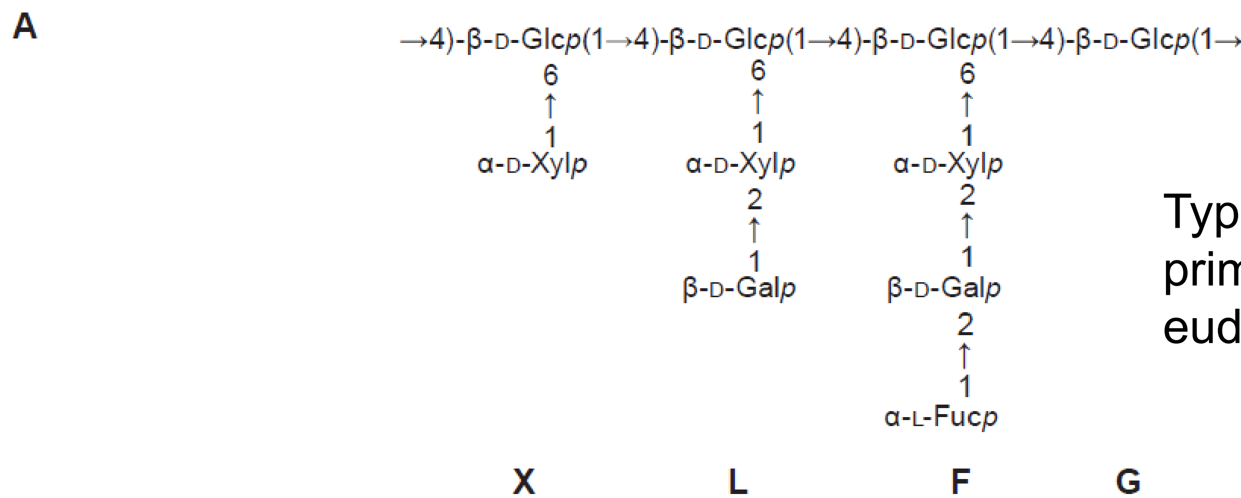
-XXFG-XXXG-



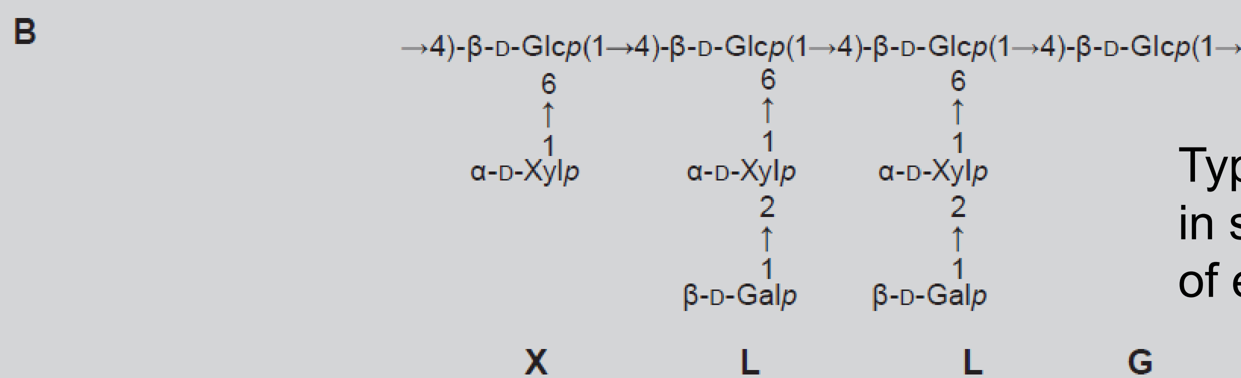
XXXG

Fry et al. *Physiol. Plant* **1993**, *89*, 1.

Structure of xyloglucan



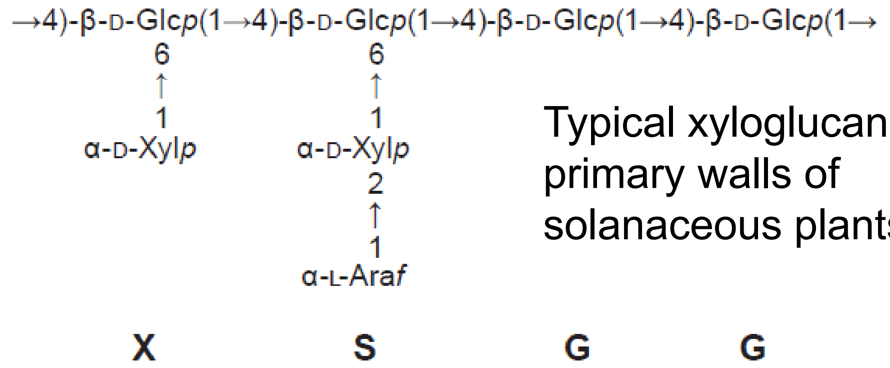
Typical xyloglucan in primary walls of eudicotyledons



Typical xyloglucan in secondary walls of eudicotyledons

Structure of xyloglucan

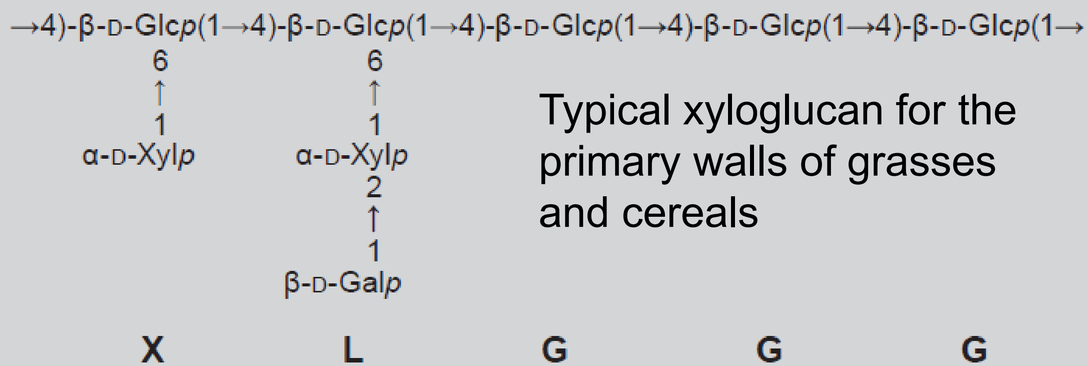
A



Typical xyloglucan for the primary walls of solanaceous plants

NOTE: no fucose units

B



Typical xyloglucan for the primary walls of grasses and cereals

Summary on basic issues

- Hemicelluloses are short chained, polysaccharides made of different monosaccharides, often with side-groups
- Hemicelluloses coexist with cellulose and other polymers in plant cell walls
- Main classes of hemicellulose are xylans, mannans, and xyloglucans

Function and occurence of hemicellulose

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Why does hemicellulose exist?

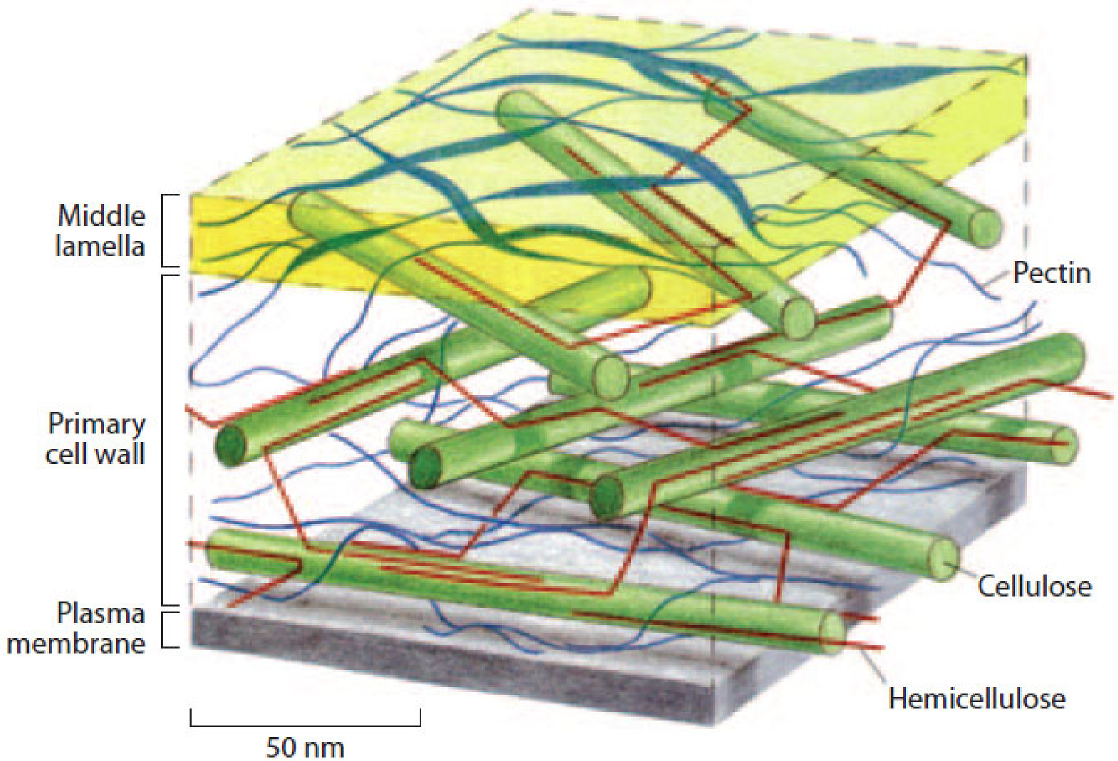
General explanation:

- The primary role of hemicelluloses is to interact with other polymers to ensure the proper physical properties of the cell wall

NOTE: Hemicelluloses are not indispensable – sometimes the cell wall contains just a few percent of hemicellulose, which is not enough to interact intimately with, e.g., cellulose microfibrils to a significant extent

Hemicellulose in primary wall

- Hemicelluloses are often seen as tethering molecules that cross link cellulose microfibrils



- Xyloglucans are proposed to be intimately hydrogen bonded to cellulose and covalently bonded to pectins

→ Xyloglucans assist cellulose in load bearing

→ Cellulose-xyloglucan network constrains cell enlargening

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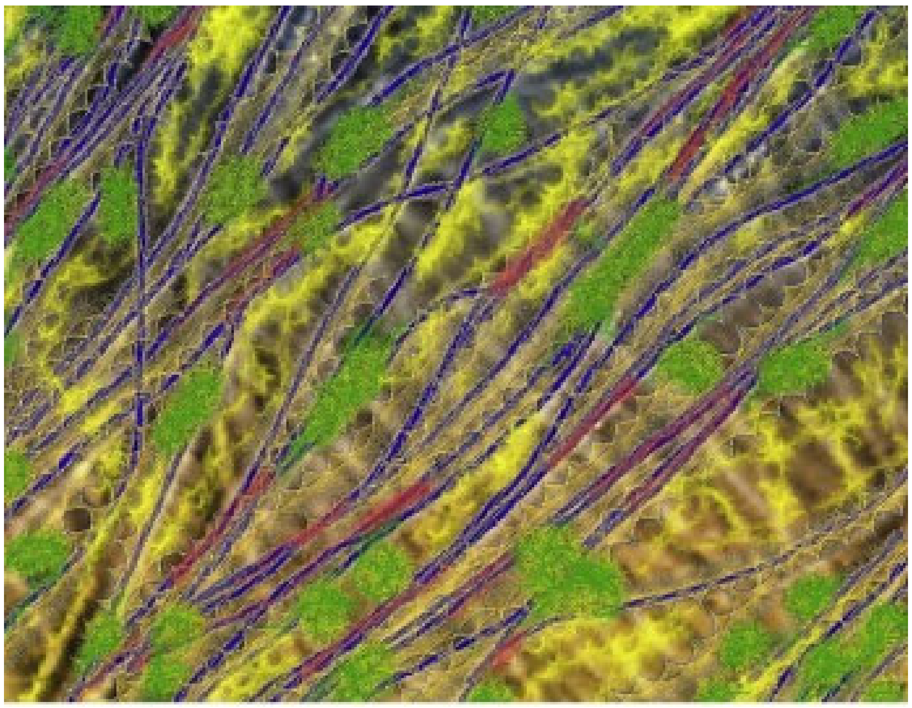
McCann and Roberts *Architecture of the primary cell wall*, In: *The cytoskeletal basis of plant growth and form*, Academic Press: New York, 1991.

Biomechanical hotspots in primary wall

New theory:

- Coiled xyloglucan (rather than extended) forms *biomechanical hotspots* in the primary wall, strengthening cellulose microfibril joints



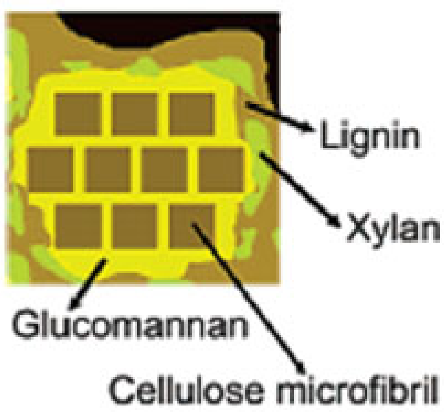
Biomechanical hotspots (red)
Cellulose microfibrils (blue)
Pectins (yellow)
Xyloglucan (green)

- The role of pectin is unclear

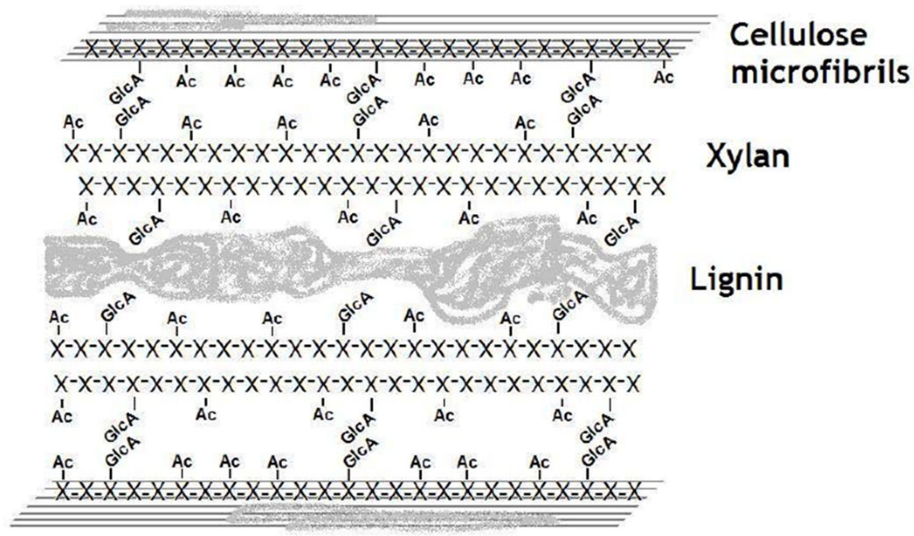
D. Cosgrove, Curr. Opin. Plant Biol. **2014**, *22*, 122.

Hemicellulose in secondary wall

Proposition for radial cross section



Proposition for longitudinal cross section



The role and arrangement of hemicellulose in the secondary wall is vaguer than in the primary wall.

Fahlén and Salmén *Biomacromolecules* **2005**, *6*, 433.
Dammström et al. *Bioresources* **2009**, *4*, 3.

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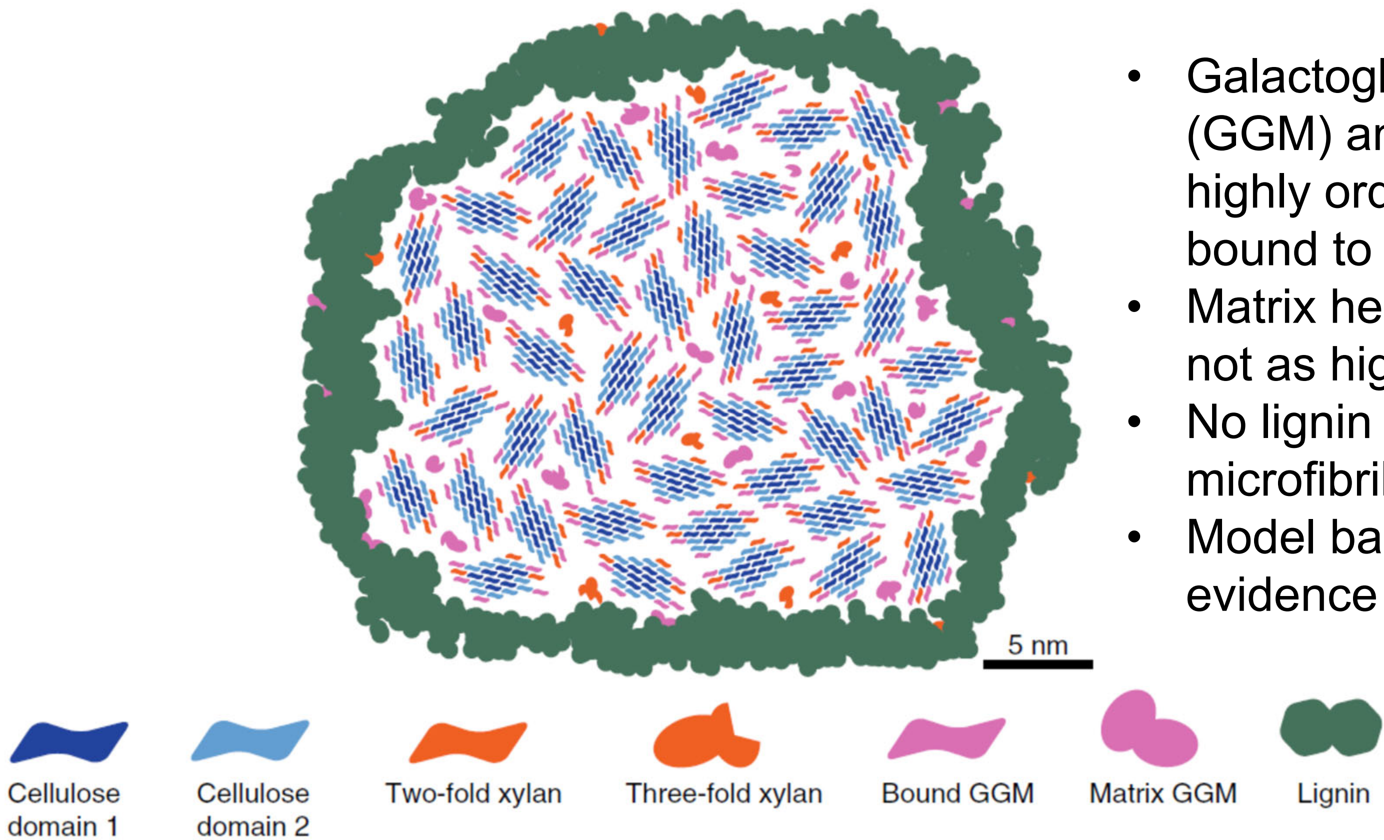
New view on secondary wall

Schematics of a cellulose microfibril bundle in softwood secondary wall



- Galactoglucomannan (GGM) and xylan both highly ordered when bound to cellulose
- Matrix hemicellulose is not as highly ordered
- No lignin inside the microfibril bundle
- Model based on NMR evidence

Terrett et al. Nature Commun. **2019**, *10*, 4978.

Occurrence of hemicellulose

Amount of polysaccharide in wall (% w/w)[a]						
	Dicot walls		Grass walls		Conifer walls	
Polysaccharide	Primary	Secondary	Primary	Secondary	Primary	Secondary
Xyloglucan	20–25	Minor	2–5	Minor	10	–[b]
Glucuronoxylan	–	20–30	–	–	–	–
Glucuronoarabinoxylan	5	–	20–40	40–50	2	5–15
(Gluco)mannan	3–5	2–5	2	0–5	–	–
Galactoglucomannan	–	0–3	–	–	+[b]	10–30
β-(1→3,1→4)-glucan	Absent	Absent	2–15	Minor	Absent	Absent

Scheller and Ulvskov *Annu. Rev. Plant. Biol.* **2010**, *61*, 263.

Characterization of hemicellulose

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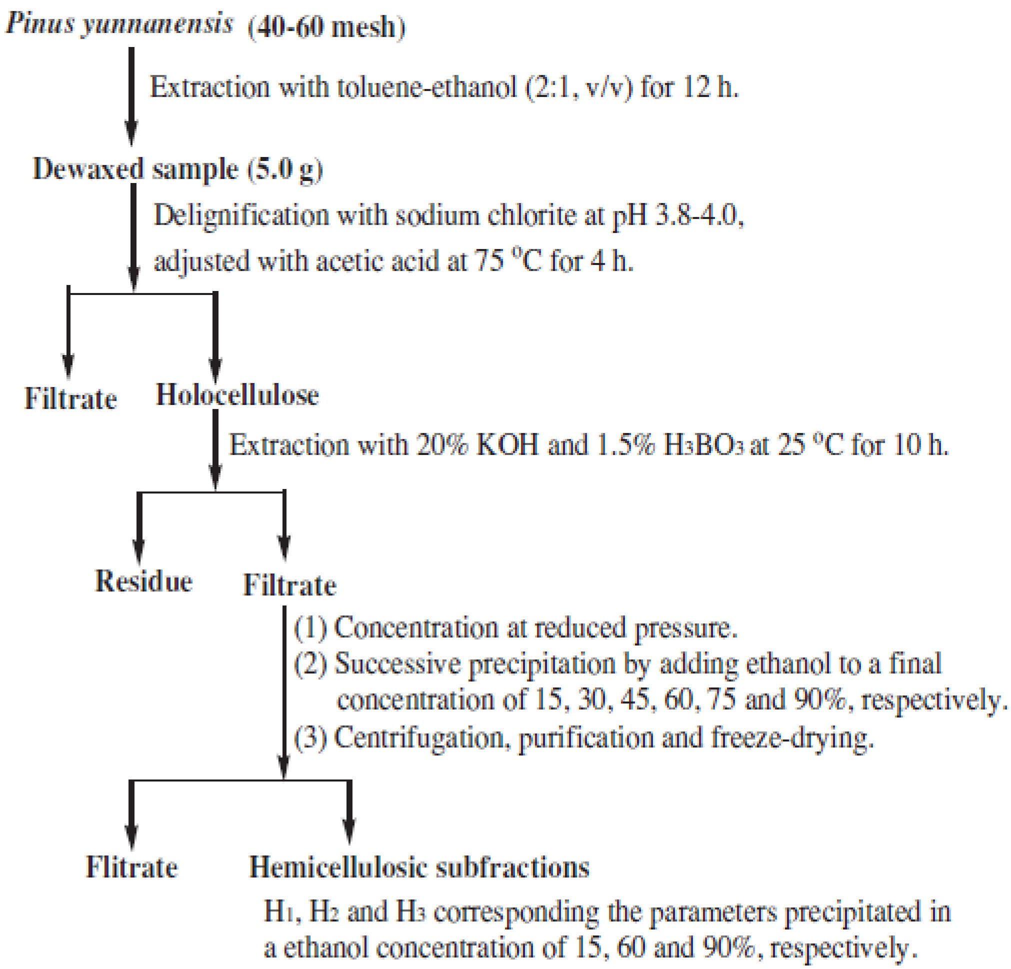
Common issues in characterization

- As a rule of thumb, hemicelluloses must be isolated from the plant matrix for reliable characterization
- Destructive characterization: breaking the hemicellulose polymer into monosaccharides and characterization by chromatography
- Non-destructive characterization: spectroscopic techniques

Example of isolation of hemicellulose



Routine procedure for determination of monosaccharide composition

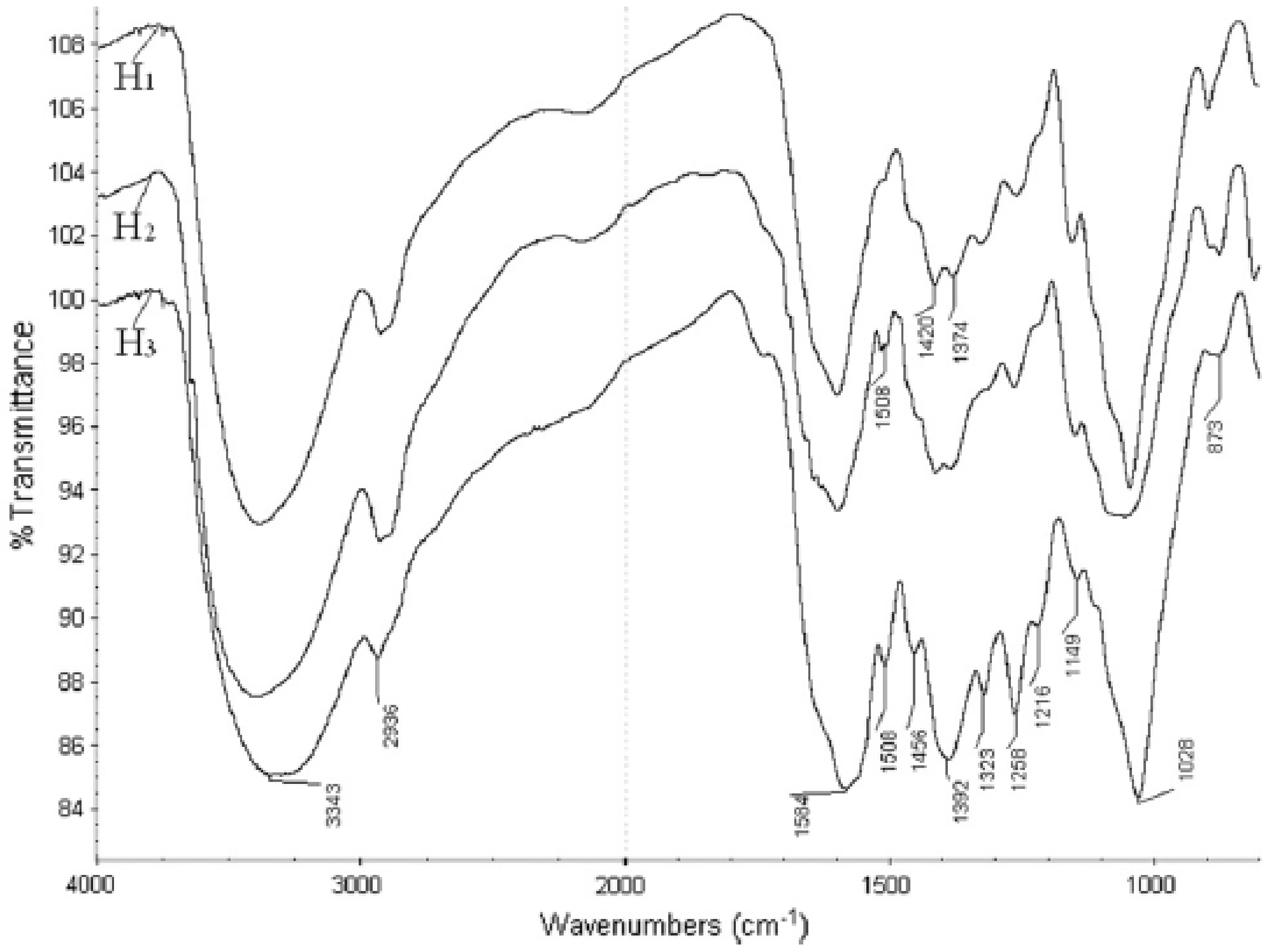
- 2.5 h hydrolysis in dilute (6%) H_2SO_4

→ Hemicelluloses are degraded to monosaccharides
→ Monosaccharide composition is determined with HPLC

If the amounts of actual hemicelluloses are desired, the results are correlated with the known ratios of monosaccharides for each hemicellulose in the sample from literature.

NOTE: This is the only way to know the exact monosaccharide composition of the plant-based sample.

Infrared spectroscopy



In general, there is too much overlap in the characteristic bands of hemicelluloses to perform reliable IR analysis.

Xue et al. *Carbohydr. Polym.* **2012**, *352*, 159.

Nuclear magnetic resonance (NMR)

Chemical shifts (ppm) can be explicitly addressed to specific carbons of specific hemicelluloses
→ powerful method

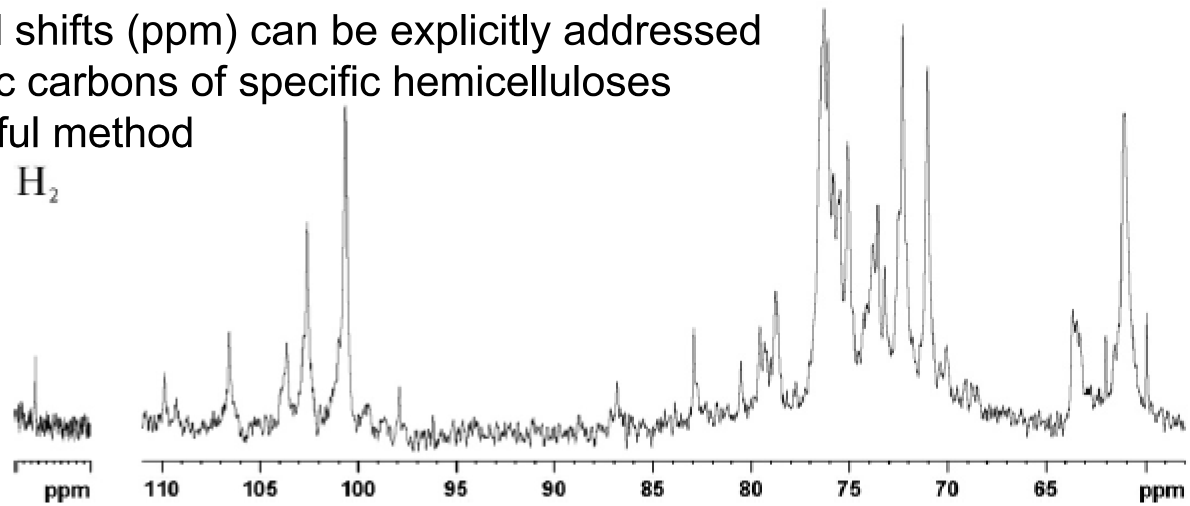


Table 3
^{13}C NMR data on the constituent monosaccharide residues in the hemicellulosic fraction H_2 (δ/ppm)

Residue	C-1	C-2	C-3	C-4	C-5	C-6
Gal	106.57(α-) 101.00(β-)	75.0–71.0	75.0–71.0	75.0–71.0	n.d [b]	63.62
Glc	103.63 (non-red[a])	73.82	78.76	79.57	75.11	63.62 (non-red)
Man	100.64	70.02	72.30	76.31	75.83	61.16 70.07
Xyl	102.60	n.d	75.47	75.83	n.d	n.d
Ara	109.85	80.52	78.76	86.83	62.04	n.d
GlcA	97.88	73.20	79.34	82.93	n.d	177.36

[a] Non-red = non-reducing units.
[b] n.d. represents not detected.

Xue et al. *Carbohydr. Polym.* **2012**, *352*, 159.

Summary on basic issues

- The structures of hemicelluloses depend on the plant or even the cell wall layer in which they were biosynthesized
- In the primary wall, xyloglucan is important in load bearing with cellulose
- In the secondary wall, the role of hemicellulose is not as clear as in the primary walls; they swell extensively in water and therefore they partially control the water content of the cell wall

Dissolution of hemicelluloses
- xylan
- mannan
- xyloglucan

General issues on hemicellulose dissolution

Rules of thumb:

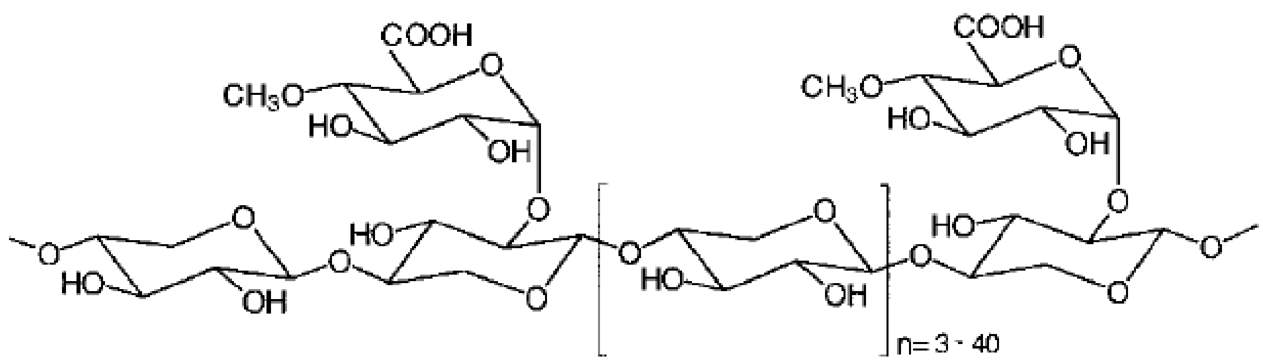
- more branched structures are easier to dissolve
- linkages with lignin reduce solubility
- linkages with cell-wall proteins reduce solubility
- acetylated structures are more water-soluble

NOTE: Many issues on hemicellulose dissolution are unresolved; systematic studies are lacking in most cases.

Dissolution of xylan

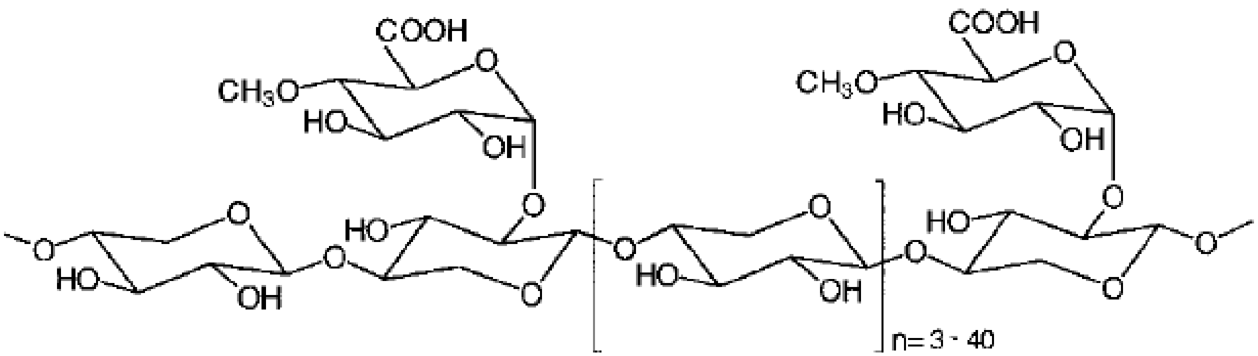
- Most xylans bear a polyelectrolyte structure through 4-O-methylglucuronic acid moieties
- However, the charged groups are generally not enough to solubilize xylan in water

→ Partial acetylation of C-2 and C-3 is required



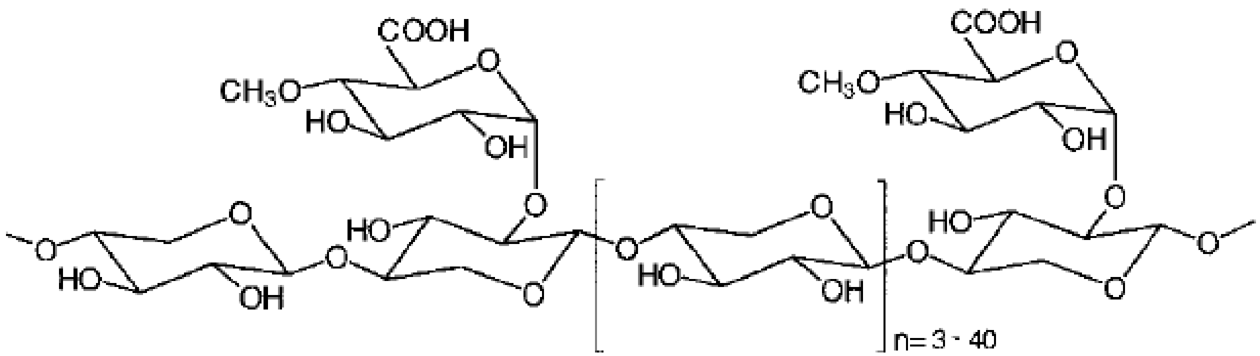
Dissolution of xylan

- Addition of alkali to aqueous solutions increases considerably the solubility of xylan
- When dissolving (isolating) xylan directly from wood cell wall, delignification considerably enhances the xylan yield



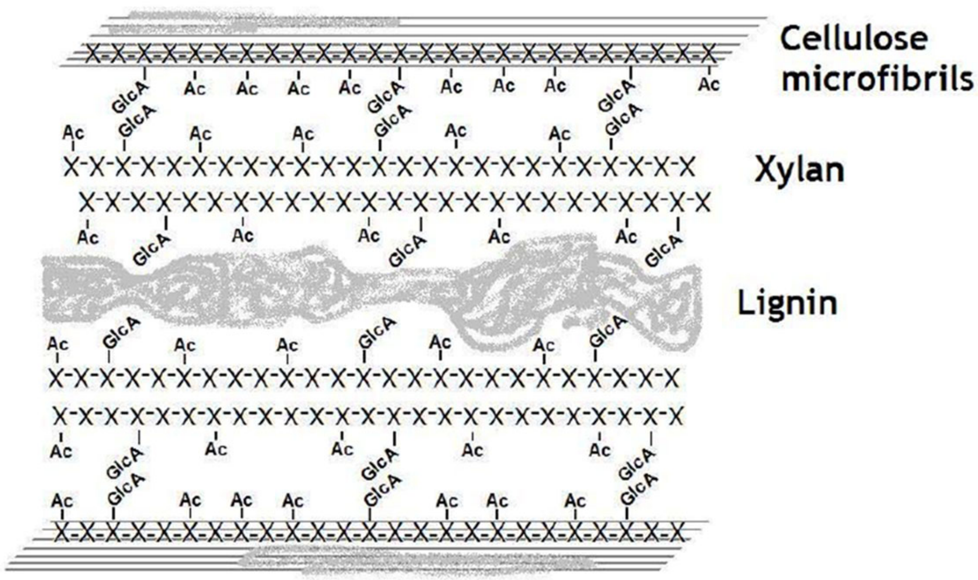
Dissolution of xylan

- Dimethylsulfoxide (DMSO) is able to efficiently dissolve most xylan grades
- Addition of water to DMSO solution enhances the solubility of low-branched xylans



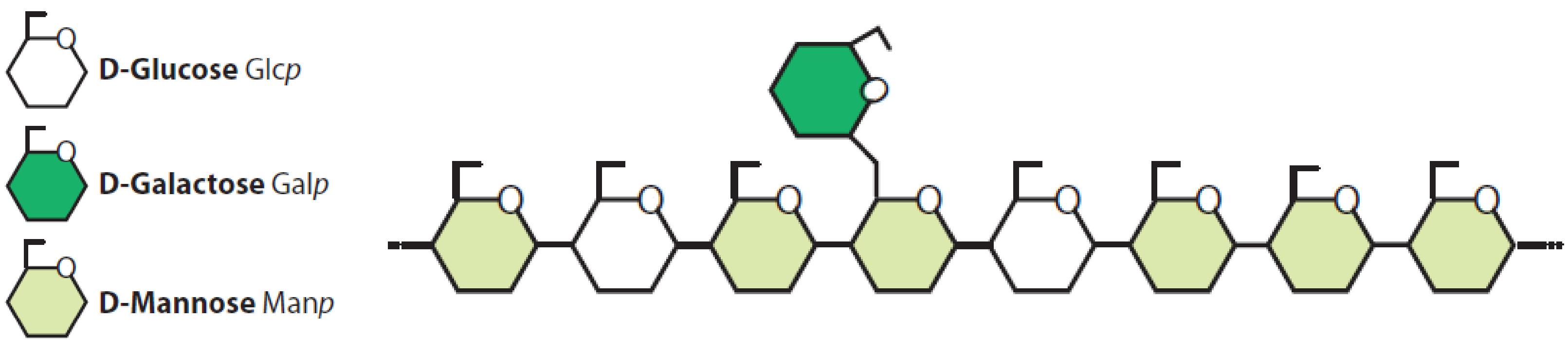
Dissolution of xylan

- When isolating xylan from the cell wall through, e.g., alkaline extraction, some of the xylan remains in the fibre
- Proposition: some of the xylan is intimately entangled with cellulose and therefore cannot be dissolved



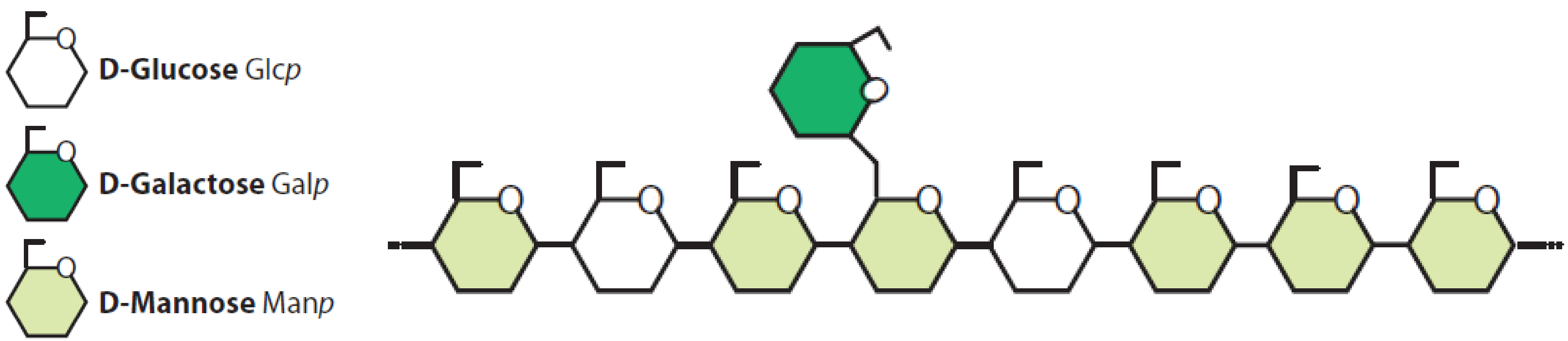
Dissolution of galactoglucomannan

- Native galactoglucomannans from softwoods are partially acetylated at C-2 and C-3 positions (DS_{Ac}=0.17-0.36)
- Acetylated galactoglucomannans are soluble in water
- A large share of galactoglucomannans dissolve during chemical and even mechanical pulping



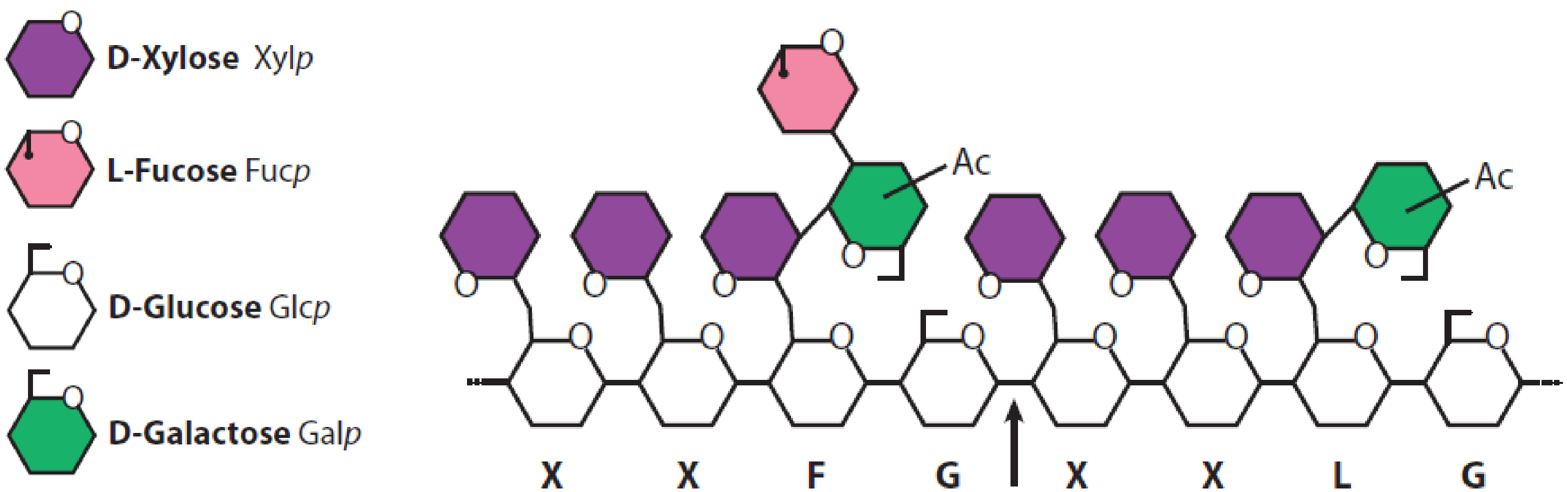
Dissolution of mannans

- Like xylans, most mannan grades (also non-acetylated) can be dissolved in dimethylsulfoxide (DMSO)



Dissolution of xyloglucans

- xyloglucans generally dissolve in water



Dissolution – summary

- Aqueous alkali usually dissolves most (acetylated) hemicelluloses
- If hemicellulose is extracted (isolated) with aqueous alkali from the fibre, part of it always remains in the fibre
- DMSO is a good solvent for hemicellulose – also the non-acetylated ones
- Many cellulose solvents dissolve also hemicellulose appreciably (the whole cell wall can dissolve)

Modification of hemicelluloses

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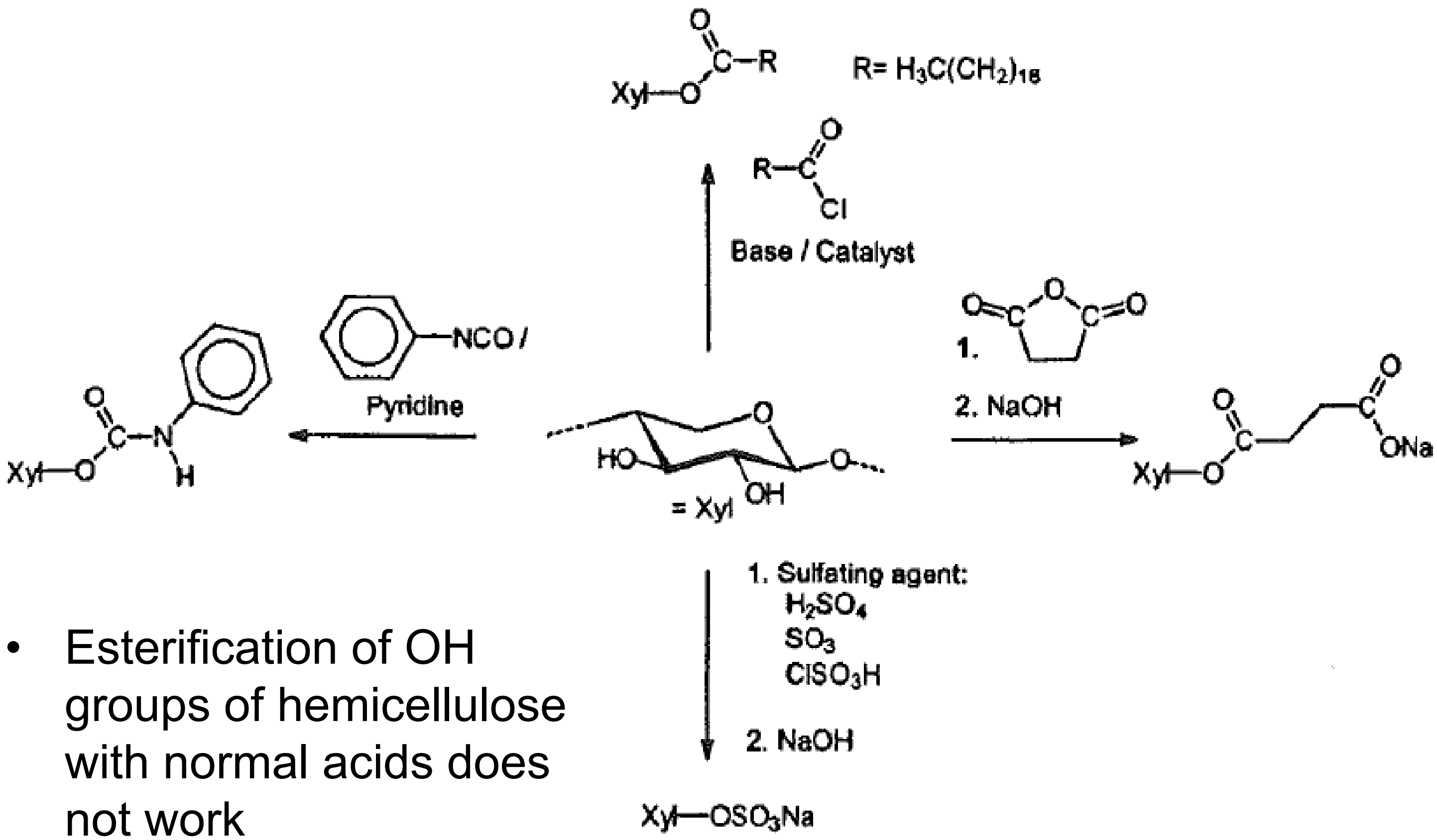
General issues on modification of hemicellulose

- Chemical modification of hemicellulose is not nearly as established an area as cellulose modification
- Heterogeneity of hemicelluloses provides obstacles for specific modification techniques
- Xylan modification is the most established field in hemicellulose modification

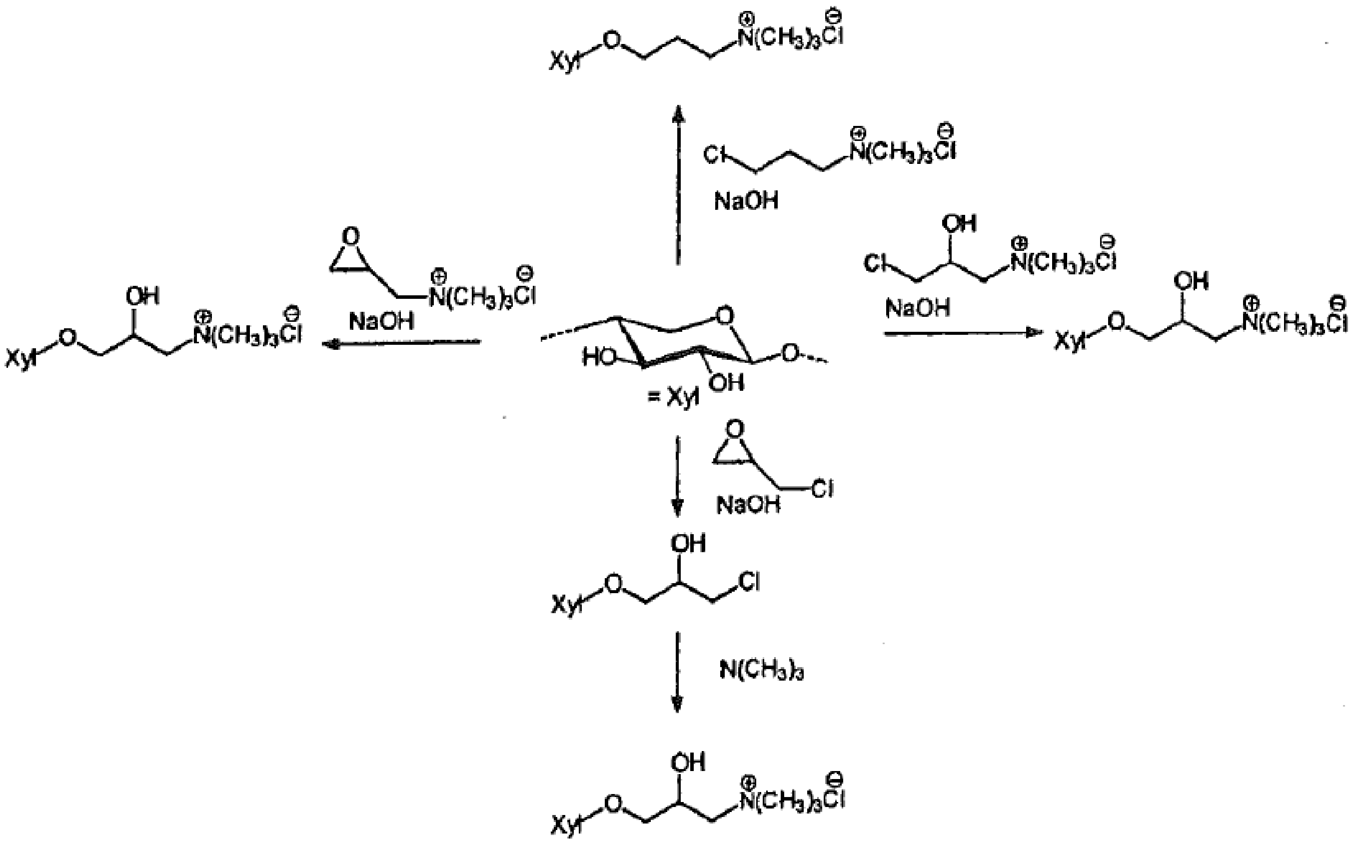
Esterification of xylan



- Esterification of OH groups of hemicellulose with normal acids does not work
- For example, acid chlorides or anhydrides are required for esterification

Ebringerova et al. *Adv. Polym. Sci.* **2005**, *186*, 1.

Cationization of xylan



- Cationizations are performed with etherifying reagents, like with cellulose

Ebringerova et al. *Adv. Polym. Sci.* **2005**, *186*, 1.

Summary on dissolution and modification

- Most hemicellulose grades dissolve in aqueous alkali
- Modification methods are most established for xylan, including many etherification and esterification reactions