Methods For Quantitative Analysis of Uronic Acids in Biomass

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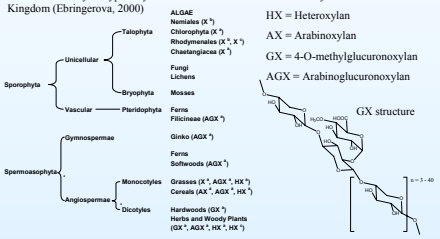
Occurrence

- Xylans occur in many structural varieties in terrestrial plants.
- GX and AGX are the main structural xylans in biomass feedstocks.

Occurrence of Xylan-type Polysaccharides in the Plant Kingdom (Ebringerova, 2000)



X = Homoxylan
HX = Heteroxylan
AX = Arabinoxylan
GX = 4-O-methylglucuronoxylan
AGX = Arabinoglucuronoxylan

Importance

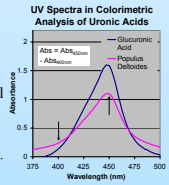
- Hardwoods contain 15-30% of hemicellulose which is predominately O-acetyl-4-O-methylglucurono-β-D-xylan.
- The xylose : 4-O-methylglucuronic acid ratio varies considerably with species and plant part from 1 – 20 : 1 (Ebringerova, 2000).
- Whereas the acetyl and xylosidic bonds are easily cleaved the uronic acid - xylose bonds are very resistant to acid hydrolysis.
- Softwoods and herbaceous plants contain arabinoglucuronoxylan. The glycosiduronic bonds in AGX are very resistant. (Sjostrom, 1981)
- 2-O-(4-O-Methyl-glucopyranosyluronic acid)-xylopyranose is hydrolyzed 20x slower than corresponding xylitol (Timell, 1964)
- Cellobiouronic acid is hydrolyzed 30x slower than cellobiose.
- Because of the resistance of the uronic acid – xylose linkage to acid hydrolysis a fraction of the xylose is not released in dilute acid pretreatment of biomass feedstocks.

Total Uronic Acids Analysis Decarboxylation

- Biomass sample decarboxylated by boiling HCl (12%). CO_2 adsorbed in ascarite and quantified by weight gain. Decarboxylation can take more than 4 hours. (Browning, 1949)
- Decarboxylation by reflux in HI. Liberated CO_2 absorbed in NaOH. CO_2 quantified by change in conductance of NaOH. Suitable for 50 – 100 mg samples containing 1-10 mg uronic acid. (Theander,1991)
- CO_2 produced from non-uronide extractives and carbohydrates complicates analysis. Even after extraction of biomass decarboxylation gave 30 – 40% higher uronic anhydride contents than colorimetry method. (Scott, 1984)

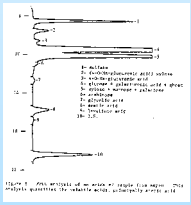
Total Uronic Acid Analysis Colorimetric Determination

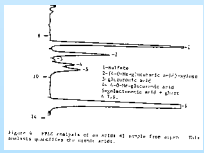
- Hydrolyzates reacted with conc. H_2SO_4 at 70 ºC converting uronic acids to 5-formyl-2-furoic acid, which is colorimetrically determined after reaction with a phenol, e.g., 3,5-dimethyl phenol
- Glucuronic acid reacts much more slowly than other uronic acids. Addition of boric acid allows its separate determination.
- Presence of lignin requires subtraction of reagent-less blank
- Different factors used depending on uronic acids present
- Reaction/measurement timing critical
- IEA round robin found 20 – 30% between-lab reproducibility in 4 feedstocks tested (UA content 1-4%). (Scott, 1979; Agblevor, 1992)



Analysis of Individual Uronic Acids Cation HPLC

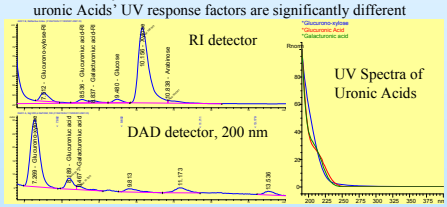
- HPLC of hydrolyzates on Bio-Rad HPX-78H column allows separation of 3 uronic acids and the aldobiuronic acid. (Kaar, 1991)
- Neutral sugars are removed from hydrolyzate using H^+ cation-exchange resin in a 7-step procedure.
  - Losses of uronic acids during hydrolysis (30 – 40%) not factored into calculations
  - Refractive Index detector used





Analysis of Individual Uronic Acids Cation HPLC

- Uronic acids absorb in the UV from 190 – 240 nm.
- With DAD detector sugar removal no longer necessary.
- Glucurono-xylose standard needed as Glucuronic and Galact-uronic Acids' UV response factors are significantly different



Analysis of Individual Uronic Acids Anion HPLC

- Anion HPLC with pulsed amperometric detection can be used to analyze for neutral, and acidic sugars.
- Acidic mono- and oligosaccharides are strongly retained by anion-exchange resin requiring sodium acetate (0.3 M) as a pusher ion in the eluent (0.1 M NaOH). (Hausalo, 1995)

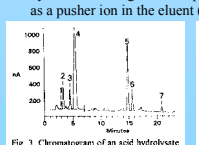


Fig. 3. Chromatogram of an acid hydrolysate of paper machine circulating water (mechanical pulp) Peaks: 1,7 Unknowns, 2 Fucose (istd), 3-4 Neutral monosaccharides, 5 GalA and 6 GlcA. [II]

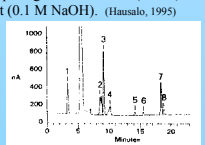


Fig. 4. Chromatogram of an enzymatic total hydrolysate of unbleached birch kraft pulp. Peaks: 1 Fucose (istd), 2-4,6 Unknowns, 5 MeGlcA, 7 HexA-X, and 8 HexA-X$_2$. [II]

Analysis of Individual Uronic Acids Derivatization and GC

- There are very many GC and derivatization methods in literature.
- Most methods give multiple peaks for each uronic acid & sugar.
- None of the methods can analyze for the uronic acid – xylose.
- Complete hydrolysis of GX or AGX is accomplished with trifluoroacetic acid or methanolic HCl. (Kardosova, 1998; Chaplin, 1982; Ha, 1988)
- Trimethylsilylated and peracetylated derivatives have been made coupled with oximation (Laine 1971), and reduction (Lehrfield, 1982; Carpita, 1984) to improve separations.
- Two methods give single peaks for each neutral and acidic sugar. TMS diethyl dithioacetals (Honda, 1979) give well resolved peaks, as do peracetylated N-hexylaldonamides (Walters, 1988).

Analysis Standards

- 4-O-methyl-glucuronic acid (MeGA) and 4-O-methyl glucurono-xylose are not commercially available.
- MeGA has been synthesized from methyl glucoside. Benzoylation is followed by methylation at the 4-position, debenzoylation, and then oxidation with TEMPO. Overall yield (74%) (Li, 1995).
- GX and AGX hemicellulose have been extracted with alkali from biomass samples and holocellulose (Timell, 1964, Ebringerova, 2000). Partial acid hydrolysis should yield 4-O-methyl glucurono-xylose.
- Without good standards it is hard to believe that much progress will be made in developing a routine analysis.

Conclusions\Future Work

- A routine method for analysis of the individual uronic acids is needed if we are to track the fraction of xylose not released in acidic biomass pretreatment.
- So far either cationic or anionic HPLC appears the most promising, however, without good standards accurate analytical data will not be possible.
- A synthesis of MeGA is underway at NREL but has not yet yielded the desired compound.
- Isolation of the uronic acid – xylose from GX extracted from biomass will also be attempted.

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