Isolation and characterization of the structural polysaccharides of Artemisia tridentata subsp. vaseyana

Wayne Bukwa

*The University of Montana*

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ISOLATION AND CHARACTERIZATION OF THE STRUCTURAL 
POLYSACCHARIDES OF ARTEMISIA TRIDENTATA SUBSP. VASEYANA

by
Wayne Bukwa

B.S., Michigan Technological University, 1964

Presented in partial fulfillment of the requirements 
for the degree of Master of Science

UNIVERSITY OF MONTANA
1969

Approved by:

[Signatures]

Chairman, Board of Examiners

Dean, Graduate School

Date

Jan. 7, 1970
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OBJECTIVES

The purpose of this study was to isolate the structural polysaccharides of mountain big sagebrush or purple sagebrush (*Artemisia tridentata* Nutt. subsp. *vaseyana* [Rydb.] Beetle) and to determine the general composition of its woody tissue. Big sagebrush (*A. tridentata*), which is widespread in the eleven Western states of this country, is a member of the highly evolved family Compositae of the dicotyledons. Little work has been done on the structural polysaccharides of the plants in the genus *Artemisia*. Most of the chemical investigations have dealt with the nutritional value of the leaf and flowering tissue and less frequently with the small edible stem tissue of the plant. This concentration of interest is due to the fact that the plant is of no great economic importance except to the livestock industry and for wildlife management.

It is of interest to determine the effect of evolution on the composition of the structural polysaccharides of these plants and the similarities or dissimilarities of these polysaccharides with those of other plants.
PREVIOUS RESEARCH ON THE POLYSACCHARIDE COMPONENTS OF ARTEMISIA AND OTHER PLANTS

Many articles have been published on the chemical composition of the leaves, stems and flowers of various species of Artemisia. These articles have been concerned especially with the amount and identity of the essential oils, and more recently with the sesquiterpene lactones present.\(^1\) \(^4\) A few studies, however, have been conducted on the carbohydrate constituents of Artemisia, but these studies have dealt with nutritional factors, including the amount of pectin, starch, and free sugars.\(^5\) \(^8\) They also have delved briefly into the structural polysaccharides present in the genus and have reported the amount of cellulose, hemicellulose, or pentosans for some species. It was reported\(^9\) that the carbohydrate complex of the roots and old stems of a wormwood consists mainly of hemicelluloses with seasonal changes in the amounts of reserve carbohydrates. The presence of cellulose in the stems of A. nova has been reported and it was observed that lignin was higher in stem base tissues than in stem tips.\(^10\) A cellulose content of 20.5 to 35.0% was reported to be in the edible tissue of A. lerchaena.\(^11\) It has been reported also that the leaves of A. saopariaeformis contain 13.3% hemicellulose, 12.7% cellulose, 7.2% lignin, and 52.1% other materials.\(^12\) The amount of pentosans in A. rigida
has been reported to be 13.48%. In nearly all of these studies the major objective was either an analysis of nutritional factors or of the neutral solvent extractives.

The major constituents of the plant cell wall in woody tissues are lignin, hemicellulose, and cellulose. Lignin, which is considered as the cementing material of the cell walls, differs sharply in the amounts present in the wood of the two main classes of seed plants, gymnosperms and angiosperms. Temperate zone hardwoods normally contain between 18 to 25% lignin and softwoods contain between 25 to 35% of this material. It is reported that tropical, arborescent angiosperms are lignified to a higher degree than temperate zone hardwoods. Lignin may be isolated in good yield by several methods, although inorganic acids are usually used to isolate lignin from ground wood.

The major structural polysaccharides of woody tissues have been extensively investigated and their constitution is approximately known. With a few exceptions, all plants contain cellulose as the basic structural polysaccharide. The exceptions include certain algae which contain "xylan" or "mannan" and some fungi which contain chitin instead of cellulose. Both hardwoods and softwoods contain about 43 ± 2% of cellulose in their normal tissue. Pure cellulose can be obtained from holocellulose by extracting the hemicelluloses with alkali.
The hemicelluloses of plants are generally considered to be those polysaccharides that are insoluble in neutral solvents and soluble in alkali. The amounts and composition of the hemicelluloses differ markedly between the gymnosperms and angiosperms, but within each class they are similar. Hardwoods have an average of 20 to 30% hemicellulose; D-xylose is the major constituent sugar. Softwoods contain 15 to 20% hemicellulose and D-mannose is the major sugar unit. Monocots in general contain fair amounts of L-arabinose and D-galactose, while hardwoods contain little or none of these two sugars (Table I).

Table I. Distribution of Wood Hemicelluloses

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Deciduous, %</th>
<th>Conifer, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-Acetyl-4-O-methyl-D-glucuronoxylan</td>
<td>80-90</td>
<td>5-15</td>
</tr>
<tr>
<td>4-O-Methyl-D-glucuronoxylan</td>
<td>0.1-1</td>
<td>15-30</td>
</tr>
<tr>
<td>Glucomannan</td>
<td>1-5</td>
<td>60-70</td>
</tr>
<tr>
<td>Galactoglucomannan</td>
<td>0.1-1 or none</td>
<td>1-5</td>
</tr>
<tr>
<td>Arabinogalactan</td>
<td>0.1-1 or none</td>
<td>0.1-30</td>
</tr>
<tr>
<td>Other galactose-containing polysaccharides</td>
<td>0.1-1</td>
<td>0.1-1 or none</td>
</tr>
<tr>
<td>Pectin and associated materials</td>
<td>1-5</td>
<td>1-5</td>
</tr>
<tr>
<td>Starch</td>
<td>0.1-1</td>
<td>0.1-1</td>
</tr>
</tbody>
</table>

aThese amounts are based on the noncellulosic carbohydrates which constitute about 15 to 20% of the softwoods and 20 to 30% of the hardwoods.
The angiosperm "xylan" is in the form of O-acetyl-4-O-methyl-\(\Delta\)-glucuronoxylan while that of gymnosperms is a 4-O-methyl-\(\Delta\)-glucuronoarabinoxylan. "Xylans" of both consist of approximately 200 \(\beta\)-\(\Delta\)-xylopyranose residues linked together by \((1 \rightarrow 4)\) glycosidic bonds with a side chain of 4-O-methyl-\(\alpha\)-\(\Delta\)-glucuronic acid attached directly to the 2 position of from every 3 to 20-\(\Delta\)-xylose units with an average of from 7 to 12 units\(^{18}\) (Formula 1).

\[\text{Formula 1. Partial structure of a 4-O-methyl-\(\Delta\)-glucuronoxylan}\]

The glucomannans of angiosperms are straight chain polymers of \(\Delta\)-glucose and \(\Delta\)-mannose residues in ratios of 1:3 to 1:1 (Formula 2). It is generally believed that this polymer is linear, but due to its ease of degradation in alkali the evidence cannot be considered conclusive.\(^{15}\)
Glucomannans and glucuronoxylans can be extracted selectively with alkali solutions or with alkali solutions as metal complexes. Sodium or lithium hydroxide is more selective for glucomannans and potassium hydroxide for glucuronoxylans. Glucomannans complex with barium hydroxide to form insoluble precipitates that can be used for resolving a mixture of the polymers. Methylsulfoxide is used to extract $\text{O}$-acetyl-$\text{D}$-glucuronoxylans. During alkaline extractions $\text{O}$-acetyl residues, which are generally attached at C-3 of $\text{O}$-acetyl-$\text{D}$-glucuronoxylans are hydrolyzed. The xylan of gymnosperms has an $\alpha$-$\text{L}$-arabinofuranose attached (1 $\rightarrow$ 3) to $\text{D}$-xylose units.

Water soluble galactoglucomannans are found in the genus *Larix*. Trace amounts of these and other hemicelluloses containing $\text{L}$-arabinose and $\text{D}$-galactose have been reported for angiosperms, but in general these two sugars are most common to gymnosperms.

Several structural plant polysaccharides have been isolated from non-woody plants and characterized. Again cellulose is the predominating polysaccharide, although it is accompanied by a larger variety of hemicelluloses.
Arabinoxylans are found primarily in cereals, grains and grasses.\textsuperscript{23,24} They also have been reported to occur in applewood as well as in white spruce bark.\textsuperscript{25} They are, however, formed as artifacts when 4-O-methyl-D-glucurono-arabinoxylans are subjected to drastic alkaline hydrolysis.\textsuperscript{25} Glucuronoxylans have been reported to occur in milkweed, jute, and ramie. A singly branched "xylan" has been reported for Esparto Grass.\textsuperscript{26} In general the types of polysaccharides characteristic of the hardwood are found in other angiosperms and those found in conifers occur in other gymnosperms.

The structural polysaccharides of big sagebrush are expected to be similar or identical to those of the other dicots which contain cellulose, O-acetyl-4-O-methyl-D-glucuronoxylan and a small amount of glucomannan. The woody tissue of big sagebrush differs, however, in one major respect from that of the other woody angiosperms. This plant develops an interxylary cork layer during the growing season which occurs between the new annual ring and that of last year.\textsuperscript{27,28} These become suberized toward the end of the growing season and lose their protoplasm. The development of this interxylary cork tissue closely parallels that of the external periderm, although it is less extensive (2 to 18 cells wide and from .03 to .275 millimeters thick).
Bark characteristically contains more phenolic polymers and less holocellulose than does wood (Table II). It also has greater quantities of extractives, and polyuronides and a fair amount of methoxyl groups. The presence of such tissue in the wood of sagebrush can be expected to influence the analysis if they cannot be completely separated from the normal wood fibers.

### Table II. Percentage Analysis of Bast Fibers and Wood of Douglas Fir.

<table>
<thead>
<tr>
<th>Component</th>
<th>Bast Fiber, %</th>
<th>Wood, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ether soluble</td>
<td>2.92</td>
<td>1.32</td>
</tr>
<tr>
<td>Alcohol soluble</td>
<td>8.65</td>
<td>5.46</td>
</tr>
<tr>
<td>Hot water soluble</td>
<td>2.58</td>
<td>2.82</td>
</tr>
<tr>
<td>Sum of three extractives</td>
<td>14.5</td>
<td>9.60</td>
</tr>
</tbody>
</table>

Values based on oven-dry extractive-free materials.

<table>
<thead>
<tr>
<th>Component</th>
<th>Bast Fiber, %</th>
<th>Wood, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>0.60</td>
<td>0.17</td>
</tr>
<tr>
<td>Klason lignin</td>
<td>44.80</td>
<td>30.15</td>
</tr>
<tr>
<td>Holocellulose</td>
<td>54.58</td>
<td>71.40</td>
</tr>
<tr>
<td>Pentosans</td>
<td>8.62</td>
<td>10.11</td>
</tr>
<tr>
<td>Methoxyl group</td>
<td>3.89</td>
<td>4.75</td>
</tr>
<tr>
<td>Acetyl group</td>
<td>2.39</td>
<td>0.59</td>
</tr>
<tr>
<td>Polyuronides</td>
<td>4.62</td>
<td>2.80</td>
</tr>
<tr>
<td>Methoxyl</td>
<td>7.16</td>
<td>15.20</td>
</tr>
</tbody>
</table>
EXPERIMENTAL

Collection and Preparation of the Specimen. Specimens of the woody tissue of big sagebrush (*Artemisia tridentata* Nutt. subsp. *vaseyana* [Rydb.] Beetle) were collected three miles west of Missoula, Montana, at Sect 10, T 13 N, R 20 W, Montana Meridian. Only stems greater than one half inch in diameter were used, and rotten and discolored tissue was excluded. The outer bark was thoroughly removed from the stem, but no attempt was made to exclude the interxylary cork from the analysis.

The fibrous woody tissue was reduced to shavings and then ground in an intermediate Wiley Mill to pass a 40 mesh screen. The wood was not dried before being ground and extracted in order to avoid changes in the cell structure that could affect the ease of extraction of the polysaccharide components or the content of the volatile compounds.\(^{29,30}\)

Extraction with Neutral Solvents. Ground big sagebrush wood (1.2 kg, dry weight) was exhaustively extracted\(^ {31,32}\) first with benzene-ethanol (2:1) (12 l) on a soxhlet extraction apparatus for 48 hours. The extractives were collected by distillation of the solvent and concentration on a rotary evaporator under reduced pressure at 45°. The red brown, thick, sticky extract was dried under
vacuum to a viscous tar and weighed; yield: 56.8 g. The
wood meal was air dried to remove excess solvent.

The once extracted wood meal was then exhaustively
extracted with 95% ethanol (12 l) for 48 hours in the
soxhlet extractor. The extractives were collected as
before, dried under vacuum to a yellowish red, crusty
mass and weighed; yield: 16.0 g.

The twice extracted wood meal was then extracted
with water (6 l) at 25° in a 16 l container. The slurry
was stirred continuously by means of an overhead stirrer.
The wood meal was filtered on a Buchner funnel over
Whatman No. 2 filter paper and washed with water (500 ml).
The water extraction was repeated once more. The combined
extracts and washings were evaporated, dried over calcium
chloride in a desiccator, and the resulting hard black
mass was weighed; yield: 13.6 g. The water extracted wood
meal was air dried.

Delignification of the Extracted Wood Meal. Delignifi-
cation was carried out using a modification of the
standard acid chlorite extraction method. Extractive-
free wood meal from sage (105 g, dry weight) was suspended
in tap water (1.8 l) at 75-85° in a 4 l Erlenmeyer flask
fitted with a magnetic stirrer. Glacial acetic acid (12 ml)
and sodium chlorite (36 g) were added in that order. The
reaction was allowed to proceed with vigorous stirring and
the temperature was kept at 80° ± 5° by means of a hot plate. After 1 hour the same amounts of reagents were added in the above order and the process continued. Five more additions of the reagent were made until a total of seven additions had been made and the product was treated for a total of 7 hours. After the oxidation was completed the remaining solid material was allowed to settle for 10 hours. The excess liquor was decanted and the product was collected in a Buchner funnel over Whatman No. 1 filter paper. The product was washed with water (1 l) until free of acid and white in color, then washed on a Buchner funnel with ethanol (250 ml) followed by acetone and dried; yield: 78.6 g. Lignin content: 3.1%.

Quantitative Analysis of the Carbohydrates in Holocellulose. For quantitative analysis of the carbohydrates in sagebrush holocellulose the sample (1.000 g, corrected for ash, moisture, and lignin) was placed in a cold flask and kept in a 20° water bath. Seventy-seven percent sulfuric acid (12.0 g) cooled to 10° was added. The sample was macerated with a glass rod, then alternately stirred and evacuated with an aspirator three times in 45 minutes. The light brown solution was then diluted with cold water (305 ml), slowly bringing the acid concentration to 3.0%. The solution was refluxed 5 1/4 hours.

After the refluxing was complete, the solution was
cooled under tap water and brought to a volume of 500.00 ml. An aliquot (50.00 ml) was withdrawn with a pipette and neutralized to pH 5.3 with saturated barium hydroxide. The mixture was centrifuged and the resulting precipitate was washed and centrifuged three times to give a negative copper reduction test (freshly prepared Fehling reagent).

The combined washings and supernates were evaporated at 45° to a thick syrup with rotary evaporator. Dry pyridine (reagent pyridine dried over phosphorous pentoxide for three days and distilled in the presence of fresh phosphorous pentoxide) (20 ml) was added to the flask and the mixture was concentrated to dryness with the rotary evaporator. Dry pyridine was added to the residue and reevaporated twice more. The water-free syrup was taken up in dry pyridine (10 ml) and refluxed for 40 minutes to equilibrate the anomeric sugar forms.

An aliquot (1.00 ml) of the cold solution was withdrawn from the flask and transferred to a dry test tube. Sorbitol standard (0.40 ml of a 10.0% solution in dry pyridine) and "Trisil" (Pierce Chemical Company product No. 489997; equal volumes of hemamethyldisilazane and trimethylchlorosilane in pyridine) (1.00 ml) was added and the solution was warmed to 75° for 2 minutes in a water bath in order to affect rapid and complete silation. A sample (3 μl) of the reaction mixture was injected into
a gas-liquid chromatograph for analysis as described later.

The areas under the peaks representing the individual sugars were measured by the tracing and weighing technique. The percentage of the anomeric forms of the sugars was derived from this data. The value for each sugar was corrected for the theoretical loss occurring during hydrolysis (Table III). The weight of each sugar was then determined by comparison with the internal standard according to the following equation:

\[
\text{Weight of individual sugars} = \frac{\text{Weight of internal standard} \times \text{Total peak area of sugars}}{\text{Peak area of internal standard}}
\]

Table III. Analysis of Known Sugar Solutions.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Amount added mg/ml</th>
<th>Amount found(^a) mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Arabinose</td>
<td>1.997</td>
<td>1.985 ± 0.080</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>1.991</td>
<td>1.835 ± 0.086</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>2.010</td>
<td>2.080 ± 0.050</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>2.005</td>
<td>2.095 ± 0.139</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>1.988</td>
<td>2.130 ± 0.200</td>
</tr>
</tbody>
</table>

\(^a\)Average of three chromatograms.

Quantitative analyses were also carried out on 0.10 g samples of extracted wood meal, the individual hemicellul-
loses and cellulose.

**Gas Liquid Chromatography.** All samples were analyzed on a temperature-programmed gas chromatograph (F and M 5750 Research Chromatograph) using a six foot, 5% silicone gum rubber, 1/4" OD, stainless steel column (Hewlett Packard SE 30 80-100S 5750) and a reference column similarly packed. Detection was by flame ionization using helium as the carrier gas. The instrument was programmed from 100° to 200° at 1° per minute and then held at 200° for 30 minutes. The conditions for the analysis were:

- **Carrier gas pressure**: 40 PSI
- **Flow rate**: 2.5 ml per min. each channel
- **Hydrogen**: 20 PSI
- **Oxygen**: 30 PSI
- **Detector temp**: 325°
- **Injection port temp**: 225°
- **Range**: 10, **Attenuation**: 8-32

**Extraction of the Hemicellulose and Isolation of the Cellulose.** Holocellulose (100.0 g) was suspended in water (1 l) in a 4 l beaker. One gram of sodium borohydride was added with stirring to reduce the reducing end of the polysaccharide chains. Amyl alcohol (20 ml) was added to control foaming. After 5 hours the product was collected by centrifugation.
Extraction of the hemicelluloses from the reduced holocellulose was carried out using a modified barium hydroxide-sodium hydroxide method. Holocellulose (850 g wet weight, 100.0 g dry weight) was slurried with continuous stirring for 20 minutes in an aqueous solution (710 g) containing barium hydroxide octahydrate (64 g). The consistency of the slurry was 6% and the concentration of barium hydroxide was 4.4%. Atmospheric oxygen was not excluded and the temperature was maintained at 25°C. Eighteen percent aqueous sodium hydroxide (1850 g) was then added to adjust the concentration of the slurry to 2.8%, the barium hydroxide concentration to 2% and the sodium hydroxide concentration to 10%. The extraction was continued for another 20 minutes with stirring. The slurry was centrifuged and the solid residue was alternately washed and centrifuged three times with a total of 500 ml of an aqueous solution of barium and sodium hydroxide of the same concentration as the extraction liquor. This gave a residue that is designated as holocellulose (B) and an extract that contained most of the "xylans."

To precipitate the "xylan" fraction, the combined washings and supernates were acidified with 10% acetic acid, while the liquid was kept cold in an ice bath. Three volumes of ethanol were then added and the mixture was allowed to stand overnight. The "xylan" precipitate was
collected by decanting off the excess liquor and centrifuging. The "xylan" was washed four times by dispersion in 70% ethanol, and dried by solvent exchange. Yield: 15.5 g; ash, 11.80%; lignin content, 2.32%.

The holocellulose (B) was further washed with water and centrifuged several times. The combined washings were acidified and treated with ethanol as above. The precipitates from these washings were combined with the final "xylan" extraction. The washed holocellulose (B) was dispersed in 10% acetic acid (1 l) and allowed to stand overnight at room temperature. The acidic slurry was decantated, centrifuged, and washed four times with water (1 l) to recover the holocellulose.

The wet holocellulose (B) (about 85 g dry weight) was dispersed in enough aqueous sodium hydroxide (2.2 l) of sufficient concentration to give a 4% slurry in a liquor containing 15% sodium hydroxide. The slurry was stirred for 20 minutes at 20°. The undissolved holocellulose (C) was collected by centrifugation and washed and centrifuged three times with 15% aqueous sodium hydroxide (500 ml). Saturated aqueous barium hydroxide (1000 ml) was added to the combined supernates to precipitate glucomannan. (This proportion of reagents kept the sodium hydroxide concentration above 10% and the barium hydroxide concentration below 0.15 M to prevent coprecipitation of "xylan.") The
recovered precipitate was dispersed in 800 ml of 2 N aqueous acetic acid and recovered with three volumes of ethanol. The glucomannan was washed four times as before and dried by solvent exchange; yield: 10.3 g; ash content, 73.8%; lignin content, 2.10%.

The supernate remaining after the precipitation of glucomannan was acidified and treated with 3 volumes of ethanol to recover the "xylan." This fraction was dried and combined with the final "xylan" fraction.

The holocellulose (C) (about 80 g dry weight) was dispersed in enough aqueous sodium hydroxide solution to give a 6% slurry and sodium hydroxide concentration of 18%. The treatment was complete after 45 minutes of continuous stirring. The last fraction of "xylan" remaining in the holocellulose was collected by centrifugation, washed twice with 18% sodium hydroxide (500 ml) then twice with water (500 ml) by alternately centrifuging and dispersing the precipitate. The liquor was acidified with 10% acetic acid and precipitated with ethanol. The product was collected and dried as before; yield: 10.0 g; ash content, 10.76%; lignin, 3.02%.

The cellulose fraction was washed with 5% acetic acid, centrifuged and collected on a Buchner funnel. It was washed with water (500 ml) then with acetone, and sucked dry to yield a fluffy white powder; yield: 55.0 g; ash
content, 4.45%; lignin content, 1.01%.

A second analytical extraction was run in identical manner on 25.000 g of extracted wood meal. Yields are given in Table IX, page 34.

A direct cellulose determination was carried out using 18% sodium hydroxide. Yield on an ash free, lignin free basis: 53.5%.

Extraction of O-Acetyl-4-O-methyl-D-glucuronoxylan.
Acid-chlorite delignified holocellulose (100 g) was treated with reagent dimethylsulfoxide (900 ml) with stirring for 3 days. The extract was recovered by filtration on a Buchner funnel. The solid residue was washed with dimethylsulfoxide (400 ml). The brown colored extract and washings were poured into ethanol (6 l) to which acetic acid (50 ml) had been added. The precipitate was collected by decanting most of the liquor and then centrifuging. The O-acetyl-4-O-methyl-D-glucuronoxylan was washed once with ethanol, once with ethanol-petroleum ether, and once with petroleum ether. The product was dried under vacuum at 25° for 4 days. Yield: 3.78 g; no ash present; lignin, 2.67%.

Drying of Samples. All samples were dried by solvent exchange or lyophilization. The "xylan" fraction (about 10 g, dry weight) recovered as a paste wet with 70% ethanol
was dispersed gradually in 95% ethanol while vigorously stirring until the paste was completely dispersed in 100 ml of ethanol. The solution was filtered over Whatman No. 2 filter paper to recover the wet paste. This paste was then dispersed gradually in absolute ethanol with vigorous stirring and recovered as before. The product was next dispersed with vigorous stirring in absolute ether, collected by filtering and dried under vacuum. The products dried to a soft, white powder.

Drying was carried by lyophilization.

**Moisture Determinations.** Samples of extracted cellulose and hemicellulose (weight variable) were dried at 105°± 3° to a constant weight. Percent moisture was determined on a wet weight basis. 43

**Lignin Analysis.** All samples were analyzed for lignin using the Ritter, et al. 44 and Adams 45 methods. Holocellulose (1.000 g dry weight) was placed in a beaker in a 20° water bath. Seventy-two percent sulfuric acid (15 ml) cooled to 10-12° was added slowly and the holocellulose was macerated with a glass rod for 1 minute. The sample was allowed to react for 2 hours with occasional stirring.

The sample was then washed into a flask with water and diluted to a 3% acid concentration (560 ml). The solution was refluxed 3 hours, the flask was cooled, and the product
was collected over a frittered glass funnel (M) with light suction. The product was washed with water to remove traces of acid, then oven dried at 105° ± 3° to a constant weight; yield: 0.0314 g.

All fractions of hemicellulose and cellulose were analyzed for lignin using the above method. Yields of lignin obtained per 1.0000 g sample were "xylan" 0.0232 g; glucomannan 0.0210 g; cellulose 0.0101 g.

**Ash Determinations.** Samples of products (0.1000 g to 1.000 g dry weight basis) were ashed in a muffle furnace by a gradual increase in temperature to 600°. After 4 hours at 600° the samples were brought to room temperature and weighed. 46

**Purification of the "Xylan."** The crude "xylan" was purified by copper complexing. 47,48 Impure "xylan" (1.5 g) was dissolved in 5% sodium hydroxide (150 ml). Freshly prepared Fehlings solution 49 was added slowly with stirring to a slight excess (100 ml). The precipitate was collected by centrifuging and the faintly blue supernate was decanted off. The precipitate was redispersed in cold water, centrifuged, and decanted again. Cold 2 N hydrochloric acid was then added slowly to decompose the copper complex. The resulting polysaccharide solution was slightly opalescent. The "xylan" was recovered by precipitation with four
volumes of ethanol. After standing overnight the precipitate was collected by decanting the excess liquor and centrifugating. The "xylan" was washed three times by first acidifying with N hydrochloric acid and then adding 80% ethanol and centrifuging. The resulting product was white. This product was dried by the solvent exchange technique followed by dessication under vacuum. Yield: 1.28 g; ash content, 1.67%; no lignin present.

Decarboxylation of the "Xylan." Decarboxylation determinations\textsuperscript{50} were made on purified "xylan" in the Browning apparatus.\textsuperscript{51} 4-O-methyl-D-glucuronoxyylan (0.2000 g) was placed in a reaction flask with 12% hydrochloric acid (20 ml) saturated with sodium chloride. Nitrogen, purified with a drying tube and a carbon dioxide trap, was passed through the reaction flask and the temperature was brought to 70° to drive out all extraneous carbon dioxide. After the ascarite collection tube reached a constant weight, the temperature was gradually increased to 135-140° (reflux temperature). The weight of the ascarite collection tube was constant after 4 hours.

The calculation for percent 4-O-methyl-D-glucuronic acid in the "xylan" was based on a molecular weight of 189 for 4-O-methyl-D-glucuronic acid and a ratio of 4.30:1 for carbon dioxide to 4-O-methyl-D-glucuronic acid units. Yield: 0.0083 g CO\textsubscript{2}, equal to 0.0356 ± 0.0008 g 4-O-methyl-
D-glucuronic acid.

**Paper and Thin Layer Chromatography.** The hemicellulose and holocellulose hydrolysates were prepared as for gas liquid chromatography but without silating (page 11). Paper chromatography was carried out on 18 inch strips of Whatman No. 1 filter paper by the descending technique. The solvent system was \( \text{N}-\text{butanol-pyridine-water} \) (10:3:3) and the development time was 48 hours at 25°. O-aminodiphenyl spray was used as a detector.

Thin layer chromatograms were made using an Eastman Kodak chromatogram thin layer chamber apparatus and fresh and dry Gelman type S.G. paper. The solvent system was pyridine-ethyl acetate-water (120:20:5). O-aminodiphenyl spray was used as a detector.

**Polarimetry.** Polarimetry measurements were taken on an O. C. Rudolph & Sons Model 80 polarimeter. The hemicelluloses samples were freed of ash before the readings. The "xylan" was freed of ash by dispersing it in 5% sodium hydroxide and centrifugating (1500 x G) for 1 hour.

**Periodate Oxidation. I. Cellulose.** Cellulose samples (1.0000 g, dry weight) were dispersed in 100 ml of 0.25 M sodium periodate solution and stored in the dark at 4° ± 1°. The solution was periodically shaken to redisperse the cellulose, which lost its fibrous nature as oxidation...
proceeded. A blank was carried out simultaneously. Con­sumption of periodate was estimated using the sodium thio­sulfate method. The thiosulfate was standardized against potassium iodate. The oxidant consumed remained constant after the sixth day. The amount consumed extrapolated to zero amounted to 1.06 M per glucose residue (Table IV).

Table IV. Periodate Consumption and Formic Acid Production of Big Sagebrush Cellulose

<table>
<thead>
<tr>
<th>Time, hours</th>
<th>24</th>
<th>48</th>
<th>70</th>
<th>95</th>
<th>110</th>
<th>134</th>
<th>150</th>
<th>176</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periodate consumption, moles per glucose unit</td>
<td>.61</td>
<td>.73</td>
<td>.95</td>
<td>1.00</td>
<td>1.05</td>
<td>1.06</td>
<td>1.06</td>
<td>1.07</td>
</tr>
<tr>
<td>Formic acid, moles per glucose unit</td>
<td>.0020</td>
<td>.0031</td>
<td>--</td>
<td>.0033</td>
<td>--</td>
<td>.0033</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

aAverage of three samples.

II. "Xylan." Several 4-O-methyl-D-glucuronoxylan samples (0.5000 g each, dry weight) were dispersed in 100 ml of 0.10 M sodium periodate solution and stored in the dark at 4° ± 1°. The solution was periodically shaken. Consumption of periodate was estimated using the thio­sulfate method as before. Consumption of oxidant remained constant after the sixth day. The amount of periodate consumed was 6.53 M per repeating 4-O-methyl-D-glucuronoxylan unit on extrapolation to zero (Table V).

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Table V. Periodate Consumption and Formic Acid Production of Big Sagebrush "Xylan"a

<table>
<thead>
<tr>
<th>Time, hours</th>
<th>7</th>
<th>44</th>
<th>92</th>
<th>128</th>
<th>155</th>
<th>175</th>
<th>190</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periodate consumption, moles per repeating 4-O-methyl-D-glucuronoxylan unit</td>
<td>1.40</td>
<td>3.50</td>
<td>5.50</td>
<td>6.20</td>
<td>6.50</td>
<td>6.52</td>
<td>6.60</td>
</tr>
<tr>
<td>Formic acid, moles per repeating 4-O-methyl-D-glucuronoxylan unit</td>
<td>.0018</td>
<td>--</td>
<td>.0024</td>
<td>--</td>
<td>.0028</td>
<td>.0028</td>
<td>--</td>
</tr>
</tbody>
</table>

a Average of five samples.

Formic Acid Production. The amount of formic acid produced upon periodate consumption was determined for both the cellulose and "xylan" samples. Samples (3.00 ml oxidized cellulose solution, 15.00 ml oxidized "xylan" solution) were quenched with ethylene glycol (1 ml). The quenching of periodate was allowed to proceed for 5 minutes. The amount of formic acid evolved was determined by titration of the quenched solution against 0.0050 N sodium hydroxide. The amount of formic acid present was 0.0033 M per glucose unit in cellulose and 0.0028 M per repeating 4-O-methyl-D-glucuronoxylan unit of "xylan." (Tables IV, V).
Infrared Measurements and X-Ray Diffraction. Infrared measurements were made on a Beckman IR5 infrared spectrophotometer. The 4-O-methyl-D-glucuronoxylan was suspended as a mull in petroleum jelly. Direct measurements could be made on the glassy air dried "xylan": moist "xylan" containing 70% ethanol was dried in a thin layer on a flat glass plate at 110° in the oven. The resulting material was a transluscent, brittle, mica like sheet.

X-ray diffraction patterns of cellulose were made on a Norelco X-ray diffraction instrument. The diffraction analysis specifications were: scale factor 8, multiplier 6, time constant 2, at 40 kilovolts and 30 milliamperes; scan time was 1° per minute and degree of span 40° to 4°. X-ray powder diagrams were made using a Cu-Ni target-filter with 6 hours exposure.

Thermal Gravimetric Analysis and Differential Thermal Analysis. These analyses were made on a DuPont Model 900-950 Thermal Analyzer, using for the TGA a 15° per minute temperature rise and a nitrogen flow of 100 cc per minute on a 10 mg sample of 40-60 mesh. The DTA was carried out at 15° per minute on a 20 mg sample of 40-60 mesh in nitrogen.
RESULTS AND DISCUSSION

The woody tissue of the stems of big sagebrush (Artemisia tridentata Nutt. subsp. vaseyana [Rydb.] Beetle) was investigated to determine the amounts of extractives and lignin present and to isolate and identify the structural polysaccharide components. The compounds present in the extractives were not determined. Determinations were made of Klason lignin in the extracted wood meal and in the holocellulose and the polysaccharide components of the holocellulose. Various physical and chemical methods were used to identify the composition of the hemicelluloses and cellulose.

Big sagebrush wood meal was extracted successively by benzene-alcohol (2:1), by alcohol, and by water. The total extractives amounted to 7.62% of the oven-dried wood meal (Table VI). The benzene-alcohol fraction constitutes 5.01% of the dry wood meal and contains most of the nonwater soluble extractives including the terpenes, phenolics, flavonoids, fatty acids, resins, and waxes. The alcohol extractives, which include the tannins, other phenolics, flavonoid compounds, and coloring materials, amounted to 1.41%. The water extractives amounted to 1.20% of the dry wood and included the water soluble carbohydrates such as pectin, starch and free sugar, and the inorganic salts.

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Table VI. Composition of Big Sagebrush Wood.

<table>
<thead>
<tr>
<th>Component</th>
<th>Composition of dry wood, %</th>
<th>Composition of extracted wood, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extractives(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene-alcohol (2:1)</td>
<td>5.01</td>
<td>---</td>
</tr>
<tr>
<td>Alcohol</td>
<td>1.41</td>
<td>---</td>
</tr>
<tr>
<td>Water</td>
<td>1.20</td>
<td>---</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>7.62</strong></td>
<td></td>
</tr>
<tr>
<td>Klason lignin(^b)</td>
<td>29.25</td>
<td>31.45</td>
</tr>
<tr>
<td>Holocellulose(^c, d)</td>
<td>66.36</td>
<td>71.40</td>
</tr>
<tr>
<td>Lignin by difference</td>
<td>26.02</td>
<td>28.60</td>
</tr>
</tbody>
</table>

\(^a\)Average of three extractions  
\(^b\)Average of four determinations  
\(^c\)Average of three delignifications  
\(^d\)Corrected for ash and lignin

None of the extractive fractions were investigated to determine their constituent compounds. The loss of volatile components in the extractives was minimized by drying the extractives under vacuum at room temperature.

The lignin content of the wood was determined by the method of Ritter, Geborg and Mitchell\(^44\) as described by Adams.\(^45\) The amount of lignin found was 31.45% of the extracted wood and 29.25% of the total wood (Table VI). This amount is high when compared to the average of 18 to
25% for the other woody dicots. The amount of lignin estimated from the difference between the whole wood and amount of holocellulose it contained was somewhat less: 28.60% of the extracted wood and 26.02% of the total wood, but was still high for a dicot. The high Klason lignin content when compared to that of hardwoods may be accounted for in part or entirely by the presence of the interxylary cork tissue between the annual rings of the wood. The discrepancy between the amount of Klason lignin and the amount determined from the difference between the holocellulose and extracted wood meal may be due to incomplete extraction resulting from the large mesh size of the particles. Sagebrush wood was ground to pass a 40 mesh screen instead of the usual 60 mesh. The percent Klason lignin from this material might also be high, since extractives are removed with difficulty from large particles and these extractives would be present in the Klason lignin.

The large amount of lignin in the wood presented a problem in delignification. The acid chlorite method of delignification using a 4 to 5 hour reaction time commonly used for delignifying hardwoods yielded a holocellulose containing 10% lignin. Hemicelluloses extracted from this holocellulose contained even larger percentages of lignin (Table VII). A more successful delignification was carried out by increasing the reaction time to 7 hours and adding
Table VII. Delignification: Percent Lignin in Components of Big Sagebrush Holocellulose

<table>
<thead>
<tr>
<th>Component</th>
<th>Lignin present after 4 hour delignification, %</th>
<th>Lignin present after 7 hour delignification, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holocellulose</td>
<td>10.7</td>
<td>3.1</td>
</tr>
<tr>
<td>Cellulose</td>
<td>1.9</td>
<td>1.01</td>
</tr>
<tr>
<td>&quot;Xylan&quot; - first fraction</td>
<td>12.1</td>
<td>2.10</td>
</tr>
<tr>
<td>&quot;Xylan&quot; - second fraction</td>
<td>9.1</td>
<td>1.02</td>
</tr>
<tr>
<td>Glucomannan</td>
<td>4.1</td>
<td>2.10</td>
</tr>
</tbody>
</table>

proportionately more reagents. The amount of lignin in the holocellulose obtained using a 7 hour extraction procedure was 3.10%.

Quantitative analysis of the holocellulose using the procedure of hydrolysis, silation, and gas liquid chromatography indicated 52.5% anhydro-D-glucose, 32.8% anhydro-D-xylose, and 0.84% anhydro-D-mannose with a total yield of 86.1% with the correction factor (Table VIII). The sugars were identified by comparing their GLC retention times with those of known sugars (Figures 1 and 2). The value for each sugar was calculated by determining the area under the respective peaks and using the results of Laver, et al. to correct for the decomposition of sugars due to hydrolysis (Table III, page 13). An initial hydrolysis time of 29
Figure 1. Gas chromatogram of trimethylsilyl ether derivatives of a known sugar mixture. (1) $\beta$-L-arabinopyranose; (2) $\alpha$-L-arabinopyranose; (3) ring isomer of L-arabinose; (4) $\alpha$-D-xylopyranose; (5) $\beta$-D-xylopyranose; (6) $\alpha$-D-mannopyranose; (7) ring isomer of D-galactose; (8) $\alpha$-D-galactopyranose; (9) $\alpha$-D-glucopyranose; (10) $\beta$-D-mannopyranose and $\beta$-D-galactopyranose; (11) ring isomer of D-mannose; (12) D-sorbitol; (13) $\beta$-D-glucopyranose.

Figure 2. Gas chromatogram of trimethylsilyl ether derivatives of big sagebrush holocellulose hydrolyzate. (1) artifacts due to lignin; (2) $\alpha$-D-xylopyranose; (3) $\beta$-D-xylopyranose; (4) $\alpha$-D-mannopyranose; (5) $\alpha$-D-glucopyranose; (6) D-sorbitol; (7) $\beta$-D-glucopyranose.
Table VIII. Analysis of Big Sagebrush Holocellulose Hydrolyzate

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Yield by Analysis, %</th>
<th>Correction factor$^a$</th>
<th>Corrected yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anhydro-D-Glucose</td>
<td>52.5</td>
<td>---</td>
<td>52.5</td>
</tr>
<tr>
<td>Anhydro-D-Xylose</td>
<td>30.2</td>
<td>.922</td>
<td>32.8</td>
</tr>
<tr>
<td>Anhydro-D-Mannose</td>
<td>0.84</td>
<td>---</td>
<td>.84</td>
</tr>
<tr>
<td>4-O-Methyl-D-glucuronic Acid$^b$</td>
<td>---</td>
<td>---</td>
<td>5.6</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td>91.7</td>
</tr>
</tbody>
</table>

$^a$Laver, et al. (1967) (Table III)$^{31}$

$^b$By decarboxylation

44 minutes and a secondary hydrolysis time of 5 1/4 hours was used to derive the maximum yield of monosaccharides (Figure 3).

![Graph](image-url)  
**Figure 3.** Hydrolysis of pulp with refluxing sulfuric acid.$^{35}$

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Quantitative analysis of the holocellulose indicated the presence of a "xylan," a glucomannan, and a large percentage of cellulose. The total yield of sugars was 92.0% when corrected for the amount of 4-O-methyl-D-glucuronic acid found by decarboxylation of the "xylan." This uronic acid component could not be detected by gas liquid chromatography. The gas chromatogram of hydrolyzed big sagebrush holocellulose (Figure 2) contained some peaks that could be due to the presence of trace amounts of L-arabinose. However, this could not be confirmed by thin layer or paper chromatography. Therefore, it is assumed that these peaks are artifacts originating from the presence of lignin. These peaks were larger on the GLC chromatogram from holocellulose containing 10.7% lignin.

Extraction of the hemicelluloses by a modified barium hydroxide-sodium hydroxide procedure (Figure 4) was chosen in order to separate the small amount of glucomannan present as indicated in the analysis of holocellulose. The holocellulose was treated initially with sodium borohydride to reduce the aldehyde end of the "xylan" and prevent its degradation in alkali. The glucomannan was complexed with barium hydroxide, rendering it insoluble in sodium hydroxide. The "xylan" was then extracted with 15% sodium hydroxide. After recovering the "xylan" the glucomannan-barium hydroxide complex was destroyed by acidification.
Holocellulose

- Reduction with NaBH₄

Reduced holocellulose

- Treatment with Ba(OH)₂ and extraction with NaOH

NaN₃ soluble NaN₃ insoluble

- "Xylan" 1 Holocellulose B

- Acidification and extraction with NaOH

NaN₃ insoluble NaN₃ soluble

- Holocellulose C Glucomannan + "Xylan"

- Extraction with NaOH

- Treatment with Ba(OH)₂

NaN₃ insoluble NaN₃ soluble

Cellulose "Xylan" 2 "Xylan" 2

Glucosyan + "Xylan" Glucosyan

Figure 4. Alkaline fractionation of the holocellulose.
The glucomannan was isolated by extracting the holocellulose (B) with 15% sodium hydroxide and then precipitating the glucomannan from the extraction liquor as its barium hydroxide complex. A second "xylan" fraction was then recovered by acidification of the liquor, from which glucomannan had been recovered, and subsequent precipitation with ethanol. The remaining holocellulose (C) was then exhaustively extracted to remove further the residual "xylan" and this final "xylan" was combined with the second "xylan" fraction. The yield of cellulose as the final insoluble product was 51.24%; the combined fractions of "xylan," 23.62%; and the glucomannan, 0.75% (Table IX).

Table IX. Components of Big Sagebrush Holocellulose Obtained by Alkaline Extraction$^{a,b}$

<table>
<thead>
<tr>
<th>Component</th>
<th>Percent obtained based on holocellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>51.24</td>
</tr>
<tr>
<td>&quot;Xylan,&quot; first fraction</td>
<td>13.28</td>
</tr>
<tr>
<td>&quot;Xylan,&quot; second fraction</td>
<td>10.34</td>
</tr>
<tr>
<td>Glucomannan$^c$</td>
<td>.75</td>
</tr>
<tr>
<td>TOTAL</td>
<td>75.61</td>
</tr>
<tr>
<td>Cellulose by ASTM Method</td>
<td>53.5</td>
</tr>
</tbody>
</table>

$^a$Average of two extractions  
$^b$Corrected for ash and lignin  
$^c$Corrected for contaminating "xylan"
Extraction of the holocellulose with 18% sodium hydroxide by the ASTM standard method for determining cellulose yielded 53.5% cellulose. This amount was 35.5% of the extracted wood. Upon analysis by gas liquid chromatography this cellulose was found to contain 99.1% D-glucose, 0.9% D-xylose, and no lignin. If hydrolysis is not carried out to completion, large peaks that do not correspond to any of the known standard sugars appear between those of D-xylose and D-glucose. Paper and thin layer chromatography indicated the presence of D-glucose only. The extraneous materials probably due to lignin did not migrate by these methods.

Several physical methods were used to ascertain the structural nature of big sagebrush cellulose. The infrared spectra of sagebrush cellulose was identical to that of a cellulose isolated in this laboratory from black cottonwood, *P. nigra* (Figure 5). The X-ray diffraction pattern indicated a crystal structure with d-values of 4.08 Å, 4.43 Å and 7.32 Å, identical to those of mercerized cellulose (Figure 6). The X-ray powder diagrams of the two celluloses were identical to those of mercerized cellulose (Figure 7). Differential thermal and thermogravimetric analyses were made on the sagebrush cellulose. These were identical to those of cotton cellulose (Figures 8 and 9). As expected, these physical analyses provided evidence that cellulose
Figure 5. Infrared spectrum of big sagebrush and black cottonwood cellulose

Figure 6. X-ray diffraction pattern of big sagebrush and black cottonwood mercerized cellulose
Figure 7. X-ray powder patterns of big sagebrush cellulose, black cottonwood cellulose and mercerized cotton cellulose (60).
TEMPERATURE, °C (CORRECTED FOR CHROMEL ALUMEL THERMOCOUPLES)

Figure 8. Thermal analysis of big sagebrush and black cottonwood cellulose

TEMPERATURE, °C (CORRECTED FOR CHROMEL ALUMEL THERMOCOUPLES)

Figure 9. Thermogravimetric analysis of big sagebrush and cotton cellulose
from big sagebrush is identical to the cellulose isolated from other seed plants.

Periodate oxidation provided chemical proof of the structure of big sagebrush cellulose. The amount of periodate consumed was constant after 5 days and amounted to 1.06 M per \( \text{D-glucose} \) unit. Allowing for slight over-oxidation, this result is in close agreement with that of cotton cellulose.\(^5\) The amount of formic acid produced was 0.0033 M per \( \text{D-glucose} \) unit, indicating a degree of polymerization (DP) of 330 \( \pm \) 40, which is somewhat shorter than the usual range of values for cellulose isolated from wood pulp.\(^6\)

The first fraction of "xylan" was relatively pure and free of hexoses, and contained 2.10% lignin. Upon hydrolysis, silation and GLC analysis, big sagebrush "xylan" yielded 97.2% \( \text{D-xylose} \) and 2.8% \( \text{D-glucose} \). The presence of lignin resulted in the appearance of peaks occurring as explained previously where \( \text{L-arabinose} \) peaks occur. These artifacts were ignored in the determination of the relative amounts of component sugars. The artifact peaks were eliminated completely after two purifications of the "xylan" by copper complexing. The amounts of sugars found after purification were 99.2% \( \text{D-xylose} \) and 0.8% \( \text{D-glucose} \). The purified "xylan" contained no Klason lignin and only \( \text{D-xylose} \) was detectable by thin layer and paper chromatography.
The second fraction of "xylan" contained 96.5% \( \delta \)-xylose and 4.5% \( \delta \)-glucose. The amount of lignin was 1.02% and the artifact peaks were proportionately less than those from the first impure "xylan" fraction.

Hydrolysis of the "xylan" fractions was carried out for 7 hours in 3% sulfuric acid. This was necessary to completely hydrolyze the "xylan." Incompletely hydrolyzed "xylan" resulted in the occurrence of a peak at 114 minutes retention time by GLC and as a fast running spot on thin layer and paper chromatograms.

The "xylan" from big sagebrush was analyzed to determine its specific rotation and infrared spectra. Polarimetry measurements on impure, first fraction, ash free "xylan" gave \([\alpha]_{D}^{25} -78.0 \pm 0.1^\circ\) (cl, 5% NaOH). The correction for lignin gave \([\alpha]_{D}^{25} -80.8 \pm 0.1^\circ\) (cl, 5% NaOH). The optical rotation measurement on purified "xylan" was \([\alpha]_{D}^{25} -81.8 \pm 0.1^\circ\) (cl, 5% NaOH). The specific rotation for elmwood 4-0-methyl-\( \delta \)-glucuronoxylan containing 12.6% 4-0-methyl-\( \delta \)-glucuronic acid was \([\alpha]_{D}^{25} -83.8^\circ\) (cl, 5% NaOH). The infrared spectra of big sagebrush "xylan" was essentially identical to that of a 4-0-methyl-\( \delta \)-glucuronoxylan isolated in this laboratory from black cottonwood (Figure 13), which contained 13.1% 4-0-methyl-\( \delta \)-glucuronic acid.

Decarboxylation\(^{47}\) of purified big sagebrush "xylan" gave 4.14% CO\(_2\), which indicates the presence of 17.8%
Figure 10. Gas liquid chromatogram of the trimethylsilyl derivatives of big sagebrush xylan hydrolyzate. (1) Artifacts due to lignin; (2) α-D-xylopyranose; (3) β-D-xylopyranose; (4) α-D-glucopyranose.

Figure 11. Gas liquid chromatogram of the trimethylsilyl derivatives of big sagebrush cellulose hydrolyzate. (1) α-D-xylopyranose; (2) β-D-xylopyranose; (3) α-D-glucopyranose; (4) β-D-glucopyranose.
Figure 12. Gas liquid chromatogram of the trimethylsilyl derivatives of big sagebrush glucomannan hydrolyzate. (1) \(\alpha\)-D-xylopyranose; (2) \(\beta\)-D-xylopyranose; (3) \(\alpha\)-D-mannopyranose; (4) \(\alpha\)-D-glucopyranose; (5) \(\beta\)-D-mannopyranose; (6) ring isomer of mannose; (7) \(\beta\)-D-glucopyranose.

Figure 13. Infrared spectrum of big sagebrush and black cottonwood 4-O-methyl-D-glucuronoxylan.
4-O-methyl-D-glucuronic acid in deacetylated "xylan." The purified "xylan" dried in vacuum from 70% ethanol gave low results. Analysis for methoxyl content yielded 2.92% methoxyl groups confirming the presence of 17.8% 4-O-methyl-D-glucuronic acid in the deacetylated "xylan" polymer.

A small amount of O-acetyl-4-O-methyl-D-glucuronoxylan was extracted from big sagebrush holocellulose with methyl-sulfoxide. The yield was 3.78% of the holocellulose. A higher yield can be obtained if acid chlorite holocellulose is impregnated with cold 5% ethanolic monoethanolamine and washed with ethanol and ether. The percent of O-acetyl in O-acetyl-4-O-methyl-D-glucuronoxylan was 12.0. The rotation was $[\alpha]_{D}^{25} = -82.0 \pm 1^\circ$ (c1, 2% NaOH) corrected for 16.0% lignin; no ash was present.

The results of decarboxylation, methoxyl and acetyl analyses (Table X) indicates that big sagebrush "xylan" is an O-acetyl-4-O-methyl-D-glucuronoxylan containing 15.7% uronic acid and 12% acetyl. It contains one 4-O-methyl-D-glucuronic acid residue for every 7 D-xylose units and about half of the D-xylose units contain an acetyl group. The distribution of the O-acetyl is not known, but it is assumed to be random and bonded at positions 2 or 3 of D-xylose as in other molecules of this type. This polymer makes up 44.0% of the holocellulose and 29.2% of the extracted wood.
Table X. Composition of Big Sagebrush O-acetyl-4-
O-methyl-D-glucuronoxylan

<table>
<thead>
<tr>
<th>Component</th>
<th>Detected by GLC Analysis, %</th>
<th>Detected by Specific Analysis, %</th>
<th>Calculated Amount in the Polymer, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Xylose</td>
<td>99.2</td>
<td>---</td>
<td>67.3</td>
</tr>
<tr>
<td>D-Glucose&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Methoxyl&lt;sup&gt;b&lt;/sup&gt;</td>
<td>---</td>
<td>2.92</td>
<td>2.58</td>
</tr>
<tr>
<td>Acetyl&lt;sup&gt;c&lt;/sup&gt;</td>
<td>---</td>
<td>12.0</td>
<td>12.0</td>
</tr>
<tr>
<td>4-O-methyl-D-glucuronic acid&lt;sup&gt;d&lt;/sup&gt;</td>
<td>---</td>
<td>17.8</td>
<td>15.7</td>
</tr>
</tbody>
</table>

<sup>a</sup>Considered to be a contaminant, not calculated into percent of polysaccharide

<sup>b</sup>Galbraith Laboratories, Knoxville, Tennessee

<sup>c</sup>Analysis on methylsulfoxide extract of holocellulose

<sup>d</sup>Determined on deacetylated "xylan"

Figure 14. O-Acetyl-4-O-methyl-D-glucuronoxylan

from Big Sagebrush

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The proposed structure of this molecule is patterned after the common type of O-acetyl-4-O-methyl-\(\alpha\)-glucuronoxylan found in dicots (Formula 3).

The validity of this structure is further supported by the results of periodate oxidation. The amount of periodate consumed was 6.52 moles per repeating 4-O-methyl-\(\alpha\)-glucuronoxylan unit. The DP of the molecule determined from the formic acid produced by the periodate reaction was approximately 70. The usual DP of hardwood 4-O-methyl-\(\alpha\)-glucuronoxylans is 160 to 200 units although fractions varying from 50 to 300 have been reported.\(^6\)

The amount of glucomannan isolated from holocellulose by alkaline extraction was 0.75% corrected for ash and lignin. The amount of \(\alpha\)-glucose to \(\beta\)-mannose by GLC analysis was 2:3, indicating that this polymer accounts for 0.56% of the \(\alpha\)-glucose found by quantitative hydrolysis. The total amount of this polymer in the holocellulose as indicated by the quantitative hydrolysis is 1.40% assuming 0.84% \(\alpha\)-mannose and 0.56% \(\alpha\)-glucose (Table VIII, Page 31).

The amount of the polysaccharides found in big sagebrush holocellulose is given in Table XI. The amount of cellulose, 51.9%, is based on the total yield of \(\alpha\)-glucose less 0.56% that makes up the glucomannan hemicellulose. This compares to the 53.5% obtained by the ASTM method.
<table>
<thead>
<tr>
<th>Polysaccharide</th>
<th>% of holocellulose</th>
<th>% of wood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>51.9</td>
<td>34.5</td>
</tr>
<tr>
<td>Acetyl-4-O-methyl-D-glucuronoxylan</td>
<td>44.0</td>
<td>29.2</td>
</tr>
<tr>
<td>Glucomannan</td>
<td>1.4</td>
<td>0.9</td>
</tr>
</tbody>
</table>
SUMMARY

The woody stem tissue of mountain big sagebrush (Artemisia tridentata Nutt. subsp. vaseyana [Rydb.] Beetle) contains about 7.62% neutral solvent extractives, 29.45% Klason lignin, and 66.36% holocellulose. Cellulose makes up 51.9% of the holocellulose and 34.5% of the extracted wood meal. It is identical to the common cellulose of other green plants and consists of (1→4)-β-D-glucose units. The major hemicellulose is an O-acetyl-4-O-methyl-D-glucuronoxylan comprising 44.0% of the holocellulose and 29.2% of the extracted wood. It contains one 4-O-methyl-D-glucuronic acid residue per 6.9 D-xylose residues and 3.48 O-acetyl residues per 6.9 D-xylose units. Glucomannan is present as a minor hemicellulose, making up 1.4% of the holocellulose and 1.2% of the extracted wood. The glucomannan contains D-glucose and D-mannose in a ratio of 2:3. The general composition of big sagebrush woody tissue is similar to that of the other wood dicotyledons.


6. N. E. Wilson, J. C. Dinsmore, and P. B. Kennedy, "Native Forage Plants and Their Chemical Composition," Nevada Agricultural Experiment Station, Bulletin 62 (1906).


