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The Effect of the Neutral Sulfite Semichemical Cook on the Hemicelluloses of Aspenwood

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THE EFFECT OF THE NEUTRAL SULFITE SEMICHEMICAL COOK ON THE HEMICELLULOSES OF ASPENWOOD

A thesis submitted by

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INTRODUCTION

PRESENTATION OF THE PROBLEM

Neutral sulfite semichemical pulp is assuming increasing importance in the pulp and paper industry. Certain of the properties of semichemical pulp such as its ease of hydration and high strength undoubtedly stem in part from the high percentage of hemicelluloses remaining in the pulp. Because of the importance of the hemicelluloses upon the characteristics of the pulp, it was felt desirable to study the effect of the neutral sulfite semichemical cook on the hemicelluloses of a typical hardwood such as aspen. An approach to the problem was selected in which emphasis was placed on a thorough study of the hemicelluloses from a typical pulp rather than a less extensive study of the hemicelluloses from a number of pulps.

STATEMENT OF THE PROBLEM

The chemical and physical properties of the hemicelluloses in the original wood (aspen) were compared with those retained in the pulp after a neutral sulfite semichemical cook, and the degradation products of the hemicelluloses in the spent liquor were studied.

HISTORICAL REVIEW

NEUTRAL SULFITE SEMICHEMICAL PULPING

Semichemical pulping, a development of the past 25 years, has as its aim the production of a low cost pulp in high yield from pulpwoods such as the hardwoods. In practice a two-step process is involved: (1) a mild chemical treatment to remove part of the lignin (a portion of the hemicelluloses are also removed), and (2) mechanical action on the treated fiber bundles to separate the fibers.

Semichemical pulp can be made by modifying nearly any presently used pulping procedure--sulfite, kraft, soda, or cold caustic. However, the most widely used is the neutral sulfite semichemical (NSSC) which consists essentially of cooking chips under pressure at about 170° in a solution of neutral sodium sulfite buffered with sodium carbonate or sodium hydroxide. Under controlled conditions this results in the solution of only 15 to 30% of the weight of the wood. It is clearly differentiated from the sulfite process by the use of a neutral or slightly alkaline liquor and produces a neutral or slightly alkaline solution of the dissolved components. The alkalinity of the cooking liquor affects the amounts of certain components that are removed. This applies particularly to the hemicelluloses (1, 2).

Neutral sulfite pulping was first suggested in 1880 in a patent by Cross (British patent 4984). In 1920 the Keebra process was introduced by Bradley and McKeefe but corrosion difficulties prevented its commercial success (3). The first commercial application was in 1925 under a process developed and patented by Rue, Wells, and Rawling (4) of the United States Forest Products Laboratory. The rapid growth in semichemical pulping has been in the last decade. In 1940 there was a daily capacity of 465 tons, in 1947 the capacity was expanded to 1490 tons and in 1951 to 2750 tons. There are now 26 semichemical mills in the United States, 22 of which use the NSSC process (5).

Perhaps 30 to 45% of the hemicelluloses and 50 to 75% of the lignin are removed from hardwoods during the NSSC cook (6, 7, 8, 9). The form taken by the carbohydrate material and lignin in the spent liquor is unknown. Aries (10) suggested that the spent liquor should contain sodium lignosulfonate, sodium salts of organic acids, sodium sulfate, sodium sulfite, and residual sodium bicarbonate. He did not mention, however, the fate of carbohydrates which were removed.

THE HEMICELLULOSES

One of the problems in the chemistry of the hemicelluloses is terminology, in that no strict definition of the term hemicelluloses seems possible. Schulze (11) originally proposed the name to describe those cell-wall components which enter into solution readily in hot dilute mineral acids with the formation of simple sugars. European chemists prefer the term polyoses; others speak of cellulosans and polyuronides, accessory carbohydrates, pentosans and hexosans, and non-cellulosic cell-wall components. The non-uniformity of the composition of the hemicelluloses increases the confusion of nomenclature. Factors such as wood species, sample source, type and method of extraction, and method of pre-treatment, if any, have a distinct bearing on the resultant hemicelluloses. Hemicelluloses are customarily extracted by some type of caustic solution and are essentially defined by the type and method of extraction. In the experimental work covered in this study the term hemicelluloses is construed to mean those substances extracted from the several starting materials under the conditions and by the caustic solutions specified in the experimental procedures and precipitated by pouring the alkaline extract into acidified ethanol.

Several excellent discussions of the chemistry of the hemicelluloses are available (12, 13, 14, 15). In this discussion it is proposed to cover only those data pertinent to the problem.

Wise, Murphy, and D'Addieco (16) studied the effect of potassium hydroxide strength on the amount of hemicelluloses extracted and the pentosan content of the residue from aspen holocellulose. They found that 24% potassium hydroxide gave a minimum pentosan value in the residue. They stated that 5% potassium hydroxide would remove a large portion of the hemicelluloses and suggested it

would be best to remove the hemicelluloses in two fractions rather than one. Wise and Ratliff (17) found that when increasing strengths (2, 5, 8, 10, 12, 16, and 24%) of potassium hydroxide (under nitrogen) were used to successively extract slash pine holocellulose, a point was reached with 16% potassium hydroxide when essentially all the hemicelluloses that could be extracted with potassium hydroxide were removed. Wethern (18), in a study of the molecular properties of black spruce hemicelluloses, prepared the hemicelluloses by extracting black spruce holocelluloses with 5% followed by 16% potassium hydroxide. Thompson and Wise (19) prepared hemicelluloses from big tooth aspen for molecular properties studies in a similar manner.

It is possible to extract more of the hemicelluloses from a partially delignified intermediate such as a holocellulose than from the original wood. Various methods are available for preparation of a holocellulose. These include the chlorine dioxide-pyridine method of Schmidt, Tang, and Jandebeur (20), the chlorite procedure of Jayme (21) and its modification by Wise, Murphy, and D'Addieco (16), the chlorine ethanolamine method of Van Beckum and Ritter (22) and the modification of the latter by Thomas (23). Wethern (18) has shown that hemicelluloses extracted from a holocellulose prepared by the Thomas method have slightly higher molecular weights than those of hemicelluloses extracted from a chlorine dioxide-pyridine or chlorite holocellulose prepared from the same material. Timell and Jahn (23a) found similar results.

Husemann (24) extracted hemicelluloses from straw and beechwood (with aqueous caustic under nitrogen) and fractionated the hemicelluloses. He found the fractions to be quite uniform as regards specific viscosity and specific rotation and suggested that the original material was relatively homogeneous. He obtained degrees

of polymerization (D.P.) of about 150 (calculated as xylan) by osmotic pressure measurements. For a spruce "mannan" he found a D.P. of 160.

Millett and Stamm (25) extracted hemicelluloses from a greatly modified Van Beckum-Ritter holocellulose extracting successively with water at 90-95°C., 1% sodium hydroxide and 5% sodium hydroxide. Their yields were quite low (about 10%) and most of their physical studies were on the water soluble fraction. They report molecular weights varying from 2,000 to 12,500 (D.P.'s of 50-95 calculated as xylan).

Wethern (18) extracted a Thomas holocellulose made from black spruce with 5% and 16% potassium hydroxide. He found an average number average D.P. of about 155. The D.P.'s varied from 114 to 195 depending on the hemicellulose fraction, type of derivative, and method of calculating as a hexosan or pentosan).

Thompson and Wise (19) extracted wood meal from big tooth aspen and a Thomas holocellulose of this wood meal with 5% and 16% potassium hydroxide. They report number average D.P.'s of 140 to 170 for the resultant hemicelluloses.

Hemicelluloses, particularly those from the hardwoods, are composed principally of xylan (26). Schoettler (27) has indicated by chromatography the presence of glucose, galactose, mannose, arabinose, xylose, and rhamnose in a sulfuric acid hydrolyzate of a hemicellulose prepared from extractive free aspenwood (Populus tremuloides). Similarly Dickey (28) has found evidence of the same sugars in a hemicellulose from black spruce.

Jones and Wise (29) isolated and identified chemically galactose, arabinose, xylose, rhamnose, and various uronic acid-containing compounds in the sulfuric acid hydrolyzate of extractive free aspenwood (Populus tremuloides).

PAPER STRIP CHROMATOGRAPHY

The use of paper in chromatography, according to some authors, dates back to Pliny (29a) who made use of papyrus impregnated with an extract of gall nuts to determine ferrous sulfate. Runge (29a) starting about 1850 developed a method of absorbing materials from their solutions directly on filter paper strips. The method was called "Kapillaranalyse". Martin and Synge (30) introduced partition chromatography in 1941 and several years later (1943) the principles were applied to the separation of free amino-acids on filter paper chromatograms by Consden, Gordon and Martin (31). Partridge (32, 33) first applied paper partition chromatography to the separation of sugars.

The exact mechanism of the process of paper partition chromatography is under some dispute. It consists in general of the partition of a solute between a mobile solvent phase and a stationary phase consisting of a cellulose-water complex. The solvent system is a mixture of water and some organic liquid along with acidic materials, basic materials, or buffers to give the desired solubilities and partition of the solute between phases. These materials also affect the speed of travel of the solvent.

For qualitative analysis, the usual procedure is to place a small amount of the unknown material on a filter paper strip and after it has been irrigated by the solvent for a suitable period, the spot or spots are identified by comparison with known substances, by color reactions with spray reagents or by comparison of R_f values (where R_f is defined as the ratio of the distance travelled by the solute to the distance travelled by the solvent front measured from the point of application of the solute).

For quantitative analysis a known amount of material which may or may not contain a reference material is chromatographed in a suitable solvent. By use of guide strips the position of the spots or bands of the substances are located. The amounts of these may be determined in several ways, such as:

(1) Chemical determination of the eluate from the spot. In the field of sugar determination Flood, Hirst, and Jones (34) determined the sugars in the eluate by use of Somogyi's reagent (35). Later they found oxidation with periodate and subsequent determination of the formic acid formed to be a more effective system (36). Hawthorne (37) proposed a similar technique but used an oxidation with alkaline iodine at controlled pH to determine the sugars.

(2) Spraying of the chromatogram with certain reagents and determining the amount of sugars in each spot by the area or the optical density of the spot. Fisher, Parsons, and Morrison (38) found that under controlled conditions the area of the spot was dependent upon the amount of sugar present. They took photographs of the chromatograms and measured the areas with a planimeter. Saarnio, Niskasaari, and Gustafsson (39) used a method similar to that of Fisher, Parsons, and Morrison except that they evaluated the density of the sugar spot photometrically from photographic negatives.

(3) Colorimetric determination of the eluate. Dimler, Schaefer, Wise, and Rist (40) found that the amount of sugar in an eluate could be determined by colorimetric analysis after adding an anthrone reagent.

(4) Other methods. These include visual color comparison, biological assay, and radioautographic or counting techniques.

EXPERIMENTAL PROCEDURES

PREPARATION OF ASPENWOOD SHAVINGS

Four aspenwood logs were taken at random from the wood lot of a local mill. These logs which had been barked in the woods were quartered and one quarter from each used to prepare shavings. The shavings were cut with a power planer which had the blades set to give a very thin shaving. The shavings were thoroughly mixed and stored in a vapor barrier bag. Shavings were selected as a starting material as it was felt they would be rapidly penetrated by chemicals and yet would have suffered relatively little damage to the fibers. The less mechanical bruising or cutting done on the original fibers, the better the comparison should by between hemicelluloses extracted from the wood and those extracted from the defibered pulp.

One hundred grams of the shavings were extracted with alcohol-benzene (1:2) for 31 hours, (rate of siphoning once every 3 hours). The shavings were removed, filtered dry and thoroughly washed with 95% ethanol. They were then extracted with 95% ethanol for 26 hours (rate of siphoning once every 3 hours). The extracted shavings were thoroughly washed with distilled water and air-dried.

The percentage of extractives was determined on 2-gram samples of the shavings by carrying out extractions identical to the above, in small Soxhlet extractors, except that the washed samples were dried at 105°C. Four hour extraction periods were used as the small extractors siphoned every 30 minutes. The percentage of extractives was obtained both by loss of weight of the material and by weight of material extracted. The former value was used for calculations as the latter did not include the material apparently lost during the washing with distilled water following the alcohol extraction.

PREPARATION OF PULPS

The experimental cooks were made in a stainless steel laboratory digester of ten pound capacity. The digester was heated by an external heat exchanger and was provided with positive liquor circulation. Temperature control was maintained by control of the steam to the heat exchanger.

The chips were prepared in the laboratory chipper from the same lot of aspenwood as was previously described. They were screened to give acceptable chips of approximately three-fourths inch size.

After the cook, the spent liquor was extracted from the chips and the chips were put through a Bauer refiner with the discs set for 0.008 inch clearance. The resulting pulp was thoroughly washed with warm water and air dried. After air drying the pulp was placed in pliofilm bags and stored in the cold room (6°).

Two 100 gram portions of the pulp were extracted for 25 hours with 95% alcohol in a large extractor. The extracted pulps were thoroughly washed with distilled water and air dried.

The percentage of alcohol extractives was determined on 2-gram samples extracted with 95% alcohol in small Soxhlet extractors. After extraction the pulp samples were washed with distilled water and dried at 105°C. to constant weight.

PREPARATION OF THE HOLOCELLULOSES

The Thomas method of preparing holocellulose as described by Wethern (18) was used to prepare holocellulose from the aspen shavings and from pulp 2 (see pages 33 and 34). Essentially, the procedure was as follows:

One hundred grams of the aspenwood shavings or pulp 2 were soaked in distilled

water and the water filtered off by suction until the material was moist but contained no excess moisture. It was then cooled to 0 to 5°C. in a 4-liter beaker and one liter of a chlorinating solution cooled to below 0° C. was added. The chlorinating solution was prepared by bubbling chlorine into carbon tetrachloride until the solution became distinctly colored. The suspension was stirred continuously for seven minutes. At the end of the chlorination period the carbon tetrachloride was removed by filtration through a Buchner funnel. The shavings or pulp were washed twice with cold and twice with hot ethanolamine solution. It was then covered with hot ethanolamine solution, consisting of three volumes of ethanolamine in 100 volumes of 95% ethyl alcohol, and allowed to stand 2 minutes. The solution was filtered off and the procedure repeated three times. The material was washed with cold alcohol, followed by an alcohol-water solution (1:1). The chlorination and extraction procedure was carried out a total of five times. After the final extraction the material was thoroughly washed with alcohol followed by acetone. After the acetone wash the holocellulose was air dried.

EXTRACTION OF THE HEMICELLULOSES

Seventy-five grams of airdry extracted shavings or pulp were placed in a 2-liter suction flask. The air was replaced with nitrogen by alternate evacuation and release of the vacuum with nitrogen. The flask and contents were cooled to 20° in a water bath. Fifteen hundred cc. of 5% potassium hydroxide were added and the air again replaced with nitrogen. The mixture was kept at 20° under nitrogen for 2 hours with occasional shaking. It was then quickly transferred to a Buchner, the alkaline extract filtered off and the residue washed with 375 cc. of 5% potassium hydroxide and 375 cc. of distilled water. The residue was immediately returned to the suction flask and after replacement of the air with

nitrogen the extraction repeated with 16% potassium hydroxide. The residue after the 16% potassium hydroxide extraction was washed with 187 cc. of 16% potassium hydroxide, 187 cc. of 5% potassium hydroxide and 375 cc. of distilled water.

The 5% potassium hydroxide filtrate (including washes) was poured into 9 liters of 95% ethanol which contained enough concentrated acetic acid to neutralize the alkali (methyl red end point). The 16% potassium hydroxide (including washes) was poured into 9 liters of 95% ethanol acidified in a similar manner. The alcohol suspensions were stirred vigorously and allowed to stand for 48 hours.

After 48 hours, the supernatant liquor was siphoned off and the hemicelluloses were separated by centrifugation. The concentrated hemicelluloses were washed four times with 95% ethanol and three times with c.p. ethyl ether. They were dried for 48 hours under vacuum at room temperature and broken up into fine powders. The residues after the final extractions were removed from the Buchner and washed with distilled water until the wash was neutral. They were then air dried.

The above procedure was used for extraction of the hemicelluloses from the holocelluloses. Fifty gram samples of the holocelluloses were used and the volumes modified accordingly.

SUGAR ANALYSIS

Paper strip chromatographic techniques were used for qualitative analysis of the sugars present and quantitative estimation of their relative and absolute amounts.

In general the following procedure was employed: A known amount of the material--aspenwood, pulp, hemicellulose, etc.--was hydrolyzed with sulfuric

acid. Either a 3% sulfuric acid solution or a portion of the filtrate from the Klason lignin determination was used. During hydrolysis with 3% sulfuric acid, the solution containing the acid and the material to be hydrolyzed was kept at the boiling point for 4 hours; constant volume was maintained by the addition of distilled water.

QUALITATIVE ANALYSIS

A portion of the lignin filtrate or hydrolyzate was heated on the steam bath, quickly neutralized with barium carbonate and immediately filtered in a Buchner funnel through two filter papers. The filtered solution which still contained traces of barium carbonate and barium sulfate was filtered with suction through a pad of purified, medium, acid-washed asbestos one-half to one cm. thick. The pad was formed in a 30 mm. coarse, fritted glass, filtering funnel. This treatment gave a very clean, clear solution. At this point the solution was very slightly basic to alkacid test paper.

After filtering, the solution was passed through a cation ion exchange column containing either Ionac C-200 or Amberlite IR-120 resin. A volume of distilled water equal to two to three times the volume of the original solution was passed through the column and combined with the solution which was now slightly acidic. The total volume was then concentrated to a thin sirup at 50° under reduced pressure. For some hydrolyzates it was found that better chromatograms could be obtained when the solution was also passed through an anion column (Duolite A-2) before concentrating.

Four paper strips were prepared from Whatman No. 1 filter paper. The strips were torn from a roll of the paper against a knife edge to give a strip 61 cm. long

(cross-machine direction) and 9 to 10 cm. wide (machine direction). A light pencil line was drawn 8 cm. from one end of the strip. On two of the strips several drops of the sirup were placed on the pencil line approximately $2\frac{1}{2}$ cm. from each edge. In the center of the strip a drop of a known sugar solution was placed on the pencil line. The known sugar solution contained all the sugars which previous studies have indicated as present in aspenwood.

On the other two strips a line of the sirup from a fine capillary was laid over the pencil line. After the sirup had dried, a second line of sirup was placed over the first one-half of the strip to insure adequate amounts for visual observation of minor constituents.

One chromatogram of each type was developed in an insulated stainless steel tank containing a solvent mixture of butanol-pyridine-water (10:3:3), and equipped with glass troughs for holding the developing solution and the chromatograms. The chromatograms were left in this system for 48 to 72 hours.

The other two chromatograms were placed in a round glass tank containing as a solvent mixture ethyl acetate-acetic acid-water(9:2:2). After the chromatograms had been developed in this system for 16 to 20 hours, they were removed and dried. The dried chromatograms were then developed in the butanol-pyridine-water system for 24 hours.

Of all the sugars found to be present--galactose, glucose, mannose, arabinose, xylose, and rhamnose--the butanol-pyridine-water system separated all except mannose and arabinose. The ethyl acetate-acetic acid-water system, on the other hand, separated all the above sugars except galactose and glucose. By use of both systems it was possible to adequately separate all the sugars present on a single

chromatogram. The R_f values of certain uronic acids in the ethyl acetate-acetic acid-water system were similar to that of rhamnose and in some cases a band was found just above xylose that gave a characteristic uronic acid color with a p-anisidine hydrochloride spray. The chromatograms developed in the ethyl acetate-acetic acid-water system were used primarily as an aid in establishing the presence or absence of mannose and arabinose in a hydrolyzate.

The two developing systems discussed above were the only ones used in this study. For brevity, the butanol-pyridine-water (10:3:3) system has been referred to as the pyridine system and the ethyl acetate-acetic acid-water (9:2:2) system has been referred to as the acid system.

QUANTITATIVE ANALYSIS

For quantitative estimation of the amounts of the various sugars in the various materials studied, a modification of the paper chromatographic procedure of Hirst and Jones (36) and Leech (41) was employed. The general procedure was as follows:

A known amount of the material was hydrolyzed with 3% sulfuric acid, or a known volume of the filtrate from the Klason lignin determination was used. To the hydrolyzate or filtrate portion was added a known amount of a reference sugar--rhamnose or ribose--in an amount roughly equal to one-half of the major sugar constituent. The solution was thoroughly mixed, heated on the steam bath and quickly neutralized with barium carbonate. It was immediately filtered through filter paper and asbestos as previously described (see page 12). Care was taken to thoroughly wash all the carbohydrate material from the precipitate and asbestos pad. The clear solution was passed through a cation ion exchange column (Ionac C-200). The column was washed with a volume of distilled water equal to 3 to 4

times that of the solution. The combined, deionized, slightly acidic solution and washings were concentrated to a thin sirup at 50° under reduced pressure.

Three paper strip chromatograms were prepared as previously described except that the width of the strips was increased to 14-16 cm. A light pencil line was drawn across the strip 8 cm. from the top. A fine line of the sirup was laid on the pencil line using a capillary tube. The chromatograms were placed in the pyridine system and allowed to develop for 24 hours. They were then removed from the tank, dried in a hood, and returned to the tank. After a second 24-hour period the drying procedure was repeated and the chromatograms again returned to the developing tank for 16 hours. This intermittent drying technique tended to compress the sugar bands and to sharpen the separation although the distance between the several bands was decreased. The technique was found particularly helpful when using ribose as a reference sugar or when spent liquor hydrolyzates were used. Ribose has an R_f value slightly greater than that of xylose. The difference in R_f value is sufficient for satisfactory separation of the two sugars in pyridine if care is taken.

After the final drying, guide strips approximately $2\frac{1}{2}$ cm. wide were cut from each side of the chromatograms. These were sprayed with either aniline hydrogen phthalate* or p-anisidine hydrochlorite* spray reagent. Both reagents give a pink

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*Preparation of Spray Reagents for Chromatographic Analysis.

1. Aniline hydrogen phthalate.

   To each 100 cc. of butanol saturated with distilled water add 1.66 grams of phthalic acid anhydride and 0.93 grams of aniline (42).

2. p-Anisidine hydrochloride.

   To 100 cc. of butanol saturated with water add 0.5 grams of p-anisidine hydrochloride. Hough, Jones, and Wadman (43) recommend a 3% solution of p-anisidine hydrochloride in butanol, but the ½% solution is very satisfactory.

color with pentose sugars, brown with hexoses, and a yellow-brown with methyl pentoses (rhamnose). The colors from the p-anisidine hydrochloride fade more rapidly than do those from aniline hydrogen phthalate. Chromatograms sprayed with the latter retain much of the color for twelve months or longer.

The sprayed strips were heated in an oven at 110° for 5 minutes to develop the colors and were then reattached to the chromatograms with scotch tape. The bands for the various sugars were marked on the unsprayed portion, and after suitable identification were cut out. A blank was also cut from a portion of the chromatogram which contained no sugar. When the intermittent drying technique was employed, it was found necessary to cut the blank strip from above the sugar bands or from between two of the bands. Blanks cut from near the bottom of the strip consumed much more periodate than those from higher up on the strip. Under the conditions of intermittent drying, the contaminants are apparently concentrated at or near the bottom of the sheet.

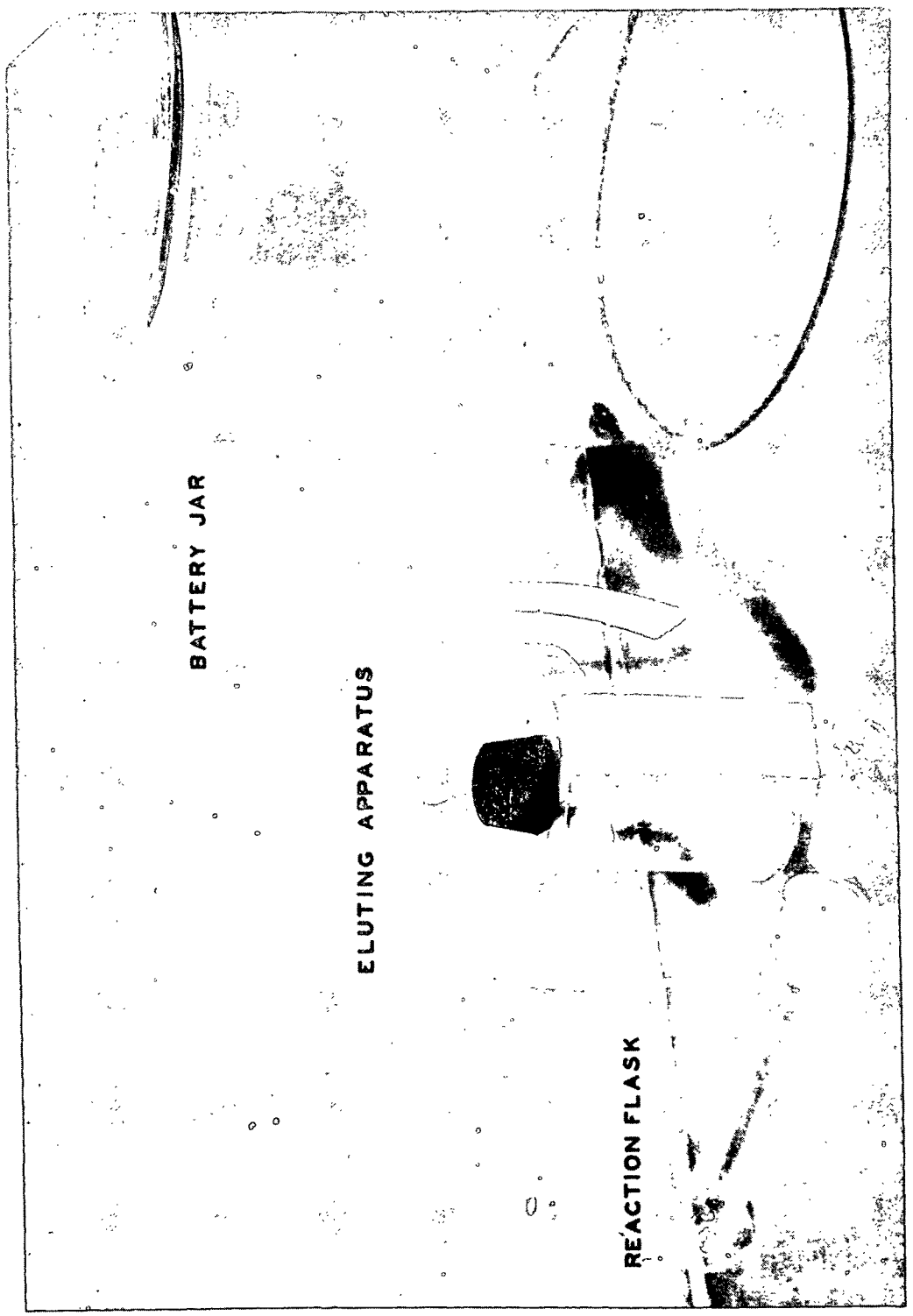
The sugar strips and the blank were placed between the ends of a pair of microscope slides which extended from an elevated receptacle containing distilled water. About 0.5 cm. of the strips were held by the slides, the remainder hung free. A battery jar was placed over the apparatus to decrease evaporation. The water moved by capillary action down the strips, maintaining a uniform front perpendicular to the length of the strip. When the front reached the bottom of the strip, a section 0.50 to 0.75 cm. in width was cut from the bottom and transferred carefully by means of tweezers to a reaction flask. The tweezers and the inner sides of the flask were carefully rinsed with distilled water.

The reaction flasks were glass tubes, with slightly enlarged bulbs at the bottom and equipped with ground glass stoppers. They were approximately 2.5 cm. in diameter and 22 cm. long. The bulb had a diameter of 4 cm. A photograph of a reaction flask and the eluting apparatus is shown on the following page (cf. Figure 1).

One cc. of a 0.25 molar solution of sodium periodate was added to each flask containing a paper section and rinsings, and the flasks were heated by direct steam in the steam bath for 12 minutes. After heating, they were immediately cooled under a cold water tap and placed in a container of cold water. To the cooled reaction solution, 0.5 cc. of ethylene glycol was added to consume any unused periodate. The flasks were shaken vigorously and allowed to stand 10 minutes for the reaction between the periodate and the glycol to be completed. The formic acid formed in the reaction was titrated with standard sodium hydroxide (ca. 0.004 N) to a greenish-yellow color, using methyl red as an indicator (0.02 g. methyl red in 60 cc. of 95% alcohol and 40 cc. distilled water).

The ratios of the weights of the various sugars to the weight of the reference sugars were calculated from the titration values after the latter were corrected for the blank and adjusted by the weight factors (see the sample calculation below). From these ratios the amounts of sugars in the sample were determined.

Theoretically hexose sugars yield five moles of formic acid per mole of sugar, pentoses four moles and methyl pentoses four moles. Hence one mole of formic acid or its equivalent of the sodium hydroxide solution used to titrate the formic acid formed in the oxidation step is equal to 36.03 g. of hexose sugars, 37.54 g. of pentose sugars, and 45.54 g. of rhamnose hydrate (rhamnose.H_2O). In order that one cc. of the titrating solution might represent the same weight of sugar in


BATTERY JAR
ELUTING APPARATUS
REACTION FLASK

every case, the volume of solution used to titrate the formic acid from the oxidation of hexoses with periodate was multiplied by 1.000, the volume of solution used to titrate the formic acid from the oxidation of pentoses was multiplied by 1.0413, and the volume of solution used to titrate the formic acid from the oxidation of rhamnose hydrate was multiplied by 1.2633.

Following is a sample calculation for the glucan and xylan in a carbohydrate material using rhamnose as a reference sugar.

DATA

Weight of material hydrolyzed, ovendry, ash free--0.2000 g.
Weight of rhamnose hydrate added to the hydrolyzate--0.0500 g.

	Original titration, cc.	Corrected for blank cc.	Adjusted for weight factor cc.	Weight ratio Sugar/rhamnose
Glucose	25.20	24.25	24.25	1.660
Xylose	18.75	17.80	18.55	1.270
Rhamnose	12.51	11.56	14.60	
Blank	0.95			

Calculations

Weight of glucan, g. 0.0500 x 1.660 x 0.90 = 0.0747

Weight of xylan, g. 0.0500 x 1.270 x 0.88 = 0.0559

(0.90 and 0.88 are the ratios of the molecular weight of a glucose and xylose anhydride unit respectively to the molecular weight of glucose or xylose.)

OTHER ANALYSES

Ash

Aspenwood shavings--Institute Method 4 (1952)
Other materials--Institute Method 422 (1952).

Lignin

Aspenwood shavings--Institute Method 13 (1952)
Other materials--Institute Method 428 (1952)
(cf. discussion of "apparent lignin" pages 69 to 78)

Pentosans

Institute Method 424 (1952).
Furfural in an approximate amount of 42% of the theoretical is given off by the uronic anhydride whereas carbon dioxide is given off in the theoretical amount (44, 45, 46). A correction to apply to the pentosan content as determined by furfural evolution was obtained from the following relationship:

$$\frac{\text{M.W. furfural}}{\text{M. W. carbon dioxide}} \times \frac{\text{M.W. pentosans}}{\text{M.W. furfural}} \times 0.42 \times \frac{1}{4} = 0.315$$

where M.W. = molecular weight

Therefore the corrected percentage of pentosans equals the percentage pentosans as determined by furfural evolution minus 0.315 times the percentage of uronic anhydride.

Uronic Anhydride

Institute Method 25 (1952).

VISCOSITIES OF THE HEMICELLULOSES

Dilute solution viscometric measurements in 10% potassium hydroxide were made of the hemicellulose by using Ostwald-Fenske viscometers at 30°C.

Thompson and Wise (19) and Wethern (18) have discussed the use of capillary viscometers to estimate the molecular weights of hemicelluloses. They pointed out that the method is not absolute but calibration for each polymer-solvent system must be made by an absolute method such as osmotic pressure, light scattering, or sedimentation equilibrium.

Molecular weights in narrow ranges, for example, from 15,000 to 30,000, can be calculated from the equation $[\eta] = K'M$. where K' is an experimentally determined

constant, M is the number average molecular weight, and $[\eta]$ is the intrinsic viscosity. $[\eta]$ is defined by $\frac{\lim}{c--0} - \frac{\eta sp}{c}$ where c is the concentration (usually given in grams per 100 cc. of solution) and ηsp is the specific viscosity, equal to $\frac{\eta solution - \eta solvent}{\eta solvent}$. Experimentally $[\eta]$ is determined by making viscosity measurements of several dilute solutions of the hemicellulose and plotting $\frac{\eta sp}{c}$ against c. Extrapolation of the curve to zero concentration of c gives $[\eta]$.

The equation $[\eta] = K'M$ can be written $[\eta] = K''(D.P.)$ where D.P. represents a number average degree of polymerization and K'' is the experimentally determined constant.

RECOVERY OF "HEMICELLULOSES" FROM THE SPENT LIQUORS

A "hemicellulosic" material was recovered from the NSSC spent liquors by a procedure developed in the present study which consisted of the following steps: (1) acidification with sulfuric acid to the methyl red end point, (2) precipitation of the larger lignosulfonic acid molecules with "Bis"* (47, 48, 49), (3) neutralization of the sulfuric acid with barium carbonate or barium hydroxide, (4) deionization after filtration and centrifugation with cation and anion ion exchange columns, (5) concentration under reduced pressure, and (6) precipitation in absolute ethanol. By varying the above conditions slightly, a precipitate practically free of lignin was obtained. It was possible to exclude step (2) and still obtain a precipitate. The precipitate, however, was dark grey in color and contained considerable lignin.

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*4,4'-bis-(dimethylaminophenyl) methane

EXPERIMENTAL RESULTS

GLOSSARY

The following symbols will be used to indicate the aspenwood, pulps, spent liquors, hemicelluloses and residues. In these symbols W designates aspenwood, P designates a pulp, SL designates a spent liquor, D and a numeral designates a hemicellulose obtained by direct extraction of the wood or pulp, H and a numeral designates a hemicellulose obtained by extraction of a holocellulose, R designates a residue obtained from a pulp or wood by extraction.

SYMBOL	MATERIAL
W	Aspenwood shavings.
W-D5	Hemicellulose extracted with 5% potassium hydroxide from aspenwood shavings.
W-D16	Hemicellulose extracted with 16% potassium hydroxide from the aspenwood shavings previously extracted with 5% potassium hydroxide.
W-DR	Residue from the aspenwood shavings after extraction with 5% and 16% potassium hydroxide.
W-H	Holocellulose prepared from aspenwood shavings by the Thomas procedure.
W-H5	Hemicellulose extracted from W-H with 5% potassium hydroxide.
W-H16	Hemicellulose extracted with 16% potassium hydroxide from W-H previously extracted with 5% potassium hydroxide.
W-HR	Residue from W-H after extraction with 5% and 16% potassium hydroxide.
P2	Pulp from NSSC cook 2.
P3	Pulp from NSSC cook 3.
P4	Pulp from NSSC cook 4.
P2-D5	Hemicellulose extracted from pulp 2 with 5% potassium hydroxide.
P2-D16	Hemicellulose extracted with 16% potassium hydroxide from pulp 2 which had been previously extracted with 5% potassium hydroxide.

P2-H	Holocellulose prepared from pulp 2 by the Thomas procedure.
P2-H5	Hemicellulose extracted from P2-H with 5% potassium hydroxide.
P2-H16	Hemicellulose extracted with 16% potassium hydroxide from P2-H which had been previously extracted with 5% potassium hydroxide.
P2-HR	Residue from P2-H after extraction with 5% and 16% potassium hydroxide.
2-SL	Spent liquor from NSSC cook 2.
3-SL	Spent liquor from NSSC cook 3.
4-SL	Spent liquor from NSSC cook 4.
2-SL-B	Hemicellulose recovered from 2-SL.
3-SL-B	Hemicellulose recovered from 3-SL.
4-SL-B1	Hemicellulose recovered from 4-SL (first sample).
4-SL-B2	Hemicellulose recovered from 4-SL (second sample).

NSSC PULPING CONDITIONS AND EXPERIMENTAL DATA

Three NSSC cooks were made during this study. The pulping conditions and experimental data for these cooks are listed in Table I.

Chromatograms were prepared of the spent liquors (2-SL, 3-SL, and 4-SL) and developed in the pyridine and in the acid tank. Portions of the spent liquors were passed through a cation ion exchange column (Ionac C-200) and through an anion column (Duolite A-2). The solutions were then concentrated to thin sirups and chromatographed as described above. There was no evidence of any free sugars or of any disaccharides or trisaccharides. By quantitative chromatographic analysis of 4-SL, sugar anhydride units equal to 4.14% of the original wood were determined to be present in spent liquor 4-SL.

TABLE I

PULPING CONDITIONS, PULP AND SPENT LIQUOR DATA

	Cook Number		
	2	3	4
Chemical			
Sodium sulfite as Na_2SO_3,%	12	12	12
Sodium carbonate as Na_2CO_3,%	5	7	5.25
Water ratic cc/g[a]	4	4	4
Maximum temperature, °C.	170	170	170
Time to max. temp., min.	120	120	120
Time at max. temp., min.	90	90	90
Maximum pressure, p.s.i.	129	121	128
Relief time, min.	15	15	15
Chip charge, ovendry, g.	3314	3314	3314
Pulp yield, ovendry, g.	2470	2453	2482
Pulp yield, %[b]	74.5	74.0	74.9
Spent liquor			
Volume, cc.	4850	5020	--
pH	6.5	9.1	7.4
Solids, g./ 100 cc.	10.3	12.0	--
Wash liquor[c]			
Volume, cc.	17,550	14,000	--
Solids, g./ 100 cc.	2.9	3.9	--

[a]Distilled water was used to make up the cooking liquors.

[b]Ovendry wood basis

[c]After extraction of the spent liquor the chips were soaked overnight in distilled water and the liquor was extracted and designated as wash liquor.

TABLE I (continued)

PULPING CONDITIONS, PULP AND SPENT LIQUOR DATA

	Cook Number		
	2	3	4
Combined spent liquor and wash liquor[d]			
Volume, cc.			46,500
Solids, g./ 100 cc.			2.97
Total solids in liquors, g.	1007	1148	1380

[d]After recovery of the spent liquor the chips were covered with distilled water and heated on the steam bath for four hours. The liquor was drained off and the chips were soaked overnight in distilled water and the liquor again drained off. The spent liquor and the two wash liquors were combined.

HEMICELLULOSE YIELDS AND ANALYSES
(OTHER THAN SUGAR ANALYSES)

The percentage yields and analytical data other than sugar analyses and viscosities for the hemicelluloses are listed in Tables II, III, and IV.

TABLE II

HEMICELLULOSE YIELDS

	Grams[a]	Percentage of wood[b]	Percentage of pulp[b]	Percentage of holocellulose[c]
W-D5	7.84	10.5		
W-D16	3.62	4.85		
W-H5	11.10	17.7		22.3
W-H16	3.04	4.8		6.1
P2-D5	9.78	9.75	13.2	
P2-D16	4.38	4.35	5.9	
P2-H5	8.69	11.0	14.9	17.4
P2-H16	2.99	3.8	5.15	6.0
W-D5/W-D16	11.46	15.35		
W-H5/W-H16	14.14	22.5		28.4
P2-D5/P2-D16	14.16	14.1	19.1	
P2-H5/P2-H16	11.68	14.8	20.1	23.4

[a]Ovendry, ash free

[b]Ovendry, extractive and ash-free basis

[c]Ovendry, ash-free basis

[a,b,c]All corrections for ash in this and subsequent tables are for the residue after ignition at red heat (ca. 600°).

TABLE III

HEMICELLULOSE ANALYSES

	Percentage ash[a]	Percentage lignin[b]	Percentage pentosans[b,c]	Percentage Uronic anhydride[b]	Color
W-D5	7.79	2.0	77.5	15.72	cream
W-D16	6.83	4.5	74.6	12.60	light tan
W-H5	9.02	0.9	71.7	20.09	grey white
W-H16	7.38	0.4	72.6	17.66	white
P2-D5	7.61	2.0	81.2	11.08	grey white
P2-D16	4.92	1.6	79.3	10.47	grey white
P2-H5	7.05	0.4	77.2	12.54	white
P2-H16	6.16	0.4	81.7	11.22	white

[a]Ovendry basis

[b]Ovendry, ash-free basis

[c]Corrected for uronic anhydride

TABLE IV

ANALYSES OF ASPENWOOD, PULP 2, HOLOCELLULOSES, AND RESIDUES

	Percentage ash	Percentage lignin	Percentage pentosans	Percentage uronic anhydride	Percentage yield
W	0.49[a]	18.0[d]	17.3[d]	6.07[d]	
W-DR	1.47[b]	21.8[c]	7.3[c]	4.32[c]	78.0[d]
W-H	0.46[b]	0.6[c]	20.3[c]	7.94[c]	79.4[d]
W-HR	0.45[b]	0.5[c]	1.3[c]	1.84[c]	62.3[e]
P2	1.40[a]	12.3[d]	17.7[f]	4.14[d]	73.6[d]
P2-DR	0.86[b]	13.7[c]	1.5[c]	3.64[c]	80.6[f]
P2-H	0.06[b]	0.4[c]	19.3[c]	3.87[c]	85.7[f]
P2-HR	0.18[b]	0.4[c]	1.2[c]	1.34[c]	75.8[e]

[a]Ovendry, extractive free basis

[b]Ovendry basis

[c]Ovendry, ash free basis

[d]Ovendry, ash free, extractive free wood basis

[e]Ovendry, ash free holocellulose basis

[f]Ovendry, ash free, extractive free pulp basis

The hemicellulose yield data in Table II show that 7.7% of the weight of the wood or 34.2% of the hemicelluloses were removed in NSSC cook 2*. The loss of pentosans (4.3% of the weight of the wood) and uronic acids (3.04% of the weight of the wood) during the cook was almost equal to the hemicelluloses removed, 7.3% as compared to 7.7% (see Table IV).

One of the interesting results shown by the data in Table II is that the hemicelluloses extracted from pulp 2 were equal to 95.3% of the hemicelluloses extracted from pulp 2 holocellulose, while under similar conditions the hemicelluloses extracted from aspenwood shavings were equal to only 68.2% of those extracted from aspenwood holocellulose. The hemicelluloses extracted from pulp 2 amounted to 91.8% of those extracted from the aspenwood shavings, but the hemicelluloses extracted from pulp 2 holocellulose amounted to only 65.8% of those extracted from aspenwood holocellulose. The hemicelluloses in pulp 2 apparently are much more accessible to caustic extraction than those in the aspenwood shavings.

More of the hemicelluloses were extracted from the holocelluloses by 5% potassium hydroxide than were extracted from the aspenwood shavings or from pulp 2 by 5% potassium hydroxide. The holocellulose preparation procedure also seems to increase the availability of the hemicelluloses to caustic extraction although the increase is not as great as for the pulp.

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*Total hemicelluloses are defined as the sum of the 5% and 16% potassium hydroxide extracts from the holocelluloses.

The hemicelluloses extracted from pulp 2 or pulp 2 holocellulose have in general a higher pentosan content and a lower uronic acid content than the corresponding ones extracted from aspenwood shavings or aspenwood holocellulose. The uronic acid fractions of the hemicelluloses are apparently more easily removed in the NSSC cook or by 5% potassium hydroxide than are the pentosan fractions. The pentosan content of the pulp was 17.7% as compared to 17.3% for the aspenwood shavings; on the other hand, the percentage of uronic anhydride in the pulp was 4.14 as compared to 6.07 in the aspenwood shavings. Likewise all the hemicelluloses extracted with 5% potassium hydroxide have higher percentages of uronic acids than do the 16% extracts from the same materials. Similar results were noted by Wise and Ratliff (17). This relationship does not hold for the pentosan contents of the hemicelluloses.

The hemicelluloses from the pulp were in general less colored than the corresponding ones from the aspenwood.

The analytical and yield data in Tables II, III, and IV indicate that:

(1) Roughly one third of the hemicelluloses were removed in the NSSC cook. The loss of pentosans and uronic acids during the cook was nearly equal to the loss of hemicelluloses.

(2) The NSSC cook rendered the hemicelluloses remaining in the pulp much more accessible to caustic extraction, 95.3% being extracted from the pulp as compared to 68.2% from the wood.

(3) The hemicelluloses from pulp 2 have a higher pentosan content, a lower uronic anhydride content, and are less colored than the corresponding ones from the wood.

QUALITATIVE SUGAR ANALYSIS

The sugars present and the amounts of each in the hemicelluloses, original aspenwood, pulp 2, holocellulose, and residues are listed in Tables V and VI. The sugars were identified by their position on paper strip chromatograms when separated alongside of known sugars. Jones and Wise (29) have adequately shown by chemical means that L-rhamnose, L-arabinose, D-xylose, and D-galactose are present in a hydrolyzate of aspenwood (Populus tremuloides). For convenience, these sugars will be referred to as rhamnose, arabinose, xylose, and galactose. Jones and Wise did not identify mannose in the aspenwood hydrolyzate by any chemical test. They did, however, tentatively identify mannose by its position on paper strip chromatograms.

The presence of mannose in aspenwood hydrolyzates has been indicated by paper strip chromatograms (27, 29). Further work to establish the presence of mannose in a hydrolyzate of aspenwood is shown on page 61.

The hemicelluloses contain predominantly xylose sugar units. W-D16 and P2-D16 contained appreciable amounts of glucose units (7.6 and 4.2% respectively); W-D16 also contained measurable amounts of galactose, mannose, and arabinose units. The other hemicelluloses contained only traces of sugar units other than xylose. The hydrolyzates of the 16% potassium hydroxide extracts contained at least traces of all six sugar units shown to be present in aspenwood. The hydrolyzates of the hemicelluloses from pulp 2 were quite similar in sugar contents to the corresponding ones from the aspenwood except for W-D16 and P2-D16. W-D16 contained appreciably more glucose, galactose, mannose, and arabinose units than did P2-D16. This is to be expected as the spent liquor hydrolyzate contained measurable amounts of each of the sugars found in aspenwood

TABLE V

SUGARS FOUND BY CHROMATOGRAPHY IN HYDROLYZATES OF THE HEMICELLULOSES[a]

	Glucose, %[b]	Galactose, %[b]	Mannose, %[b]	Xylose, %[b]	Arabinose, %[b]	Rhamnose, %[b]
W-D5				100-		slight trace
W-D16	7.6	3.0	1.0[c]	87.4	1.0[c]	slight trace
W-H5	trace	trace		100-	trace	slight trace
W-H16	trace	trace	slight trace	100-	slight trace	slight trace
P2-D5				100-		slight trace
P2-D16	4.2	slight trace	slight trace	95.8	slight trace	slight trace
P2-H5	trace	trace		100-	slight trace	slight trace
P2-H16	trace	trace	slight trace	100-	slight trace	slight trace

[a] Does not include the sugar units, such as xylose, which may constitute part of the aldobiuronic acid molecules.

[b] Percentage as a pentosan or hexosan of the total pentosans and hexosans present. Percentages were calculated from the quantitative analysis data.

[c] Mannans and arabans were determined together and each was arbitrarily assumed to constitute half of the total.

TABLE VI

SUGARS FOUND BY CHROMATOGRAPHY IN HYDROLYZATES OF ASPENWOOD SHAVINGS, PULP 2, HOLOCELLULOSES, RESIDUES, AND SPENT LIQUORS[a]

	Glucose, %[b]	Galactose, %[b]	Mannose, %[b]	Xylose, %[b]	Arabinose, %[b]	Rhamnose, %[b]
W	68.5	trace	1.3[c]	29.0	1.3[c]	trace
W-DR	88.3	slight trace	1.1[c]	9.5	1.1[c]	slight trace
W-H	70.0	1.2	1.4[c]	25.9	1.4[c]	slight trace
W-HR	100-		trace	trace	slight trace	
P2	76.0	slight trace	trace	24.0	slight trace	slight trace
P2-DR	97.7		slight trace	2.3		
P2-H	74.2	slight trace	0.9	24.9	slight trace	slight trace
P2-HR	96.4		1.6	2.0		
2-SL	4.1	8.9	2.9	75.3	6.2	2.6
3-SL	8.1	9.5	2.3	66.6	7.6	5.8
4-SL	6.4	9.8	3.5	69.9	6.1	4.3

[a] Does not include the sugar units, such as xylose, which may constitute part of the aldobiuronic acid molecules.

[b] Percentage as a pentosan or hexosan of the total pentosans and hexosans present. Percentages were calculated from the quantitative analysis data.

[c] Mannans and arabans were determined together and each was arbitrarily assumed to constitute half of the total.

hydrolyzates. In fact, the galactose, mannose, arabinose and rhamnose units appeared to be extracted almose preferentially in the cook. On the other hand, they appeared to be resistant to alkali extraction at room temperature, inasmuch as they were found in the 16% potassium hydroxide extracts to a greater extent than in the 5% extracts. This was particularly true of W-D16.

The rhamnose units were almost completely removed in the cook. There was a slight trace found in all the hemicelluloses and the other materials except the residues left after the extraction of the hemicelluloses from the wood holocellulose and the pulp holocellulose.

W-D5 and P2-D5 contained only xylose units except for a trace of rhamnose. W-H5 and P2-H5 contained traces of all the sugar units except mannose. This would seem to be in agreement with the yield data which indicate that the holocellulose preparation procedure makes the hemicelluloses more accessible to caustic extraction. It should be noted, however, that glucose units are apparently not easily extracted from the holocellulose, inasmuch as hydrolyzates of the 16% potassium hydroxide extracts from the holocelluloses (W-H16 and P2-H16) contained only traces of glucose while the corresponding extracts from the wood and pulp (W-D16 and P2-D16) contained 7.5 and 4.6% glucose respectively.

The sugar data indicate that:

(1) In general, the hydrolyzates of the hemicelluloses from pulp 2 contain the same sugars in approximately the same proportions as the hydrolyzates of the corresponding hemicelluloses from aspenwood.

(2) The galactose, mannose, arabinose, and particularly rhamnose units appear to have been removed proportionately more fully than have the xylose and glucose units in NSSC cook 2.

QUANTITATIVE SUGAR ANALYSIS

EVALUATION OF THE METHOD OF ANALYSIS

The results of the quantitative sugar determinations are listed in Tables V, page 32, VI, page 33, and VII, page 36. The "percentage of the adjusted sample" in Table VII was calculated by dividing the weight of the sugar anhydride units obtained by chromatographic analysis by an adjusted sample weight. (The adjusted sample weight was determined by subtracting the weight of lignin and uronic anhydride from the ovendry, ash free sample weight.) The adjusted sample weight was not corrected for the pentose or hexose sugar units combined with the uronic acids to form difficultly hydrolyzable compound uronic acids. Jones and Wise (29) have identified by chemical means the presence of an aldobiuronic acid, 2-α-(4-methyl D-glucuronosyl) α-D-xylose, in the acid hydrolyzate of aspenwood. They found that very drastic conditions were required to produce xylose and 4-methyl-D-glucuronic acid from the aldobiuronic acid. It is not probable that xylose would be obtained from such an aldobiuronic acid under the comparatively mild hydrolysis used in preparing the materials for chromatographic analysis.

The sum of the weights of xylan and araban as determined by chromatographic analysis was multiplied by factors calculated from Krŏbers' tables in order that they would be on the same basis as the pentosans determined by furfural evolution. This sum was then multiplied by 100 and divided by the ovendry, ash free sample weight to give a chromatographically determined pentosan value for comparison with the pentosan value obtained by furfural evolution. Rhamnose was used as a reference sugar in most of the quantitative sugar analyses. It was replaced by ribose in later analyses although both sugars were used simultaneously in

TABLE VII

QUANTITATIVE SUGAR DETERMINATIONS

Material Hydrolyzed	Type of Hydrolysis	Reference Sugar	Adjusted Sample, %	Pentosans (calculated), %	Pentosans by Furfural Determination, %[a]
W-D5	lignin filtrate	rhamnose	70.9	64.7	77.5
W-D5	lignin filtrate	rhamnose	76.2	69.5	77.5
W-D5	3% sulfuric acid	rhamnose	93.0	84.6	77.5
W-D5	3% nitric acid	rhamnose	69.8	63.5	77.5
W-D16	lignin filtrate	rhamnose	87.2	69.8	74.6
W-H5	lignin filtrate	rhamnose	65.7	57.9	71.7
W-H5	3% sulfuric acid	rhamnose	65.8	57.5	71.7
		ribose	80.0	69.8	71.7
W-H16	lignin filtrate	rhamnose	77.6	70.2	72.6
		ribose	98.2	88.9	72.6
P2-D5	lignin filtrate	rhamnose	72.5	69.7	81.2
P2-D5	lignin filtrate	rhamnose	74.5	71.5	81.2
		ribose	80.0	76.6	81.2
P2-D5	2% sulfuric acid	rhamnose	80.8	77.8	81.2
P2-D5	1% sulfuric acid	rhamnose	71.6	69.0	81.2
P2-D5	1% nitric acid	rhamnose	69.9	67.2	81.2
P2-D16	lignin filtrate	rhamnose	102.7	95.6	79.3
P2-D16	3% sulfuric acid	rhamnose	76.4	74.3	79.3
		ribose	86.3	83.0	79.3
P2-H5	lignin filtrate	rhamnose	72.6	69.6	77.2
P2-H5	3% sulfuric acid	rhamnose	72.0	69.0	77.2
		ribose	102.2	98.0	77.2
P2-H16	lignin filtrate	rhamnose	77.6	74.5	81.7
		ribose	87.7	85.6	81.7

[a] Corrected for uronic anhydride

TABLE VII (Continued)

QUANTITATIVE SUGAR DETERMINATIONS

Material Hydrolyzed	Type of Hydrolysis	Reference Sugar	Adjusted Sample, %	Pentosans (calculated), %	Pentosans by Furfural Determination, %[a]
W	lignin filtrate	rhamnose	66.5	16.8	17.3
W	lignin filtrate	rhamnose	67.0	16.2	17.3
W-DR	lignin filtrate	rhamnose	93.0	7.5	7.3
W-H	lignin filtrate	rhamnose	86.0	22.4	20.3
W-HR	lignin filtrate	rhamnose	94.5		1.3
P2	lignin filtrate	rhamnose ribose	82.7 96.0	17.0 19.8	17.7 17.7
P2-DR	lignin filtrate	ribose	81.4	1.7	1.5
P2-H	lignin filtrate	rhamnose	81.8	21.6	19.3
P2-HR	lignin filtrate	rhamnose	73.2	1.8	1.2
P2-HR	lignin filtrate	rhamnose	76.9	1.4	1.2
4-SL-B1	3% sulfuric acid	ribose	74.5	57.8	64.7
4-SL-B2	3% sulfuric acid	ribose	75.7	62.7	69.6

[a] Corrected for uronic anhydride

a number of analyses to provide a basis of comparison of the relative merits of each as a reference sugar. Ribose was shown to be a much better reference sugar than rhamnose for the analysis of the type of materials studied in this investigation. Furthermore, although not shown by the tabulated data, more precise results between samples were obtained with ribose than with rhamnose.

The quantitative sugar analyses did not give completely satisfactory results. This is shown by the percentage of "adjusted sample" data, where with rhamnose as a reference sugar, the percentage of the "adjusted sample" varied from 65 to 102.7%. The results with ribose were much better, with 75 to 102.2% of the adjusted sample being accounted for.

Sugar analyses were made for the most part on lignin filtrates. Several strengths of sulfuric acid (1, 2, and 3%) and nitric acid (1 and 3%) were investigated as hydrolytic agents. Nitric acid appeared to give poorer results than sulfuric acid and had to be neutralized on an anion ion exchange column. Three per cent sulfuric acid seemed to be the most effective hydrolytic agent for hemicelluloses, although 1 and 2% sulfuric were nearly as effective. Treatment with 72% sulfuric acid (as in the lignin determination) seemed essential for complete hydrolysis of the materials which contained cellulose. Cellobiose and cellotriose appeared to be present in some of the hydrolyzates even under these more severe hydrolytic conditions.

Following are listed some of the possible drawbacks to the method of quantitative sugar analysis as used in this study:

1. Degradative effects of hydrolysis step on certain of the sugar groups--xylan in particular.

2. Incomplete hydrolysis of cellulose to glucose. This is suggested by the low percentage of adjusted sample for the analyses of the aspenwood and by the presence of spots, which have been tentatively identified as cellobiose and cellotriose, on the chromatograms of certain lignin filtrates from materials containing large amounts of cellulose.

3. The materials analyzed were not pure carbohydrates but were heterogeneous materials containing lignin, ash and uronic acids. Even the hemicelluloses which are essentially 100% xylan (W-D5 and P2-D5) contain about 5% of material which is not accounted for by a summation of the analyses--ash, lignin, uronic acid and pentosans.

RHAMNOSE AS A REFERENCE SUGAR

As previously stated, rhamnose was not as effective as a reference sugar as was ribose. The data of Hirst and Jones (36) were examined and no reason for this behavior of rhamnose in the present experimental work could be explained by their data.

The following examination of rhamnose as a reference sugar was made. Chromatograms were prepared from a hydrolyzate of W-H16 which contained both ribose and rhamnose as reference sugars, and from which ribose gave much better quantitative results (see Table VII). The chromatograms were developed for 48 hours in the pyridine developing system. The rhamnose sections were eluted from several of these, the eluate concentrated and re-chromatographed for 19 hours in the acid system along with the concentrated rhamnose eluate from a known sugar mixture containing glucurone. When the chromatograms were dried and sprayed with p-anisidine hydrochloride spray reagent, a spot which from its color and position appeared to be a uronic acid was found above both

the rhamnose spots from the hydrolyzate and from the known sugar solution.

To check the possibility that the reference rhamnose might be contaminated, a sirup was made of rhamnose taken from the same bottle from which the reference rhamnose was obtained. Chromatograms of this sirup showed no traces of uronic acid or other carbohydrate impurities. It appears that when a uronic acid containing material is hydrolyzed and chromatographed in the pyridine system, a portion of the uronic acid, probably in lactone form, moves down to approximately the same position as does rhamnose. This uronic acid, if present, would be eluted with the rhamnose and consume periodate in the oxidation step.

XYLOSE DEGRADATION DURING HYDROLYSIS

The analytical data suggest that xylose undergoes degradation in the hydrolysis step. That there was some degree of degradation was indicated by the high apparent lignin values (cf. discussion on apparent lignin, pages 52 to 61). Sohn (50) has shown that xylose solution with an initial pH of 6.5 when refluxed forms increasing amounts of furfural as the time of refluxing is increaces. Rollinson and Wise (51) suggest that sugar degradation products may contribute to the apparent lignin in a lignin filtrate. They found that filtrates from "lignin determinations" on pure xylose and Yundt's xylan (52) had sharp maxima in their optical densities at 275 mmu. The ultraviolet absorption curves of these filtrates resembled a typical furfural ultraviolet absorption curve.

To study the degradation of xylose under the conditions of the hydrolysis procedures used for sugar analysis, the following experiments were carried out.

a. A solution of xylose, rhamnose and ribose (0.1545 g. xylose, 0.0589 g. ribose, and 0.0678 g. rhamnose) was prepared and divided into two portions. One portion was immediately concentrated at 50°. Quantitative chromatograms were made and developed in the pyridine system. The sugars were eluted, oxidized, and the resultant formic acid titrated in the usual manner. The second portion was acidified to the methyl red end point with 6N sulfuric acid and immediately neutralized with barium carbonate. The barium sulfate and excess barium carbonate were removed by filtration and the filtrate was passed through a cation ion exchange column. The sugar analysis was then completed as described for the first portion.

b. A solution of xylose and rhamnose (0.2091 g. xylose and 0.1251 g. rhamnose) was acidified with sulfuric acid to the methyl red end point and immediately neutralized with barium carbonate. The barium sulfate and excess barium carbonate were removed by filtration and the filtrate was passed through a cation ion exchange column. The resulting solution was analyzed for sugar content as described in a.

c. Xylose (0.2003 g.) was boiled in 260 cc. of 3% sulfuric acid for four hours at constant volume. Rhamnose (0.1086 g.) was added to the solution and the solution analyzed for sugar content in the usual manner.

d. Xylose (0.2018 g.) was treated as described in c except that both rhamnose (0.0617 g.) and ribose (0.0656 g.) were added as reference sugars.

e. A sample (0.0469 g.) of Yundt's xylan (52) was hydrolyzed with 3% sulfuric acid for four hours at constant volume. Rhamnose (0.0261 g.) and ribose (0.0246 g.) were added as reference sugars and the quantitative analyses completed as was previously described.

The results of these experiments are listed in Table VIII.

TABLE VIII

QUANTITATIVE SUGAR DETERMINATIONS
OF KNOWN SUGARS AND YUNDT'S XYLAN

	Type of Hydrolysis	Reference Sugar	Percentage of Adjusted Sample
Xylose	none	rhamnose	97.6
		ribose	101.3
Xylose[a]	none	rhamnose	95.9
		ribose	100.3
Xylose[a]	none	rhamnose	97.5
Xylose	3% sulfuric acid	rhamnose	79.1
Xylose	3% sulfuric acid	rhamnose	82.8
		ribose	93.7
Yundt's xylan	3% sulfuric acid	rhamnose	85.3
		ribose	98.9

[a]Acidified with 6N sulfuric acid, immediately neutralized with barium carbonate, filtered and put through a cation column before being concentrated.

These experiments show that:

(1) apparently no xylose is lost by degradation in the neutralization, filtering, deionizing, or chromatographic steps. If xylose is lost the reference sugars are lost proportionally.

(2) Less than 10% of the xylose is destroyed when it is boiled with sulfuric acid for four hours. It should be noted that for most of the materials with less than 20% pentosans (see Table VII), the percentage pentosans as determined by sugar analyses were within 10% of the percentage as determined by furfural evolution.

(3) Less than 5% of Yundt's xylan is destroyed when it is hydrolyzed with 3% sulfuric acid for four hours.

(4) Quite consistent results were obtained for like samples and conditions.

(5) Ribose gave better results as a reference sugar than did rhamnose. It should be noted that rhamnose gives poorer results with hydrolyzed material than with sugar solutions or acidified sugar solutions even though the material hydrolyzed was xylose or Yundt's purified xylan.

CONCLUSIONS FROM THE QUANTITATIVE SUGAR ANALYSES

The following conclusions are indicated by the quantitative sugar analysis data.

1. Better results were obtained with ribose as a reference sugar than with rhamnose.

2. Some loss of xylose occurs in the hydrolysis step, but the loss is probably less than 10%.

3. The hydrolysis step appears to be responsible for much of the inaccuracy of the method.

4. The quantitative sugar analysis as used in this study has certain inaccuracies and more study should be made of the procedure to "sharpen up" the analysis. However, the present method gives a relatively rapid estimation of the sugar contents of carbohydrate containing materials and gives reasonable precision.

VISCOSITY MEASUREMENTS OF THE HEMICELLULOSES

The intrinsic viscosities of the hemicellulose prepared from aspenwood shavings, aspenwood holocellulose, pulp 2, and pulp 2 holocellulose, the calculated degrees of polymerization (D.P.'s) and molecular weights (M.W.'s) are shown in Table IX.

TABLE IX

INTRINSIC VISCOSITIES, DEGREES OF POLYMERIZATION AND MOLECULAR WEIGHTS OF THE HEMICELLULOSES

Hemicellulose	Intrinsic Viscosity	Degree of Polymerization[a,b]	Molecular Weight[a]
W-D5	0.54	123	16,200
W-D16	0.67	152	20,100
W-H5	0.59	134	17,700
W-H16	0.76	173	22,800
P2-D5	0.63	143	18,900
P2-D16	0.68	155	20,400
P2-H5	0.68	155	20,400
P2-H16	0.71	161	21,300

[a]Number average

[b]As a pentosan

The D.P's of the hemicellulose fractions are quite similar to those reported by Thompson and Wise (19) who extracted similar fractions from big tooth aspen. They extracted aspenwood meal with 5% followed by 16% potassium hydroxide and extracted a Thomas holocellulose made from the wood meal in like manner. They found D.P.'s of from 140 to 170 for these hemicelluloses.

The data in Table IX suggest that the shorter chain hemicelluloses are preferentially removed in the pulping procedure, for the D.P.'s of the 5% potassium hydroxide extracts from pulp 2 or pulp 2 holocellulose are higher than the corresponding 5% extracts from the aspenwood shavings or aspenwood holocellulose. Also the difference in D.P.'s between the 5% extract and the 16% extract is much greater for the hemicelluloses

prepared from aspenwood shavings or aspenwood holocellulose than the corresponding hemicelluloses prepared from pulp 2 or pulp 2 holocellulose.

The hemicelluloses extracted from the wood holocelluloses had higher D.P.'s than those extracted directly from the wood shavings. Likewise the hemicelluloses extracted from pulp 2 holocellulose had higher D.P.'s than those extracted directly from pulp 2. The yield of hemicelluloses was greater from the holocelluloses than from the wood or pulp (on an ovendry, extractive free, ash-free wood basis; the yields were 22.5% from the wood holocellulose, 15.4% from the wood, 14.8% from pulp 2 holocellulose and 14.1% from pulp 2). It appears that in a Thomas holocellulose either because of the preparation procedure or because of the removal of most of the lignin, the longer-chain hemicelluloses become more accessible for extraction. That there is an increase in caustic accessibility in the holocellulose is further substantiated by the fact that 78.5% of the hemicelluloses extracted from the aspenwood holocellulose were extracted by the 5% potassium hydroxide solution while only 68.4% of the hemicelluloses extracted from the aspenwood shavings were extracted with 5% potassium hydroxide.

The same relationship was found for the hemicelluloses extracted directly from pulp 2 and from pulp 2 holocellulose. From the holocellulose, 74.4% of the hemicellulose were extracted by 5% potassium hydroxide while 69.0% of the hemicelluloses were extracted by the same strength caustic from the pulp.

The data in Table IX do not take into account the relative amounts of the hemicelluloses extracted by 5% and by 16% potassium hydroxide. Wales, Williams, Thompson, and Ewart (53) have shown that for a sample of polystyrene, a summation of the weight of each of 12 fractions times its intrinsic viscosity was

essentially equal to the weight of the unfractionated sample times its intrinsic viscosity. If the assumption is made that this relationship would hold true for the sum of the two hemicellulose fractions (5% potassium hydroxide extract and 16% potassium hydroxide extract) from each starting material, then an intrinsic viscosity can be calculated for each "summative hemicellulose". These data are shown in Table X.

TABLE X

INTRINSIC VISCOSITY, DEGREE OF POLYMERIZATION, AND MOLECULAR WEIGHTS FOR SUMMATIVE HEMICELLULOSES

Hemicellulose	Intrinsic Viscosity	Degree of Polymerization[a,b]	Molecular Weight[a]
W-D5/W-D16 (extd. from aspenwood)	0.58	132	17,400
W-H5/W-H16 (extd. from aspenwood holocellulose)	0.63	143	18,900
P2-D5/P2-D16 (extd. from pulp 2)	0.64	146	19,200
P2-H5/P2-H16 (extd. from pulp 2 holocellulose)	0.69	157	20,700

[a]Number average

[b]Calculated as pentosan

The data in Table X show that the D.P. of the "summative hemicelluloses" extracted directly from pulp 2 or extracted from pulp 2 holocellulose are significantly higher than the corresponding ones from aspenwood shavings or aspenwood holocellulose.

The viscosity data indicate that:

(1) Hemicelluloses extracted from pulp 2 or from pulp 2 holocellulose have a higher D.P. than the corresponding ones extracted from aspenwood shavings or aspenwood holocellulose.

(2) The shorter chain hemicellulose molecules are preferentially removed by the NSSC cook.

(3) The NSSC pulping conditions used in preparing pulp 2 apparently did not degrade the hemicelluloses remaining in the pulp and left a substantial portion of the hemicelluloses removed from the pulp in a relatively undegraded state.

(4) In the preparation of the Thomas holocelluloses, the hemicelluloses become more accessible to the 5% potassium hydroxide solution.

"HEMICELLULOSES" PRECIPITATED FROM THE SPENT LIQUORS

"Hemicelluloses" were precipitated from the spent liquors, 2-SL, 3-SL, and 4-SL. In the case of spent liquor 4-SL two were prepared.

The "hemicelluloses" were analyzed for moisture, ash, uronic anhydride, pentosans, sugar contents of hydrolyzates, and viscosities. In addition the material not precipitated by alcohol was recovered, hydrolyzed and the hydrolyzate analyzed for sugar content. These data are shown in Tables XI, XII, and XIII.

TABLE XI

ANALYSES OF THE HEMICELLULOSES PRECIPITATED BY ALCOHOL FROM THE SPENT LIQUORS

	2-SL-B	3-SL-B	4-SL-B1	4-SL-B2
Yield, %[a]	1.35	1.04	1.95	1.61
Moisture, %[b]	10.98	11.04	7.56	7.48
Ash, %[c]	3.12	5.41	8.75	9.26
Uronic anhydride, %[d]			13.20	14.15
Pentosans, %[d]			64.7	69.6
Residue on hydrolysis, %[d]			3.7	negligible
Yield of carbohydrate not precipitated by alcohol[a]			0.1	0.2

[a] Ovendry wood basis

[b] Determined by loss in weight at 60°, in vacuo.

[c] Ovendry basis

[d] Ovendry, ash-free basis

TABLE XII

SUGARS FOUND BY CHROMATOGRAPHY IN THE HYDROLYZATES OF THE "HEMICELLULOSES" PRECIPITATED BY ALCOHOL FROM THE SPENT LIQUORS

	Glucose, %[b]	Galactose, %[b]	Mannose, %[b]	Xylose, %[b]	Arabinose, %[b]	Rhamnose, %[b]
2-SL-B	trace	present	--	91.4	8.6	--
3-SL-B[c]						
4-SL-B1	4.6	5.7	--	81.7	8.0	--
4-SL-B2	4.6	6.7	--	80.5	8.2	trace
4-SL-B1 (portion not precipitated by alcohol)	45.7	8.6	possible	9.4	36.3	trace
4-SL-B2 (portion not precipitated by alcohol)	24.6	16.8	possible	26.0	32.6	trace

[a] Does not include the sugar units such as xylose, which may constitute part of the aldobiuronic acid molecules.

[b] Percentage as a pentosan or hexosan of the total pentosans or hexosans present. Percentages were calculated from the quantitative analysis data.

[c] 3-SL-B contained xylose, arabinose, galactose, glucose, and mannose.

TABLE XIII

INTRINSIC VISCOSITIES AND DEGREES OF POLYMERIZATION OF THE "HEMICELLULOSES" PRECIPITATED BY ALCOHOL FROM THE SPENT LIQUORS

	Yield %[a]	Intrinsic Viscosity	Degree of Polymerization[b,c]
2-SL-B	1.35	0.48	109
3-SL-B	1.04	0.54	123
4-SL-B1	1.95	0.44	100
4-SL-B2	1.61	0.48	109

[a]Ovendry wood basis

[b]Number average

[c]Calculated as a pentosan

Tables XI and XII show that in general the analytical data of the "hemicelluloses" from the spent liquor are similar to those of the hemicelluloses extracted from the aspenwood and pulp 2. The spent liquor hemicelluloses contain less xylose units and more arabinose, glucose and galactose units than do most of the hemicelluloses from the wood and pulp. Particularly is this true of arabinose; the spent liquor hemicelluloses contain about 8% arabinose as compared to only trace amounts in all the extracted hemicelluloses except W-D16 which contains approximately 1%. The carbohydrate material in the spent liquor which was carried through the procedure and was not precipitated by alcohol contained appreciably larger proportions of glucose and arabinose units than did the precipitated material.

By chromatographic analyses of the spent liquor of cook 4, sugar anhydride units equal to 4.14% of the original wood were obtained. This is slightly more than twice the sum of carbohydrate material obtained by precipitation with alcohol from the spent liquor and the carbohydrate material not precipitated by alcohol.

The difference in the amount of carbohydrate material obtained from the spent liquor by precipitation and that obtained by chromatographic analysis of the spent liquor may be due to (1) mechanical loss of the material during the preparation of the "hemicelluloses" from the waste liquor, or (2) a lignin-carbohydrate bond, which in the unhydrolyzed sample could cause the carbohydrate material to be retained with the ligno-sulfonates on the anion ion exchange column. Care was taken to thoroughly wash all precipitates, filter pads and ion exchange columns during the procedure used in preparing the "hemicelluloses" from the spent liquor. It is unlikely that a very large fraction of the carbohydrate material would be lost mechanically during the procedure used. It appears more probable that the loss was caused by the retention of carbohydrate material on the anion ion exchange column--due to some type of lignin-carbohydrate bond.

The relationship between the intrinsic viscosities and yields of "hemicelluloses" from the spent liquor in Table XIII should be noted. In general the greater the yield, the lower was the intrinsic viscosity. Probably the hemicelluloses obtained in greater yield contain more of the shorter chain hemicelluloses.

Comparison of the viscosities of the "hemicelluloses" from the spent liquor with the viscosities of the hemicelluloses extracted with 5% potassium

hydroxide from aspenwood and from pulp 2 (W-D5= 123; P2-D5 = 143) indicate that approximately 25% of the hemicelluloses removed from the pulp during the cook are removed relatively undegraded. These data and the viscosity data for the hemicelluloses indicate that the NSSC cook preferentially removes the shorter chain hemicellulose molecules.

In general the experimental data on the "hemicelluloses" precipitated from the spent liquor show that:

(1) These "hemicelluloses" are similar to those extracted from aspenwood or from pulp 2. They do, however, contain more glucose, galactose, and arabinose than those from aspenwood or from pulp 2.

(2) Twice as much carbohydrate material can be shown present in a hydrolyzate of the spent liquor by chromatographic analysis as can be obtained from the same spent liquor as "hemicellulose". It is suggested that this is due to a lignin-carbohydrate bond.

(3) Approximately 25% of the hemicelluloses removed from the pulp are removed essentially undegraded.

(4) The shorter chain hemicellulose molecules are removed preferentially during the cook.

"APPARENT LIGNIN" IN THE FILTRATES FROM LIGNIN DETERMINATIONS

"Klason" lignin determinations were made on all the materials studied, including the original wood, pulps, holocelluloses, hemicelluloses, and residues. The procedure (Institute Method 428) was modified for the hemicelluloses by increasing the relative amounts of 72% sulfuric acid and decreasing the time of contact with the strong acid. Dilution to 3% acid strength and 4 hours boiling

at constant volume was followed in each case. The results of these determinations are shown in Table XIV.

The ultraviolet absorption at 280 mmu of the filtrates from the lignin determinations was determined and the "apparent lignin" in the filtrates calculated from the following relationship

$a = \frac{A}{bc}$ where a = specific extinction coefficient

A = optical density

b = internal cell length in centimeters

c = concentration in grams per liter

These data are included in Table XIV. A value of 15 was used for a (54).

It can be readily seen that the "apparent lignin" values in Table XIV are out of proportion to the lignin determined as Klason lignin. This is particularly true in the case of the hemicelluloses. Therefore, further investigation of the ultraviolet absorption curves for these and related materials was undertaken. The graphs on pages 73 to 76 show ultraviolet absorption curves for the materials listed in Table XV. The concentration c used in calculating the specific extinction coefficient in the graphs was the concentration in grams per liter of the material before hydrolysis.

TABLE XIV

LIGNIN CONTENTS OF THE ASPENWOOD, PULP 2, HOLOCELLULOSES, HEMICELLULOSES AND RESIDUES

	Klason Lignin, %[a]	Apparent Lignin in the Filtrate, %[a]	Total, %[a]
W	18.0	5.6	23.6
W-D5	2.0	7.3	9.3
W-D16	4.5	7.4	11.9
W-DR	21.8	6.0	27.8
W-H	0.6	7.0	7.6
W-H5	0.9	7.6	8.5
W-H16	0.4	5.5	5.9
W-HR	0.5	3.4	3.9
P2	12.3	7.1	19.4
P2-D5	2.0	7.3	9.3
P2-D16	1.6	7.6	9.2
P2-DR	13.7	5.1	18.8
P2-H	0.4	4.4	4.8
P2-H5	0.4	8.3	8.7
P2-H16	0.4	6.5	6.9
P2-HR	0.4	3.1	3.5
Aspen native lignin[b]	91.7	4.4	96.1
Xylose[c]		6.9	6.9
Yundt's xylan[c]		4.2	4.2

[a] Ovendry, ash-free basis

[b] Ovendry basis

[c] Boiled with 3% sulfuric acid for 4 hours

TABLE XV

ULTRAVIOLET ABSORPTION DATA FOR LIGNINS, LIGNIN FILTRATES, HYDROLYZATES, AND OTHER SOLUTIONS

Starting Material	Solution	Curve	Maximum, mmu	Minimum, mmu
Aspenwood (W)	lignin filtrate	1	280	258
W-DR	lignin filtrate	2	280	255
W-HR	lignin filtrate	3	283	248
P2-DR	lignin filtrate	4	281	259
P2-H	lignin filtrate	5	280	245
W-D16	lignin filtrate	6	275	250
W-H5	lignin filtrate	7	275	248
P2-D5[a]	2% sulfuric acid hydrolyzate	8	277	254
P2-D5[a]	1% sulfuric acid hydrolyzate	9	278	260
P2-H16	lignin filtrate	10	277	245
W-D5[a]	3% nitric acid hydrolyzate	11	215	
Xylose[a]	3% sulfuric acid hydrolyzate	12	275	245
Yundt's xylan[a]	3% nitric acid hydrolyzate	13	300	262
Yundt's xylan[a]	3% sulfuric acid hydrolyzate	14	270	245
Aspen native lignin	lignin filtrate	15	255	227
P2-D5[a]	1% nitric acid hydrolyzate	16	301	262
Aspen native lignin (54)	dioxane	17	275[b]	
Spruce native lignin (54)	dioxane	18	280	260
Furfural (50)	10% alcohol	19	278	245
Reductic acid (50)	10% alcohol	20	270	220

[a] Hydrolysis consisted of boiling for 4 hours at constant volume.

[b] Slight inflection point only.

Figure 2

ULTRAVIOLET ABSORPTION CURVES

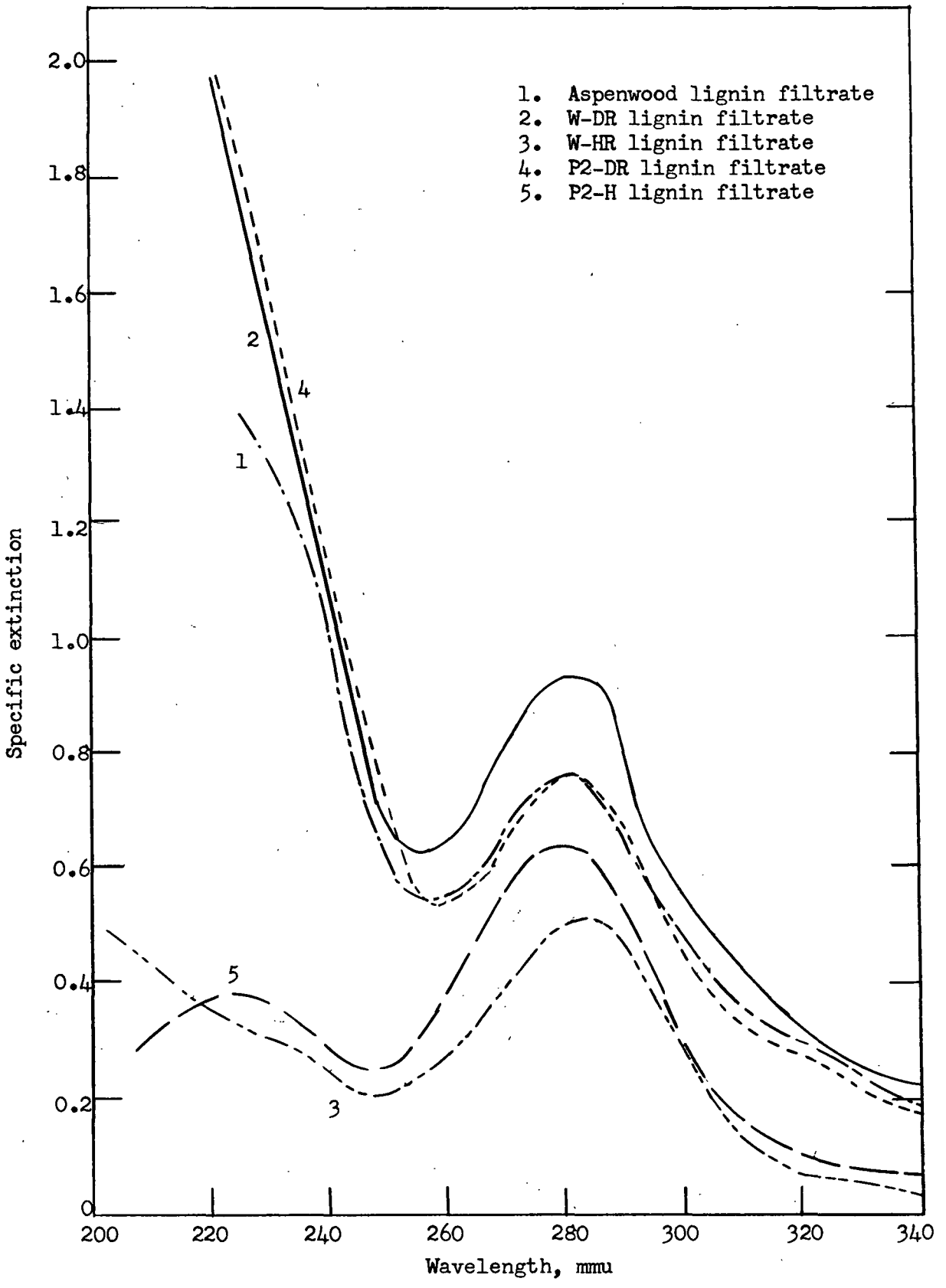


Figure 3

ULTRAVIOLET ABSORPTION CURVES

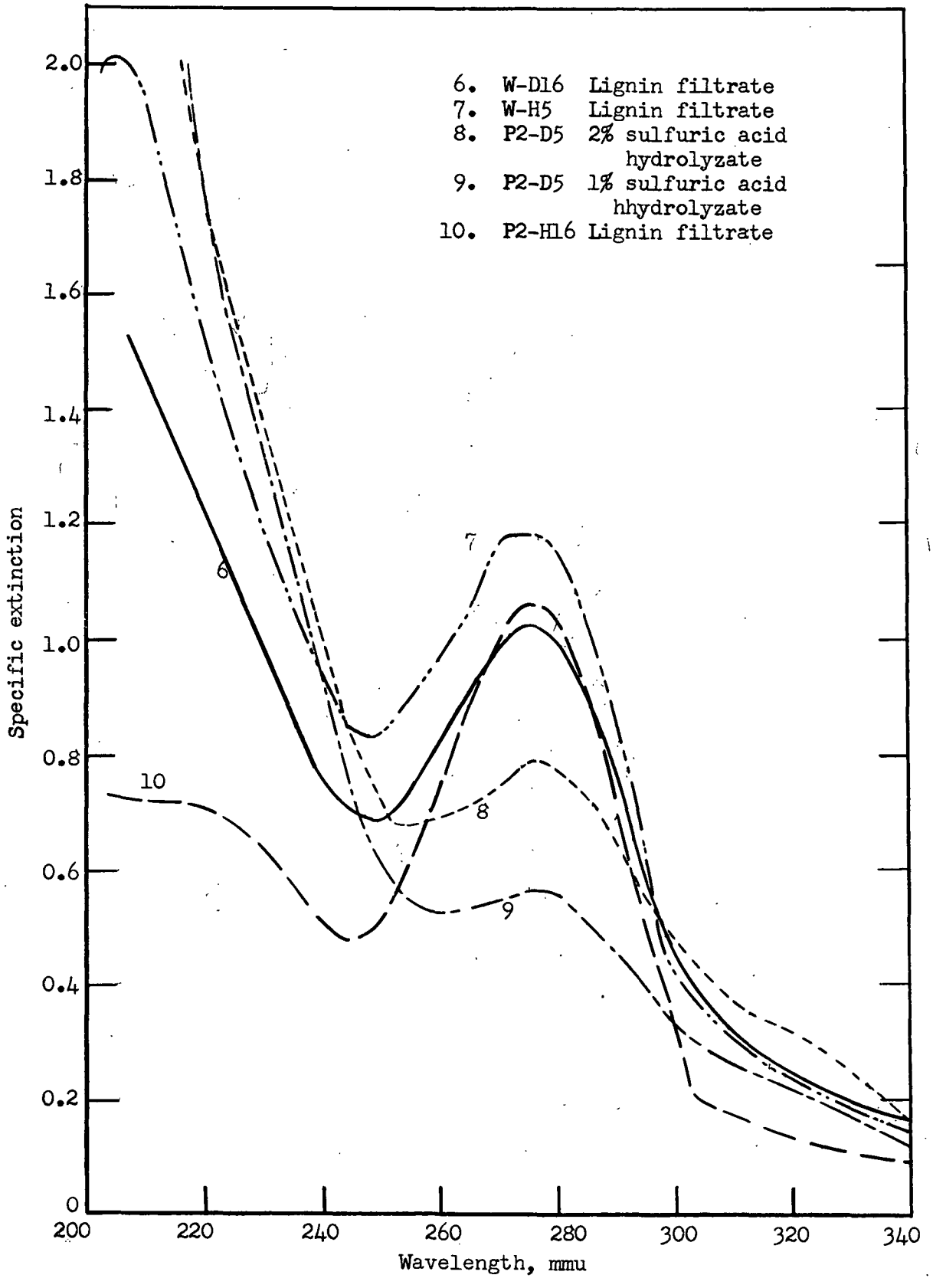


FIGURE 4

ULTRAVIOLET ABSORPTION CURVES

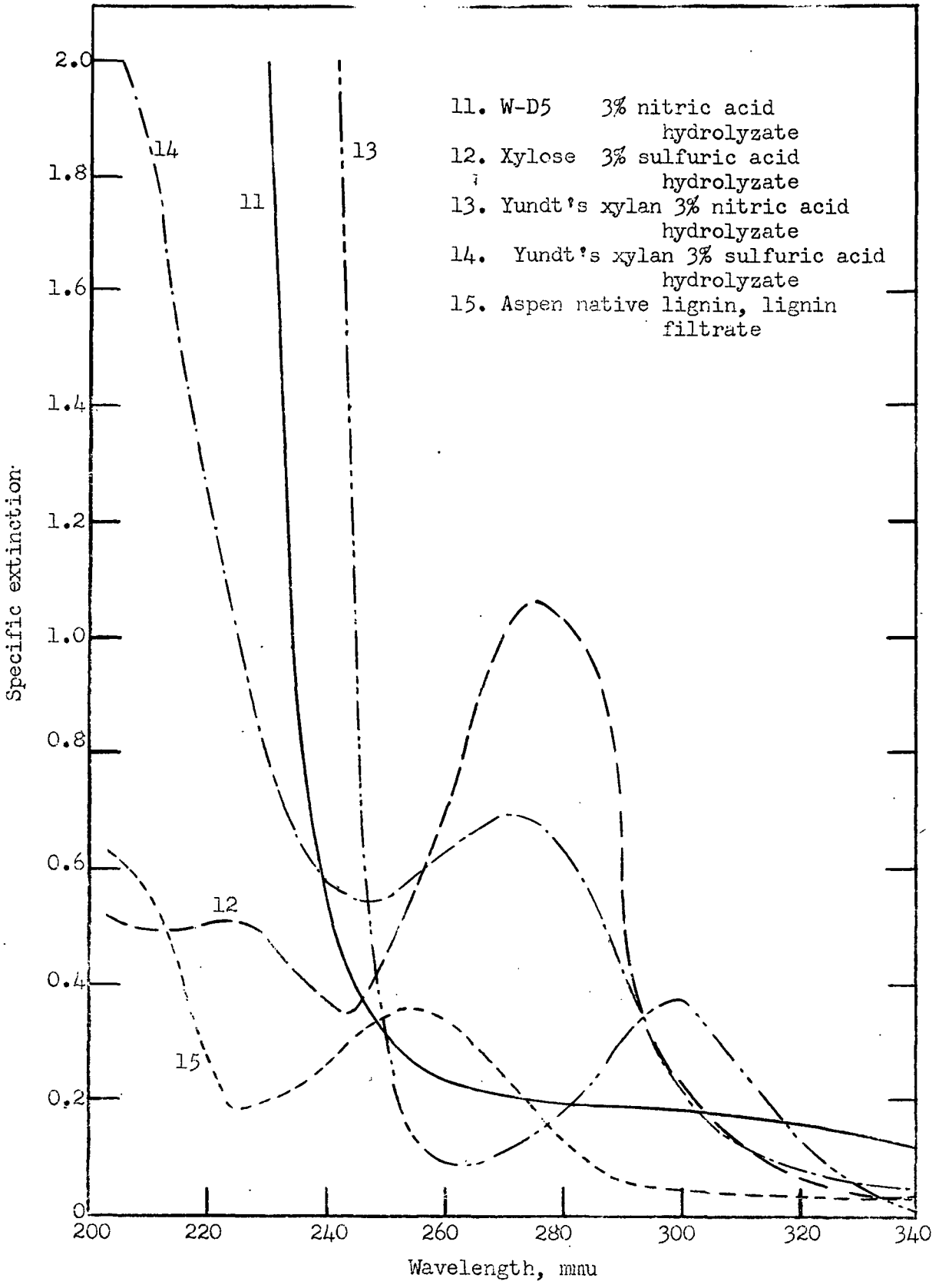
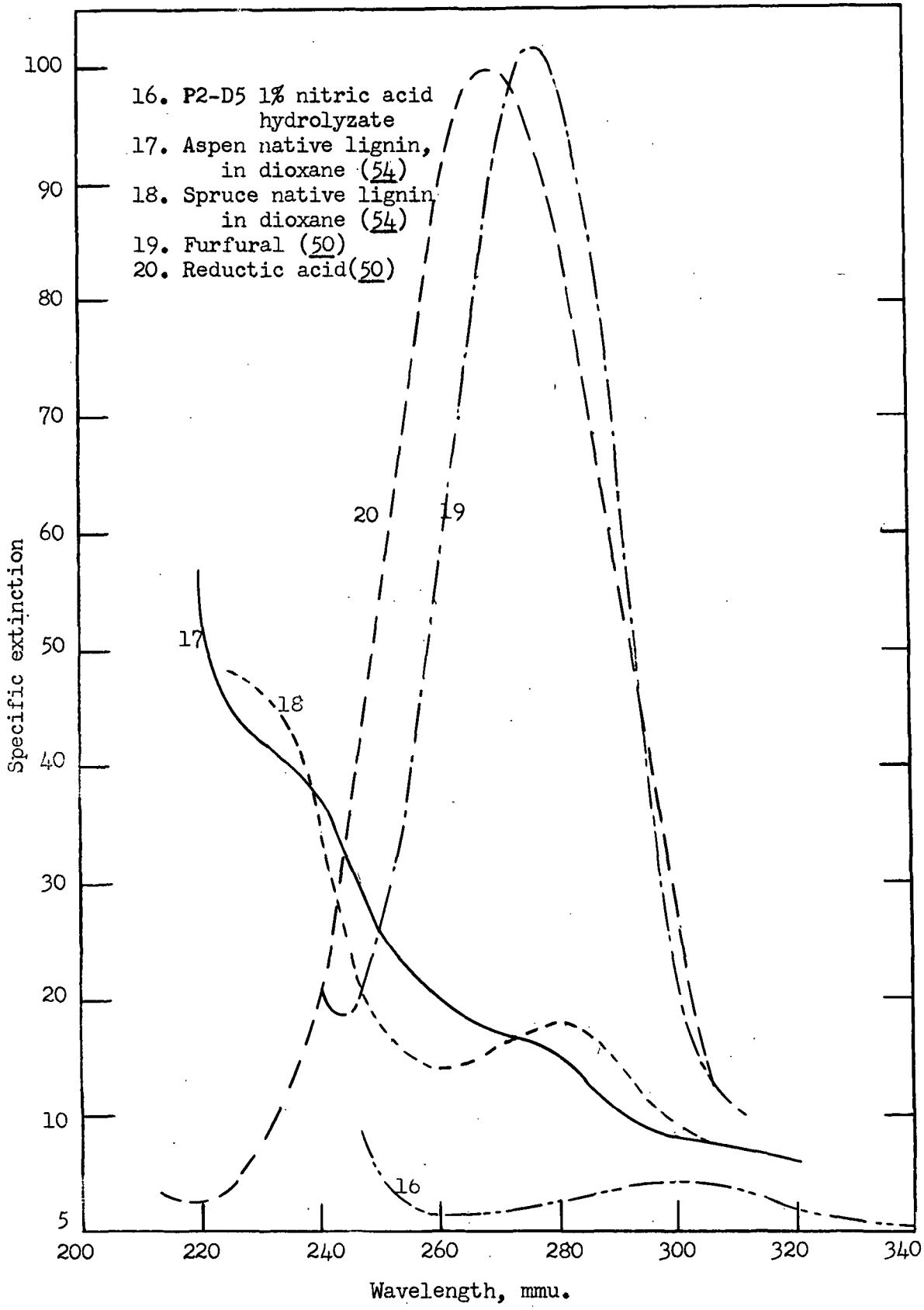


Figure 5

ULTRAVIOLET ABSORPTION CURVES



It can be seen that neither aspen native lignin nor the filtrate from a Klason lignin determination on the native aspen lignin have the characteristic maximum at 280 mmu or minimum at 260 mmu as does spruce native lignin. The curves for the hemicelluloses resemble those of furfural and reductic acid rather than that of the aspen native lignin. Further, the curve for the 3% sulfuric acid hydrolyzate of xylose is similar to those of the hemicelluloses and shows an apparent lignin content of 6.9%. Similar results were obtained by Rollinson and Wise (51). Sohn (50) refluxed a 0.0033 molar solution of xylose with an initial pH of 6.5 for 19 hours, and found that the optical density at 280 increased steadily with the time of refluxing. He obtained curves similar to curve 12.

The large specific extinction coefficients at 280 mmu for furfural (100) and reductic acid (87) should be noted. When these are compared to the specific extinction coefficient for aspen native lignin (15) at 280 mmu it can be seen that a small amount of either compound in a hydrolyzate would have a large effect on its optical density and hence upon the apparent lignin content.

The above data indicate that:

(1) Hydrolysis with nitric acid shifts the maximum to around 300 mmu (except for curve 11).

(2) Degradation products of xylose and other pentosans such as furfural and reductic acid are probably responsible for most of the ultraviolet absorption at 280 mmu of high-pentosan, low-lignin containing materials, such as hemicelluloses, when these materials are hydrolyzed with sulfuric acid (as in the Klason lignin determination). Since the nitric acid hydrolyzates have maxima at 300 mmu instead of 280 mmu this may or may not be true for nitric acid hydrolyzates.

(3) Aspen lignin does not appear to give the same ultraviolet absorption curve in acid solutions as does spruce lignin.

(4) Extreme caution must be used in correcting Klason lignin values for acid soluble lignin, particularly in the case of a hardwood such as aspen.

IDENTIFICATION OF MANNOSE

Mammose has been tentatively identified in hydrolyzates of aspenwood by paper strip chromatography (27, 29). It was felt desirable to make a chemical derivative of mannose in order to provide more definite evidence of its presence in aspenwood.

A sulfuric acid hydrolyzate of P2-HR (residue from pulp 2 holocellulose after extraction with 5 and 16% potassium hydroxide) was selected. It contained 96.4% glucose, 1.6% mannose, and 2% xylose determined by paper partition chromatographic analysis). The hydrolyzate was filtered, concentrated to a volume of 2 cc. and cooled under the cold water tap. Two cc. of phenylhydrazine reagent (2 volumes of phenylhydrazine, 1 volume of acetic acid and 3 volumes of water), which had been previously cooled, were added to the hydrolyzate. After thorough mixing the mixture was allowed to stand in the refrigerator for 48 hours. The precipitate was recovered by filtration, washed with cold water, cold absolute ethanol and cold c.p. ether. The material was then recrystallized from hot ethanol and again washed with cold ethanol and cold ether. After drying the precipitate had a decomposition point of 193-195°. Mannose phenylhydrazone has a decomposition point of 195-200 (55, 56). The crystals had a yellow tinge. Only a very small amount was obtained, hence recrystallization could not be repeated.

SUMMARY AND CONCLUSIONS

A neutral sulfite semichemical cook was made of aspenwood. Holocelluloses were prepared from the original wood and from the pulp according to the Thomas procedure. Eight hemicelluloses were prepared by extracting the original wood, the pulp, and the two holocelluloses with 5% potassium hydroxide followed by 16% potassium hydroxide. In addition "hemicelluloses" were recovered from the spent liquors. The hemicelluloses, the holocelluloses, the pulp, the original wood and all residue were analyzed for lignin, pentosans, uronic anhydride, ash, sugars and sugar contents. Also dilute solution viscosity measurements were made on the hemicelluloses.

The following conclusions were made from the experimental data:

1. Hemicelluloses

The NSSC cooking conditions as used in preparing pulp 2 did not appear to cause much degradation of the hemicelluloses. The hemicelluloses extracted from the pulp had higher viscosities than those extracted from the original wood. However, the viscosity data indicate that proportionately more of the shorter chain hemicelluloses have been removed from the pulp so that the viscosities of the hemicelluloses from pulp 2, if not degraded by the cook, should be somewhat higher.

One third of the hemicelluloses were removed from the pulp during the cook. The loss of pentosans and uronic acids during the cook was nearly equal to the loss of hemicelluloses. It was possible to account for 50% of the hemicelluloses removed from the pulp by sugar analysis of the spent liquor and to recover 25% of the hemicelluloses removed from the pulp by precipitation from

the spent liquor. The intrinsic viscosities of the recovered hemicelluloses were only slightly lower than that of those extracted from the aspenwood with 5% potassium hydroxide.

The hemicelluloses in the pulp are more accessible to caustic extraction (or more soluble in caustic). This is shown by the fact that 95% of the total hemicelluloses were extracted from the pulp directly with caustic solutions (5% followed by 16% potassium hydroxide) while only 68% were extracted from the original aspenwood under the same conditions.

The hemicelluloses from the pulp had a higher pentosan content, a lower uronic anhydride content, and were less colored than are the corresponding ones from the original aspenwood. The "summative" hemicelluloses from the pulp had higher viscosities than the "summative" hemicelluloses from aspenwood. This is probably due to the removal of shorter chain hemicelluloses predominantly during the cook.

In general, the sugar contents of the hydrolyzates of the hemicelluloses from the pulp are similar to those of the corresponding hemicelluloses from the original aspenwood. The hydrolyzates of the "hemicelluloses" which were recovered from the spent liquor contained appreciably more glucose, galactose, and arabinose than did those of the hemicelluloses extracted from aspenwood or pulp 2. The hydrolyzates of the spent liquors contained larger amounts of the minor sugar units--galactose, mannose, arabinose, and rhamnose than did those of the aspenwood of pulp 2. Apparently the hemicelluloses containing sugar units other than xylose are more soluble in the pulping

liquor or the chain molecules containing these sugar units are more susceptible to rupture and subsequent solubilization by the cooking liquor.

Twice as much carbohydrate material (50% of the hemicelluloses lost from the pulp during the cook) can be shown present in a hydrolyzate of the spent liquor by chromatographic analysis as can be obtained from the spent liquor as "hemicellulose." It is suggested that this is due to a lignin-carbohydrate bond.

Examination of the experimental data with regard to the presence or absence of glucose units in the hemicelluloses leads to no clear conclusion. The 16% potassium hydroxide extracts from aspenwood (W-D16) and from pulp 2 (P2-D16) contained 7.5% and 4.2% glucan, respectively. The "hemicelluloses" precipitated from the spent liquors had up to 4.6% glucan. The 5% potassium hydroxide extracts from aspenwood and pulp 2 and all the hemicelluloses extracted from the aspenwood holocellulose and from pulp 2 holocellulose contained only trace amounts of glucan. According to the definition for hemicelluloses as used in this study (see page 3), the glucan in W-D16 and P2-D16 would be part of the hemicellulose. Actually, the glucan could have come from cellulose molecules whose chain length for some reason was short enough to be soluble in 16% potassium hydroxide. The glucan in the spent liquor hemicellulose could have come from cellulose that was solubilized by the NSSC cooking liquor but which still retained sufficient molecular magnitude to be precipitated by alcohol. The glucan in the spent liquor did not come from starch, as tests for starch on the original aspenwood were negative.

Since the hemicelluloses from the holocelluloses contain no glucan, the data suggest that if aspenwood hemicelluloses are defined as the carbohydrate

material removed from an aspenwood holocellulose by 5% followed by 16% potassium hydroxide, then the hemicelluloses do not contain glucan.

2. Presence of free sugars in the spent liquor

Chromatographic analysis of three NSSC spent liquors indicated that there were no free sugars, disaccharides, or trisaccharides in these spent liquors.

3. Quantitative sugar analysis

The method of quantitative sugar analysis as used in this work has certain inaccuracies. These appear to be due primarily to the losses during hydrolysis step, probably because of the degradation of certain sugar units such as xylose and because of incomplete hydrolysis of the cellulose. Experiments with xylose and Yundt's xylan indicate that for pure xylose the loss is less than 10% and occurs principally in the hydrolysis step and that for Yundt's xylan the loss in the hydrolysis step is less than 5%.

Ribose is a much better reference sugar for the materials considered in this study than is rhamnose.

The quantitative method, despite the inaccuracies involved, provides a rapid, fairly accurate means of estimation of the sugars in the hydrolyzates of carbohydrate containing materials such as wood, holocelluloses and hemicelluloses. Precision between separate analyses is quite good.

4. "Apparent lignin"

The "apparent lignin" as defined by the optical density at 280 mmu of the filtrates from Klason lignin determinations of high pentosan, low lignin

containing materials such as hemicelluloses, appears to be due primarily to sugar degradation products. Aspen lignin does not give the same type of ultraviolet absorption curve in acid solution as does spruce lignin.

The experimental data indicate that caution should be used in correcting the Klason lignin values of hardwoods such as aspen for acid soluble lignin when the latter is determined by the ultraviolet absorption of the filtrate.

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