[CONTRIBUTION FROM THE FOREST PRODUCTS LABORATORY,[1] FOREST SERVICE, U. S. DEPARTMENT OF AGRICULTURE]

Spruce Holocellulose and the Composition of its Easily Hydrolyzable Fraction[2]

BY E. F. KURTH AND GEO. J. RITTER

Holocellulose, the total carbohydrate material in extractive-free wood, has been isolated previously[3] from maple, a hardwood, by the chlorination–pyridine–alcohol and the chlorine dioxide methods. The authors have found that neither of these methods can be used for isolating holocellulose quantitatively from spruce, a softwood. To obtain quantitative yields of holocellulose from spruce the previously published chlorination–pyridine–alcohol procedure[3] has been modified. In general, the modifications consist of (1) a change in the concentration from 15.0 to 50.0% of pyridine in the pyridine–alcohol solution employed for extracting chlorinated lignin from the holocellulose, and (2) an omission of a bleach treatment of the delignified spruce holocellulose. If the spruce holocellulose is bleached according to the procedure previously published,[3] it suffers a loss ranging from 1 to 2%. The method as modified can be used successfully for preparing quantitatively unbleached spruce holocellulose and bleached or unbleached maple holocellulose.

This article discusses spruce holocellulose and the composition of its easily hydrolyzable hemicelluloses.

Procedure for Isolation of Spruce Holocellulose

Weigh approximately 1.7 g. of air-dry extracted 60–80 mesh sawdust in an alundum crucible, moisten the material with distilled water, and remove the excess moisture by suction. Transfer the sawdust to a 250-cc. beaker and treat the material with chlorine gas in a chlorinating chamber for four minutes; remove from the chamber and add a few cubic centimeters of 50% pyridine–alcohol solution to the contents in the beaker in order to neutralize the acid formed during chlorination; transfer to the alundum crucible and remove the excess solution by suction. Transfer the crucible with its contents to a Soxhlet apparatus and extract with a 50% solution of pyridine in alcohol for two and one-half hours; remove from the extractor and wash with cold distilled water. Repeat the chlorination and extractive treatments five times or until the loss in weight is equal to the lignin content. When the lignin is approximately all removed the material upon chlorination is pale yellow as contrasted with an orange color when lignin is present. When the lignin-free material is extracted with pyridine–alcohol it is grayish tan in color.

Should a white product be desired, the material may be bleached by the method described for maple holocellulose.[3]

Characterization of Holocellulose

The unbleached spruce holocellulose was characterized by subjecting it to the following chemical analyses. Lignin was determined by the 72% sulfuric acid method;[4] uronic acids by the 12.0% hydrochloric acid method;[5] methoxyl by the Zeisel method;[6] acetyl by the toluene–sulfonic acid method;[7] pentosans by the Tollens method;[6] and Cross and Bevan cellulose by the method proposed by Ritter.[8] The results of these analyses made it possible to compare the material with Cross and Bevan cellulose, with other wood fractions, and with the spruce wood freed from its extractives, all of which were subjected to the same analyses as was the holocellulose. The results are listed in Table I.

TABLE I

RESULTS OF PARTIAL ANALYSIS OF EXTRACTIVE-FREE SPRUCE WOOD AND SOME OF THE WOOD FRACTIONS

Percentage composition based on weight of oven-dry (105°) extractive-free wood

Material	Yield, %	Lignin	Pentosans	Methoxyl	Carbon dioxide	Volatile acids as acetyl
Spruce wood extracted with alcohol–benzene and hot water	100.00	28.45	12.60	5.46	0.81	1.80
Spruce holocellulose	71.30	Sl. trace	11.80	0.67	.80	1.79
Residue after holocellulose was hydrolyzed with 1.0% sulfuric acid	61.00	0.00	7.93	.34	.42	0.97
Spruce Cross and Bevan cellulose	61.50	.00	7.90	.21	.46	.98
Spruce lignin	28.45	...	0.68	4.64	..	..

Discussion of the Characteristics of Holocellulose

The results of Table I show the main difference in composition between the holocellulose and the extractive-free wood is the absence of lignin in

(1) Maintained at Madison, Wis., in coöperation with the University of Wisconsin.

(2) Presented at a meeting of the American Chemical Society at Cleveland, Ohio, September 10–14, 1934.

(3) Geo. J. Ritter and E. F. Kurth, *Ind. Eng. Chem.*, **25**, 1250 (1933).

(4) Geo. J. Ritter, R. M. Seborg and R. L. Mitchell, *Ind. Eng. Chem., Anal. Ed.*, **4**, 203 (1932).

(5) A. Dickson, H. Otterson and K. Link, THIS JOURNAL, **52**, 775 (1930).

(6) M. W. Bray, *Paper Trade J.*, **87**, 59–68 (1928).

(7) K. Freudenberg, *Ann.*, **433**, 230–237 (1923).

(8) Geo. J. Ritter, *Ind. Eng. Chem.*, **16**, 947 (1924).

the former material. This difference in lignin content accounts for all except 0.25% of the difference in yields of the two materials.

A discrepancy of 0.80% between the pentosans of the holocellulose and those of the wood may be noted. This difference is approximated by a "pentosan" value listed in the analysis of the lignin. When lignin is subjected to the pentosan determination it liberates formaldehyde[9] which, like furfural, forms a precipitate with phloroglucinol. Thus when pentosans are determined in wood, which contains both lignin and pentosans, the value obtained is slightly high.

The holocellulose has a methoxyl content only slightly over 12.0% of that of the wood. All except 0.15 of the difference between the methoxyl contents of the two materials is recovered in the spruce lignin. Characterized by their content of carbon dioxide, pentosans and volatile acids calculated as acetyl, the holocellulose and the spruce wood are similar in composition. Agreement of the carbon dioxide content of the two materials indicates that no oxidation of the holocellulose occurs during its isolation. Presence in the holocellulose of a part of the methoxyl groups and all of the acetyl groups that are in the extractive-free wood confirms the results reported on maple holocellulose. The percentage of the acetyl groups in the holocellulose is not in harmony with that of Hägglund,[10] who reports 60.0% of the total acetyls recovered in the carbohydrates. The low acetyl content of the carbohydrates isolated by Hägglund may be due to his use of an alkaline calcium hypochlorite for bleaching, thereby removing some acid groups that are calculated as acetyl.

The volatile acids listed in Table I were further analyzed. The total percentage of the volatile acids calculated as acetyl was determined by the method of Freudenberg.[7] In the mixture of volatile acids, formic acid was determined quantitatively by means of mercuric chloride, and acetic acid was determined by the difference between the total volatile acids and the formic acid.

The presence of acetic acid was proved by hydrolyzing 100 g. of the extractive-free spruce wood with 2.5% sulfuric acid, filtering, neutralizing with barium hydroxide and barium carbonate, filtering to remove the precipitated barium sulfate, concentrating the filtrate to a small volume, and then forming the *p*-nitrobenzyl ester of acetic acid, m. p. 78°. The quantitative results are given in Table II.

(9) K. Freudenberg and M. Harder, *Ber.*, **60B**, 581 (1927).
(10) E. Hägglund, *Svensk Kem. Tidskrift*, **46**, 83–87 (1934).

TABLE II

COMPOSITION OF VOLATILE ACIDS IN EXTRACTIVE-FREE SPRUCE WOOD AND SPRUCE HOLOCELLULOSE

Percentage composition based on weight of oven-dry (105°) holocellulose

Material	Total volatile acids as acetic	Formic acid	Acetic acid (by diff.)
Extractive-free spruce wood	2.53	0.56	1.97
Spruce holocellulose	2.50	.56	1.94

Spruce holocellulose treated for thirty minutes with 1.0% sulfuric acid lost 10.3% on the basis of the wood, leaving a residue of 61.0% (line 3, Table I). A comparison of the analysis of this residue with that of the Cross and Bevan cellulose (line 4, Table I) shows that these residues are similar in yields and in chemical characteristics. Since the two residues are approximately the same, the hemicellulose fraction that dissolved with the lignin during the isolation of the Cross and Bevan cellulose must be approximately the same as that constituting the 10.3% removed from the holocellulose by hydrolysis with 1.0% acid. Thus, by first isolating holocellulose and then hydrolyzing the material with dilute acid, it is possible to separate for study a wood fraction that under the older methods of analysis has always been mixed with other wood constituents. This wood fraction is composed of several hemicellulosic constituents, a discussion of which appears in the following paragraphs.

Composition of the Easily Hydrolyzable Hemicelluloses in Holocellulose

The easily hydrolyzable wood fraction contains pentosans and substituent groups comprising methoxyl, carboxyl, acetyl and formyl as may be determined by subtracting from the analysis of the holocellulose (line 2, Table I) that of the residue obtained by hydrolyzing the holocellulose with 1.0% acid (line 3, Table I). It is by this means of difference that the substituent groups were determined in the easily hydrolyzable fraction. Pentosans and other carbohydrate constituents were determined directly on the sugar solution prepared from the easily hydrolyzed fraction.

Preparation of Sugar Solution.—Approximately 300 g. of spruce holocellulose was heated for thirty minutes with 8 liters of 1% sulfuric acid at the boiling temperature. The hot solution was decanted and the residue was washed well with distilled water. The concentration of sulfuric acid was then increased to 2.5% and the solution boiled for an additional five hours in order to hydrolyze completely the more complex carbohydrates. After this the solution was cooled, made neutral with calcium carbonate, filtered, and concentrated to a thin sirup under diminished pressure.

The sirup was filtered and then poured into 5 volumes of ethanol. A voluminous precipitate of the calcium salts of uronic acid was obtained; it was filtered, dried and set aside for further study.

The filtrate, after removing the alcohol by evaporation, was clarified with neutral lead acetate, deleaded with hydrogen sulfide and filtered. Next it was boiled in the presence of charcoal and again filtered. It was then diluted to approximately 4.5% reducing sugar and analyzed.

Analyses of Sugar Solution

Mannose.—The percentage of mannose was determined following the method of Bourquelot and Hérissey[11] and was isolated as mannose phenylhydrazone, m. p. 196°.

Fructose.—Seliwankoff's color test[12] and titration with iodine and sodium hydroxide showed that fructose was present only in negligible quantities.

Glucose.—Oxidation of an aliquot of the sugar solution with nitric acid followed by neutralization with potassium carbonate gave shining rhombic crystals of acid potassium saccharate.

The percentage of glucose was determined through fermentation studies with yeast cultures, *Torula dattila*. This culture ferments mannose, fructose and glucose but does not ferment galactose or the pentoses.[13] Since the percentage of mannose was known and fructose was absent, the amount of glucose present was obtained by subtracting the percentage mannose from the difference in reducing sugars present before and after fermentation.

Galactose.—Oxidation of an aliquot of the sugar solution with nitric acid gave mucic acid, m. p. 217°. The percentage of galactose present was determined through the selective fermentation of *Torula dattila* and *Sacch. cerivisiae* (top and bottom varieties). *Sacch. cerivisiae* ferments galactose while *T. dattila* does not.

Arabinose.—A portion of the residual liquor from the fermentation of the hexoses was concentrated, filtered, clarified and then evaporated to a thick sirup. When this sirup was treated with diphenylhydrazine according to the method of Neuberg and Wohlgemuth,[14] arabinose diphenylhydrazone, m. p. 204 to 205°, was obtained A confirmatory check on this value was obtained by fermentation tests made in conjunction with xylose and described under the heading "Xylose."

Xylose.—The residual liquor from the arabinose determination was refluxed with formaldehyde to decompose the soluble xylose diphenylhydrazone. Xylose was identified in the liquor through the formation of the *p*-bromophenylosazone, m. p. 207°, and the methylphenylhydrazone, m. p. 110°. A quantitative estimation of the amount of xylose present was not determinable by chemical means. Fermentation tests with pentose fermenting bacteria were therefore tried.

The sugar solution remaining after the yeast fermentation was concentrated to one-half the original volume, was divided into three parts, and sterilized. An excess of sterile calcium carbonate was added. The previous growth of the yeast rendered these solutions suitable for the development of lactic acid bacteria. One solution was inoculated with a mixture of pentose fermenting bacteria cultures Nos. 19 and 36. The second solution was inoculated with the single culture No. 19 and the third with a single culture No. 36. Culture No. 19 ferments arabinose but not xylose; culture No. 36 ferments xylose but not arabinose. The inoculated solutions were incubated at 37.5° for twelve days.

Culture No. 19 removed 16.9% of the initial reducing material and culture No. 36 removed 28.1% of the initial reducing material. The unfermented residue in the solution containing both organisms was 10.15% of the reducing material. The percentage of reducing material removed previously through fermentation with *T. dattila* and *S. cerivisiae* totaled 45.1%. The sum of the above values constitutes 100.25% of the reducing material.

Cultures Nos. 19 and 36 do not readily ferment the methylpentose, rhamnose. A control solution containing 1.58% rhamnose and 5% yeast extract inoculated with these cultures still contained 1.59% reducing material after incubation for twelve days at 37.5°.

Unfermented Residue.—The liquor from the pentose fermentation tests was concentrated on a steam-bath to a small volume. It was then treated with phenylhydrazine hydrochloride and sodium acetate for one-half hour in a boiling water-bath. A flocculent precipitate of very fine yellow needles gradually separated after cooling the solution. These were filtered off as they separated and recrystallized several times from a hot alcohol–water solution. They were next filtered and dried in a desiccator over calcium chloride. When dried in this manner the crystals lost their crystalline structure and changed to a brown amorphous mass. The melting point of this mass was indefinite, the greater portion melting at 107 to 110°. Since no phenylhydrazine derivatives having the above melting points are known, it was concluded that the residue contained no additional sugars.

Uronic Acid Fraction.—The precipitate containing the calcium salts of the uronic acid that was formed during the preparation of the sugar solution gave a well-defined qualitative test for glucuronic acid with naphthoresorcin. Oxidation with bromine in a solution of hydrobromic acid gave no mucic acid,[15] which indicates that galacturonic acid is absent.

Percentage Composition.—The composition of the easily hydrolyzable hemicelluloses of the spruce holocellulose is given in Table III.

TABLE III

PERCENTAGE COMPOSITION OF EASILY HYDROLYZABLE HEMICELLULOSES IN SPRUCE HOLOCELLULOSE

Hemicellulose[a]	Basis of hydrolyzed material	Basis of wood
Mannose anhydride	17.7	1.8
Glucose anhydride	8.0	0.8
Galactose anhydride	7.8	.8
Arabinose anhydride	12.5	1.3
Xylose anhydride	20.9	2.2
Methoxyl	3.2	0.3
Uronic acid anhydride (glucuronic acid)	14.6	1.5
Volatile acids (formyl and acetyl groups)	8.0	0.8
Undetermined	7.3	.8
Total	100.0	10.3

[a] Total material dissolved, 10.3% of the extractive-free wood.

(11) E. Bourquelot and H. Hérissey, *Compt. rend.*, **129**, 339–341 (1899).

(12) G. M. Kline and S. F. Acree, *Ind. Eng. Chem., Anal. Ed.*, **2**, 413–415 (1930).

(13) A. J. Kluyver, "Biochemische Suikerbepalingen," Doctor's thesis, Delft, Holland, 1914.

(14) C. Neuberg and J. Wohlgemuth, *Z. physiol. Chem.*, **35**, 31–40 (1902).

(15) M. Heidelberger and W. F. Goebel, *J. Biol. Chem.*, **74**, 613 (1927).

Acknowledgment

The authors wish to thank E. B. Fred, Department of Agricultural Bacteriology, University of Wisconsin, for furnishing the fermenting cultures and for his helpful suggestions.

Summary

A procedure for isolating holocellulose from spruce and maple wood is given. The chemical characteristics of spruce holocellulose are compared with those of other wood fractions and with extractive-free spruce wood. Holocellulose, after hydrolysis with 1.0% sulfuric acid, leaves a carbohydrate residue comparable to Cross and Bevan cellulose.

By hydrolyzing the holocellulose with dilute acid there has been removed an easily hydrolyzable hemicellulosic fraction similar to a fraction that under the older methods of analysis has always been mixed with other wood constituents. It is composed of constituents that contain methoxyl, carboxyl, acetyl and formyl groups and that may be hydrolyzed to mannose, glucose, galactose, arabinose and xylose.

MADISON, WISCONSIN RECEIVED SEPTEMBER 19, 1934

[CONTRIBUTION FROM THE LABORATORY FOR PURE RESEARCH OF MERCK & CO., INC.]

Action of Aspergillus Niger on Normal 1,2-Diols

BY A. WALTI

The study of the action of *Aspergillus Niger* on sugars and other substrates has received a great impetus in recent years. This work is conveniently summarized by Bernhauer.[1] Since this fungus thrives particularly well on sugar solutions it was thought desirable to investigate its action on a series of homologous substances which resemble a hexose molecule in part of their molecules. We have in the molecule of glucose the grouping $CH_2OHCHOH$— in the open form as well as in the γ-form, at carbon atoms five and six. The simplest substances containing this grouping are the normal glycols of the type $CH_2OHCHOH$—R, *i. e.*, propane-, butane-, pentane- and hexanediol-1,2.

These glycols were prepared by hydrolyzing the corresponding 1,2-oxides. The oxides were obtained from the respective chlorohydrins,[2] which in turn were prepared by allowing chloroacetaldehyde to react with alkylmagnesium bromides. While butene-1,2-oxide and to some extent pentene-1,2-oxide was readily obtained from 1-chlorobutanol-2 and 1-chloropentanol-2, respectively, with the aid of hot aqueous alkali, such was not the case with the higher homolog hexene-1,2-oxide. To avoid this difficulty the chlorohexanol was refluxed in anhydrous ether solution with powdered potassium or sodium hydroxide with mechanical stirring. Under these conditions hexene oxide was obtained in a good yield. The racemic hexene oxide was transformed either into hexanediol-1,2 or into bromohexanol. Part of the pentene oxide was added to aqueous dimethylamine whereby 1-dimethylaminopentanol-2 was readily formed. With methyl iodide the latter compound yielded β-propylcholine iodide. This iodide was compared with a sample made in this Laboratory from 1-chloropentanol-2 directly.[3] Their melting points and mixed melting points agreed perfectly.

Propane-, butane-, pentane- and hexanediol-1,2 were subjected to the action of a known strain of *Aspergillus Niger*. The technique employed was the one recommended by several authors and consisted of subjecting the sterile substrates to well-developed mycelia of the fungus. With the first three glycols mentioned above in each case a substance was formed which reduced Fehling's solution very readily. These reducing substances were found to be the ketones formed by the oxidation of the secondary hydroxyl group. From propanediol-1,2, hydroxyacetone (acetol) was shown to be formed, from butanediol-1,2, 1-hydroxybutanone-2, and from pentanediol-1,2, 1-hydroxypentanone-2. These hydroxy ketones could be separated readily from the respective

(1) K. Bernhauer, "Die oxydativen Gärungen," Verlag Julius Springer, Berlin, 1932.

(2) (a) 1-Chlorobutanol-2, Helferich and Speidel, *Ber.*, **54**, 2634 (1921); (b) 1-chloropentanol-2, Levene and Haller, *J. Biol. Chem.*, **77**, 555 (1928); (c) 1-chlorohexanol-2, Levene and Haller, *ibid.*, **79**, 475 (1928); also Koelsch and McElvain, THIS JOURNAL, **52**, 1164 (1930).

(3) To be published shortly.