*Chemical Studies on Wood Hemicelluloses. III. On the Hemicellulose of Ganpi Bast Fibers. II**

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In the previous paper, the hemicellulose from the Ganpi plant (*Wichstroemia sikokiana* Franch. et Sav.) was studied in connection with Japanese paper making[1]. It was concluded that a complete picture of the chemical nature of hemicellulose is essential to explain the excellent characteristics of the hand-made paper of Japan.

The present investigation is an attempt in this direction, and the results reported here relate mainly to uronic acid which is the most important component of the hemicellulose. The chemical structure of the hemicellulose was also investigated.

The hemicellulose obtained from holocellulose of Ganpi bast fibers by alkaline extraction gave upon hydrolysis a mixture of an aldobiouronic acid, 4-*O*-methyl-D-glucuronic acid and D-xylose together with small amounts of D-galacturonic acid, D-mannose, D-galactose, L-arabinose and L-rhamnose.

The acidic components were separated from

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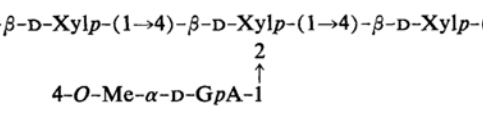

Fig. 1. Structure of Ganpi Hemicellulose.

the hydrolyzate by the use of an anion-exchange resin. By means of chromatography on a column of cellulose[2], 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid and an aldobiouronic acid were isolated from the acidic components.

The 4-*O*-methyl-D-glucuronic acid was characterized after reduction of its ester glycoside with lithium aluminum hydride followed by hydrolysis, as 4-*O*-methyl-D-glucose[3].

The aldobiouronic acid was designated as 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose by the following evidences: a paper chromatographic study proved that the R_X value and color reaction of the acid were the same as for 2-*O*-(4-*O*-methyl-D-glucopyranosyluronic acid)-D-xylopyranose[4], and the methoxyl group content and average equivalent weight corresponded to the calculated value of the acid. A high positive specific optical rotation presumed the presumed of an α-glycosidic linkage in the molecule[5]. The aldobiouronic acid hydrolyzed with considerable difficulty and concomitant destruction.

After conversion to the methyl ester methyl glycoside by methanolysis, however, it was comparatively easily hydrolyzed and afforded 4-*O*-methyl-D-glucuronic acid and D-xylose in equal amounts. Reduction of the ester glycoside of the aldobiouronic acid with lithium aluminum hydride followed by hydrolysis readily yielded 4-*O*-methyl-D-glucose and D-xylose. The aldobiouronic acid isolated here is identical with that obtained by partial hydrolysis of a large number of wood hemicelluloses[6].

The D-galacturonic acid was identified chromatographically after being conversed into D-galactose. But the D-galacturonic acid is believed to originate in pectic substances in the bast fibers. As has previously been reported, pectic substances are contained in the bast fibers of the Ganpi plant, and the hemicellulose isolated from the fibers is unavoidably contaminated more or less with the pectic substances[7]. The D-galacturonic acid is not a component of the hemicellulose molecule.

By oxidation of the Ganpi hemicellulose with 0.27 M sodium periodate[8], it was shown that the periodate uptake was about one mole per pentose unit. The reaction, however, was not and it was found that a small amount of D-xylose unid in the hemicellulose remained unoxidized. It is believed that the D-xylose unit had been attached by a 4-*O*-methyl-D-glucuronic acid side chain in the main chain of the molecule.

From the above consideration, it seems justifiable to assign the main chain of the Ganpi hemicellulose to the structure as Fig. 1. As was reported in the previous paper, the Ganpi hemicellulose has basically a linear structure built up from about 160 xylose residues with, on the average, every sixth xylose units carrying a uronic acid residue[1].

The xylopyranose residues are jointed together with 1, 4-β-linkages. The side chain is formed from single 4-*O*-methyl-D-glucuronic acid residue which is linked to the main chain by a 1, 2-α-linkage.

The constitution of the hemicellulose of the Ganpi bast fibers is similar, albeit not identical, to that of other sorts of wood hemicelluloses[6]. But it is noticeable that this hemicellulose molecule has a comparatively longer chain and has more uronic acid groups along the chain than other wood hemicelluloses.

The studies on the methylation of the hemicellulose will be reported in the next paper.

Experimental

All evaporation were carried out under reduced pressure at a bath temperature not exceeding 40°C. Optical rotations were determined at 16°C and in water unless otherwise stated. In paper chromatographic studies, the sugar and uronic acid mixtures were separated on a sheet of Toyo Roshi No. 50 filter paper by the ascending method, using one of the following solvents in the volume ratios as indicated: (A) ethyl acetate-acetic acid-formic acid-water (18 : 3 : 1 : 4), (B) *n*-butanol-ethanol-water (40 : 11 : 19), (C) ethyl acetate-pyridine-water

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3) B. Lythgoe and S. Trippett, ibid., **1950**, 1983; F. Smith, ibid., **1951**, 2646.
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7) S. Machida and S. Nishikori, *Bull. Fac. Text. Fibers, Kyoto Univ. Ind. Arts & Text. Fibers*, **3**, 94 (1960).
8) S. K. Chanda, E. L. Hirst, J. K. N. Jones and G. V. Percival, *J. Chem. Soc.*, **1950**, 1289.

TABLE I. FRACTION OF ACID COMPONENT OF HEMICELLULOSE

Fraction	Yield (%) from Hemicellulose	$[\alpha]_D^{16}$	Average equivalent weight	R_x[a]	Coloration[b] after five min.	Coloration[b] after a few days
A	1.9	75.6	202	1.60	Red	Yellowish brown
B	5.2	94.7	332	0.74	Red	Red
C	1.2	69.8	200	0.48	Red	Brown

a) Solvent A.
b) 3% Butanol solution of *p*-anisidine hydrochloride.

(2 : 1 : 2). All sugars and uronic acids were located by spraying the paper with an aqueous solution of *p*-anisidine hydrochloride unless otherwise stated. R_x represents the rate of movement on the paper relative to D-xylose.

Separation of the Acid Component of Ganpi Hemicellulose.—The hemicellulose was prepared by the extraction of holocellulose of bast fibers of the Ganpi plant with 5% sodium hydroxide solution in the manner described in the previous paper[1].

The hemicellulose (10 g.) was dissolved in 1 N sulfuric acid (500 ml.) and heated on a water bath for 8 hr. The cooled solution was neutralized with barium hydroxide solution to a pH of 3, and barium sulfate was removed by filtration. The filtrate was passed through a column of Amberlite IR-120 resin to remove barium ions. The acid eluate was passed through a column of Amberite IR-4B resin and the column was washed until the washings gave a negative Molish test. The acid component was eluted from the resin with 1 N sodium hydroxide solution (25 ml.), the alkaline solution passed through Amberlite IR-120 and the eluate therefrom concentrated to dryness, giving a glassy solid (1.1 g.). On analysis, the average equivalent weight by titration was 314 and the methoxyl group content was 6.2%; theoretical values calculated for $C_{12}H_{20}O_{11}$ are 340 and 9.1% respectively. Paper chromatography using solvent A showed the fraction to be a mixture of 4-*O*-methyl-D-glucuronic acid (R_x 1.60), an aldobiouronic acid (R_x 0.72) and D-galacturonic acid (R_x 0.48).

Fractionation of the Acid Component of Ganpi Hemicellulose.—The mixture (1 g.) was fractionated on a column of cellulose[2] (3 cm. diameter, 25 cm. long) with, as eluant, ethyl acetate-acetic acid-water (9 : 2 : 2).

The normal rate at which the eluant passed through the column was about 0.25 ml. per min. The effluent was collected on an automatic fraction collector and, after examination of paper chromatograms, was grouped into three separated fractions. Each fraction was extracted with an equal volume of water, the aqueous phase separated and the organic phase discarded. The aqueous solution was extracted four times with an equal volume of ether to remove acetic acid-ethyl acetate, and then evaporated to dryness. Table I shows the yields and properties of the three fractions.

Characterization of the Fractions. — *Fraction A*: Paper chromatography showed the fraction to be mainly 4-*O*-methyl-D-glucuronic acid[3]. To confirm this, a portion of the glassy matter (110 mg.) was refluxed with 1.8% methanolic hydrogen chloride (50 ml.) for 6 hr. After neutralization with silver carbonate, filtration, and evaporation, the methyl ester methyl glycoside was obtained. It was dissolved in tetrahydrofuran (150 ml.) and reduced with a solution of lithium aluminum hydride (200 mg.) in the same solvent (50 ml.). After half an hour the excess of lithium aluminum hydride was decomposed by the addition of ethyl acetate and the mixture was diluted with water. The organic solvents were evaporated, the solution filtered and the filtrate was deionized with Amberlite resins IR-4B and IR-120. The neutral solution was evaporated to syrup. On paper chromatograms, as is shown in Table II, it gave three spots corresponding to 4-*O*-methyl-D-glucose, D-xylose and D-galactose. The spots of the last two sugars were very faint. From the data shown in Tables I and II, the Fraction A was found to be

TABLE II. PAPER CHROMATOGRAPHY OF THE REDUCING SUGARS FROM REDUCTION OF THE FRACTION A

Sugar detected	R_x value Solvent A	Solvent B	Solvent C
4-*O*-Methyl-D-glucose	1.30	1.20	1.14
D-Xylose	1.00	1.00	1.00
D-Galactose	1.41	0.76	0.61

4-*O*-methyl-D-glucuronic acid accompanying traces of D-xylose and D-galacturonic acid. D-Galactose was probably derived from D-galacturonic acid[7].

When the fraction A was preserved in a desiccator for a long time, a part of the 4-*O*-methyl-D-glucuronic acid was found to change to its lactone, which gave a pink spot (R_x 1.60) on a paper chromatogram in solvent A.

Fraction B; Paper chromatography showed the fraction to be an aldobiouronic acid. As is shown in Table I, R_x value in solvent A, average equivalent weight and optical rotation corresponded to those of 2-*O*-(4-*O*-methyl-D-glucopyranosyluronic acid)-D-xylopyranose[4,5].

A portion of the aldobiouronic acid (500 mg.) was converted to its ester methyl glycoside by methanolysis with 5% methanolic hydrogen chloride (25 ml.). After removing the solvent, a syrup was yielded. The syrup was dissolved in 1 N hydrochloric acid, and the solution was heated on a boiling water bath for 25 hr., the course of hydrolysis being followed by iodometric titrations. The solution was neutralized with silver carbonate, filtered and removed from silver ion with hydrogen sulfide. Filtration and evaporation of the solution yielded a syrup (300 mg.) which, by paper

chromatographic examination, was found to consist mainly of 4-*O*-methyl-D-glucuronic acid and D-xylose in equal amounts. A trace of aldobiouronic acid unreacted was also detected as a faint pink spot in the chromatogram. The syrup of the hydrolyzate was fractionated by chromatography on ten sheets of Toyo Roshi No. 50 filter paper (30× 60 cm.) using solvent A. The fraction corresponding to 4-*O*-methyl-D-glucuronic acid was extracted from the appropriate strips of paper with methanol in a Soxhlet apparatus and syrup (70 mg.) was yielded by evaporating the extract. The fraction corresponding to D-xylose was extracted with water. The extract was deionized by passing down a column of Amberlite IR-4B resin and evaporated to a syrup (190 mg.) which on cooling gave crystalline D-xylose. After recrystallisation from methanol the pure D-xylose had m. p. 142~143°C, $[\alpha]_D^{16}$ +17.6°.

Another portion of the aldobiouronic acid (100 mg.) was converted to its methyl ester glycoside by refluxing with 1.8% methanolic hydrogen chloride (50 mg.) for 8 hr. Neutralization with silver carbonate and evaporation yielded a syrup which was dissolved in tetrahydrofuran (50 ml.) and reduced with lithium aluminum hydride (200 mg.). Hydrolysis of the neutral disaccharide with 1 N sulfuric acid, neutralization with barium carbonate, and concentration yielded a clear syrup which yielded two spots of equal intensity on the paper chromatogram in solvent A, corresponding in color reaction and position to 4-*O*-methyl-D-glucose and D-xylose.

Fraction C. Paper chromatography showed the fraction to be D-galacturonic acid[7]. A portion of the fraction was converted to its ester methyl glycoside by methanolysis and reduced with lithium aluminum hydride in the manner described above. Only D-galactose was obtained. This was confirmed by paper chromatography.

Periodate Oxidation of Ganpi Hemicellulose.— The hemicellulose (160 mg.) was shaken with water (25 ml.), and to it was added 0.27 M sodium metaperiodate solution (25 ml.) at room temperature in the dark. At intervals portions (5 ml.) were withdrawn and the periodate contents were determined by the arsenite method. The results obtained are shown in Table III. After the oxidation, the excess of periodate was destroyed by adding ethylene glycol. The solution was dialyzed against tap water to remove inorganic ions, evaporated to a syrup, and hydrolyzed with 1 N sulfuric acid for 6 hr. Neutralization with barium hydroxide solution, filtration and then evaporation of the filtrate, yielded a syrup. Examination by paper chromatography indicated the presence of D-xylose only.

TABLE III. PERIODATE UPTAKE OF GANPI HEMICELLULOSE

Time, hr.	17	65	141.5	232.5
$NaIO_4/C_5H_8O_4$	0.98	1.02	1.18	1.17

Summary

The hemicellulose of Ganpi bast fibers (*Wickstroemia sikokiana* Franch. et Sav.) gave upon hydrolysis a mixture of an aldobiouronic acid, 4-*O*-methyl-D-glucuronic acid and D-xylose together with small amounts of D-galacturonic acid, D-mannose, D-galactose, L-arabinose and L-rhamnose.

The acidic components were separated, and by means of chromatography on a column of cellulose, 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid and 2-*O*-(4-*O*-methyl-D-glucopyranosyluronic acid)-D-xylopyranose were isolated and characterized.

A structure was proposed for the hemicellulose which has a straight chain of about 160 1,4-linked-β-D-xylopyranose residues with, on the average, every sixth residue carrying a terminal 4-*O*-methyl-α-D-glucopyranosyluronic acid residue linked through position 2.

The D-galacturonic acid is believed to originate in pectic substance and not a component of the hemicellulose.

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