Studies on Japanese Hand-made Paper.

VI. On the Structure of Xylan in the Purified Bark of Mitsumata (*Edgewortia Papirifera* Sieb.).

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So-called xylan is abundantly distributed in the plant kingdom as a cellular cement, being found in roots, stems, leaves, seeds, and in some marine algae. In corn cobs, corn stalks, grain hulls, and stems, it occupies the amount ranging from 15 to 30%. Hard-wood contains 20 to 25% of xylan whereas soft-wood contains 7 to 12%. Up to the present, wood xylans have not been isolated in high degree of purity, and usually they contain L-arabinose or uronic acids as their minor components. The notable researches of Haworth and his collaborators[1),3)] on esparto xylan obtained from esparto grass have thrown considerable light on the structure of it. Thus it has been shown that esparto xylan contains small quantities of L-arabofuranose residues, which are easily removed by the action of 0.2% oxalic acid at 100°C. And so it may be concluded that they are located at the terminals of the xylan chains.

The degree of polymerization of xylans measured by the use of osmometric, viscometric method and of the end-group assay are shown below;
40 for wheat straw xylan,[2)] 75 for esparto xylan,[3)] 115 for pear wood xylan.[4)]
Generally the degree of polymerization of xylans does not exceed 200.

The glycosidic linkages of D-xylopyranose residues in xylans are concluded, as in cellulose, as β-1 : 4 bonds from the studies of methylation,[1),2),3),4)] and the isolation and the identification of crystalline xylobiose[5)] (4-O-β-D-xylopyranosyl-D-xylopyranose) from the partial hydrolysate of xylan by fuming hydrochloric acid at low temperature.

In the previous papers,[6),7)] the authors have established the composition of the xylan of Mitsumata (*Edgewortia Papirifera* Sieb.) and the present paper is concerned with the structure of this xylan. Xylan was obtained from the holocellulose (H.C.-9)* by the extraction with dilute sodium hydroxide solution followed by the addition of acid and the precipitation by acetone. As shown previously,[7)] crude xylan extracted directly from holocellulose contains D-xylose, D-glucose, and L-arabinose

* See ref. 7).

in the proportion of 24～27 : 1～3 : 1, respectively. Arabinose and glucose residues, however, could be eliminated by the purification using Fehling's solution in the analogous procedure employed in the purification of wheat straw xylan.[8] Two successive purifications gave pure xylan (xylan-II), which on hydrolysis gave D-xylose as a single component.

Crude xylan showed an optical rotation of $[\alpha]_D$ -107° in 2% sodium hydroxide solution as an ash free basis.

In order to determine the mode of the linkage of the residues of the sugar in this xylan, it was methylated ten times with 45% potassium hydroxide solution and dimethyl sulfate. After purification, the methylated xylan was obtained as a white powder and showed methoxyl content (OCH_3) of 35.7%. Further methylations with methyl iodide and silver oxide gave completely methylated xylan (OCH_3 : 38.8%). Methanolysis of this product with 1.5% methanolic hydrogen chloride, followed by hydrolysis with 0.5N hydrochloric acid, gave a mixture of methylated sugars. Paper chromatographic examination of the hydrolysate with n-butanol—ethanol—water (5 : 1 : 4, v/v) as a developing solvent indicated the presence of 2 : 3 : 4-tri-O-methyl-D-xylose, 2 : 3-di-O-methyl-D-xylose, monomethyl-D-xylose and a trace of free D-xylose or oligosaccharide(s). The proportion of the sugars on the paper chromatogram was determined by the method of Hirst, Hough and Jones,[9] and was found to be a) trimethylxylose 2.7, b) dimethylxylose 94.9, c) monomethylxylose 2.4 molar percents.

Being treated with fuming hydrochloric acid at low temperature, xylan-I gave, after the neutralization and the concentration under reduced pressure, a syrup containing D-xylose and oligosaccharides. When this syrup was chromatographed on a charcoal—Celite column, D-xylobiose was obtained as a crystalline mass which had identical physical constants with the specimen isolated from corn cob xylan by Whistler and Tu.[5]

The molecular weight of the fully methylated sample was determined by measuring the osmotic pressure of dilute solution and gave a value of $(2.7\pm0.08)\times10^4$, which corresponded to the chain of 170±6 of xylose residues. The results of the periodic acid oxidation of xylan-II at 0°C would lead to the conclusion that one mole of formic acid liberated from 16 to 17 xylose residues.

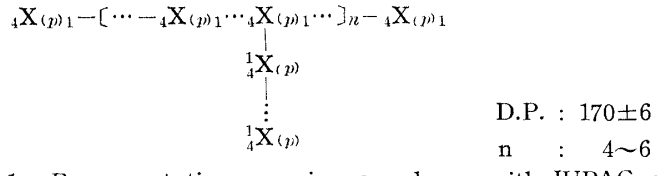

Fig. 1. Representations are in accordance with IUPAC rules.

From the results mentioned above, it seems reasonable to assume that the

xylan from Mitsumata has a structure containing about 170±6 D-xylopyranose residues linked by 1 : 4-β bonds with a few branching points (probably the numbers are ranging 4 to 6), the mode of branching is not yet determined (see the above formula).

Experimental Parts.

Preparation of xylan.

Holocellulose (H.C.-9) was prepared as reported previously,[7] and it (30 g., dry) was placed in a three-necked flask and extracted with sodium hydroxide solution (5%, 750 ml.) for 24 hours in a hydrogen atomosphere. After 24 hours, acetic acid was added to the filtrate and the solution was adjusted to pH 5.0. In this acidic solution, twice the volume of acetone was added and the solution was allowed to stand over a night. The precipitated xylan was collected, washed with acetone-water mixture (2 : 1, v/v), acetone and ether successively, and dried over phosphorous pentoxide *in vacuo*. The resulted xylan (xylan-I) was white powder : yield, 4.6 g. ; ash, 0.4% ; pentosan content, 93.7% ; $[\alpha]_D$-107° (in 2% sodium hydroxide, as ash free). It was found that xylan-I had D-xylose, L-arabinose, and D-glucose as its component sugars, in the proportion of 24～27 : 1 : 1～3, respectively.

To a 5% sodium hydroxide solution (300 ml.) of xylan-I (5 g.) an equal volume (or less) of freshly prepared Fehling's solution was added with stirring. Immediately, the xylan was precipitated as copper complex, and this precipitate was collected by the centrifuge, washed with water, and poured into ice-water. 1N-Hydrochloric acid was poured into this mixture with stirring and xylan-copper complex was decomposed. After the decomposition was completed, twice the volume of acetone was added and xylan was reprecipitated, washed with acetone-water mixture (2 : 1, v/v) containing a few drops of acetic acid, and acetone successively, and dried. After the second purification with Fehling's solution was achieved in the same manner as mentioned above, the xylan was washed with acetone and ether successively, and dried over phosphorous pentoxide *in vacuo*. The xylan thus treated (xylan-II) was obtained as white powder : yield, 3.5 g. ; ash 0.5% ; pentosan content, 97.9%. Xylan-II gave, on hydrolysis, D-xylose as a single component.

Methylation of xylan.

In a three-necked flask, xylan-II (5 g., dried over phosphorous pentoxide) was pasted with water (10 ml.), then the air was replaced by hydrogen. Hydrogen gas was gently streamed during the period of methylation. Potassium hydroxide solution (83 ml., 45%) was added and the mixture was stirred vigorously until the

complete dissolution was done. Then dimethy sulfate (50 ml.) was added dropwise with mechanical stirring for 5 hours, the flask being kept in an ice-water bath. A few drops of iso-amyl alcohol was added if the mixture foamed. After the mixture was stirred for 1 hour more, the flask was immersed in a boiling water bath for 1 hour and cooled. Then potassium hydroxide solution was added and the second methylation was carried out with the addition of dimethyl sulfate as described above. After ten successive methylation, the mixture was treated with dilute sulfuric acid until it was made faintly alkaline to litumus, added with boiling water and boiled on a water bath, whereupon the methylated xylan was separated. After filtrated in hot state, the precipitate was washed successively with hot water and dried. The aqueous filtrate and the washings were combined and extracted with a half volume of chloroform, and just then the remaining methylayed xylan was separated as white gum which was combined to the main bulk. The combined methylated xylan was dissolved in methyl-cellosolve or 1 : 4-dioxane and diluted with distilled water to about 200 ml. and dialyzed against distilled water. The dialysis needed a week or more. The dialyzed solution was evaporated to a few mililiters under a reduced pressure and an equal volume of alcohol was added. Into this solution an excess volume of ether was poured, then the methylated xylan jellified. When this jellified material was treated with absolute alcohol, white solid was obtained. The solid was dissolved in methyl-cellosolve, diluted with alcohol, reprecipitated by adding an excess volume of ether, and dried over phosphorous pentoxide *in vacuo*. Yield, about 2 g. ; OCH_3, Found. 35.7%, Calc. for $C_5H_6O_2$, 38.75%.

A portion of the methylated xylan (1.8 g.) was dissolved in warm methyl-cellosolve (3 ml.), which was subsequently treated with methyl iodide (20 ml.) and silver oxide (10 g.) at 40°C. After stirring for 8 hours, the reaction mixture was retreated for 8 hours with methyl iodide and silver oxide as mentioned above. Silver iodide and remaining silver oxide were filtered off with the aid of Super cel, and water (20 ml.) was added to the filtrate. The whole solution was passed throngh a deionizing column (Amberite IR-120 and IR-45), decolorized with charcoal, and concentrated to a syrup under a reduced pressure. With the addition of alcohol and ether to this syrup, white product precipitated, which was filtered and dried over phosphorous pentoxide *in vacuo*. The product was white powder : yield was about 1.2 g. ; OCH_3, Found., 38.8%, Calc. for $C_5H_6O_2(OCH_3)_2$, 38.75%.

Hydrolysis of methylated xylan and chromatographic separation of methylxyloses.

The methylated xylan (150 mg., dried over phosphorous pentoxide) was treated with methanolic hydrogen chloride (4 ml., 1.48%) in a sealed tube at 100°C for 8 hours. After removal of the solvent *in vacuo*, hydrochloric acid (4 ml., 0.5N) was

added to the syrupy residue and the solution was hydrolysed at 100°C. After 7 hours, the hydrolysate was neutralized with silver carbonate, filtered, and passed througth a deionizing column (Amberite IR-120 and IR-45). The resulted neutral solution was concentrated to a syrup under a reduced pressure. This syrup showed, on paper chromatogram using n-butanol—ethanol—water (5 : 1 : 4, v/v) as a developing solvent, three distinct spots (corresponding to trimethylxylose, dimethylxylose, monomethylxylose, respectively) and two faint spots (probably xylose and/or oligosaccharide(s)). For the quantitative estimation, 300 × 400 mm. filter paper (Toyo Roshi Co. Ltd., No. 50) was used, on which a portion of the syrup was spotted to be a thick line in 200 mm. length and two spots for guide trails on each side of the paper. After developing, the strips containing the sugars were cut off and extracted with boiling water (7 ml.) for 40 minutes. Then the extract was cooled, and the sugar was estimated by the method of Hirst, Hough and Jones[9] using hypoiodite in Menzel's buffer solution. The results are tabulated in Table 1.

Table 1.

Sugar	Molar composition, % (shown as a average value).
2 : 3 : 4-Tri-O-methyl-D-xylose	2.7
2 : 3-Di-O-methyl-D-xylose	94.9
Monomethyl-D-xylose	2.4

Isolation and identification of D-xylobiose.

Xylan-I (5 g., dry) was dissolved in fuming hydrochloric acid (500 ml., d=1.22) previously cooled to −15°C. The dissolution needed about 30 minutes. The flask was then placed in an ice-water bath and the hydrolysis was carried out. At suitable intervals, the samples (10 ml.) were pipetted out and analysed for reducing values. When the reducing power of the solution was reached to a value of 66% of the theoretical completion, the solution was immediately neutralized with sodium bicarbonate. After standing over night, the precipitated salts were filtered off and the filtrate was concentrated to about 100 ml. *in vacuo*. The solution thus obtained was placed on the top of a 40×290 mm. column of a mixture of charcoal (Darco G-60) and Celite-535 (1 : 1, w/w) which was previously washed successively with water and 5% ethanol. Then the column was washed with distilled water and after the elute has been free from salt and negative to Benedict's reagent, the column was developed with 5% aqueous ethanol until it was negavive to Benedict's reagent. The 5% aqueous ethanol portion was collected and concentrated to a syrup *in vacuo*. The syrup obtained (400 mg.) was dissolved in hot water (1 ml.) and methanol (4 ml.) was added into this solution, then the white solid was soon precipitated. This solid was filtered and dried. Yield, 300 mg.; m.p., 184~5°C after recrystallization from ethanol. Whistler and Tu[5] reported m.p. 185~6°C for their D-xylobiose.

D-Xylobiose (250 mg.) thus obtained was heated to 100°C with a mixture of acetic anhydride (9 ml.), acetic acid (1 ml.) and anhydrous sodium acetate (1 g.) for 30 minutes. The the solution was poured into ice-water with stirring. After standing over night, the precipitated acetate was collected and washed with water and the crude acetate was recrystallized from ethanol. Yield, 120 mg.; m.p., 157°C after twice recrystallizations; molecular weight (Rast), 544, 549, Calc. for $C_{10}H_{12}O_3(AcO)_6$, 534. Whistle and Tu[5] reported m.p. 155.5~6.0°C for their D-xylobiose hexaacetate.

Oxidation of xylan with sodium metaperiodate.

Dry xylan-II (2.68 m mol.) was suspended in water (250 ml.) and to this mixture sodium metaperiodate (about 11 m mol.) and water (250 ml.) were added. The oxidation mixture was kept in dark at 0°C and at suitable intervals a portion of this mixture was pipetted out. The amount of consumption of periodate was determined by the method of Fleury and Lange,[10] and the liberated formic acid by the titration with 0.00382N sodium hydroxide solution (phenolphthalein as an indicator) after the addition of excess 10% ethylene glycol solution. The ammount of formic acid liberated was practically constant after 145 hours, corresponding to 1 mole formic acid per 16 to 17 xylose residues.

Table 2.

Time (hours).	3	6	12	25	53	4 days	6〃	8〃	11〃	25〃
Formic acid produced, moles per $C_5H_8O_4$.	0.023	0.035	0.042	0.045	0.051	0.056	0.059	0.060	0.061	0.060
Periodate consumption, moles per $C_5H_8O_4$.	0.150	0.215	0.290	0.355	0.453	0.512	0.572	0.647	0.669	0.744

In other two experiments, the oxidations were carried out in dark at 10°C and at room temperature (about 30°C), respectively. In each case, a considerable over oxidation was observed.

Molecular weight determination.

A Zimm-Meyerson osmometer was employed for the determination of the molecular weight of the methylated xylan. Methyl-cellosolve—chloroform was chosen as a solvent. A value of $(2.7\pm0.08)\times10^4$ was obtained for the molecular weight of the methylated xylan. It correponded to the degree of polymerization of 170 ± 6.

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要　　旨

三椏（*Edgewortia Papirifera* Sieb.）白皮から得られるホロセルロース中には，D-キシロース，L-アラビノース，D-グルコース（構成比；24～27：1：1～3）から構成される粗キシラン〔キシラン－Ⅰ：ペントーザン含量，93.7%；灰分，0.4%；$[\alpha]_D$－107°（2%カ性ソーダ）〕が含まれているが，このキシランは，フェーリング溶液を用うる精製法によつてD-キシロースだけからなるキシラン〔キシラン－Ⅱ：ペントーザン含量，97.9%；灰分，0.5%〕を与える。キシラン－Ⅱをジメチル硫酸およびカ性カリを用いて10回連続メチル化することによつてメチル化キシラン（OCH_3, 35.7%）が得られるが，このものはさらにヨウ化メチルおよび酸化銀を用いてメチル化を行うと，完全メチル化キシラン（白色粉末；OCH_3, 38.8%）を与える。完全メチル化キシランは浸透圧法による重合度（D.P.）測定によると D.P.＝170±6 であり，メタノリシス後に加水分解するとメチルキシロースを含むシロップを与える。このシロップ中の糖含有比は，次亜ヨウ素酸塩滴定によると Table 1 に示すようなものであり，約35ケのD-キシロース残基当り1ケの末端基が存在することを示している。

さらに，キシラン－Ⅱを水溶液中で過ヨウ素酸酸化を行つた結果，0° における酸化ではギ酸の生成は145時間で一定値を示し，16～17ケのD-キシロース残基当り1モルのギ酸の生成が認められる（Table 2 参照）。10°および室温における酸化では，いずれの場合もかなりの過酸化が見られる。

キシラン－Ⅰの発煙塩酸による冷時部分加水分解液を，Darco G-60—Celite 535 のカラムを用いてクロマトグラフィ分離を行つたところ，D-キシロビオース〔m.p. 184～5°；そのヘキサアセタート，m.p. 157°, M.W., 544, 549 (Rast)〕が得られるが，これは Whistler, Tu[5] の 4-O-(β-D-キシロピラノシル)-D-キシロースと同一物質であると考えられる。

以上の結果から，三椏白皮に含まれているキシランは，Fig. 1 に示すような構造をもつものと考えられる。

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