Studies on Hemicellulose of Bamboo*

Eiichi MAEKAWA**

Contents

I. Introduction
II. Isolation and Purification of Hemicellulose from Culms of Bamboo
III. Structural Studies on a Xylan from Bamboo
IV. Isolation of a Xylan containing Native Acetyl Groups from Bamboo
V. Discussion and Conclusion

Introduction

The somewhat vague term "hemicellulose" was initially proposed and used by Schulze[1] to designate those polysaccharide components which are readily hydrolyzed with acid and extractable from plant cell walls with aqueous alkali. Since then, the term, which was neither systematic nor definitive but for want of a more appropriate name, has been retained even now through the common usage to designate the broad group of non-cellulosic polysaccharides which are found in close association with cellulose, especially in lignified tissues.

The hemicelluloses, next to cellulose, are one of the most abundant naturally occurring organic materials along with lignin on the earth and are widely distributed in both terrestrial plants and marine algae[2,3]. However, polysaccharides such as exudate gums, pectic substances and plant mucilages are usually not included in the hemicelluloses. Recently, with the possibility that other groups such as a water-soluble arabinogalactan from larchwood, β-glucans of barley and oats, and some mannans and galactomannans of seeds may be regarded as hemicelluloses, the distinction of hemicelluloses has become vague and indifinitive in these days[4].

Bamboos are widely distributed in the Oriental region, being found in Japan, China and South-East Asian countries. Some of them grow wild but others are cultivated for food as *Takenoko* and various manual bamboo wares. In some South-East Asian countries, bamboo culms provide very important materials for house and furniture construction. In recent years, much attention has been denoted to bamboo materials from the economic importance as potential natural resources of pulp

* This Review Article constitutes a part of a thesis for a doctor's degree.
** Division of Wood Chemistry.

and paper. From such practical and industrial points of view, behaviors and properties of hemicellulose of bamboo in sulfite and kraft pulping processes have been studied[5]. On the other hand, an academic investigation concerning isolation of a xylan from bamboo materials was initially carried out by Lüdtke[6]. Matsuzaki *et al.*[7] later studied its properties after isolation and fractionation of hemicellulose from bamboo, and proposed a chemical structure of the hemicellulose. Since that time, bamboo hemicellulose was established to be a xylan, but further investigation has not been carried out in detail. Thus, since information concerning the constitution and properties of hemicellulose from bamboo was relatively few, this investigation was undertaken to elucidate the chemical structure of a xylan present as the predominant component of bamboo hemicellulose and to compare with some properties of certain xylans found in hardwoods and softwoods.

Bamboo was formerly included in the Gramineae, but now it is classified to Bambusaceae which includes Sasa. Early investigations were concerned with the hemicelluloses from the Gramineae, especially, with the elucidation of the structure of esparto grass xylan. Afterwards, xylans from agricultural sources such as wheat[8] and oats[9] straw, barley husk[10], corn cob[11] and corn hull[12] were studied by numerous investigators. As the result of the extensive studies, the main structural features of the xylan group were established and characterized[13~15]. The ordinary xylans found in the higher land plants are composed of the basic structure having essentially a linear backbone of 1,4-linked D-xylopyranosyl residues. In many cases they are characterized by the presence of L-arabinofuranose usually attached to C-3 D-xylose residues as single side chains and of single side chains of 4-*O*-methyl-D-glucuronic acid or D-glucuronic acid linked to C-2 of D-xylose residues. Hence, xylans containing only D-xylopyranose are considered to be rare in nature. Such polysaccharides have hitherto been isolated only from a few plant sources such as esparto grass, tamarind seed, etc., and the majority of xylans from natural sources is known as heteroglycans.

However, further definitive evidence for determinating the sequence of L-arabinofuranosyl residues in xylans has not been presented. In recent years, it has been elucidated by the skillful methods using enzymic hydrolysis[16] and periodate oxidation[17] that the side chains of xylans are arranged in an irregular manner.

This Review Article is concerned with the isolation and strucural studies of a xylan as the predominating component of bamboo hemicellulose, and with the isolation of a bamboo xylan containing native acetyl groups.

II. Isolation and Purification of Hemicellulose from Culms of Bamboo

This part[18] deals with the following subjects: preparation of a bamboo ho-

locellulose by Wise's chlorite method[21], extraction of hemicellulose with aqueous alkali, isolation and purification through fractional precipitation with Fehling's solution, and various properties of the fractions obtained.

EXPERIMENTAL

Preparation of the experimental material

Culms (6~8 cm, diameter) of a green bamboo, Ma-dake (*Phyllostachys reticulata* C. Koch or *P. bambusoides* Sieb. et Zuce.), was cut into the suitable segments (length, 1~1.5 meter), and the bamboo was made into shavings and air-dried for 3 days. The fully air-dried shavings were ground in a Wiley mill and the bamboo meal prepared to 40~60 mesh was submitted to this work. The general analytical results of the bamboo meal were as follows:(%) holocellulose 67.4, α-cellulose 44.3, pentosan 22.1, Klason lignin 21.6, 1% NaOH extract 27.0, methanol and benzene (1:2, v/v) extract 3.8, uronic anhydride 4.7, ash 1.8 (silicate 1.0)

Analytical methods

Paper chromatography was carried out on Toyo Roshi No. 51 filter paper sheet with the following solvent systems; 1) n-butanol-pyridine-water (6:4:3, v/v), 2) ethyl acetate-pyridine-water (8:2:1, v/v), 3) n-butanol-ethanol-water (4:1:5, v/v), 4) benzene-ethanol-water (200:47:45, v/v), 5) ethyl acetate-acetic acid-formic acid-water (18:4:1:3, v/v). The development was conducted exclusively by a descending method. The spray reagent used most commonly to detect the reducing sugars and their methylated derivatives was aniline hydrogen phthalate. The general analyses used in this work mainly followed the Tappi standard methods. Uronic anhydride was determined by the modified method[19] based on decarboxylation in mineral acid solution. Silicate content in ash was determined according to the colorimetric method[20] described by Snell. Lignin content was determined as Klason lignin.

Preparation of bamboo holocellulose

Air-dried bamboo meal was extracted with a mixed solvent of methanol and benzene (1:2, v/v) and then used for the preparation of a chlorite holocellulose according to the method[21] described by Wise *et al.* The defatted bamboo meal (200 g) was suspended in water (4.5 liters) and heated to 70~75°C in a water-bath, then, sodium chlorite (60 g) and glacial acetic acid (24 ml) were added gradually with mechanical stirring. After 1.5 hr, the reaction mixture was allowed to react for 3 hr by addition of the same amount of reagents. The reaction mixture was transferred into a large Büchner funnel, filtered through a cloth and washed with a large amoout of cold water and ethanol, successively. The pale yellowish product was air-dried at the ventilated place. Yield of the holocellulose was 165 g, corresponding

to 82.5% of the original defatted bamboo meal. Klason lignin content of the holocellulose was 4.5~5.0%.

Isolation of hemicellulose by direct extraction with aqueous alkali

The defatted bamboo meal (200 g) was extracted directly with 5% aqueous sodium hydroxide (2.5 liters) without prior delignification. The extract was filtered and acidified with acetic acid, then the hemicellulose fraction was precipitated by addition of three or four volumes of ethanol. The precipitate was collected by centrifugation and washed successively with 50% ethanol, anhydrous ethanol and ethyl ether. The product was dried over phosphorus pentoxide under a vaccum to give a pale brownish powder (Fl). The yield was 16 g, corresponding to 39.8% of hemicellulose extractable with aqueous alkali from bamboo holocellulose. Lignin content of this product was 6.5%.

Isolation and purification of bamboo hemicellulose

Bamboo holocellulose (200 g) prepared as described above was extracted twice with 5% aqueous sodium hydroxide (3 liters) under a nitrogen atmosphere at room temperature overnight. The extract was filtered through a cloth on a Büchner funnel and acidified with acetic acid, then the crude hemicellulose fraction was precipitated by addition of three or four volumes of ethanol. The product was treated as described previously, giving a pale brownish powder (F2).

The residue after treatment with 5% aqueous sodium hydroxide was extracted successively twice with 18% aqueous sodium hydroxide (1.5 liter) under the same conditions, and the extract was treated in the same manner, giving a hemicellulose fraction (F3) as a colorless powder.

The crude hemicellulose (F2) was dissolved in 4% sodium hydroxide solution (1.2 liter), to which newly prepared Fehling's solution was added gradually until the copper complex was formed completely (*ca.* 400 ml). The gel-complex insoluble in water was collected by centrifugation, washed with distilled water, and decomposed with stirring in ice-water by soaking in 50% ethanl containing 3% hydrochloric acid until the complex gave a colorless product. The precipitate was collected by filtration on a cloth, and washed successively with distilled water and ethanol until the washings gave a negative chloride test. The product was dried in the same manner, giving 37.8 g of a colorless amorphous powder (F2A). ash: 0.38%, silicate in ash: 0.21%. The filtrate recovered from fraction F2A was neutralized with acetic acid and dialyzed against running water for 2 days. The dialyzed solution was concentrated to a small volume (*ca.* 100 ml), and then addition of four volumes of ethanol gave a precipitate. The precipitate was collected by centrifugation and obtained as a grayish powder (F2B, 2.4 g). Furthermore, the hemicellulose fraction

(F3) was also treated with Fehling's solution in the same manner, and the fraction (F3A) was obtained as a colorless amorphous powder (7.3 g). However, fraction corresponding to F3B was negligible.

Acid hydrolysis of hemicellulose

Acid hydrolysis of the individual hemicellulose fractions was carried out under conditions similar to that of Saeman *et al.*[22] Two ml of 72% H_2SO_4 was added to each (0.1 g) of the hemicellulose, and the mixture was allowed to stand at room temperature for one hr with frequent mixing. After dilution to 3% acid concentration with distilled water, the mixture was boiled under reflux for 5 hr. The hydrolyzate was neutralized to pH 5.5 partially with a saturated solution of barium hydroxide and followed with barium carbonate. Barium sulfate produced was removed by filtration through a Celite layer. The filtrate and washings were desalted through Amberlite IR 120(H^+) exchange resin, filtered and concentrated to a syrup under a reduced pressure.

Quantitative determination of sugar components

Toyo Roshi No. 51 filter paper sheet (13 × 55 cm) containing a localization guide was used for quantitative determination of sugars[23]. All sugars present were found to be separated very successfully with a descending method as follows; The first development was performed with solvent 1 at room temperature for 24 hr. After air-drying, the second development was performed with solvent 2 for 20~24

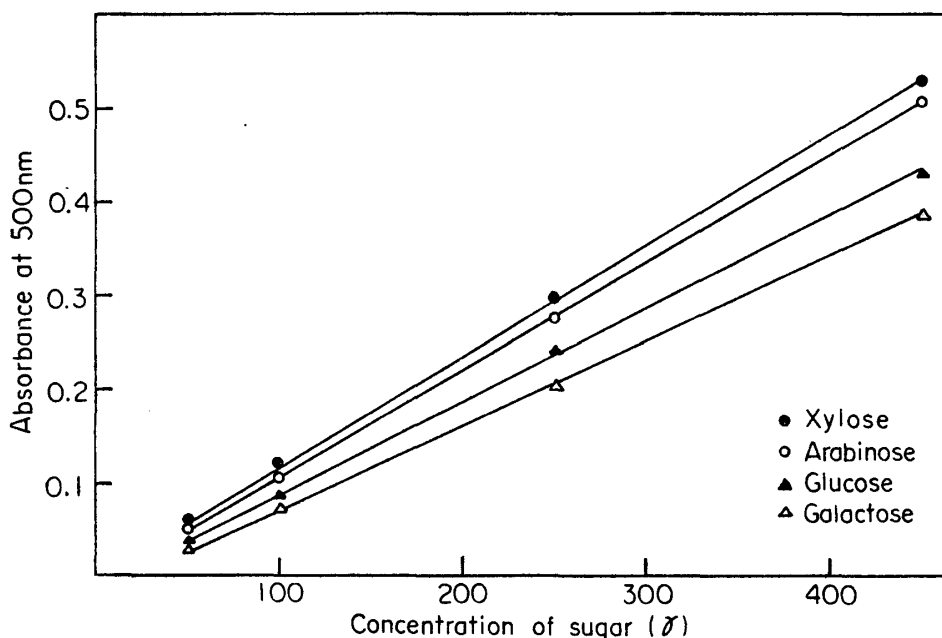

Fig. 1. Calibration curves for the determination of sugars (by Nelson-Somogyi colorimetric method).

hr. The syrup was diluted with 0.1 ml of distilled water, and applied with a micropipet to the determined line drawn on the paper. A guide strip was cut out and the individual sugars were located by spraying with aniline hydrogen phthalate and heating at 105°C for 5 min. Parts corresponding to the individual sugars were cut out and extracted in warm water for 10 min. An adequate amount (contains sugars in the range of 5～500 γ) from each extract was pipetted out and analyzed according to the colorimetric method[24] of Nelson-Somogyi. The absorbance at 500 nm was read against a blank solution using a spectrometer. The amount of sugars was determined from the linear calibration curves between the absorbance and the amount for each standard sugar (Fig. 1).

RESULTS AND DISCUSSION

Pulverized air-dried bamboo was extracted beforehand with a mixed solvent of methanol and benzene (1:2, v/v) to remove coloring matter and waxy materials, and it was delignified by Wise's sodium chlorite method. The holocellulose which still contains 4.5～5.0% of lignin was used for isolation of hemicellulose in order to avoid the degradation of polysaccharides with sodium chlorite.

Hemicellulose was isolated almost completely by successive extraction with 5% and 18% aqueous sodium hydroxide from the holocellulose. Each of the hemicellulose extracts was recovered as precipitates by addition of a large amount of ethanol containing acetic acid, and purification by fractional precipitation with Fehling's solution gave bamboo xylans of F2A and F3A, respectively. Since the fractional precipitation using Fehling's solution has often been used for the fractionation of alkali-soluble polysaccharides containing D-mannose or D-xylose[25], the precipitant was applicable most effectively to the fractionation of bamboo hemicellulose.

A large portion of bamboo hemicellulose thus isolated was found to correspond to the fractions(F2A and F3A) forming precipitates by addition of Fehling's solution. The yield corresponded to 95% of bamboo hemicellulose. On the other hand, fraction F1 was obtained by direct extraction with aqueous alkali of an air-dried defatted bamboo meal without prior delignification, but the yield was only 39.8% of hemicellulose obtained by extraction with aqueous alkali after delignification. This fraction contained a considerable amount of non-carbohydrates(6.5%). The yields and properties of the individual fractions are summarized in Table I. Each fraction of F1, F2A and F3A may be regarded as the similar xylan fractions, as revealed by analysis of carbohydrate composition. Therefore, the predominating part of bamboo hemicellulose is characterized as a xylan containing the residues of L-arabinose, D-glucose, minute quantities of D-galactose and D-glucuronic acid. Other fraction of F2B was obtained as polysaccharides which involved relatively

Table I. Yields, specific rotations and sugar compositions of hemicellulose fractions isolated from bamboo.

Fraction	Yield* (%)	$[\alpha]_D^{25}$ (c, 1.0, 4% NaOH)	Uronic anhydride (%)	OMe (%)	Sugar composition (%)				Xyl./Ara.
					Xyl.	Ara.	Glu.	Gal.	
F1	8.0	−83°	5.16	1.92	92.9	5.6	1.3	0.2	17.9
F2	16.7	−92°	5.62		91.1	5.6	2.4	0.9	16.2
F2A	15.6	−98°	4.46	1.10	90.3	4.8	3.9	1.0	18.8
F2B	1.0	−74°	6.33		62.1	10.1	22.6	5.3	6.1
F3	3.4	−93°	4.67		88.4	5.9	5.5	0.3	15.5
F3A	3.0	−92°	3.75		89.9	4.5	4.3	1.3	20.0

* Percent based on the original air-dried defatted bamboo meal
Xyl. xylose; Ara. arabinose; Glu. glucose; Gal. galactose.

higher amounts of glucan and arabinan, corresponding to approximately 5% of hemicellulose in a yield.

Bamboo culms are known to contain significant amounts of silicate in the ash. For confirmation of the fact, preliminary silicate analysis[26] in the ash revealed that *ca.* 55-60% of ash was silicate. Bamboo hemicellulose prepared also contained silicate in the ash. However, further examination of the silicate was not carried out.

III. Structural Studies on a Xylan from Bamboo

This part[18] is concerned with the examination of acid components prepared from a bamboo xylan and with the structural studies of the xylan by means of partial hydrolysis, methylation, periodate oxidation and acetolysis.

Analytical methods

The method for paper chromatography and the developing solvents were described in the previous part. Methoxyl content[27] was determined by a modified semi-micro method. Demethylation of methylated sugars was carried out using borontribromide according to the method[28] of Bonner *et al.* Detection of sugar alcohols[29] on paper chromatogram was made by spraying with 1% sodium metaperiodate solution and after air-drying for 30 min, by subsequent spraying with benzidine HCl-ethanol solution.

Examination of acid components from bamboo xylan

Thirty grams of bamboo xylan (F2A) was heated in 3% H_2SO_4 (350 ml) on a boiling water-bath for 5 hr. After cooling, the solution was neutralized exactly to pH 5.5 with barium carbonate, and barium sulfate produced was removed by filtration through a Celite layer. The pale yellow filtrate which was previously treated with Amberlite IR 120(H^+) exchange resin was passed through a column

of Dowex 1x4(50～100 mesh, acetate form), eluted with distilled water until Molish test became negative, and then the acid components were eluted with 30% acetic acid. After removal of the acetic acid by shaking with ethyl ether, the aqueous layer was evaporated under a reduced pressure to a syrup (1.6 g), corresponding to 5.3% of the original bamboo xylan. On the other hand, cold 72% H_2SO_4 (150 ml) was poured into newly prepared bamboo holocellulose (100 g), which was allowed to stand at room temperature for one hr with frequent mixing. The hydrolyzate was diluted to 3% acid concentration and heated under reflux for 4 hr. The acid components were obtained as a syrup (2.4 g) through the same procedure as that prepared from hemicellulose. Both acid components were examined by paper chromatography in solvent 5 and the examination revealed the occurrence of the same acid components in both acid products. Then both acid components were put together and applied further to a column of Dowex 1x4 (100～200 mesh, 3×50 cm, acetate form), which was eluted with distilled water. The acid components absorbed were then eluted with 0.1～3.0N acetic acid, stepwisely. The eluate was collected in 10 ml portions with an automatic fraction collector. The main fraction eluted with 2.0-2.5N acetic acid was collected and concentrated to a pale yellow syrup(1.9 g) under a reduced pressure. Further separation of the fraction was carried out on a cellulose column (2×50 cm) packed with Whatman cellulose powder using solvent 5. Each of the eluate collected in 10 ml portions was evaporated and examined by paper chromatography in solvent 5. Paper chromatographic examination revealed that the acid components were separated into four fractions as follows; Fraction I (73 mg) ; syrup, a mixture of acid components, $[\alpha]_D+16°$ (c=0.75, H_2O), OMe 4.1%. Fraction II (87 mg); pale brownish powder, R_x 1.05 (solvent 5), $[\alpha]_D$ +46 (c=1.0, H_2O). OMe 14.5%, acid equivalent 203 (Calcd. for $C_7H_{12}O_7$; OMe 14.9%, molecular weight 205). Fraction III (610 mg); colorless powder, R_x 0.72 (solvent 5), $[\alpha]_D+97°$ (c=1.0,H_2O), Anal. found: C, 42.32; H, 5.95, OMe 9.42%, acid equvalent 355 (Calcd. for $C_{12}H_{20}O_{11}$; C, 42.36; H, 5.92, OMe 9.12%, molecular weight 340). Fraction IV (46 mg); colorless powder, R_x 0.67 (solvent 5), $[\alpha]_D+57°$ (c=1.0, H_2O), OMe 6.16%, acid equivalent 517 (Calcd. for $C_{17}H_{28}O_{15}$: OMe 6.54 %, molecular weight 472). R_x values are expressed as relative mobility to that of D-xylose (1.00).

Hydrolysis of the fractions containing uronic acid

Each of Fraction III and IV (*ca.* 10 mg) was hydrolyzed with 1N H_2SO_4 (2 ml) in a sealed tube for 15 hr at 100°C. The hydrolyzate was neutralized with barium carbonate, and barium sulfate produced was removed by filtration. After treatment of the filtrate with Amberlite IR 120(H^+) exchange resin, evaporation of the solution gave a syrup. Paper chromatographic analysis in solvent 1 revealed the

presence of D-xylose and 4-O-methyl-D-glucuronic acid.

Identification of 4-O-methyl-D-glucuronic acid

The acid Fraction II (65 mg) was converted to the methyl ester glycoside by heating under reflux with 3% methanolic hydrogen chloride (10 ml) for 8 hr. The product (70 mg) was dissolved in dry tetrahydrofuran (10 ml) and reduced with lithium aluminum hydride (100 mg) according to Adams' method[30]. The reaction mixture was heated under reflux gently for one hr. After cooling, the excess lithium aluminum hydride was decomposed by addition of ethyl acetate and water. The solution which was desalted with exchange resins of Amberlite IR 45(OH^-) and IR 120(H^+) was concentrated under a reduced pressure to give the neutral methyl glycoside as a glassy solid (52 mg). Acid hydrolysis with 1N H_2SO_4 (5 ml) at 100°C for 10 hr yielded a yellow syrup (41 mg). $[\alpha]_D+52°$ ($c=0.5$, H_2O) [lit.[31] value for 4-O-methyl-D-glucose, $[\alpha]_D+53°$ (H_2O)] Paper chromatographic analysis in solvent 1 revealed the presence of 4-O-methyl-D-glucose (R_x 1.14). Furthermore, identification of 4-O-methyl-D-glucose was made by converting to its crystalline phenylosazone derivative giving the melting point of 158~9°C in the usual way.

Examination of 2-O-(4-O-methyl-a-D-glucopyranosyl uronic acid) D-xylose

The acidic Fraction III (400 mg) was converted to the methyl ester methyl glycoside by refluxing with 3% methanolic hydrogen chloride (40 ml) for 10 hr. The methyl ester methyl glycoside (397 mg) dissolved in dry tetrahydrofuran (50 mg) was reduced with lithium aluminum hydride (800 mg) to the corresponding neutral disaccharide (373 mg) by the same procedure as described above. The disaccharide was dissolved in methanol (5 ml) and methylated by the simultaneous dropwise addition of dimethyl suflate (15 ml) and 30% aqueous sodium hydroxide (20 ml) under a nitrogen atmosphere, and then with methyl iodide (5 ml) and silver oxide (2.0 g). The reaction mixture was heated at 95°C for 30 min. After cooling, it was acidified with 6N H_2SO_4 and extracted with chloroform. Evaporation of the solvent which previously dried over anhydrous sodium sulfate left a syrup (351 mg). Anal. found; OMe 52.3% (Calcd. for $C_{18}H_{34}O_{10}$: OMe 52.9%). The fully methylated product (340 mg) was dissolved in 5% methanolic hydrogen chloride (10 ml) and heated gently under reflux 15 hr. After removal of the solvent, acid hydrolysis was carried out in 0.5N hydrochloric acid (10 ml) for 8 hr at 100°C. The hydrolyzate was neutralized with silver carbonate and filtered. The filtrate was desalted with Amberlite IR 120 (H^+) and IR 45 (OH^-) exchange resin, and then concentrated under a reduced pressure to give a yellow syrup (233 mg). Chromatographic examination of the syrup using solvent 3 revealed two spots corresponding to 2,3,4,6-tetra-O-methyl-D-glucose and 3,4-di-O-methyl-D-xylose. The methylated sugars were separated on a

thick Toyo Roshi No. 514 filter paper sheet with solvent 3 to give 2,3,4,6-tetra-*O*-methyl-D-glucose (137 mg) and 3,4,-di-*O*-methyl-D-xylose (88 mg).

Identification of 2,3,4,5-tetra-O-methyl-D-glucose

The pale yellow syrup recovered from the elution of the filter paper was crystallized from ethyl ether, and it was obtained as needles having mp and mixed mp 94~6°C and $[\alpha]_D+83°$ ($c=0.85$, H_2O). A methanolic solution of the sugar (*ca.* 100 mg) was heated under reflux with aniline (0.5 ml) for 2 hr to convert to 2,3,4,6-tetra-*O*-methyl-N-phenyl-D-glucosylamine. Crystallization from ethanol gave a compound having mp 134~6°C and $[\alpha]_D+235°$ ($c=0.5$, $CHCl_4$).

Identification of 3,4-di-O-methyl-D-xylose

This methylated sugar showed a specific rotation of $[\alpha]_D+21°$ ($c=1.0$, H_2O) and paper chromatographic examination in solvent 4 gave R_g value of 0.38 corresponding to that of 3,4-di-*O*-methyl-D-xylose. It was distinguished from 2,3,-di-*O*-methyl-D-xylose (R_g 0.33). R_g values show a relative mobility for 2,3,4,6-tetra-*O*-methyl-D-glucose (1.00). The sugar (*ca.* 70 mg) dissolved in water (2 ml) containing barium carbonate (55 mg) was oxidized with bromine (10 drops) in the dark for 48 hr. The bromine was removed by aeration, and the solution was extracted with chloroform. The solvent was evaporated under a reduced pressure to a syrup (56 mg). The syrup was crystallized on standing, and recrystallization from ethyl ether yielded 3,4-di-*O*-methyl-D-xylonolactone, giving mp 67°C and $[\alpha]_D$ $-22°$ ($c=0.55$, H_2O).

Further examination of Fraction IV was not performed because of a small quantity of the acid component. On the basis of the molecular weight and the results of chemical analyses the Fraction was presumed to be an aldotriouronic acid.

Partial hydrolysis with weak acid

One gram of bamboo xylan(F2A) was heated with stirring in 0.2% oxalic acid (120 ml) for 6 hr at 85~90°C. After cooling, the mixture was separated into the hydrolyzate and the insoluble portion by centrifugation. The hydrolyzate was concentrated to a syrup by removal of crystalline deposits. Paper chromatographic analysis of the syrup revealed the spot corresponding to arabinose in addition to spots of a few xylo-oligosaccharides. The insoluble portion was dissolved in a dilute sodium hydroxide solution. The addition of four volumes of ethanol containing acetic acid to the solution precipitated a xylan (0.72 g). $[\alpha]_D$ $-84°$ ($c=1.0$, 4% NaOH) Chromatographic analysis of the hydrolyzate with 1N H_2SO_4 showed the presence of only D-xylose and aldobiouronic acid; arabinose was no longer detectable.

Methylation of bamboo xylan

Twelve grams of bamboo xylan (F2A) was dissolved in 5% aqueous hydroxide and methylated with stirring by the dropwise addition of dimethyl sulfate (150 ml)

and 40% aqueous sodium hydroxide (200 ml) at 35~40°C under a nitrogen atmosphere during the period of 6 hr. The reaction mixture was heated for one hr at 95~100°C to decompose the excess dimethyl sulfate. After cooling, it was neutralized with 6N H_2SO_4 to the acidic range. The partially methylated product separated as insoluble portion was collected by filtration, and second methylation was repeated in the same manner under the presence of a small amount of dioxane. After such five treatments, the partially methylated product (6.8 g) was methylated with methyl iodide (60 ml) and silver oxide (45 g) in N,N-dimethyl formamide accoring to Kuhn's method[32], and then with methyl iodide (110 ml) and silver oxide (40 g) to give the fully methylated product (5.66 g). $[\alpha]_D^{20}$ −86.5° (c=0.74, $CHCl_3$), found: OMe 36.1% Its infrared spectrum showed no absorption of the hydroxyl group.

Hydrolysis of the methylated product and separation of the methylated sugars

The methylated product (5.64 g) was heated under reflux with 5% methanolic hydrogen chloride (550 ml) for 6 hr until optical rotation became constant. After cooling, the solution was neutralized with silver carbonate, filtered, and the solvent was evaporated. The methyl glycoside obtained as a syrup (6.28 g) was hydrolyzed in 0.5N hydrochloric acid (600 ml) for 8 hr until optical rotation was constant. The cooled solution was neutralized with silver carbonate, and silver chloride produced was removed by filtration. After treatment with Amberlite IR 120(H^+) exchange resin, the filtrate was concentrated to a syrup (5.10 g) under a reduced pressure. Paper chromatographic examination of the syrup revealed the presence of at least six methylated sugars (Table II). A part of the syrup was separated quantitatively on paper chromatogram using solvent 3. The individual parts corresponding to the reducing methylated sugars were cut out and extracted with distilled water. The

Table II. Paper chromatography of methylated sugars.

	R_G	
	A	B
2, 3, 4, 6-Tetra-*O*-methyl-D-glucose	1.00	1.00
2, 3, 4-Tri-*O*-methyl-D-xylose	0.97	1.91
2, 3, 5-Di-*O*-methyl-L-arabinose	0.93	0.85
2, 3-Di-*O*-methyl-D-xylose	0.83	0.34
3-*O*-Methyl-D-xylose	0.49	0.20
2-*O*-Methyl-D-xylose	0.36	0.12
Partially methylated aldobiouronic acid	0.24	0.10

R_G values of methylated sugars indicate rates of movement relative to 2, 3, 4, 6-tetra-*O*-methyl-D-glucose.
A : n-Butanol : ethanol : water (4 : 1 : 5, v/v).
B : Benzene : ethanol : water (200 : 47 : 15, v/v).

amount of *O*-methylated sugars was determined by alkaline hypoiodite method[33]. A large portion of the remaining syrup (4.96 g) was applied to a column (3.6×70 cm) packed with a mixture of Whatman cellulose and prepared hydrocellulose (1:1, v/v)[34]. The syrup was dissolved in a small amount of methyl ethyl ketone saturated with water and added on the upper layer of the column, which was eluted with the same solvent. The eluate was collected in 10 ml portions with an automatic fraction collector. The methylated sugars in the fraction tubes were evaporated and examined by paper chromatography in solvent 3. However, since the methylated sugars were not fractionated perfectly through cellulose column chromatography, further separation and purification were performed on a thick filter paper sheet using solvent 3. The following methylated sugars were confirmed by examination of various derivatives.

Fraction I: The syrup (389 mg), showing a specific rotation of $[\alpha]_D$ +28.9° (*c* =1.04, H_2O), was a mixture of 2,3,5-tri-*O*-methyl-L-arabinose ($[\alpha]_D$ −35°), 2,3,4-tri-*O*-methyl-D-xylose ($[\alpha]_D$ −18°) and 2,3,4,6-tetra-*O*-methyl-D-glucose ($[\alpha]_D$ +84°). These sugars were confirmed on paper chromatogram and their separation on a thick paper sheet using solvent 4 gave fraction Ia, Ib and Ic. Demethylation of fraction Ia gave arabinose and the sugar was identified as 2,3,5-tri-*O*-methyl-L-arabinose by its conversion into 2,3,5-tri-*O*-methyl-L-arabonamide, giving mp and mixed mp 133~4°C (lit[35]. mp 137°C). Demethylation of fraction Ib gave xylose, which was identified as 2,3,4-tri-*O*-methyl-D-xylose by preparation of aniline derivative, having mp and mixed mp 99~100°C (lit[36]. mp 102°C). Demethylation of fraction Ic gave glucose and the position of the spot on paper chromatogram coincided with that of the authentic sample, but the preparation of the sugar derivative was unsuccessful.

Fraction II: The syrup (762 mg) gave a single spot of R_G 0.83 (solvent 3), 0.34 (solvent 4) and had a specific rotation of $[\alpha]_D$ +25.3° (c=1.46, H_2O). The sugar was characterized as 2,3-di-*O*-methyl-D-xylose by its conversion into 2,3-di-*O*-methyl-D-xylonamide, having the melting point of 130-2°C (lit.[37] mp 132~4°C), and into the aniline derivative of the melting point 121~2°C (lit.[38] mp 123~5°C),

Fraction III: The syrup (21.5 mg) which showed a specific rotation of $[\alpha]_D$ +53.1° (*c*=1.10, MeOH) was converted into the methyl ester methyl glycoside with 3% methanolic hydrogen chloride and then reduced with lithium aluminum hydride in ethyl ether. After acid hydrolysis with 1N H_2SO_4, the hydrolyzate was neutralized, filtered and concentrated to give 2,3,4-tri-*O*-methyl-D-glucose on paper chromatogram. Therefore, the methylated sugar was regarded as 2,3,4-tri-*O*-methyl-D-glucuronic acid methyl ester.

Fraction IV: The syrup (98.1 mg) showed R_G 0.49 (solvent 3) and $[\alpha]_D$

+17.8 (c=1.01, H_2O). Demethylation of the syrup gave only xylose, and it corresponded to mono-*O*-methyl-D-xylose. The sugar was identified to be 3-*O*-methyl-D-xylose by its conversion into the corresponding lactone, having mp 91~2°C and $[\alpha]_D$ +61° (c=0.18, H_2O). [lit.[39] mp 94°C and $[\alpha]_D$ +76~40° (H_2O)]

Fraction V: The syrup (33.8 mg) was chromatographically (solvent 3) and electrophoretically (in borate buffer) distinguished from that of Fraction IV. It was crystallized on standing in a desiccator. The methylated sugar was identified as 2-*O*-methyl-D-xylose by showing the melting point of 132-5°C and the specific rotation of $[\alpha]_D$ +34.3° (c=1.60, H_2O). [lit.[40] mp 133~4°C, $[\alpha]_D$ +35° (H_2O)]

Fraction VI: The syrup (130.5 mg) was heated under reflux with 3% methanolic hydrogen chloride for 5 hr. After neutralization with silver carbonate, the reaction mixture was filtered and condensed to the syrup which was reduced with lithium aluminum hydride in ethyl ether. The excess reductant was composed with water and then the reaction mixture was extracted with chloroform. Evaporation of the solvent gave a partially methylated product(128 mg). A portion of the syrup(*ca.* 10 mg) was hydrolyzed with 1N H_2SO_4 (10 ml) for 5 hr. The hydrolyzate was neutralized with barium carbonate, and the barium sulfate was filtered. Treatment of the filtrate with Amberlite IR 120(H^+) exchange resin, and evaporation of the resulting solution gave a syrup. Paper chromatography of the hydrolyzate in solvent 3 revealed the presence of 2,3,4-tri-*O*-methyl-D-glucose (R_G 0.86) and 3-*O*-methyl-D-xylose (R_G 0.48). The remaining syrup was methylated with methyl iodide and silver oxide to give the fully methylated product, the hydrolysis of which gave two components which were chromatographically distinguished from 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose. The two components were separated by chromatography on a thick paper sheet in solvent 3 to give fraction VIa and VIb.

Fraction VIa (60 mg) was identified as 3,4-di-*O*-methyl-D-xylose by oxidation to 3,4-di-*O*-methyl-D-xylonolactone, mp 66~7°C. Fraction VIb (56 mg) was identified as 2,3,4,6-tetra-*O*-methyl-D-glucose by its conversion into the corresponding aniline derivative, having mp 136~7°C.

Periodate oxidation of bamboo xylan

A solution of the bamboo xylan (F2A, 53.45 mg) dissolved in 4% aqueous sodium hydroxide (20 ml) was neutralized with dilute sulfuric acid. To this turbid suspension was added a solution of 0.1M sodium metaperiodate (20 ml), and the total volume was adjusted to 50 ml with distilled water. The suspension was allowed to stand, being kept at 5°C in the dark. A solution containing no sample was prepared at the same time. A constant aliquot (1 ml) was pipetted out at intervals from both solutions and analyzed for periodate comsumption according to Fleury-Lange method[41]. After 216 hr, the periodate consumption became constant at the value

of 1.09 mole per anhydroxylose residue. Formic acid liberated after 228 hr showed the value of 0.05 mole per anhydroxylose residue by titration with 0.01N NaOH using phenolphthalein as an indicator.

	6	24	50	72	216	228 (hr)
Periodate consumed	0.33	0.53	0.69	0.72	1.09	1.05 mole/xylose

The Smith degradation of bamboo xylan

Bamboo xylan(2.0 g) was dissolved in 4% aqueous sodium hydroxide solution and the solution was neutralized with dilute sulfuric acid. The turbid suspension was diluted with distilled water to 200 ml of volumes and oxidized with 0.05M sodium metaperiodate at 5°C in the dark. After 240 hr, ethylene glycol was added to decompose the excess periodate, and then the insoluble portion was removed by centrifugation from the suspension which was allowed to stand for 30 min. The solution was dialyzed against running water to remove inorganic salts. The dialyzed solution was reduced with sodium borohydride (3.0 g) at room temperature. The excess borohydride was decomposed with acetic acid and the solution was again dialyzed against running water. The dialyzed solution was concentrated to a half volume under a reduced pressure, and conc. hydrochloric acid was added so as to give a concentration of approximately 0.5 normal. Acid hydrolysis was allowed to proceed for 24 hr at room temperature. A precipitate produced at this stage was collected by centrifugation and led to an amorphous powder. Acid hydrolyzate of the powder gave only xylose as well as trace of glucose. The powder was obtained as a xylan which was not attcked by periodate, showing a specific rotation of $[\alpha]_D$ $-72°$ ($c=1.0$, 2% NaOH). The hydrolyzate from which the precipitate was removed was neutralized with Amberlite IR 45 (CO_3^{--}) exchange resin, and then concentrated to a dryness (0.85 g) under a reduced pressure.

Separation of the Smith degradation products

The Smith degradation products thus obtained were dissolved in a small amount of water (10 ml) and applied to a column (3.5×50 cm) packed with Whatman cellulose powder. The column was eluted with a solvent of n-butanol-acetic acid-water(5:1:2, v/v) and the eluate was collected in 10 ml portions with an automatic fraction collector. After the organic solvents of the fraction tubes were removed by shaking with ethyl ether, the aqueous layer of the individual tubes was evaporated, and the sugar alcohols present were examined by paper chromatography in the same solvent. Further separation and purification were carried out on a thick paper sheet. The Smith degradation products were separated to the following six components, as shown in Table III.

Table III. Components obtained from the Smith degradation products.

Components	Yield (mg)	$R_{xyl.}$		Sugar alcohols
		A	B	
1	31.0	2.16	1.71	ethylene glycohol
2	334.0	1.61	1.32	glycerol
3	67.8	1.10	1.07	2-*O*-β-D-xylopyranosyl glycerol
Xylose	—	1.00	1.00	
4	24.1	0.57	0.45	unidentified, composed of glycerol, xylose, glucose
5	76.9	0.45	0.32	unidentified
6	98.0	—	—	unknown

$R_{xyl.}$ values are expressed as relative mobility of D-xylose (1.00).
A: n-Butanol: acetic acid: water (5:1:2, v/v).
B: Ethyl acetate: pyridine: water (5:2:7, v/v) upper layer.

Identification of glycerol

Component 2 (*ca.* 100 mg) was dissolved in dry pyridine (10 ml) and heated at 80-90°C for 2 hr with *p*-nitrobenzoyl chloride (2 g). After cooling, the reaction mixture was neutralized with 5% aqueous sodium bicarbonate and extracted with chloroform. The chloroform layer was washed with distilled water, dried over anhydrous sodium sulfate, and evaporated under a reduced pressure to give a crystalline product. Recrystallization from chloroform and ethanol yielded tri-*p*-nitrobenzoate of glycerol. mp 197~8°C, lit.[42] mp 195~6°C, found: N, 7.66% Calcd. for $C_{24}H_{17}O_{12}N_3$; N, 7.79%.

Identification of 2-O-a-D-xylopyranosyl glycerol

Component 3 gave a specific rotation of $[\alpha]_D^{20}$ $-33.3°$ (c=2.4, H_2O). This component (15 mg) was hydrolyzed with 1N H_2SO_4 (10 ml) in a sealed tube for 10 hr at 100°C. Paper chromatographic analysis of the hydrolyzate revealed the presence of D-xylose and glycerol. The component was also converted into the corresponding derivative of penta-*p*-nitrobenzoate in the same manner to give a crystalline product. Recrystallization from ethyl acetate and methanol yielded penta-*p*-nitrobenzoate of 2-*O*-α-D-xylopyranosyl glycerol. mp 103~4°C, $[\alpha]_D$ $-37.3°$ (c=0.9, AcOEt), lit.[42] mp 105~6°C, $[\alpha]_D$ $-36°$ (lutidine), found: N, 7.10%, Calcd. for $C_{43}H_{31}O_{22}N_5$; N, 7.23%

Acetolysis of bamboo xylan

Acetolysis was performed according to the method[43] described by Whistler *et al.* A mixture of acetic anhydride (30 ml), glacial acetic acid (20 ml) and conc. sulfuric acid (2.5 ml) was cooled to 0°C, and then bamboo xylan (5 g) was added with stirring. Since the xylan was not soluble in the acetolysis reagents, the reaction

temperature was allowed to rise gradually to room temperature (20~25°C), and the mixture was allowed to stand with stirring for 3 days. After the insoluble portion was removed by centrifugation, the black brown product was poured into ice water (500 ml) and neutralized with sodium bicarbonate. The acetates were extracted with chloroform from the aqueous layer. The extract was washed with cold water, dried over anhydrous sodium sulfate, and evaporated to give a pale brown syrup (7.25 g). The syrup was applied to a column (3.4×45 cm) packed with a mixture of magnesium silicate and Celite 535 (10:1, v/v). The column, which was kept in equilibrium beforehand with a mixture of benzene and tertiary butanol (100:1, v/v), was eluted with the same solvent (1.5 liter). The absorbing part of the column was extruded and extracted with acetone. The acetone was evaporated to give a syrup (1.98 g). On treatment of the syrup with ethanol at 0°C, the solid produced was recrystallized repeatedly from cold ethanol. A part of the product was obtained as crystalline compound. mp 52°C, $[\alpha]_D$ −3.8° (c=3.16, $CHCl_3$), Anal. Calcd. for $C_5H_6O_6(CH_3CO)_6$; C, 48.57; H, 5.75, Acetyl 61.44%, found: C, 48.77; H, 5.76, Acetyl 61.2% (Kunz and Hudson method)[44]. The remaining syrup was deacetylated with 0.1N sodium methoxide (150 ml) at room temperature. After desalting with Amberlite IR 120(H^+) exchange resin, the filtrate was concentrated to give a pale yellow syrup. Paper chromatographic examination showed that the syrup contained only monosaccharides of xylose and arabinose. Therefore, the preparation of xylo-oligosaccharides were unsuccessful under this acetolysis conditions.

DISCUSSION

This bamboo xylan contained 4 to 5% of uronic acid residues. Examination of the acid components of the bamboo xylan (F2A) by controlled acid hydrolysis gave 4-*O*-methyl-D-glucuronic acid, aldobiouronic acid which was identified as 2-*O*-(4-*O*-methyl-α-D-glucopyrinosyl uronic acid) D-xylose, and a small quantity of aldotriouronic acid which was supposed to be *O*-4-methyl-α-D-glucopyranosyl uronic acid (1→2) *O*-β-D-xylopyranosyl (1→4) D-xylose. The aldobiouronic acid was confirmed to be the same one with a widely observed acid component of xylans isolated from other sources such as hardwoods and softwoods. Therefore, it seems probable that uronic acid residues are present as 4-*O*-methyl ether. The finding, however, gives the different information from that the presence of D-glucuronic acid together with 4-*O*-methyl-D-glucuronic acid has often been pointed out in the acidco mponents of hemicelluloses isolated from certain Gramineae[45]. Bishop[46] reported previously that D-glucuronic acid residues in hemicellulose from wheat straw were attached by 1→3 linkage to the main chain of the xylose unit. Matsuzaki[7] inferred that, from its taxonomical similarity of bamboo, uronic acid residues of bamboo

xylan also linked to the xylose unit by the same 1→3 likage. In the present investigation, however, the isolation of 1,2-linked aldobiouronic acid and identification of 3-*O*-methyl-D-xylose as the predominating mono-*O*-methyl-D-xylose gave evidence to support the attachments of 1→2 linkage.

The hemicellulose fractions (F2A and F3A) purified with Fehling's solution still contained approximately 4% of D-glucose residues, and the acid hydrolyzates of F2A and F3A gave equal amounts of glucose on paper chromatogram. Moreover, a small amount of methylated glucose was detected in the methylated products of bamboo xylan(F2A). However, no conclusive evidence was obtained to determine whether glucose residues are the constitutional sugar of the xylan or they originated from the contaminating glucan.

Methanolysis and hydrolysis of the fully methylated products of the bamboo xylan (F2A) afforded 2, 3, 4-tri-*O*-methyl-D-xylose, 2, 3, 5-tri-*O*-methyl-L-arabinose, 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,-di-*O*-methyl-D-xylose, and mono-*O*-methyl-D-xylose (mainly 3-*O*-methyl-D-xylose). The methylated sugars separated by cellulose column chromatography and paper chromatography on a thick paper sheet are shown in Table IV. The molar ratio of the methylated sugars is also shown in Table V. 2,3,4-Tri-*O*-methyl-D-xylose is originated from the terminal end group of the xylan chain. Identification of 2,3,5-tri-*O*-methyl-L-arabinose indicates that

Table IV. Methylated sugars obtained from fully bamboo xylan.

Fraction	Yield (mg)	$[\alpha]_D$ (H_2O) degrees	Methylated sugars identified
I	389	+28.9	2, 3, 4-Tri-*O*-methyl-D-xylose
			2, 3, 5-Tri-*O*-methyl-L-arabinose
			2, 3, 4, 6-Tetra-*O*-methyl-D-glucose
II	762	+25.3	2, 3-Di-*O*-methyl-D-xylose
III	21.5	+53.1	2, 3, 4-Tri-*O*-methyl-D-glucuronic acid methyl ester
IV	98.1	+17.8	3-*O*-Methyl-D-xylose
V	33.8	+34.3	2-*O*-Methyl-D-xylose
VI	130.5	+ 3.8	Methylated aldobiouronic acid

Table V. Molar ratio of methylated sugars.

Methylated sugars	Molar ratio
2, 3, 4-Tri-*O*-methyl-D-xylose	1.2
2, 3, 5-Tri-*O*-methyl-L-arabinose	1.6
2, 3, 4, 6-Tetra-*O*-methyl-D-glucose	0.4
2, 3-Di-*O*-methyl-D-xylose	35.8
Mono-*O*-methyl-D-xylose	2.6

the bamboo xylan contains L-arabinofuranose units linked glycosidically to C-2 or C-3 of D-xylose residues as a non-reducing end group. Isolation of 2,3-di-*O*-methyl-D-xylose as the predominating methylated sugar suggests that the bamboo xylan consists of a comparatively linear framework of 1,4-linked β-D-xylopyranosyl residues.

On periodate oxidation, the production of formic acid is regarded to be arised from the end group in the 1,4-linked structure. Additional evidence for a linear chain structure was also obtained by estimation of a small amount of formic acid liberated on the consumption of periodate. In addition, isolation and identification of large amounts of glycerol from the Smith degradation products support the 1,4-linked linear structure of the bamboo xylan.

This bamboo xylan consequently seems to be a linear polysaccharide which, on the average, contains one L-arabinose residue for 18-9 D-xylose units, and one 4-*O*-methyl-D-glucuronic acid residue for 24-5 D-xylose units. The finding is nearly in accord with the result estimated from mono-*O*-methyl-D-xylose of the methylation products. Since no positive evidence was found for the presence of the branching in the xylan chain, it seems most reasonable to consider that the bamboo xylan has a linear structure with almost no branching.

Thus, the predominant part of bamboo hemicellulose will be characterized as a 4-*O*-methyl-D-glucurono-arabino-xylan. However, arabino-(4-*O*-methyl-D-glucurono) xylans[15] found in softwoods have relatively higher amounts of L-arabinose and 4-*O*-methyl-D-glucuronic acid as the single side units than bamboo xylan. In addition, it has been pointed out that softwood xylans are slightly branched and not linear, having no native acetyl groups[47]. On the other hand, the predominant 4-*O*-methyl-D-glucurono-xylans[15] of hardwoods, having no arabinose residues, are usually characterized by having higher amounts of glucuronic acid than those of the Gramineae and by containing native acetyl groups[48]. Generally, most xylans from hardwoods such as beech and birch contain one 4-*O*-methyl-D-glucuronic acid residue for 10-15 D-xylose units, whereas those of the Gramineae such as oats and wheat straw contain one or two arabinose residue and one glucuronic acid residue for approximately 30 D-xylose units.

In view of these results, it is concluded that the structural features of bamboo xylan may be essentially similar to various xylans from the Gramineae with respect to mode of linkage of its main chain and attachments of single side units, but it is somewhat different from xylans from the Gramineae.

IV. Isolation of a Xylan containing Native Acetyl Groups from Bamboo

This part is described concerning the isolation and characterization of a xylan containing native acetyl groups from bamboo.

EXPEPIMENTAL AND RESULTS

Preparation of a xylan containing native acetyl groups from bamboo

Commercial DMSO (dimethyl sulfoxide, 1.5 liter) was poured into a completely air-dried bamboo holocellulose (100 g) newly prepared according to the method described in the previous part. The mixture was allowed to stand at room temperature with frequent shaking for 6 days. The brownish extract was filtered through a cloth on a Büchner funnel, and five volumes of ethanol was added to the filtrate to produce a precipitate. The precipitate was collected by centrifugation, washed successively with ethanol and ethyl ether, and dried *in vacuo* over phosphorus pentoxide. The product was dissolved in water with slightly warming, and after removal of the insoluble portion, the solution was concentrated to a volume of *ca.* 50 ml under a reduced pressure, then a polysaccharide was reprecipitated by addition of ethanol(to 75~80% EtOH). The polysaccharide was applied to gel-filtration on Sephadex G-75, and the main portion eluted on gel-filtration was collected and recovered as an amorphous powder (3.70 g). The yield corresponded to 3.1% on the basis of the original defatted bamboo meal. The powder (AX-I) showed the properties as follows; $[\alpha]_D$ $-59°$ ($c=1.0$, DMSO), $-68°$ ($c=1.0$, 4% NaOH), acetyl 6.53%, OMe 0.97%, sugar composition analysis(%): Xyl. 90.6, Ara. 5.8, Glc. 3.1, Gal. 0.4, uronic anhydride 4.5%. Acetyl group was determined according to the method[49] described by Whistler *et al.*. Other analyses followed the methods described in the previous part. The fact that this xylan contains native acetyl groups was also indicated by infrared spectrum (Fig. 2), giving a characteristic absorption at 1755 cm^{-1} due to acetyl carbonyl streching. Infrared spectrum of the xylan containing native acetyl groups is shown in Fig 2, compared with those of a chlorite holocellulose and other various xylans prepared from bamboo.

The residue after extraction with DMSO was extracted twice with hot water (1.5 liter). The extract was filtered, concentrated to a volume of *ca.* 100 ml, and precipitated by addition of a large amount of ethanol. The precipitate was collected and obtained as a grayish amorphous powder (2.38 g). The powder (AX-II) showed the properties as follows; $[\alpha]_D$ $-31.7°$ ($c=1.0$, DMSO), $-42°$ ($c=1.0$, 4% NaOH), acetyl 3.17%, OMe 2.07%, sugar composition analysis (%); Xyl. 88.7, Ara. 6.0, Glc. 4.4, Gal. 0.9, Uronic anhydride 5.2%.

Gel-filtration

Gel-filtration was carried out with a column packed with Sephadex G-75. The column was prepared to a gel bed volume of 3.5×45 cm. Each (*ca.* 0.1 g) of AX-I and AX-II was dissolved in water (10 ml) with slight warming and applied on the upper of the gel layer. The column was eluted continuously with distilled water,

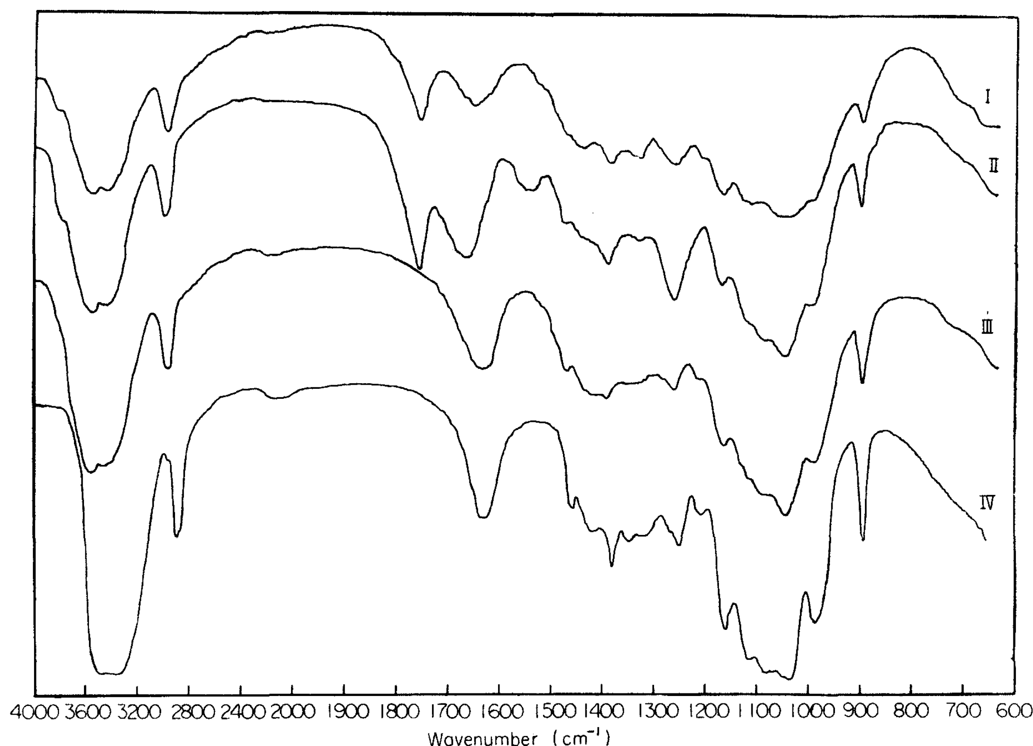

Fig. 2. Infrared spectra for a chlorite holocellulose and some xylans prepared from bamboo.
I : chlorite holocellulose, II : xylan (AX-I) containing native acetyl groups, III : xylan obtained by extraction with alkaline solution, IV : xylan treated with 0.05% oxalic acid (at 120°C in autoclave for 5 hr)

and the eluates in 10 ml portions were analyzed for polysaccharides by the phenol-sulfuric acid method[50]. The absorbance at 490 nm was read against a solution containing no sample. Carbohydrate content in the fraction tubes was determined by reference to the linear calibration curves of standard monosaccharides (commercial Guaranteed Reagents, glucose and xylose) which were likewise performed by the phenol-sulfuric acid method[50], and at the same time commercial Dextran T-20 (M_W 22,300, M_n 15,000) was applied to the same gel layer to estimate the molecular weights of AX-I and AX-II. These elution curves are shown in Fig. 3.

Electrophoresis

Electrophoresis was carried out with a Hitachi's Tiselius electrophoresis apparatus (HTB-2A type). The electrophoretic pattern was observed on bamboo xylan (AX-I), which was prepared by dissolving in 1/15 M borate buffer [ionic strength (μ) 0.19, pH 9.4] and dialyzing against the same buffer solution for 48 hr at 5°C. The electrophoretic patterns are shown in Fig. 4.

Viscosity

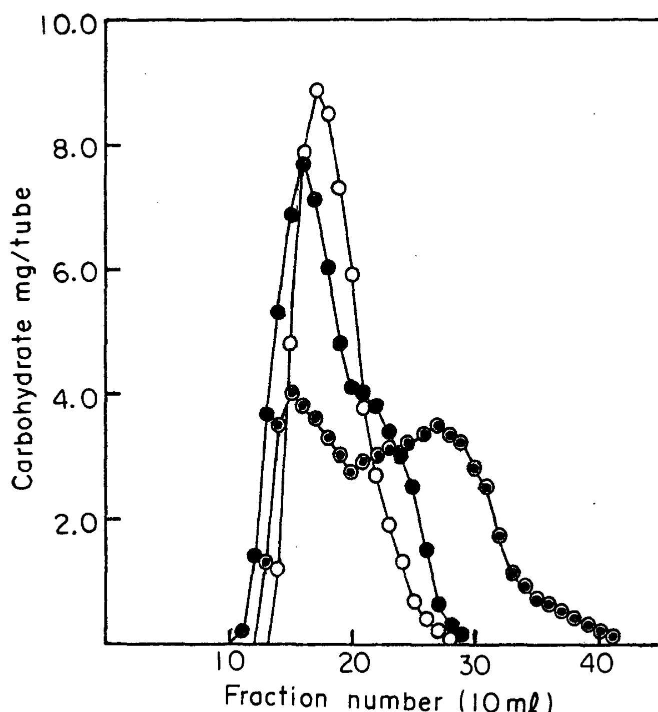

Fig. 3. Elution curves of bamboo xylans (AX-I, AX-II) and Dextran T-20 obtained by gel-filtration on Sephadex G-75.
● Bamboo xylan (AX-I),
◉ Bamboo xylan (AX-III),
○ Dextran T-20.

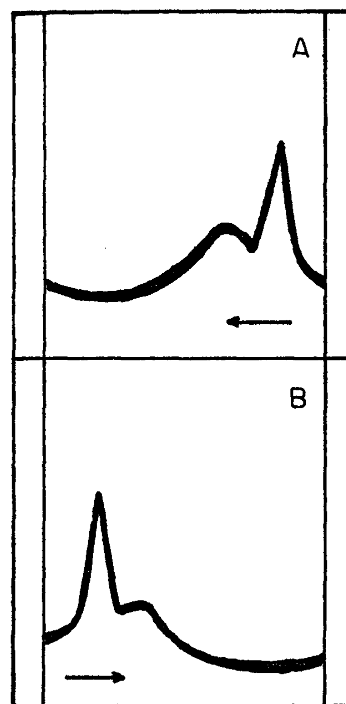

Fig. 4. Electrophoretic paterns of bamboo xylan (AX-I) in 1/15M borate buffer (μ=0.19, pH=9.4) after movement for one hr. (A) Descending, (B) Ascending

Viscosity measurements were made at 25±0.01°C with a Cannon-Fenske viscometer. Dimethyl sulfoxide (DMSO) and 0.5 M cupriethylene diamine (CED) were used as the solvents. 0.5 M CED was prepared according to the Tappi standard method T 230-63[51]. The solution and the solvent were clarified through a glass filter prior to measurement. Viscosity was determined at four different concentrations in the individual solvents. Reduced viscosities(η_{sp}/c) were plotted against various concentrations, and intrinsic viscosities(η) were estimated by extrapolation to zero concentration. Intrinsic viscosities[η] obtained against various concentrations(c) are shown in Fig. 5.

Sedimentation

Bamboo xylan (AX-I) was characterized by sedimentation to determine the sedimentation coefficient and the homogeneity. Spinco model E analytical ultracentrifuge equipped with a schlieren optical system was used for determination of sedimentation velocity. Sedimentations in DMSO and in 0.15 M KCl were measured at the rotor speed of 59,780 rpm and 52,640 rpm, respectively.

Sedimentation coefficient(s) is calculated using the following equation(1);

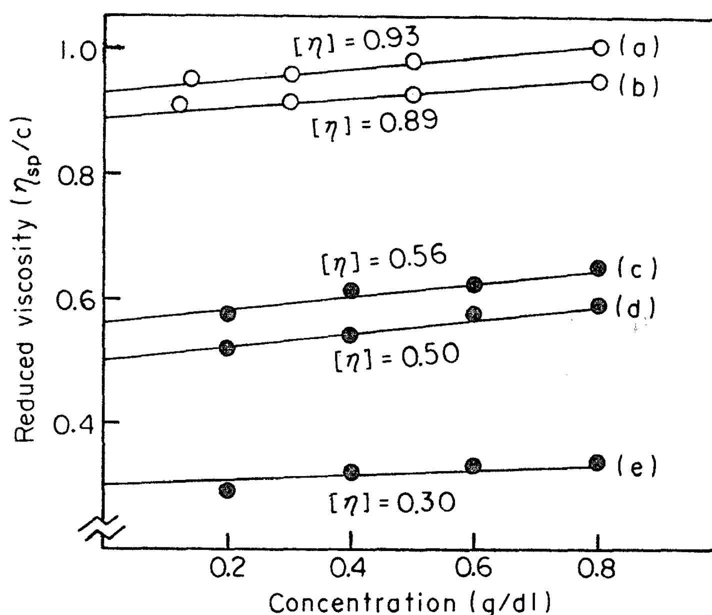

Fig. 5. Relation ship of η_{sp}/c vs. concentration (c) for various bamboo xylans in DMSO and CED.
○ CED (cupriethylene diamine), ● DMSO (dimethyl sulfoxide)
(a): F2A, (b), (c): AX-I, (d): DMSO-soluble xylan, (e): AX-II.

$$s=\frac{2.303}{60\omega^2}\cdot\frac{d\log_{10}x}{dt} \qquad \cdots\cdots(1)$$

where ω is the rotor speed or the angular velocity and x is the distance from the center of the rotor.

From linear gradient of $\log_{10}x$ against time(min.) as shown in Fig. 6 and 7,

$$\frac{d\log_{10}x}{dt}=0.47\times10^{-4}\ (\mathrm{min}^{-1}\ \text{in DMSO})$$

and

$$\frac{d\log_{10}x}{dt}=1.31\times10^{-4}\ (\mathrm{min}^{-1}\ \text{in } 0.15\,\mathrm{M}\ \mathrm{KCl})$$

were obtained respectively.

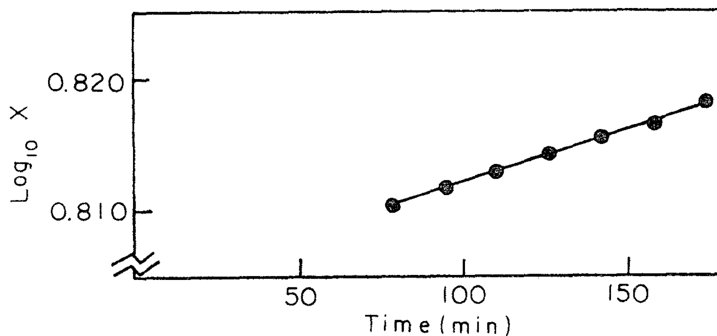

Fig. 6. Sedimentation velocity data of $\log_{10}$ x vs. time (min) in DMSO.

Fig. 7. Sedimentation velocity data of $\log_{10}$ x vs. time (min) in 0.15 M KCl.

Fig. 8. Sedimentation patterns of bamboo xylan (AX-1) in 0.15 M KCl. Sedimentation proceeds from right to left. The photographs were taken at indicated times after reaching full speed at 52,640 rpm.

Accordingly, sedimentation coefficient($s_{25,w}$) in DMSO was calculated as 0.462S and that ($s_{20,w}$) in 0.15 M KCl was also calculated as 1.66S ($S=10^{-13}$). Sedimentation patterns in 0.15 M KCl are shown in Fig. 8.

DISCUSSION

A xylan containing 6.5% of native acetyl groups was prepared in a yield of 3.1% based on the original defatted bamboo meal by extraction of bamboo holocellulose prepared by Wise's chlorite method with DMSO. Bamboo xylan prepared by extraction with aqueous alkali was almost insoluble in water, but xylans of AX-I

and AX-II were soluble in water and DMSO. The latter xylans showed the similar sugar composition as the former, except that they had native acetyl groups.

The assessment of homogeneity of AX-I was conducted by examination of ultracentrifugation and electrophoresis, but the polysaccharide was not electrophoretically homogeneous. On the other hand, it was shown by examination of gelfiltration that the fraction of AX-II contained two components having broad molecular weight distribution.

The presence of native acetyl groups in hemicellulose portion has hitherto been pointed out and verified[52]. However, isolation of a xylan containing native acetyl groups from bamboo is the first finding. The fact seems to be of interest from points of the distribution and significance of native acetyl groups in nature. The location of acetyl groups in the xylose residues of hardwood xylans was established by Bouveng *et al.*[53] On the other hand, it was proved by Koshijima and Meier[54] that O-acetyl groups in softwoods were attached to mannose units of a glucomannan. However, the significance of the acetyl groups in hemicellulose is not yet clear. Stewart *et al.*[55] suggested a physiological role, indicating that O-acetyl groups are hydrolyzed in the living tissues according as the tree grows older, producing acetic acid. Katz[56] pointed out from some observations of polarization microscopy, X-ray diffraction and electron microscopy that O-acetyl groups in hemicellulose portion were effective in preventing molecular orientation and subsequent development of lateral order in cell wall. There is hitherto nevertheless no conclusive evidence to account for the fact.

Weight average molecular weight (M_W) of bamboo xylan (AX-I) which was determined from sedimentation equilibrium at 20,410 rpm by the short column method[57] using multichannel cell was 27,200 as apparent molecular weight. Number average molecular weight (M_n) estimated from osmotic pressure measurement was 25,700 for the acetate of bamboo xylan (F2A). M_W from sedimentation equilibrium of a birch xylan was reported to be 25,000, and M_n from osmotic pressure measurement was 22,500[58]. In recent years, M_n of a native hardwood xylan of trembling aspen was determined and reported to be in the order of 30,000, corresponding to degrees of polymerization of 150~200[59,60]. Therefore, molecular weight of the native bamboo xylan may also be expected to be in the range of that of hardwood xylans.

V. Discussion and Conclusion

Bamboo holocellulose was prepared from a defatted preparative meal of bamboo culms according to the sodium chlorite method. Hemicellulose of bamboo was isolated exhaustively by extraction with aqueous alkali of the resulting holocellulose. The hemicellulose was recovered as a precipitate by addition of acetic acid

and large amount of ethanol, and then it was purified by fractional precipitation as a copper complex with Fehling's solution. A xylan which was obtained as the fraction forming a precipitate on addition of Fehling's solution was found to comprise more than 90% of bamboo hemicellulose. The examination of acid components prepared from the bamboo xylan and the structural investigations by means of methylation analysis and periodate oxidation (the Smith degradation) gave the evidences supporting a 1, 4-linked linear structure of D-xylopyranose residues and attachments of single side units such as the residues of L-arabinose and 4-*O*-methyl-D-glucuronic acid. Therefore, the bamboo xylan was characterized as a 4-*O*-methyl-D-glucuronic acid, L-arabinose and D-xylose in a molar ratio of 1.0: 1.3: 25, respectively.

Furthermore, a xylan containing 6.5% of native acetyl groups was first prepared in a yield of 3.1% (on the defatted bamboo meal) by extraction with DMSO from bamboo holocellulose and its properties were characterized. This xylan showed the similar sugar composition as xylan prepared by extraction with aqueous alkali, except that it had native acetyl groups. That bamboo xylan has native acetyl groups is characteristic in common with that hardwood xylans originally have native O-acetyl groups.

From discussion based on the experimental results, bamboo xylan seems to have properties similar to xylans from hardwood, but in respect to have arabinose residues which are characteristic in softwood xylans, bamboo xylan is rather close to softwood xylans. Therefore, considerations about above described lead to a conclusion that bamboo xylan would have a structural feature as an intermediate between hardwood xylans and softwood xylans.

This work has been carried out in the Division of Wood Chemistry, Wood Research Institute, Kyoto University.

The author wishes to thank to Emeritus Professor Koichiro Kitao for his valuable suggestions and encouragement during the course of this work. The author is also indebted to Professor Tetsuo Koshijima, Wood Research Institute of Kyoto University, for his helpful advice and discussion on this work.

References

1) E. SCHULZE, Ber., **24**, 2277 (1891).
2) "Comparative Phytochemistry", ed. by T. Swain, Academic Press, London and New York, 1966, p. 139.
3) T. E. TIMELL, "Cellular Ultrastructure of Woody Plants", Syracuse Univ. Press, Syracuse, 1965, p. 127.
4) E. L. HIRST, Proceedings of the Wood Chemistry Symposium, Montreal, Canada, London Butterworth, 1962, p. 53.

5) T. R. NAFFIZIGER, R. S. MATUSZEWSKI. T. F. CLARK and I. A. WALFF, Tappi, **43**, 591 (1960); M. G. KARNIK, A. J. MORAK and J. R. KYLE WARD, ibid., **46**, 130 (1963).
6) M. LÜDTKE, Ann., **466**, 27 (1928).
7) K. MATSUZAKI, M. MORIYA and H. SOBUE, Kogyo Kagaku Zasshi, 63, 638 (1960); ibid., 65, 987 (1962).
8) C. T. BISHOP, Can. J. Chem., **31**, 134 (1953); G. O. ASPINALL and E. G. MEEK, J. Chem. Soc., **1956**, 3830.
9) G. O. ASPINALL and K. C. B. WILKIE, J. Chem. Soc., **1956**, 1072.
10) G. O. ASPINALL and R. J. FERRIER, ibid., **1957**, 4188.
11) R. L. WHISTLER and G. E. LAUTERBACK, J. Amer. Chem. Soc., **77**, 6328 (1955).
12) R. MONTGOMERY, F. SMITH and H. C. SRIVASTAVA, ibid., **78**, 2837, 6169 (1956); ibid., **79**, 695, 698 (1957).
13) R. L. WHISTLER, "Advances in Carbohydrate Chemistry", Vol. 5, Academic Press Inc., New York, 1950, p. 269.
14) G. O. ASPINALL, ibid., Vol. 14, Academic Press Inc., New York, 1959, p. 429.
15) T. E. TIMELL, ibid., Vol. 19, Academic Press Inc., New York, 1964, p. 247; ibid., Vol. 20, 1965, p. 409.
16) H. R. GOLDSCHMID and A. S. PERLIN, Can. J. Chem., **41**, 2272 (1963).
17) C. M. EWALD and A. S. PERLIN, ibid., **37**, 1254 (1959); G. O. ASPINALL and K. M. ROSS, J. Chem. Soc., **1963**, 1681.
18) E. MAEKAWA and K. KITAO, Agr. Biol. Chem., **37**, 2073 (1973).
19) B. L. BROWNING, Tappi, **32**, 119 (1949); G. G. MAHER, Anal. Chem., **21**, 1142 (1949).
20) F. D. SNELL and T. C. SNELL, "Colorimetric Methods of Analysis", Vol. II, Van Nostrand, New York, 1959, p. 691.
21) L. E. WISE, M. MURPHY and A. A. D'ADDIECO, Paper Trade J., **122**, 35 (1946).
22) J. F. SAEMAN, W. E. MOORE, R. L. MITCHELL and M. A. MILLET, Tappi, **37**, 336 (1954).
23) B. L. BROWING, "Methods of Wood Chemistry", Vol. II, Interscience Pub., New York-London-Sydney, 1967. p. 589.
24) "Methods in Carbohydrate Chemistry", Vol. I, ed. by R. L. Whistler and M. L. Wolfrom, Academic Press, New York and London, 1962, p. 383.
25) "The Carbohydrate", Vol. IIA, ed. by W. Pigman and D. Horton, Academic Press, New York and London, 1970, p. 449.
26) E. MAEKAWA, unpublished paper.
27) F. VIEBOCK and C. BRECHNER, Ber., **63**, 3207 (1930).
28) T. G. BONNER, E. J. BOURNE and S. MCNALLY, J. Chem. Soc., **1960**, 2929.
29) J. A. CIFONELLI and F. SMITH, Anal. Chem., **26**, 1132 (1954).
30) G. A. ADAMS, Can. J. Chem., **37**, 29 (1959).
31) J. MUNRO and E. G. V. PERCIVAL, J. Chem. Soc., **1935**, 873.
32) R. KUHN, H. TRISCHMANN and I, LOW, Angew. Chem., **67**, 32 (1959).
33) "Methods of Biochemical Analysis", Vol. III, ed. by D. Glick, Interscience Pub., New York, 1963, p. 179.
34) J. D. GEERDES, B. A. LEWIS, R. MONTGOMERY and F. SMITH, Anal. Chem., **26**, 264 (1954).
35) J. FRIED and H. E. STAVELY, J. Amer. Chem. Soc., **74**, 5461 (1952).
36) R. A. LAIDLAW and E. G. V. PERCIVAL, J. Chem. Soc., **1949**, 1600.
37) G. O. ASPINALL, E. L. HIRST, R. W. MOODY and E. G. V. PERCIVAL, ibid., **1953**, 1631; I. EHRENTHAL, R. MONTGOMERY and F. SMITH, J. Amer. Chem. Soc., **76**, 5509 (1954).
38) C. CROON and T. E. TIMELL, ibid., **82**, 3416 (1960).
39) R. A. LAIDLAW and E. G. V. PERCIVAL, J. Chem. Soc., **1950**, 528.
40) E. L. HIRST, E. G. V. PERCIVAL and C. B. WYLAM, ibid., **1954**, 189.
41) "Methods of Biochemical Analysis", Vol. III, ed. by D. Glick, Interscience Pub., New York, 1963, p. 124.

42) I. J. GOLDSTEIN, G. W. HAY, B. A. LEWIS and F. SMITH, "Methods in Carbohydrate Chemistry", Vol. V, Academic Press, New York and London, 1965, p. 361.
43) R. L. WHISTLER, E. HEYNE and J. BACHRACH, J. Amer. Chem. Soc., **21**, 1476 (1949).
44) "Methods in Carbohydrate Chemistry", Vol. I, ed. by R. L. Whistler and M. L. Wolfrom, Academic Press, New York and London, 1962, p. 448.
45) G. A. ADAMS and C. T. BISHOP, J. Amer. Chem. Soc., **78**, 2842 (1956).
46) C. T. BISHOP, Can. J. Chem., **33**, 1073 (1955).
47) M. ZINBO and T. E. TIMELL, Svensk Papperstid., **70**, 597, 695 (1967).
48) "Glucuronic Acid", ed. by G. J. Dutton, Academic Press, New York, 1966, p. 160.
49) R. L. WHISTLER and A. JEANES, Ind. Eng. Chem., Anal. Ed., **15**, 317 (1943).
50) M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS and F. SMITH, Anal. Chem., **28**, 350 (1956).
51) "Methods in Carbohydrate Chemistry", Vol. III, ed. by R. L. Whistler and M. L. Wolfrom, Academic Press, New York and London, 1963, p. 77.
52) T. E. TIMELL, J. Amer. Chem. Soc., **82**, 5211 (1960).
53) H. O. BOUVENG, P. J. GAREGG and B. LINDBERG, Acta Chem. Scand., **14**, 742 (1960); H. O. BOUVENG, ibid., **15**, 96 (1961).
54) T. KOSHIJIMA, Mokuzai Gakkaishi, **6**, 194 (1960); H. MEIER, Acta Chem. Scand., **15**, 1381 (1961).
55) C. M. STEWART, J. F. KOTTEK, H. E. DADSWELL and A. J. WATSON, Tappi, **44**, 798 (1961).
56) G. KATZ, ibid., **48**, 34 (1965).
57) D. A. YPHANTIS, Ann. N. Y. Acad. Sci., **88**, 586 (1960).
58) R. G. LEBEL, D. A. I. GORING and T. E. TIMELL, J. Polymer Sci., part C, **2**, 9, 29 (1963).
59) T. KOSHIJIMA, T. E. TIMELL and M. ZINBO, ibid., **11**, 265 (1965).
60) R. W. BRYANT, T. E. TIMELL and M. ZINBO, Cellulose Chemistry and Technology, **2**, 269 (19-68).