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STRUCTURAL INVESTIGATION OF A β-D-GLUCAN AND A XYLOGLUCAN FROM BAMBOO-SHOOT CELL-WALLS*

YOJI KATO†, RYOJI SHIOZAWA, SOGO TAKEDA, SHUZO ITO, AND KAZUO MATSUDA
Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, Tsutsumidori, Sendai 980 (Japan)

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ABSTRACT

Cell-wall polysaccharides of the bamboo shoot of Moso-chiku were fractionated by successive extraction with ammonium oxalate–oxalic acid, 4% potassium hydroxide, and 24% potassium hydroxide. A β-D-glucan and a xyloglucan were obtained from the 4% potassium hydroxide extract and the 24% potassium hydroxide extract, respectively. Methylation analysis and enzymic-degradation studies of the polysaccharides showed that the former was built up predominantly of repeating-oligosaccharide units of 3-*O*-β-cellobiosyl-D-glucose and 3-*O*-β-cellotriosyl-D-glucose in 2:1 molar ratio, and the latter had repeating-oligosaccharide units of α-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-D-glucose, α-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-D-glucose and cellobiose.

INTRODUCTION

To obtain accurate information on the structure and ubiquitous distribution of glucose-containing polysaccharides in plant cell-walls, we have studied cell-wall polysaccharides of several seedlings belonging to the Leguminosae and Gramineae, which are representative of dicotyledon and monocotyledon, respectively. We have obtained firm evidence, by isolation and structural characterization of the polymers, that non-cellulosic glucose residues in cell walls of *Phaseolus aureus*[1–3], *Glycine max*[4], and *Vigna sesquipedalis*[4] seedlings are derived from xyloglucan, and those in cell walls of *Hordeum distichum*[5,6] and *Oryza sativa*[7] seedlings are from both linear β-(1→3); β-(1→4)-glucan and xyloglucan.

The present work was conducted to elucidate whether or not cell walls of the bamboo shoot of Moso-chiku, which is markedly different from grasses in all other subfamilies of Gramineae, possess these polysaccharides and, if so, to determine

*Dedicated to Professor Sumio Umezawa on the occasion of his 73rd birthday and the 25th anniversary of the Microbial Chemistry Research Foundation.
†Present address: Department of Botany, Iowa State University, Ames, Iowa 50011, U.S.A.

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whether structural features of the cell-wall polysaccharides are similar to those of β-D-glucans and/or xyloglucans from other plants.

RESULTS AND DISCUSSION

Polysaccharides were extracted from cell walls of the bamboo shoot of Mosochiku successively with 0.25% ammonium oxlate–oxalic acid, 4% potassium hydroxide, and 24% potassium hydroxide. After removal of contaminant starch in each extract by treating with salivary alpha amylase followed by dialysis, seven fractions (PS-A, PS-B, HC-IA, HC-IB, HC-IIA, HC-IIB, and CL) were prepared. A high proportion of non-cellulocic polysaccharides was obtained in alkali extracts (fractions HC-IB and HC-IIB, Table I).

When fraction HC-IB was resolved into three subfractions by chromatography

TABLE I

YIELDS, TOTAL SUGAR CONTENT, AND NEUTRAL-SUGAR COMPOSITION OF FRACTIONS OBTAINED FROM THE CELL WALLS OF BAMBOO SHOOTS

Fraction	*Yield*[a] *(g)*	*Total sugar content (g)*[b]	*Neutral-sugar composition (mol%)*			
			Ara	*Xyl*	*Glc*	*Gal*
PS-A	1.14	0.80	9.7	trace	84.5	5.8
PS-B	1.52	1.23	19.5	2.4	48.7	29.5
HC-IA	2.02	0.33	23.5	45.6	19.4	11.5
HC-IB	24.30	19.29	19.9	33.6	37.2	9.2
HC-IIA	0.22	0.17	16.8	51.6	19.9	11.8
HC-IIB	14.30	14.92	23.2	51.4	15.7	9.7
CL	22.20	19.14		n.d.[c]		

[a]From 80.0 g of crude cell-wall materials. [b]Expressed as the glucose polymer equivalent. [c]Not determined.

TABLE II

YIELDS AND NEUTRAL-SUGAR COMPOSITION OF SUBFRACTIONS OBTAINED FROM FRACTION HC-IB BY CHROMATOGRAPHY ON DEAE-SEPHADEX A-25

Subfraction	*Yield*[a] *(mg)*	*Neutral-sugar composition (mol%)*			
		Ara	*Xyl*	*Glc*	*Gal*
HC-IB-I	1728	16.4	26.8	51.7	5.0
HC-IB-II	149	35.9	48.0	1.5	14.6
HC-IB-III	54	32.9	60.3	3.6	3.2

[a]From 2.5 g of fraction HC-IB.

on DEAE-Sephadex A-25, most of glucose-containing polysaccharides were recovered in the neutral polysaccharide fraction, fraction HC-IB-I (Table II). G.l.c. of the alditol acetates obtained from the hydrolyzate of methylated HC-IB-I showed that 2,3,5-tri-*O*-methyl-L-arabinose (15.2%), 2,3-di-*O*-methyl-D-xylose (11.7%), 2,4,6-tri-*O*-methyl-D-glucose (13.8%), 2,3,6-tri-*O*-methyl-D-glucose (36.4%), and 2- and/or 3-mono-*O*-methyl-D-xylose (14.8%) were prominent structural components of the methylated HC-IB-I. The degrees of hydrolysis of fraction HC-IB-I by *Bacillus circulans* endo-(1→3)-β-D-glucanase, *Trichoderma viride* cellulase, and *Streptomyces* sp. endo-(1→4)-β-D-xylanase were 6.0, 15.0, and 7.0%, respectively. These results suggest that fraction HC-IB-I consisted primarily of two kinds of polysaccharide: a β-linked polymer containing both (1→4)- and (1→3)-linked glucosyl residues and an arabinoxylan having a linear backbone-chain of (1→4)-β-D-xylose residues, ~56% of which are substituted at the 2 and/or 3 position by arabinose.

Isolation of β-D-glucan from a mixture of hemicellulosic polysaccharides is performed conventionally by graded ethanol precipitation[8,9] or by complexing with Fehling's solution[10]. However, attempts to isolate a glucan from fraction HC-IB-I by these methods were unsuccessful. The glucan in the present study was ultimately isolated by graded precipitation with ammonium sulfate[5,11]. The glucan preparation finally obtained contained glucase, xylose, galactose, and arabinose in the molar ratio of 87.5:5.4:3.8:3.2. Small proportions of arabinose, xylose, and galactose residues were considered to be derived from an arabinoxylan-like contaminant in the glucan. The glucan isolated accounted for 34% of fraction HC-IB-I.

The glucan was insoluble in water, but was soluble in M sodium hydroxide. It gave no color with iodine reagent (0.02% of I_2 in 0.2% of KI), and was not attacked by salivary alpha amylase. The presence of β-D-linked residues was indicated by the low specific rotation: $[\alpha]_D$ −5.5° (*c* 1.0, in M sodium hydroxide). G.l.c. of the alditol acetates obtained from a hydrolyzate of the methylated glucan showed that 2,3,6-

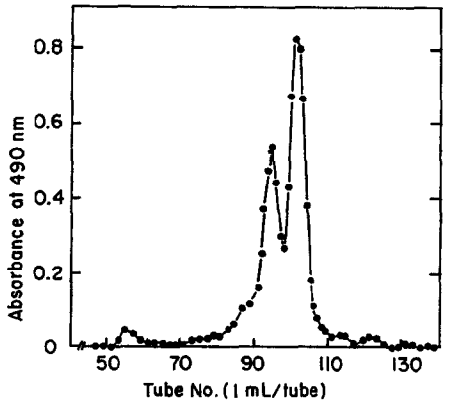

Fig. 1. Separation by gel filtration on Bio-Gel P-2 of the enzymic hydrolyzate of bamboo-shoot β-D-glucan. Tubes 91–97 (oligosaccharide-II) and 99–105 (oligosaccharide-I) were separately combined and freeze-dried. Details are given in the text.

TABLE III

METHYLATION ANALYSIS OF OLIGOSACCHARIDES DERIVED FROM THE GLUCAN BY ENZYMIC HYDROLYSIS

Methylated sugar[a]	*Relative retention-times*			*Oligosaccharide*	
	Alditol acetate	*Methyl glycosides*		*OS-I*	*OS-II* (%)
2,3,4,6-Me_4-Glc	1.00	1.00	1.46	34.0	26.2
2,4,6-Me_3-Glc	1.96	3.60	5.45	29.0	22.8
2,3,6-Me_3-Glc	2.48	3.80	5.33	37.0	51.0

[a]2,3,4,6-Me_4-Glc = 2,3,4,6-tetra-*O*-methyl-D-glucose and so on.

tri-*O*-methyl-D-glucose, 2,4,6-tri-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-glucose were present in the ratio of 2.6 : 1.0 : trace.

Several workers[12-15] have indicated that the alpha amylase preparation of *Bacillus subtilis* (Sigma Type IIIA) contains an enzyme that can hydrolyze the glucosyl linkage at C-1 of the 3-substituted constituent in linear (1→3); (1→4)-β-D-glucan (lichenan, oat glucan, and so on). Hydrolysis of the isolated glucan with this enzyme preparation, followed by separation of the hydrolyzate by gel filtration on Bio-Gel P-2, afforded mainly two types of oligosaccharide, OS-I and OS-II in the molar ratio

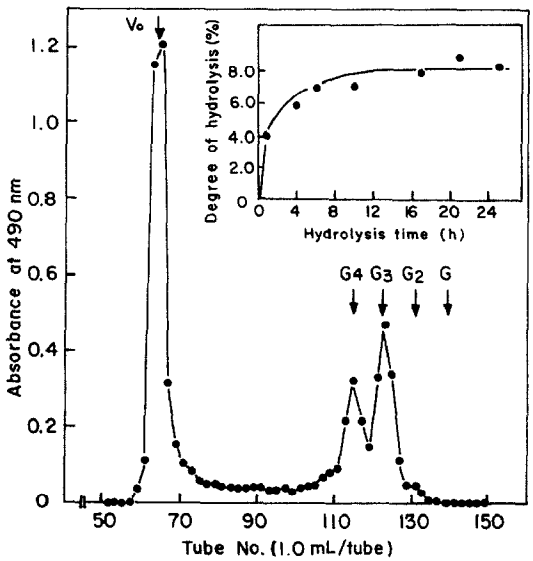

Fig. 2. Separation by gel filtration on Bio-Gel P-2 of *B. subtilis* alpha amylase preparation-hydrolyzate of fraction HC-IB. Fraction HC-IB in sodium phosphate buffer was incubated at 40° with alpha amylase preparation of *B. subtilis*. At intervals, the degree of hydrolysis was calculated (inset). After 24 h of incubation, the hydrolyzate was resolved by gel filtration on Bio-Gel P-2. The letters V_0 and G–G_4 indicate the elution positions of Blue Dextran and cello-oligosaccharides used for column calibration. Details are given in the text.

of 2.0:1.0 (Fig. 1). Both oligosaccharides appeared homogeneous in paper chromatography (OS-I, R_{Glc} 0.40; OS-II, R_{Glc} 0.16). G.l.c. of the alditol acetates and the methyl glycosides obtained from a hydrolyzate of the methylated oligosaccharides showed that the molar ratio of 2,3,4,6-tetra-, 2,4,6-tri-, and 2,3,6-tri-*O*-methyl-D-glucose were ~1:1:1 and ~1:1:2 for OS-I and OS-II, respectively (Table III). After and before reduction by sodium borohydride, OS-I and OS-II were partially hydrolyzed by acid and the hydrolysis products analyzed by paper chromatography. The following oligosaccharides were identified: OS-I, glucose, laminarabiose, cellobiose, and 3-*O*-β-cellobiosyl-D-glucose; the reduced OS-I, glucose and cellobiose; OS-II, glucose, laminarabiose, cellobiose, cellotriose, 3-*O*-β-cellobiosyl-D-glucose, and 3-*O*-β-cellotriosyl-D-glucose; and the reduced OS-II, glucose, cellobiose, and cellotriose. From these results, OS-I and OS-II were assigned the structures 3-*O*-β-cellobiosyl-D-glucose and 3-*O*-β-cellotriosyl-D-glucose, respectively.

The proportion of (1→3) linkages in the polysaccharide is 30% on the basis of the molar ratio of OS-I and OS-II. This value is consistent with that (28%) calculated from the results of methylation analysis of the polysaccharide.

When the hydrolyzate of fraction HC-IB by a *B. subtilis* alpha amylase preparation was subjected to gel filtration on Bio-Gel P-2 (Fig. 2), a polysaccharide fraction eluted at the void volume was almost devoid of glucose (arabinose:xylose:galactose:glucose = 30.4:57.8:7.0:4.7). Furthermore, two oligosaccharides released from fraction HC-IB were attributed as 3-*O*-β-cellobiosyl-D-glucose and 3-*O*-β-cellotriosyl-D-glucose, respectively. This result indicates that the glucose residues in fraction HC-IB are derived from a linear (1→3); (1→4)-β-D-glucan.

G.l.c. of the alditol acetates obtained from the hydrolyzate of the methylated HC-IIB showed that 2,3,5-tri-*O*-methyl-L-arabinose (16.1%), 2,3,4-tri-*O*-methyl-D-xylose (5.1%), 2,3-di-*O*-methyl-D-xylose (18.4%), 2,4,6-tri-*O*-methyl-D-glucose (3.4%), 2,3,6-tri-*O*-methyl-D-glucose (19.1%), 2- and/or 3-mono-*O*-methyl-D-xylose (18.8%), and 2,3-di-*O*-methyl-D-glucose (11.8%) were prominent structural units in the methylated HC-IIB. Degrees of hydrolysis of fraction HC-IIB by *B. circulans* endo-(1→3)-β-D-glucanase, *Streptomyces* sp. endo-(1→4)-β-D-xylanase, and salivary

TABLE IV

YIELDS AND NEUTRAL-SUGAR COMPOSITION OF SUBFRACTIONS OBTAINED FROM FRACTION HC-IIB BY CHROMATOGRAPHY ON DEAE-SEPHADEX A-25

Subfraction	*Yield*[a] *(mg)*	*Neutral-sugar composition (mol%)*			
		Ara	*Xyl*	*Glc*	*Gal*
HC-IIB-I	1960	21.9	47.4	24.9	5.7
HC-IIB-II	200	31.5	35.1	3.4	30.0
HC-IIB-III	138	26.6	51.2	12.8	9.4

[a]From 2.43 g of fraction HC-IIB.

TABLE V

YIELDS AND NEUTRAL-SUGAR COMPOSITION OF SUBFRACTIONS OBTAINED FROM FRACTION HC-IIB-I BY CHROMATOGRAPHY ON SEPHAROSE CL-6B

Subfraction	*Yield*[a] *(mg)*	*Neutral-sugar composition (mol%)*			
		Ara	*Xyl*	*Glc*	*Gal*
HC-IIB-I-1	909	26.9	57.9	10.3	4.9
HC-IIB-I-2	425	18.9	42.5	32.2	6.4
HC-IIB-I-3	374	8.2	44.7	42.6	4.5

[a]From 1.95 g of fraction HC-IIB-I.

alpha amylase were 1.3, 8.1, and 0%, respectively. Furthermore, when fraction HC-IIB was subjected to hydrolysis with Sanzyme 1000 (*A. oryzae* enzyme preparation) followed by paper chromatography of the hydrolyzate, isoprimeverose (6-*O*-α-D-xylopyranosyl-D-glucose), which is the minimum repeating unit of various xyloglucans[2,3,6,7,16-27], was detected on the chromatogram, in addition to xylose, arabinose, glucose, and galactose. These results suggest that fraction HC-IIB consists mainly of an arabinoxylan and a xyloglucan, in addition to a small proportion of polysaccharide containing (1→3)-β-D-glucosidic linkages [(1→3); (1→4)-β-D-glucan].

Fraction HC-IIB was resolved into three subfractions by chromatography on DEAE-Sephadex A-25. Most of the glucose-containing polysaccharides were recovered in the neutral polysaccharide fraction, fraction HC-IIB-I (Table IV). This fraction was next separated into three subfractions by chromatography on Sepharose CL-6B. Fraction HC-IIB-I-3, which became insoluble in water after the fractionation, contained a large proportion of glucose and xylose residues (Table V).

G.l.c. of the alditol acetates obtained from a hydrolyzate of the methylated HC-IIB-I-3 showed that 2,3,5-tri-*O*-methyl-L-arabinose (10.1%), 2,3,4-tri-*O*-methyl-D-xylose (12.9%), 2,3-di-*O*-methyl-D-xylose (18.6%), 2,3,6-tri-*O*-methyl-D-glucose (29.2%), 2- and/or 3-mono-*O*-methyl-D-xylose (9.4%), and 2,3-di-*O*-methyl-D-glucose (15.0%) were prominent structural units in the methylated HC-IIB-I-3. This result suggests that fraction HC-IIB-I-3 contains two kinds of polysaccharide, one (~40% of the fraction) being an arabinoxylan having a linear backbone chain of (1→4)-β-D-xylose residues, ~34% of which are substituted at 2 and/or 3 position with arabinose residues, the other (~60% of the fraction) being a xyloglucan having a linear backbone chain of (1→4)-β-D-glucose residues, ~31% of which are branched through O-6 by xylose residues. Attempts to remove the arabinoxylan from the fraction by several methods were unsuccessful. Because fraction HC-IIB-I-3 was free from such (1→3)-β-D-glucosidic linkage-containing polysaccharides as (1→3); (1→4)-β-D-glucan, fragmentation of xyloglucan in fraction HC-IIB-I-3 followed by analyses of the fragment oligosaccharides was conducted.

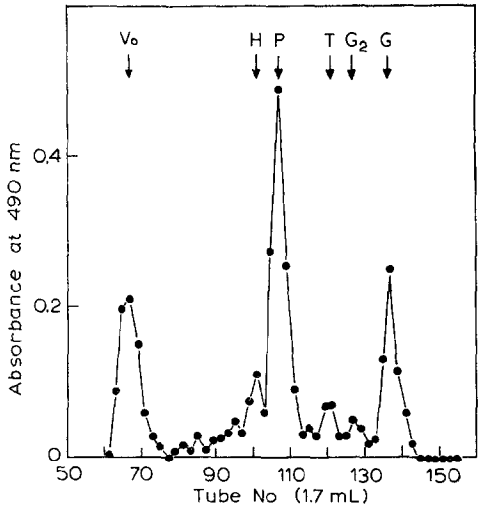

Fig. 3. Separation by gel filtration on Bio-Gel P-2 of the enzymic hydrolyzate of fraction HC-IIB-I-3. Tubes 61–71 (fraction 7), 97–102 (fraction 6), 103–112 (fraction 5), 113–117 (fraction 4), 118–123 (fraction 3), 124–131 (fraction 2), and 132–143 (fraction 1) were separately combined, concentrated, and subjected to rechromatography on the same column. Tube numbers at which Blue Dextran (V_0), hexasaccharide (H), pentasaccharide (P), and trisaccharide (T) from barley xyloglucan, cellobiose (G_2), and glucose (G) appeared are indicated in the figure by arrows.

Fraction HC-IIB-I-3 was hydrolyzed with a partially purified cellulase from *T. viride*. The resultant mixture was resolved by gel filtration on Bio-Gel P-2 (Fig. 3). Each fraction shown in Fig. 3 was rechromatographed on the same column. Fraction 1, corresponding to the elution position of monosaccharides, contained mainly glucose. Fraction 2 showed two spots (R_{Glc} 0.78 and 0.64). The major component having R_{Glc} 0.64 was obtained by preparative paper-chromatography. Fractions 3, 4, and 5

TABLE VI

YIELDS AND PROPERTIES OF OLIGOSACCHARIDES DERIVED FROM FRACTION HC-IIB-I-3 BY ENZYMIC HYDROLYSIS

Oligosaccharide	*Yield (mg)*	R_{Glc}	*D.p.*[a]	*Molar ratio of component sugars* Glucose: Xylose	A. oryzae *β-glucosidase-hydrolysis product*[b]
1	15.7	1.00	1	1.0	
2	2.9	0.64	2	2.0	G
3	4.9	0.52	3	2.0:0.7	G:IP = 1:1
4	trace	0.38	4	2.0:1.7	IP
5	28.5	0.28	5	3.0:1.8	G:IP = 1:2
6	5.3	0.16	6	4.0:1.8	G:IP = 2:2

[a]D.p. (degree of polymerization) was determined by gel filtration on Bio-Gel P-2. [b]Molar ratio of glucose (G) and isoprimeverose (IP).

TABLE VII

METHYLATION ANALYSIS OF OLIGOSACCHARIDES DERIVED FROM FRACTION HC-IIB-I-3 BY ENZYMIC HYDROLYSIS

Methylated sugar[a]	*Relative retention-times*		*Oligosaccharide*					
	Alditol acetate	*Methyl glycosides*	*6*	*5*	*R-5*[b]	*3*	*R-3*[b]	*2 (%)*
2,3,4,6-Me_4-Glc	1.00	1.00, 1.46						51.0
2,3,4-Me_3-Xyl	0.69	0.48, 0.61	30.0	38.7	36.1	34.0	38.5	
2,3,4-Me_3-Glc	2.48	2.85, 4.19	17.5	21.8	26.3	30.9	34.0	
2,3,6-Me_3-Glc	2.48	3.80, 5.33	31.5	19.2		35.1		49.0
2,3-Me_2-Glc	4.83		21.0	20.3	26.0			
1,2,3,5,6-Me_5-glucitol	0.37				11.6		27.5	

[a]2,3,4,6-Me_4-Glc = 2,3,4,6-tetra-*O*-methyl-D-glucose and so on. [b]R-5 and R-3 denote the reduced oligosaccharide 5 and 3, respectively.

appeared homogeneous in paper chromatography. Fraction 6 was found to be composed of two kinds of oligosaccharide (R_{Glc} 0.22 and 0.16), the major one (R_{Glc} 0.16) being isolated by preparative paper chromatography. Fraction 7, eluted at the void volume, consisted of arabinose, xylose, glucose, and galactose in the molar ratio of 31.4:58.6:7.8:2.2. Because this fraction was considered to be derived from an accompanying polysaccharide, arabinoxylan, it was not studied further. Table VI shows the yields and several properties of the oligosaccharides.

Oligosaccharide 5 (OS-5) was the major component of the cellulase-hydrolysis products. It consisted of glucose and xylose in the molar ratio of ~3:2 (Table VI). Methylation analysis showed that OS-5 contained two nonreducing terminal xylose residues, one 6-substituted glucose residue, one 4-substituted glucose residue, and one 4,6-disubstituted glucose residue (Table VII). Methylation analysis of the reduced OS-5 showed the appearance of one 4-substituted glucitol residue and the concomitant disappearance of one 4-substituted glucose, indicating that the 4-substituted glucose residue is present at the reducing terminal of OS-5. The i.r. spectrum of OS-5 showed absorption bands at 895 cm^{-1} (indicating the presence of a β-D-glucopyranosyl residue) and at 760 and 940 cm^{-1} (indicating the presence of an α-D-xylopyranosyl residue). On hydrolysis with *A. oryzae* β-glucosidase, OS-5 yielded isoprimeverose and glucose in the molar ratio of ~2:1 (Table VI). OS-5 also had the same paper-chromatographic mobility as oligosaccharide-B from barley xyloglucan[6]. From the foregoing results, OS-5 is proposed to have the structure α-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-D-glucose.

Oligosaccharide 3 (OS-3) consisted of glucose and xylose in the molar ratio of ~2:1 (Table VI). Methylation analysis showed that OS-3 consisted of one non-

reducing, terminal, xylose residue, one 6-substituted glucose residue, and one 4-substituted glucose residue (Table VII). Methylation analysis of the reduced OS-3 showed that the 4-substituted glucose residue was present at the reducing terminal of this oligosaccharide. The i.r. spectrum of OS-3 showed absorption bands at 760, 895, and 940 cm^{-1}. Hydrolysis of OS-3 with *A. oryzae* β-glucosidase yielded isoprimeverose and glucose in the molar ratio of ~1:1 (Table VI). OS-3 also had the same paper-chromatographic mobility as oligosaccharide-C from barley xyloglucan[6]. From these results, the structure of OS-3 is proposed to be α-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-D-glucose.

Oligosaccharide 6 (OS-6) consisted of glucose and xylose in the molar ratio of ~2:1. Methylation analysis showed it to contain two non-reducing terminal xylose residues, one 6-substituted glucose residue, two 4-substituted glucose residues, and one 4,6-substituted glucose residue (Table VII). Hydrolysis of OS-6 with *A. oryzae* β-glucosidase, yielded isoprimeverose and glucose in the molar ratio of ~1:1 (Table VI). OS-6 also had the same paper chromatographic mobility as oligosaccharide A from barley xyloglucan[6]. On the basis of these results, OS-6 is proposed to be α-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-D-glucose.

Oligosaccharide 4 (OS-4) contained of glucose and xylose in the molar ratio of ~1:1. On hydrolysis with *A. oryzae* β-glucosidase, it gave only isoprimeverose. Therefore, OS-4 is probably α-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-D-glucose.

Oligosaccharide 2 was assigned as cellobiose from the results of acid and enzymic hydrolysis and of methylation analysis (Tables VI and VII).

OS-2 and OS-6, obtained in significant quantities, may be considered as the intermediate products, convertible into D-glucose and into OS-5 and D-glucose, respectively, on prolonged incubation with the partially purified cellulase preparation. This consideration leads to the conclusion that the xyloglucan from cell walls of bamboo shoots consists mainly of repeating-units of OS-5 with some additional glucose residues, and OS-3 with some glucose residues.

The foregoing studies on fractions HC-IB and HC-IIB offer firm evidence for the presence of a linear (1→3); (1→4)-β-D-glucan and a xyloglucan in the cell walls of the bamboo shoot of Moso-chiku. There have been several studies on the structure of linear (1→3); (1→4)-β-D-glucans in various kinds of monocotyledons[2,8]. The general properties and the β-(1→3)/β-(1→4) ratio of the bamboo-shoot β-glucan are in fairly good agreement with those of the glucans isolated from other monocotyledons[5,7-10]. However, the enzymic-degradation study of the bamboo-shoot β-D-glucan shows that molar ratio of repeating oligosaccharide units (trisaccharide/tetrasaccharide) is slightly different from those of repeating oligosaccharide units of β-D-glucans in cell walls of other young grass species[5,7,29-31].

On the other hand, it is well known that xyloglucan is one of the major matrix polysaccharides of the cell walls of dicotyledonous plants. Recent studies on cell-wall polysaccharides from young monocotyledons have revealed that oat coleoptile[32],

rice endosperm[33], barley seedling[6], or rice seedling[7] cell-walls contain a xyloglucan. As compared with xyloglucans from dicotyledons and to those from other monocotyledons, the bamboo-shoot xyloglucan is very similar to barley[6] or rice[7] xyloglucan.

It was confirmed, in a manner almost identical with that described for fraction HC-IB, that the glucose residues in fractions PS-A and PS-B were derived from a (1→3); (1→4)-β-D-glucan (data not shown). We have suggested that xyloglucans in young plants are strongly bound to cellulose microfibrils by a bond stable to 4% potassium hydroxide but labile to 24% potassium hydroxide at room temperature[1,2,4,6,7,34]. This seems to be also the case with bamboo-shoot xyloglucan, because most of the xyloglucan was obtained by extraction with 24% potassium hydroxide.

From our previous[6,7] and present studies and the recent literature[32,33], it may be concluded that xyloglucans occur widely in Gramineae as well as in Leguminosae.

EXPERIMENTAL

General methods. — Concentration by rotary evaporation was performed under diminished pressure at 40–45°. Paper chromatography, both analytical and preparative, was performed on Toyo No. 50 filter paper by the multiple ascending method with 6:4:3 (v/v) butanol–pyridine–water. Neutral sugars on the chromatogram were detected with alkaline silver nitrate[35]. Optical rotation was determined with a Nippon Bunko Model DIP-SL polarimeter. Gas–liquid chromatography (g.l.c.) was effected on a glass column (0.4 × 200 cm) with a Yanagimoto Model G-80 gas chromatograph equipped with a flame-ionization detector at a nitrogen flow of 15 mL/min. The column packing was 1.5% QF-1 on Chromosorb W (column *a*), 10% poly(diethylenglycol succinate) on Diasolid-L (column *b*), or 3% OV-210 on Supelcoport (column *c*). Total carbohydrate was determined by the phenol–sulfuric acid method[36]. Reducing power was measured by the Nelson–Somogyi method[37,38]. Enzymic hydrolysis of poly- or oligo-saccharides was performed in the presence of a few drops of toluene to prevent microbial infection. Dialysis was conducted against distilled water at 4°.

Analysis of neutral sugars in poly- or oligo-saccharides. — The polysaccharide (1–2 mg) was hydrolyzed in a sealed tube with M sulfuric acid for 5–6 h at 100°, and oligosaccharides (0.2–1 mg) were hydrolyzed with 0.5M sulfuric acid for 3–4 h at 100°. Each hydrolyzate was made neutral with barium carbonate, filtered, the filtrate treated with Amberlite IR-120 (H^+) resin to remove barium ions, and then concentrated. Sugars were analyzed by g.l.c. on column (*a*) at 140° after converting them into their alditol trifluoroacetates[39].

Methylation analysis of poly- or oligo-saccharides. — Polysaccharide (10 mg) in dimethyl sulfoxide (2 mL) was methylated 4 times with methylsulfinyl carbanion (1 mL) and methyl iodide (0.5 mL) by the method of Hakomori[40]. The methylated polysaccharide, which was extracted into chloroform followed evaporation of the extract, was hydrolyzed by the 90% formic acid–0.25M sulfuric acid method[41]. The hydrolyzate was made neutral with barium carbonate, deionized with Amberlite

IR-120 (H^+) resin, and evaporated. The methylated sugars were analyzed by g.l.c. on column (*c*) at 172° as their corresponding alditol acetates[41] and the identities of the components were established by comparison with authentic compounds. Retention times of alditol acetate of 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, 2,4,6-tri-*O*-methyl-D-glucose, 2,3,6- and 2,3,4-tri-*O*-methyl-D-glucose, 2- and 3-mono-*O*-methyl-D-xylose, and 2,3-di-*O*-methyl-D-glucose relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol were 0.51, 0.69, 1.47, 1.96, 2.48, 2.71, and 4.83.

Oligosaccharides (1–2 mg) in dimethyl sulfoxide (0.5 mL) were methylated with methylsulfinyl carbanion (0.5 mL) and methyl iodide (0.2 mL) by the method of Hakomori[40], before or after treatment overnight with sodium borohydride. The methylated oligosaccharide was hydrolyzed, and the hydrolyzate converted into the corresponding alditol acetates for g.l.c. analysis in a manner identical with that for polysaccharides. Portions (0.5–1 mg) of the methylated oligosaccharides were methanolyzed with 5% methanolic hydrogen chloride for 10–12 h at 100°. The methyl glycosides formed were analyzed by g.l.c. on column (*b*) at 180°.

Preparation of cell-wall material from bamboo shoots of Moso-chiku. — Bamboo shoots (30–50 cm) of Moso-chiku (*Phyllostachys edulis*) were collected in May 1977 in the Katos' garden, Kagitori, Sendai. The edible portion (2.9 kg) of bamboo shoots were cut into slices, homogenized 3 times in 2 L of 0.05M Tris–HCl buffer (pH 7.6) with a Waring blender, and filtered through three layers of cheese cloth. The insoluble material was washed successively with water and ethanol, and then treated with 2:1 benzene–ethanol for 3 h at 50° to remove lipids. After this treatment, the material was washed with acetone and dried at 40° to give crude cell-wall material (yield 91.2 g).

Fractionation of the cell-wall polysaccharides. — The crude cell-wall material (80 g) was extracted sequentially 8 times with 1 L of 0.25% ammonium oxalate–oxalic acid, 5 times with 1 L of 4% potassium hydroxide, and 5 times with 1 L of 24% potassium hydroxide. Ammonium oxalate–oxalic acid extraction was conducted for 1 h at 75–80° and alkali extraction for 18–20 h at room temperature under nitrogen. After each extraction, the residue was separated from the supernatant solution by centrifugation. Ammonium oxalate–oxalic acid extract (pectic substances fraction, fraction PS), 4% potassium hydroxide extract (hemicellulose-I fraction, fraction HC-I), and 24% potassium hydroxide extract (hemicellulose-II fraction, fraction HC-II) were separately dialyzed and freeze-dried. Each fraction was suspended in 0.02M sodium phosphate buffer (pH 6.0) and incubated for 48 h at 40° with salivary alpha amylase[5] (0.1 mg/100 mg of the material). After incubation with the alpha amylase, each solution was heated for 10 min in a boiling-water bath to inactivate the enzyme, dialyzed, and centrifuged. Each soluble product (fraction B) in the supernatant solution was recovered by freeze-drying. Each insoluble product (fraction A) obtained as a precipitate was washed successively with water, ethanol, and acetone, and dried *in vacuo*. The residue (cellulose fraction, fraction CL) remaining after

alkali extraction was washed successively with dilute acetic acid, water, ethanol, and acetone, and dried *in vacuo*.

Resolution of fraction HC-IB by chromatography on DEAE-Sephadex A-25. — Fraction HC-IB (2.5 g) was dissolved in 100 mL of 0.01M sodium phosphate buffer (pH 6.0) and centrifuged. The supernatant solution was applied to a column (4.3 × 20 cm) of DEAE-Sephadex A-25 (Pharmacia Fine Chemicals) pre-equilibrated with the same buffer and first eluted with the same buffer (1 L, fraction HC-IB-I) and then stepwise with 0.5M sodium phosphate buffer (pH 6.0) (1 L, fraction HC-IB-II), and 0.5M sodium hydroxide (1 L, fraction HC-IB-III). Each fraction was made neutral, dialyzed, concentrated, and finally freeze-dried.

Enzymic hydrolysis of fraction HC-IB-I. — Fraction HC-IB-I (10 mg) in 10 mL of 0.02M McIlvaine buffer (pH 5.8) was incubated for 48 h at 40° with 1 mg of *Bacillus circulans* endo-(1→3)-β-D-glucanase[5]. Fraction HC-IB-I (10 mg) in 10 mL of 0.02M sodium acetate buffer (pH 5.0) was incubated for 48 h at 45° with 0.2 mg of *Trichoderma viride* cellulase[5]. Fraction HC-IB-I (10 mg) in 10 mL of 0.02M McIlvaine buffer (pH 5.7) was incubated for 48 h at 40° with 0.05 mg of *Streptomyces* sp. endo-(1→4)-β-D-xylanase[5]. After the incubation, each solution was heated in a boiling-water bath for 10 min and assayed for total carbohydrate and reducing power.

Isolation of β-D-glucan from fraction HC-IB-I. — Fraction HC-IB-I was resolved into five fractions by the procedure illustrated in Fig. 4. Fractions HC-IB-I-1,

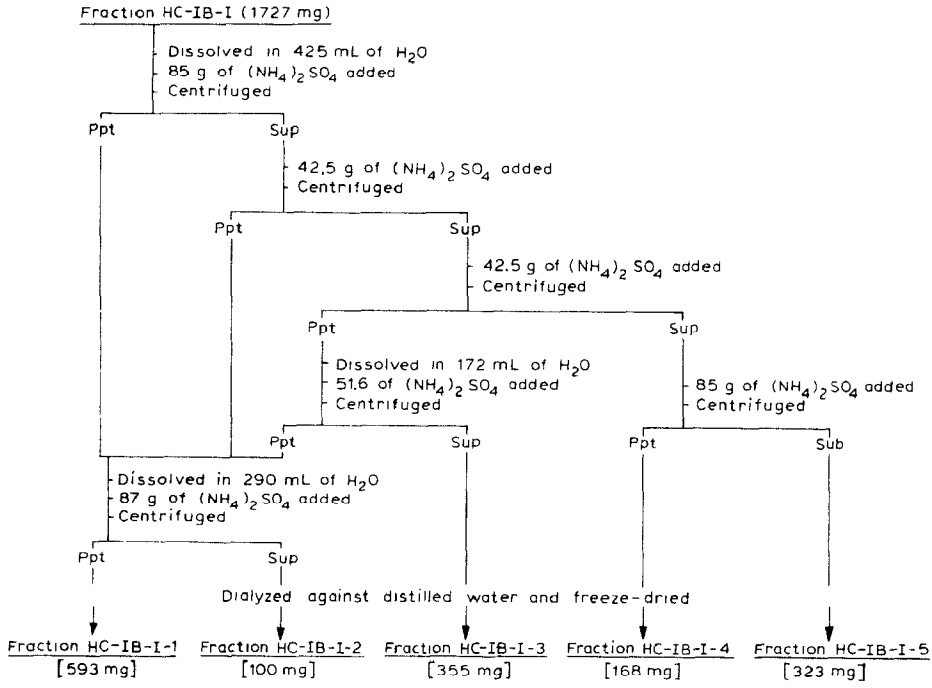

Fig. 4. Isolation procedure for glucan from fraction HC-IB-I. The values in branckets give the yield.

-2, -3, -4, and -5 consisted of arabinose, xylose, glucose, and galactose in molar ratio of 3.2:5.4:87.5:3.8, 21.2:42.4:30.7:5.8, 26.6:56.0:10.9:6.4, 38.5:51.5:7.0:3.0, and 34.0:43.4:9.1:13.4, respectively. Fraction HC-IB-I-1 was further characterized as a glucan preparation.

Hydrolysis of the glucan preparation with Bacillus subtilis *alpha amylase preparation and separation of the hydrolyzate.* — The glucan preparation (100 mg) was suspended in 50 mL of 0.02M sodium phosphate buffer (pH 6.3) and incubated for 24 h at 40° with 4 mg of *Bacillus subtilis* alpha amylase preparation (Sigma Type IIIA)[5]. After incubation, the solution was heated in a boiling-water bath for 10 min and centrifuged. The supernatant solution was concentrated to low volume and applied to a column (1.5 × 150 cm) of Bio-Gel P-2 (200–400 mesh, Bio-Rad) operated at 50°, followed by elution of the column with water (Fig. 1). Tubes 91–97 and 99–105 were separately combined, concentrated, and freeze-dried to give oligosaccharide-II (OS-II, 26.5 mg) and oligosaccharide-I (OS-I, 40.0 mg). Purities of the oligosaccharides were checked by paper chromatography.

Partial acid-hydrolysis of OS-I and OS-II. — Each oligosaccharide (5 mg) was hydrolyzed under reflux with 1 mL of 0.1M hydrochloric acid in a boiling-water bath for 2 h. The acid in each hydrolyzate was removed by evaporation. Another 5-mg portion of each oligosaccharide was reduced conventionally with sodium borohydride, followed by hydrolysis with 0.1M hydrochloric acid under the same conditions as already described. The hydrolysis products were analyzed by paper chromatography. Identification was made by comparison with the following standards, which are given with their respective R_{Glc} values: cellotetraose, 0.09; 3-*O*-β-cellotriosyl-D-glucose, 0.16; cellotriose, 0.25; 3-*O*-β-cellobiosyl-D-glucose, 0.39; laminaratriose, 0.34; cellobiose, 0.56; and laminarabiose, 0.71.

Hydrolysis of fraction HC-IB with the alpha amylase of Bacillus subtilis. — Fraction HC-IB (100 mg) in 10 mL of 0.02M sodium phosphate buffer (pH 6.3) was incubated at 40° with *Bacillus subtilis* alpha amylase preparation[5] (1 mg). At intervals, a portion of the solution was assayed for total carbohydrate and reducing power. After 24 h of incubation, the solution was heated for 10 min in a boiling-water bath and concentrated to low volume. The concentrate was applied to a column (1.5 × 150 cm) of Bio-Gel P-2 operated at 50°, followed by elution of the column with water (Fig. 2). Tubes 59–71 (polysaccharide fraction), 111–119 (tetrasaccharide fraction), and 121–127 (trisaccharide fraction) were separately combined and freeze-dried. The sugar composition of the polysaccharide fraction was investigated, and the structures of both oligosaccharide fractions were studied in a manner almost identical with that described for OS-I and OS-II.

Enzymic hydrolysis of fraction HC-IIB. — Fraction HC-IIB (10 mg) in 10 mL of 0.02M McIlvaine buffer (pH 5.8) was incubated for 48 h at 40° with 1 mg of *B. circulans* endo-(1→3)-β-D-glucanase[5]. Fraction HC-IIB (10 mg) in 10 mL of 0.02M McIlvaine buffer (pH 5.7) was incubated for 48 h at 40° with 0.05 mg of *Streptomyces* sp. endo-(1→4)-β-D-xylanase[5]. Fraction HC-IIB (10 mg) in 10 mL of 0.1M sodium phosphate buffer (pH 6.9) was incubated for 48 h at 40° with 0.01 mg of salivary

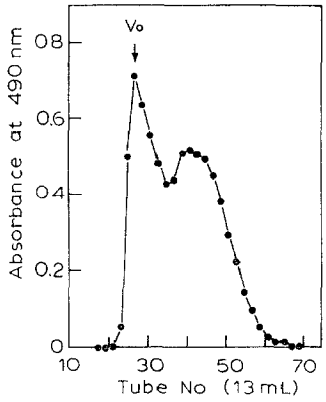

Fig. 5. Fractionation by gel filtration on Sepharose CL-6B of fraction HC-IIB-I. Details are given in the text.

alpha amylase[5]. After incubation, each solution was heated in a boiling-water bath for 10 min and assayed for total carbohydrate and reducing power.

Fraction HC-IIB (2 mg) in 3 mL of distilled water was incubated for 12 h at 40° with 0.8 mg of Sanzyme 1000 (*A. oryzae* enzyme preparation[2], Sankyo Co. Ltd., Japan). After incubation, the solution was heated in a boiling-water bath for 10 min and then concentrated. The concentrate was subjected to paper chromatography. Glucose, xylose, arabinose, galactose, and isoprimeverose (6-*O*-α-D-xylopyranosyl-D-glucose)[2] from xyloglucan of mung bean were used as standard sugars.

Resolution of fraction HC-IIB by chromatography on DEAE-Sephadex A-25. — Fraction HC-IIB (2430 mg) was dissolved in 200 mL of 0.01M sodium phosphate buffer (pH 6.0) and centrifuged. The supernatant solution was applied to a column (3.7 × 17.5 cm) of DEAE-Sephadex A-25 pre-equilibrated with the same buffer and eluted with it (800 mL, fraction HC-IIB-I) and then stepwise with 0.5M sodium phosphate buffer (pH 6.0) (800 mL, fraction HC-IIB-II), and 0.5M sodium hydroxide (800 mL, fraction HC-IIB-III). Each fraction was made neutral, dialyzed, concentrated, and finally freeze-dried.

Resolution of fraction HC-IIB-I by chromatography on Sepharose CL-6B. — Fraction HC-IIB-I (1950 mg) was dissolved in 200 mL of 0.1M sodium hydroxide. A portion (20 mL) of the solution was applied to a column (3 × 119 cm) of Sepharose CL-6B (Pharmacia Fine Chemicals) pre-equilibrated with 0.1M sodium hydroxide, followed by elution with 0.1M sodium hydroxide (Fig. 5). Tubes 23–35 were combined, made neutral, and dialyzed. The dialyzate was concentrated and freeze-dried to give fraction HC-IIB-I-1. Tubes 36–60 were combined and made neutral. The neutral solution was centrifuged at 10,000*g* for 30 min. The supernatant solution was dialyzed, concentrated, and freeze-dried to give fraction HC-IIB-I-2. The precipitate was washed successively with water, ethanol, and acetone, and dried to give fraction HC-IIB-I-3. Ten such fractionation procedures of fraction HC-IIB-I were performed.

Hydrolysis of fraction HC-IIB-I-3 with the cellulase of T. viride. — Fraction

HC-IIB-I-3 (110 mg) was suspended in 50 mL of 0.2M sodium acetate buffer (pH 5.0) and incubated with a partially purified *T. viride* cellulase[6] (~1.5 units) for 48 h at 40°. After incubation, the mixture was heated in a boiling-water bath for 15 min and centrifuged. The supernatant solution was treated with Amberlite IR-120 (H^+) resin, concentrated, and freeze-dried. The material in 2 mL of water was applied to a column (1.5 × 150 cm) of Bio-Gel P-2, followed by elution with water at 50° (Fig. 3).

Hydrolysis of oligosaccharides with the β-D-glucosidase of A. oryzae. — Each oligosaccharide (0.5–1 mg) in 3 mL of water was hydrolyzed for 16 h at 40° with 0.1–0.2 mL of *A. oryzae* β-glucosidase[2]. The enzymic hydrolyzate was subjected to paper chromatography. After chromatography, the filter paper was cut into strips. Carbohydrates on the strips were extracted with 1 mL of distilled water and the carbohydrate content in each solution was determined. Glucose, xylose, and isoprimeverose were used as standard sugars.

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