Studies on the Constituents of the Leaves of the Bamboo Grass, *Sasa* sp. (sect. *Sasa*)

Isolation of Hemicelluloses and Preparation of Carboxymethyl Hemicelluloses*

By

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サ サ の 葉 の 成 分 の 研 究

ヘミセルロースの単離とカルボキシメチル・ヘミセルロースの調製

太 田 路 一**

CONTENTS

Introduction

It has been recognized that the polysaccharide materials of the leaves of bamboo grass showed the marked inhibitory effect against some kind of tumor[1)~5)]. Some of them were very crude extract mixtures and the others were purified polysaccharides. The former so far known contained non-carbohydrate constituents such as chlorophyll, vitamins, amino acids, lignin, etc. except carbohydrates, and the latter did not essentially contain them except traces.

Recently, the report was made that the anti-tumor polysaccharides were isolated from the crude, saturated lime-water extract of the leaves of the Yakushima

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bamboo grass (*Sasa senanensis*), in homogeneous state with both of DEAE-cellulose ion-exchange chromatography and starch-block zone electrophoreses and their anti-tumor effect might be strongly due to the arabinoxylan, the common moiety through them[5]. This finding motivated us to attempt to investigate the hemicelluloses of the leaves of the bamboo grass which was distributed abundantly and widely in Hokkaido.

In the first part of the present paper, the isolation of hemicelluloses from the bamboo grass leaves preextracted with a mixture solvent of ethanol and benzene with special reference to its successive direct extraction is described. It would be possibly assumed that such an extraction might yield hemicelluloses with a little modifications in both of chemical and physical natures. In the course of the investigation, it was found that the water-soluble hemicelluloses were small in quantity and the arabinoxylan was the common moiety through all of the hemicelluloses obtained.

With this facts, the another impetus that led us to prepare a water-soluble polysaccharide, carboxymethyl hemicellulose A, came from the recent experiment by HAMURO and his co-workers who demonstrated that Pachymaran derived chemically from the Pachyman (the natural glucan) obtained from *Poria cocos* Wolf (Bukuryo), was converted into the water-soluble polysaccharide, "Carboxymethylpachymaran" and this substance had still the marked anti-tumor effect[7]. Thus, in the second part, we describe the preparation of the carboxymethyl hemicellulose A in which the alkoxide formation greatly catalyzed by the methylsulfinyl anion in dimethyl sulfoxide, followed by etherification with sodium chloroacetate was employed with the hope of possible lowering of degradations of carbohydrates which encountered in such a reaction of polysaccharide and sodium hydoxide in aqueous solution or aqueous organic solvents as has been used usually[8)~10)].

Results and Discussion

Hemicellulose A from chlorite holocellulose

The air-dried leaves of the bamboo grass, *Sasa* sp. (sect. *Sasa*) were ground in a Wiley mill and the meals were used for a series of experiments without screenings. The analytical data of the sample meals is presented in Table 1. The meals preextrated exhaustively in a soxhlet extraction apparatus with a mixture solvent of ethanol and benzene (1 : 2, v/v) were subjected to two treatments with sodium chlorite to yield the chlorite holocellulose (yield 80.6%, ash 13.2%, lignin 4.8%, pentosan 32.6%).

Table 1. Analytical data of the leaves of the bambo grass, *Sasa* sp. (sect. *Sasa*)

Sample	Ash	Lignin	Pentosan	Methyl pentosan	Holocellulose	Extracts				
						Alcohol-benzene	Cold water	Hot water	Saturated lime-water	1% NaOH
Leaves	12.3	22.2	21.7	1.3	57.6	11.5	12.1	18.5	16.9	58.3

All values in per cent, based on original sample leaves.

The hemicellulose was extracted from the chlorite holocellulose by the modified method of WISE and co-workers in which aqueous NaOH was used in stead of aqueous KOH[11]. Two extractions with aqueous 5% NaOH, followed by dialysis, were carried out in order to reduce both of lignin and ash. The hemicellulose A thus obtained contained lignin 0.9% and ash 1.6%. The yield and properties of the hemicellulose A are presented in Table 2.

Table 2. Analytical data of holocellulose and hemicellulose A

Sample	Yield	Ash	Lignin*1	Pentosan*1	Reducing*1 sugar	Uronic*1 anhydride	$[\alpha]_D$ *4 degrees	$[\eta]$*5	Sugar composition (molar ratio)			
		(%)	(%)	(%)	(%)	(%)			Ara.	Xyl.	Gal.	Glu.
Holocellulose	73.6*2	12.7	5.2	32.6								
Hemicellulose A	15.7*3	1.6	0.9	88.3	81.7	6.3	−88	0.61	1	6.2	trace	trace

*1 based on ash-free holocellulose and—hemicellulose A.
*2 based on ash- and extractives-free sample leaves, and ash- and lignin-free holocellulose.
*3 based on ash-free holocellulose and—hemicellulose A.
*4 c=1 in 10% KOH
*5 dℓ/g in 10% KOH.
Ara.; arabinose, Xyl.; xylose, Gal.; galactose, Glu.; glucose.

The hemicellulose A obtained with the yield of 15.7% (based on ash-free holocellulose) contained about 42% of the pentosan of the holocellulose. Therefore, it can be considered that the hemicellulose A is a representative portion of the hemicallulose of the leaves of the bamboo grass used here. It contained the sugar residues of L-arabinose, D-xylose, uronic acid in addition to traces of hexose, among which the molar ratio of D-xylose to L-arabinose was 6.2.: 1. The oxalic acid partial hydrolysis of it was attempted to confirm a linkage between L-arabinose and D-xylose residues. The sugar composition of the hydrolysis residues recovered after a given time of hydrolysis was determined. The results are presented in Table 3. It can be seen apparently that L-arabinose residue was hydrolyzed much more easily than D-xylose residue and thus linked in a furanose form to D-xylose skeltal chain in the hemicellulose A.

Further evidence for this fact is that on paper chromatography of the supernatant separated by centrifugation of the hydrolysis mixtures, any detectable spots of D-xylose were not observed until 90 min of time of hydrolysis.

The bamboo grass belongs to Gramineae plant such as wheat, corn, bamboo, etc. of which the hemicelluloses are composed of the Arabinoxylan[12)~14)]. They have the similar general chemical structure in which they contain L-arabinose and D-glucuronic acid residues attached to the β-1, 4-linked D-xylose skeltal chain. Therefore, the hemicellulose A may be supposed to have such an overall structural feature. For the trace of hexose, further work to confirm whether they are of structural elements or impurities was not conducted.

Table 3. Yields and sugar compositions of oxalic acid hydrolysis residues of the hemicellulose A

Time of hydrolysis (min)	Yield*1	Sugar composition*2	
		Ara.	Xyl.
0	51	1	6.2
10	48	1	7.1
30	38	1	10.7
60	37	1	12.9
90	35	1	12.6
120	31	1	18.8
180	25	1	29.0

*1 mg/10 mℓ

*2 presented in a ratio of peak areas on gas chromatogram.

Extraction with various solvents

In order to examine the amouts of substances extracted with common various solvents from the sample meals before and after preextraction with a mixture solvent of ethanol and benzene (1 : 2, v/v), the experiments were conducted. The following solvents were used; cold water, hot water, saturated lime-water, and aqueous 1% NaOH.

As seen in Table 4, aqueous 1% NaOH extraction yielded the notable large amount of extract, 49.6 and 52.8% for the sample meals before and after the preextration, respectively, which might contain most of the hemicellulose and lignin of the meals as well as organic extractives. The solubility of lignin in the solvents used can be seen in Table 5; especially, in aqueous 1% NaOH, lignin dissolved so much. It is apparent that the substances extracted with these solvents before the preextraction may contain some extractives soluble in a mixture solvent of ethanol and benzene. In order to confirm it further, the experiment was made in which the amounts of substances soluble in a mixture solvent of ethanol and benzene were determined for the meal residues extracted with these solvents before the preextraction. The results were as follows; 6.4, 5.1, 5.4, and 3.4% for

Table 4. Amounts of organic substances extracted with various solvents before and after alcohol-benzene preextraction

Solvent	Extracts (%)	
	Before it	After it
Cold water	9.3	4.2
Hot water	12.2	5.3
Saturated lime-water	10.8	11.9
1% HaOH	52.8	49.6

Ash was corrected for both of starting and residual sample leaves.

Table 5. Lignin and ash of residues after each extraction of alcohol-benzene preextracted leaves

Sample	Lignin*1 (%)	Ash in lignin (%)	Ash in residues (%)	Silica in residues*2 (%)
Original	24.3	30.0	12.4	not tested
Cold water	19.6	32.2	10.1	9.0
Hot water	19.0	22.8	7.5	5.9
Saturated lime-water	19.6	28.3	9.9	7.7
1% NaOH	7.3	8.5	1.1	0.9

*1 Ash was corrected for both of lignin and residues.
*2 Silica was determined from residues remained after dissoluing ash in aqueous HCl (1:1, v/v).

the cold water-, hot water-, saturated lime-water-, and aqueous 1% NaOH extracted residues, respectively. Thus, it can be said that much of organic extractives dissolved into these solvents in such extractions without the preextraction. Where the extractions described above are conducted without the preextraction, it can be obviously anticipated that such organic extractives become possible contaminations of polysaccharides isolated from these extracts. In conclusion, the preextraction step can not be avoided in isolation of the hemicelluloses which have been possibly assumed to play an important role in the tumor-inhibiting phenomeon as described above.

Preliminary successive extraction

The extraction was carried out in order to examine an amount and sugar composition of the polysaccharides of the substances which were extracted from the preextracted meals successively with hot water and then saturated lime-water

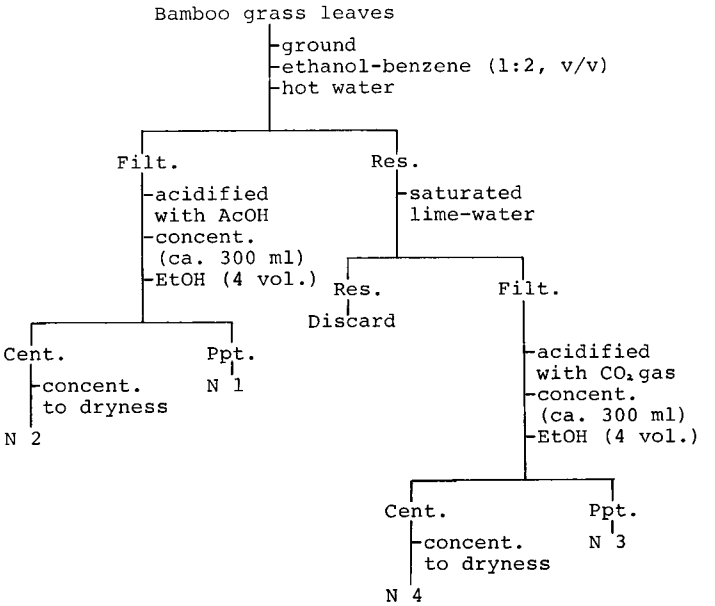

Fig. 1. Extraction scheme in preliminary successive extraction.

As seen in Fig. 1, each of the extracted substances was separated into two fractions, both of fractions precipitable and soluble in aqueous ethanol, by addition of ethanol into the solutions.

The analytical data are presented in Table 6. As calculated with the base of data obtained, it was found that the yields of the polysaccharides (as reducing sugars) of the fractions of from N 1 to N 4 were 0.31, 0.61, 0.18 and 0.23%, respectively, based on the ash-free preextracted meals. Thus, the predominated constituents of each fraction were apparently non-carbohydrate substances. All of the fractions were soluble easily in cold water. They contained the sugar residues of L-arabinose, D-xylose, D-rhamnose, D-mannose, D-galactose, and D-glucose. Their presence in all fraction were detected on both of gas chromatogram and paper chromatogram. The ratios of D-xylose to L-arabinose of the polysaccharides in each fraction except fraction 4 were a little less than 1.0. The ratios of hexose to pentose residues of the polysaccharides of the fractions of from N 1 to N 4 were found to be 2.2, 3.5, 1.4 and 0.9, respectively. With the fact of these ratios, the sugar compositions may suggest that the polysaccharids of all of the fractions would be of plant mucilage.

Table 6. Analytical data of each fraction in preliminary successive extraction

Fraction	Yield*1 (%)	Ash (%)	Lignin (%)	Reducing sugar*2 (%)	Sugar composition*3					
					Ara.	Xyl.	Rham.	Man.	Gal.	Glu.
N 1	1.8	37.6	4.8	26.9	1	0.8	0.2	0.5	2.2	1.1
N 2	5.3	25.6	12.3	15.5	1	0.9	0.2	0.6	1.4	4.3
N 3	0.6	17.3	5.4	37.5	1	0.8	0.3	0.3	1.3	0.7
N 4	1.9	25.6	13.6	14.9	1	1.9	0.2	0.2	0.6	1.7

*1 based on ash-free preextracted leaves.
*2 based on ash-free fractions.
*3 Ratios of peak areas on gas chromatogram.
Rham., rhamnose, Man., mannose.

Successive direct extraction with aqueous NaOH

As seen in Fig. 2, this extraction scheme was chosen with the aim of avoiding possible degradations of polysaccharides incident to delignification and also with the base of the findings obtained in the previous sections in connection with a solubility of lignin and a presence of plant mucilage.

The extraction was conducted without previous removal of lignin successively with hot water, aqueous 0.5% ammonium oxalate, aqueous 1%, 5%, and 17.5% NaOH. The former two extractions were conducted in order to remove previously both of water-soluble- and pectic substances from the preextracted meals before doing successive aqueous NaOH extractions. In this step, it is apparent that almost all extraneous substances were removed from the cell wall of the meals except some proteins and thus the remaining cell wall constituents are essentially lignin and polysaccharides with some proteins. It was found that on a whole ap-

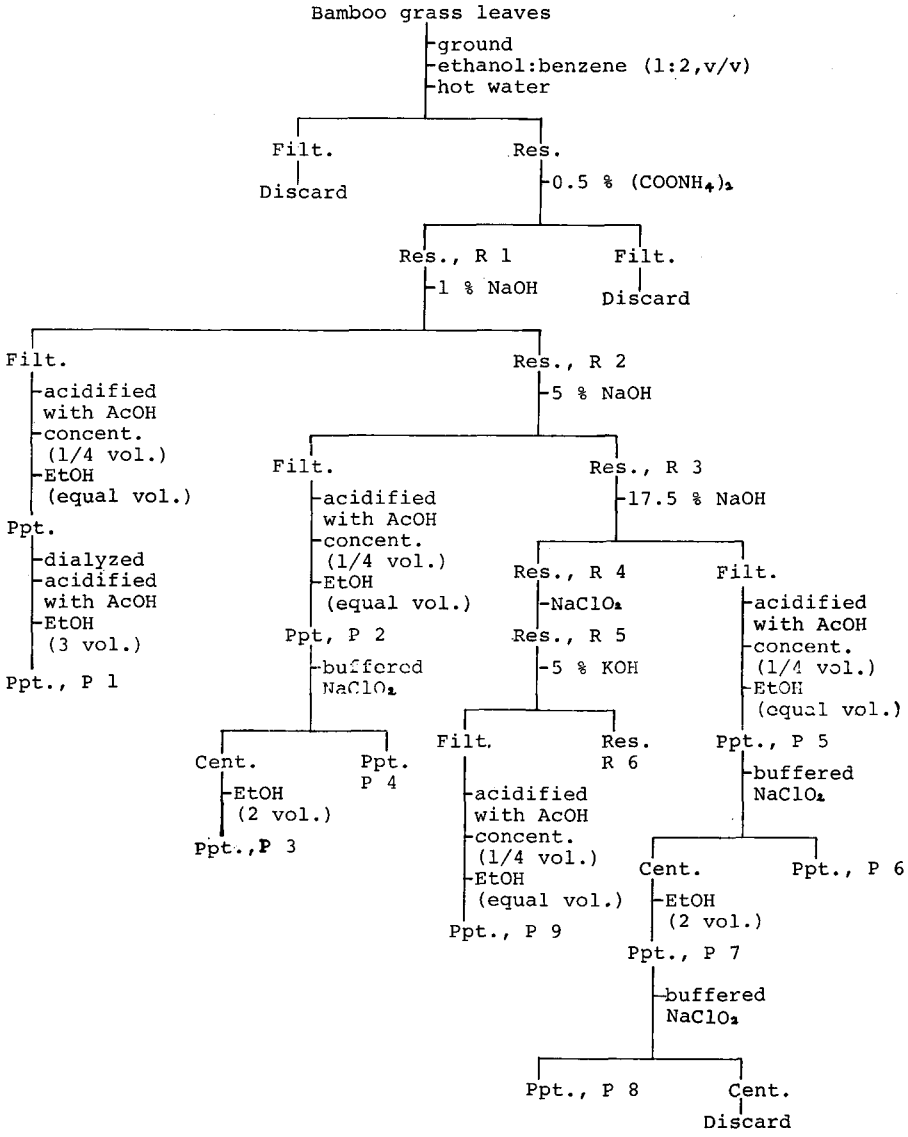

Fig. 2. Extraction scheme in successive direct extraction.

proximately one third of the hemicellulose (as pentosan) of the preextracted meals were recovered through this extraction, as calculated with the base of the data in Table 7 and 8. The buffered sodium chlorite treatment on crude hemicelluloses can remove much of lignin with least degradations due to an oxidative reaction according to the method of CLAYTON, as demonstrated in the experiment by DUTTON and co-workers[15),16)]. Thus, the treatment was employed to remove lignin of the fractions of P 2, P 5 and P 7. As seen in Table 7, however, the fractions of P 3, P4, P6, P7 and P8 still contained 2.3, 4.8, 1.7, 8.4, and 5.9% Klason lignin, respectively and thus further mild treatment with aqueous alkali would be necessitated to reduce it less than one per cent. However, such a work was not conducted

Table 7. Analytical data of each fraction in successive direct extraction

Fraction	Yield (g)	Ash (%)	Lignin*1 (%)	Pentosan*2 (%)	Hexosan*3 (%)	Uronic anhydride*4 (%)	$[\alpha]_D$ *5 degrees	$[\eta]$*6
P 1	0.7*	1.2	3.6	83.2		8.1	−103	
P 2	9.6	2.6	10.7	88.5				
P 3	5.3*	1.5	2.3	81.9	7.6	6.5	−105	0.59
P 4	2.6*	2.7	4.8	92.3	10.9	6.4		
P 5	7.3	4.9	16.2	76.8				
P 6	1.9*	1.3	1.7	70.7	22.9	7.7	−95	0.95
P 7	4.3*	3.4	8.4	83.5	8.2			
P 8	4.0	3.8	5.9	82.3		5.0		
P 9	4.4	9.7	1.5	74.5		8.3	−83	0.64

* corrected with the base on the assumpton that all amounts of preceding fraction would be used for the treatments.
*1 based on ash-free fractions.
*2 based on ash-and lignin-free fractions and methyl pentosan was corrected.
*3 based on ash- and lignin-free fractions and presented as glucan.
*4 based on ash- and lignin-free fractions and presented as glucuronic acid anhydride.
*5 c=1 in 10% KOH.
*6 dℓ/g in 10% KOH.

Table 8. Analytical data of each residue in successive direct extraction

Residue	Ash (%)	Lignin*1 (%)	Pentosan*2 (%)	Pentosan*3 (%)
R 1	11.2	22.4	36.6	28.5
R 2	5.6	18.8	35.2	28.6
R 3	5.8	16.6	28.1	23.4
R 4	5.6	16.4	20.4	17.1
R 5	5.9	8.0	not tested	

*1 Ash was corrected for both of lignin and residues.
*2 based on ash- and lignin-free residues.
*3 based on ash-free residues.

in the present paper, because one of the present aims was to describe the preliminary picture of an amount and nature of the hemicellulose thus obtained. After the extraction with aqueous 17.5% NaOH, the resulting residue R 4 contained 16.4% Klason lignin and still 17.1% pentosan. It appeared that both of the remaining lignin and pentosan in the residue R4 were not available to aqueous NaOH under the conditions used. Thus, it was treated with sodium chlorite under the usual conditions, followed by aqueous 5% KOH extraction to yield another fraction,

P 9. The major constituent of carbohydrate moiety all through fractions was pentosan, ranging from 71 to 92%. There was the apparent decreasing intendency in pentosan content among the fractions of P 2, P5, and P9, that is, 88.5, 76.8, and 74.5%, respectively. As described before, both fractions of P 3 and P 7 contained much of lignin, even though they were obtained from the supernatant solutions by centrifugation. It seems that this fact provided the interesting problem as to whether lignin macromolecules were co-precipitated with hemicelluloses or present as lignin-carbohydrate complexes. The presence of uronic acid in the fractions was detected on paper chromatogram of their hydrolyzates. Thus, analysis of uronic acid was conducted by the colorimetric method for the fractions which contained relatively small amounts of lignin. It was in the range of from 5.0 to 8.3%.

The values of specific rotation of both fractions of P1 and P3, $[\alpha]_D = -103°$ (c=0.96, in 10% KOH), were almost same as that of the P-Fr. 1-(A), $[\alpha]_D = -102.7°$ (c=1.0, in H_2O), described as the homogeneous hemicellulose by SUZUKI and co-workers[5]. The hemicellulose, P-Fr, 1-(A), was isolated and purified from the saturated lime-water extract of Yakushima bamboo grass (*Sasa senanensis*) leaves. As described later, the molar ratios of D-xylose to L-arabinose of both fractions of P 1 and P 3, 3.2 : 1 and 4.3 : 1, respectively, were also very close to that of the P-Fr, 1-(A), 3.7 : 1 (calculated by us from the per cent values reported). However, there is the discrepancy in a kind of hexose and an amount of hexose and uronic acid. Nevertheless, such an apparent coincidence may suggest that there are some potential similarities in chemical and physical features among these polysaccharides. An intrinsic viscosity was determined tentatively for the fractions of P 3, P 6 and P 9, since it appeared that lignin contents of them did not affect its measurements. The value of the fraction P 6 was 0.95 dℓ/g (in 10% KOH), which was much higher than those of the others, 0.59 and 0.64 dℓ/g It is clear that the fraction P 6 contains the greater polysaccharide in a molecular weight than the others. The sugar composition of a neutral polysaccharide moiety of the fractions is presented in Table 9. They were composed of L-arabinnose, D-xylose, D-galactose, D-glucose, and uronic acid residues. The former two sugar residues were the predominant constituents and the others were small or trace. The molar ratio of D-xylose to L-arabinose residues was in the range of from 3.2 : 1 to 14.2 : 1 through all fractions and also increased as a whole as the aqueous NaOH extraction and buffered chlorite treatment proceeded successively as seen in Fig. 2. It is considered reasonably that this wide range of the ratio may be due to the fact that the fractions obtained would be present in heterogeneous state with the varied ratios but not of products fragmented by degradations encountered in each extraction, decause the fractions were isolated under N_2 gas atmosphere by the direct extraction without prior oxidative delignification and thus possible degradations of polysaccharide could be neglected to such an extent that they would not affect essentially the ratios[16]. The ratio of the fraction P 7 was 14.2 : 1 exceptionally large among those of the other fractions. This ratio seems to be reasonable in magnitude, since that of the fraction P 8 which was derived from the fraction P 7,

Table 9. Sugar composition of each fraction in successive direct extraction

Fraction	Sugar composition (molar ratio)			
	Ara.	Xyl.	Gal.	Glu.
F 1	1	3.2	0.3	trace
F 3	1	4.3	0.3	0.2
F 4	1	6.0	trace	trace
F 5	1	6.2	0.2	0.1
F 6	1	4.0	0.4	0.2
F 7	1	14.2	trace	trace
F 8	1	10.4	trace	trace
F 9	1	8.4	trace	trace

showed 10.4. : 1 The ratios of the fractions of P 6 and P 7 were 4.0 : 1 and 14.2 : 1, respectively, even though both fractions were derived from the same preceding fraction P 5. There seems to be two possible hypothetical explanations for this large discrepancy; First, the fraction P 5 would be an aggregative mixture of both fractions of P 6 and P 7 with each the ratio. The buffered chlorite treatment could affect a solubility of them, giving both fractions soluble and insoluble in the solution. Second, both fractions of P 6 and P 7 would be cross-linked through a bridge of lignin moiety, giving the original fraction P 5. The buffered chlorite treatment could break up such a bridge and thus the fraction P 5 would be separated into two portions, the fractions of P 6 and P 7. Both explanations can give the same result in which as calculated from the data presented in Tables 7 and 9, a fraction supposedly prepared from both fractions of P 6 and P 7, would have a molar ratio of D-xylose to L-arabinose, 7.2 : 1. The ratio thus calculated is very close to that of the original fraction P 5. Therefore, it can be said conclusively that such a discrepancy was not caused by a preferential elimination of L-arabinofuranose residue but essentially due to the original presence of both fractions with each the ratio in the original hemicellulose. In conclusion, the presented results show that the arabinoxylan (D-xylan) is the common structural moiety through all fractions in which the ratio of D-xylose to L-arabinose was in the range of from 3.2 : 1 to 14.2 : 1.

With reference to its ratio of the hemicelluloses of culms of bamboo grass and bamboo so far studied, there are the papers in which as demonstrated the former and latter plant had the ratios of 7–16 : 1 and 15 : 1, respectively[17),18)]. Besides such sugar residues, they also contained small amounts of either or both of D-galactose and D-glucose residues and uronic acid residue; in the case of the bamboo grass culm, a presence of D-galactose residue was not demonstrated. However, this dicrepancey between two parts in the same plant, bamboo grass, can not be said conclusively in any sence as to whether it is true or not before a careful examination will be done. Further, the problem as to whether either or both of

D-galactose and D-glucose residues would be an integral part of these hemicelluloses or an possible contaminations mixed aggregatively with each together remains to be inquired.

Preparation of the carboxymethyl hemicellulose A

Carboxymethly acidic ethers of polysaccharide materials have been prepared by the interaction of polysaccharide alkoxides and chloroactic acid or sodium chloroacetate in aqueous solution or aqueous organic solvent[7),8),19),20)]. The former polysaccharide alkoxides have been formed through the interaction of polysaccharides and alkali. However, original polysaccharides were suffered from severe damages on their chemical and physical natures in the reaction used, even though resulting products have been utilyzed favorably in their own modified natures for many purposes. With the aim of possibly reducing such damages, therefore, the method of the alkoxide formation described by HAKOMORI was employed[9)], because the hemicellulose A was able to dissolve in dimethyl sulfoxide and thus the reaction was expected to proceed in homogeneous state all through the carboxymethylation. As presented in Table 10, the reaction conditions were chosen essentially according to those of CONRAD except those of the substitution reaction[10)]. In the formation of the methylsulfinyl anion base, the amount of base formed was calculated as 2.45–3.03 in a molar ratio of base to anhydro pentose, based on the assumption that the hemicellulose A is composed of only pentose residue.

Table 10. Reaction conditions of the carboxymethylation of the hemicellulose A

Experiment No.	Alkoxide formation			Substitution reaction		
	Base : anhydro pentose ratio*1	Reaction temp.	Reaction time (hr)	Salt : anhydro pentose ratio*2	Reaction temp. (°C)	Reaction time (hr)
1	2.45	R. T.*3	4	2.34	50	2
2	2.66	R. T.	4	5.00	50	2
3	2.74	R. T.	4	5.03	50 (27)*4	2 (13)*4
4	2.71	R. T.	4	5.05	63	2
5	3.03	R. T.	4	9.95	50	2

*1 Ratios of methylsulfinyl anion to hemicellulose A in moles per mole of anhydro pentose. Amounts of the hemicellulose A were 0.5 g in all experiments.

*2 Ratios of sodium chloroacetate to hemicellulose A in moles per mole of anhydro pentose.

*3 Room temperature.

*4 Reaction followed in the condition shown inside parenthesis.

In the formation of the hemicallulose A alkoxide, the amount of the methylsulfinyl anion base was 2.45–3.03 in a molar ratio of base to anhydro pentose, based on the same assumption as above. In the substitution reaction with sodium chloroacetate, its amount added was in the range of 2.34–9.95 in a molar ratio of salt to anhydro pentose, based on the same supposition as above. Both of the

reaction tempreature and -time were selected tentatively.

The reaction mixture which was diluted with water and then acidified to pH 2～3 with aqueous 2*N* HCl, was dialyzed overnight against running tap water and then further overnight against deionized water. In some experiments, the latter dialysis was replaced by the dialysis against deionized water containing a cation-exchange resin Dowex 50W-X4. In such cases, addition of ethanol failed to form either precipitates or white cloudy solution. Therefore, the following procedure was attempted to produce white precipitates; the aqueous ethanol solution were concentrated to about 20 mℓ at 40～50°C *in vacuo* and then ethanol was added. Where any precipitates were still failed to form by this procedure, the dropwise careful addition of aqueous 2*N* HCl was attempted to attain the optimum pH in which the rapid formation of precipitates occured. The yields and properties of the products thus obtained were listed in Table 11. It can be seen that the reaction used was able to yield the products soluble in cold water in 5% concentration which the tentative values of D.S. were in the range of 0.41～0.67. The values of D.S. were tentatively determined by the titration method and thus not of true ones, because almost complete removal of ash from the products was not conducted except the product 4 (ash 0.90%). The product 4 was obtained in such a way that after removing almost all of dimethyl sulfoxide used as solvent and by-products from the reaction mixture by the dialysis against running tap water, the solution was further dialyzed against water containing a cation-exchange resin, Dowex 50 W-X 4. However, such a removal of it appeared to render the product less soluble in cold water. It can be further observed that the single reaction failed to yield a fully carboxymethylated product, even though it proceeded in homogeneous state all through the reaction steps.

Table 11. Yields and properties of the carboxymethyl hemicellulose A

Experiment No.	Yield (g)	Ash (%)	Solubility*1 in water	Degree of substitution	$[\alpha]_D$ *2 degrees	Cation-exchange resin treatment*3
1	0.59	10.5	+	0.41	−81	−
2	0.63	10.0	+	0.61	−76	−
3	0.55	5.6	+	0.67	−77	+
4	0.34	0.9	−	0.83		+
5	0.63	12.6	+	0.41	−82	−

*1 Plus (+) shows products soluble in 5% concentration and minus (−) products partially insoluble in the same concentration.

*2 c=1% in water.

*3 Plus (+) shows the presence of cation-exchange resin (Dowex 50 W-X4) in the dialysis and minus (−) the absence of it.

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Experimental

The leaves of the bamboo grass used, *Sasa* sp. (sect. *Sasa*) were collected in the Uryu Experiment Forest, one of the College Experiment Forests in Hokkaido University. The air-dried leaves were ground in a Wiley mill and the meals were used for a series of experiments without screenings.

Analytical procedures

Acid hydrolysis of the hemicelluloses which were isolated directly from the preextracted meals or from the chlorite holocellulose, was carried out in a closed Pyrex tubing with 4% H_2SO_4 at 120°C for 2 hr. The hydrolyzates were neutralized with an anion-exchange resin, Dowex 44 (CO_2 form, 20~50 mesh). After the solution was filtered through a sintered glass filter (1 G 2) and the resin was washed with water, the filtrate and washings were evaporated to small volume *in vacuo* at 40~50°C. A part of the sugar solution was used for qualitative paper chromatography and the remaining one for quantitative gas chromatographic analysis of sugar composition as follows; after inositol as an internal standard was added to the sugar solution, sugars present in it were reduced with sodium borohydride to give alditols, followed by acetylation with acetic anhydride and pyridine (1 : 1, v/v) to give alditol acetates. The alditol acetates were analyzed quantitatively by a dual column gas chromatography (Yanaco G 8 type, YANAGIMOTO MFG. CO. LTD.) equipped with a flame ionization detector and separated on a column (0.3 × 200 cm) packed with 3% ECNSS-M on Gas Chrom Q, 100/120 mesh. The operating conditions were as follows; column temp. 190°C, injection temp. 200°C, detector temp. 250°C, flow rate of N_2 gas as a carrier gas 10.5 mℓ/min. The paper chromatography was conducted as follows; solvents used for separating sugars were (a) n-butanol-benzene-pyridine-water (10 : 2 : 5 : 5, v/v) and (b) n-butanol-acetic acid-water (4 : 1 : 5, v/v).

Separations were carried out on Toyo Filter Paper No. 50 by the descending method. Aniline hydrogen phthalate was used for the detection of reducing sugars. An uronic acid was determined according to the colorimetric method in which the Carbazole-H_2SO_4 was employed[22]. A neutral hexose was determined according to the colorimetric method in which the Anthrone method was employed[22].

A non-carbohydrate was detemined as Klason lignin. The other analytical procedures were carried out essentially according to the standard method of wood analysis[23].

A specific rotation was determined in aqueous 10% KOH at room temperature

(19~22°C) and its value was in an equilibrium. An intrinsic viscosity was determined in the Ostwald type viscometer and the reduced viscosities were estimated at four different concentrations in aqueous 10% KOH and linearly extraporated to zero concentration. A degree of substitution of carboxymethyl hemicellulose A was determined as follows; sample (100 mg) was added to deionized water (10 mℓ) and then aqueous 0.1 *N* NaOH (20 mℓ) was added with stirring. After the solution appeared completely dissolved, the solution was then back-titrated with aqueous 0.1 *N* HCl to a phenolphthalein end point: D.S.=0.132 A/(1−0.058 A), where, A=milliequivalents of sodium hydroxide required per gram of sample.

Preparation of chlorite holocellulose

The meals were exhaustively extracted with a mixture solvent of ethanol and benzene (1 : 2, v/v) (extractives; 11.5%). The analytical data of the preextracted meals are presented in Table 1. The preextracted meals (98.4 g, base on a moisture-free) were delignified by two treatments with sodium chlorite (60×2 g). The product thus obtained (80.1 g, based on a moisture-free), chlorite holocellulose, contained lignin of 4.8% and ash of 13.2%, and used for isolation of the following hemicellulose A.

Isolation of the hemicellulose A

Isolation was conducted essentially according to the method of WISE and coworkers in which aqueous NaOH was used in stead of aqueous KOH[10]. The chlorite holocellulose (78 g, based on a moisture-free) was extracted with aqueous 5% NaOH (1.6 ℓ) at room temperature under N_2 gas for 110 min. The mixture was filtered with suction through a cloth on Büchner funnel into a flask containing an excess of glacial acetic acid (225 mℓ). The residue was washed with aqueous 5% NaOH (800 mℓ) and deionized water (2.4 ℓ). The filtrate and washings were added by equal volume of ethanol with stirring and stood in refrigerator overnight. The resulting precipitate was centrifuged off and washed in succession with aqueous 80% ethanol, 100% ethanol, and ether. The material was dried *in vacuo* over P_2O_5 for two days to give light brown powder (yield 19.5 g, lignin 3.2%, ash 10.9%). It was dissolved again in aqueous 5% NaOH (0.5 ℓ) under N_2 gas and the solution was acidified with glacial acetic acid, followed by addition of equal volume of ethanol with stirring. The resulting precipitate was recovered as described above to give almost white powder (yield 14.7 g, lignin 1.9%, ash 10.2%). Further, this material (13.3 g) was put into a seamless cellulose tubing (Visking Co.) containing deionized water (0.5 ℓ) and then dialyzed against running tap water for five days and then deionized water for two days. The aqueous mixture of the solid material and liquor in the tubing was recovered into a beaker as much as possible and the tubing was linsed with deionized water. Ethanol (1 ℓ) was added into the beaker with stirring. The resulting precipitate was worked up as described above to give white powder (yield 10.8 g, lignin 0.87%, ash 1.56%), corresponding to 15.5% of the holocellulose with the base of ash-free holocellulose and—product. This material was designated here as hemicellulose A.

Oxalic acid partial hydrolysis of the hemicellulose A

The hemicellulose A (500 mg) was suspended in aqueous 0.02 *N* oxalic acid (100 mℓ). The mixture was gently refluxed in a glycerine bath controlled at 105°C for a given time. After a temperature of the mixture reached to the boiling point, several aliquots (each 10 mℓ) were pipetted into centrifuging bottles in the following time intervals; 10, 30, 60, 90, 120, and 180 min. The residual materials were centrifuged off and washed in succession with aqueous 80% ethanol and ether, and then dried *in vacuo* over P_2O_5. The yields of the recovered materials were as follows; 48, 38, 37, 35, 31 and 25 mg/10 mℓ, respectively. Their sugar compositions were determined as described above.

Preliminary successive extraction with hot water and subsequent saturated lime-water

The preextracted meals (353 g, based on a moisture-free) were suspended in hot water (4 ℓ) and refluxed gently for 2 hr. The mixture was filtered with suction through a cloth on Büchner funnel and the residue was washed with hot water until filtrates appeared almost colorless. The filtrate (pH 6.2) and washings were adjusted to pH 5.2 with dilute acetic acid and then concentrated to ca. 300 mℓ *in vacuo* at 40~50°C. The resulting precipitate was centrifuged off and the supernatant was readjusted to ca. pH 4.0. Four times volume of ethanol was added in the supernatant with stirring and the solution was allowed to stand in a refrigerator overnight. The resulting precipitate was centrifuged off and worked up as described above to give the light brown powder (5.7 g), fraction N 1. The supernatant was concentrated to dryness as described above to give the brown powder (16.3 g), fraction N 2. Then, the meal residue was also suspended in hot saturated lime-water (0.9 g CaO in 4 ℓ H_2O) and refluxed gently for one hour. The mixture was filtered and washed as described above. The filtrate and washings were adjusted to pH 5.2 with CO_2 gas and concentrated to ca. 300 mℓ *in vacuo* at 40~50°C. The solution was worked up as described above to give two fractions, precipitable fraction, N 3 (1.8 g, light brown) and soluble fraction, N 4 (6.0 g, orange).

Successive direct extraction with aqueous NaOH

The meals were preextracted in succession with a mixture solvent of ethanol and benzene (1 : 2, v/v), hot water, and then aqueous 0.5% ammonium oxalate as follows; the meals extracted exhaustively with ethanol-benzene solvent (122 g, based on a moisture-free) were refluxed gently with water (1.4 ℓ) for 2.5 hr. After filtering and washing the mixture, the meals residue was heated gently with aqueous 0.5% ammonium oxalate (1.7 ℓ) at 90°C for 2.5 hr. After filtering and washing the mixture, the meal residue thus obtained, R 1, was then subjected to successive direct extraction with aqueous NaOH as follows; it was extracted with aqueous 1% NaOH (1 ℓ) under N_2 gas at room temperature for 24 hr. The mixture was filtered through a cloth on Büchner funnel into a flask containing aqueous 50% acetic acid (60 mℓ) and the residue, R 2, was washed with deionized water until filtrates appeared light brown. After being allowed to stand overnight, the resulting precipitate was centrifuged off and the supernatant was concentrated to one

fourth of its original volume *in vacuo* at 40～50°C to which equal volume of ethanol was added. The resulting precipitate was centrifuged off and washed in succession with aqueous 80% ethanol, 100% ethanol, and then ether, and dried *in vacuo* over P_2O_5 for two days to give almost white powder (2.97 g, ash 40.43%, lignin 4.87%). This material (2.2 g) was put into a seamless cellulose tubing containing deionized water and dialyzed against running tap water for three days and then against deionized water for one day. After the solution was acidified to pH 3 with aqueous 10% acetic acid, three times volumes of ethanol were added. The resulting precipitate was worked up as described above to give a water-soluble grayish white powder, P 1 (0.66 g, ash 1.17%, lignin 3.63%). The residue R 2, was extracted with aqueous 5% NaOH under the same conditions as described above. The mixture was worked up as described above to give the filtrate and washings and the residue, R3. To the former, equal volume of ethanol was added. The resulting precipitate was centrifuged off, washed and dried as described above to give cream-colored powder, P2 (9.57g, ash 2.55%, lignin 10.73%). This material, P2 (4.1g), was subjected to the buffered sodium chlorite delignification. It was put into the solution (100 mℓ) of sodium acetate three hydrate (1.7 g), glacial acetic acid (2 mℓ), and sodium chlorite (3 g) and stirred at room temperature for 17 hr. The mixture was separated by centrifugation into two fractions, the precipitate and supernatant. The former was washed and dried as described above to give grayish white, P 4 (1.1 g, ash 2.74%, lignin 4.78%) and the latter supernatant was added by two times ethanol of original solution. The resulting precipitate was worked up as described above to give almost white powder, P 3 (2.3 g, ash 1.53%, lignin 2.29%). The residue, R 3, was extracted with aqueous 17.5% NaOH (1 ℓ) under the same conditions as described above. The mixture was filtered and washed as described above to give the filtrate and washings and the residue, R 4. The former solution was worked up as descrbed above to give light brown powder, P 5 (7.29 g, ash 4.89%, lignin 16.21%). This material (5.5 g) was subjected to the same delignification as described above in which the amounts of reagents proportionate to those of the reagents used above were employed, and the reaction time and -temperature were 20 hr and room temperature, respectively. The mixture was worked up as described above to give two fractions, P 6 (1.42 g, white, ash 1.32%, lignin 1.69%) and P 7 (3.24 g, cream-colored, ash 3.43%, lignin 8.35%). Further, the latter fraction, P 7 (2.2 g), was treated in the same manner as the fraction P 5 with the exception of amounts of each reagent used, to give one fraction, P 8 (2.1 g, white, ash 3.77%, lignin 5.86%). The residue, R 4, was subjected to the chlorite deligification in which two treatments with sodium chlorite was conducted in the following condition; that is, water (700 mℓ), glacial acetic acid (29×2 mℓ), sodium chlorite (23×2 mℓ), reaction temperature (70～80°C), and reaction time (1 hr). The residue thus obtained, R 5, still contained lignin of 8.03%. It was then extracted with aqueous 5% KOH (0.6 ℓ) under N_2 gas room temperature for two hr. The mixture was subjected to the work-up procedures as described above to give one fraction P 9 (4.39 g, white, ash 9.73%, lignin 1.54%).

Preparation of carboxymethyl hemicellulose A

The carboxymethylation reaction employed was conducted in the two steps in which first, the hemicellulose A was treated with the methylsufinyl anion to form the hemicellulose alkoxide and second, its alkoxide was reacted with sodium chloroacetate. The methylsufinyl anion was prepared as follows; into a dry 200 mℓ three-necked round-bottom flask fitted at one neck with an inlet tubing and containing a magnetic stirring bar was weighted 0.5 g sodium hydride in oil (ca. 50%, in "Bayol" 85)[9]. The sodium hydride was washed three times by stirring with 14 mℓ portions of n-pentane and decanting the wash. After the third wash, the flask was fitted with a thermometer and the residual pentane was removed through one open neck by gentle flushing of N_2 gas through the inlet tubing. The flask was then fitted with a dropping funnel containing dimethyl sulfoxide of 8 mℓ (Extra pure reagent grade) under N_2 gas atmosphere. After the dimethyl sulfoxide was transferred into the flask, the dropping funnel was then removed and a condenser was fitted to the flask. The flask was placed in a heating mantle and stirred with a magnetic stirrer at 50°C for 45 min and N_2 gas was passed continuously through the inlet tubing. For the formation of hemicellulose A alkoxide, the dry hemicellose A (0.5 g) was added to dimethyl sulfoxide of 24 mℓ in a dropping funnel and its suspension was shaked until the hemicellulose A was completely dissolved. The dropping funnel was then fitted to the flask containing the methylsulfinyl anion chilled to room temperature in stead of the condenser and the dimethyl sulfoxide solution of the hemicellulose A was added slowly and stirred under N_2 gas at room temperature for four hr. The amount of the anion base used was in the range of 2.45~3.03 in a molar ratio of base to anhydro pentose unit, based on the assumption that the hemicellulose A is composed of only pentose residue. Upon addition to the anion, a gel formed immediately but became gradually liquidized. For the carboxymethylation, sodium chloroacetate, dried at 100°C overnight, was added to the stirred solution through the neck for the condenser which was taken off and fitted again before and after its addition, respectively. The amount of sodium chloroacetate added was in the range of 2.34~9.95 in a molar ratio of salt to anhydro pentose unit, based on the same assumption as above. In most of the experiments, the solution was stirred under N_2 gas at 50°C for two hr and in the other, the temperature and time of reaction as listed in Table 10 was taken. The reaction mixture was diluted with deionized water and acidified with 2 *N* HCl and then dialyzed against running tap water for one day and subsequently against deionized water for another one day.

In some experiments, the second dialysis was conducted against deionized water containing a cation-exchange resin, Dowex 50 W-X 4. After acidifying the solution to ca. pH 3 with aqueous 2 *N* HCl, equal volume of ethanol was added with stirring. The resulting precipitate was centrifuged off and washed in succession with 100% ethanol and ether to give a white powder. Where this procedure could not produce any precipitate, the solution was concentrated to ca. 20 mℓ at 40~50°C *in vacuo* and then four times volume of ethanol was added and aqueous 2 *N* HCl

was carefully added until the solution became turbid and white floculent materials appeared. The white powder was dried *in vacuo* over P_2O_5 at room temperature.

Summary

The first object of the present study was to fractionate and characterize briefly the hemicellulose of the leaves of the bamboo grass, *Sasa* sp. (Sect. *Sasa*) with the hope of contributing to provide useful informations for future preparation of polysaccharides with an anti-tumor effect. The second one was to prepare a water-soluble polysaccharide, carboxymethyl hemicellulose A from the hemicellulose A with the same hope as above.

The results obtained so far are summerized in due order as follows;

1) Chemical analysis of the bamboo grass leaves showed the following results; ash 12.8%, lignin 22.2%, holocellulose 57.6%, pentosan 21.7%, methyl pentosan 1.3%, alcohol-benzene extractives 11.5%, cold-water extract 12.1%, hot-water extract 18.5%, saturated lime-water extract 16.9%, 1% NaOH extract 58.3%. Thus, it suggested reasonably that a direct extraction of the hemicellulose of the sample leaves with aqueous alkali would be possible.

2) Amount of ethanol-benzene extractives of the bamboo grass leaves extracted previously with cold water, hot water, saturated lime-water, and aqueous 1% NaOH without prior removal of such extractives, were 6.4, 5.1, 5.4, % and 3.4%, respectively. Thus, prior removal of substances soluble in an ethanol-benzene mixture solvent should be conducted in order to avoid possible contaminations of the hemicelluloses.

3) The hemicellulose A (yield 16%, ash 1.6%, lignin 0.9%) obtainted from the chlorite holocellulose contained about 42% of the pentosan of the chlorite holocellulose, the hemicellulose A can be considered to be of a representative portion of the hemicelluloses of the bamboo grass leaves. The hemicellulose A had the following properties; pentosan 88.3%, reducing sugar 81.7%, uronic acid 6.3%, $[\alpha]_D = -88°$ (c=1 in 10% KOH), $[\eta] = 0.61$ dℓ/g (in 10% KOH), and sugar composition of L-arabinose, D-xylose, D-galactose, and D-glucose in a molar ratio of 1 : 6.2 : trace : trace. The oxalic acid partial hydrolysis of the hemicellulose A revealed that L-arabinose residue was hydrolyzed much more easily than D-xylose residue and thus probably linked in a furanose form to D-xylose skeltal chain.

4) In the preliminary successive extraction, both of the hot water extract and saturated lime-water extract were separated into each two fractions by addition of ethanol into the solutions. The precipitates of the former and latter extract were 1.8 and 0.6% in yield, respectively and contained polysaccharides of 27 and 38% as a reducing sugar, respectively. The polysaccharide moiety of the former precipitate were composed of L-arabinose, D-xylose, D-rhamnose, D-mannose, D-galactose, and D-glucose in a ratio of 1 : 0.8 : 0.2 : 0.5 : 2.2 : 1.1 and that of the latter in a ratio of 1 : 0.8 : 0.3 : 0.3 : 1.3 : 0.7.

5) The successive direct extraction with aqueous NaOH yielded nine fractions, among which four fractions were obtained through the buffered chlorite treatment

on two solid extracts and the another one through the usual chlorite treatment on the final residue.

The overall yield of the hemicellulose (as pentosan) extracted all through nine fractions were approximately one third of that of the bamboo grass leaves used. The single buffered chlorite treatment used failed to reduce lignin content less than one per cent. A lignin and pentosan content of aqueous NaOH extracts were in the range of 3.6～16.2% and 76.8～88.5%, respectively. A lignin and pentosan content of the fractions obtained by the buffered chlorite treatment on above extracts were in the range of 1.7～8.4% and 70.7～92.3%, respectively. An uronic acid was determined for the fractions of small lignin contents to give the range of 5.0～8.3%.

A specific rotation and intrinsic viscosity were also measured for the fractions of small lignin contents; the former values were in the range of $[\alpha]_D = -83° \sim -103°$ (c=1 in 10% KOH) and the latter ones in the range of $[\eta] = 0.59 \sim 0.95$ dℓ/g (in 10% KOH).

All nine fractions contained the sugar residues of L-arabinose, D-xylose, D-galactose and D-glucose. The predominating sugar residues were both of L-arabinose and D-xylose and the others were small or trace in amount. Therefore, the common polysaccharide moiety all through nine fractions was the arabinoxylan in which a molar ratio of D-xylose to L-arabinose ranged from 3.2 : 1 to 14.2 : 1. Among the fractions, the two fractions showed an apparent coincidence in the molar ratio of D-xylose to L-arabinose and specific rotation value with one of the anti-tumor polysaccharides demonstrated by SUZUKI and co-worker (1968).

6) The carboxymethyl hemicellulose A, water-soluble polysaccharide, was prepared by the modified carboxymethylation in which the hemicellulose A alkoxide was formed by the reaction of the methylsulfinyl anion base and hemicellulose A in dimethyl sulfoxide and the overall reaction proceeded in homogeous state. The following reaction conditions were tentatively found to be proper in order to obtain a water-soluble polysaccharide with possible least degradations; in the alkoxide formation, hemicellulose A (as anhydro pentose unit) one mole, methylsulfinyl anion 2.7 moles, reaction temperature room temperature, reaction time 4 hr, and in the substitution reaction, sodium chloroacetate 5.0 moles, reaction temperature 50°C, recation time 2 hr. The carboxymethyl hemicellulose A thus obtained showed D.S. of 0.61 and specific rotation of $-76°$ (c=1 in H_2O).

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摘　　要

ササの葉のヘミセルロースを抽出し，その特性をしらべ，将来における抗腫瘍多糖類の製造に寄与する事を第一の目的とした。第二にホロセルロースから抽出したヘミセルロースAから水溶性多糖類，カルボキシメチル・ヘミセルロースAを同じ目的で改良法により調製した。以上の結果は次のように要約される。

1) 試料の葉の分析結果は次のようであった。灰分12.8%，リグニン22.2%，ホロセルロース57.6%，ペントサン21.7%，メチルペントサン1.3%，アルコール・ベンゼン抽出物11.5%，冷水抽出物12.1%，温水抽出物18.5%，飽和石灰水抽出物16.9%，1%カセイソーダ抽出物58.3%

2) エタノール・ベンゼン混合溶媒による抽出前処理をしない原試料葉の冷水抽出，温水抽出，飽和石灰水抽出，1%カセイソーダ抽出を行ない，それぞれの抽出残渣のアルコール・ベンゼン抽出物はそれぞれ6.4%，5.1，5.4，3.4%であった。従って，ヘミセルロース抽出の際の夾雑物となる可能性を除去するために，アルコール・ベンゼン抽出処理を前処理として実施する必要がある。

3) ホロセルロースは32.6%のペントサンを含有しており，5%カセイソーダ抽出及び透析処理により16%のヘミセルロースA（ホロセルロースのペントサンの約42%）が得られた。従って，このヘミセルロースAは試料葉のヘミセルロースの代表的な部分と考えられる。ヘミセルロースAの化学的性質は次のようであった。灰分1.6%，リグニン0.9%，ペントサン88.3%，還元糖81.7%，ウロン酸6.3%，比旋光度 $-88°$（1%水溶液），極限粘度 $[\eta]=0.61$ dℓ/g（10% KOH溶液），構成糖比（モル比）；アラビノース：キシロース：ガラクトース：グルコース＝1：6.2：trace：trace。シュウ酸部分加水分解によりアラビノース残基はキシロース残基より容易に水解され，従ってキシロース残基主鎖にフラノース型で結合している事が予測された。

4) 温水と飽和石灰水による予備的連続抽出で，それぞれの抽出溶液にエタノールを添加し沈殿物を得た。収量はそれぞれ1.8%と0.6%で，27%と38%の還元糖を含有していた。その構成糖比は次のようであった。すなわち，温水抽出物ではアラビノース：キシロース：ラムノース：マンノース：ガラクトース：グルコース＝1：0.8：0.2：0.5：2.2：1.1，飽和石灰水抽出物では1：0.8：0.3：0.3：1.3：0.7であった。

5) カセイソーダ溶液による連続直接抽出（1%，5%，17.5% NaOH）により9フラクションを得た。そのうち，4フラクションは2フラクションの温和な亜塩素酸ソーダ処理により得られ，17.5% NaOH抽出残渣に通常の亜塩素酸ソーダ処理を行ないさらに1フラクションを得た。9フラクションのヘミセルロース合計量はペントサンとして試料葉の約1/3であった。温和な亜塩素酸ソーダの1回処理により粗ヘミセルロースのリグニン含量は1%以下に減少しなかった。カセイソーダ抽出物の粗ヘミセルロースのリグニンとペントサンの含量はそれぞれ

3.6～16.2%, 76.8～88.5% の範囲にあり，温和な亜塩素酸ソーダ処理をして得られた粗ヘミセルロースのリグニンとペントサンの含量はそれぞれ1.7～8.4%，70.7～92.3% であった。ウロン酸の定量はリグニン含量の低いフラクションについて行ない，5.0～8.3% の範囲であった。比旋光度及び極限粘度は同じくリグニン含量の低いフラクションについて定量を行ない，前者は −83°～−103° (濃度1%, 10% KOH 溶液)，後者は $[\eta]=0.59\sim0.95$ dℓ/g (10% KOH 溶液) であった。9フラクションの構成糖はアラビノース，キシロース，ガラクトース，グルコースで，前二糖が主構成糖で，他二糖は少量或いは微量であった。従って，9フラクションで共通している多糖類はアラビノキシランで，キシロースとアラビノースの構成糖比 (モル比) は3.2:1～14.2:1の範囲であった。9フラクションのうち，2フラクションの粗ヘミセルロースはアラビノースとキシロースのモル比及び比旋光度において鈴木等 (文献5) の報告した抗腫瘍多糖類のそれらと明らかな一致を示した。

6) ヘミセルロースAより水溶性多糖類，カルボキシメチル・ヘミセルロースAを改良カルボキシメチル化法により調製した。すなわち，ヘミセルロースAのアルコキシドはヘミセルロースAとメチルスルフィニル塩基 (methylsulfinyl anion) のジメチルスルホキシド溶液中で調製し，ナトリウムモノクロル酢酸を加え均一系で反応が進行した。使用した反応条件は次の通りであった。(1) アルコキシド生成；ヘミセルロースA (ペントース単位として) 1モル，メチルスルフィニル塩基2.7モル，反応温度50°C，反応時間4時間。(2) 置換反応；ナトリウムモロクロル酢酸5.0モル，反応温度50°C，反応時間2時間。このカルボキシメチル・ヘミセルロースAの置換度は0.61で，比旋光度は −76° (1% 水溶液) であった。