ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 325 (2000) 230–232

CARBOHYDRATE RESEARCH

Note

Isolation of hemicellulose from a sorghum, *Andropogon sorghum* Brot, Kumadake no. 263, and determination of its constituent sugars

Takashi Kitayama [a,*], Miwa Morimoto [a], Masahiro Takatani [a], Yasuo Tamura [b], Masaru Takumi [c], Tadashi Okamoto [a]

[a] *Department of Agricultural Chemistry, Faculty of Agriculture, Kinki University, 3327-204, Naka-machi, Nara 631-8505, Japan*

[b] *Institute of Wood Technology, Akita Prefectural University, Noshiro, Akita 016-0876, Japan*

[c] *Koyo Sangyo Co., 1181-155, Fujita, Minaminasu, Tochigi 321-0532, Japan*

Received 6 July 1999; accepted 14 December 1999

Abstract

Hemicellulose was isolated from the stem of sorghum, *Andropogon sorghum* Brot, Kumadake no. 263, and the constituent sugars of this isolate were determined. The xylose concentration in the hemicellulose of SV263 was extremely high.

Keywords: Sorghum; Hemicellulose; Stem; Xylose; Arabinose

Polyols are important substances that have versatile uses in industry. However, it is very difficult for some polyols, especially their polymers, to be prepared synthetically. To develop a simple means of obtaining polyols, we focused on hemicellulose from natural resources such as that in the stem of sorghum, *Sorghum* sp., which is an agricultural waste discarded in large quantities in China.

The hemicellulose concentration and the sugar composition of hemicellulose from sorghum [1–6] are known to vary in response to differences in the cultivar, maturity, plant tissue, and also the analytical method [1–3]. Nandra et al. described how the hemicellulose content and its xylose proportion in *Sorghum bicolor* increased with maturity [1]. Kawamura et al. studied the effect of the hemicellulose constituent sugars on the digestibility of *S. bicolor* (L.) Moench stored in silages by sheep and found that differences in the relative proportions of hemicellulose constituent sugars affected the digestibility by sheep [2]. It is important during studies of the characterization of hemicellulose to isolate the hemicellulose and determine its constituent sugars. We report here the extraction of hemicellulose from sorghum, *Andropogon sorghum* Brot, Kumadake no. 263 (SV263), and the determination of its constituent sugars.

As shown in the flow chart for the separation in Procedure 1 (Fig. 1), the stems of

* Corresponding author. Tel.: +81-742-43-1511; fax: +81-742-43-1445.

E-mail address: kitayama@nara.kindai.ac.jp (T. Kitayama)

0008-6215/00/$ - see front matter

PII: S0008-6215(00)00002-1

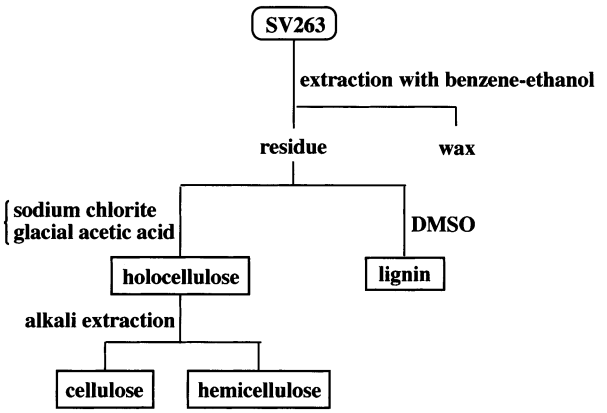

Fig. 1. Procedure 1: flow chart for the extraction of hemicellulose.

SV263 (with pith/without pith) were ground and the wax was extracted from the mixture using 1:1 benzene–ethanol to obtain a beige powder. In the attempted extraction with dimethyl sulfoxide (DMSO) after exclusion of wax, a lignin fraction was extracted without contamination by hemicellulose (Fig. 1).

Lignin was further removed from the ground mixture or the DMSO extract residue by stirring with a mixture of sodium chlorite and glacial acetic acid to obtain holocellulose. Hemicellulose was extracted from the holocellulose using various aqueous alkalis. As shown in Table 1, white hemicellulose that was insoluble in water was obtained in a yield of 14% by extraction of lignin-free holocellulose with 24% KOH for 24 h. Brown hemicellulose containing some contaminants was obtained by direct extraction of the ground mixture with alkali without delignification. Delignification improved the yield as well as the purity of hemicellulose extracted in comparison with extraction using a lignin-containing sample. From the pith of SV263, hemicellulose was hardly extracted by alkali extraction.

Isolated hemicellulose was hydrolyzed with sulfuric acid to afford monosaccharides, which were reduced by $NaBH_4$ to alditols. Then, the alditols were thoroughly acetylated in sulfuric acid to afford alditol hexaacetates [7,8]. In the same manner, alditol hexaacetates of rhamnose, arabinose, xylose, mannose, galactose, and glucose as standard sugars and *myo*-inositol as an internal standard were prepared and analyzed by gas chromatography

Table 1
Extraction of hemicellulose using an alkaline solution [a]

Lignin	Extraction time (h)	Yield (%)	Color
No	6	12	White
	20	9	
	22	11	
	24	14	
Yes	3	13	Brown
	20	16	
	24	15	

[a] 24% aqueous KOH.

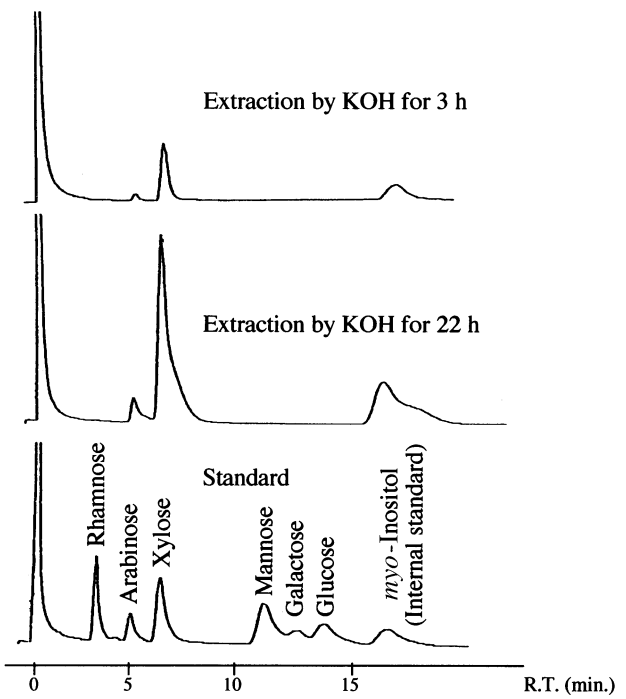

Fig. 2. Gas-chromatographic analyses of peracetates of constituent sugars of hemicellulose SV263.

using a column of Gaschrom Q (100–120 mesh; injection temperature, 220 °C; column temperature, 210 °C; carrier gas, N_2; detector, FID) and ^1H NMR spectroscopy. The result of gas-chromatographic analyses of the acetates shows that hemicellulose from SV263 consists of only two monosaccharides: 91% xylose and 9% arabinose (Fig. 2).

As described above, the hemicellulose content and sugar composition in sorghum are known to vary in response to differences in the cultivar, maturity, plant tissue, the analytical method, and the xylose proportion in *S. bicolor* increased with maturity [1]. Nandra et al. [1] used the whole plant of forage sorghum

and found that the neutral sugar of the hemicellulose comprised four monosaccharides: 5.3% arabinose, 84.0% xylose, 1.8% galactose, and 7.6% glucose, and the hemicellulose content of the sorghum was 15% 100 days after sowing. In the study of the effect of the hemicellulose constituent sugars on the digestibility of *S. bicolor* (L.) Moench stored in silages by sheep, Kawamura et al. [2] found that the neutral sugar of the hemicellulose comprised four monosaccharides: 9.5% arabinose, 79.4% xylose, 2.2% galactose, and 8.9% glucose, and the hemicellulose concentration of the sorghum was approximately 20%. SV263 in our study was a wild type and matured at harvest. It was difficult to compare precisely the conditions of cultivar and maturity of SV263 with those of other species. Compared with the reported results, however, it is notable that the xylose content of the hemicellulose of SV263 was 91%, which is extremely high. The hemicellulose was found to comprise two monosaccharides, xylose and arabinose. The hemicellulose content of the sorghum was 14%, which is similar to the levels reported in other reports. From these findings, it may be concluded that xylose and arabinose are the main monosaccharide residues of SV263, and SV263 has a higher concentration of xylose as a constituent sugar of hemicellulose than other sorghums.

1. Experimental

General methods.—Gas-chromatographic analyses were performed using a Shimadzu gas chromatograph model GC-12A equipped with an Gaschrom Q column.

Plant material.—*A. sorghum* Brot, Kumadake no. 263 was collected at Liaoning pendi, China, and matured at harvest.

Separation.—SV263 stem (60 g) with pith was ground and the wax was extracted from the mixture by Soxhlet extraction using 120 mL of 2:1 benzene–ethanol overnight to obtain a beige powder. Lignin was removed from the crushed powder (20 g) by stirring continuously for 3 h in water (1 L) at 75 °C in the presence of glacial AcOH (1.2 mL) and sodium chlorite (15 g) to obtain holocellulose (14.2 g). Hemicellulose was extracted in a 14% yield from holocellulose using 24% aq KOH.

Preparation of acetates for GLC analyses.—Hemicellulose (0.3 g) was added to 72% H_2SO_4 (3 mL) and stirred at 30 °C for 1 h. The mixture was poured into water (84 mL), and the solution was treated in an autoclave at 120 °C for 1 h. *myo*-Inositol (0.1 g) was added as the internal reference, the pH was adjusted to 5.5 with dilute aq barium hydroxide, and the resulting suspension was centrifuged. Sodium borohydride (0.4 g) was added to the supernatant solution, and the mixture was stirred at room temperature (rt) for 2 h. Glacial AcOH was added to the mixture until the evolution of hydrogen ceased, and the solvent was evaporated under reduced pressure to afford a syrupy oil.

The oil was added to a mixture of Ac_2O (15 mL) and concd H_2SO_4 (1 mL), and then the mixture was stirred at 60 °C for 1 h. The solution was poured into water (200 mL) at rt, and the mixture was extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with satd $NaHCO_3$ (3×50 mL) and satd aq NaCl (3×50 mL), dried over anhyd Na_2SO_4, and evaporated under reduced pressure to afford alditol hexaacetates. The products were analyzed by gas chromatography using a column of Gaschrom Q (100–120 mesh; injection temperature 220 °C; column temperature 210 °C; carrier gas N_2; detector FID).

References

[1] K.S. Nandra, B.K. Gupta, A.K. Chopra, *J. Sci. Food Agric.*, 34 (1983) 962–964.
[2] O. Kawamura, S. Tanaka, T. Miaki, K. Shinkai, *Bull. Fac. Agric. Miyazaki Univ.*, 39 (1992) 63–65.
[3] M. Goto, A.H. Gordon, A. Chesson, *J. Sci. Food Agric.*, 54 (1) (1991) 47–60.
[4] J. Miron, D.B. Ghedalia, R. Solomon, *J. Dairy Sci.*, 80 (1) (1997) 144–151.
[5] C.L. Wedig, E.H. Jaster, K. Moore, *J. Dairy Sci.*, 72 (1) (1989) 104–111.
[6] C.L. Wedig, E.H. Jaster, K.J. Moore, *J. Dairy Sci.*, 72 (1) (1989) 112–122.
[7] J.H. Sloneker, *Methods Carbohydr. Chem.*, 6 (1972) 20–24.
[8] H.N. Englyst, J.H. Cummings, *J. Assoc. Off. Anal. Chem.*, 71 (1988) 808–814.