Studies on Mannan in Wood Pulp

Part VII. The Constitution of a Glucomannan

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The glucomannan isolated from holocellulose pulp of Akamatsu (*Pinus densiflora* SIEB. et Zucc.) as its triacetate was methylated and the following methylated sugars were obtained by hydrolysis: the 2,3,4,6-tetra-O-methyl ethers of D-glucose and D-mannose (1 part) and the 2,3,6-tri-O-methyl ethers of D-mannose and D-glucose (34-37 parts). Periodate oxidation of the glucomannan showed that 1.00 mole of periodate was consumed per mole of hexose unit and 3 moles of formic acid liberated for every 33 hexose units.

Akamatsu wood (*Pinus densiflora* SIEB. et Zucc.), the most widely distributed coniferous wood in Japan, has been the most excellent pulp wood for dissolving pulp, although the deciduous wood is also used at the present time as pulp source. Throughout a series of the experiments on "mannan" in wood pulp, the commercial dissolving sulfite pulp^{1,2)} or holocellulose pulp³⁻⁶⁾ prepared from Akamatsu wood has been used as a source of glucomannan. The present paper is concerned with the constitution of Akamatsu glucomannan. The glucomannan was isolated from Akamatsu holocellulose pulp prepared under a mild condition of delignification by the procedure described previously and purified as triacetate⁴⁾. The triacetate used for methylation showed an optical rotation of $[\alpha]_D -30.7^\circ$ (chloroform); $[\eta]$ 0.470 dl/g in chloroform. It was composed of 3.6 parts of mannose and one part of glucose; the other sugars were absent. Methylation of the glucoman-

nan triacetate with methyl sulfate and sodium hydroxide by the procedure of Haworth and Percival⁷⁾ and completion of the methylation with silver oxide and methyl iodide⁸⁾ afforded the corresponding fully methylated glucomannan, $[\alpha]_D -19.6^\circ$ (chloroform). Fractionation of the methylated glucomannan indicated that the polysaccharide was essentially homogeneous except the lower molecular part. The intrinsic viscosity of a solution of the methylated glucomannan in chloroform was 0.475 dl/g. This shows that no degradation of the glucomannan molecule has occurred in the methylation process. After hydrolysis with 5% methanolic hydrogen chloride followed by 5% hydrochloric acid, the specific optical rotation of the glucomannan rose from -19.6° to a constant value of $+19.3^\circ$ and this shows that β -configuration is predominant in the glucomannan molecule. The following methylated sugars were separated by chromatography on a cellulose column: a) 2,3,4,6-tetra-O-methyl-D-glucose, b) 2,3,4,6-tetra-O-methyl-D-mannose, c) 2,3,6-tri-O-meth-

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2) T. Koshijima and I. Tachi, *ibid.*, **22**, 11 (1958).

3) T. Koshijima, *J. Japan Wood Res. Soc.*, **6**, 194 (1960).

4) T. Koshijima, This Journal, **24**, 687 (1960).

5) T. Koshijima, *J. Agr. Chem. Soc. Japan*, in press.

6) T. Koshijima, *ibid.*, in press.

7) W.N. Haworth and E.G.V. Percival, *J. Chem. Soc.*, **1932**, 2277.

8) T. Purdie and J.C. Irvine, *ibid.*, **83**, 1021 (1903).

yl-D-mannose, d) 2,3,6-tri-O-methyl-D-glucose, e) Di-O-methyl sugars. Components a) and b) could not be separated each other. They were identified by the R_F value of the first component (see experimental part) and by demethylation of the component to glucose and mannose. Components c) and d) were characterized as their 1,4-di-O-*p*-nitrobenzoates⁹⁾. Component e) consisted of a mixture of di-O-methyl hexoses and was not identified. The ratio of c) to d) was calculated to be approximately 4:1 by the specific optical rotation of the second and the third effluent sirup from the cellulose column (see experimental part). The consumption of alkaline hypoiodite¹⁰⁾ by the methylated sugars separated on silica gel chromatostrip¹¹⁾ indicated that one non-reducing end group was present for every 34-37 hexose units in the glucomannan chain molecule: the non-reducing end group consisted of mainly glucose (87%). A part of the glucomannan triacetate used for methylation study was deacetylated and oxidized with sodium metaperiodate at 2°C. The consumption of 1.00 mole of periodate by the glucomannan is consistent with an essentially linear structure for the polymer and therefore isolation of di-O-methyl hexoses seemed to be attributed to demethylation during hydrolysis. The liberation of 3 moles of formic acid for every 33 hexose units indicates also that the polymer has no branching point. These data described support the view that the glucomannan present in Akamatsu wood consists of about 35 units joined by 1→4 linkages in the β -configuration and is made up of linear chain of D-mannose (4 parts) and D-glucose (1 part). Recently the glucomannan from white birch¹⁴⁾ is reported

to contain approximately equal quantities of D-mannose and D-glucose, and number average D.P. of 70. It should be noted that each of the glucomannan present in coniferous and deciduous wood has a lower D.P. in comparison with arabino-4-O-methylglucuronoxylan (100)¹²⁾ or 4-O-methylglucuronoxylan (120)¹³⁾ in wood.

EXPERIMENTAL

Isolation of the Acetylated Glucomannan.

A glucomannan extracted with 10% aqueous sodium hydroxide from Akamatsu (*Pinus densiflora*) holo-cellulose pulp was dialyzed through gel-cellophane, treated with dimethylsulfoxide and made free from the water-insoluble matter as described in part IV⁴⁾. The resultant water solution of the glucomannan (12 g in 2 l) was concentrated in vacuo, precipitated from 3 volumes of methanol, and the white precipitate recovered by filtration. The glucomannan, while still containing some methanol (54 g), was acetylated with acetic anhydride in pyridine. After the second acetylation, the acetate (16 g) showed a specific optical rotation of $[\alpha]_D^{25} -30.0^\circ$ in chloroform (c, 1.0). It was dissolved in chloroform (1.2 l) and fractions A₁ (1.1 g), A₂ (2.9 g), A₃ (3.0 g), B (5.9 g), and C (0.2 g) were obtained by adding increasing amounts of petroleum ether in the usual manner. Fractions A₃ and B, showing $[\alpha]_D^{25} -30.7^\circ$ (c, 1.0, chloroform), $[\eta]$ 0.470 dl/g (chloroform) were the pure glucomannan triacetate⁴⁾ which was composed of 3.6 parts of mannose and one part of glucose. The infrared adsorption in hydroxyl region was absent in this acetate.

Methylation of the Glucomannan Acetate.

The acetate (6.0 g) was dissolved in acetone (100 ml) and stirred at 50°C for one hour under nitrogen stream. To a solution of acetate, 30% sodium hydroxide (250 ml) and dimethylsulfate (100 ml) were added in ten portions with vigorous stirring over a period of one hour at 50°C. The reaction was allowed to continue for another hour at this temperature and then at 80°C for thirty minutes. After cooling the suspension of the partly methylated glucomannan was neutralized with 6 N-sulfuric acid and the precipitate separated by decantation. The liquid was through-

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11) J.G. Kirchner, J.M. Miller and G.J. Keller, *Anal. Chem.*, **23**, 420 (1951).

14) T.E. Timell, *TAPPI*, **43**, 844 (1960).

12) S.K. Banerjee and T.E. Timell, *TAPPI*, **43**, 849 (1960).

13) C.P.J. Glaudemans and T.E. Timell, *Svensk Papperstidn.*, **60**, 869 (1957).

ly extracted with chloroform and then added to 2.5 volumes of methanol to remove sodium sulfate produced. The filtered clear solution was evaporated in vacuo to a sirup which was combined with the precipitate of the methylated glucomannan remaining in the original flask. The chloroform extract was evaporated to dryness and the acetone solution of this residue was also combined with the precipitate; and the second methylation was carried out as before. Five more methylation was performed in the same manner. After the final methylation the solution and the precipitate were extracted with chloroform, which was dried with anhydrous sodium sulfate and evaporated; yield, 5.1 g; OMe, 36.3%. The methylated product (5.0 g) was treated with methyl iodide (100 g), silver oxide⁸⁾ (5 g), and anhydrous calcium sulfate (5 g) under reflux with stirring for fifteen hours. The product, recovered by extraction with chloroform, was dissolved in methyl iodide and subjected to the second Purdie methylation as before; yield, 5.0 g.

Fractionation of the Methylated Glucomannan.

The methylated glucomannan (4.9 g) was dissolved in chloroform (200 ml) and the following fractions obtained by adding petroleum ether. The precipitate of each fraction was dissolved in chloroform and recovered by pouring into a large amount of petroleum ether. The results of the fractionation were:

Fraction	(1)	(2)	(3)	(4)	(5)	(6)
$[\alpha]_D^{20}$ in CHCl_3	—	-19.6°	-19.8	-19.5	-19.5	-2.8
OMe, %	—	44.8	45.2	44.8	39.0	—
Yield, g.	0.623	0.944	0.718	0.287	0.038	0.936
Total petroleum ether added, ml	1000	1000 ^a	2070	2770	b	c

a) Precipitated after standing overnight.

b) Obtained by evaporation of the supernatant from fraction 4 to 50 ml and pouring it into 10 volumes of petroleum ether.

c) Recovered by evaporation of the supernatant from fraction 5.

Fraction 2, 3 and 4 were combined and an intrinsic viscosity of the chloroform solution was measured at $20 \pm 0.01^\circ$ using Ostward viscometer, $[\eta]$ 0.475 dl/g.

Hydrolysis of the Methylated Glucomannan.

The methylated glucomannan (1.39 g, fraction 2, 3 and 4) was dissolved in 5% methanolic hydrogen chloride (100 ml) and heated at 100°C for ten hours, $[\alpha]_D^{20} + 63 \pm 1^\circ$. The solution was concentrated under reduced pressure to a sirup and after addition of

water and boiling for four hours, again concentrated. The treatment was repeated once more and then the solution neutralized with silver carbonate and deionized with Amberlite IR-120 (H). It was evaporated to a sirup (1.43 g), which showed $[\alpha]_D^{20} + 19.3$ (c, 1.2, chloroform).

Quantitative Examination of the Mixture of Methylated Sugars by "Chromato-strip".

Paper chromatography using ethylacetate-pyridine-water 10:4:3 showed the presence of four components with R_F values of 0.87, 0.84, 0.79 and 0.47 respectively in the mixture. For the quantitative analysis, the methylated sugar mixture was separated on the thin layer (1~1.5 mm) of silica gel fixed on glass plate¹¹⁾ (25 × 240 mm) with butanone-water (89:11) as the solvent. After three steps of developments, separation of tetra- tri-, and di-O-methyl sugars from each other was complete. Three strips were combined and, from the silica gel mixture, tetra-O-methyl sugars were eluted by chloroform and the others by butanone-water (89:11) separately. They were evaporated to dryness and oxidized with alkaline hypoiodite¹⁰⁾. One milliliter of 0.1 N-iodine was used for the tri- and 0.3 ml for the tetra- and di-methyl fractions: oxidation was allowed to proceed for twenty hours and after acidification the excess of iodine was titrated with 0.01 N-sodium thiosulfate. The results are:

Methylated sugar	Consumption of 0.01 N- I_2 , ml.			Corresponding mol. ratio		
	Tetra-	Tri-	Di-	Tetra-	Tri-	Di-
1st.	0.20	7.30	0.87	1	37	4
2nd.	0.22	7.40	0.46	1	34	2

This indicates that the glucomannan molecule has one non-reducing end group for every 34 to 37 hexose units. The amount of di-O-methylhexoses varies from 2 to 10% of the methylated glucomannan. It is thought, however, from the results of periodate oxidation of the glucomannan that di-O-methylhexoses probably arise from demethylation during hydrolysis.

Isolation of Methylated Sugar.

A portion (1.20 g) of the methylated sugar sirup was separated on a cellulose column (3.7 × 41 cm) with butanone-water (89:11)¹⁵⁾ as a solvent and using an automatic fraction collector. Collection time

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for each fraction of 10 ml was ten minutes. The first fraction (24 mg) with R_F of 0.87 and $[\alpha]_D^{18} + 73.0$ (c, 0.4, water) was tetra-O-methyl sugar. The *N*-phenylglycosylamine of the sugar, however, failed to crystallize from the ether-petroleum ether solution. A portion of the tetra-O-methyl sugar was dissolved in 48% hydrobromic acid (sealed tube)¹⁶ and after heating for five minutes in boiling water-bath, the solution was neutralized with silver oxide, deionized with Amberlite IR-120 (H), concentrated and paper-chromatographed as above. Glucose and trace of mannose were detected with partly demethylated products. The rotation of this fraction indicated that it contained 87 parts of 2,3,4,6-tetra-O-methyl-D-glucose, $[\alpha]_D + 84^\circ$ (water)¹⁷ and 13 parts of 2,3,4,6-tetra-O-methyl-D-mannose, $[\alpha]_D + 2.4^\circ$ (water)¹⁸.

The second effluent sirup (235 mg) with R_F value of 0.84 showed an optical rotation of $[\alpha]_D^{18} + 47.2^\circ$ (c, 1.3, water). A portion of the sirup was dissolved in absolute methanol containing 1% hydrogen chloride⁹. The specific optical rotation changed as follows: $+51.8^\circ$ (initial value); $+45.4^\circ$ (after 2 hrs); $+32.2^\circ$ (after 4 hrs); -3.4° (after 18 hrs); -6.0° (after 21 hrs). After eighteen hours, the solution was neutralized with silver carbonate, filtered and concentrated to a sirup which was separated on a cellulose column with butanone-water (89:11) as the solvent. The first effluent was collected and the solvent removed by evaporation. This was hydrolyzed with 0.1 N-sulfuric acid, neutralized with barium carbonate and the deionized solution again evaporated in vacuo. After drying over phosphorus pentoxide, 2,3,6-tri-O-methyl-D-glucose 1,4-di-O-*p*-nitrobenzoate⁹ was prepared as described below. m.p. 185°C , $[\alpha]_D^{20} - 32^\circ$ (c, 1.0, chloroform). *Anal.* Found: N, 5.3. Calcd. for $\text{C}_{23}\text{H}_{24}\text{O}_{12}\text{N}_2$: N, 5.4%.

had an optical rotation of $[\alpha]_D^{20} + 27.7^\circ$ (c, 1.0, ethanol) and was 2,3,6-tri-O-methyl-D-mannose. When 25 mg of the sirup was heated with 120 mg of *p*-nitrobenzoyl chloride and 1.6 ml of dry pyridine at 75°C for thirty minutes and then allowed to stand overnight at room temperature, 1,4-di-O-*p*-nitrobenzoate⁹ was obtained. After recrystallization from methanol the product showed m.p. 186°C and $[\alpha]_D^{20} + 32^\circ$ (c, 0.7, chloroform). *Anal.* Found: N, 5.4. Calcd. for $\text{C}_{23}\text{H}_{24}\text{O}_{12}\text{N}_2$: N, 5.4%. From the optical rotation of the second and the third effluents it was shown that trimethyl fraction was composed of 4 parts of 2,3,6-tri-O-methyl-D-mannose and 1 part of 2,3,6-tri-O-methyl-D-glucose.

The last fraction (20 mg) with R_F value of 0.47 was dimethyl sugar. *Anal.* Found: OMe, 28.8. Calcd.: OMe, 29.8%.

Periodate Oxidation of Glucomannan.

The glucomannan used in this experiment was prepared from the purified triacetate showing an optical rotation of $[\alpha]_D^{18} - 30.7^\circ$ (c, 1.0, chloroform). A series of suspension of the glucomannan (30 mg) in water (2 ml) was allowed to stand overnight and the calculated amounts (10% excess) of sodium metaperiodate solution (0.163 M) was added to the suspension, which was shaken at room temperature until the solution of glucomannan was complete (forty-five minutes). They were placed in a refrigerator at 2°C in the dark. A portion (0.5 ml) was taken for analysis of periodate at intervals: to the solution 5 ml of phosphate buffer (pH, 7.4), and 2 ml of 10% potassium iodide were added and the solution titrated with 0.025 N-arsenous oxide. The residual oxidizing solution was treated with ethylene glycol (0.2 ml), allowed to remain at room temperature for one hour and titrated with 0.01 N-barium hydroxide using methyl red as an indicator. The results were as follows:

Time, hrs.	3	18	43	91	187	355	499	643
IO_4^- consumed, M/162 g	0.444	0.555	0.652	0.780	0.920	1.00	0.980	1.00
HCOOH liberated, M/162 g	0.038	0.049	0.060	0.072	0.083	0.090	0.104	0.114

The third effluent (664 mg) with R_F value of 0.79

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