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GLUCOMANNAN FROM THE FIBRE OF SUNN HEMP (*Crotalaria juncea* LINN)

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ABSTRACT

A glucomannan, isolated by fractionation of the alkali-soluble hemicelluloses of sunn-hemp fibre (*Crotalaria juncea* Linn), had $[\alpha]_D^{32}$ −45° and, on hydrolysis, yielded glucose, mannose, and xylose in the molar ratios 1.2:1:0.07. The methylated polysaccharide contained an average of ~45 hexose residues, whereas the corresponding value for the nitrate derivative was 49. Structural studies showed that the glucomannan has a main chain of (1→4)-linked β-D-glucopyranosyl and -mannopyranosyl residues. The molecule appears to have an average of one branch point, through position 6 of both glucose and mannose residues of the main chain. Both D-glucopyranosyl (87%) and D-mannopyranosyl (13%) residues were present as non-reducing end groups. D-Glucose and D-mannose occur in the chain both as alternate and contiguous units.

INTRODUCTION

Sunn-hemp fibre was obtained by retting the stem of the plant *Crotalaria juncea* Linn. Unlike jute[1] and other bast[2–4] and leaf fibres[5–7], sunn-hemp fibre contains more mannose than xylose. The structure of the glucomannan isolated as the major hemicellulose component of sunn-hemp fibre is now reported.

RESULTS AND DISCUSSION

Sunn-hemp fibre was cut into small pieces, extracted with alcohol–benzene, and then delignified by the acid–chlorite method[8]. The holocellulose was then extracted successively with 5% and 24% aqueous sodium hydroxide. The extracts were neutralised and alcohol was added to the extracts to precipitate hemicellulose fractions I (2.87%) and II (4.87%). Fraction I had 1.3% of OMe and 18.5% of uronic anhydride and, on acid hydrolysis, released galactose, glucose, mannose, xylose, arabinose, and rhamnose in the molar ratios 1:1.9:1.3:2.5:0.2:0.1. This material is being further investigated.

Fraction II, on acid hydrolysis, released galactose, glucose, mannose, xylose, and rhamnose, in the molar ratios 1:17.7:11.7:2.8:0.2, and also uronic acid. Further fractionation of fraction II six times with barium hydroxide[9] gave a glucomannan fraction (4%) which had $[\alpha]_D^{32}$ −45° (*c* 0.8, 10% sodium hydroxide) and, on acid hydrolysis, gave glucose, mannose, and xylose in the molar ratios of 1.2:1:0.07.

The glucomannan was nitrated with a mixture[10,11] of nitric acid, phosphoric acid, and phosphorus pentaoxide which is reported to cause no degradation. The molecular weight of the nitrated glucomannan, as determined by osmometry, was 15,600, which corresponds to a d.p. of 49.

The glucomannan was methylated successively by the methods of Haworth[12], Kuhn[13], and Purdie[14], and the product showed no i.r. absorption for hydroxyl groups. The methylated polysaccharide was fractionated by graded dissolution in boiling mixtures of chloroform and light petroleum (b.p. 60–80°) and gave the fractions shown in Table I. Fraction 4 consisted of methylated xylan and was not studied further. Fraction 2 was the main fraction and was studied in detail. The d.p. of fractions 2 and 3 were determined by osmometry.

TABLE I

FRACTIONATION OF METHYLATED GLUCOMANNAN

Fraction	*Chloroform–light petroleum*	*Yield* (%)	$[\alpha]_D^{32}$ *(degrees)*	*Mol. wt. (D.p.)*
1	0:100	32.5	−4.8	—
2	10:90	46.5	−19.4	9,900 (48)
3	20:80	17.7	−56.8	11,800 (58)
4	30:70	1.2	—	—

The methylated glucomannan (fraction 2) was hydrolysed first with methanolic hydrogen chloride and then with aqueous hydrochloric acid. Paper chromatography of the product mixture revealed tetra-*O*-methyl, tri-*O*-methyl, and di-*O*-methyl derivatives of glucose mixed with the corresponding methylated mannose derivatives. The proportions of the tetra-*O*-methyl-, tri-*O*-methyl-, and di-*O*-methyl-hexoses were determined by hypoiodite oxidation (Table II).

TABLE II

PROPORTION OF METHYLATED SUGARS

Component	*Weight* (*g*)	*Molar ratio*	*Molar ratio (column)*	*Molar ratio (hypoiodite oxidation)*
2,3,4,6-Tetra-*O*-methyl-D-glucose	0.105	3.7	2.1	2.2
2,3,4,6-Tetra-*O*-methyl-D-mannose	0.015	0.54		
2,3,6-Tri-*O*-methyl-D-glucose	1.167	44.0	41.5	47.0
2,3,6-Tri-*O*-methyl-D-mannose	1.033	39.0		
2,3-Di-*O*-methyl-D-glucose	0.025	1.0	1.0	1.0
2,3-Di-*O*-methyl-D-mannose	0.025	1.0		

The mixture of methylated sugars (2.3 g) was fractionated by elution from a cellulose column with butanone half saturated with water. 2,3,4,6-Tetra-*O*-methyl-D-mannose was obtained contaminated with 2,3,4,6-tetra-*O*-methyl-D-glucose; the proportions were estimated from the magnitude of the optical rotation of the mixture and they were identified as their anilides. 2,3,6-Tri-*O*-methyl-D-glucose and -D-mannose were identified as the 1,4-bis(*p*-nitrobenzoates). A part of the 2,3,6-tri-*O*-methyl-D-glucose and 2,3,6-tri-*O*-methyl-D-mannose was obtained as a mixture; the proportions were estimated from the magnitude of the optical rotation of the mixture, and also from the change in optical rotation when the mixture was treated with methanolic hydrogen chloride. A mixture of 2,3-di-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-mannose was also obtained, and this was fractionated by p.c. The proportions of the various methylated sugars are given in Table II.

The methylation data indicate an average d.p. of ~45, which is in good agreement with the d.p. of the nitrated glucomannan (49) and methylated glucomannan (48) determined by osmometry. The molar ratio of methylated glucose to methylated mannose is 1.2:1. Thus, the glucomannan consists of 25 glucopyranose and 20 mannopyranose residues linked through (1→4) positions by β-glycosidic bonds. The molecule appears to have an average of one branch through position 6 of both glucopyranose and mannopyranose residues of the main chain. The chains were variously terminated at the non-reducing end by both the hexose residues, 87% of which were D-glucopyranosyl units.

The results of periodate oxidation corroborate the simplified structure given above. The glucomannan reduced 1.08 mol. of periodate per hexose residue, with the liberation of 1 molecule of formic acid per 22 hexose residues. Acid hydrolysis of the reduced oxopolysaccharide released mainly erythritol together with a small proportion of glycerol. Intact hexose was not detected in the hydrolysate.

Hydrolysis of fractions 1 and 3 of the methylated hemicellulose (Table I), with subsequent chromatography, revealed the formation of types of sugars similar to those obtained from fraction 2. The proportions of the di-*O*-methyl-, tri-*O*-methyl-, and tetra-*O*-methyl-hexoses, as estimated by hypoiodite oxidation, were 1:11.6:1.3 and 1:20:1.5, respectively. Thus, fractions 1–3 are similar in nature, but differ in the d.p. and in the presence of a different number of branch-points in the molecule.

Partial hydrolysis of the glucomannan with aqueous formic acid[15] gave a mixture of mono- and oligo-saccharides, which was fractionated on charcoal–Celite by gradient elution with aqueous ethanol. The following oligosaccharides were isolated: 4-*O*-β-D-mannopyranosyl-D-mannose, cellobiose, 4-*O*-β-D-glucopyranosyl-D-mannose, 4-*O*-β-D-mannopyranosyl-D-glucose, *O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-glucopyranose, *O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-D-mannopyranose. The isolation of these oligosaccharides shows that glucose and mannose are present both as alternate and contiguous units in the chain.

The sunn-hemp fibre, like soft woods[16], contains a glucomannan as the main hemicellulose component. But the glucomannan in this case contains more glucose

than mannose (1.2:1) and, considering the nature of the products of partial hydrolysis and the non-reducing end groups, the structure of the glucomannan is more akin to that of hardwood glucomannan[17]. Glucomannans have been obtained from Western hemlock[18] and Western red alder[19] having glucose to mannose ratios of 2:1 and 1:0.9, respectively. The sunn-hemp glucomannan is branched in a manner similar to that of the glucomannan from Eastern hemlock[20], Scots pine[21], and Norway spruce[22].

EXPERIMENTAL

P.c. was carried out on Whatman Nos. 1 and 3 MM papers, using *A* 1-butanol–ethanol–water (4:1:5, upper layer), *B* butyl acetate–pyridine–ethanol–water (8:2:2:1), *C* butanone–water azeotrope, *D* butanone two thirds saturated with water, *E* ethyl acetate–pyridine–water (10:4:3), and detection with alkaline silver nitrate and aniline oxalate. Concentration was conducted under diminished pressure at 40–45°. Melting points are uncorrected. Methylated sugars were demethylated with hydrobromic acid. Polysaccharides were hydrolysed by the method of Jeffery *et al.*[23]. Hydrolysates were neutralised with barium carbonate, and the sugars in the hydrolysate were estimated by the Somogyi[24] method.

Sunn-hemp fibre. — Sunn-hemp fibre was cut into small pieces, exhaustively extracted with ethanol–benzene (1:2), and then dried in air. The following data were obtained by standard analytical procedures: α-cellulose, 78.3%; lignin, 4.0%; pentosan, 3.6%; ash, 0.3%; acetyl, 1.5%; uronic anhydride, 1.7%; galactose, 2.4%; glucose, 78.5%; mannose, 7.0%; arabinose, 1.0%; xylose, 2.0%; and rhamnose, 0.4%.

Isolation of the hemicellulose fractions. — Extractive free fibre was delignified by treatment with sodium chlorite (0.5%) in acid medium[8]. The resulting holocellulose (900 g, 91.3%) was treated in an atmosphere of nitrogen with 5% aqueous sodium hydroxide (8 l) at room temperature for 2 h to give hemicellulose fraction I (25.8 g). Further treatment of the residue for 36 h with 24% sodium hydroxide (8 l) gave hemicellulose fraction II (43.8 g), which was the source of the glucomannan. Acid hydrolysis of fraction II yielded galactose, glucose, mannose, xylose, and rhamnose in the molar ratios 1:17.7:11.7:2.8:0.2.

Purification of the glucomannan. — The crude glucomannan (30 g) was purified by treatment[9] with barium hydroxide to give material (24.6 g) having $[\alpha]_D^{32}$ −45° (*c* 0.8, 10% sodium hydroxide). Hydrolysis of the glucomannan, followed by p.c. (solvent *B*), gave glucose, mannose, and xylose in the molar ratios 1.2:1:0.07.

Methylation of the glucomman. — The glucomannan (11.2 g) was methylated successively by the methods of Haworth[12], Kuhn[13], and Purdie[14], to give the methylated glucomannan (OMe, 45.5%) which showed no i.r. absorption for hydroxyl. The product (7.5 g) was fractionated by graded dissolution in a boiling mixture of chloroform and light petroleum (b.p. 60–80°) to give the four fractions shown in Table I.

Hydrolysis of the methylated glucomannan. — The methylated glucomannan (2.5 g) was boiled under reflux for 12 h with 3% methanolic hydrogen chloride (250 ml), and the resulting glycosides were hydrolysed with 0.5M hydrochloric acid

(350 ml) for 14 h. The hydrolysate was neutralised with silver carbonate, and the silver ions were removed as the sulphide. Concentration of the filtrate gave a yellow syrup (2.3 g) which p.c. (solvent *C*) indicated to contain di-, tri-, and tetra-*O*-methyl derivatives of glucose and mannose.

The hydrolysate (2.3 g) was eluted with solvent *D* from a column (45 × 4 cm) of cellulose to give six fractions.

Fraction A was a syrup (93 mg) which contained 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-mannose. Demethylation gave glucose and mannose. Using $[\alpha]_D$ values of +84° and +2.5° for 2,3,4,6-tetra-*O*-methyl-D-glucose and -D-mannose, respectively, the optical rotation of the syrup, $[\alpha]_D^{28}$ +71° (*c* 0.5, water), indicated the presence of 84% of 2,3,4,6-tetra-*O*-methyl-D-glucose. The 2,3,4,6-tetra-*O*-methyl-D-glucose and -D-mannose were separated and identified as their anilides[18], m.p. 134–136° and 142–143°, respectively.

Fraction B (27 mg) crystallized on storage and, when recrystallized from light petroleum, gave 2,3,4,6-tetra-*O*-methyl-D-glucose, m.p. 85–87°, $[\alpha]_D^{28}$ +84° (*c* 1.22, water). Demethylation gave glucose.

Fraction C (310 mg) was recrystallized from ethyl ether–light petroleum (1:1) to give 2,3,6-tri-*O*-methyl-D-glucose, m.p. 121°, $[\alpha]_D^{28}$ +69° (*c* 1.5, water) The 1,4-bis(*p*-nitrobenzoate) had m.p. 188–189°. Demethylation gave glucose.

Fraction D (1.52 g) was a mixture of 2,3,6-tri-*O*-methyl-D-glucose and 2,3,6-tri-*O*-methyl-D-mannose. Demethylation gave glucose and mannose. Using $[\alpha]_D$ values of +70° and −10° for 2,3,6-tri-*O*-methyl-D-glucose and -D-mannose, respectively, the optical rotation of the mixture, $[\alpha]_D^{30}$ +35° (*c* 1, water), indicated the presence of 56.3% of 2,3,6-tri-*O*-methyl-D-glucose. The change in optical rotation of the mixture in methanolic 3% hydrogen chloride, $[\alpha]_D^{30}$ +36.7 → −21.7° (*c* 1.77), indicated the presence of 56.8% of 2,3,6-tri-*O*-methyl-D-glucose.

Fraction E (367 mg), $[\alpha]_D^{28}$ −4.6° (*c* 1.5, water), was 2,3,6-tri-*O*-methyl-D-mannose. Demethylation gave mannose. The 1,4-bis(*p*-nitrobenzoate) had m.p. 186–187°.

Fraction F (50 mg), $[\alpha]_D^{30}$ +16.3° (*c* 0.5, water), was a mixture of 2,3-di-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-mannose. Demethylation gave glucose and mannose. P. c. of the mixture (solvent *C*) showed nearly equal amounts of the two sugars which were identified by comparison with authentic samples.

Determination of molar proportions of the methylated sugars. — Methylated glucomannan (150 mg) was hydrolysed with 1.2% methanolic hydrogen chloride (5 ml) in a sealed tube at 100° for 21 h and then with 0.5M hydrochloric acid (10 ml) on a boiling water-bath for 7 h. The products were fractionated by p.c. (solvent *C*) into three spots corresponding to tetra-, tri-, and di-*O*-methylhexoses. The sugars were eluted from the paper with water and estimated with alkaline hypoiodite solution[25]. The proportions of tetra-, tri-, and di-*O*-methylhexose were 2.2:47:1.

Similar treatment of methylated fractions 1 and 3 (Table I) gave the corresponding ratios 1.3:11.6:1 and 1.5:20:1, respectively.

Partial hydrolysis of the glucomman. — The glucomannan (6 g) was hydrolysed

with aqueous formic acid[15] to give a mixture of monosaccharides and oligosaccharides. A solution of the mixture (4.64 g) in water (85 ml) was added to a column (50 × 6.5 cm) of charcoal–Celite. Elution with water yielded a mixture of glucose, mannose, and xylose which was not examined further. Elution with a water–ethanol gradient gave the following oligosaccharides which were further purified, if necessary, by p.c. (solvents *B* and *E*).

4-*O*-β-D-Mannopyranosyl-D-mannose (120 mg), m.p. 198–199° (from methanol), $[\alpha]_D^{30}$ −8° (*c* 1.6, water). Hydrolysis gave only mannose.

4-*O*-β-D-Glucopyranosyl-D-mannose (210 mg), m.p. 137–138°, $[\alpha]_D^{30}$ +13 → 6° (*c* 1, water). Hydrolysis gave equal amounts of glucose and mannose. Hydrolysis, after reduction with sodium borohydride, gave only glucose.

4-*O*-β-D-Mannopyranosyl-D-glucose (174 mg), $[\alpha]_D^{30}$ +22° (*c* 0.5, water), gave equal amounts of mannose and glucose on hydrolysis. After reduction with sodium borohydride, hydrolysis gave only mannose.

4-*O*-β-D-Glucopyranosyl-D-glucose (84 mg), m.p. 225–226° (from aqueous methanol), $[\alpha]_D^{30}$ +18 → +35° (*c* 0.4, water). Hydrolysis gave only glucose.

O-β-D-Glucopyranosyl-(1 → 4)-*O*-β-D-mannopyranosyl-(1 → 4)-D-glucose (103 mg) gave glucose and mannose in the ratio of 2:1 on hydrolysis. After reduction with sodium borohydride, hydrolysis gave equal amounts of glucose and mannose. The optical rotation, $[\alpha]_D^{30}$ −8° (*c* 0.6, water), is different from that (+15°) of trisaccharide *O*-β-D-Man-(1→4)-*O*-β-D-Glc-(1→4)-D-Glc from the European-larch glucomannan[26].

O-β-D-Glucopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1→4)-D-mannose (118 mg), m.p. 209–210°, $[\alpha]_D^{30}$ −24.5° (*c* 1.1, water), yielded glucose and mannose in the ratio of 3:1 on hydrolysis. After reduction with sodium borohydride, hydrolysis gave only glucose. Partial hydrolysis with 0.25M sulphuric acid gave cellobiose, glucose, and mannose. The d.p. was estimated[27] to be 4.3.

Periodate oxidation of the glucomannan. — The glucomannan (900 mg) was oxidised in the dark with 100 ml of 0.1M sodium metaperiodate. The consumption of periodate was measured by the excess arsenite method. Extrapolation to zero time indicated a consumption of 1.08 mol. of periodate per hexose residue.

The glucomannan (740 mg) was oxidised with periodate (100 ml) according to the method of Halsall *et al.*[28], and the formic acid liberated was monitored with 0.01M sodium hydroxide, using Methyl Red as indicator. Extrapolation to zero time indicated the liberation of 0.045 mol. of formic acid per hexose residue.

The glucomannan (220 mg) was oxidised with 0.1M sodium metaperiodate (100 ml) for 10 days. Excess of periodate and iodate ions were precipitated with barium hydroxide, the solution was filtered, the oxopolysaccharide was reduced with sodium borohydride[29], and the polyalcohol was hydrolysed. P.c. (solvent *A*) revealed erythritol as the main component together with a small proportion of glycerol. No intact hexose was detected in the hydrolysate.

Nitration[11] *of the glucomannan.* — The dry glucomannan (1 g) was added in

small portions to a nitration mixture[10] (100 ml) at 0° composed of nitric acid, phosphoric acid, and phosphorus pentaoxide (64:26:10). The nitrogen content of the product, estimated according to the procedure of Timell[30], was 14.0%.

Determination of number-average molecular weight. — The number-average molecular weight of the nitrated glucommannan was determined by measuring the osmotic pressure of its solution in butyl acetate at 35 ±0.01°. An osmometer as described by Hellfritz[31] was employed together with a regenerated cellulose membrane SM 11536 (Sartorius Membranfilter GmbH, Gottingen). The membrane was conditioned to butyl acetate according to the procedure of Wagner[32]. Measurement was made by the static method, and equilibrium was reached within 6–8 h; $\Delta h/C$ was plotted against C, where Δh is the osmotic height in cm and C is the concentration (g/l) of the solution. The molecular weight was calculated from the equation

$$M_n = 29{,}600 \Big/ \left(\frac{\Delta h}{C}\right)_{C=0}.$$

The molecular weight of the nitrated glucomannan was estimated to be 15,600. The molecular weights of methylated fractions 2 and 3 in butyl acetate were 9,900 and 11,800, respectively.

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