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Preparation of mannodextrins and their separation by gel chromatography

The preparation of the straight-chain mannodextrins, *i.e.*, linear polymers of β-(1→4)-linked D-mannopyranose units, is described. Mannose occurs most commonly as a constituent of the complex polysaccharides of plant cell walls, for example, the galactoglucomannans in softwoods and the glucomannans in hardwoods. Ivory nut mannan is a hemicellulose made up of mannose as the sole constituent, in which the chains are devoid of branch points. Branched chains built up of mannose units form a constituent of the cell walls of many yeasts.

The mannodextrins were required for investigations of the properties of tightly cross-linked networks. The separations of the mannodextrins on dextran and polyacrylamide gels are compared with those of the corresponding straight-chain cellodextrins[1] and xylodextrins[2].

Experimental

Hydrolysis of mannan. Ivory nut mannan* (12 g) was added in small portions under stirring to 750 ml of fuming hydrochloric acid at 0°. The latter was prepared by passing hydrogen chloride gas for 2 h through the concentrated acid cooled in an ice-water bath. The mannan dissolved slowly (*ca.* 30 min). After dissolution, the solution was allowed to warm up to room temperature (17°) and the hydrolysis allowed to proceed for 45 min. The hydrolysis was stopped by adding the mixture to 1.2 kg of sodium bicarbonate in 1 kg of ice. A few milliliters of *n*-octanol were added to reduce frothing on stirring. The final pH was *ca.* 6. The mixture was filtered after standing overnight and the solution applied directly to the active carbon–Celite column.

A second hydrolysis was carried out as above, but with a hydrolysis time of 2 h at room temperature.

Fractionation of mannan hydrolysate. The fractionation technique was as previously described[2]. The dimensions of the charcoal–Celite column were 19.6 cm^2 × 80 cm (settled bed dimensions).

Following introduction of the hydrolysate, the column was washed with 20 l of distilled water to remove salts and mannose. The oligosaccharides were obtained by gradient elution using 10 l of deionized water in one container connected through a siphon to an equal volume of 50% aq. ethanol in a second container. A hydrostatic head of 2.5 m was applied. Aliquots (0.1 ml) of every 10th fraction were analyzed for carbohydrate content using the orcinol reagent (3 ml of 0.2% orcinol in 70% sulphuric acid).

The fractions were also analyzed by thin-layer chromatography (TLC) (see below). The fractions were pooled according to these results and each pool concentrated to about 200 ml in a rotary evaporator at 45°. The residues were freeze-dried.

The first hydrolysis gave only mannotetraose (M4) to mannoheptaose (M7). Either the hydrolysis was insufficient or alternatively the lower oligomers had not adsorbed on the column packing. The second hydrolysis was thus carried out for the extended period and, following introduction of the hydrolysate on to the column,

* Kindly donated by Professor TORE TIMELL, State University College of Forestry, Syracuse, N.Y., U.S.A.

the latter was not washed with water to remove salts but all eluent was collected instead. Mannose to mannotriose eluted with the bulk of the salt, although even the higher oligomers were salt-contaminated.

It was found that use of a 1% stearic acid solution to partially deactivate the active carbon–Celite mixture (instead of the 2.5% solution employed) gave good fractionation of M1 to M7, following removal of salts by exhaustive elution with water. Corresponding fractions from both hydrolyses were consequently combined and refractionated on this column.

Thin-layer chromatography. Kieselguhr F_{254} plates (Merck, Darmstadt, G.F.R.) were used. The developing solvent system was: isopropanol–ethyl acetate–water (42:35:23). The spray detection liquid was: 100 ml of acetic acid containing 2 ml of conc. sulphuric acid and 1 ml of anisaldehyde.

The cellodextrins and xylodextrins were run in parallel with the mannodextrins. Values of the migration parameter, R_F, are given in Table I. Fig. 1 shows the function $\log (1 - R_F)/R_F$ plotted *versus* molecular weight.

Gel chromatography. The preparation of columns and the detection set-up employed have been previously described[1,2].

TABLE I

VALUES OF THE MIGRATION PARAMETER R_F IN TLC[a]

Mannodextrins	R_F	*Cellodextrins*	R_F	*Xylodextrins*	R_F
M1	0.66_8	G1	0.63_6	X1	0.75_0
M2	0.44_1	G2	0.48_0	X4	0.29_2
M3	0.25_1	G3	0.33_6	X5	0.15_8
M4	0.12_4	G4	0.20_4	X6	0.08_7
M5	0.05_0	G5	0.12_0	X7	0.04_4
M6	0.01_9	G6	0.06_7	X8	0.02_2

[a] Kieselguhr, F_{254} (Merck). Development: isopropanol–ethyl acetate–water (42:35:23).

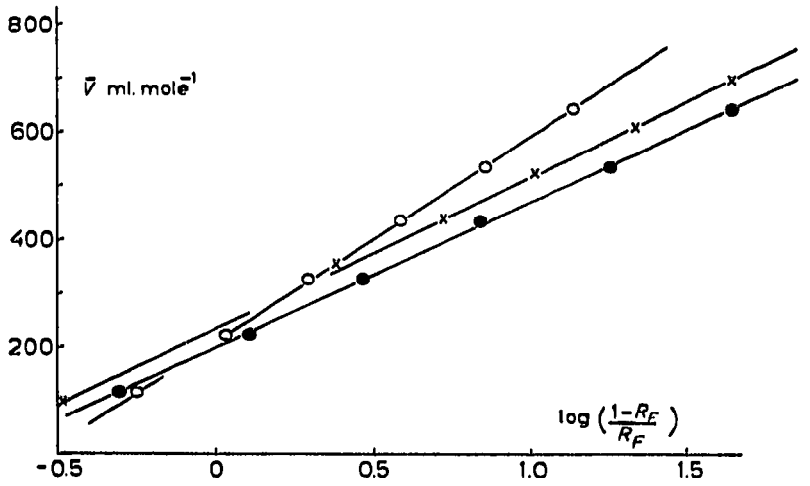

Fig. 1. TLC data: relationship between the partial molar volume and $\log \left(\frac{1-R_F}{R_F}\right)$ for mannodextrins (●), cellodextrins (○) and xylodextrins (×).

Results and discussion

Dextran gel. Elution volumes and partition coefficients for the mannodextrins, M_1 to M_7, obtained with Sephadex G-15 and 0.1 *M* sodium chloride as eluant, are included in Table II.

TABLE IIa

GEL CHROMATOGRAPHY DATA FOR MANNODEXTRINS (0.1 *M* NaCl)

Mannodextrins	*Polyacrylamide P-2*		*Sephadex G-15*	
	V_e[a]	K_{av}	V_e[b]	K_{av}
M1	42.7$_0$	0.80$_2$	35.2$_0$	0.53$_7$
M2	39.4$_0$	0.68$_0$	31.2$_5$	0.40$_1$
M3	37.0$_0$	0.60$_6$	28.3$_5$	0.30$_1$
M4	33.9$_0$	0.51$_8$	26.0	0.21$_0$
M5	32.2$_0$	0.45$_8$	24.2$_5$	0.15$_8$
M6	31.1$_0$	0.40$_1$	23.2$_5$	0.12$_0$
M7	29.3$_0$	0.33$_8$	22.1$_5$	0.08$_6$

[a] $V_0 = 19.5_4$; $V_T = 48.4_0$.
[b] $V_0 = 19.7_0$; $V_T = 48.5_0$.

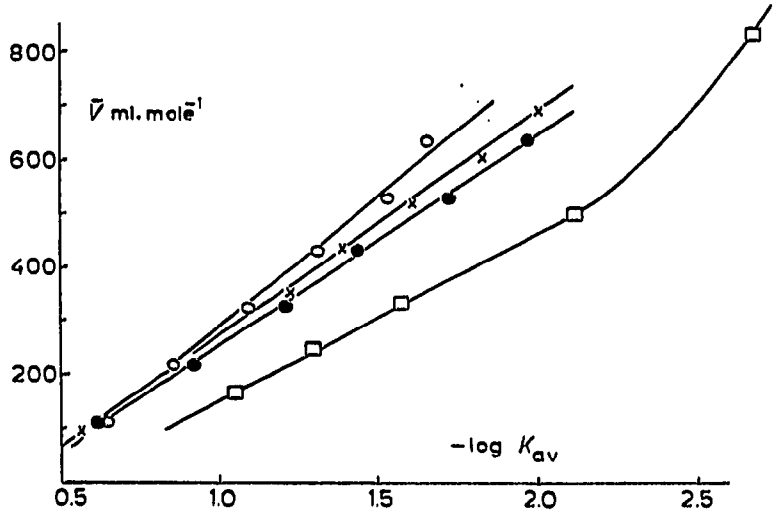

Fig. 2. Dextran gel chromatography: relationship between the partial molar volume and −log K_{av} for mannodextrins (●—●), cellodextrins (○—○), xylodextrins (×—×) and polyethylene oxides (□—□). Sephadex G-15; 0.1 *M* NaCl.

Fig. 2 shows a plot of −log K_{av} as a function of molar volume, with data included for the cellodextrins and xylodextrins. Each type of oligosaccharide separates characteristically, the differences in the partition coefficient for oligomers of a given chain length reflecting the relative affinities of the solute for the gel interface and the solvent, respectively. The partitioning is then found to bear an inverse relationship to the liquid–liquid partition coefficient in TLC. Thus a plot of the TLC data *versus* gel chromatography data results in a common line for the different oligosaccharides (Fig. 3).

Polyacrylamide gel. Data for the mannodextrins on polyacrylamide P-2 with 0.1 *M* sodium chloride as eluant are given in Table II. Fig. 4 shows plots of −log K_{av}

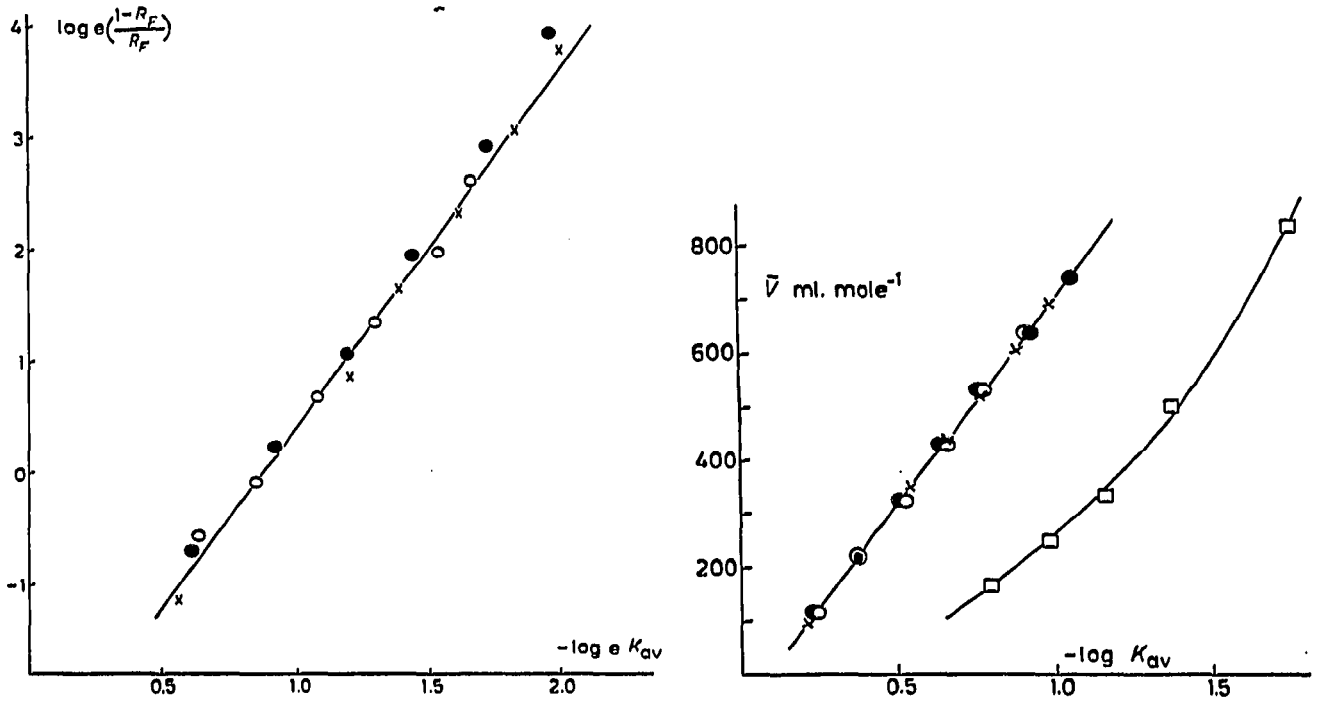

Fig. 3. Correlation between TLC data and dextran gel chromatography data (Sephadex G-15) for mannodextrins (●—●), cellodextrins (○—○) and xylodextrins (×—×).

Fig. 4. Polyacrylamide gel chromatography: relationship between the partial molar volume and $-\log K_{av}$ for mannodextrins (●—●), cellodextrins (○—○), xylodextrins (×—×) and polyethylene oxides (□—□). Polyacrylamide P-2; 0.1 *M* NaCl.

TABLE IIb

PARTITION COEFFICIENTS FOR CELLODEXTRINS AND XYLODEXTRINS

Oligo saccharide	*Polyacrylamide P-2* K_{av}	*Sephadex G-15* K_{av}
G1	0.78_8	0.52_8
G2	0.68_8	0.42_7
G3	0.59_3	0.33_6
G4	0.52_2	0.27_2
G5	0.46_0	0.21_5
G6	0.42_1	0.18_0
X1	0.81_4	0.57_0
X4	0.58_5	0.29_7
X5	0.51_8	0.24_0
X6	0.47_0	0.19_8
X7	0.42_0	0.15_0
X8	0.37_0	0.13_4

as a function of molar volume for the various oligomers. Thus $-\log K_{av}$ is a linear function of the partial molar volume and there is a common relationship for the different oligosaccharides. Nevertheless, there are substantial solute–gel interactions in this system as shown by their retardation compared to the polyethylene oxides. The importance of gel–solute interactions is demonstrated by the finding that with a hydrophobic gel(polystyrene) the polyethylene oxides elute considerably later than the oligosaccharides[4].

It would appear that the specific interactions of oligosaccharides with the dextran gel surface (and, similarly, with hydroxyethylcellulose and cellulose gel surfaces) is related to the relative abilities of the solutes to couple with the hydroxyl groups at the surface. The separations in TLC are, as noted, the inverse of those in dextran gel chromatography, *i.e.* on the basis of their partial molar volumes the mannodextrins are the most hydrophilic and elute first in dextran gel chromatography. The TLC separations should consequently reflect true liquid–liquid partitioning based on solubility behaviour rather than the relative affinities of the solutes for the Kieselguhr surface. The cellodextrins being least hydrophilic have the strongest interactions with the gel surface. When compared on a chain-length basis the xylodextrins are the least hydrophilic, however, and are the most retarded in gel chromatography. The partition coefficients are considered to be most meaningfully expressed as functions of the partial molar volume, however. The reversal of the separations of the oligomers in comparison with the monomers is noteworthy. This is presumably because intramolecular hydrogen bonding involving the C2 hydroxyl groups is more favoured in the oligomeric cellodextrins, resulting in substantially decreased solubility in the series in comparison with the mannodextrins. It is relevant that the temperature dependence of the solute–gel interactions is reversed with the dextran and polyacrylamide gels[3]. The interactions increase with increasing temperature for the dextran gel but decrease with increasing temperature for the polyacrylamide gel. This is a direct consequence of the reversed solvent–gel interactions in the two cases. It has also been observed[4] that with deionized water as eluent the polyacrylamide–solute interactions increase, whereas the opposite applies for the dextran gel. With salt present the amount of physically bound water at the dextran gel surface should be reduced and the activity of the surface hydroxyl groups thereby be increased. With polyacrylamide, on the other hand, the activity of the amide group as an adsorption site is presumably reduced in the presence of salt.

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