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Structural and solution properties of corn cob heteroxylans

A. Ebringerová,* Z. Hromádková, J. Alföldi

Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Czechoslovakia

&

G. Berth

Central Institute of Nutrition, 1505 Potsdam-Rehbrücke, Germany

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The main structural features of the L-arabino-(4-O-methyl-D-glucurono)-D-xylan (AGX) from corn cobs were evaluated by means of methylation analysis and ^{13}C-NMR spectroscopy. The heteroxylan consisted of water-soluble and water-insoluble xylan molecules differing in the amount and proportion of non-regularly distributed side chains. Single α-(1 → 2)-linked 4-O-methyl-D-glucopyranosyl uronic acid units and α-(1 → 3)-linked L-arabinofuranose residues, as well as 2-O-β-D-xylopyranosyl-α-L-arabinofuranose moieties constituted the side chains. GPC analysis of the water-soluble fractions coupled with viscometry and light-scattering was used to characterise the molecular weight distribution (MWD) and to establish the Mark-Houwink relationship. The flow properties of aqueous dispersions of the water-soluble and water-insoluble fractions were discussed in relation to their structural properties.

INTRODUCTION

The heteroxylan of corn cobs, owing to its high abundance (Whistler, 1950; Wilkie, 1979) is of particular interest, not only as a pentose-rich resource (Reilly, 1981; Kusakabe *et al.*, 1983), but also as a biopolymer with potential technical applications in the pharmaceutical, textile printing and pulp and paper industries (Náterová *et al.*, 1986; Bartoš *et al.*, 1990; Lichnerová *et al.*, 1991).

Pursuing an interest in the relationship between structure and properties we decided to study the structure and solution properties of the corn cob heteroxylan, which in contrast to earlier research (Aspinall, 1959) was prepared without the sodium chlorite delignification step. This paper reports the results with emphasis on the water-soluble fraction.

*To whom correspondence should be addressed.

MATERIALS AND METHODS

The heteroxylan was produced from milled corn cobs at a pilot plant of the Institute of Chemistry (Bratislava) according to the method of Ebringerová *et al.* (1988), using extraction with 5% aqueous sodium hydroxide at 60°C. The crude polysaccharide (AGX) was isolated from the alkaline extract by ethanol precipitation. During dialysis of the aqueous dispersion of AGX, an insoluble portion (water-insoluble AGX) settled down and was separated by centrifugation. From the supernatant, the soluble fraction (water-soluble AGX) was obtained by freeze drying. A part of the alkaline corn cob extract was treated with sodium hypochlorite (Ebringerová *et al.*, 1988). The subsequent dialysis of the ethanol-precipitated polysaccharide yielded the water-soluble fraction (AGX-H), dried by lyophilisation. Dextran T 2000 and dextran sulphate were obtained from Pharmacia (Sweden).

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The methods for chemical and physico-chemical analysis were described in previous papers (Hromádková *et al.*, 1987; Odonmažig *et al.*, 1990). The ^{13}C-NMR spectra (75·47 MHz) were measured at 40°C on the Bruker AM-300 Spectrometer in the inverse gated decoupling mode. The dried polysaccharides (100–200 mg/ml) were dissolved in DMSO-d_6 and/or D_2O using methanol (δ 50·15 ppm of Me_4Si) as internal standard. The methylation analysis of AGX was performed with a slightly modified Ciucanu method (Ciucanu & Kerek, 1984). Osmometry was carried out on the membrane osmometer (Knauer, Germany).

GPC was performed on two coupled columns Sepharose 2B and Sepharose 4B (together approximately 380 ml, ascendent direction, flow rate about 10 ml/h). As solvent and eluent 0·037 M phosphate buffer, pH 6·5, with an addition of 1 mMol/l Na_2 EDTA was used. 15 ml sample volume were injected for each run. The loading concentration was 2 mg/ml for AGX-H or less (water-soluble AGX). The concentration was monitored by means of a differential refractometer (Knauer, Germany) calibrated with glucose and dextran, respectively. Fractions (10 ml) were collected to determine the intrinsic viscosity (η) at 25·00 ± 0·01°C (Viscomatic Fica, France) and the molecular weight M_w by light scattering (Sofica, Fica, France) (using $\delta n/\delta c$ = 0·15 ml/g). More details of light scattering measurements are given elsewhere (Berth *et al.*, 1990). AGX-H dissolved rapidly and quantitatively at ambient temperature, whereas the water-soluble AGX gave a distinct insoluble portion after heating at 60°C for 1 h, which was removed by centrifugation. The reported GPC data for water-soluble AGX concern only the soluble portion.

The flow curves were obtained using the rheoviscometer Rheotest 2 (2–50 Hz, VEB MLW Prüfgerate Medingen, Germany). The dispersions were prepared at ambient temperature using lyophilised samples which were swollen in distilled water for 1 h and subsequently dispergated under thorough stirring for 1 h. Then they were left to rest for 24 h at 10°C. The shear rates were changed stepwise and applied until a constant shear stress value was obtained. Apparent viscosities (η_a) of the aqueous dispersions were calculated according to the equation $\eta_a = \tau/D$ and were obtained at constant shear rate D 145/s (20°C).

RESULTS AND DISCUSSION

The crude L-arabino-(4-O-methyl-D-glucurono)-D-xylan (AGX) consists of a mixture of water soluble and water insoluble polymers differing mainly in the content of arabinose and uronic acid (Table 1). Low amounts of protein and phenolic substances were found in AGX and its fractions. The water-soluble fraction (AGX-H) obtained after alkaline hypochlorite treatment was free of protein and Klason lignin, and had a higher arabinose content.

The structural features of AGX evaluated by methylation analysis (Table 2) are reflected to different extents in the ^{13}C-NMR spectra of the xylan fractions (Fig. 1a-c). The spectra were interpreted (Table 3) on the basis of reported data for structurally defined glucuronoxylan-type and arabinoxylan-type polymers (Kováč *et al.*, 1982; Azuma & Koshijima, 1983; Hromádková *et al.*, 1987; Bengtsson & Åman, 1990; Odonmažig *et al.*, 1990). Single α-(1 → 2)-linked MeGlcA units; preferentially as the 4-O-methyl derivative and α-(1 → 3)-linked L-Araf units, respectively, represented the main side-chains of the xylan molecules, which was evident in the spectrum of AGX-H (Fig. 1c). In contrast to AGX isolated from Echinaceae (Proksch & Wagner, 1987), wheat straw (Toman & Chimidcogzol, 1988) and ponderosa pine (Duckart *et al.*, 1988), the spectrum of AGX-H is characterised by a multiplicity of signals related to C-1, C-2, and C-4 of the α-Araf residues, which suggests differences in the localisation and

Table 1. Characteristics of corn cob heteroxylans

Analysis	AGX	Water-insoluble AGX	Water-soluble AGX	AGX-H
Neutral sugars[a] (%)				
Arabinose	6·1	2·8	7·6 (16·1)[b]	14·5
Xylose	88·3	92·6	85·5 (79·4)	81·4
Galactose	2·5	3·1	4·2 (2·5)	3·0
Glucose	3·1	2·5	2·8 (2·0)	1·1
Uronic acid[c] (%)	4·2	3·5	7·3 (5·2)	7·7
Klason lignin (%)	3·1	2·3	1·1	0·0
Nitrogen (%)	0·15	0·09	0·16	0·0
$(\alpha)_D^{20}$ (degrees)[d]	−79·8	−79·0	−83·1	−90·5
$(\eta)_{DMSO}$ (cm^3/g)	83	86	78	76

[a]Determined as alditol trifluoro acetates on OV-225.
[b]Sugar compositin of the portion soluble in the phosphate buffer.
[c]As 4-O-methyl-D-glucuronic acid unit.
[d]c = 0·5, in 0·5 M NaOH.

Table 2. Methylation analysis of AGX

Sugar[a]	Linkage position	Mol (%)
Arabinofuranosyl	Terminal	6·5
	2	1·6
	5	0·6
Xylopyranosyl	Terminal	2·0
	4	71·4
	3, 4 and 2, 4	12·3
	2, 3, 4	1·9
Glucopyranosyl	4	0·9
Galactopyranosyl	Terminal	2·8

[a]Determined as partially methylated alditol acetates on SP 2340.

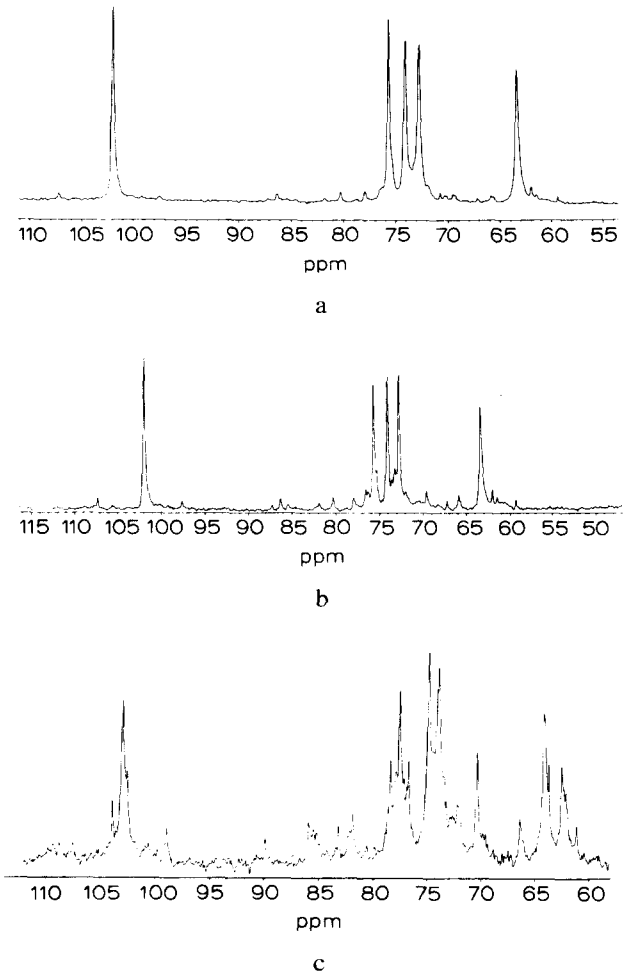

Fig. 1. ^{13}C-NMR spectra of the water-insoluble AGX (a) and water-soluble AGX (b) in DMSO-d_6, and AGX-H in D_2O (c).

substitution pattern of this constituent. The disaccharide side-chain, 2-O-β-D-xylopyranosyl-α-L-arabinofuranose which was proved to be attached at position O-3 to the main chain of corn cob xylan by Kusakabe *et al.* (1983) was indicated by the presence of a nearly equivalent proportion of 2-linked Araf and terminal Xylp residues (Table 2). This feature is more abundant in AGX-H than in water-soluble AGX (Fig. 1). The signals of C-1 and C-2 of the 2-linked Araf were shifted up-field to δ 107·49 and down-field to δ 89·81, respectively (Gorin & Mazurek, 1976). The signals at δ 70·25, and 66·36 were assigned to C-4, and C-5 of the terminal β-Xylp units. Splitting of the signal for C-1 at δ 103·73 (103·50) indicated two different positions for this unit. The minor 5-linked Araf (Table 2) was detectable by its C-5 resonance at δ 67·10 (Cartier *et al.*, 1987) in the spectra of both water-soluble AGX and water-insoluble AGX, but there was no evidence of a corresponding signal in AGX-H. In view of the compositional changes observed after the hypochlorite treatment of AGX, it can be assumed that these residues were involved in native linkages between the heteroxylan and phenolic substances (Neukom & Devel, 1958; Morrison, 1974).

As can be seen from Table 1, there are no substantial differences in the intrinsic viscosities (η) of the xylan fractions. The corresponding (LeBel & Goring, 1963) DP_w values varied between 176 and 200. To determine the molecular weight distribution (MWD) of both water-soluble AGX and AGX-H, the buffer salt containing polysaccharide solutions were fractionated by GPC on Sepharose 2B/Sepharose 4B column combination coupled with capillary viscosimetry and light scattering measurements. The elution profiles obtained are given in Fig. 2. The carbohydrate recoveries were 96% in the case of AGX-H, and only 32% in case of the water-soluble AGX related to the original matter. Obviously the latter had partly lost its solubility during storage for several years as was already observed for other acidic polysaccharides stored in the freeze-dried form (Anderson *et al.*, 1969). One may suggest an irreversible aggregation of lower substituted xylan molecules, as the soluble portion revealed an increase of the arabinose component (Table 1). The insoluble portion was removed by centrifugation prior to GPC and not included in the further consideration.

The elution profiles of both soluble fractions differed strongly. Whereas the positions of the concentration maxima were not too distant from each other; carbohydrate elution started at the void volume for AGX-H but roughly 100 ml later for the water-soluble AGX, indicating a narrowed size distribution for the latter.

In Figs 3 and 4 the results of viscosity and light scattering measurements were plotted against the elution volume (V_e) yielding the common universal calibration line in Fig. 5. This fact is sufficient to indicate the reliability of the individual data although the slope of the universal calibration line for arabinoxylans was found to be different from that of dextran T 2000 and dextran sulphate, which were measured under the same conditions. This might be due to the varied heterogeneities and/or polydispersities of identical elution volume slices dependent on the nature of the polymer studied, which can play a role in connection with light scattering molecular

Table 3. Main features of the ^{13}C-NMR spectra[a] of corn cob xylan fractions

	C-1	C-2	C-3	C-4	C-5	C-6
Water-soluble AGX[b]						
β-D-Xylp						
4-linked	101·83	72·64	73·97	75·59	63·31	
2, 4-linked	101·35	76·25	72·64	75·40	63·31	
3, 4-linked	101·35	73·16	76·58	73·58	63·31	
Terminal	101·90		74·75	69·59	66·01 (65·80)	
α-L-Araf						
Terminal (f)	107·21	80·21	77·96	86·18	61·98 (61·60)	
2-linked	105·72	87·21				
α-MeGlcA (terminal)	97·81	71·85	72·00	81·82[c]	69·59	173·10
AGX-H[d]						
β-D-Xylp						
4-linked	102·73	73·79	74·75	77·43	64·05	
2, 4-linked	102·39	77·95	73·40	77·05	63·60	
3, 4-linked	102·39	74·00	79·10 (78·34)	74·75 (74·35)	63·60	
Terminal	103·73 (103·50)	74·00	76·64	70·25	66·36	
α-L-Araf						
Terminal (f)	108·70	81·80	78·34	85·85	62·00	
	109·25	82·00		85·11	62·25	
	109·70	82·40		85·40	62·40	
2-linked	107·49	89·81				
α-MeGlcA (terminal)	98·84	72·15	72·59	83·12[c] (77·64)	70·25 (71·88)	171·90

[a]Chemical shifts, δ (ppm).
[b]In DMSO-d_6.
[c,e]OMe, δ 59·30 and 61·26, respectively.
[d]In D_2O.
[f]α-Araf linked to 0–3 of the xylan chain (Reilly, 1981; Hromádková *et al.*, 1987).
( ) signals in parentheses are of low intensity and not definite.

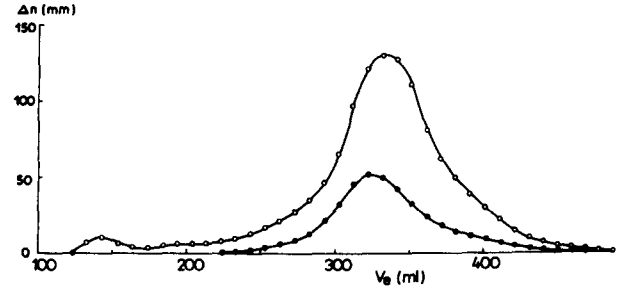

Fig. 2. Elution profile of water-soluble AGX (●) and AGX-H (○) on Sepharose 2B/Sepharose 4B.

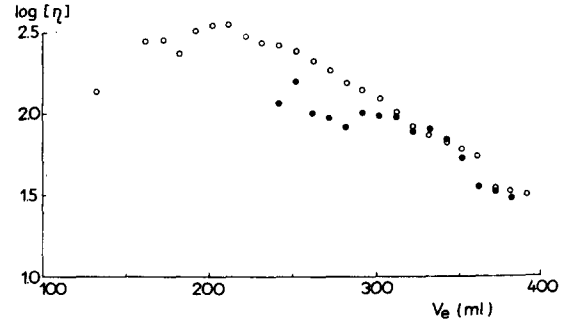

Fig. 3. Dependence of $[\eta]$ on the elution volume for water-soluble AGX (●) and AGX-H (○).

weight detection where especially high molecular weight species within a population are emphasised.

The MWD curves (Fig. 6) were derived from the gel chromatography data, using f(M) in percent, which were calculated from the size distribution (Fig. 2) and the molecular weight (M), taken from the distribution curve on Fig. 4. In contrast to the enormous differences in the size distribution in Fig. 2, the MWD curves were similar in the region of $10^4 < \overline{M}_w < 10^6$ as well as were the average intrinsic viscosities $[\eta]$ and the number average molecular weight ($\overline{M}_n$) from membrane osmometry (Table 4). The great differences in the high molecular weights ($\overline{M}_w$) result mainly from differences in the high molecular weight tail of the MWD.

The corresponding Mark-Houwink plot is presented in Fig. 7. In spite of the scattered data we conclude that both fractions fit the common $[\eta]$-M_w relation in the

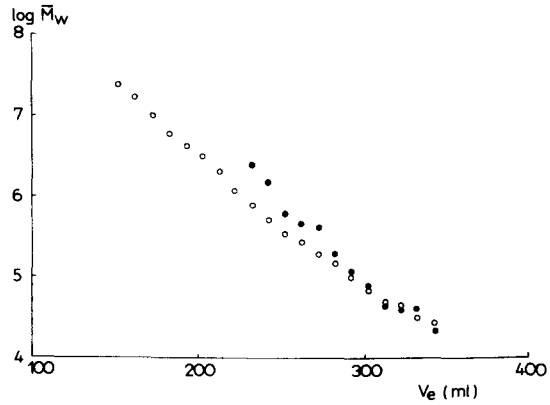

Fig. 4. Dependence of M_w on the elution volume for water-soluble AGX (●) and AGX-H (○).

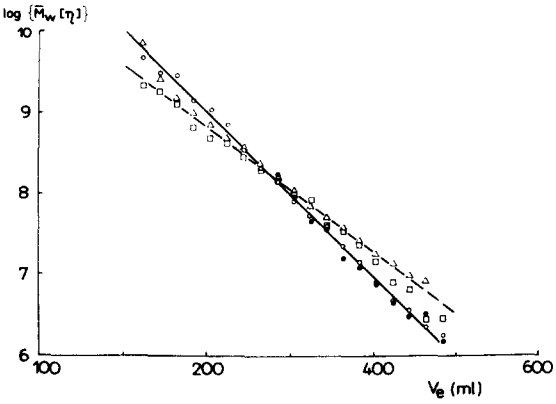

Fig. 5. Universal calibration plot for water-soluble AGX (●), AGX-H (○), on Sepharose 2B/Sepharose 4B; for comparison corresponding data for dextran T 2000 (□) and dextran sulphate (△) are included.

molecular region up to approximately 350 000. The exponent a = 0·50 is characteristic of molecules in unperturbed coil-shaped structures. AGX-H exhibited this feature for molecules with 10-fold higher M_w values. For the rye-flour pentosan in the same solvent (Anger *et al.*, 1986), the exponent a = 0·98 was found which is characteristic of polymer chains with restricted flexibility. The differences in the hydrodynamic properties of the compared heteroxylans can be explained by their different structural features. Whereas

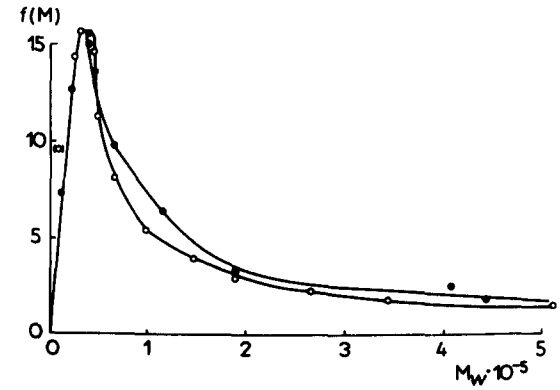

Fig. 6. Molecular weight distribution curves of AGX-H (○) and water-soluble AGX (●); f(M) is given in percent and calculated from Fig. 2; for molecular weights (M) see Fig. 4.

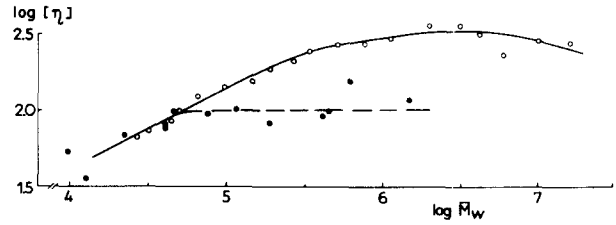

Fig. 7. Mark-Houwink plot for water-soluble AGX (●) and AGX-H (○); data were taken from Figs. 3 and 4.

the rye-flour xylan chain is highly branched (>50%) preferentially at position 0-3 with single Araf side chains (Bengtsson & Åman, 1990), the water-soluble AGX fractions studied had a lower degree of substitution (<30%), and acidic, as well as neutral mono- and disaccharide side chains.

Apart from the molecularly dispersed portion which was indicated by the proportionality of [η] and M_w; representing 90–95% and 65–70% of the water-soluble AGX and AGX-H, respectively, both fractions contained a high molecular weight matter for which no corresponding increase in [η] was observed. The lowered 'a' exponent of the Mark-Houwink relation reveals alternations in the gross conformation of macromolecules and can be interpreted as transition from coil-like towards sphere-like structures. This

Table 4. Molecular parameters[a] of water-soluble AGX and AGX-H

Sample	[η][b] (cm³/g)	$\bar{M}_n \times 10^{-3}$	$\bar{M}_w \times 10^{-3}$ [b]	$\bar{M}_w \times 10^{-3}$ [c]
Water-soluble AGX	75	26·9[d]	87·3	162·7[e]
AGX-H	86	25·5	1 415·7	1544·2

[a]Measured in the phosphate buffer.
[b]Calculated from the GPC fractions.
[c]Calculated from the Guinier-diagram.
[d]Original water-soluble AGX in water.
[d]After ultracentrifugation.

might be a result of either aggregation or steadily increasing branching up to compact particles or, at last, a rising level of branched matter. Without additional studies we cannot distinguish between aggregates and highly branched molecules. A particulate component was reported for the aqueous solution of glucuronoxylan of *Agave sisalana* (Mabusela & Stephen, 1989) as well as for the rye-flour pentosan (Anger *et al.*, 1986) and citrus pectin (Berth *et al.*, 1990), respectively. Whereas for the former glucuronoxylan it was ascribed to aggregates with $\overline{M}_n$ approximately equal to 150 000, for the later two polysaccharides, highly branched native structures were proposed.

We conclude that the results obtained coincide with the conception of aggregated molecular structures in the heteroxylan dispersions (preserved from the dry state and/or formed in the solution) rather than of extensively branched molecules. Aggregation is favoured (Dea *et al.*, 1973; Morris *et al.*, 1977) by the linear extended regions of the xylan chains which are non-uniformly substituted, as inferred from γ-radiolytic fragmentation analysis (Ebringerová *et al.*, 1990).

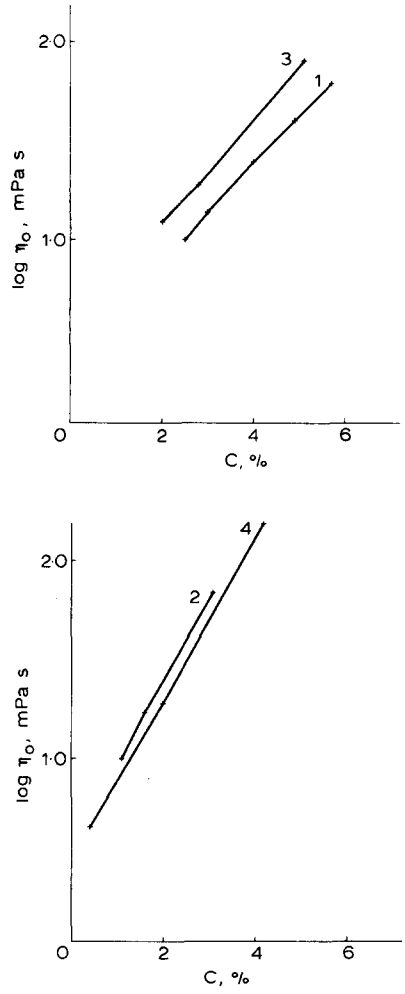

Fig. 8. Apparent viscosity (η_a) of aqueous dispersions of water-soluble AGX (1) and water-insoluble AGX (2) as a function of concentration. Viscosity data were obtained at constant shear rate D = 145/s (at 20°C). For comparison, corresponding data for water-soluble beechwood glucuronoxylan (3) and water-insoluble glucuronoxylan (4) are included.

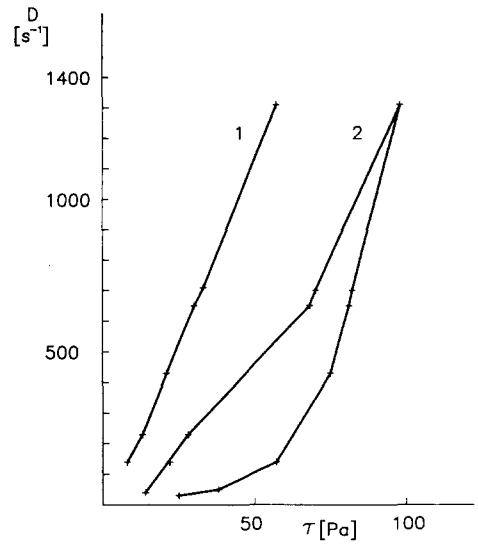

Fig. 9. Flow curves of aqueous dispersions of water-soluble AGX (1) for concentration 5·2% w/w and water-insoluble AGX (2) for concentration 4·2% w/w measured at 20°C.

The reported structural features of the AGX fractions mark not only the character of dilute solutions, but also that of semi-dilute aqueous dispersions of the polymers. Figure 8 shows that the increase of the apparent viscosity (η_a) of aqueous dispersions with increasing concentration was lower for water-soluble AGX than for water-insoluble AGX. A similar behaviour was found for beechwood glucuronoxylan fractions (Hromádková, 1991; Hromádková & Ebringerová, 1991). The flow curve (Fig. 9) of an approximately 5% dispersion of water-soluble exhibits very weak pseudoplastic behaviour without thixotropy which is characteristic of water-soluble glucuronoxylan dispersions (Hromádková & Ebringerová, 1991). The water-insoluble AGX formed a suspension of highly swollen particles which shows at approximately 4% distinct plastic behaviour. The interactions between the insoluble but swollen particles of water-insoluble AGX seem to produce a stronger intrinsic structure than the soluble water-soluble AGX molecules. A more detailed study of these properties is desirable as it may provide information not only about the biological function of the heteroxylan complex in the corn cob cell walls, but also for potential technical applications of heteroxylans, in common.

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