DISTRIBUTION PATTERN OF URONIC ACID UNITS IN 4-O-METHYL-D-GLUCURONO-D-XYLAN OF BEECH (*Fagus sylvatica* L.)

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4-O-Methyl-D-glucurono-D-xylan isolated from beech sawdust was characterized: its main chain consists of D-xylopyranose units linked by a glycosidic β(1 → 4) bond; uronic acid units are bound to $C_{(2)}$ of D-xylose units of the main chain as monomeric side units by a glycosidic α(1 → 2) bond. The distribution pattern of uronic acid units along the macromolecule chain was investigated in the water-soluble polysaccharide fraction of the D-xylose to uronic acid 6·1 to 1 molar ratio. The method based on interpretation of the activity coefficient of calcium counterions $\gamma_{Ca^{2+}}$ as determined with the calcium salt of glucuronoxylan was applied. The activity coefficient is a function of the linear charge density of the acid polysaccharide. It has been evidenced that the 4-O-methyl-D-glucurono-D-xylan under investigation is irregularly branched; it consists of longer segments of the macromolecule, each second D-xylose unit of which binds a uronic acid unit as a monomeric side terminal; these segments alternate with those composed of only D-xylan units.

Determination of distribution pattern of anionic groups in the molecule of acid polysaccharides by a standard structural analysis, *i.e.* by partial acid or enzyme hydrolysis followed by fractionation and characterization of oligomeric fragments is time consuming and results in ambiguous conclusions. A method based on protection of free carboxyl groups of pectin by glycolation and hydrolysis of parts of molecules including esterified uronic acid units by a mixture of pectolytic enzymes could be employed only for structural analysis of pectic substances[1-3]. Papers dealing with elucidation of distribution pattern of uronic acids in the hemicellulose macromolecule are quite rare[4-7]. It was generally assumed that the monomeric side uronic acid units are randomly distributed in the hemicellulose macromolecule[8]. Shimizu and coworkers[4] isolated, however, a tetramer containing two vicinal units of 4-O-methyl-D-glucuronic acid linked to $C_{(2)}$ of D-xylobiose from the hydrolysates of hemicelluloses of neutral sulfite waste liquor of larch. A relatively considerable amount of similar fragments was also isolated from the glucuronoxylan hydrolysate of larch[5]. The distribution pattern of these vicinal uronic acid units along the D-xylan chain has not been cleared up.

Another possibility to contribute to the knowledge of detailed structure of acid polysaccharides is the determination of physicochemical properties or constants,

which are functions of linear charge density of the macromolecule, as *e.g.* interaction of carboxyl groups of the acid polysaccharide with Ca^{2+} ions[7,9,10], affinity of the macromolecule towards an anion exchanger[11-13], activity of a specific enzyme cleaving glycosidic bonds of the acid polysaccharide[14,15] *etc.* (*cf.* review[2]).

As we have already shown, determination of the single-ion activity coefficient of calcium counterions ($\gamma_{Ca^{2+}}$) in solutions of calcium salts of acid polysaccharides containing carboxyl groups can provide a deeper view on the distribution pattern of anionic groups in the macromolecule. This technique was employed for structural analysis of acid polysaccharides of peach gum[10], 4-O-methyl-D-glucurono-D-xylan from the white willow bark[7] and for elucidation of the mechanism of action of various pectinesterases of both plant and microbial origins[15-17]. A block-wise distribution of uronic acid units in the macromolecule was proved with the acid polysaccharide of peach gum[10] and glucuronoxylan of white willov bark[7] in contrast to earlier conceptions on structure of these compounds[8]. The polysaccharide chain contained longer segments rich in uronic acid units, which alternated with those consisting of only neutral saccharide units.

This paper is aimed to throw more light on the distribution pattern of uronic acid units in the macromolecule of 4-O-methyl-D-glucurono-D-xylan isolated from beech sawdust.

EXPERIMENTAL

Isolation and Characterization of the Polysaccharide. 4-O-Methyl-D-glucurono-D-xylan was isolated from beech sawdust according to a patented procedure[18]. The sodium chlorite delignified sawdust (acid medium, 70°C) was extracted subsequently with 1%-NH_4OH and 5%-NaOH at 20°C. The glucuronoxylan was precipitated from the second extract by addition of a two-fold volume of ethanol. The salts were removed by washing the coagulated polysaccharide with 75%-ethanol, the precipitate was suspended in water and the polysaccharide was obtained by freeze-drying in 16·9% yield. Structure of the polysaccharide was estimated by methylation analysis and by ^{13}C NMR spectroscopy[19]. Content of 4-O-methyl-D-glucuronic acid was determined by the carbazole method[20] and compared with the methoxyl group content; both methods afforded consistent results. The mean molecular mass $\overline{M}_n$ was determined osmometrically after equilibration with a NaCl 0·1 mol l^{-1} solution.

A sample of the glucuronoxylan was transformed into H^+ form by washing with 90%-ethanolic HCl 0·1 mol l^{-1}, neutral 90%- and 95%-ethanols and ether; finally, the sample was air-dried at an ambient temperature (86·9% dry substance). Removal of Cl^- ions was checked argentometrically by potentiometric titration with the $AgNO_3$ 0·01 mol l^{-1} solution using silver electrode. The dry matter of preparations was determined at atmospheric pressure and 105°C. Content of carboxyl groups of samples in H^+ form was determined alkalimetrically by potentiometric titration with KOH 0·05 mol l^{-1} or $Ca(OH)_2$ 0·021 mol l^{-1}.

Determination of the Mean Distance b of Neighbouring Carboxyl Groups in the Polysaccharide Molecule

Distribution pattern of 4-O-methyl-D-glucuronic acid units in the linear macromolecule of D-xylan was considered from the values of activity coefficient of calcium counterions ($\gamma_{Ca^{2+}}$) according

to our method[10,7]. The glucuronoxylan (H^+ form) was neutralized with KOH 0·05 mol l^{-1} to the point of equivalence. Suspension of the polysaccharide ((COOK)-concentration approximately 0·005 mol l^{-1}) was centrifuged at 20 000 *g* for 30 min. One part of the supernatant was employed for determination of the molar ratio of D-xylose and 4-O-methyl-D-glucuronic acid in the soluble fraction of the polysaccharide and for determining the circular dichroism spectra of its potassium and calcium salts. The second part of the supernatant was percolated through a Dowex 50 W × × 2(H^+) column; the resulting solution of the polyacid was neutralized with $Ca(OH)_2$ 0·021 mol . . l^{-1} to the point of equivalence. The activity of Ca^{2+} counterions was then determined in the solution of calcium salt of 4-O-methyl-D-glucurono-D-xylan ($(COOCa_{0.5})$ final concentration 0·003 mol l^{-1}) by the metallochromic indicator method (tetramethylmurexide)[21,22]; the solution did not contain any additional electrolyte. The corresponding single-ion activity coefficient $\gamma_{Ca^{2+}}$ was calculated from the determined value for activity $a_{Ca^{2+}}$. The mean distance *b* (nm) of two neighbouring carboxyl groups of the polysaccharide in their perpendicular projection on the axis of the D-xylan linear chain was then estimated from the analytical curve[10,7] ($\gamma_{Ca^{2+}} = f(b)$, Fig. 1).

Apparatuses and reagents: Gas chromatograph Hewlett–Packard, model 5700 A (England), Jeol FX-100 spectrometer (^{13}C NMR) (Japan), CD spectrometer Jobin Yvon, Mark III (France), compensation spectrophotometer Hilger (England), potentiometer Radiometer PHM 64 (Denmark), Membranosmometer Knauer (West Berlin), carbonate-free KOH 0·05 mol l^{-1}, saturated $Ca(OH)_2$ 0·021 mol l^{-1}, chemicals of *p.a.* grade, and redistilled carbonate-free water.

RESULTS AND DISCUSSION

Hemicelluloses isolated from beech can be considered as a pure 4-O-methyl-D-glucurono-D-xylan, since D-xylose represents 98% of the total amount of neutral saccharides in the preparation. The main polysaccharide chain consists of D-xylopyranose units bound by a β(1 → 4) glycosidic linkage. The 4-O-methyl-D-glucuronic acid units are bound to D-xylose units of the main chain as monomeric terminals by an α(1 → 2) glycosidic bond. Characterization of the polysaccharide (Na salt, Table I) refers to anhydro units of the saccharides.

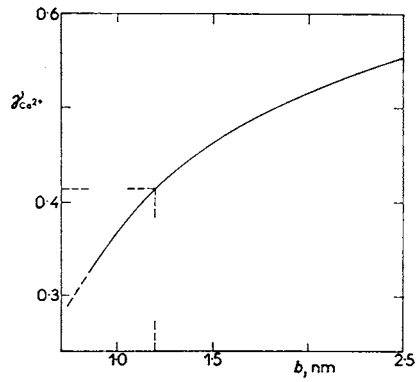

FIG. 1

Dependence of the single-ion activity coefficient $\gamma_{Ca^{2+}}$ on the mean distance *b* of adjacent carboxyl groups in solutions of calcium salts of linear acid polysaccharides. Concentration of uronic acids ($(COOCa_{0.5})$ 0·003 mol l^{-1}

TABLE XI
(*Continued*)

$\lambda = 1{\cdot}25$		$\lambda = 2{\cdot}0$		$\lambda = 2{\cdot}75$		$\lambda = 3{\cdot}0$	
η	$\beta P/\varrho$	η	$\beta P/\varrho$	η	$\beta P/\varrho$	η	$\beta P/\varrho$
—	—	0·6063	34·5	0·548	24·9	0·5445	24·8
—	—	0·6021	33·2	0·5550	26·8	—	—
—	—	0·6199	40·3	0·5655	29·2	—	—
—	—	—	—	0·5760	32·5	—	—
—	—	—	—	0·5891	35·3	—	—

Concerning the system of infinitely thin platelets (an interesting system of particles with zero volume), Eppenga and Frenkel[56] focused in their simulations mainly on the nematic phase and nematic-isotropic phase transition. The equation of state (for both isotropic and nematic phases) was presented as a graph and is not therefore included in our tables.

Homonuclear diatomics (HOMO DB). This is the simplest FHS model and it has been therefore very intensively studied. In many cases, however, only structural properties have been evaluated. Older data on EOS are due to Freasier[77],Freasier and coworkers[72], and Streett and Tildesley[120]. Data of Aviram and coworkers[78] were shown[51] to be incorrect and must be therefore discarded. Newer data have been obtained by Freasier[71] and Tildesley and Streett[98] and are in mutual agreement. The latter authors performed extensive simulations at 45 state points covering elongations from 0·2 to 1·0 and packing fractions up to 0·47. These are the representative data of HOMO DB and are listed in Table XIV. Tildesley and Streett also parametrized these data by means of a Carnahan–Starling-type equation,

$$\beta P/\varrho = [1 + (1 + UL + VL^3)\eta + (1 + WL + XL^3)\eta^2 - (1 + YL + ZL^3)\eta^3]/(1 - \eta)^3 , \qquad (4.29)$$

where

$$U = 0{\cdot}37836 \quad V = 1{\cdot}07860 \quad W = 1{\cdot}30376$$
$$X = 1{\cdot}80010 \quad Y = 2{\cdot}39803 \quad Z = 0{\cdot}35700 . \qquad (4.30)$$

The average difference between the simulation data and Eq. (*4.29*) is 0·4 per cent and the maximum difference is 1·1 per cent. Since the estimated accuracy of the MC data is 2·0 per cent, Eq. (*4.29*) enables one to determine accurately the compressibility

TABLE XII

Compressibility factors of the isotropic oblate ellipsoid fluids taken from ref.[119]. The compressibility factors are claimed to be accurate, approximately, to within 1 per cent

$\lambda = 0{\cdot}80$		$\lambda = 0{\cdot}50$		$\lambda = 0{\cdot}3636$		$\lambda = 0{\cdot}333$	
η	$\beta P/\varrho$	η	$\beta P/\varrho$	η	$\beta P/\varrho$	η	$\beta P/\varrho$
0·210	2·49	0·197	2·66	0·189	2·78	0·183	2·87
0·281	3·73	0·268	3·91	0·252	4·15	0·251	4·18
0·332	4·73	0·316	4·97	0·304	5·16	0·295	5·33
0·370	5·67	0·351	5·96	0·335	6·26	0·327	6·41
0·390	6·71	0·379	6·92	0·364	7·19	0·379	8·29
0·410	7·65	0·394	7·98	0·367	7·14	0·404	10·4
0·432	8·48	0·414	8·86	0·381	8·24	0·419	11·2
0·446	9·39	0·428	9·79	0·396	9·26	0·431	12·2
0·460	10·2	0·439	10·7	0·412	10·2	0·439	13·1
0·471	11·1	0·457	11·5	0·424	11·1	0·453	13·9
0·483	11·9	0·466	12·4	0·438	11·9	0·466	14·6
0·493	12·8	0·478	13·1	0·449	12·8	0·4712	15·6
0·500	13·6	0·481	14·1	0·458	13·7	0·4712	15·6
0·511	14·4	0·493	14·9	0·4650	14·6	0·481	16·3
0·521	15·1	0·502	15·6	0·4712	14·9	0·4817	16·5
0·526	15·9	0·507	16·5	0·471	14·9	—	—
0·537	16·6	0·515	17·3	0·4812	15·2	—	—
0·541	17·4	0·514	17·3	0·4817	15·9	—	—
0·544	18·3	0·518	18·2	0·482	15·8	—	—
0·547	19·1	0·522	18·1	0·485	16·2	—	—
	—	0·526	18·9	0·4890	17·1	—	—
	—	0·528	18·8	0·4922	17·0	—	—
	—	0·530	19·8	0·492	16·9	—	—
	—	0·533	19·7	0·502	17·7	—	—
	—	0·543	20·3	0·495	18·0	—	—
	—	0·534	20·6	0·5027	18·3	—	—
	—	0·542	21·2	0·508	18·6	—	—
	—	0·544	21·2	0·509	18·5	—	—
	—	0·548	22·0	0·513	18·3	—	—
	—	0·551	22·8	0·516	19·3	—	—
	—	0·555	23·6	0·5236	21·5	—	—
	—	0·563	24·2	0·5341	22·6	—	—
	—	0·5624	25·0	0·5445	24·5	—	—
	—	0·563	25·1	0·5550	27·1	—	—
	—	0·5760	26·6	—	—	—	—
	—	0·5849	27·9	—	—	—	—
	—	0·5891	30·3			—	—
	—	0·5927	31·0	—	—	—	—
	—	0·6074	36·3	—	—	—	—
—	—	0·6168	40·6	—	—	—	—

factor of the fluid of HOMO DB with any elongation ($L \leqq 1$) and at any packing fraction.

TABLE XIII
Compressibility factors of the oblate spherocylinder fluids from computer simulations (ref.[33])

$\varphi(=\gamma - 1)$	η	$\beta P/\varrho$
0·5	0·35	5·41 ± 0·04
1·0	0·10	1·58 ± 0·01
	0·25	3·35 ± 0·07
	0·35	5·79 ± 0·07
	0·45	10·56 ± 0·09
		10·53 ± 0·07
1·5	0·35	6·23 ± 0·07
	0·45	11·35 ± 0·12
2·0	0·25	3·83 ± 0·05
	0·35	6·79 ± 0·05
		6·81 ± 0·08
	0·45	12·30 ± 0·10
		12·09 ± 0·16
2·5	0·35	7·37 ± 0·09
	0·45	13·00 ± 0·12

TABLE XIV
Compressibility factors of the homonuclear diatomic fluids from computer simulations (ref.[98])

η	$\beta P/\varrho$				
	$L = 0·2$	$L = 0·4$	$L = 0·6$	$L = 0·8$	$L = 1·0$
0·1047	1·56 ± 0·03	1·59 ± 0·03	1·63 ± 0·03	1·70 ± 0·03	1·79 ± 0·03
0·1571	2·01 ± 0·04	2·04 ± 0·04	2·13 ± 0·04	2·26 ± 0·05	2·46 ± 0·05
0·2094	2·59 ± 0·05	2·64 ± 0·05	2·78 ± 0·06	3·01 ± 0·06	3·36 ± 0·07
0·2618	3·36 ± 0·07	3·49 ± 0·07	3·67 ± 0·06	4·05 ± 0·08	4·62 ± 0·09
0·3142	4·45 ± 0·09	4·59 ± 0·09	4·95 ± 0·10	5·48 ± 0·11	6·40 ± 0·13
0·3665	5·95 ± 0·12	6·21 ± 0·12	6·69 ± 0·13	7·52 ± 0·15	8·95 ± 0·18
0·4189	8·02 ± 0·16	8·42 ± 0·17	9·23 ± 0·18	10·54 ± 0·21	12·64 ± 0·25
0·4451	9·44 ± 0·19	9·91 ± 0·20	10·89 ± 0·22	12·50 ± 0·25	15·12 ± 0·30
0·4712	11·17 ± 0·22	11·67 ± 0·23	12·87 ± 0·26	14·88 ± 0·30	18·06 ± 0·36

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