Controlled Depolymerization of 4-*O*-Methyl-D-glucurono-D-xylan Isolated from Wood of Beech (*Fagus sylvatica* L.)

[a]E. LATTOVÁ, [a]A. EBRINGEROVÁ, [b]R. TOMAN, and [a]M. KAČURÁKOVÁ

[a]*Institute of Chemistry, Slovak Academy of Sciences, CS-842 38 Bratislava*

[b]*Institute of Virology, Slovak Academy of Sciences, CS-842 46 Bratislava*

Received 4 June 1990

Dedicated to Dr. Ing. Š. Bauer, DrSc., in honour of his 70th birthday

In order to obtain 4-*O*-methyl-D-glucurono-D-xylan fractions in the range of relative molecular masses 7000—8500, optimal conditions of oxidative-acidic depolymerization in the medium of sulfuric acid and hydrogen peroxide were elaborated. The used methods for isolation of degradation products, *i.e.* gel filtration, dialysis, and ultrafiltration were evaluated from the point of view of yield, properties of product, and primary structure of polysaccharide fractions.

The controlled depolymerization of polysaccharides and the isolation of fragments with required molecular mass is a key-problem in the preparation of biologically active derivatives on the basis of plant polysaccharides. Unlike cellulose, depolymerization of D-xylan type polysaccharides was studied only in relation to the preparation of chemically and molecularly defined fractions necessary for the characterization of molecular properties (especially polymolecularity) and determination of molecular masses of these polysaccharides. In this respect, *Wikström* [1] had studied hydrolysis of glucuronoxylan isolated from the birch wood in phosphoric acid solutions. *Lebel* and *Goring* [2] degraded similar xylan in the medium of dimethyl sulfoxide and hydrochloric acid at pH = 1.

In our work, oxidative-acidic degradation of 4-*O*-methyl-D-glucurono-D-xylan isolated from wood of beech (*Fagus sylvatica* L.) [3] was studied with the aim to obtain polysaccharide fractions with M_r in the range of 7000—8500.

The course of degradation of xylan in the medium of diluted sulfuric acid with an addition of hydrogen peroxide in dependence on reaction temperature followed according to the amount of nondialyzable portion of reaction mixture, is depicted in Fig. 1. With increasing time of reaction, the yield of nondialyzable portion representing polymeric part of degraded xylan decreases, namely, at 90 °C two times faster than at 70 °C. Oxidative function of hydrogen peroxide in acidic medium is manifested by a decrease of viscosity average molecular mass of xylan (Table 1) which is the most expressive in the course of the first 60 min of reaction, when $\bar{M}_v$ decreases from initial 18064 to 8023. Owing to progressive destruc-

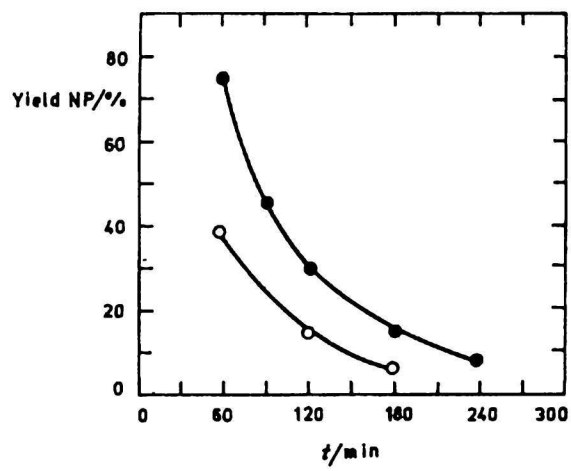

Fig. 1. The course of degradation of 4-*O*-methyl-D-glucurono-D-xylan in the medium of sulfuric acid and hydrogen peroxide in dependence on time and temperature. -●- Degradation at 70 °C, -○- degradation at 90 °C. NP — nondialyzable portion.

Table 1. Fractions of Degraded 4-*O*-Methyl-D-glucurono-D-xylan at 70 °C Isolated by Dialysis

Fraction	t / min	Yield / %	$\bar{M}_v$	$[\alpha]$/° (D, ρ = 20 g dm^{-3}, H_2O)	w(UA)[a] / %
1	60	75	8023	− 78.4	17.2
2	90	45	7860	− 75.8	18.4
3	120	30	7500	− 72.8	17.6
4	180	15	7448	− 71.9	16.9
5	240	8	7371	− 72.3	17.1

a) Expressed as a unit of 4-*O*-methyl-D-glucuronic acid.

Table 3. Fractions of Degraded 4-*O*-Methyl-D-glucurono-D-xylan at 70 °C Obtained by the Combination of Ultrafiltration and Gel Filtration Methods on Sephadex G-25

Fraction	t / min	Yield / %	$\bar{M}_v$	$\bar{M}_N$	$[\alpha]$/° (D, ρ = 20 g dm^{-3}, H_2O)	w(UA)[a] / %
11	60	40	8456	7560	− 61.8	16.3
12	90	38	8560	7580	− 75.7	17.0
13	120	27	8360	7437	− 61.2	15.5

a) As in Table 1.

tion of xylan to low-molecular dialyzable portions, the yield of nondialyzable portion decreases after 240 min down to 8 % at its decreasing value of $\bar{M}_v$ by ≈ 1000.

The change of molecular mass of xylan is also accompanied by a change of its polymolecularity. This is documented by the results of gel filtration of reaction mixture (Table 2) in comparison with the results of dialysis (Table 1). After 60 min of degradation, high-molecular xylan fraction *6* with $\bar{M}_v$ = 7500 is obtained in 70 % yield relating to the starting xylan, which represents 93 % of yield of nondialyzable portion *1* indicating low polymolecularity of this fraction. After 90 min of degradation, the yield of high-molecular xylan fraction *7* isolated by gel filtration decreases expressively, representing 70 % of yield of nondialyzable portion *2*. From the reaction mixture after 90 min of degradation, polymeric fractions with essentially lower molecular mass (fractions *8* and *9*, Table 2) were also obtained by gel filtration, indicating higher molecular heterogeneity of nondialyzable portion of xylan. Under the mentioned conditions, about 50 % of polysaccharide was decomposed to the low-molecular destruction products. The obtained results indicate that regarding the yield of high-molecular xylan fraction with relative molecular mass in the range of 7000—8500, the method of dialysis of xylan degraded at 70 °C for 60 min is the most suitable. Longer action causes a substantial decreasing of yield and the product with low-molecular heterogeneity can be obtained only by application of gel filtration or its combination with ultrafiltration (Table 3), where more effective separation of required fractions is achieved.

Table 2. Fractions of Degraded 4-*O*-Methyl-D-glucurono-D-xylan at 70 °C Obtained by Gel Filtration on Sephadex G-25

Fraction	t / mih	Yield / %	$\bar{M}_v$	$\bar{M}_N$	$[\alpha]$/° (D, ρ = 20 g dm^{-3}, H_2O)	w(UA)[a] / %
6	60	70	7500	6772	− 69.8	16.5
7	90	30	7400	6816	− 71.2	16.0
8		8	–	5417	− 52.3	8.0
9		12	–	3177	0	3.6
10	120	22	7100	6164	− 68.3	15.5

a) As in Table 1.

Interesting knowledge is a fact that all high-molecular fractions of degraded xylan have, independently of the method of isolation, approximately the same contents of 4-*O*-methyl-D-glucuronic acid which forms side chains on the (1→4)-β-D-xylan chain and its α-glycosidic bond affects the value of optical rotation of xylan [4]. Also, the small changes of optical rotation of compared xylan fractions are in accordance with contents of uronic acid. In the case of polymeric fractions with lower molecular mass (Table 2), the proportion of 4-*O*-methyl-D-glucuronic acid is essentially lower and optical rotation exhibits expressive decrease in contrast with the expected increase of the negative value of optical rotation at the increase of relative proportion of β-glycosidic bonds in xylan molecule. We suppose that this fact is connected with heterogeneous chemical character of these fractions, where besides neutral and acidic xylodextrins, products of oxidative destruction are also cumulated and these together contribute to the resulting value of optical rotation of fractions. Otherwise hardly explainable zero value of the specific optical rotation of fraction *9* could have been an accidental result of all these factors. In the case of degradation of structurally similar xylan isolated from birch wood by using conventional method of acid hydrolysis [2], a tendency of increasing of uronic acid contents in fractions with lower degree of polymerization was observed. In our previous papers [5, 6] we have shown that units of 4-*O*-methyl-D-glucuronic acid are not regularly distributed in xylan chain. They are concentrated rather in blocks on an average on each second D-xylose unit, producing sections of chain with a high degree of substitution alongside the linear sections. In connection with the known different stability of glycosidic

bonds of both saccharidic components of 4-*O*-methyl-D-glucurono-D-xylan in acidic medium [7], which decreases in the sequence: (1→2)-α bond of uronic acid, (1→4)-β bond of D-xylose substituted by uronic acid units, (1→4)-β bond of unsubstituted D-xylose, during the hydrolysis, obviously glycosidic bonds of unsubstituted D-xylan chain are preferentially split. Our results indicate different course of depolymerization of 4-*O*-methyl-D-glucurono-D-xylan in acidic medium in the presence of hydrogen peroxide. Oxidative-acidic splitting of glycosidic bonds is less dependent on their kind and position. Not only intact proportion of xylose and uronic acid in high-molecular fractions of degraded xylan but also more regular distribution of 4-*O*-methyl-D-glucuronic acid units supports this fact [8].

It is necessary to remark that oxidation effect of hydrogen peroxide does not confine itself only to glycosidic bonds of polymer. Hydroxyl groups are also attacked under formation of carbonyl groups as well as conjugated double-bond systems, which is manifested by the presence of absorption bands at $\tilde{\nu} = 1720$ and 1529 cm^{-1} in the IR spectra of degraded xylan (Fig. 2). As can be seen, the occurrence of these new functional groups is evident only in the case of fraction with lower molecular mass, while IR spectra of high-molecular fraction and initial xylan are practically identical. With regard to the high molar absorption coefficient of the mentioned groups, their proportion registered by IR spectroscopic analysis does not influence basic structural features of compared polymeric fractions of degraded xylan, determined by ^{13}C NMR analysis. The spectra of fractions *6* and *8* include all characteristic signals of the starting 4-*O*-methyl-D-glucurono-D-xylan [9], identified on the basis of spectral analysis of suitable oligosaccharide models [10, 11]. These are summarized in Table 4. In addition to this, in the region of resonances of carbonyl and conjugated structures, the increments of sig-

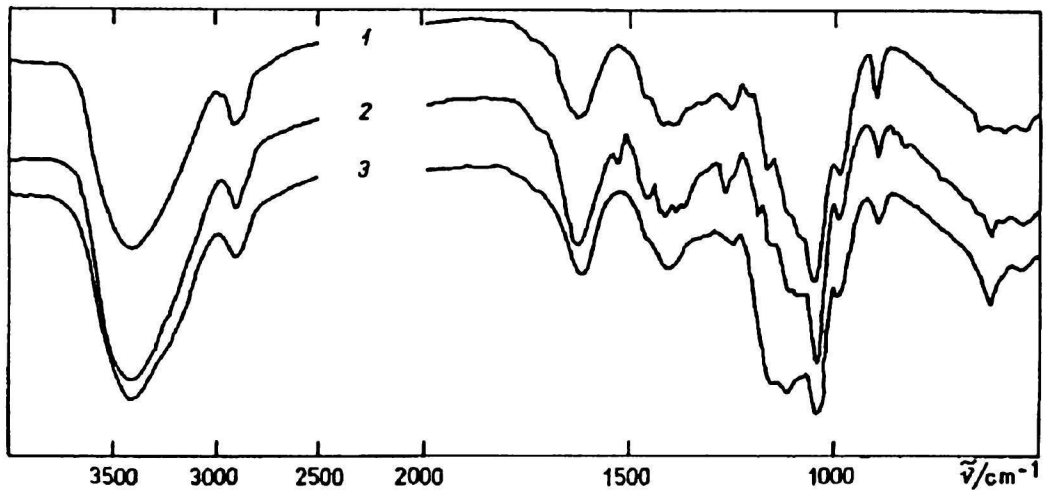

Fig. 2. IR spectra of initial 4-*O*-methyl-D-glucurono-D-xylan (*1*) and fractions *8* (*2*) and *6* (*3*).

Table 4. ^{13}C NMR Data of Fractions *6* and *8* of Degraded 4-*O*-Methyl-D-glucurono-D-xylan

Fraction	Structural unit	Chemical shift δ						
		C-1	C-2	C-3	C-4	C-5	C-6	OMe
6	-4-β-D-Xylp-1-	102.78	73.94	74.90	77.49	64.09		
	-4-β-D-Xylp-1-	102.40	77.99	73.47	77.15	63.99		
	2 | 1							
8	4-OMe-α-D-GAp	98.66	72.48	73.23	83.58	70.44	177.78	61.0
	-4-β-D-Xylp-1-	102.91	73.97	74.91	77.62	64.21		
	-4-β-D-Xylp-1-	102.62	77.80	73.45	76.86	64.21		
	2 | 1							
	4-OMe-α-D-GAp	98.70	72.49	73.23	83.50	70.31	177.93	61.0

nals in the spectrum of low-molecular fraction *8* are not distinguished. The presence of oligomeric portions in this fraction is confirmed by expressive resonances of both anomeric carbon atoms of reducing terminal D-xylose unit at δ = 97.7 and 93.3 and by the high proportion of signals of nonreducing (1→4)-β-bonded terminal D-xylose unit at δ = 103.1, 70.48, and 66.47 corresponding to the C-1, C-4, and C-5. A part of these units is substituted by 4-*O*-methyl-D-glucuronic acid. The signals at δ = 78.2 and 75.2 assigned to the C-2 and C-3 [12] suggest this fact.

In conclusion, it can be stated that the oxidative-acidic depolymerization of 4-*O*-methyl-D-glucurono-D-xylan in the medium of diluted sulfuric acid and hydrogen peroxide enables one to obtain fractions with required relative molecular mass in the range of 7000—8500 with the retained content of uronic acid of initial polysaccharide, while the yield is affectable by reaction time, temperature, and method of isolation from the reaction mixture.

EXPERIMENTAL

As a starting material, 4-*O*-methyl-D-glucurono-D-xylan from beech wood [3] with average molecular masses $\bar{M}_N$ = 16 600, $\bar{M}_v$ = 18 064, [α] (D, H_2O) = − 74°, containing 17.5 % of 4-*O*-methyl-D-glucuronic acid, was used.

Optical rotations were measured on a polarimeter 141 (Perkin—Elmer) in 0.5 % aqueous solution at 22 °C. Infrared spectrum of polysaccharide was taken on a spectrometer 9836 (Perkin—Elmer). The number average molecular mass $\bar{M}_N$ was measured osmometrically at 30 °C in water on a membrane osmometer (Knauer) equipped with Zweischicht—Membrane (Knauer). The viscosity average molecular mass $\bar{M}_v$ was determined by viscosimetric measurement of DMSO solutions in an Ubbelohde viscosimeter [2]. The content of 4-*O*-methyl-D-glucuronic acid was determined by using the carbazole method [13].

^{13}C NMR spectra of xylan fractions solutions (ρ = 10 g dm^{-3} in D_2O) were measured at 30 °C on a spectrometer AM-300 (Bruker). Chemical shifts referred to methanol (δ_{TMS} = 50.15) which was used as an internal standard.

Individual fractions were identified by a continuous-flow refractometer 5100 (Knauer).

Depolymerization of Xylan

4-*O*-Methyl-D-glucurono-D-xylan (1 g) was suspended in distilled water (50 cm^3) and the pH of the solution was adjusted to 0.3 by the use of concentrated sulfuric acid. Under stirring, hydrogen peroxide (30 %, 10 cm^3) was added and the reaction mixture was heated on a water bath for definite time at 70 °C and 90 °C. When the reaction was over, the solution was neutralized with 1 M solution of sodium hydroxide. Dialysis of the reaction mixture was performed in dialysis tubings towards distilled water and the degradation material was obtained as a nondialyzable residue. Gel filtration was performed on a column (7 cm x 100 cm) of Sephadex G-25 and distilled water was used as an eluent at column flow rate of 0.8 $cm^3\ min^{-1}$. Fractionation of the reaction mixture using combination of ultrafiltration and gel filtration methods was first performed on an instrument 7018 (Amicon) applying dialysis Cartridge HLP 3-20 in order to remove low-molecular fractions of degraded xylan. The obtained portion was finally fractioned by gel filtration similarly to that of foregoing case. In all cases, the fractions were obtained by the use of lyophilization of aqueous solutions.

REFERENCES

1. Wikström, R., *Sv. Papperstidn. 71*, 399 (1968).
2. Lebel, R. G. and Goring, D. A. J., *J. Polym. Sci., C 2*, 29 (1963).
3. Ebringerová, A. and Toman, R., *Czechoslov.* 231686 (1986).
4. Marchessault, R. H., Holava, H., and Timell, T. E., *Can. J. Chem. 41*, 1612 (1963).
5. Kohn, R., Hromádková, Z., Ebringerová, A., and Toman, R., *Collect. Czechoslov. Chem. Commun. 51*, 2243 (1986).
6. Toman, R., Kohn, R., Malovíková, A., and Rosík, J., *Collect. Czechoslov. Chem. Commun. 46*, 1405 (1981).
7. Timell, T. E., *Adv. Carbohydr. Chem. Biochem. 19*, 247 (1964).
8. Lattová, E. and Malovíková, A., unpublished results.
9. Ebringerová, A., *Drevársky výskum 109*, 21 (1986).
10. Kováč, P., Alföldi, J., Kočiš, P., Petráková, E., and Hirsch, J., *Cellulose Chem. Technol. 16*, 261 (1982).
11. Hirsch, J., Kováč, P., and Petráková, E., *Carbohydr. Res. 106*, 203 (1982).
12. Utille, J. P., Kováč, P., Sauriol, F., and Perlin, A. S., *Carbohydr. Res. 154*, 251 (1986).
13. Bitter, T. and Muir, H. M., *Anal. Biochem. 4*, 330 (1962).

Translated by M. Kóš