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4-O-Methyl-D-Glucurono-D-Xylan Degradation by γ-Irradiation. Part 1 — Dose Effects on the Macromolecular Properties

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ABSTRACT

The degree of polymerization and molecular weight distribution of 4-O-methyl-D-glucurono-D-xylan is compared before and after γ-irradiation. With increasing the radiation dose, the profile of the distribution curve becomes broadened and the molecular weight peaks are shifted to lower values. The radiation-chemical yield of cleaved glycosidic bonds G *was found to be* 8 ± 1 *scissions per 100 eV of energy absorbed. The polydispersity index* $\bar{M}_w/\bar{M}_n$ *increased from 1·5 to 1·9, thus indicating a random scission of the xylan chain.*

INTRODUCTION

Cellulose and xylans constitute the major part of higher plant cell wall materials, and the study of the mechanism of their radiation-chemical transformations is a matter of acute scientific and practical importance. The ionizing radiation treatment of cellulose results in its destruction and in the change of its molecular weight distribution (Kusama *et al.*, 1976; Adam, 1983; Burczak *et al.*, 1983; Ershov & Klimentor, 1984; Fischer *et al.*, 1987). In comparison to cellulose, the radiolysis of xylan has received only little attention (Beynart *et al.*, 1978; Fischer *et al.*, 1987; Ebringerová *et al.*, 1989).

In a previous paper (Ershov *et al.*, submitted), γ-radiation induced radical formation in beech wood 4-O-methyl-D-glucurono-D-xylan was reported. The present paper deals with changes in its solubility and molecular weight parameters.

EXPERIMENTAL

Materials

The 4-O-methyl-D-glucurono-D-xylan sample was obtained by fractional extraction of beechwood holocellulose (Ebringerová, 1979) and applied in the freeze-dried Na^+ form. Its analytical characteristics are summarized in Table 1. The neutral sugars were released by two-stage hydrolysis (Saeman *et al.*, 1954) and analysed by gas-liquid chromatography of the alditol trifluoro acetates (Hromádková & Ebringerová, 1987). Methoxyl groups were determined according to Viebeck and

TABLE 1

Analytical Characteristics of the Beechwood Xylan

D-Xylose in the neutral sugar fraction of the hydrolysate	95·5%
Methoxyl group	2·5%
Carboxyl group	0·77 mM/g
Uronic acid[a]	14·6%
Molar ratio of xylose to uronic acid	8·4 : 1
$[\alpha]_D^{20}$, (c, 0·53 in H_2O)	−70°
Ash	2·5%
DP_v in Cadoxen (Wikström, 1968)	133
DP_v in DMSO (LeBel & Goring, 1963)	129
Water extract[b]	35·7%

[a] Expressed as the 'anhydro form' of the 4-O-methyl-D-glucuronic acid, calculated from the carboxyl group content.

[b] Determined by the fractionation method used for the irradiated xylan (see Experimental).

Brecher (1930) and carboxyl groups alkalimetrically by potentiometric titration.

Radiation treatment and fractionation of the irradiated xylan

Irradiation of the xylan was performed at room temperature in air using a 60°C source (30 kGy h^{-1}) as described in a previous paper (Ershov *et al.*, 1989). The irradiated xylan (0·5–1 g) was stirred in distilled water (50–100 ml) at room temperature for 2 h. The water-insoluble portion (WIS-fraction) was separated from the water extract by centrifugation and further dried by ethanol and acetone. From the extract the soluble polysaccharidic portion (WS-fraction) was precipitated by addition of ethanol (1 : 3, v/v) and after filtration dried using ethanol and acetone.

Methods

The degree of polymerization (DP_v) of the original and irradiated xylan samples was obtained from viscometric measurements in cadoxen (Wikström, 1968) and dimethylsulfoxide (LeBel & Goring, 1963). The molecular distribution curve (MWD) was estimated by size-exclusion chromatography (SEC). Measurements were performed on a Laboratory Instrument Work model GPC (Czechoslovakia) equipped with a Rheodyne 7125 fixed loop (20 μl) injector, using Separon S HEMA 1000 column (Tessek, Czechoslovakia). Operating conditions were: eluent, dimethylformamid-0·03 M/litre H_3PO_4–0·03 M/litre LiCl; flow rate 0·5 ml/min; detection by refractometry. Xylan samples (10 mg) were dissolved in 2 ml of DMSO-0·03 M/litre H_3PO_4–0·03 M/litre LiCl. The column was calibrated using sharp fractions of a birchwood 4-*O*-methylglucuronoxylan characterized by viscometry in cadoxen (Eremeeva & Khinoverova, submitted). The number-average ($\bar{M}_n$), weight-average ($\bar{M}_w$), and viscosity-average ($\bar{M}_v$) molecular weights were calculated according to eqns (1)–(3):

$$\bar{M}_n = \Sigma h_i / \Sigma (h_i / M_i) \tag{1}$$

$$\bar{M}_w = \Sigma h_i . M_i / \Sigma h_i \tag{2}$$

$$\bar{M}_v = (\Sigma h_i^{\alpha} . M_i / \Sigma h_i)\, 1/\alpha \tag{3}$$

where α is the Mark-Houwink exponent (0·94 for DMSO (LeBel & Goring 1963). From the average-molecular weight value $\bar{M}_n$, the appropriate degree of polymerization DP_n was calculated using the monomer unit M_0 of the xylan with an uronic acid content (UA) 14·6% (Table 1):

$$M_0 = 132 \times 100/(100\% \, \mathrm{UA}).$$

RESULTS AND DISCUSSION

The most evident recognizable effect caused by the action of γ-radiation on the xylan is the decrease of DP_v combined with an increase in water extractibility (Table 2). At the highest radiation dose applied, the water extract of the irradiated xylan was nearly twice as high as that of the original xylan. A more pronounced radiation effect was found (Beynart *et al.*, 1978) on a birch xylan, where about 98% of the sample became water soluble after a ten times lower (30 kGy) radiation dose and its *DP* decreased from 104 to 38. No further changes were observed with increasing radiation doses up to 600 kGy.

The experimental chromatograms of the unfractionated xylan before and after γ-irradiation at various radiation doses are presented in Fig. 1. As is seen, the peak shifts to the lower molecular weight region. Moreover, the polydispersity increased (Table 2).

Plotting the *DP* obtained by SEC and viscometry (Table 2) via irradiation dose, bended curves resulted (Fig. 2), with the same curvature. A slight increase in *DP* can be noted at the highest applied radiation dose.

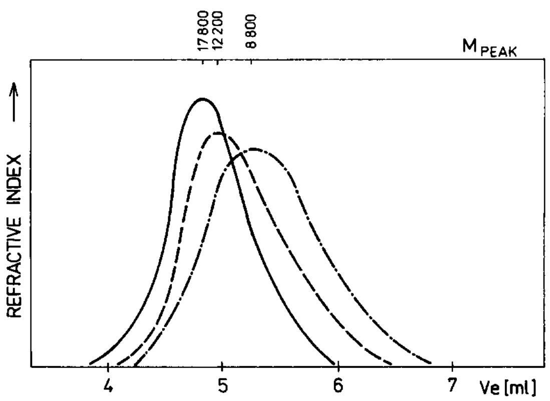

Fig. 1. Experimental chromatograms of the xylan before (——) and after γ-irradiation at 50 kGy (- - -), and 125 kGy (—·—·—).

TABLE 2
Solubility and Molecular Weight Parameter Changes in Irradiated Xylan

Sample No.	*Radiation dose (kGy)*	*Water extract (%)*	*SEC*				*Viscometry*	
			$\bar{M}_w \times 10^{-3}$	$\bar{M}_n \times 10^{-3}$	$\bar{M}_v \times 10^{-3}$	$\bar{M}_w/\bar{M}_n$	DP_v	
							Cadoxen	*DMSO*
1	0	35·7	22·1	14·4	21·8	1·53	133	129
2	12·5	40·7	20·1	12·6	19·8	1·59	117	—
3	25	51·2	18·5	11·2	18·2	1·65	107	105
4	50	49·6	16·0	9·4	15·8	1·70	101	93
5	87·5	49·1	12·7	7·2	12·5	1·76	85	—
6	125	54·5	12·2	6·4	12·0	1·90	76	68
7	300	61·0	12·6	6·6	12·4	1·91	84	71

If the destruction occurs accidentally, eqns (4) and (5) will be valid (Charlesby, 1962):

$$1/DP_n - 1/DP_n^{\circ} = G.D./100.N_A, \tag{4}$$

$$1/DP_v - 1/DP_v^{\circ} = 1/2\ G.D./100.N_A, \tag{5}$$

where DP_n° and DP_n are number-average degrees of polymerization of the xylan before and after γ-irradiation, DP_v° and DP_v are the corresponding viscosity average degree of polymerization, (measured in Cadoxen), D is the absorbed dose in eV, N_A is the Avogadro number, and G is the radiation-chemical yield, which expresses the number of scission per 100 eV of the energy absorbed. As shown in Fig. 3, eqns (4) and (5) well fit the experimental data in the range up to 125 kGy. The obtained G-value is 8 ± 1 scissions/100 eV. Different G-values were reported for γ-irradiated cellulose (Adam, 1983; Burczak *et al.*, 1983; Fischer *et al.*, 1987) ranging from 1 to 15 depending on the used type of cellulose (McLaren, 1978).

The polydispersity index $\bar{M}_w/\bar{M}_n$ changes from 1·5 (original xylan) to 1·9 after irradiation (Table 2). Actually, the ratio 2·0 is approached, which corresponds to the most probable or exponential distribution of the molecular weight. Such MWD can be obtained during a random (accidental) scission of an endless polymer chain. The pattern of the

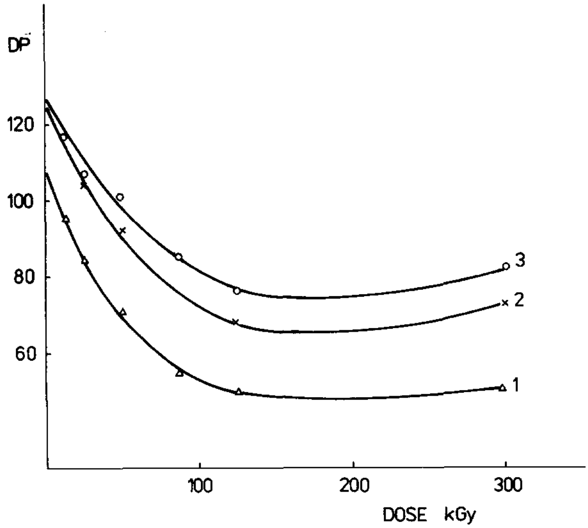

Fig. 2. Depolymerization curve of the xylan in dependence on the radiation dose. (1) DP_n from SEC; (2) DP_v in DMSO; and (3) DP_v in Cadoxen. Values from Table 2.

MWD changes after xylan irradiation proves to be similar to that observed for native cellulose (Kusama *et al.*, 1976), with polydispersity index increasing from 1·26 to 2·0 at absorbed doses higher than 30 kGy. However, this can be compared to a medium-viscosity cellulose (Fischer *et al.*, 1987), where the index decreased from higher values to ~2·0. A distinct behaviour was noted for schizophyllan, a water-soluble polysaccharide with periodic structural sequences, during sonication (Yanaki, 1983). In this case, the $\bar{M}_w/\bar{M}_n$ ratio decreases to values near unity indicating that the chains tend to break at middle portions. From these results it can be concluded that the destruction of both the xylan and cellulose chains occurs randomly and is markedly influenced by their structural and ultrastructural features.

Crosslinking was indicated by a slight increase of *DP* together with a decrease of xylan chain fission observed at the highest radiation dose applied; this was found to proceed also with cellulose as a result of free radical coupling (Burczak *et al.*, 1983, Fischer *et al.*, 1987).

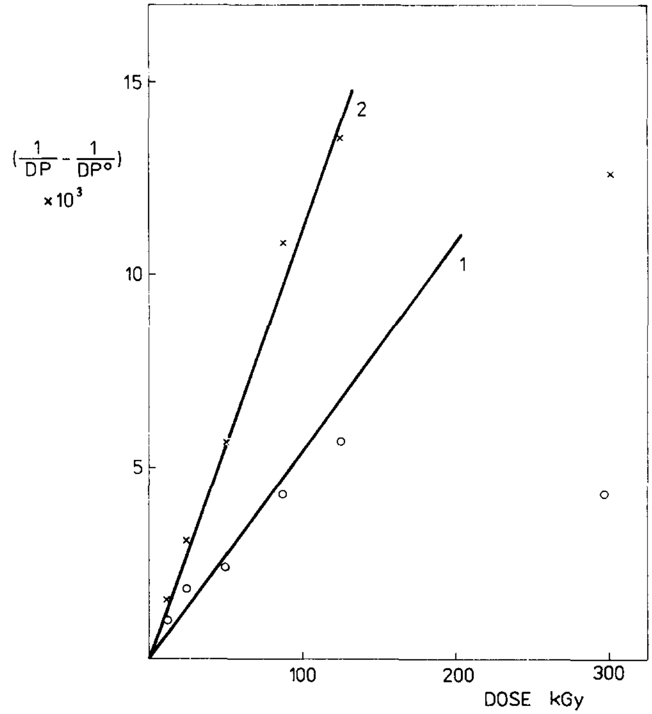

Fig. 3. Degree of scission of the xylan chain in dependence on the radiation dose. (1) DP_v (Cadoxen); (2) DP_n (SEC).

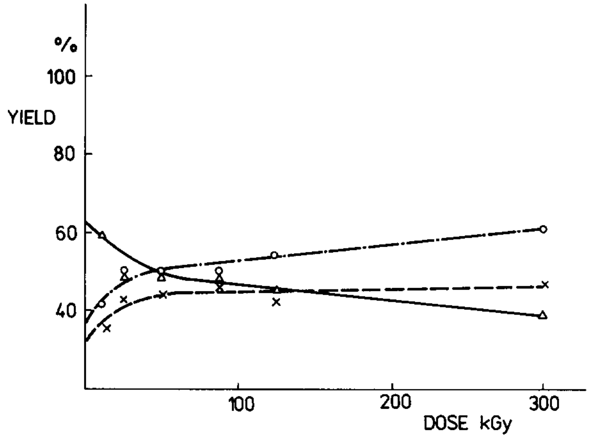

Fig. 4. Influence of the radiation dose on the water extract of xylan and the yield of WS and WIS fractions. (—·—·—) Water extract; (- - -) WS-fraction; (———) WIS-fraction.

The original and irradiated xylan samples were fractionated into WS- and WIS-polymeric fractions. As it is seen in Fig. 4, the yield of both fractions follows the radiation dose dependent 'levelling off' trend of depolymerization (Fig. 2) up to 125 kGy. Whereas at doses higher than 25 kGy the water extract and the WIS-fraction show distinct yield changes, the yield of the WS-fraction remained almost unchanged. This supports the aforementioned hypothesis of crosslinking. In contrast to cellulose where crosslinking leads to formation of gels, in the case of the xylan the present uronic acid side chains in their ionized form might solubilize to a limited extent the crosslinked chains, thus increasing the molecular weight without substantial change in the yield of the WS-fraction.

The study of free radical formation and degradation of xylan submitted to high-energy treatment (Ershov *et al.*, submitted) shows that its destruction, like that of cellulose, occurs not only in the form of glycosidic bonds scission, but also of monomer unit decomposition. This process is accompanied by the formation of carbonyl and carboxyl groups and by release of carbon dioxide and hydrogen. Further investigations in this direction will be reported in a separate paper.

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