Hemicelluloses form Twigs of the White Willow (*Salix alba* L.) (III) Wood Xylans

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Purification of three hemicellulose fractions isolated by fraction extraction of holocellulose obtained from wood of white willow twigs yielded electrophoretically homogeneous fractions of (4-*O*-methylglucurono)-xylans, possessing common structural features, but differing in molecular and equivalent weights. The molecular weights of the single fractions of (4-*O*-methylglucurono)-xylans were 8300, 12 600 and 12 500, while the ratio between 4-*O*-methyl-D-glucuronic acid and D-xylose was found to be 1 6, 1 8 and 1 10, respectively.

In the preceeding paper [1] the isolation of the crude fractions of hemicelluloses was reported. The isolated fractions DA, DH 1 and DH 2/1 and DH 2/2, containing a xylan-type polysaccharide as a main component, were purified by precipitation with Fehling solution and solution of barium hydroxide. Three homogeneous fractions of (4-*O*-methylglucurono)-xylans were obtained by this procedure. On the basis of the identification of acidic oligosaccharides obtained as a result of a partial hydrolysis of polysaccharides, the results of periodate oxidation and hydrolysis of the methylated polysaccharides, their optical rotation data and equivalent weights, the following structural features of (4-*O*-methylglucurono)-xylans of wood of white willow twigs could be suggested:

$$-[\rightarrow 4\text{-}\beta\text{-D-Xylp-1}-]_{n}\rightarrow 4\text{-}\beta\text{-D-Xylp-1}-[\rightarrow 4\text{-}\beta\text{-D-Xylp-1}-]_{m}\rightarrow$$

$$\begin{matrix} & 2 \\ & \uparrow \\ & 1 \end{matrix}$$

4-*O*-Me-α-D-GlcpUA

DP of the backbone of the single polysaccharides is 80, 80 and 50, and D-Xylp : 4-*O*-Me-GlcpUA ratio 6 1, 8 1 and 10 1, respectively.

Experimental Part and Results

Melting points were determined on a Kofler hot-stage. Optical rotation was measured at 24 °C; in the case of polysaccharides 1 % solution in 2 % NaOH was used, while saccharides and their derivatives were measured as 1 % aqueous solutions, if not otherwise stated.

Free electrophoresis was carried out in a borate buffer solution (pH 11) in 2 % concentration and potential gradient 47.6 V/cm, using Kern LK 30 apparatus.

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For paper chromatography descending technique and the following systems were used:

S 1: ethylacetate—acetic acid—water 18 : 7 8,
S 2: ethylacetate—pyridine—water 8 : 2 : 1,
S 3: *n*-butanol—ethanol—water 40 : 11 19.

For qualitative paper chromatography Whatman No 1 and for preparative purposes Whatman No 3 papers were used.

Detection of polysaccharides was performed by anilinium hydrophthalate [2]. Evaporations were carried out on a rotatory evaporator in vacuo at 40 °C.

Methoxyl groups were determined by the method of Viebeck and Becher [3].

Carboxyl group determination was carried out by decarboxylation method [4].

Molecular weights were measured osmometrically by the static method in osmometer of Zimm—Mayerson type, using membranes Allerfeinst, Göttingen in 1 % NaOH solution. The consumption of oxidation agent and the amount of formic acid liberated by the oxidation of saccharides with sodium metaperiodate was determined iodometrically [5, 6].

The equivalent weight of aldouronic acids was determined iodometrically, according to the amount of iodine liberated from KIO_3 and KI by the known amount of aldouronic acid. The nature of the linkage between 4-*O*-methyl-D-glucuronic acid and D-xylose in the isolated aldouronic acids was established by periodate oxidation.

Purification of the Crude Fractions of Hemicelluloses

The fractions DA and DH 1 were purified by precipitation with Fehling solution [7]. From fractions DH 2/1 and DH 2/2 hexosan containing polysaccharides were removed by complexing with Ba^{2+} ions [8], centrifuged and from the supernantant, after removing the excess of barium hydroxide with carbon dioxide, precipitation with Fehling solution afforded (4-*O*-methylglucurono)-xylan. The isolated polysaccharides (*I* — obtained from fraction DA, *II* — from DH 1 and *III* from DH 2/2), according to a free electrophoresis were shown to be homogeneous. The polysaccharide isolated from fraction DH 2/2 was found to be identical with polysaccharide *III*. After hydrolysis, from the neutral monosaccharides by paper chromatography, comparing with the authentic sample, only D-xylose was proven to be present.

The summary of the results of the analysis of homogeneous (4-*O*-methylglucurono)-xylans is given in the Tab. 1.

Table 1

The results of characterisation of (4-*O*-methylglucurono)-xylans

(4-*O*-methyl-glucurono)-xylan	Yield of the homogeneous fraction g	Molecular weight	$[\alpha]_D^{24}$	—OCH_3	—COOH	Equivalent weight		xyl : 4-*O*-Me-GlcUA	
						1	*2*	*1*	*2*
I	12	8 300	—57°	3,15	4,7	984	957	5,9	5,7
II	14	12 600	—72°	2,4	3,6	1288	1250	8,2	7,9
III	0,5	12 500	—88°	2,0	3,1	1550	1452	10,1	9,4

1. Calculated from —OCH_3 contents,
2. calculated from —COOH contents.

Oxidation of (4-O-Methylglucurono)-xylans

The polysaccharides *I* and *II* were oxidised in the dark in 100 ml of 0.05 M sodium metaperiodate solution, both at 5 °C and room temperature for 250 hours. The consumption of oxidising agent and the amount of formic acid formed were determined iodometrically every 24 hours. Periodate consumption determined by extrapolation to $t = 0$ was in all cases the same (approximatively 1 mol IO_4^-/mol of D-xylose) and temperature independent. Temperature influenced the rate of oxidation only. The formation of formic acid did not terminate after 250 hours of oxidation, when oxidation has been finished. Oxidation of the polysaccharides was practically finished after 150 hours.

Methylation of (4-O-Methylglucurono)-xylans

The polysaccharides *I*, *II* and *III* were methylated with dimethylsulfate in acetone [9] and the partially methylated product with methyl iodide in the presence of Ag_2O [10]. The totally methylated product in the IR spectrum in the region 3600—3400 cm^{-1} exhibited minimal absorption. The contents of methoxyl groups — 39.1, 38.8 and 38.6 % — has not increased on repeated methylation.

Hydrolysis of the methylated polysaccharides was performed with 72 % H_2SO_4 [11]. In the hydrolyzates on comparison by paper chromatography in S 3 with the authentic samples mono-*O*-methyl, di-*O*-methyl and tri-*O*-methyl derivatives of D-xylose were found. Di-*O*-methyl derivative of D-xylose, which formed the main part of the hydrolyzates of the three fractions of the methylated (4-*O*-methylglucurono)-xylans was 2,3-di-*O*-methyl-D-xylose. This was isolated from the hydrolyzates by preparative paper chromatography in S 3 and identified through anilide-(2,3-di-*O*-methyl-*N*-phenyl-D-xylo-pyranosylamine), m. p. 145 °C and $[\alpha]_D^{24} = +181°$ (*c* 1.5, in ethylacetate). Two isomorphic structures of 2,3-di-*O*-methyl-*N*-phenyl-D-xylo-pyranosylamine exist [12]: one with m. p. 126 °C and the second one with m. p. 145 °C. For both isomorphic structures the same optical rotation $[\alpha]_D^{25} = +180°$ (in ethylacetate) was reported.

Partial Hydrolysis of (4-O-Methylglucurono)-xylans

a) Preparation of aldouronic acids

The polysaccharide *II* (10 g) had been mixed with 1 l of hot distilled water, where 50 g of washed and dried ion exchanger Dowex 50 WX4 (200—300 mesh, in H^+ form) was added. The mixture was warmed on a steam bath under stirring for 18 hours. This time was experimentally shown to be optimal for partial hydrolyzis of used polysaccharide.

After the reaction mixture was cooled, ion exchanger was filtered off and without any additional treatment was worked up to obtain the acidic and neutral portions, by trapping the acidic part on ion-exchanger Amberlit IRA-402 (20—50 mesh, in acetate form). The neutral saccharides were washed out with distilled water to negative phenol-test [13]. The eluate was distilled off and after drying over P_2O_5 the yield of the neutral fraction was 7.8 g. By paper chromatography in S 1 it was shown, that the neutral oligosaccharides are forming an homological sequence (Fig. 1). Their isolation and further characterisation has not been carried out.

The acidic oligosaccharides were washed out from the ion-exchanger by 6 N acetic acid. The eluate was distilled off and after drying over P_2O_5 the yield of the acidic fraction was 2.9 g. The acidic polysaccharides are forming an homological sequence as well (Fig. 2).

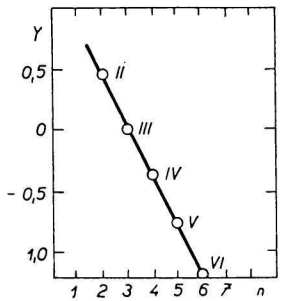

Fig. 1. Homological series of the neutral oligosaccharides.

$$Y = \log \frac{R_x}{1 - R_x},$$

where R_x stands for chromatographic mobility in S 2, reffering to D-xylose; n number of units.

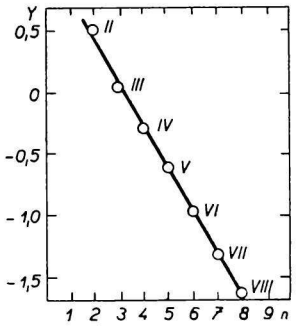

Fig. Homological series of the acidic oligosaccharides.

$$Y = \log \frac{R_m}{1 - R_m},$$

where R_m stands for chromatographic mobility in S 1, reffering to 4-*O*-methyl-D--glucuronic acid; n number of units.

b) Isolation of aldouronic acids

The single acidic oligosaccharides were isolated by paper chromatography in S 2. The isolated aldouronic acids were rid off cations originated from a chromatographic paper by percolation through Dowex 50 WX4 column (100—200 mesh in H^+ form). After evaporation and drying over P_2O_5 the samples were weighted.

c) Partial hydrolysis of the aldouronic acids

Partial hydrolysis of the aldouronic acids was carried out using Dowes 50 WX4 (200 — —400 mesh, in H^+ form) in sealed vials for 6 hours at 100 °C. In all instances by paper chromatography in S 1 and S 2 in hydrolyzates 4-*O*-methyl-D-glucuronic acid, D-xylose and small amount of the starting material were found. From the higher aldouronic acids by the partial hydrolysis all lower homologues of the acidic and neutral oligosaccharides were formed.

d) Reduction of aldouronic acids

The single isolated acidic oligosaccharides were transformed to methyl ester methyl glycoside [10], reduced with $LiAlH_4$ and after hydrolysis of the resulting neutral oligosaccharide the presence of D-xylose and 4-*O*-methyl-D-glucose was demonstrated by the paper chromatography in S 3, by comparing the mobility with the authentic specimen with the substances in hydrolyzate.

e) The equivalent weight of the aldouronic acids

To the aqueous solution of 5—25 mg of the sample a surplus of KIO_3 and KI was added and liberated iodine was titrated with N/100 solution of sodium thiosulfate. The equivalent point was indicated amperometrically.

f) Identification of the aldouronic acids

Oxidation of aldobiuronic acid (24.2 mg) was performed in 0.05 M aqueous solution of sodium metaperiodate at 5 °C. Consumption after 48 hours was 2.2 moles of IO_4^-

per mole of aldobiuronic acid and no formic acid was formed. Theory for 2-*O*-(4-*O*-methyl-D-glucuronopyranosyl)-D-xylose requires 2 moles of IO_4^-/mol, with no formic acid formed.

Acetylation of methyl ester methyl glycoside of aldobiuronic acid was carried out according to reference [14]. Isolated crystalline methyl ester methyl 2-*O*-(2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucuronopyranosyl)-3,4-di-*O*-acetyl-D-xylopyranoside has m. p. 200 °C, $[\alpha]_D^{24} = 103°$ (*c* 1, in chloroform). In literature [14, 15] m. p. 200—202 °C and $[\alpha]_D = 100 - 106$ °C are reported.

Higher aldouronic acids (15 — 30 mg) were transformed to the respective methyl ester methyl glycoside by treatment with 2.5 % HCl in methanol for 10 days at room temperature [16]. After the reaction mixture was made neutral with Ag_2CO_3, methyl ester methyl glycoside of oligosaccharide was subjected to oxidation in 0.05 solution of $NaIO_4$ in the dark at room temperature. The samples were taken and analysed. Periodate consumptions were in the case of aldotriuronic, aldotetrauronic, aldopentauronic and aldohexuronic acid 3.01, 3.95, 5.06 and 6.1 moles of periodate per mole of substance, respectively. By the oxidation, there was no formic acid formed. Periodate consumption required for the oxidation of one mole of methyl ester methyl glycoside of the above given aldouronic acids with 4-*O*-methyl-D-glucuronic acid attached at C-2 of D-xylose, the latter being at the same time a not-reducing unit of the molecule, are 3, 4, 5 and 6 moles of periodate. In this case formic acid should not be formed by oxidation.

Table 2

Summary of the results of the isolated aldouronic acids

No	Isolated substance	Yield mg	R_m**	—OCH_3 %		Equivalent weight		$[\alpha]_D^{24}$	m. p. °C
				found	required	found	required		
I	4-*O*-methyl-D-glucuronic acid	67	1	14,7	14,9	195	208	+80	
II	2-*O*-(4-*O*-methyl-α-D-glucuronopyranosyl)-D-xylopyranose	380	0,77	9,3	9,1	322	340	+92	
III	*O*-(4-*O*-methyl-α-D-glucuronopyranosyl)-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranose	440	0,52	6,2*	5,9*	470	472	+51	173—179*
IV	*O*-(4-*O*-methyl-α-D-glucuronopyranosyl)-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranose	212	0,33		5,13	610	604	+26	
V	4-*O*-methyl-glucuronopyranosyl-xylotetraose	155	0,2	4,3	4,2	743	736	+ 1	
VI	4-*O*-methyl-glucuronopyranosyl-xylopentaose	110	0,09	3,7	3,7	855	868	—10	
VII	4-*O*-methyl-glucuronopyranosyl-xylohexaose	92	0,04	3,15	3,08	1020	1000	—22	
VIII	4-*O*-methyl-glucuronopyranosyl-xyloheptaose	80	0,02	2,62	2,72	1158	1132	—28	

* Crystalline trihydrate,

** R_m chromatographic mobility reffering to 4-*O*-methyl-D-glucuronic acid in S 1.

The summary of the other results of identification of the obtained aldouronic acids is listed in Tab. 2.

On the partial hydrolysis of the polysaccharides *I* and *III* the same homological series of oligosaccharides were formed, as on the partial hydrolysis of polysaccharide *II*.

Discussion

Purification of three rough hemicellulose fractions isolated from wood of white willow twigs, the main part of which was polysaccharide of xylan-type, afforded three polysaccharides, which were shown to be homogeneous on free electrophoresis and after hydrolysis from the neutral components yielded D-xylose only. On their partial hydrolysis a homological series of neutral and acidic oligosaccharides were formed. The acidic polysaccharides were composed from D-xylose, and 4-*O*-methyl-D-glucuronic acid only. The single (4-*O*-methylglucurono)-xylans were differing by the equivalent weights and consequently by the D-xylose—4-*O*-methyl-D-glucuronic acid ratio. This in polysaccharide *I* was found to be 6 1, in polysaccharide *II* 8 1 and in polysaccharide *III* 10 1. The number average degree of polymerisation of the backbone, calculated from the molecular and equivalent weights were as follows:

1. (4-*O*-methylglucurono)-xylan *I* 50,
2. (4-*O*-methylglucurono)-xylan *II* 80,
3. (4-*O*-methylglucurono)-xylan *III* 80.

From the obtained results it could be suggested, that by fraction extraction, the more concentrated alkaline solution was used for extraction, the less acidic (4-*O*-methylglucurono)-xylans were isolated.

The results of periodate oxidation of the homogeneous fractions of (4-*O*-methylglucurono)-xylans which established the consumption of oxidation agent approximatively 1 mole of periodate for 1 mole of D-xylose, together with isolation of 2,3-di-*O*-methyl-D-xylose, originating after hydrolysis of the totaly metylated polysaccharide indicated, that the backbone of these polysaccharides was composed of D-xylose units linked together by (1→4) glycosidic bonds. By oxidation of the polysaccharides with sodium periodate more formic acid was liberated, as required by theory for unbranched (4-*O*-methylglucurono)-xylan. Similar discrepancies, however, were observed for the polysaccharides with uronic acid units attached [17, 18].

Xylan-type polysaccharides exhibited as a consequence of a β-D-xylopyranose composed main chain negative values of optical rotation. For the neutral xylan the value $[\alpha]_D = -110°$ was given [19]. 4-*O*-Methyl-D-glucuronic acid attached, shifts the optical rotation to the more positive values, which proves its α-glycosidic bond. (4-*O*-Methylglucurono)-xylan *III* was the less acidic (the ratio between D-xylose and 4-*O*-methyl-D-glucuronic acid being 10 1) and exhibited so the highest optical rotation (−88°); the lowest negative rotation (−57°) was observed for (4-*O*-methylglucurono)-xylan *I*, which is the most acidic one (D-xylose—4-*O*-methyl-D-glucuronic acid ratio 6 1).

Summarising the presented results, it can be stated, that the polysaccharides from wood of willow twigs are (4-*O*-methylglucurono)-xylans. Their backbone is formed by β-(1→4)-D-xylopyranose units α-(1→2) branched by glycosidically linked 4-*O*-methyl-D-glucuronic acid.

The structure of the studied (4-*O*-methylglucurono)-xylans from wood of white willow (*Salix alba* L.) twigs is similar to the structures of polysaccharides previously found in hornbeam (*Carpinus betulus* L.) [20], birch (*Betula papyrifera* MARSCH) [21], trembling poplar (*Populus tremuloides* MICHX) [22] and other hardwoods, except that the number average degree of polymerisation were mostly significantly higher. Lower degrees of polymerisation of (4-*O*-methylglucurono)-xylans from wood of white willow twigs are probably due to the nature of the starting material and the methods of isolation used.

The fraction extraction [1] enabled the isolation of (4-*O*-methylglucurono)-xylans differing by their molecular weight. It is possible, that in wood of hardwoods, series of (4-*O*-methylglucurono)-xylans of different acidities are generally present. This fact, however, remain unrecognised if hemicellulose were isolated by one-step alkaline extraction. An evidence for our suggestion has been presented by C. P. J. Glaudemans and T. E. Timell in 1957 [21], who reported the isolation of (4-*O*-methylglucurono)-xylan from holocellulose of birch (*Betula papyrifera* MARCH) by the extraction with 24 % KOH. The ratio of 4-*O*-methyl-D-glucuronic acid to D-xylose in this case was 1 11, while I. Croon [23] obtained by the stepwise extraction of holocellulose from the same species (4-*O*-methylglucurono)-xylans with different contents of uronic acids.

HEMICELULÓZY Z VETVIČIEK VŔBY BIELEJ (*Salix alba* L.) (III) XYLÁNY Z DREVNEJ ČASTI

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Tri hrubé frakcie hemicelulóz, získané z holocelulózy dreva vetvičiek vŕby bielej (*Salix alba* L.) frakčnou extrakciou, prečistili sa zrážaním Fehlingovým roztokom a roztokom hydroxidu bárnatého. Získali sa tak tri polysacharidy typu (4-*O*-metylglukurono)xylánov. Hlavný reťazec týchto polysacharidov je zložený z β-(1→4) viazaných jednotiek D-xylopyranóz a je vetvený α-(1→2) viazanou kyselinou 4-*O*-metyl-D-glukurónovou. Molekulová váha jednotlivých elektroforeticky homogénnych (4-*O*-metylglukurono)-xylánov je 8300, 12 600 a 12 500. Pomer medzi D-xylózou a kyselinou 4-*O*-metyl-D-glukurónovou v jednotlivých polysacharidoch je 1 6, 1 8, resp. 1 10.

ГЕМИЦЕЛЛЮЛОЗЫ ИЗ ВЕТВЕЙ ИВЫ (*Salix alba* L.) (III) КСИЛАНЫ ДРЕВЕСИНЫ

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Три фракции гемицеллюлоз, первоначально полученные фракционированной экстракцией из голоцеллюлозы древесины ветвей ивы (*Salix alba* L.), были очищены осаждением при действии раствором Фелинга и раствором гидроокиси бария. При

этом получили три полисахарида типа (4-*O*-метилглюкуроно)ксиланов. Основная цепь этих полисахаридов состоит из β-(1→4)-связанных звеньев D-ксилопираноз и разветвляется α-(1→2)-связанной 4-*O*-метил-D-глюкуроновой кислотой. Молекулярный вес отдельных электрофоретически гомогенных (4-*O*-метилглюкуроно)ксиланов равен 8300, 12 600 и 12 500. Соотношение между D-ксилозой и 4-*O*-метил-D-глюкуроновой кислотой в отдельных полисахаридах составляет 1 6, 1 8 и 1 10.

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REFERENCES

1. Kováč P., Rendoš F., *Chem. zvesti* ***20***, 862 (1966).
2. Partridge S. M., *Nature* ***164***, 443 (1949).
3. Jureček M., *Organická analysa*, 338. Nakladatelství ČSAV, Praha 1967.
4. Johansson A., Lindberg B., Theander O., *Svensk Papperstidn.* ***57***, 41 (1954).
5. Whistler R. L., Hickson J. L., *J. Am. Chem. Soc.* ***76***, 1671 (1954).
6. Babor K., Kaláč V., Tihlárik K., *Chem. zvesti* ***18***, 9113 (1964).
7. Adams G. A., *Methods in Carbohydrate Chemistry* ***5***, 170 (1965).
8. Meier H., *Methods in Carbohydrate Chemistry* ***5***, 45 (1965).
9. Hirst E. L., Percival E., *Methods in Carbohydrate Chemistry* ***5***, 287 (1965).
10. Adams G. A., *Can. J. Chem.* ***35***, 556 (1957).
11. Garegg D. J., Lindberg B., *Acta Chem. Scand.* ***14***, 871 (1960).
12. Erenthal I., Rafique M. C., Smith F., *J. Am. Chem. Soc.* ***74***, 1341 (1952).
13. Dubois M., Gilles K. A., Hamilton J. K., Rebers P. A., Smith F., *Anal. Chem.* ***28***, 350 (1956).
14. Timell T. E., *Can. J. Chem.* ***36***, 827 (1959).
15. Bremner J., Wilkie K. C. B., *Carbohydrate Res.* ***2***, 24 (1966).
16. Hamilton K. J., Thompson N. S., *J. Am. Chem. Soc.* ***79***, 6464 (1957).
17. Zinbo M., Timell T. E., *Svensk Papperstidn.* ***68***, 647 (1965).
18. Chanda S. K., Hirst E. L., Percival E. G. V., *J. Chem. Soc.* ***1951***, 1240.
19. Guttman P. J., Timell T. E., *J. Polymer Sci.*, Part C, No 11, 281 (1965).
20. Rendoš F., Pavlovová E., Kováč P., *Cellulose Chemistry and Technol.* ***1***, 711 (1967).
21. Glaudemans C. P. J., Timell T. E., *J. Am. Chem. Soc.* ***80***, 1209 (1958).
22. Koshijima T., Timell T. E., Zinbo M., *J. Polymer. Sci., Part C*, No 11, 265 (1965).
23. Croon I., Enström B. F., *Tappi* ***44***, 870 (1961).

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