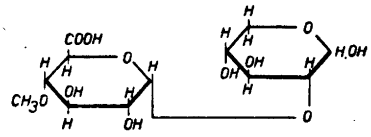
ACTA CHEMICA SCANDINAVICA 8 (1954) 825—828

Structure of an Acidic Xylan Isolated from Birch Wood Holocellulose

JOUKO SAARNIO, KRISTINA WATHÉN and CHARLEY GUSTAFSSON

Oy Keskuslaboratorio — Centrallaboratorium Ab, Helsinki, Finland

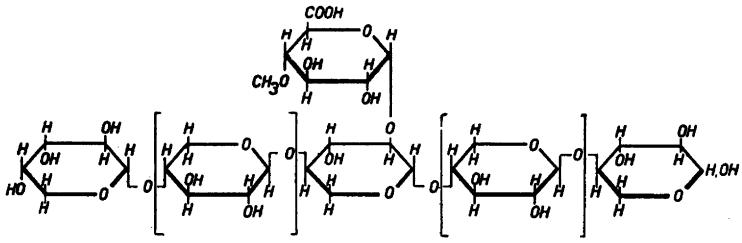
In a previous work Gustafsson *et al.*[1] have proved chromatographically that the carbohydrates from birch wood (*Betula verrucosa*) are built up chiefly from glucose (58 %) and xylose (38 %) and from small amounts of galactose, arabinose, mannose and rhamnose all together about 4 %. It was found that birch wood also contains other compounds similar to carbohydrates, which however, were not identified. To investigate the nature and origin of these compounds holocellulose was prepared from birch wood according to Chanda *et al.*[2]. The holocellulose was extracted with *N* sodium hydroxide and an acidic xylan fraction was isolated from the extract by precipitating with acetic acid. The raw xylan was purified as a copper complex. The purified xylan fraction was hydrolysed, and a paper chromatographic analysis showed that the hydrolysate contained xylose and monomethylaldobiuronic acid only. The monomethylaldobiuronic acid was isolated, after a graded hydrolysis, by elution from a cellulose column. A paper chromatographic study proved that the R_F values and colour reactions of the acid were the same as for 2-*O*-(4-*O*-methyl-α-D-glucuronosido)-D-xylose, which has been isolated by Jones[3] from spruce wood. The methoxyl and carbonyl content was determined and found to be in agreement with the theoretical values. It was thus considered that the aldobiuronic acid is identical with 2-*O*-(4-*O*-methyl-α-D-glucuronosido)-D-xylose I.



The 4-*O*-methylaldobiuronic acid mentioned above has previously been isolated not only from spruce wood but also from aspen wood (*Populus tremuloides*)[4] and from *Eucalyptus regnans*[5]. 4-*O*-methyl-glucuronic acid has been found in several plant gums such as mesquite[6] and myrrh[7] gums.

By oxidation of the xylan fraction in question, with 0.3 *M* sodium periodate according to Chanda [2] it was shown that the periodate uptake was about one mole per $C_5H_8O_4$ unit. The reaction however, was not complete, and one xylose unit per 22 $C_5H_8O_4$ units remained unoxidized. The formic acid release from the periodate oxidation of xylan was determined in principle according to Halsall *et al.* [8], and the yield of formic acid produced was 0.142 moles per $C_5H_8O_4$ unit. A viscometric determination in formamide of the molecular weight according to Husemann [9] ($K_m = 8 \cdot 10^{-3}$) gave a DP value of 24—25 units. Titration of the xylan with sodium hydroxide gave an equivalent weight o 3 140, which corresponds to 24 $C_5H_8O_4$ units. Uronic acid determination according to Whistler *et al.*[10] gave 0.06 moles of CO_2 per $C_5H_8O_4$ unit, and a methoxyl micro determination according to Zeissel [11] 0.06 moles per $C_5H_8O_4$ unit.

From the above consideration it seems justifiable to assign the acidic xylan fraction the following structure II:



The xylan forms a straight chain built up from about 22 xylopyranose units. The xylopyranose units are joined together with 1,4-β linkages. In the main chain there is a single branching point, and the side chain is formed from one 4-methylglucuronic acid residue only, which is linked to the main chain by a 1,2 bond. No conclusions as to the position of the branching point on the main chain can be drawn.

This xylan fraction is only a part of the birch wood hemicelluloses, which probably contain other types of xylans too. From the literature on wood hemicelluloses it is evident that the xylans from plants belonging to different botanical systematic groups vary considerably in respect to their structure and composition. On the other hand it seems probable that *e. g.* all hardwoods contain xylan of the same type as the acidic birch wood xylan.

EXPERIMENTS

Preparation of birch holocellulose. 200 g (dry-matter 89.5 %) of birch wood (*Betula verrucosa*) flour extracted with acetone was suspended in 8 800 ml of water containing 1 000 g of acetic acid (98 %) and 1 000 g of sodium chlorite. The mixture was heated to 60° C and kept at that temperature for one minute. Sodium acetate (40 g) was added, and the reaction mixture was transferred to a thermostat at 30° C and kept there with occasional shaking for 20 hours. The brownish yellow mother liquor was filtered off through a glass filter, and the holocellulose was washed with cold water to remove the salts. After that the water was displaced by acetone. The yield was 77.5 % of the original wood.

	Ash %	Pentosan %[12]	CO_2 %[10]	$-OCH_3$ %[11]	Lignin %[13]
Birch wood	0.33	24.7	0.84	6.8	20.4
Birch holocellulose	0.98	29.7	1.35	3.23	4.0

Isolation and purification of xylan. 100 g (dry-matter 88.9 %) of holocellulose was extracted with *N* sodium hydroxide in a nitrogen atmosphere for 20 hours at room temperature. The mixture was filtered, and the residue was washed with water. The filtrate and the water used for washing were poured together and the pH of the solution was adjusted to 5.5 with acetic acid. Part of the dissolved polysaccharides was thereby precipitated. The precipitate was centrifuged and washed twice with ethanol-water (1 : 1) and then twice with ethanol in order to displace the water. Finally the ethanol was displaced with ether. The precipitate was dried *in vacuo* at 50° C. The yield was 18.5 g (Xyl. I).

10 g (Xyl. I) was dissolved in *N* sodium hydroxide solution and was precipitated with Fehling's solution as a copper complex. The polysaccharide copper complex was filtered off, and the complex was decomposed with 1.5 *N* hydrochloric acid. The undissolved part was centrifuged off, and washed first with ethanol : water : hydrochloric acid (45 : 40 : 10) to remove the copper salts, then with ethanol : water mixtures in with the ethanol content was increased for each washing, and finally with pure ethanol and ether. The precipitate was dissolved in *N* sodium hydroxide and reprecipitated with acetic acid. The precipitate was washed and dried as before. Yield 5.9 g (Xyl. II).

	Ash %	Lignin %[13]	$-OCH_3$ %[11]	CO_2 %[10]	$[\alpha]_D^{20}$ (NaOH)
Xyl. I	—	+	1.84	1.78	−80.0 (c = 0.5)
Xyl. II	0.39	0	1.65	2.01	−77.0 (c = 2.5)

Isolation of 4-methylglucuronosidoxylose. 3 g (Xyl. II) was dissolved in 72 % sulfuric acid and the solution was diluted with water to form a 4 % solution. The solution was boiled for 6 hours and neutralized with barium carbonate. Ba^{++} ions were then removed by an ion-exchange resin (Amberlite IRC 50). The solution was evaporated to dryness, and the residue was extracted with boiling methanol. The clear methanolic solution was concentrated to a yellowish syrup. Examination with different solvents on paper chromatogram such as butanol : ethanol : water (4 : 1 : 5), butanol : pyridine : benzene : water (5 : 3 : 1 : 3) and ethyl acetate : acetic acid : formic acid : water (18 : 3 : 1 : 4), showed, on development with aniline oxalate and *p*-anisidine hydrochloride, the presence of xylose and 4-methylglucuronosidoxylose only. As substances for comparison pure specimens of D-xylose and 4-methylglucuronosidoxylose were used. Aniline oxalate gave an orange spot with 4-methylglucuronosidoxylose, the spot having an intensive orange fluorescence in ultraviolet light.

The 4-methylglucuronosidoxylose was isolated by elution from a cellulose column, ø 22 mm and 340 mm high. Butanol : ethanol : water (2 : 3 : 5) was used as eluant, and the fractions were collected in test tubes by using an automatic fraction collector. The contents of the tubes that indicated only 4-methylglucuronosidoxylose on paper chromatogram were then grouped and the solvent was distilled off *in vacuo*. The residue was light yellow and hygroscopic. Hypoiodite oxidation of the isolated substance calculated as $C_{12}O_{11}H_{20}$ indicated a purity of 97 %. Hydrolysis with *N* sulfuric acid for 6 hours gave only traces of xylose and unhydrolysed 4-methylaldobiuronic acid, as was the case with the substance used for comparison. (Found: OCH_3 8.87. Calc. for $C_{12}O_{11}H_{20}$ (340): OCH_3 9.10.)

Periodate oxidation of xylan. 300 mg (Xyl. II) was suspended in 25 ml of water and treated in the dark with 25 ml of 0.3 *M* sodium metaperiodate at room temperature. Samples were drawn from the solution at intervals. The rotation and amount of periodate consumed (per $C_5H_8O_4$) were determined. The following results were obtained:

Time	20.5 h	90.0 h	164.0 h	228.0 h
$[\alpha]_D^{20}$	64.7°	83.3°	89.7°	85.3°
$\frac{NaJO_4}{C_5H_8O_4}$	0.73	1.11	1.25	1.39

A similar oxidation was also carried out at pH 3.8 in a solution buffered with sodium acetate. The periodate consumed after 91 hours per $C_5H_8O_4$ unit was 1.043 moles of $NaJO_4$.

After oxidation the sodium periodate was destroyed with ethylene glycol. The solution was dialysed against tap water, concentrated to a syrup, and hydrolysed. Analysis by paper chromatography showed the presence of 4.4 % xylose calculated on the original xylan, which means that approximately one xylose unit per 22 $C_5H_8O_4$ units had escaped oxidation.

300 mg (Xyl. II) was suspended in 30 ml of water and 10 ml of 16 % potassium chloride solution and 20 ml 0.3 *M* sodium metaperiodate were added. The oxidation was carried out in the dark at room temperature. Samples were taken at intervals and the remaining sodium periodate was destroyed with ethylene glycol. The formic acid liberated was titrated with 0.01 *N* sodium hydroxide and found to be constant after 120.0 h *i.e.* 0.142 moles per $C_5H_8O_4$ unit.

Equivalent weight by titration. The xylan (Xyl. II) was titrated after dialysis. 100.0 mg (Xyl. II) consumed 3.12 ml of a 0.0102 *N* sodium hydroxide solution, corresponding to an equivalent weight of 3 140.

Viscosity determination. The viscosities of xylan (Xyl. II) in formamide were measured in an Ubbelohde viscometer at 20° C.

C g/100 ml	Solution	Solvent	$\eta sp/C$	DP
0.0958	422.4	414.9	0.19	24
0.1868	430.3	414.9	0.20	25

SUMMARY

An acidic xylan fraction was isolated from birch wood *(Betula verrucosa)* holocellulose. By hydrolysis it gave xylose and 4-*O*-methyl-(2-*O*-glucurono-sido)-D-xylose only. The xylan forms a straight chain of about 22 xylopyranose units. The xylopyranose units are joined together with 1,4-β linkages. In the main chain there is a single branching point, and the side chain is formed from one 4-methyl-glucuronic acid residue only. This has been linked to the main chain by a 1,2 bond.

The authors are indebted to Dr J. K. N. Jones (Bristol) for the sample of 4-methyl-glucuronosidoxylose.

REFERENCES

1. Gustafsson, Ch., Ollinmaa, P. J. and Saarnio, J. *Acta Chem. Scand.* **6** (1952) 1299.
2. Chanda, S. K., Hirst, E. L., Jones, J. K. N. and Percival, G. V., *J. Chem. Soc.* **1950** 1289.
3. Jones, J. K. N., *Private Communication.*
4. Jones, J. K. N. and Wise, L. E. *J. Chem. Soc.* **1952** 2750, 3389.
5. Stewart, C. M. and Foster, D. H. *Nature* **171** (1953) 792.
6. White, E. V. *J. Am. Chem. Soc.* **70** (1948) 367.
7. Hough, L., Jones, J. K. N. and Wadman, W. H. *J. Chem. Soc.* **1952** 796.
8. Halsall, T. G., Hirst, E. L. and Jones, J. K. N. *J. Chem. Soc.* **1947** 1427.
9. Husemann, E. *J. prakt. Chem.* **155** (1940) 13.
10. Whistler, R., Martin, A. and Harris, M. *J. Research Natl. Bur. Standards* **24** (1940) 13.
11. Pregl, F. „*Die quantitative organische Microanalyse*", Berlin 1935, p. 211.
12. Oy Keskuslaboratorio — Centrallaboratorium Ab. *Testing Methods* (in Finnish) (1941) No. A 4061.
13. Oy Keskuslaboratorio — Centrallaboratorium Ab. *Testing Methods* (in Finnish) (1941) No. A 4051.

Received February 18, 1954.