LXXXIX. THE HEMICELLULOSES OF THE WOOD OF ENGLISH OAK

IV. THE STRUCTURE OF HEMICELLULOSE A

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PREVIOUS work [O'Dwyer, 1934] has shown that the hemicellulose A of oak wood is a mixed polysaccharide containing anhydroxylose and uronic anhydride units and that the sapwood product differs from that obtained from heartwood in that it gives with iodine the blue colour which is characteristic of starch. It has since been shown [O'Dwyer, 1937] that the blue colour reaction given by the sapwood product is accounted for by the presence of anhydroglucose units in the molecular structure and that glucose can be obtained therefrom quantitatively on hydrolysis with takadiastase under controlled conditions. The present communication records a further stage in the fractionation of hemicellulose A isolated from both sapwood and heartwood.

EXPERIMENTAL

(1) *Further fractionation of hemicellulose A by takadiastase*

A series of hydrolyses by means of takadiastase at 38–40° and pH 4.5 [O'Dwyer, 1937] was carried out (a) on hemicellulose A from sapwood after previous removal of its inherent anhydroglucose residues and (b) on hemicellulose A from heartwood after previous digestion with water at 100° for 24 hr. [O'Dwyer, 1934]. Both starting materials showed $[\alpha]_D^{20} = -97.5^\circ$ (ash-free) in 2 % NaOH ($c=0.4$) and they did not reduce Fehling's solution. 2.5 g. samples were used as before, and the products of hydrolysis were examined after intervals of 46–210 hr. The percentage weight of hemicellulose dissolved at each stage is shown in Table I. The unhydrolysed residue in each case gave a specific rotation of -97.5° . It was observed that the efficiency of the takadiastase became considerably impaired after 95 hr. at 38–40° when approximately 55 % of each hemicellulose had been dissolved. For the succeeding stages it was accordingly found expedient to use a fresh supply of enzyme.

In each case the combined residues (4.60 g.) from four separate 2.5 g. samples which had reached the 95 hr. stage were collected, dried and subsequently digested with fresh takadiastase for an additional 45 hr., making the total time of digestion 140 hr. After determining the loss in weight and the specific rotation of the residue (-97.5°) the hydrolysis was extended in stages to 185 and finally to 210 hr., by which time approximately 94 % of each hemicellulose had been dissolved. The ultimate residues included traces of insoluble matter from the takadiastase.

The material brought into solution by the enzyme consisted in every case of (1) a soluble polysaccharide which showed $[\alpha]_D^{20} = -51.2^\circ$ (ash-free) (in water, $c=2$) and (2) a reducing sugar. Separation of the mixture was difficult and the

sugar fraction frequently contained some of the soluble polysaccharide together with traces of residual takadiastase and its specific rotation accordingly varied, values ranging from $+10^\circ$ to $+17.2^\circ$ being recorded. The sugar was estimated as glucose by the method of Lane & Eynon [1934] and identified as *d*-xylose in that the characteristic boat-shaped crystals of cadmium bromide-cadmium xylonate and xylose phenylosazone, m.p. 163° [Fischer & Paulus, 1935], were obtained. The presence of no other sugar could be detected. It is noteworthy that at all stages of the enzymic hydrolysis xylose and soluble polysaccharide were produced in the constant proportion of 3 parts by weight of the former to 2 parts of the latter.

Table I. *Action of takadiastase on hemicellulose A from oak wood*

Time of hydrolysis hr.	Loss in wt. % hemicellulose from	
	Sapwood (after removal of anhydroglucose residues)	Heartwood
48	24.77	25.0
70	37.8	36.5
76	40.0	42.6
95	55.0	54.1
140	73.8	Not determined
144	Not determined	74.9
185	86.2	85.1
193	88.0	Not determined
210	94.3	93.7

(2) *Hydrolysis by dilute mineral acids*

In order to determine whether the results obtained with takadiastase could be reproduced by means of mild hydrolysis with mineral acids the following experiments were carried out on hemicellulose A of $[\alpha]_D^{20} = -97.5^\circ$. The results have been calculated on an ash-free basis.

(1) A 1.2530 g. sample, on hydrolysis with 200 ml. $N/200$ HNO_3 at 100° for 1 hr. according to the method of Bywater *et al.* [1937], lost 56 % of its weight, 36.65 % being accounted for as soluble polysaccharide of $[\alpha]_D^{20} = -51.2^\circ$ and 12.62 % as *d*-xylose. The specific rotation of the unhydrolysed residue was -97.5° .

(2) 1.4941 g. hydrolysed with 200 ml. 0.05 % H_2SO_4 for 1 hr. lost 58 % of its weight, 37.5 % being accounted for as soluble polysaccharide of $[\alpha]_D^{20} = -51.2^\circ$ and 12.29 % as *d*-xylose. Neutralization of the acid was effected in this case by BaCO_3 instead of Na_2CO_3 . The unhydrolysed residue gave, as before, $[\alpha]_D^{20} = -97.5^\circ$.

(3) Hydrolysis of a 2.0 g. sample with 200 ml. 1 % H_2SO_4 for 1 hr. at 100° resulted in the solution of 80 % of the hemicellulose. 20 % was accounted for on neutralization as a Ba salt ($[\alpha]_D^{20} = +49^\circ$) and 52 % as *d*-xylose ($[\alpha]_D^{20} = +18.9^\circ$). The Ba salt was acid to phenolphthalein and gave a positive naphthoresorcinol test for uronic acids and their anhydrides. A CO_2 estimation (6.99 %) on the Ba salt indicated that it was probably derived from a dixylonodimethyluronic acid [Sands & Gary, 1933]. The specific rotation of the unhydrolysed residue was -95.3° in 2 % NaOH .

(4) A 1 hr. hydrolysis with 200 ml. $N/200$ HNO_3 at 100° of 1.50 g. sapwood hemicellulose A of $[\alpha]_D^{20} = -65^\circ$ (in 2 % NaOH , $c=0.4$) which had previously been partially fractionated with water at 100° [O'Dwyer, 1937] and which gave the blue colour reaction with iodine, resulted in the liberation and solution of 62 % of the starting material. The unhydrolysed residue still gave the blue

colour reaction with iodine and the soluble polysaccharide formed on hydrolysis which represented 57 % of the starting material gave a reddish purple colour with iodine, indicating that it probably contained partially hydrolysed anhydroglucose residues. It had $[\alpha]_D^{20} = -35^\circ$ (in water, $c=0.4$). No sugar was isolated in this case.

(5) 1 % H_2SO_4 under the same conditions as in experiment (4) caused the liberation of 70 % of a 2.0 g. sample of hemicellulose. The residue, which gave a green colour with iodine indicating that it still contained anhydroglucose residues, had a specific rotation of -80° in 2 % NaOH ($c=0.4$). A Ba salt of $[\alpha]_D^{20} = +37.5^\circ$ representing 20 % of the starting material was obtained from the hydrolysate on neutralization. The hydrolysate reduced Fehling's solution strongly and gave a positive naphthoresorcinol test. 35.7 % of the starting material was accounted for as reducing sugars estimated as glucose [Lane & Eynon, 1934]. The specific rotation of the sugar solution was $+29^\circ$ which suggests that both glucose and xylose were present.

(3) Analysis of soluble polysaccharide

The soluble polysaccharide obtained from hemicellulose A of $[\alpha]_D^{20} = -97.5^\circ$ on hydrolysis with takadiastase, $N/200$ HNO_3 and 0.05 % H_2SO_4 respectively, was isolated as an impalpable white powder and purified by solution in water and reprecipitation with excess EtOH. The purified product gave no colour reaction with iodine and only induced faint reduction of Fehling's solution. Since the products obtained by means of all three hydrolysing agents had the same specific rotation (-51.2°) they were assumed to be identical. The analysis of a combined sample is given in Table II.

Table II. Analysis of soluble polysaccharide obtained from hemicellulose A on hydrolysis

	Uronic anhydride	Methoxyl	Xylan	Ash %	Specific rotation
	% ash-free				
	17.90	3.08	81.75	0.3	-51.2 (in water $c=0.5$)
Theoretical molecule consisting of six anhydroxyloses and one methylhexuronic acid [Sands & Gary, 1933]	17.83	3.14	80.24	—	—

The yield of CO_2 obtained on hydrolysis with 12 % HCl was determined by the method described by Dickson *et al.* [1930] in an improved form of apparatus evolved at this Laboratory [Campbell *et al.* 1938]. Campbell *et al.* [1938] have recently found that, when this modified apparatus is used, a number of carbohydrates, including xylose, give small but significant yields of CO_2 , so that the result for uronic anhydride containing one methoxyl group (Table II) may be somewhat high.

Xylan was estimated by the Tollens procedure after correcting for the phloroglucide arising from uronic anhydride residues by the method of Lefèvre & Tollens [1907]. Modification of the method of these latter authors has been suggested by Norris & Resch [1935], but since the general question of the estimation of pentosans and related bodies in plant materials is still under investigation in several quarters, the original method of calculation has been adhered to in the present study. The methoxyl content was determined by Zeisel's method.

(4) *Hydrolysis of soluble polysaccharide*

4.8406 g. soluble polysaccharide of $[\alpha]_D^{20} = -51.2^\circ$ were hydrolysed with 200 ml. 1 % H_2SO_4 for 3 hr. at 100° . The precipitate obtained after hydrolysis (0.0160 g.) was filtered off and the clear filtrate neutralized with BaCO_3 and treated as previously described [O'Dwyer, 1934]. It was found to contain 1.65 g. of a Ba salt upon removal of which crystalline *d*-xylose (2.58 g.) was obtained as before [O'Dwyer, 1934]. Found $[\alpha]_D^{20} = +19.0$ (in water, $c=4$), M.P. 153° , and xylosazone, M.P. 163° (Fischer & Paulus, 1935). The characteristic boat-shaped crystals of cadmium bromide-cadmium xylonate were also obtained. No sugar other than *d*-xylose could be detected. The analysis of the Ba salt after purification by dissolving in water, reprecipitating with excess of alcohol and drying as before [O'Dwyer, 1934] is given in Table III. The figures for the Ba salt are similar to those previously obtained for this substance [O'Dwyer, 1934].

Table III

	Uronic anhydride %	Ba %	Methoxyl %	Free aldehyde %	Specific rotation + 70° (in water $c=0.4$)	Methoxyl groups per uronic acid % 1.0 (approx.)
	43.10	17.1	7.11	6.9		
Theoretical for Ba salt of xylonomethylaldobionic acid	43.19	16.86	7.61	7.12	—	1.00

The free aldehyde groups were estimated by Cajori's method [1922], 3 hr. being required for complete oxidation by iodine in alkaline solution. The percentage of Ba was determined by Pregl's micro-method.

DISCUSSION OF RESULTS

In conjunction with the findings of previous work the results in Table I afford interesting evidence which has not hitherto been adduced concerning the constitutional relationship which exists between hemicellulose A isolated from oak sapwood on the one hand and heartwood on the other. It had already been established [O'Dwyer, 1937] that anhydroglucose units form a definite proportion of the sapwood product and since that time the results of a number of experiments have convinced the author that such units are absent from the heartwood product. It is now observed that the complete removal of the glucose residues from sapwood hemicellulose A by hydrolysis with takadiastase at pH 4.5 is accompanied by a change in specific rotation from an initial value of -65° (in 2 % NaOH) to -97.5° for the unhydrolysed residue. It is noteworthy that this latter value is the same as that obtained for heartwood hemicellulose A as ordinarily isolated. Apart from this close similarity in rotatory power there is further strong evidence that sapwood hemicellulose A when freed of its inherent glucose residues is chemically identical with the heartwood product. Takadiastase under controlled conditions affects both products in precisely the same manner. The losses in weight (Table I) at corresponding stages of hydrolysis are closely comparable and the products of hydrolysis at all stages are the same.

It is observed that, chiefly on account of its greater severity, mild acid hydrolysis does not afford the same degree of insight into the structure of hemicellulose A as does the enzymic hydrolysis. Part of the products of hydrolysis is decomposed, presumably to furfuraldehyde, by the acids, and the removal of

glucose residues from the sapwood product by such reagents is accompanied by pronounced decomposition of this type.

It would now appear to be conclusive that the only constitutional change in hemicellulose A which is involved in the transformation of sapwood into heartwood consists in the removal of anhydroglucose units. It is further established that the simplest building units which are common to hemicellulose A from both sources are xylose and a monomethylhexuronic acid. It is calculated that the recurring unit of heartwood hemicellulose A probably consists of eleven of the former and one of the latter. The corresponding unit of the sapwood product is calculated to consist of one glucose, one methylhexuronic acid and eleven xylose residues.

The next step towards establishing the constitution of hemicellulose A should naturally consist in the determination of the nature of the linkages between the units which have here been identified. For instance it is of importance to determine whether the glucose units in the sapwood product are of the α or β configuration in order to decide whether hemicellulose A is structurally related to starch on the one hand or cellulose on the other. The colour reaction of the sapwood product with iodine, coupled with the observation that glucose is obtained on hydrolysis with takadiastase, tend to indicate that α -glucose residues are present, but it is freely admitted that this evidence is in itself insufficient to warrant a definite conclusion.

SUMMARY

1. Hemicellulose A of oak sapwood after removal of anhydroglucose residues by takadiastase has been found to be chemically identical with the product from heartwood.

2. Prolonged hydrolysis with takadiastase results in a complete fractionation of hemicellulose A into a soluble polysaccharide and xylose, 2 parts by weight of the former being formed for every 3 parts of the latter.

3. Mild hydrolysis of hemicellulose A by dilute mineral acids gives rise to the same substances as takadiastase digestion, but the yields are smaller and the proportions of sugar and soluble polysaccharide are not constant.

4. The soluble polysaccharide gives rise on dilute mineral acid hydrolysis to a monomethylhexuronate and xylose, and its determined composition agrees with that of a theoretical molecule consisting of six anhydroxylose units and one methylhexuronic anhydride unit.

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REFERENCES

- Bywater, Haworth, Hirst & Peat (1937). *J. chem. Soc.* p. 1983.
Cajori (1922). *J. biol. Chem.* **54**, 617.
Campbell, Hirst & Young (1938). *Nature, Lond.*, **142**, 912.
Dickson, Otterson & Link (1930). *J. Amer. chem. Soc.* **52**, 775.
Fischer & Paulus (1935). *Arch. Pharm., Berl.*, **273**, 83.
Lane & Eynon (1934). Determination of reducing sugars by Fehling's solution with methylene blue as indicator. London: Norman Rodger.
Lefèvre & Tollens (1907). *Ber. dtsch. chem. Ges.* **40**, 4513.
Norris & Resch (1935). *Biochem. J.* **29**, 1590.
O'Dwyer (1934). *Biochem. J.* **28**, 2116.
— (1937). *Biochem. J.* **31**, 254.
Sands & Gary (1933). *J. biol. Chem.* **101**, 573.