XXXVI. THE HEMICELLULOSES OF THE WOOD OF ENGLISH OAK

III. THE FRACTIONATION OF HEMICELLULOSE A

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(Received 24 December 1936)

IN a former paper [O'Dwyer, 1934] it was shown that hemicellulose A from English oak sapwood gives the blue coloration with iodine which is characteristic of starch. Although the presence of glucose among the hydrolysis products was not established the possibility that anhydroglucose units might nevertheless be present in the hemicellulose was not excluded. The formulation of an interesting hypothesis by Campbell [1935, 2] concerning the relationship between starch and hemicellulose in oak wood has now led to the examination of hemicellulose A along somewhat different lines and certain results have been obtained which appear to support the above author's contention.

EXPERIMENTAL

Fractionation of hemicellulose A by takadiastase

(a) *Hemicellulose A from sapwood.* Since enzymic hydrolysis offered a prospect of a less complex mixture of products than is obtained by the action of dilute acid on hemicellulose A, a series of hydrolyses was carried out by means of takadiastase according to the method of Denny [1934], which has been applied by Campbell [1935, 1] to the starch and related products from oak sapwood. Fourteen separate 2.5 g. (air-dry) samples of the hemicellulose isolated from sapwood dried in various ways [O'Dwyer, 1934] were digested at 38–40° with 40 ml. of water, 25 ml. of a 4 % aqueous solution of dialysed takadiastase and 25 ml. of acetate buffer solution at pH 4.5 for 44 hours.

After boiling in the usual way to destroy the enzyme it was observed that a considerable proportion of the starting material had not been attacked, but owing to the colloidal nature of the system this unattacked residue could not be recovered directly by filtration. On adding 1.5 volumes (approximately 140 ml.) of hot 95 % alcohol to each flask, however, the residue settled in the form of a gelatinous precipitate and the hot mixture was readily filtered through an alundum crucible of porosity RA 360. The residue in each case was washed with water, alcohol and ether and then dried at 105° to constant weight. The average loss in dry weight of the 14 samples of original hemicellulose was 30 %. None of the residues reduced Fehling's solution or gave a blue coloration with iodine but all gave a positive naphthoresorcinol test for uronic acids and their anhydrides. In Table I the composition of a typical residue is compared with that of the starting material.

In each case the portion of hemicellulose rendered soluble during the enzymic hydrolysis was found to reduce Fehling's solution strongly. Glucose was

Table I. *Effect of takadiastase on the composition of hemicellulose A from air-dry oak sapwood [O'Dwyer, 1934]*

	Original hemicellulose	Residue
Loss in weight	30.0% oven-dry	—
CO ₂ as uronic anhydride	9.8% "	8.71% oven-dry
Pentosan as xylan (by Tollens's method)	83.8% "	84.94% "
$[\alpha]_D^{20}$ in 2% NaOH, $c=0.8$	-65.2°	-81.7°

identified by means of the following three crystalline derivatives prepared by the method of Fischer & Paulus [1935]: glucosazone m.p. 224°, *p*-nitrophenyl-osazone m.p. 256° and *p*-bromophenylhydrazone m.p. 146–148°. No other sugar could be detected and estimations by the method of Lane & Eynon [1934] indicated that 9–10% of the parent hemicelluloses consisted of anhydroglucose residues. Thus glucose only accounted for approximately one-third of the soluble material remaining after hydrolysis. The remaining two-thirds was eventually found to consist of a polysaccharide soluble in 50% but insoluble in 80% alcohol. This latter was isolated in the usual manner as an impalpable white powder. It did not reduce Fehling's solution immediately on boiling but a slight reduction took place on standing. The indications are therefore that this substance may have arisen from the parent hemicellulose as the result of the severance of an aldehydic linkage. Qualitative analyses of a number of samples of this polysaccharide indicated the presence of pentosan and uronic acid residues. Found $[\alpha]_D^{20}$ (in water) -30° for the product obtained from the hemicellulose A of air-dry sapwood. Found $[\alpha]_D^{20}$ (in water) +48° for the sugar obtained from the same preparation.

Various small-scale experiments indicated that prolongation of the enzymic hydrolysis of hemicellulose A up to 80 hours at 38–40° did not increase the yield of sugar or other soluble products and that for periods under 40 hours the hydrolysis was incomplete.

(b) *Hemicellulose A from heartwood.* Hemicellulose A from oak heartwood gives no change of colour with iodine. Approximately 23% of a number of preparations were dissolved during digestion with takadiastase under the same conditions as those applied to the sapwood products. The soluble material was found to consist almost entirely of a polysaccharide of $[\alpha]_D^{20}$ -46° in water and -51.6° in 2% NaOH as compared with a specific rotation of -30° in water for the product from sapwood. The presence of uronic acid residues was confirmed by the naphthoresorcinol test and the substance reduced Fehling's solution slightly on standing. Sugar estimations indicated that the original hemicellulose contained approximately 1% of anhydroglucose residues. The undissolved residue from the enzymic hydrolysis had the same general properties as the original hemicellulose.

Since it has been found [Campbell, 1935, 1] that starch and certain related products can be extracted from oak sapwood by means of hot water, it is conceivable that the glucose found among the hydrolysis products of the hemicellulose preparations referred to above might have been derived from traces of starch which had not been completely removed during the preliminary purification of the parent wood substance. In order to test this point a sample of oak sapwood sawdust was extracted with water at 100° with changes of water at 3-hour intervals for a total period of 105 hours. At this stage the iodine test revealed no starch in the residual wood substance but the hemicellulose A prepared from this residue in the usual way [O'Dwyer, 1931] gave a pronounced

blue coloration with iodine. The analytical data in Table II indicate a close similarity in composition between this hemicellulose and previous preparations [O'Dwyer, 1934].

Table II. *Composition of hemicellulose A isolated from oak sapwood after exhaustive extraction with water at 100°*

Pentosan as xylan	82.40%	oven-dry
CO ₂ as monomethyluronic anhydride	9.79%	"
OMe	2.02%	"
Mineral ash	2.9%	"
$[\alpha]_D^{20}$ (in 2% NaOH), $c=0.8$	-65°	

Fractionation of hemicellulose A by digestion with water

A series of attempts was now made to fractionate various samples of oak hemicellulose A without the aid of an enzyme. Prolonged soaking in water at room temperature or at 38° for 44 hours induced no change in the polysaccharides, but in every case it was found that digestion with water at 100° for 24 hours effected a separation into a soluble and an insoluble portion. The soluble portion could be removed by filtration after the insoluble portion had been coagulated by the addition of alcohol. The water-soluble portion was in each case precipitated by an excess of alcohol and on the average amounted to approximately 20% of the starting material. The water-soluble portion of sapwood hemicellulose A gave no change of colour with iodine, but in every case the insoluble residue gave the characteristic blue colour obtained with the starting material. It is significant that the proportion of the original sapwood hemicellulose dissolved by hot water represents the difference between the respective amounts of total dissolved material and sugars obtained after the action of takadiastase. An extension of the treatment with hot water to 30 hours produced no difference in the percentage dissolved or in the general properties of the fractions obtained. Similar treatment of hemicellulose A from heartwood also yielded both a soluble and an insoluble fraction.

DISCUSSION

The object of the foregoing experiments has been in the first instance to obtain evidence concerning the significance of the blue coloration given by the hemicellulose A of oak sapwood with iodine-potassium iodide solution. The identification of glucose among the products of the enzymic hydrolysis of the polysaccharide now makes it clear that the portion of the complex which is responsible for the coloration consists of anhydroglucose residues. When these latter are removed the residual hemicellulose fails to give the blue coloration with iodine. The conclusion that anhydroglucose residues form integral parts of a polysaccharide chain in oak hemicellulose A is supported by at least two separate pieces of evidence. In the first place the product prepared from wood which had previously been exhaustively extracted with water to remove the more readily dispersible polysaccharides still gave a blue coloration with iodine-potassium iodide solution. Secondly, although wood starch and certain closely related bodies [Campbell, 1935, 1, 2] are readily dispersed by hot water, digestion of hemicellulose A with water at 100° for periods up to 30 hours failed to disperse the portion which contains anhydroglucose residues. It is therefore obvious that a close constitutional similarity exists between part of the hemicellulose A of oak sapwood and the polysaccharides already isolated by Campbell [1935, 1, 2] and, although it is impossible at this stage to draw definite conclusions, the

results appear to afford strong support for the hypothesis advanced by the above author [1935, 2] concerning the derivation of hemicellulose A. It is hoped that further light may be thrown on this important question as a result of the investigations of the water-soluble portion of hemicellulose A and also of hemicellulose B which are in progress.

It has been shown above that hemicellulose A from the heartwood of English oak is constitutionally sharply differentiated from the product obtained from sapwood. In amplification of previous work [O'Dwyer, 1934] it has now been shown that the proportion of anhydroglucose units in hemicellulose A decreases in marked degree during the transition from sapwood to heartwood.

SUMMARY

Hemicellulose A from sapwood and heartwood of English oak, on treatment with takadiastase at pH 4.5 is split up into two polysaccharide fractions. Glucose corresponding to about 10% of the starting material has also been obtained by this method from the hemicellulose A of sapwood.

Digestion of hemicellulose A with water at 100° results in a fractionation of a somewhat similar character except that glucose residues are not split off.

Anhydroglucose units apparently form a definite proportion of hemicellulose A and considerable support is given to the hypothesis put forward by Campbell [1935, 2] as to the origin of these substances in wood.

The author is deeply indebted to Mr W. G. Campbell for valuable suggestions in connexion with the work. Her best thanks are also due to Sir James C. Irvine for criticism of the manuscript and to Mr W. A. Robertson, for permission to publish this communication.

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