XLIX. PRELIMINARY INVESTIGATIONS ON THE CONSTITUTION OF THE HEMICELLULOSES OF TIMBER.

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INTRODUCTION.

FOLLOWING on the work described in a previous communication [O'Dwyer, 1926], an attempt has been made in the present investigation to obtain further information regarding the chemical constitution of the hemicelluloses of timber, and thereby, if possible, to gain a better knowledge of their function in plant life.

The first section of the work has been carried out on a small quantity of the so-called *hemicellulose A* from beech wood, the preparation and hydrolysis products of which have already been described [O'Dwyer, 1926]. This substance is obtained by treating sawdust in the cold with 4 % sodium hydroxide, after the removal of the water-soluble constituents, the pectic substances and the protein. Hemicellulose A bears very little resemblance to cellulose in physical properties or, so far as can be judged, in chemical constitution.

The general method of investigation employed was to subject the hemicellulose to mild acetylating reagents which would be unlikely to cause profound depolymerisation on hydrolysis. In this way it was expected that a uniform acetate would be obtained, which, owing to its solubilities, could be purified from extraneous compounds and permit of more accurate study. Some idea of the complex nature of the investigation may be gathered from the fact that the acetate prepared from this material can be separated into three different fractions, and that whereas 80 % of the hemicellulose from beech wood, already referred to, could be readily acetylated, only 20 % of hemicellulose A from an older beech and from oak heartwood responded to the same treatment. This resistance to acetylation is not attributable to the accumulation of inorganic constituents in the fraction of the hemicellulose unaffected by the reagents and must be regarded as a specific property. Even the application of the more drastic acetylating mixture used in the acetolysis of cellulose [Irvine and Robertson, 1926] had little effect and the conclusion might have

been drawn that no hydroxyl groups were present. That such is not the case was shown by subjecting the material which could not be acetylated to the effect of methyl sulphate and alkali. Normal methylation then took place until the value $\text{OMe} = 15.7\%$ had been obtained, but thereafter a sodium derivative was produced and this interfered with carrying the alkylation to its extreme limit.

A detailed examination of hemicellulose B has not been possible at this stage owing to the low percentage of that substance obtained from oak heartwood, due, probably, to the age of the wood. In the course of the work it was found that hemicellulose, even when purified exhaustively from pectin, contains methoxyl and reference may now be made to this unexpected observation.

A series of experiments showed that about 50 % of the methoxyl present in hemicellulose A could be readily de-esterified, but the remainder proved absolutely resistant even when drastic methods of hydrolysis were employed. It is, therefore, reasonable to conclude that methoxyl is present in two forms, both as a methyl ester and as a methoxy-derivative. The occurrence of methoxyl in the hemicelluloses can hardly be ascribed to the pectic substances present in wood, for these substances are found in very small quantities in lignified tissue and any which had not been removed by previous treatment of the sawdust [O'Dwyer, 1926] would be de-esterified by the 4 % sodium hydroxide used for the extraction of the hemicellulose. In addition, as pectic substances are soluble in water they are unlikely to be present as an impurity in hemicellulose A.

The possibility that lignin may be the source of the methoxyl detected in hemicellulose has not been overlooked. Mehta [1925], and Dorée and Barton-Wright [1927] found that a soluble form of lignin (termed by the latter authors "metalignin") could be isolated in relatively large amount by extracting wood sawdust for 1 hour with 4 % sodium hydroxide under a pressure of from 8 to 10 atmospheres. Further, Hawley and Campbell [1927] record that, after the extraction of Sitka spruce sawdust with 1 % sodium hydroxide for 1 hour on a boiling water-bath, the lignin content of the wood had fallen from 29.3 % to 27.7 %.

The hemicelluloses described in the present paper were obtained by extracting sawdust in the cold with 4 % sodium hydroxide for 48 hours, the mixture being stirred mechanically, and it is conceivable that not only would some metalignin pass into solution during this treatment but that it would be precipitated with the hemicellulose on the subsequent addition of acetic acid, despite the fact that metalignin is soluble in that reagent. Metalignin is, however, likewise soluble in alcohol and as the precipitated hemicelluloses are purified by immersion in alcohol of gradually increasing concentrations for a period of from two to three weeks and finally washed several times on the pump with absolute alcohol before being dried, it seems unlikely that any considerable amount of metalignin would be present in the final product.

As the methoxyl content of metalignin is 17.4 % [Dorée and Barton-Wright, 1927] 24 % of this compound must be assumed to be present in the hemicellulose A examined, if the methoxyl is to be attributed to the former alone. The history of the material makes this supposition unlikely, and it seems evident therefore, that little, or none, of the methoxyl in hemicellulose can be ascribed to the presence of lignin.

The initial work on beech hemicellulose was of value in suggesting methods for the more detailed investigations which were later carried out on the corresponding product from oak. Unfortunately even in this second instance the work has been hampered by insufficient quantities of material and for this reason has had to be suspended, for the moment, at an interesting stage.

Although this investigation has, so far, not produced sufficient evidence on which to assign a chemical constitution to the hemicelluloses it has nevertheless yielded valuable results and has afforded further evidence that, as stated in the previous communication, these substances are apparently more akin to pectin than to cellulose and that they may even bear some slight resemblance to lignin.

Von Fellenberg [1918] and Ehrlich [1927] have already suggested the possibility of a transition in the plant from the pectic substances to lignin. If such be the case, it is conceivable that the hemicelluloses may prove to be transition products in this important natural transformation. As has been previously pointed out [O'Dwyer, 1926], such unignified tissue as has been examined contains a much larger proportion of pectic substances than of hemicellulose, whereas experiments conducted on lignified tissue have shown the opposite to be the case. Again, in unignified tissue, hemicellulose B occurs in larger quantities than hemicellulose A, and in lignified tissue the reverse holds true. The timber examined so far has shown that the percentage of hemicellulose B tends to diminish with the age of the wood, and a point might conceivably be reached at which this substance would disappear altogether. Hemicellulose A might also become much modified as lignification proceeds further.

EXPERIMENTAL.

Acetylation of beech hemicellulose. The first set of experiments was carried out on a small quantity of hemicellulose A from beech wood 80 years old, containing 3.7 % methoxyl and 2.7 % ash. The hydrolysis products obtained from this substance have already been examined [O'Dwyer, 1926].

An endeavour was made to obtain an acetate by the method of Barnett [1921] using certain modifications suggested by Irvine and Hirst [1922]. Several experiments were carried out with small quantities of hemicellulose and in each case a white, amorphous product was obtained which was not completely soluble in either chloroform or pyridine and was insoluble in other organic solvents. Fractional precipitation of the chloroform solution with

alcohol gave a second substance soluble in both chloroform and pyridine. Table I shows the results obtained.

Table I.

Experi- ment	Quantity of hemi- cellulose taken g.	Temperature and time of experiment	Amount of acetate obtained g.	Acetyl groups before frac- tionation %	Specific rotation	
					Fraction I	Fraction II
1.	5	65-70° 36 hours	5.5	49.5	$[\alpha]_D - 36^\circ$ (pyridine) (cloudy) $c = 1.774$	$[\alpha]_D - 52^\circ$ (pyridine) (clear) $c = 2$
2.	5	65-70° 75 hours	5.4	49.0	$[\alpha]_D - 19^\circ$ (chloroform) $c = 1.5$ $[\alpha]_D - 20^\circ$ (pyridine) $c = 1.8$ (cloudy)	$[\alpha]_D + 28^\circ$ (chloroform) $c = 2.03$ $[\alpha]_D + 20^\circ$ (pyridine) $c = 2.00$ (clear)
3.	5	45-50° 36 hours	3.4	48.2	$[\alpha]_D - 52^\circ$ (chloroform) (cloudy) $c = 0.9520$	$[\alpha]_D - 60^\circ$ (chloroform) (clear) $c = 1.070$ $[\alpha]_D - 55^\circ$ (pyridine) (clear) $c = 0.9300$
4. Residue from 3.	1.6	45-50° 36 hours	1.5	45.04	$[\alpha]_D - 54^\circ$ (pyridine) (clear) $c = 1$ $[\alpha]_D - 60^\circ$ (chloroform) (clear) $c = 1.02$	$[\alpha]_D - 10^\circ$ (chloroform) (clear) $c = 0.992$ $[\alpha]_D - 3.8^\circ$ (pyridine) (clear) $c = 1.634$

From these results it seems evident that the acetates contain at least two and possibly three fractions and that prolonged acetylation at a temperature of 65-70° results in de-polymerisation of the substance. A sixth acetylation was carried out on 8.3 g. of hemicellulose A from an older beech wood (130 years) at 40° for 3 days. Only 1.1 g. of acetate was obtained and no further yield was possible by treating the hemicellulose at 50° and at 65-70°. This substance therefore shows marked differences as regards acetylation compared with hemicellulose A from younger beech.

Hydrolysis of acetate from beech with acid methyl alcohol. 2.4706 g. containing both fractions of the acetate obtained at 45-50° were treated in a sealed tube at 100-105° by the method of Irvine and Hirst [1922] with 30 cc. pure methyl alcohol containing 0.5 % dry hydrogen chloride. At the end of 115 hours 14 % of the substance still remained undissolved. This residue consisted of a greyish amorphous powder, volatile on ignition, free from nitrogen, sulphur and carbohydrates. The ash content was negligible. It contained 2.4 % methoxyl.

After filtering off the residue, the acid methyl alcohol solution was subjected to further treatment by Irvine and Hirst's method. 1.0346 g. of uncrystallisable syrup was obtained: $[\alpha]_D$ (in acid methyl alcohol) + 92°, $c = 2.06$. The

syrup was then extracted with ether and 0.1412 g. of a substance, soluble in that reagent but insoluble in water, was obtained. It had $[\alpha]_D$ (in alcohol) $+ 26^\circ$, $c = 0.5624$. As the residue insoluble in ether still failed to crystallise, the methoxyl content was determined and 19.9 % OMe obtained.

Hydrolysis of the syrup with hydrochloric acid. For this purpose 0.4709 g. was treated with 25 cc. 3 % hydrochloric acid [Irvine and Hirst, 1924]. The syrup was not completely soluble in the acid, so that a cloudy solution resulted of which the rotation could not be taken. After heating at 100° for 30 minutes a brown precipitate separated. This was filtered off and the hydrolysis continued. The residue weighed 0.0378 g. It melted at 166° .

The following polarimetric results were obtained during the progress of the hydrolysis.

Table II.

Time of hydrolysis ($c = 1.852$)	30 mins.	75 mins.	120 mins.	165 mins.	200 mins.	245 mins.
α	$+0.55^\circ$	$+0.42^\circ$	$+0.25^\circ$	$+0.18^\circ$	$+0.15^\circ$	$+0.15^\circ$
$[\alpha]_D$	$+60^\circ$	$+45.8^\circ$	$+27.3^\circ$	$+21.4^\circ$	$+18.4^\circ$	$+17.8^\circ$

The last three sets of figures were calculated on the weight of the sugar formed during hydrolysis [*vide* Irvine and Hirst, 1922]. The hydrolysed solution was neutralised, taken to dryness under reduced pressure, and the residue dissolved in hot absolute alcohol. Weight of sugar formed 0.9460 g. M.P. 146° . $[\alpha]_D + 18^\circ$ (in water), $c = 3.65$. The sugar is therefore xylose.

HEMICELLULOSE A (OAK).

Such information as had been gained by the study of hemicellulose A from beech was now applied to 60 g. of the same substance from American oak heartwood, the age of which is not definitely known. This substance was prepared in a similar manner to the beech hemicellulose, and contained 4.8 % OMe, 2.4 % ash (mostly silica) and a very small quantity of phosphorus.

(a) *Extraction of oak hemicellulose A with alcohol.* Two extractions with boiling absolute alcohol were carried out in order to effect further purification of the substance. On taking the alcoholic extract to dryness a small amount of residue was obtained having the odour of vanillin, insoluble in water, M.P. 162° (approx.) and containing 5 % OMe. The hemicellulose after the alcoholic extraction still contained 4.1 % OMe.

(b) *Examination of methoxyl groups in oak hemicellulose A.* In order to ascertain whether any of the methoxyl was present in the form of an ester, a series of experiments was carried out on small quantities of the hemicellulose after purification. Amounts varying from 2 to 3 g. were treated with 0.5 N sodium hydroxide at temperatures ranging from 65° to 85° for 2 hours. The hemicellulose was recovered as a white powder, after dialysis of the solution with distilled water, by the addition of acetic acid and alcohol, and was dried in the usual way [O'Dwyer, 1926]. In each case a methoxyl content of about 2 % was obtained for this substance. It is evident, therefore, that about 50 % of the methoxyl present in the hemicellulose resists de-esterification and

is present in the substance in a very stable form. Some additional experiments on the hemicellulose with different strengths of ammonia showed that the methoxyl content again dropped to 2 % after the substance had stood for some time in the cold with 10 *N* ammonium hydroxide, but that 4 *N* ammonium hydroxide under similar conditions had apparently no de-esterifying effect.

(c) *Acetylation of oak hemicellulose A*. Barnett's method was employed with modifications as in the case of beech hemicellulose. The first estimation was carried out at 40–45° for 36 hours, as these conditions apparently gave the best results in the acetylation of beech hemicellulose. As, however, the yield of acetate from this experiment was only a little over 10 %, the time was extended to 100 hours in a second experiment. The amount of acetate obtained, however, was only about 20 %. Raising the temperature in subsequent experiments to 80° produced no more acetate, and it became evident that the acetylation could be carried no further.

Under these circumstances a more drastic method was employed [Irvine and Robertson, 1926]. 1 g. of the hemicellulose which remained as a residue after acetylation was carefully added to a mixture consisting of 1 cc. sulphuric acid and 10 cc. acetic anhydride. The resulting mixture was kept at 0° for 1 hour to prevent charring, and then at room temperature for 3 days. It was next plunged into a large volume of water, the precipitate filtered off and dried. It weighed 0.79 g. and proved to be unchanged hemicellulose. A very small quantity of acetate was obtained. The experiment was repeated with 5 g. of hemicellulose residue.

Yield of acetate	0.1 g. (grey in colour)
Residue (unchanged hemicellulose)	4.69 g.
OMe in residue	2.16 %
Ash in residue	2.9 %

Table III shows the results obtained from the first series of acetylations.

(d) *Hydrolysis of acetate from oak with acid methyl alcohol*. The fractions of the acetate soluble in chloroform were combined and treated with acid methyl alcohol in the same manner and under the same conditions as in the case of beech acetate.

Amount of substance taken	1.5673 g.
„ syrup obtained	0.5692 g.
„ residue obtained	0.0978 g.

On treatment of the syrup with hot absolute alcohol fine white crystals were obtained. Wt. 0.2375 g., M.P. 150°, $[\alpha]_D$ (in alcohol) – 62°, $c = 2$. These crystals were apparently those of β -methylxyloside. Owing to the small yield it was impossible to recrystallise.

(e) *Methylation of unacetylated hemicellulose*. As the acetylation of this substance could be carried no further, it was decided to attempt the methylation of the unacetylated residue by dimethyl sulphate and sodium hydroxide. For this purpose a small quantity of hemicellulose, which had been submitted to acetylation by both processes, was employed. 3 g. were dissolved in 30 cc.

Table III.

Experiment	Quantity of substance taken g.	Temp. and time of experiment	Amount of acetate obtained g.	Acetyl groups in combined fractions %	Specific rotation of Fraction II. (Fraction I is insoluble in organic solvents)
I (hemicellulose)	5	40-45° 36 hours	0.6	43.0	$[\alpha]_D - 75^\circ$ (pyridine) (cloudy) $c = 2$
I (a) Residue from I	4.2	60° 30 hours	0.9	43.9	$[\alpha]_D - 74^\circ$ (pyridine) (cloudy) $c = 0.67$
II (hemicellulose)	10	40-45° 100 hours	2.06	42.5	$[\alpha]_D - 76^\circ$ (pyridine) (very cloudy) $c = 2$ (approx.)
II (a). Acetate from II	1.6	70° 10 hours (Slight discoloration of substance)	0.8	49.0	$[\alpha]_D - 43^\circ$ (chloroform) (cloudy) $c = 2.012$
III (hemicellulose)	10	60-65° 36 hours	1.85	42.0	$[\alpha]_D - 28^\circ$ (pyridine) (less cloudy) $c = 2.0$
IV (hemicellulose)	10	70-75° 36 hours	2.3	44.8 After fractionation	$[\alpha]_D - 72^\circ$ (pyridine) (cloudy) $c = 1.2$
				Frac. I. Frac. II 43.0 50.0 After fractionation	$[\alpha]_D - 76^\circ$ (chloroform) (cloudy) $c = 0.1938$
				Frac. I Frac. II 43.1 50.0 Insol. in org. solvents Sol. in chloroform	Specific rotation after further fractionation
V (hemicellulose)	14	65-70° 36 hours	4.8 Separated into two fractions by chloroform		Frac. II a Frac. II b $[\alpha]_D - 64^\circ$ (chloroform) (clear) $c = 2.075$ $[\alpha]_D - 35.6^\circ$ (chloroform) (clear) $c = 2.083$

The effect of acetylation on the acetate soluble in chloroform seems to be de-polymerisation, unless—as suggested by the higher acetyl value of Fraction II in the above experiment—a further separation might be effected and a third substance obtained. The proportion of material insoluble in chloroform in these acetates is much greater than in those obtained from beech.

of 2 *N* sodium hydroxide. The temperature was then raised to 37° and 27 cc. of 30 % sodium hydroxide and 8 cc. of dimethyl sulphate were run in slowly with stirring, the temperature during this process being kept at 37°. It was then raised to 100° for 30 minutes after which the mixture was cooled, acidified with hydrochloric acid and made faintly alkaline with solid sodium carbonate. It was then taken to dryness, extracted with boiling chloroform and the liquid filtered off and evaporated to dryness. The amount of the substance soluble in chloroform or in alcohol was negligible. In order to free it from inorganic matter the mixture was dialysed for several days and a precipitate obtained from the colloidal solution remaining in the dialyser by the addition of acetic

acid and alcohol. This precipitate was dried in a similar manner to that employed in the case of hemicellulose. It was a fine white powder (1.8480 g.) having the same chemical properties as hemicellulose A and containing 13.7 % OMe and 1.2 % ash.

As the methoxyl content of the substance had been raised to a considerable extent by the action of dimethyl sulphate and sodium hydroxide, 20 g. were methylated under the same conditions. The resulting product was similar to that obtained in the last experiment. It weighed 13 g., contained 15.7 % OMe, and 2.2 % ash which was identified as silica.

10 g. of the substance obtained from the last experiment were now submitted to a second methylation under the same conditions as in the first experiment. On taking the chloroform extract to dryness a very small amount of substance, 0.2790 g., was found to be soluble in that reagent. This product was obtained, not as a syrup but as an amorphous, fine, yellow powder, giving a methoxyl content of 30.2 %. The remainder of the material obtained as a result of the methylation was dialysed as before and a precipitate obtained by the addition of acetic acid and alcohol. A considerable loss occurred during the methylation process as only 3.5 g. of a greyish powder were obtained. This was sparingly soluble in sodium hydroxide and much less hygroscopic than that obtained after the first methylation. It contained 14.76 % ash. The methoxyl content of the ash-free substance was 27.8 %. An attempt to determine the rotation in 2 % sodium hydroxide showed that the substance was slightly laevorotatory, but an exact figure could not be obtained.

Table IV.

Substance	Percentages of				Specific rotation	Soluble in	Insoluble in
	C	H	OMe	Ash			
a. Hemicellulose A	44.68 44.56	6.51 6.43	4.1	1.85	$[\alpha]_D - 125^\circ$ (in 2 % NaOH) $c = 0.240$	Dilute alkali	Dilute acids, water and organic solvents
b. Acetate from hemicellulose A — fraction I	41.18 40.99	6.32 5.98	nil	1.28	Unobtainable		All organic solvents and acetic acid and cold alkali
c. Acetate from hemicellulose A — fraction II	46.38 46.52	6.37 6.05	nil	negligible	$[\alpha]_D - 75^\circ$ (in chloroform) $c = 2$	Chloroform and pyridine	Water, alcohol and most other organic solvents
d. Residue hemicellulose A after acetylation	44.12 43.88	5.90 5.91	2.2	1.52	$[\alpha]_D - 75^\circ$ (in 2 % NaOH) $c = 0.20$	Similar to hemicellulose A	
e. Product obtained from methylation of (d)	43.71 43.70	6.56 6.83	15.7	2.26	$[\alpha]_D - 101^\circ$ (in 2 % NaOH) $c = 0.2167$	Similar to hemicellulose A	
f. Product obtained from methylation of (e)	42.94 43.23	7.04 7.25	27.8	14.76	Small laevorotation, exact figure unobtainable	Sparingly in dilute alkali otherwise solubilities similar to hemicellulose A	
g. Product obtained after attempted acetylation of (f)	47.57 47.75	7.26 7.37	28.2	5.4	$[\alpha]_D - 5.2^\circ$ (in chloroform) $c = 1.632$	Chloroform	Alcohol and water

The carbon content of (f) is obscured by the high percentage of ash. All results were calculated on an ash-free basis.

The high percentage of ash—a rise of about 12·5 % on that obtained after the first methylation—suggests the formation of a sodium compound during methylation. A substance, for instance, having the composition $C_6H_5O_5Na$ would contain 12·5 % sodium and this figure corresponds almost exactly to the increase noted in the ash.

(f) *Attempted acetylation of the methylated product.* An attempt was made to acetylate the product obtained after a second methylation by Barnett's method. This was not successful, but after neutralising the filtrate from the acetylation with barium carbonate, filtering, evaporating the filtrate to dryness and extracting the residue with boiling chloroform 0·7182 g. of a substance soluble in that reagent, and insoluble in water and alcohol, was obtained.

Table IV gives the ultimate analysis, methoxyl content, percentage of ash, specific rotation and solubilities of the substances obtained during the investigations on oak hemicellulose.

HEMICELLULOSE B (OAK).

About 5 g. of this product was obtained from 2 kg. of the oak heartwood. It was similar in character to that found in beech and described in a previous communication [O'Dwyer, 1926].

The following results were obtained from this substance after extraction, as in the case of hemicellulose A, with boiling absolute alcohol.

Percentages of				<i>Specific rotation</i> [α] _D - 66° (in 2 % NaOH) c = 0·2100
C	H	OMe	Ash	
37·27	6·67			
		3·4	5·8	
37·34	6·37			

These results were calculated on an ash-free basis.

SUMMARY.

Preliminary investigations into the constitution of the hemicelluloses of timber have been carried out on hemicellulose A from beech wood and from American white oak.

Some idea of the complex nature of these investigations may be gathered from the fact that the acetates obtained contain apparently three different substances.

80 % of hemicellulose A from beech wood 80 years old could be readily acetylated, but only 20 % of the same substance from an older beech and from oak heartwood responded to the same treatment. Hence the nature of the hemicelluloses depends, apparently, on the age of the wood.

The hemicelluloses from both beech and oak contain a certain percentage of methoxyl of which about half exists in the form of an ester. The remainder seems to bear a different character as it resists drastic methods of de-esterification. The determination of the source of the methoxyl presents interesting possibilities.

Methylations have been carried out on the residue from acetylation in the case of oak, and a considerable rise in the methoxyl content has been noted, thereby confirming the presence of unsubstituted hydroxyl groups. This, however, was accompanied by a large increase in ash after re-methylation of the substance, due to the formation of a sodium compound.

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