CXLIH. STUDIES ON HEMICELLULOSES.

IV. THE PROXIMATE ANALYSIS OF BOX-WOOD, AND THE NATURE OF ITS FURFURALDEHYDE- YIELDING CONSTITUENTS.

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INTRODUCTION.

As a preliminary to an investigation of the nature of the hemicelluloses of box-wood, it was desired to obtain as complete an idea as possible of the constitution of the wood, and with the minimum of overlapping among the constituents estimated.

Preliminary experiments had indicated that the furfuraldehyde-yielding constituents of the wood could be separated into a number of groups. Thus, hot 0.5 % ammonium oxalate solution removed from the sawdust both pectin and a small amount of non-pectic substance capable of yielding furfuraldehyde. Again, extraction of the residue from this treatment with boiling alcoholic sodium hydroxide solution dissolved in addition to lignin a substance which was reprecipitated on rendering the extract acid. This product, here described as "lignosaccharide," appeared to resemble the hemicelluloses closely in properties and constitution. Extraction with 4 % sodium hydroxide solution at room temperature removed "free hemicelluloses," but a portion of the hemicellulosic substance still remaining in association with the tissue residue required more drastic treatment for its removal. This "combined hemicellulose" was dissolved out by hot 4 % sodium hydroxide solution, but an amount of "other pentosans" remained undissolved, and this was shown to consist largely, if not entirely, of xylan.

Means were found for estimating separately all the above groups with the exception of the non-pectic substance soluble in ammonium oxalate solution. This substance was also found to be soluble in hot water and is included in the results of analysis under the heading "hot water-soluble." In addition, a number of other determinations were made, including ash, alcohol-ether-soluble substances, protein, lignin and cellulose, and it is thought that no constituent of the wood present in significant amount escapes inclusion under these headings.

EXPERIMENTAL.

A. PROXIMATE ANALYSIS.

Methods of analysis.

Furfuraldehyde-yielding constituents. The estimation of hemicelluloses is a matter of some difficulty. The alkali-soluble "furfuroids" of Cross, Bevan and Smith [1895] would correspond roughly to hemicelluloses, but the figures obtained by these authors can only be approximations. König and Becker [1919] determined hemicelluloses in wood by heating with 0.4 % sulphuric acid at arbitrary pressures, the sugars arising being estimated in the resultant hydrolysis liquid. Dore [1920] determined the loss in weight of woods on extraction with 5 % NaOH. Other workers have distinguished "pentosans in cellulose" and "pentosans not in cellulose."

A method for the determination of hemicelluloses in cereal straws has recently been described by Norman [1929], it being assumed that the only furfuraldehyde-yielding constituents of the straws are pectin, free hemicelluloses and xylan associated with cellulose. Attempting to apply this method of estimation to box-wood, the following figures were obtained.

Table I.

Total furfuraldehyde on dry wood	...	...	...	11.86 %
Calcium pectate yield	...	...	...	0.41
Cellulose (Cross and Bevan)	...	...	...	49.35
Furfuraldehyde yield % of cellulose (Cross and Bevan)	...	...	...	10.35
% xylan in cellulose	...	...	...	16.04
% xylan calculated to dry wood	...	...	...	7.92

It may be pointed out that a knowledge of the carbon dioxide yield of wood and hemicellulose, obtained by the method of Nanji, Paton and Ling [1925], is not necessary to the calculation.

100 parts calcium pectate yield 19.5 parts furfuraldehyde.

100 parts xylan yield 64.5 parts furfuraldehyde.

Hence, furfuraldehyde arising from hemicellulose is given by

$$11.86 - \{(0.41 \times 0.195) + (7.92 \times 0.645)\} = 6.67 \%$$

The yield of furfuraldehyde from the hemicellulose being 55.14 %, the percentage of hemicellulose on the dry wood should be given by the quotient of 6.67 and 0.5514, *i.e.* 12.09 %. However, carefully carried out preparations of the hemicellulose mixture gave yields amounting to no more than two-thirds of this value, and similar discrepancies were found with other woods. It is therefore apparent that this method of determination is not applicable to woods.

It was decided, therefore, in the present work to determine the hemicelluloses by actual preparation, by a method which is in essentials that described by Norris and Preece [1930].

20–25 g. of the wood are extracted on the boiling water-bath with 300 cc. of 0.5 % ammonium oxalate solution for $1\frac{1}{2}$ hours, the mass after cooling being filtered on paper at the pump. The residue is washed back into the extraction flask and extraction with ammonium oxalate is repeated. The filtered residue is then treated for $1\frac{1}{2}$ hours with 300 cc. of boiling 50 % alcohol containing 1 % of sodium hydroxide. After further filtration through paper at the pump, the residue is submitted to two further extractions with 300 cc. portions of 50 % alcohol. The three filtered alcoholic extracts are combined and reserved.

The filtered residue, thus freed from pectin and much of its lignin, is allowed to stand at room temperature with 300 cc. of 4 % NaOH solution for not less than 6 hours. The extract is then filtered at the pump through a circle of calico previously boiled with 4 % NaOH. The wood residue is repeatedly further extracted with 150 cc. portions of NaOH solution until the filtered extract no longer gives a precipitate on neutralising with glacial acetic acid and adding an equal volume of acetone.

The combined filtrates are rendered acid by addition of glacial acetic acid, and a volume of acetone equal to that of the acid liquid is added. The precipitate is allowed to settle, the supernatant liquor syphoned off and the hemicellulose treated with graded strengths of alcohol, drying being continued *in vacuo* after filtering at the pump. The weight of the product is ascertained, its moisture content determined on a small portion by oven drying, and the yield further corrected for ash content. The yield of dry, ash-free hemicellulose is calculated as a percentage of the dry wood, being returned as “free hemicellulose,” *i.e.* hemicellulose soluble in 4 % sodium hydroxide solution in the cold.

By this method, the losses of material resulting during the estimation can be reduced to negligible proportions. It is suggested that the free hemicellulose content of woody materials can be more accurately determined in this way than by other methods.

The wood residue, after removal of free hemicellulose, is treated with 300 cc. of boiling 4 % NaOH solution for 1 hour, the extract being filtered clear on calico as before. The residue is then repeatedly extracted with 150 cc. portions of boiling 4 % NaOH solution, till the filtered extract on cooling no longer gives any appreciable amount of precipitate on neutralising with glacial acetic acid and adding an equal volume of acetone.

The combined filtered extracts are neutralised and acetone added as above. The precipitate obtained is taken up to dryness in the usual way and is designated “combined hemicellulose.”

It should be understood that the terms “free” and “combined” hemicellulose are employed relatively, rather than literally, to emphasise the more drastic treatment necessary to remove the latter.

The alkaline alcoholic filtrates reserved from above are rendered acid by the addition of glacial acetic acid, and 95 % alcohol may be further added to assist precipitation. The precipitate which separates out after prolonged standing is re-dissolved in 1 % NaOH, the solution is acidified, and alcohol

added to give a concentration of 75 %. The precipitate is allowed to settle, the liquid syphoned off and the product taken to dryness in the usual way.

This product, which appears to possess many properties in common with the hemicelluloses, is here described as "lignosaccharide," indicating its extraction with alcoholic sodium hydroxide, a solvent which readily extracts a portion of the lignin of woody tissues.

It is inconvenient to deduce the lignosaccharide content of the tissue from the yield obtained in this preparation, as the possibility of loss of product appears to be great. Consequently, the furfuraldehyde yield of the substance is determined, and its amount present in the wood calculated by the method shown below.

The results to this point are set out in Table II.

Table II.

Calculated on dry wood:	free hemicellulose	7.68 %
	combined hemicellulose	6.43
Furfuraldehyde yield from:	free hemicellulose	47.35 %
	combined hemicellulose	51.44
	lignosaccharide	38.10

The second stage of the estimation involves the treatment of 3–5 g. of the material with 200 cc. of 0.5 % ammonium oxalate solution at 85° for 24 hours. The solution is filtered, the residue washed with further ammonium oxalate solution, and the filtrate and washings are made up to 250 cc. The tissue residue is reserved. 100 cc. of the extract are treated for the estimation of total pectin by the method of Nanji and Norman [1928]. A second 100 cc. portion of the extract is evaporated gently to 50 cc., and 25 cc. of concentrated hydrochloric acid are added. The whole is washed into a distilling flask with 12 % HCl, and furfuraldehyde distillation carried out in the usual way. The furfuraldehyde arising is estimated and calculated to a percentage of the original dry wood.

This furfuraldehyde arises from pectin and from non-pectic substances simultaneously removed. The amount of pectin present being known, the non-pectic furfuraldehyde may be calculated by difference from the fact that 100 parts of calcium pectate yield 19.5 parts of furfuraldehyde.

The reserved tissue residue is extracted twice with 100 cc. portions of boiling 50 % alcohol containing 1 % of NaOH, the periods of extraction each being 1½ hours. The alcoholic filtrates are combined, and the tissue is finally washed with hot 50 % alcohol, the washings being added to the combined filtrates. To the extract is added glacial acetic acid so that the mixture remains just alkaline, the alcohol is evaporated off, and the total volume adjusted to 50 cc. 25 cc. of concentrated HCl are added, and the mixture is washed into a distilling flask, and furfuraldehyde again estimated and calculated to the dry wood.

Knowing now the amount of furfuraldehyde obtainable from the alcoholic

sodium hydroxide extract, and also the furfuraldehyde yield from the lignosaccharide, the percentage of this constituent in the tissue can be calculated. Thus, in the case of box-wood, the figures were 0.70 % of furfuraldehyde from the extract, and 38.10 % from the lignosaccharide, whence this wood contains 0.70/0.381 or 1.83 % of lignosaccharide.

If now a total furfuraldehyde determination is carried out on the wood itself, a complete furfuraldehyde balance sheet can be drawn up as shown in Table III.

Table III.

	% of dry wood
Furfuraldehyde from substances soluble in:	
(i) 0.5 % ammonium oxalate (a) pectin ...	0.08
(b) non-pectin ...	0.18
(ii) Alcoholic sodium hydroxide	0.70
Furfuraldehyde from:	
(iii) Free hemicellulose	3.64
(iv) Combined hemicellulose	3.31
(v) Other sources (by difference)	3.95
Total furfuraldehyde found directly	11.86

It was shown that the material remaining after removal of hemicelluloses from box-wood still retained a considerable amount of xylan.

Some 2 g. of the wood residue was treated with 72 % sulphuric acid according to the method of Monier-Williams [1921]. The dried products of hydrolysis were extracted with absolute alcohol. The bulk of the sugar produced was dissolved in this way and proved to be a mixture of glucose and xylose. Thus it yielded cadmium xylonobromide and a mixture of glucosazone, melting at 205°, and xylosazone melting at 155°. Other sugars could not be detected.

Consequently, the furfuraldehyde described as from "other sources" is converted to xylan, using the factor 1.55, the figure appearing as "other pentosans" in Table IV corresponding to this result.

Cellulose. This constituent was determined by chlorination, after a preliminary treatment of the tissue with 100 cc. of 1 % NaOH solution for 20 minutes. Chlorination was carried out in a Gooch crucible for periods of 20 and 15 minutes, followed by two further treatments of 10 minutes each. The resulting crude cellulose in every case gave large yields of furfuraldehyde on distillation with 12 % HCl. The yield of crude cellulose was therefore corrected, as suggested by Norman [1929], on the assumption that this furfuraldehyde is derived from xylan associated with the cellulose, the factor 1.55 being used for conversion of furfuraldehyde yield to xylan.

A further correction might conceivably be made assuming that the difference between furfuraldehyde yield from the crude cellulose, calculated back to the dry wood, and the furfuraldehyde from "other sources" of Table III represents hemicellulose not removed by chlorination. However, it is not unlikely that different portions of the hemicellulose complex might show

different degrees of resistance to removal by chlorination, and further correction on these lines was not attempted.

Lignin. It was recognised by Mehta [1925] that lignin estimation by alkaline treatment gave results which were very much lower than those obtained by the use of strong mineral acids.

Undoubtedly, some change is induced in lignin by acid treatment. Thus, lignin obtained by using 72 % sulphuric acid may still contain small amounts of carbohydrate material, while evidence has been obtained by Mehta that sulphonation may also take place. On the other hand, methoxyl and acetyl groups may be lost during the treatment, and on the whole it is not unlikely that the various errors tend to balance one another [*cf.* Euler, 1923].

In the present work, two methods of lignin estimation are used. The first is Mehta's method for free and combined lignin, these being returned separately. The second, carried out on wood previously extracted with alcohol, employs the 72 % H_2SO_4 -HCl mixture of Schwalbe [1925] in the manner described by Norman [1929]. The probable relative value of the two methods is further discussed below.

Other constituents. Ash content is determined in the usual way. The loss in weight of the wood when exhaustively extracted with alcohol and ether successively is also found. If the acid method of lignin estimation is used, the value for total "alcohol-ether-soluble" is returned directly. If free and combined lignin are determined by alkaline treatment and returned separately, the value for free lignin must be subtracted from total alcohol-ether-soluble, and the difference returned as "free fat, pigment, *etc.*" The residue after alcohol-ether extraction is used for the determination of protein, the factor 6.25 being employed.

Finally, hot water-soluble substances are determined as described by Schorger [1926, p. 506]. If overlapping of results is to be avoided, "hot water-soluble" may not be returned directly. Thus, hot water dissolves some of the ash constituents, possibly protein, and some of the material soluble in alcohol and ether. Accordingly, these determinations are repeated on wood extracted with hot water, and the value for "hot water-soluble" is corrected. In some cases, the pectin soluble in hot water might also have to be taken into account, but it was not thought necessary to attempt this further correction in the case of woods.

Results of analysis.

The results obtained by the above methods of analysis are set out in Table IV. Two columns are given, allowing the values obtained by the two methods of lignin estimation to appear in separate columns, together with the correction where necessary of "alcohol-ether-soluble" to "free fat, pigment, *etc.*"

The summation of 87.78 % obtained when lignin is estimated by sodium hydroxide treatment is most unsatisfactory, since it is inconceivable that any constituent present in appreciable amount has escaped determination. Using

Table IV.

	% of dry wood	
Ash	1.22	1.22
Alcohol-ether-soluble ...	—	3.91
Free fat, pigment, etc. ...	2.05	—
Protein	2.81	2.81
Hot water-soluble (corrected)	0.71	0.71
Free lignin (NaOH) ...	1.86	—
Combined lignin (NaOH) ...	15.23	—
Lignin ($\text{H}_2\text{SO}_4\text{-HCl}$) ...	—	26.45
Pure cellulose	41.43	41.43
Calcium pectate number ...	0.41	0.41
Free hemicellulose	7.68	7.68
Combined hemicellulose ...	6.43	6.43
Lignosaccharide	1.83	1.83
Other pentosans (as xylan)...	6.12	6.12
Total estimated	87.78	99.00

the acid method, on the other hand, the summation of 99.00 % represents a very satisfactory figure.

On this evidence, it is reasonable to conclude that figures for lignin content obtained by the alkali method cannot be accepted as reliable, and it may be mentioned that figures further supporting this view have been obtained with other woods.

B. NATURE OF THE FURFURALDEHYDE-YIELDING CONSTITUENTS OF BOX-WOOD.

There remain to be discussed the results of a qualitative study of the furfuraldehyde-yielding constituents of the wood.

Water-soluble constituents. 100 g. of the sawdust were boiled for several hours with 1 litre of water. The filtered extract was cooled and concentrated to a bulk of 50 cc. 200 cc. of absolute alcohol were added, and after settling the supernatant liquor was poured off and reserved. The precipitate was heated with absolute alcohol to denature any protein present, and the residue was extracted with hot water, and the solution filtered. Four volumes of absolute alcohol were added to the filtrate, this treatment bringing down a very small amount of precipitate.

The product was readily soluble in hot water, the solution being non-reducing. It gave a blue-green colour with iodine solution, suggesting the presence of a little starch or amylo-hemicellulose [*cf.* Ling and Nanji, 1925]. On treating a little of the solution with Bial's orcinol reagent [see Browne, 1912, p. 382] the reaction for pentoses gave a negative result, but on boiling the solution a green colour developed, suggesting the presence of uronic anhydride. Possibly the substance responsible for this reaction is analogous to the ϵ -galactan of Trimble [1898] and of Schorger and Smith [1916], who isolated from western larch a substance yielding furfuraldehyde, but containing no pentosan. Their product was probably uronogalactan. The amount arising from box-wood was insufficient for examination.

The mother-liquor reserved from above was evaporated just to dryness *in vacuo* and the residue taken up in water. The solution, which had strong reducing properties, gave no coloration with iodine and was free from furfuraldehyde-yielding substances. Free simple sugars were evidently absent, since a crystalline osazone could not be obtained; the product instead was gelatinous, and its properties resembled those of phenylhydrazine derivatives prepared from the dextrinous products of starch conversions.

Lignosaccharide. The method of preparation of this substance has already been described, 100 g. of box-wood yielding 1.5 g. of lignosaccharide. The product was a fine white powder, somewhat soluble in cold water, completely in hot water to an opalescent, non-reducing solution. Lignosaccharide dissolves to a clear solution in 4 % NaOH solution and is not precipitated by Fehling's solution. It is precipitated, however, from the mixture on addition of acetone, thus behaving analogously to hemicelluloses B2 and C2 isolated from other sources.

Box-wood lignosaccharide gave 38.10 % of furfuraldehyde, and 6.65 % of carbon dioxide, the latter corresponding to a uronic anhydride content of 26.60 %.

This product showed a marked degree of resistance to hydrolysis. Thus, boiling for 2 hours with 5 % sulphuric acid produced a liquor in which free simple sugars could scarcely be detected.

Boiling for $7\frac{1}{2}$ hours proved more successful, sugar being produced equivalent in amount to the hydrolysis of rather less than one-half of the lignosaccharide.

Two-thirds of this sugar was dissolved by absolute alcohol and proved to be chiefly xylose.

The sugar soluble in 80 % alcohol was treated with nitric acid for the preparation of mucic acid, but with negative results, so that the only constituents of the lignosaccharide which have been identified are uronic anhydride and anhydro-xylose.

Free hemicellulose. The wood residue remaining after removal of water-soluble substances, pectin, lignin, and lignosaccharide was treated with 4 % NaOH solution at room temperature, and the filtered extract was neutralised with glacial acetic acid and submitted to acetone fractionation. Only one fraction was obtained, corresponding to crude hemicellulose B, the yield from 100 g. of sawdust being 6.2 g. This was purified by copper treatment, the whole being precipitated from alkaline solution by Fehling's solution. Thus, the only free hemicellulose obtainable from box-wood sawdust is hemicellulose B1.

The purified product is a white, amorphous powder, easily soluble in hot water, the solution giving a faint opalescence on cooling. The solution is non-reducing and gives a faint greenish tinge with iodine. The hemicellulose is precipitated from aqueous solution by basic, but not by neutral, lead acetate. Three-quarter saturation with ammonium sulphate also gives a precipitate.

The hemicellulose is easily soluble in cold dilute alkali and in hot dilute mineral acids, the latter causing hydrolysis. The solution in 4 % NaOH solution gives for $c = 3.00 [\alpha]_D^{20} - 62.3^\circ$.

An aqueous solution of this hemicellulose is hydrolysed by the action of taka-diastrase. After 24 hours, a precipitate is no longer given on rendering the solution alkaline and adding Fehling's solution, but the subsequent addition of acetone gives a thick precipitate. Iodine absorption corresponding to hydrolysis of 11 % of the hemicellulose takes place at this stage. At the end of 7 days, 40 % of the hemicellulose is apparently hydrolysed, and an equilibrium is reached at a little in excess of this value after 10 days. At the end of this time, the amount of precipitate given by the solution, either with acetone or a mixture of Fehling's solution and acetone, is extremely small. Free simple sugars cannot be detected in the final hydrolysis liquor.

The following analytical figures were obtained with hemicellulose B1, being calculated to a dry, ash-free basis:

Yield of furfuraldehyde	...	...	55.14 %
„ carbon dioxide	...	...	4.25 %
Hence, uronic anhydride	...	...	17.00 %

Since, as is shown below, the only sugar which can be detected amongst the products of hydrolysis of this substance is xylose, these figures may be interpreted as follows:

Furfuraldehyde from uronic anhydride	2.83 %
Hence, furfuraldehyde from xylan	52.31 %

The composition of the hemicellulose may now be calculated:

Uronic anhydride	...	...	...	17.00 %
Xylan	...	...	...	81.08 %
Total accounted for	...	...	...	98.08 %

This composition corresponds approximately to a molecule

which would yield 55.80 % of furfuraldehyde, and 4.55 % of carbon dioxide.

1.31 g. of the hemicellulose was hydrolysed with 75 cc. of 1 % sulphuric acid for $3\frac{1}{2}$ –4 hours. The sulphuric acid was neutralised with the calculated quantity of 0.25 *N* barium hydroxide solution, and the filtered liquid, free from barium sulphate, titrated with 0.25 *N* barium hydroxide till it remained just acid to phenolphthalein. 4.7 cc. were required to effect this, equivalent to $0.176 \times 0.25 \times 4.7 = 0.207$ g. of uronic anhydride, *i.e.* 15.7 % calculated on the hemicellulose. But the hemicellulose contains 17.0 % of uronic anhydride; hence, a practically quantitative liberation of the free carboxyl groups had been achieved. No insoluble barium salt was thrown down during this neutralisation of carboxyl groups.

The solution was taken just to dryness *in vacuo* and the residue extracted

several times with absolute alcohol. Xylose was isolated from the alcoholic extracts. The dry product weighed 0.55 g., corresponding to a yield of some 45 % of the theoretical. The xylose obtained gave $[\alpha]_D^{20} + 18.6^\circ$, and the usual derivatives were prepared.

It has been shown in the case of the hemicelluloses of maize cobs [Preece, 1930] that 90 % of the theoretical amount of xylose can be obtained by the action of 1 % sulphuric acid. Hence, it appears fairly certain that acid of this strength does not cause a complete hydrolysis of box-wood hemicellulose; indeed, it seems to liberate only about one-half of the total possible amount of xylose.

Accordingly, the formula given above may be amended to

where one-half of the anhydro-xylose exists in a form of combination differing from that of the rest.

To test these suggestions further, a known weight of hemicellulose was treated with 1 % sulphuric acid, portions of the hydrolysis liquor being removed every half-hour, and the sugar present estimated iodimetrically by the method of Baker and Hulton [1920]. The results were calculated as apparent xylan and further expressed as a percentage of the hemicellulose hydrolysed. In a typical experiment, 1.473 g. of dry, ash-free hemicellulose was heated with 100 cc. of 1 % sulphuric acid.

Table V A.

Time from commencement hrs.	Apparent xylose produced g.	Equivalent weight xylan g.	Xylan as % of hemicellulose
$\frac{1}{2}$	0.515	0.453	30.7
1	0.883	0.777	52.7
$1\frac{1}{2}$	1.024	0.901	61.2
2	1.065	0.938	63.7
$2\frac{1}{2}$	1.059	0.932	63.3

It is apparent from this table that an equilibrium is reached when there is a production of apparent xylose equivalent to the hydrolysis of some 64 % of the hemicellulose. However, the percentage of free xylose liberated in the first experiment described corresponds to a hydrolysis of xylan calculated on the hemicellulose of only 41 %. The difference between 64 % and 41 %, therefore, must be attributed to a liberation of free aldehydic groups, capable of reacting with alkaline iodine, but not present in the form of free xylose.

In continuation of the above hydrolysis, the volume of hydrolysis liquor

Table V B.

Time from commencement hrs.	Apparent xylose produced g.	Equivalent weight xylan g.	Xylan as % of hemicellulose
3	0.799	0.703	79.5
$3\frac{1}{2}$	0.827	0.728	82.4
4	0.840	0.740	83.7
$4\frac{1}{2}$	0.875	0.771	87.2

remaining (60 cc.) was treated after $2\frac{3}{4}$ hours with 40 cc. of 10 % sulphuric acid, the resultant concentration of acid thus being 4.6 %, and 100 cc. of hydrolysis liquor now being equivalent to 0.883 g. of hemicellulose (see Table V B).

Thus, the increased concentration of acid caused an increase in the amount of hydrolysis, the rate, however, diminishing after the first few minutes.

Next the course of hydrolysis was followed, using 4.6 % sulphuric acid from the commencement. The equivalent of 0.7237 g. of dry, ash-free hemicellulose was treated with 50 cc. of 4.6 % sulphuric acid.

Table VI.

Time from commencement hrs.	Apparent xylose produced g.	Equivalent weight xylan g.	Xylan as % of hemicellulose
$\frac{1}{2}$	0.473	0.416	57.5
1	0.652	0.574	79.3
$1\frac{1}{2}$	0.652	0.574	79.3
2	0.641	0.564	77.9
$2\frac{1}{2}$	0.613	0.540	74.6
3	0.673	0.592	81.8
$3\frac{1}{2}$	0.684	0.602	83.1
4	0.652	0.574	79.3

Here again the hydrolysis at first proceeds rapidly, but after 1 hour further action is slow, and eventually shows a falling off. This last effect is probably to be attributed to a destruction of uronic acid. Thus, O'Dwyer [1926] found that uronic acids tend to decompose during hydrolysis. This effect masks for a time the liberation of sugar, which, however, eventually re-asserts itself. It should be noted that the point at which the hydrolysis slackens—79.3 %—is almost exactly the same as the point of retardation found in the previous experiment—approximately 79.5 %.

Hydrolysis of the hemicellulose with an intermediate strength of sulphuric acid, namely 2.8 %, gave results agreeing closely with those obtained with 1 % acid, equilibrium being reached at an apparent hydrolysis of about 66 % of the hemicellulose.

Hydrolysis with either 1 % or 2.8 % acid, therefore, causes the rapid apparent hydrolysis of 64–66 % of the hemicellulose, subsequent action being absent or very slow. 4.6 % acid causes the rapid apparent hydrolysis of approximately 80 % of the hemicellulose, the action proceeding slowly beyond this point, when its effect may be at least partially masked by a destruction of uronic acid.

The results obtained may be best interpreted as follows. Suppose the structure of the hemicellulose is represented by the formula

It is to be assumed that the following changes take place. I is rapidly hydrolysed by 1 % H_2SO_4 to free xylose. II is converted to a disaccharide of

the form $C_5H_9O_4 \cdot O \cdot C_5H_9O_4$ or, since it contains a free aldehydic group, $CHO \cdot C_4H_8O_3 \cdot O \cdot C_5H_9O_4$. III is converted to a similar disaccharide derivative, containing one free aldehydic group, and one free carboxyl group. This would account for the liberation of the total acidity without formation of free uronic acid. It appears likely that the uronic acid concerned is glycuronic acid, and indeed free glycuronic acid could not be detected amongst the products of hydrolysis of the hemicellulose by 1 % acid.

The suggestion that a disaccharide derivative of the type of a xylosido-uronic acid is formed may be compared with the observation of Butler and Cretcher [1929] that hydrolysis of arabic acid, obtained from gum arabic, with 2 % sulphuric acid yields *d*-galactosido-*d*-glycuronic acid.

Hydrolysis of the hemicellulose in this way would result in the liberation of three molecules of xylose, one free carboxyl group, and two free additional .CHO groups.

4.6 % acid causes a further liberation of .CHO groups. Suppose the portion III of the molecule is, along with I, readily hydrolysed to its components by this stronger acid, while II is still only slowly attacked. Then a total of 4 molecules of free xylose, one of uronic acid (now susceptible to the destructive action of the mineral acid), and one additional free .CHO group would be liberated.

Assuming that 90 % of the free xylose produced is isolated as such, the theoretical yields on the foregoing assumptions and the yields actually obtained are given in Table VII.

Table VII.

	1 % H_2SO_4		4.6 % H_2SO_4	
	Free xylose from 1 g. of hemicellulose	Total apparent hydrolysis*	Free xylose from 1 g. of hemicellulose	Total apparent hydrolysis*
Theory	0.42 g.	68 %	0.56 g.	82 %
Actual	0.42	65	0.52	80

* Calculated as a percentage of xylan on the hemicellulose.

Thus, there appears to be good evidence for assuming that the hemicellulose is a polymerised substance of the formula given above.

Combined hemicelluloses. The tissue residue remaining after removal of free hemicelluloses was extracted several times with boiling 4 % NaOH solution, the extracts obtained being filtered and combined. Neutralisation of the cooled extracts with glacial acetic acid caused a precipitation of combined hemicellulose fraction A. This was centrifuged off, and half a volume of acetone was added to the filtered centrifugate. Fraction B was thus precipitated. The filtrate from this gave a further small amount of precipitate on adding more acetone, but the yield of fraction C arising in this way was too small for examination.

Heated with water, fraction A gives a silky suspension, which is non-reducing and gives a greenish tinge with iodine. This hemicellulose becomes

soluble in cold 4 % NaOH, and the bulk of it is precipitated from alkaline solution on addition of Fehling's solution. A separation on these lines into its components A1 and A2 was not attempted, owing to the very small amount of A2 which would thus have been obtained.

Fraction B is soluble in hot water; the solution gives a pale green colour with iodine and is non-reducing. This fraction also dissolves in cold 4 % NaOH solution and is partially re-precipitated on addition of Fehling's solution. Again, a separation was not made.

The usual analytical data were obtained, as shown in the following table.

Table VIII.

Hemicellulose fraction	A	B
Furfuraldehyde yield	36.57 %	48.29 %
Carbon dioxide yield	3.22	4.33
Uronic anhydride content	12.88	17.32

In each case hydrolysis was carried out by boiling for 2 hours with 5 % sulphuric acid. After removal of sulphuric acid, the hydrolysis liquid was evaporated just to dryness *in vacuo*, and the sugar extracted successively with absolute and 80 % alcohol. Several extractions were made with alcohol of each strength. The filtered extracts were worked up for the preparation of dry sugar fractions.

Some 65 % of the sugar obtained from combined hemicellulose A was dissolved by absolute alcohol. This product had $[\alpha]_D^{20} + 17^\circ$ and represented an impure sample of xylose. The sugar dissolving in 80 % alcohol gave $[\alpha]_D^{20} + 26^\circ$, but sugars other than xylose could not be detected even in this fraction.

In the case of combined hemicellulose B also the bulk of the sugar produced on hydrolysis was dissolved by absolute alcohol. The sugar of this fraction was again impure xylose.

80 % alcohol dissolved sugar having $[\alpha]_D^{20} + 49^\circ$. The osazone prepared from the sugar of this fraction was a mixture of indefinite character and low melting-point, but certainly contained much xylosazone. An attempt to prepare cadmium xylonobromide was successful. From the figure quoted and the fact that xylose is present to an appreciable extent in the sugar of this fraction, it would seem that the second sugar of this fraction is one with a high specific rotation. However, mucic acid tests gave negative results, while the absence of arabinose from the hydrolysis products is indicated by the low specific rotation of the sugar of the absolute alcohol-soluble fraction, namely $[\alpha]_D^{20} + 20^\circ$. Thus the nature of the second sugar must remain at present undetermined.

The tissue residue. The wood residue remaining after removal of combined hemicelluloses was examined. Attempted estimations of mannan and galactan were carried out by the methods described by Schorger [1926, pp. 537, 538], but both these substances were found to be absent. As has been shown above,

the pentosan constituent of the residue is xylan. It was also found that boiling dilute acid—5 % sulphuric acid—had but little hydrolytic effect on the wood residue.

DISCUSSION.

Since the amount of pectin present in the wood is 0.41 %, the proportion of anhydro-arabinose is less than 0.1 %. Anhydro-xylose, on the other hand, amounts to approximately 17 % of the wood, while the total uronic anhydride content is of the order of 3 %. Unidentified anhydro-hexose or anhydro-methylpentose constituents of the hemicelluloses and lignosaccharide account for some 2 % of the wood.

It is apparent from the work described that there is little difference in nature between the combined hemicelluloses isolated from box-wood and the free hemicelluloses from box-wood and from the other sources dealt with previously [Norris and Preece, 1930; Preece, 1930, 1931]. The lignosaccharide, though more resistant to hydrolysis, is also of a similar nature. These constituents, consequently, can be grouped together as "structural hemicelluloses," the name "hemicellulose" being retained in preference to that of "polyuronide" suggested by Candlin and Schryver [1928].

It is desirable to distinguish between the hemicelluloses and the xylan of the wood residue. This xylan resists extraction by the relatively drastic action of boiling 4 % NaOH solution and is also resistant to the hydrolytic action of boiling dilute mineral acids. In short, its properties approach more nearly those of cellulose than those of the hemicelluloses. Accordingly, it is proposed to make such a distinction by the re-introduction of the term "para-xylan" for this resistant form of xylan. Thus, the final wood residue may be said to consist of cellulose and para-xylan, together with some unextracted lignin.

Although it has been found possible to assign a comparatively simple formula to the free hemicellulose of box-wood, it is not claimed that the fractions described in this and previous communications represent individual hemicelluloses [see also Preece, 1930]. It has been shown by O'Dwyer [1928] that the complexity of a hemicellulose seems to vary with the age of the wood from which it is obtained. If change in complexity is regularly taking place, this must lead to the presence of a large number of true hemicellulose units of different composition. Since the present method of separation of free hemicelluloses cannot give more than six fractions, it would seem that these fractions must, generally speaking, be mixtures, representing aggregates of molecules of similar physical properties.

A similar argument might, it may be supposed, be applied to lignosaccharide and combined hemicelluloses. An inter-relationship between these three subdivisions of the structural hemicelluloses might conceivably, if shown to exist, lead to a revision of the above view.

SUMMARY.

1. A method of analysis of woody tissues giving a very satisfactory summation is described, various types of furfuraldehyde-yielding substance being estimated separately.

2. The alkali method for lignin estimation gives results which cannot be accepted.

3. Free pentoses are absent from box-wood, but hot water extracts some non-pentosan furfuraldehyde-yielding substance.

4. Lignosaccharide and free and combined hemicelluloses have been isolated from box-wood, and their nature examined. Xylose and uronic acid arise on hydrolysis of each of these substances.

5. The composition of the free hemicellulose is completely accounted for as urono-xylan of relatively simple constitution. The hydrolysis of this substance has been closely studied, and different parts of the molecule shown to possess different degrees of resistance to hydrolysis by dilute sulphuric acid.

6. The wood residue remaining after extraction of the above constituents consists chiefly of cellulose, para-xylan, and some unextracted lignin.

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