LXXXVI. THE HEMICELLULOSES. PART IV.

THE HEMICELLULOSES OF BEECH WOOD.

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In a previous communication by the author [1923] it was pointed out that the term "hemicellulose," as defined by Schulze [1892], was applied to a class of substances soluble in alkalis, from which solution they could be precipitated by acids or alcohol, and much less resistant to the action of hydrolysing agents than cellulose.

The investigation described in this paper indicates that the hemicelluloses are not as originally thought true carbohydrates (pentosans, hexosans, etc.) but contain acid groups (glycuronic and galacturonic acids) and are, therefore, more nearly allied to the pectic substances than to cellulose.

The hemicelluloses from different sources, furthermore, have been found to vary to a considerable extent, especially as regards the products of hydrolysis, and, indeed, two hemicelluloses have been obtained from beech wood itself, for on adding a large excess of acid to a sufficiently concentrated alkaline extract of the hemicellulose it is possible to obtain a precipitate, the clear filtrate from which gives on the addition of two volumes of alcohol (95 %) a second precipitate. These two precipitates are essentially different both in appearance and properties. That which is directly precipitated by acid is more resistant to hydrolysis, giving, after several hours' treatment with dilute sulphuric acid, xylose and apparently 11 % glycuronic acid. On treatment with 12 % hydrochloric acid by the method of Tollens 50 % of furfural is obtained.

The second precipitate, on the other hand, seems to be still more closely allied to the pectic substances. It gives, on hydrolysis, apparently about 63 % of galacturonic acid together with some arabinose and a small amount of galactose. The amount of furfural obtained is 42·7 %. This precipitate also yields small amounts of mucic acid on oxidation with nitric acid.

The amount of the second precipitate obtained from beech wood is small in comparison with that obtained by direct precipitation with acid. Workers in these laboratories on the hemicellulose of non-lignified tissue find, however, that the opposite is the case in the substances which they have examined. They also have obtained two hemicelluloses of which that not precipitated directly by acid is in predominant quantity.

The whole subject of the relation between the hemicelluloses and the pectic substances in lignified and unlignified tissue seems to open up a very fruitful field for investigation. The pectic substances occur in unlignified tissue in much greater amounts than the hemicelluloses [Norris and Schryver, 1925]; in lignified tissue the opposite is found to be the case [O'Dwyer, 1925].

The obtaining of sufficient quantities of the "uronic" acids for the purpose of identification has been tedious work owing to their decomposition during the course of hydrolysis. However, the method of estimating the carbon dioxide given off in such cases by treatment with 12 % hydrochloric acid, advanced by Nanji, Paton and Ling [1925], has proved very helpful as showing the existence of these substances as products of hydrolysis of the hemicellulose of beech wood, and in giving the amounts of these acids which might be obtained from each precipitate if their decomposition during hydrolysis could be avoided.

Before the separation of the two precipitates had been effected, a sugar acid was obtained after hydrolysis of the mixed precipitate from sawdust 93 years old with 1 % sulphuric acid. When, however, hemicellulose from beech wood 130 years old was treated in the same manner, apparently no sugar acid was present in the hydrolysed solution. It was thought at the time that the age of the wood was responsible for its non-appearance, but further experiments showed that, while in the earlier stages of hydrolysis a sugar acid appeared which gave a barium salt soluble in water, hydrolysis for a further period produced an acid giving an insoluble barium salt. When the hydrolysis was carried still further both acids apparently disappeared, as after the addition of the amount of barium hydroxide necessary for the neutralisation of the sulphuric acid the solution was no longer acid.

These results were extremely puzzling, and it was only after repeated experiments on the preparation and hydrolysis of hemicellulose had been carried out, that it was found that the material originally employed was a mixture of two hemicelluloses, one of which could be obtained from the alkaline solution by direct precipitation with acid, while the other separated out from the acid filtrate on addition of alcohol. These two substances were then isolated in as pure a condition as possible, and, when they were subjected to hydrolysis under the same conditions as before, it was found that the sugar acid giving an insoluble barium salt was a product of hydrolysis of the hemicellulose directly precipitated by acid, whilst that forming a soluble barium salt came from the second precipitate, which, as before stated, is less resistant to hydrolysis. They were subsequently found to be respectively glycuronic and galacturonic acid. The isolation of the acid depends therefore on the conditions of hydrolysis and not on the age of the tree, although experiments showed that age has apparently some bearing on the amount of each hemicellulose obtained.

Norris and Schryver [1925] have isolated two hemicelluloses from non-lignified tissue of a single plant material (turnips). One of these is water-soluble and the other is precipitated from the soda extract by acid. Their work will be made the subject of a further communication.

PREPARATION OF THE HEMICELLULOSES.

Separation of two hemicelluloses. 4500 g. of sawdust from beech wood 80 years old were used in the preparation of these hemicelluloses, and an endeavour was made to obtain as pure a product as possible. After thorough extraction with hot water, the sawdust was treated for a short time with 0·2 % sodium hydroxide in order to remove the small amount of protein (0·7 to 0·8 %) previously found present by Kjeldahl's method. This weak alkaline solution did not apparently affect the hemicelluloses which were subsequently extracted with 4 % sodium hydroxide. Lime water was then added to the soda extract, to bring down any pectic acid present, and the mixture filtered. It was found possible, by adding a sufficient excess of glacial acetic acid to the filtrate, to effect a complete separation of the precipitate which had previously been obtained in a colloidal condition. The clear filtrate obtained after the removal of this precipitate was concentrated, and two volumes of 95 % alcohol were added. A second precipitation now occurred. These two precipitates were also obtained from a soda extract of beech sawdust which had previously been treated with hot 0·5 % ammonium oxalate.

The hemicellulose obtained by direct precipitation with acetic acid was now washed with small quantities of distilled water until the filtrate became colourless, dissolved again in 4 % sodium hydroxide and re-precipitated with acid. It was then electro-dialysed for 10 days in order to effect the removal of sodium acetate and other inorganic matter which might be present. After treatment with graded strengths of alcohol and dry ether, it was finally dried to constant weight in a vacuum desiccator over phosphorus pentoxide. The yield was a little over 5 % of the weight of the sawdust.

The second hemicellulose, precipitated from the acid filtrate by the addition of alcohol, which was soluble in water, was found on dialysis to pass through the membrane of the dialyser to some extent. It was accordingly, after filtering, re-dissolved in water, the solution concentrated and the hemicellulose re-precipitated with alcohol. After repeating this process, a substance was obtained, which, after drying with graded strengths of alcohol and then with dry ether, was found to contain only a small amount of inorganic matter. This product was dried to constant weight in a vacuum desiccator over phosphorus pentoxide. The yield was about 1 % of the weight of the sawdust.

Properties of the two hemicelluloses. The hemicellulose obtained by direct precipitation of the soda extract with acetic acid is an amorphous substance, almost white in colour, insoluble in dilute acids and alcohol and soluble in alkalis. When freshly prepared, it is dispersed by water to a small extent, but after drying is insoluble in cold water. It forms, on treatment with hot water and cooling, a somewhat gelatinous mass. A greenish coloration is obtained with iodine. It does not reduce Fehling's solution, and is laevo-rotatory $[\alpha]_D = -107°$, the determination being made in a 1 % sodium hydroxide solution. The furfural yield, obtained by Tollens' method, reckoned on the ash-free substance was 50·0 %. The ash amounted to 4·0 % and was found to be mostly ferric oxide.

The following figures were obtained by combustion, the results being given for the ash-free substance:

(*a*) 0·1605 g. gave 0·2615 g. CO_2 and 0·1218 g. H_2O.
C = 44·43 %, H = 8·43 %, O = 47·47 %.

(*b*) 0·1415 g. gave 0·2288 g. CO_2 and 0·1025 g. H_2O.
C = 44·10 %, H = 8·05 %, O = 47·85 %.

On oxidation with nitric acid (sp. gr. 1·15) by the method of Tollens [Norris and Schryver, 1925] no mucic acid was obtained.

In order to ascertain the relation between the time of hydrolysis and the reducing sugars present, 0·5 g. of the substance was hydrolysed with 1 % sulphuric acid for different periods.

Bertrand's method was employed to ascertain the reducing power of the hydrolysed solution in each case.

Duration of hydrolysis (hours)	Reducing sugar as glucose (%)
1	20·5
2	45·3
4	62·5
6	86·5
8	88·7

The hemicellulose brought down from the acid filtrate by alcohol is amorphous, dark in colour, and is, as before stated, soluble in water. It is also dissolved by dilute acids and alkalis but is insoluble in alcohol and ether. On the addition of iodine, a slight decoloration of the iodine solution was observed.

The substance does not reduce Fehling's solution and has a specific rotation in 0·5 % sodium hydroxide of – 120°. The furfural yield is 42·7 %.

5·84 % of ash was present and consisted largely of ferric oxide, with a small amount of carbonates.

The following figures were obtained by combustion of the ash-free substance:

(*a*) 0·2400 g. gave 0·3843 g. CO_2 and 0·1570 g. H_2O.
C = 43·66 %, H = 7·27 %, O = 49·07 %.

(*b*) 0·2410 g. gave 0·3890 g. CO_2 and 0·1568 g. H_2O.
C = 44·10 %, H = 7·23 %, O = 48·67 %.

(The author is indebted to Mr B. D. Wakeford for the combustion figures and also for those for ash and furfural.)

The mucic acid estimation gave the following result:

0·5388 g. gave 4·40 % mucic acid ≡ 4·80 % galactan.
0·4950 g. gave 4·30 % mucic acid ≡ 4·76 % galactan.

The relation between the time of hydrolysis and the amount of reducing sugars was ascertained as before, and the following figures were obtained:

After 1 hour's hydrolysis ...	28·5 %
„ 2 hours' „ ...	60·0 %
„ 4 „ „ ...	74·2 %
„ 6 „ „ ...	80·5 %

It is proposed to call the hemicellulose obtained by direct precipitation with acid hemicellulose A, and that obtained by adding alcohol to the acid filtrate, hemicellulose B.

HYDROLYSIS OF THE TWO HEMICELLULOSES.

(a) *Determination of carbon dioxide evolved on treatment with strong acid.*

As carbon dioxide is given off when hemicellulose is submitted to hydrolysis by acids, the method adopted by Nanji, Paton and Ling [1925] for its estimation was followed. Accordingly estimations were made on the acid and alcohol precipitates of hemicellulose just described, and also on a hemicellulose containing both precipitates. Hemicellulose from which the pectic substances had not been removed was also used in these experiments, as it was expected that the percentages of "uronic" acids would be slightly higher in such cases.

In each experiment carbon dioxide was given off, and the following results have been obtained:

Hemicellulose (description of)	Amount taken (g.)	% CO_2 on decarboxylation	% "uronic" acids (calc.)
(*a*) Mixed precipitate containing pectic substances	0·5849 0·6500	3·31 3·52	13·24 14·08
(*b*) Mixed precipitate free from pectic substances	0·5676 0·4977	3·00 2·91	12·0 11·64
(*c*) Acid precipitate free from pectic substances	0·4207 0·4130	2·74 2·76	10·96 11·04
(*d*) Alcohol precipitate free from pectic substances	0·4705 0·4590	15·98 16·02	63·92 64·08

From the above results it is evident that the alcohol precipitate gives a large amount of carbon dioxide on decarboxylation, and resembles pectic acid in this respect. The slight differences between the yields of carbon dioxide from hemicellulose free from, and that containing, pectic substances are accounted for by the small percentage of such substances in beech wood. The yield of carbon dioxide from the acid precipitate shows again that this substance is quite unlike that brought down by alcohol.

(*b*) *Hydrolysis with dilute sulphuric acid.*

The following hydrolyses were now carried out on the two hemicelluloses:

(i) 15·5 g. of hemicellulose A were hydrolysed for 2½ hours with 200 cc. of 1 % sulphuric acid. After adding the requisite amount of baryta to neutralise the sulphuric acid, the hydrolysed solution was no longer acid, so apparently no sugar acid is present.

(ii) 11·8 g. of the same hemicellulose were hydrolysed for 6 hours with 150 cc. of 2½ % sulphuric acid. The hydrolysed solution after the addition of the requisite amount of baryta was found still strongly acid. On the addition of more baryta a salt insoluble in water was obtained. After washing and drying to constant weight, the amount of the salt was found to be 0·1480 g.

(iii) 10·7 g. of the same material were hydrolysed for 5 hours with 1 % sulphuric acid. A very small amount of the insoluble barium salt was obtained.

(iv) 3·74 g. of hemicellulose B were treated with 200 cc. of 1 % sulphuric acid for 2½ hours. After adding baryta as before, the solution was still acid, but, on the addition of more baryta, no further precipitation occurred and a barium salt soluble in water was obtained.

(v) 4·52 g. of the second hemicellulose were hydrolysed for 4½ hours with 200 cc. of 1 % sulphuric acid. The solution was still acid after adding baryta and a soluble barium salt was again formed.

From these results it appears evident that the acid which gives a barium salt insoluble in water is a hydrolysis product of the hemicellulose directly precipitated by acid, or hemicellulose A, while the barium salt which is soluble in water is obtained from a sugar acid produced on hydrolysis from hemicellulose B. The insoluble barium salt was in each case filtered off, washed, and dried to constant weight. The yield from the various hydrolyses was 5·3527 g.

The barium salt which is soluble in water was separated from the sugars present in the solution in the manner described at a later stage.

(*c*) *Identification of the sugars produced by the hydrolysis of the two hemicelluloses.*

Hemicellulose A. The filtrates which were obtained after precipitation of the insoluble barium salt were concentrated under reduced pressure, taken up with hot alcohol, and the solution again concentrated. The syrup thus obtained was boiled under a reflux condenser, evaporated to dryness under reduced pressure, taken up with a little hot alcohol, and the solution again evaporated down and left to dry in a vacuum desiccator over sulphuric acid.

The amorphous mass thus obtained was treated with absolute alcohol under a reflux condenser and filtered while hot. This process was repeated several times, and it was found that the greater part of the sugar present came out of the solution on cooling. It proved to be practically pure xylose, giving a melting point of 148–150° and a specific rotation of + 18°. Bertrand's reagent [1891] was employed as a further means of identification for xylose.

To 1 g. of the sugar, 1·5 g. of bromine and 3 g. of cadmium carbonate were added, and the characteristic crystals of the double cadmium salt of xylose $(C_5H_9O_6)_2Cd + CdBr_2 + H_2O$ obtained.

From a third portion of the sugar *l*-xylose diformal $C_5H_6O_5(CH_2)_2$ was obtained [de Bruyn and Van Eckenstein, 1903].

The residue obtained after treatment with hot absolute alcohol was dissolved in hot 80 % alcohol. Crystals separated out on cooling, and, after drying, 1 g. was treated with 2 g. of phenylhydrazine hydrochloride and 3 g. sodium acetate in a test tube in a boiling water-bath [Mulliken, 1905]. An osazone appeared after 10 minutes' heating. It consisted of an orange-yellow mass, with oily drops at the surface. Under the microscope, the crystals appeared to be those of the osazone of xylose. They gave a melting point of 160° on recrystallising from 50 % alcohol.

As oily drops appearing on the surface of an osazone are an indication of the presence of arabinose, an attempt was made to obtain the diphenylhydrazone by treating a little of the sugar with an excess of diphenylhydrazine in alcoholic solution [Neuberg, 1900, 1904]. When the hydrazone had separated out it was dried and weighed. The crystals melted at 199° but a very small amount of the diphenylhydrazone, less than 1 %, was obtained. As the amount of arabinose present is so small it may be regarded as an impurity, probably from an incomplete separation of the two hemicelluloses. As will be seen later, it occurs in fairly large quantities as a product of the hydrolysis of the other hemicellulose.

Hemicellulose B. As this hemicellulose gives on hydrolysis a soluble barium salt, the hydrolysed solution was concentrated under reduced pressure and evaporated to dryness. It was then extracted successively with absolute, 80 % and 60 % alcohol. A precipitate was obtained on the cooling of the filtrate in each instance, but the bulk of the sugar was dissolved by 80 % alcohol, a very small amount being obtained from absolute alcohol, whilst the residue from 60 % alcohol proved to be a little of the barium salt itself with a trace of sugar.

The sugar obtained in this case by solution in hot absolute alcohol was found to be arabinose. It melted at 160°, but, owing to the small amount obtained, the specific rotation could not be taken. Treatment with diphenylhydrazine gave the diphenylhydrazone of arabinose, melting at 202°.

A mixture of sugars was obtained from the hot 80 % alcohol solution. This gave a melting point of 143–145° and a specific rotation of $+ 95°$ ($c = 4$ %). The diphenylhydrazone of arabinose was again obtained and also the phenylhydrazone of galactose. To obtain this 1 g. of the mixture was treated with a slight excess of phenylhydrazine in the cold. After a short time colourless crystals separated out. They were dissolved in a very little water and recrystallised from a small amount of absolute alcohol; M.P. 156°. Galactose phenylhydrazone melts at 158°. The following table shows the amounts of sugar extracted by various strengths of alcohol from the hydrolysis products.

Hemicellulose A.

11·8 g. taken for hydrolysis.

Concentration of alcohol	Sugar g.	Percentage on hemicellulose
(*a*) Absolute	4·220	35·79
(*b*) 80 %	2·6885	22·78
(*c*) 60 %	Nil	—

Hemicellulose B.

7·5 taken for hydrolysis.

(*a*) Absolute	0·4959	6·80
(*b*) 80 %	1·8984	25·0
(*c*) 60 %	Trace	—

(*d*) *Examination of the sugar acids.*

Hemicellulose A. This, as stated before, yielded a barium salt insoluble in water. It was found that 0·6188 g. of the barium salt contained 0·0342 g. of barium sulphate and that, after allowing for this, the barium content was 27·0 %. As barium glycuronate contains theoretically 26·19 % of barium, it was decided to add sufficient sulphuric acid to decompose the remainder of the salt and obtain the free acid, if possible. The mixture was then filtered free from barium sulphate and the filtrate concentrated under reduced pressure and finally left for some time in a vacuum desiccator over sulphuric acid. On drying, a substance with M.P. 160–165° was obtained. These figures approximate to the melting point of glycuronic acid and it was decided to try and obtain the cinchonine salt, which had been used by both Neuberg [1900] and Schwalbe and Feldtmann [1925] as a means of identifying glycuronic acid. This was obtained in the form of white needles, melting at 201° and having a specific rotation of + 139°. The cinchonine salt of glycuronic acid melts at 204° and has a specific rotation of + 138·6°.

As a further means of identification a little of the acid was dissolved in water and treated with 5 g. of *p*-bromophenylhydrazine and an equal amount of glacial acetic acid [Neuberg, 1899]. A product was obtained, which after recrystallisation from 60 % alcohol melted at 234° and had a specific rotation of − 365° when dissolved in a mixture of 6 cc. absolute alcohol and 4 cc. pyridine. The *p*-bromophenylhydrazide (or hydrazone) of glycuronic acid, according to Neuberg [1899], melts at 236° and has a specific rotation of − 369°. There is therefore no doubt that the acid hydrolysis product of hemicellulose A is glycuronic acid.

Hemicellulose B. This hemicellulose yields, as before stated, a barium salt soluble in water. An estimation of the barium present gave a figure a good deal lower than the theoretical amount of barium in barium galacturonate. It was found that a little sugar was also present. This was dissolved in 80 % hot alcohol, and the examination of the salt proceeded with. The cinchonine salt was prepared, as in the case of glycuronic acid, and was found to agree closely with that obtained by Ehrlich [1917] from galacturonic acid. It was more soluble than the salt obtained from glycuronic acid. Crystals were obtained by evaporating to dryness and taking up with a very little water. They melted at 157° and had a specific rotation of + 137°. According to Ehrlich, the cinchonine salt of galacturonic acid melts at 158° and has a specific rotation of + 134°.

The naphthoresorcinol test [Tollens, 1908] was carried out on each of these acid solutions by heating with a little naphthoresorcinol in the presence of an equal volume of hydrochloric acid (sp. gr. 1·19). A bluish, turbid solution was obtained in each case, and on treating the deposit with ether, a violet blue coloration appeared. There is little doubt that the acid obtained on hydrolysis of hemicellulose B is galacturonic acid. Owing to the small amount of this acid (it being remembered that the yield of hemicellulose B is only a small one), a more complete examination was not possible.

SUMMARY.

Hemicelluloses are not, as originally thought, true carbohydrates (hexosans, pentosans, etc.) but contain acid groups.

Two hemicelluloses have been isolated from beech wood, each giving different physical and chemical properties. One can be directly precipitated by acid, hemicellulose A, and the other, hemicellulose B, is obtained on precipitation from the acid filtrate by alcohol.

Hemicellulose A yields, on hydrolysis, xylose and an amount of carbon dioxide corresponding to 11 % of glycuronic acid, whereas hemicellulose B yields arabinose and an amount of carbon dioxide corresponding to 63 % of galacturonic acid, together with small amounts of galactose.

The acids were obtained only in small amounts, as they decompose during the hydrolysis. Their presence or absence amongst the hydrolysis products was found to be due not to differences in the age of the wood, method of drying, etc., but was determined solely by the method of hydrolysis employed. Similar mixtures of hemicelluloses have been obtained from non-lignified tissue by workers in these laboratories.

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