LXI. THE HEMICELLULOSES. III. THE HEMICELLULOSE OF AMERICAN WHITE OAK.

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Introduction. The term "hemicellulose" was employed by E. Schulze [1892] and B. Schulze [1894] to denote a substance somewhat similar in character to cellulose, which is easily hydrolysed by weak acids.

E. Schulze [1892] isolated this substance from leguminous seeds by treatment with dilute alkali, and then precipitating the filtrate obtained with dilute acid. He also obtained it from wheat bran and rye bran by the same method. On hydrolysing the product obtained from all three sources, he found that it yielded, in each instance, arabinose and xylose.

Schulze and Tollens [1892] obtained xylose from wheat straw and maize stalks by the same method. From beech wood sawdust Wheeler and Tollens [1889] obtained xylose, and Allen and Tollens [1890] found the same sugar in cherry wood.

Ulander and Tollens [1906] obtained from the hemicellulose of lichens on hydrolysis, glucose, mannose and galactose; while Tottingham, Roberts and Lepkowsky [1920] found xylose, glucose and small amounts of galactose in the alcohol-soluble fraction of the products from the acid hydrolysis of the hemicellulose of apple wood.

Castoro [1906] found that hemicellulose from the seeds of *Ruscus aculeatus* yielded mannose and a small quantity of arabinose on hydrolysis. From the husks of the same seed he obtained galactose.

Again Castoro [1907] found that the husks of *Cucurbita Pepo* seeds contained xylan, as xylose crystals were isolated from the syrup obtained after the husks had been hydrolysed with sulphuric acid, and that a galactan was also present, as the mother liquor from the xylose crystals yielded mucic acid when oxidised.

E. Schulze and Pfenninger [1910] obtained some interesting results from the pods of *Pisum sativum* and of *Phaseolus vulgaris*. Both of these were found to be rich in hemicellulose, the amount increasing as maturation advanced. Thus the unripe pods of *Pisum* gave 16–19 %, whilst the ripe pods yielded 48.6 %. On hydrolysis, galactose and arabinose were obtained, but the hemicellulose was not actually isolated.

Although isolated instances of this kind occur throughout the literature,

no systematic attempt seems to have been made, so far, to examine the "hemicelluloses" obtained from various sources, and to compare the results obtained in each case. Most of the estimations seem to have been carried out on seeds.

Before the work described in this paper was begun experiments carried out in this laboratory by Miss E. M. Thomas [Schryver, 1922] on small amounts of American White Oak sawdust, showed that alkali extracted substances in amounts varying greatly in different specimens of timber. These substances appeared to be somewhat similar to those obtained by E. Schulze [1892], and called by him the "hemicelluloses."

It was then decided to endeavour to isolate the product, or products, from large quantities of the American White Oak, and to investigate its properties and the products of hydrolysis. The sawdust employed was obtained directly from the cooperage of the brewery of Messrs Whitbread and Co., through the courtesy of Mr H. C. Sweetman. No further information as to its origin was available beyond the fact that it was from American White Oak. It is hoped later to continue the investigation of the hemicelluloses from timbers, the origins of which are authenticated, with the object of determining the distribution and functions of these substances.

Method of Preparation. In the first instance, 600 g. of the sawdust were extracted several times with hot water and dried. 6 litres of 4 % caustic soda were then added to the dried material and the mixture was left standing for two days, during which time it was well stirred with a mechanical stirrer. It was next filtered through fine muslin on a Buchner funnel, the sawdust being well washed with weak alkali. A slight excess of acetic acid was added to the filtrate and washings, and a greyish-white precipitate of a sticky character which was found to be partly re-distributed in water, was formed. The filtrate appeared to be of a colloidal nature and precipitation was not complete until an equal volume of 95 % alcohol had been added. The precipitate brought down by the alcohol was a flocculent one. The whole of the precipitate was then collected on a filter, washed with 50 % alcohol, and purified by re-dissolving in 4 % caustic soda, filtering through paper pulp, and re-precipitating with glacial acetic acid, and 95 % alcohol. After washing with various grades of alcohol and finally with ether, the resulting product was dried first in the air, and then in the oven at 105° and weighed. It was found that 62.45 g. of the products were obtained from 600 g. of sawdust, a yield of a little over 10 %.

50 g. of this material were now further purified by a method described by Baker and Pope [1900]. It was first extracted with 2 % caustic soda on the water-bath, and filtered through glass wool. An excess of Fehling's solution was then added, and the voluminous blue precipitate was washed several times with very dilute alkali. Cold dilute acetic acid was then added to decompose the copper compound, and the mixture washed first with very dilute acid and then with dilute alcohol. The precipitate was next treated with boiling absolute alcohol and washed with cold 95 % alcohol to get rid of the

last traces of acid. After washing with alcohol and then with ether, it was allowed to dry, first in the air, and then in the oven at 105°.

Properties. The product obtained by this method consists of a fine greyish-white amorphous mass, free from nitrogen and from inorganic matter. It is soluble in boiling water, forming a thin gelatinous mass on cooling. It does not dialyse through parchment to any extent. On addition of iodine to the solution, a slightly greenish-blue coloration is given. It does not reduce Fehling's solution and is laevo-rotatory. After purification of the product by the copper method as described above, the rotation $[\alpha]_D = -75^\circ$, in 1 % caustic soda solution, was found. The following figures were obtained by combustion:

(1) 0.1874 g.; 0.3070 CO₂; 0.1089 H₂O; C = 44.67 %, H = 6.40 %.

(2) 0.1974 g.; 0.3239 CO₂; 0.1130 H₂O; C = 44.74 %, H = 6.35 %.

The examination of the hydrolysis products shows that the substance is probably a mixture of nearly 70 % xylan + araban, and 30 % mannan + galactan, a very small percentage of material remaining undissolved after hydrolysis. A mixture of 70 % araban + xylan (C₅H₈O₄) and 30 % mannan + galactan (C₆H₁₀O₅) requires C = 45.14 % and H = 6.12 %.

On hydrolysing 0.4 g. of the product with 12 % hydrochloric acid and treating the distillate with phloroglucinol, the amount of furfural phloroglucide precipitated was found to be 0.2730 g., which, reckoned as pentose, gives a yield of 70 %. On treating the contents of the crucible containing the furfural phloroglucide with alcohol, according to the method described by Tollens and Ellett [1905], for the identification of methylpentose, no loss in weight occurred, and the alcoholic filtrate showed no change in colour. It is indicated therefore that there is no methylpentose present.

In order to ascertain the relation between the time of hydrolysis of the product with dilute sulphuric acid and the amount of reducing sugars present, 0.5 g. of the product was subjected to hydrolysis with 1 % sulphuric acid for periods of two, three, four and six hours. Bertrand's method was employed to ascertain the reducing power of the hydrolysed solution in each case.

It was found that:

After 2 hours' hydrolysis 43.74 %			
„	3	„	51.24 „
„	4	„	59.0 „
„	6	„	61.74 „

of reducing sugar, reckoned as glucose was obtained.

THE ISOLATION OF THE HYDROLYSIS PRODUCTS.

Preliminary Experiments. These preliminary experiments were carried out with a product which had not been purified by the copper method.

20 g. of the product were hydrolysed with 200 cc. of 1 % sulphuric acid for six hours. The hydrolysed solution at the end of this time still contained a small proportion of insoluble matter. The solution was filtered and a suffi-

cient quantity of barium hydroxide added exactly to neutralise the solution, which was then filtered and concentrated to small bulk under reduced pressure. The solution was now in a syrupy condition. Some 95 % alcohol was added to get rid of any barium sulphate remaining, and after filtering, the solution was again concentrated as before. A little more alcohol was then added and the syrup was allowed to evaporate slowly over sulphuric acid. The crystalline mass which slowly formed was then heated for a long time on the water-bath with 95 % alcohol [E. Schulze, 1892] and left to stand over concentrated sulphuric acid. After some time a white crystalline mass formed. The mother liquor was then filtered off and to 20 cc. of this, 2 g. phenylhydrazine hydrochloride and 3 g. of sodium acetate were added. The mixture was heated in a test-tube in a boiling water-bath for ten minutes and at the end of that time an orange-yellow osazone separated out. This was filtered off, and on again heating the mixture in the water-bath for some time indications of a second osazone appeared, with the formation of some brownish oily drops.

The first formed osazone was now washed with water and dried and gave a melting point of 156° . On recrystallisation from 60 % alcohol the constant melting point was found to be 160° . It appeared to be the osazone of xylose. As a means of further identification Bertrand's reaction [1891] was now carried out on another portion of the solution from which the osazone was formed. To 2 g. of the sugar syrup, 2.5 g. of bromine and 5 g. of cadmium carbonate were added. The mixture was then gently warmed in a test-tube and after corking loosely set aside for twenty-four hours. At the end of that time the solution was evaporated almost to dryness, taken up with water and again evaporated almost to dryness. On the addition of a little alcohol a crystalline mass formed which at first appeared somewhat amorphous under the microscope, but on recrystallisation from alcohol, the characteristic boat-shaped needles of the double cadmium salt of xylose $(C_5H_6O_6)_2Cd + CdBr_2 + 2H_2O$ were deposited. A third portion of the same sugar syrup was boiled with some paraformaldehyde and after a time a white mass of crystals separated out. These were found to have a melting point of 55° . *l*-Xylose diformal melts at 56° and has the formula $C_5H_6O_5(CH_2)_2$ [Lobry de Bruyn and Van Eckenstein, 1903]. The osazone of *l*-xylose, the double cadmium salt of the same sugar, and *l*-xylose diformal have therefore been obtained from different portions of the same sugar syrup.

The mass of crystals which had been filtered off from the mother liquor were now dried and examined. 1 g. was treated as before with 2 g. of phenylhydrazine hydrochloride, 3 g. of sodium acetate and 20 cc. of water [Mulliken, 1905] and after heating in a test-tube in a boiling water-bath for a very short time some colourless crystals separated out. After more than fifteen minutes an orange-yellow osazone formed and brownish-yellow oily drops appeared at the surface. After filtering off the osazone, washing as before, and recrystallising from 60 % alcohol, it was found to have a melting point of 160° . As the osazones of both xylose and arabinose give this melting point, further

means of identification had to be sought. An attempt was therefore made to obtain the diphenylhydrazone of arabinose by treating the syrupy solution of the sugar with slight excess of diphenylhydrazine [Neuberg, 1900, 1904; Browne, 1912]. Sufficient alcohol was added to form a perfectly clear solution and the mixture was boiled for half an hour in a water-bath in a flask connected with a reflux condenser. After standing for some time a white crystalline mass separated out from the solution. The crystals were washed with a very little cold alcohol and dried in the water oven. They gave a m.p. of 196° . After recrystallisation a m.p. of 199° was obtained with rapid heating. Tollens and Maurenbrecher [1905] observed a m.p. of $204^{\circ}-5^{\circ}$ for the diphenylhydrazone of arabinose, but Haar [1920] points out that it is difficult to obtain a constant m.p. higher than 200° .

Second Hydrolysis. 1200 g. of sawdust were now subjected to the same treatment as before, and yielded 121.05 g. of the product; 60 g. of which were hydrolysed with 300 cc. of 1 % sulphuric acid. The hydrolysed solution was again neutralised with barium hydroxide and concentrated as before. Four volumes of 95 % alcohol were then added and the solution, after filtering, was concentrated a second time to very small bulk and decolorised with norite. After further addition of alcohol and a third concentration, the resulting syrup was divided up into two portions. Absolute alcohol was added to the first portion and a large amount of precipitate formed. This, after drying, was treated with hot 80 % alcohol and filtered. As mannose is soluble in hot 80 % alcohol it was hoped to obtain the greater part of this sugar, if present, in the filtrate. This filtrate was again divided into two portions, one of which was left standing over concentrated sulphuric acid. To part (a) of the remainder, one volume of phenylhydrazine and one volume of 50 % acetic acid were added. After the mixture had stood for a short time, colourless rhombic crystals were formed. These were left in the solution over the week-end and had disappeared on the following Monday morning. On warming the solution for a short time a mass of sheaf-like crystals, entirely different from those first formed, appeared. They closely resembled crystals of glucosazone under the microscope. After recrystallisation from 60 % alcohol they gave a constant melting point of 204° . This melting point is that of the osazone of mannose.

Part (b), the remainder of the second portion of the last filtrate, was treated with phenylhydrazine and 50 % acetic acid as before. After standing for a short time the rhombic crystals again appeared. Some colourless needles were also seen under the microscope. The crystal mass was collected after twenty-four hours, dried and recrystallised from hot water. The melting point before recrystallisation was found to be 178° with rapid heating; after repeated recrystallisations from hot water a fairly pure hydrazone was obtained, melting at 196° . The low melting point obtained in the first instance is probably due to the fact that some crystals of the hydrazone of galactose were present. $[\alpha]_D$ (in pyridine) = $+22^{\circ}$. Haar [1920] gives a value for mannose phenylhydrazone $[\alpha]_D = +26.61^{\circ}$.

That portion of the solution obtained with hot 80 % alcohol which had been left standing over sulphuric acid, was now examined. Part of it was found to have separated out as a hard white amorphous mass. On drying this and treating with 90 % alcohol under reduced pressure crystals were formed after the syrup had stood a short time. These were dried on a suction filter and washed with small amounts of alcohol and ether, and then recrystallised from alcohol. They gave a constant melting point of 136° . $[\alpha]_D$ (in 10 % aqueous solution) = $+15.3^{\circ}$. On adding phloroglucinol to the sugar solution no coloration was given. The crystals are evidently mostly those of mannose, with probably a little galactose also present. The rest of the syrup obtained from the second hydrolysis with 1 % sulphuric acid was used later on for a rough qualitative estimation of two of the sugars which had previously been identified.

Third Hydrolysis. 20 g. of the purified product were hydrolysed as before. There was still present a small insoluble residue which after drying and weighing, was found only to amount to 0.45 %. The hydrolysed solution was neutralised as before with barium hydroxide, treated with alcohol and evaporated to a syrup. After concentrating and taking up with alcohol three times an attempt was made to separate the sugars, which previous experiments had shown to be present, by their differences in solubility in alcohol and in water. The results have been fairly satisfactory and it is hoped that further work along these lines may be even more successful. The sugar syrup was decolorised with norite, and, after concentration the third time to very small bulk under reduced pressure, was dried over sulphuric acid, precipitated with hot absolute alcohol and filtered immediately. As xylose is soluble in hot absolute alcohol to a much greater extent than mannose, arabinose or galactose, it was hoped to obtain this sugar in a fairly pure condition in the filtrate. The filtrate was again concentrated under reduced pressure and left to crystallise out over sulphuric acid. After some time crystal aggregates consisting for the most part of fine needles appeared. These were sucked dry on the filter, washed with a little alcohol and ether and then recrystallised from alcohol. They gave a melting point of 156° . $[\alpha]_D$ (in 10 % aqueous solution) = $+20^{\circ}$. On heating a solution of the sugar with phloroglucinol, a greenish-black precipitate appeared. According to Mulliken [1905] the melting point of xylose is $150-153^{\circ}$ and its specific rotation $+18.7^{\circ}$.

The precipitate obtained by adding hot absolute alcohol was left dry over sulphuric acid and then just sufficient water was added to dissolve up the crystals. After concentrating to a slight extent under reduced pressure, the solution was left over sulphuric acid. Crystals began to separate out from the aqueous solution. These were collected and dried on the suction filter, the mother liquor being again treated with absolute alcohol and left over sulphuric acid. The crystals, on drying, gave a melting point of 116° . On recrystallisation from absolute alcohol a mass of fine hexagonal crystals formed. These melted at 167° . A solution of the crystals gave a very slight coloration with phloro-

glucinol. Galactose [Mulliken, 1905] melts with rapid heating at 168° and has a specific rotation of $+80.3^{\circ}$. A value for $[\alpha]_D$ of $+82^{\circ}$ was obtained in 5 % aqueous solution. Owing to the small amount of the sugar present, the mucic acid estimation [Fernau, 1909] could not be carried out at this stage, but it was done later on a solution containing a mixture of the sugars. The phenylhydrazone was obtained by dissolving some of the sugar crystals in a little water and adding phenylhydrazine and 50 % acetic acid as before. After the mixture had stood for about two hours the hydrazone separated out in the form of fine colourless needles, similar to those seen when the hydrazone of mannose was prepared. These were washed with ether and then with a little alcohol and ether. After drying, these were found to have a melting point of 156° . The specific rotation was not obtained. The mother liquor from the last filtration which, it was thought, would probably contain arabinose, some mannose, and perhaps a little galactose was concentrated under reduced pressure and treated several times with hot absolute alcohol. Mannose is slightly soluble in hot absolute alcohol, and galactose is a little less insoluble in the same reagent than arabinose, hence after repeated extractions with hot absolute alcohol, it was hoped to obtain a fairly pure specimen of arabinose. The precipitate which remained after these extractions was dried on the suction filter after standing over sulphuric acid and recrystallised from hot absolute alcohol. The crystals were filtered off, washed with a little alcohol and ether and dried over sulphuric acid. They gave a constant melting point of 160° . $[\alpha]_D$ (in 10 % aqueous solution) = $+103^{\circ}$. On treatment of the sugar solution with phloroglucinol a purplish-black precipitate appeared.

The sugar is therefore identified as *l*-arabinose.

In order to obtain an approximate idea of the amounts of xylose and arabinose present, the diphenylhydrazone of arabinose was obtained by the method described in a previous part of this paper. The mixed sugar syrup from the second hydrolysis, after being treated with alcohol, and concentrated under reduced pressure was allowed to crystallise out over sulphuric acid. 2 g. of the mixed sugar crystals were then dissolved in a little water and 2.5 g. diphenylhydrazine added. When the hydrazone had separated out, the crystals were filtered into a weighed Gooch crucible, washed with a very little alcohol, dried in the water oven, and weighed. It was found that 0.780 g. of diphenylhydrazone $C_{17}H_{20}O_4N_2$ was obtained from 2 g. of the mixed sugars. In order to obtain the amount of arabinose ($C_5H_{10}O_5$) which this figure represents, it was multiplied by $150/316 = 0.4747$.

$$0.780 \times 0.4747 = 0.3703 \text{ g. arabinose} = 18.5 \% \text{ (approx.).}$$

As the total amount of pentose present was found to be 70 % the percentage of xylose may be roughly calculated as 51.5 %.

An estimation of the amount of mucic acid formed from the mixed sugars was now attempted in order to obtain some idea of the relative amount of galactose, and hence of mannose present. A modification of the Tollens method

described by Fernau [1909] was employed for this purpose. It was found that 4.89 g. of mixed sugar crystals gave 0.3640 g. of mucic acid, or

$$0.3640 \times 1.33 = 0.4841 \text{ g. galactose.}$$

If the sugar from which the mucic acid was obtained were pure galactose, this would represent 70 % of the galactose present, according to Fernau. The percentage of the sugar present would, therefore, be 6.9, approximately. In a mixture of sugars, however, the percentage of galactose obtained by the mucic acid estimation would probably be much lower, and besides it is not likely that mannose is present in any great amount, as it is found mostly in gymnosperms [Schorger, 1917]. Pinoff's test [1905] for fructose, gave a negative result.

It was not found possible to form either the hydrazone or the osazone of glucose from the mixed sugar crystals. On treating the crystals with diphenylhydrazine only the diphenylhydrazone of arabinose was formed. It is therefore assumed that glucose is not present.

SUMMARY.

Apparently the chief product of hydrolysis of the hemicellulose of American White Oak is *l*-xylose. According to Browne [1912, p. 553], xylan, with a little araban, makes up about 15–25 % of the dry matter of deciduous trees. Arabinose is apparently present in much smaller quantities than xylose in American White Oak. A rough quantitative estimation gave the amount of xylose yielded by the hemicellulose of this wood as 51.5 % and that of arabinose as 18.5 %.

Hexoses apparently form about 30 % of the products of hydrolysis. These have been identified as mannose and galactose. A mucic acid estimation of the galactose gave approximately 7 %, but as the estimation was made on the mixed sugars, the true figure is probably very much higher, as the amount of mannose present appears to be very small.

It is impossible to say in what combination these products exist in the wood. Probably in the form of an arabo-xylan and a manno-galactan.

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