IX. STUDIES ON HEMICELLULOSES.

I. THE HEMICELLULOSES OF WHEAT BRAN.

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INTRODUCTION.

THE term hemicellulose was first proposed by Schulze [1891] as a group name for substances entering into the composition of the cell membrane, which, though in some respects similar to cellulose, yet differ from it in being soluble in dilute alkalis and comparatively easily hydrolysed by dilute mineral acids, yielding pentoses in addition to hexoses.

Although the true physiological significance of the hemicelluloses is for the most part obscure, it would seem that they represent partly structural and partly reserve materials. Thus, reserve hemicelluloses occur in the kernels of seeds, especially such as are poor in their content of starch and fat. In such cases, hexoses predominate among the products of hydrolysis. Hemicelluloses fulfilling probably mainly structural functions occur in seed and fruit husks, wood, straw, etc. The results obtained by earlier workers seemed to indicate that such hemicelluloses were largely composed of pentose residues, xylose and arabinose being the chief hydrolysis products isolated. The recent work of O'Dwyer (see p. 60), however, has shown that the furfuraldehyde-yielding "uronic" acids occur in some, at least, of these hemicelluloses, and it may be that preponderance of pentose residues is not a universal property of substances of this group.

A typical method of attack was that used by Schulze and Tollens [1892], who extracted wheat straw and maize stalks with dilute alkali, precipitating the filtrate with dilute acid. Hydrolysis of the product gave xylose in each case. Schulze [1892] similarly obtained both arabinose and xylose from the brans of wheat and rye. Ulander and Tollens [1906] obtained *d*-mannose and *d*-galactose from certain reindeer mosses which contained no lichenin. Pentosans and methylpentosans were also present. Schulze and Pfenniger [1910], investigating the pods of *Pisum sativum* and *Phaseolus vulgaris*, found them to be rich in hemicelluloses, the amounts of which increased as the pods matured. The products of hydrolysis were galactose and arabinose.

Baker and Pope [1900] obtained mannose and galactose from the hemicellulose of the Indian clearing nut, while the hemicellulose of the ivory nut they showed to be laevulo-mannan. Both these hemicelluloses are to be regarded as reserve substances.

A careful study of the hemicellulose of the wood of American white oak has been carried out by O'Dwyer [1923]. Sawdust from this wood was extracted with 4 % sodium hydroxide, the extract neutralised with glacial acetic acid, and the hemicellulose precipitated by alcohol. The product was purified by precipitation from 2 % sodium hydroxide solution as a copper compound, as described by Baker and Pope [1900], this then being decomposed by treatment with dilute acetic acid. The hemicellulose was shown to contain some 70 % of pentose residues, xylan and araban, the former preponderating. The hexoses, mannose and galactose, were also identified amongst the hydrolysis products.

O'Dwyer [1926], following a method indicated by Clayson, Norris and Schryver [1921], later separated from beech wood two hemicelluloses with distinct physical and chemical properties. Hemicellulose A, obtained by acid precipitation of a sodium hydroxide extract, yielded on hydrolysis xylose and carbon dioxide, evolved under the conditions described by Nanji, Paton and Ling [1925], equivalent to 11 % of glycuronic acid. Hemicellulose B, obtained from the acid filtrate from the above by alcohol precipitation, gave arabinose and carbon dioxide equivalent to 63 % of what was shown to be galacturonic acid.

These results, showing that uronic acids, in some cases at least, enter into the composition of hemicelluloses, and also the possibility that the hemicellulose from a particular source may be split up into a number of fractions, indicate a need for revision of much of the earlier work on these substances.

Much confusion in nomenclature exists with reference to these so-called hemicelluloses. This is largely due to the great variety of plant material in which they are to be found and also to the doubt which exists as to their exact function. Schryver recognised the necessity for improvement in the nomenclature of these substances, and recently suggested the term "polyuronide" to describe substances in which uronic acids are found in conjugation with sugars, especially pentoses [Candlin and Schryver, 1928]. The class of "polyuronides" would therefore include pectin and the structural "hemicelluloses" such as those described by O'Dwyer. The older term "hemicellulose" might then be reserved for products which yield no uronic acids on hydrolysis. On this basis of classification, some of the products described in this paper are more correctly described as "polyuronides."

The work herein described aimed at a somewhat detailed investigation of the hemicelluloses of wheat bran. Owing to the ease with which such products as hemicelluloses may become contaminated with other colloidal cell-wall substances, special care is necessary if a pure product is to be obtained. To this end, such substances as pectin and lignin were removed from the tissue, by appropriate extractives, prior to the extraction of the hemicelluloses.

EXPERIMENTAL.

Preparation.

400 g. of the bran are allowed to stand for an hour with 2 litres of water. The mixture is then poured into an equal volume of 1 % ammonium oxalate solution, previously heated on the water-bath to 90°. The temperature is taken back to 90° and is maintained at this level for 2–3 hours. The mixture is allowed to cool, filtered through muslin, and extraction of pectin from the residue is repeated with 0.5 % ammonium oxalate. After filtering, as much liquid as possible is removed at the press.

Removal of lignin is carried out by two extractions with 3 litres of 50 % alcohol containing 1 % of NaOH, each extraction being carried out under reflux for 2 hours. After the second extraction, the filtered tissue is further treated under reflux with 3 litres of neutral alcohol (50 %) for 1 hour. After filtration through muslin, excess liquid is again removed at the press.

The first extraction of hemicellulose is carried out with 3 litres of 4 % NaOH solution. Two or three further extractions with 4 % NaOH should be made from the residue. The combined extracts are filtered carefully, several times, through glass wool.

To the filtered extract is added rather more glacial acetic acid than is necessary to neutralise it. The precipitate obtained at this stage (hemicellulose A) must be separated at the centrifuge. The mother liquor is reserved. The precipitate is repeatedly washed at the centrifuge with water, the early washings being added to the mother liquor. The precipitate is then treated with gradually increasing strengths of alcohol, in the usual way for obtaining a dry product. Drying is completed *in vacuo* over phosphorus pentoxide.

The mother liquor from the above is filtered through paper pulp, after which half a volume of acetone is added to it. This precipitates hemicellulose B, which, after standing, may be filtered off. The filtrate is reserved. The precipitate is redissolved in 4 % NaOH solution, the solution neutralised with glacial acetic acid, and the hemicellulose reprecipitated with half a volume of acetone. After several re-solutions and reprecipitations in this way, the final product is taken up to dryness in the usual manner.

To the filtrate reserved from the above is added a volume of acetone equal to that added previously. The precipitate—hemicellulose C—is purified by dissolving in water and precipitating with an equal volume of acetone, re-solution and reprecipitation being carried out several times and the final product then being taken to dryness.

Yields of the crude hemicelluloses obtained in a typical preparation from 400 g. of bran, calculated on the dry weight of the products, were: hemicellulose A, 8.0 g.; B, 4.5 g.; C, 4.5 g.

Purification.

Hemicellulose A. The crude product is dissolved in 4 % sodium hydroxide solution (1000 cc.), and any undissolved matter is removed at the centrifuge or by filtration through paper pulp. 250 cc. of mixed Fehling's solution are added to the clear liquid, and the copper compound formed is filtered off and decomposed by treatment with dilute hydrochloric acid. Precipitation of the hemicellulose is effected or assisted by the addition of acetone. The product is washed free from acid and copper chloride by three or four changes of acetone, after which drying is completed with alcohol. The product obtained in this way is contaminated with a little starch, but means have not been devised for eliminating this.

Hemicellulose B. Some 5 g. of the crude product are dissolved with constant stirring in boiling water, and the solution is boiled for a further 5 minutes. Any small amount of insoluble residue is filtered off through glass wool and is neglected. The clear solution is cooled, and NaOH is added to give a final concentration of 4 %. 250 cc. of mixed Fehling's solution are added with stirring, and after standing the precipitate—B1 copper complex—is removed by pouring through muslin. After further filtration through paper pulp, half a volume of acetone is added to the filtrate, and the second precipitate—derived from hemicellulose B2—filtered off. The two precipitates, B1 and B2, are decomposed separately with dilute hydrochloric acid, and acetone is added to effect or assist precipitation of the hemicelluloses. After further washings with acetone, and finally with alcohol, the process of purification described is repeated with each product to ensure complete separation.

Hemicellulose C. This product is dissolved in 4 % NaOH and mixed Fehling's solution added. Although the viscosity of the solution increases greatly, no precipitate is obtained; consequently half a volume of acetone is added, and the precipitate—designated C2 by analogy with B2 above—filtered off, decomposed with hydrochloric acid, and the hemicellulose C2 precipitated, washed, and dried as usual.

General properties of the hemicelluloses.

The four hemicellulose fractions were obtained in the form of white amorphous powders of low ash content (0.3–2.0 %). Hemicellulose A was somewhat soluble in boiling water, while fractions B1, B2 and C2 were almost completely dissolved. The aqueous solution of C2 was laevorotatory. No iodine colorations were obtained, except in the case of fraction A, a suspension of which in water gave a blue colour owing to the presence of a little starch.

Furfuraldehyde estimations were carried out on all fractions, and also decarboxylations by the method of Nanji, Paton, and Ling [1925]. Mean values for a number of determinations are given in the following table, the results quoted being calculated in all cases on the dry, ash-free substance.

Hemicellulose ...	A	B 1	B 2	C 2
	%	%	%	%
Furfuraldehyde	55.0	48.4	3.2	49.0
Carbon dioxide	Nil	1.85	Nil	1.98
Uronic anhydride	„	7.40	„	7.92

The crude hemicellulose B yielded 31.1 % of furfuraldehyde. Consequently, fractions B1 and B2 are present approximately in the ratio 2 : 1.

Preliminary hydrolysis.

The yields of hemicelluloses obtained were not sufficient for more than preliminary investigations to be made as to the nature of the sugars liberated on hydrolysis.

The procedure followed in all cases was to boil the hemicellulose under reflux for 4 hours with 1 % sulphuric acid, 50 cc. of the acid being used per g. of hemicellulose. At the end of this time, the hydrolysis liquor was neutralised while still hot with the theoretical volume of a standard strong solution of baryta. The precipitate of barium sulphate formed was filtered off, and the filtrate suitably concentrated *in vacuo*.

Hemicellulose A. The concentrated liquor obtained thus from 5 g. of hemicellulose A was divided into several portions. One portion treated with diphenylhydrazine yielded a white crystalline precipitate of arabinose diphenylhydrazone, melting, after two recrystallisations, at 202°.

From another portion, cadmium xylonobromide was obtained, indicating the presence of xylose.

Repeated attempts to obtain mucic acid, by nitric acid oxidation of some of the sugar syrup, failed, nor was it possible to prepare mannose hydrazone. Likewise, the Pinoff-Selivanoff test for ketohexoses gave a negative result.

An osazone was prepared, which was found to be a mixture. A little of it was insoluble in hot water; examined microscopically this was found to be glucosazone, doubtless derived from the contaminating starch. Examination of the osazone soluble in hot water showed it to be a mixture of xylosazone and arabinosazone, the characteristic form of each being clearly seen.

Thus, it may be supposed that the only sugars obtainable from hemicellulose A on hydrolysis are xylose and arabinose. The detection of these two sugars as the products of hydrolysis of the hemicellulose of wheat bran, which is precipitable by acid, thus confirms the finding of Schulze [1892], whose "wheat bran hemicellulose" corresponds to this fraction.

Hemicellulose B1. The chief product of hydrolysis of this substance would appear to be xylose. A large amount of cadmium xylonobromide was obtained on treating a portion of the hydrolysis liquor according to Bertrand's method. Another portion of hydrolysis liquor gave a very small amount of arabinose diphenylhydrazone, melting after recrystallisation at 202–203°.

An osazone was prepared, and a little of it, too small in amount for further examination at this point, was found to be insoluble in hot water. The residue was practically pure pentosazone, melting after several recrystallisations at 155°.

In an endeavour to ascertain the nature of the insoluble osazone, the hydrolysis liquid from a further 5 g. of B1 was evaporated *in vacuo*, just to dryness, and the sugar extracted twice with 50 cc. portions of absolute alcohol. The residue was then extracted with 80 % alcohol. The combined absolute alcohol extracts and the 80 % extract were separately evaporated, and the sugars taken to dryness by repeated treatment with absolute alcohol, drying being completed *in vacuo*. The bulk of the sugar was extracted by the absolute alcohol, and the dry product from this gave $[\alpha]_D^{20} = 26^\circ$ for $c = 1.6$.

The osazone obtained from this fraction was a mixture. The portion insoluble in hot water was repeatedly extracted with hot water to remove pentosazone. It was recrystallised from 50 % alcohol, and under the microscope was seen to crystallise in clusters of thick yellow needles, quite distinct in appearance from the osazones of glucose and galactose. The melting point of the purified osazone was 158° . The pentosazone, doubtless xylosazone, melted after recrystallisation at 155° .

It seems not improbable that the insoluble "osazone" here obtained was derived from glycuronic acid. Mayer [1900] found that glycuronic acid treated with excess of phenylhydrazine yielded a compound melting at $159-164^\circ$. Again, Giemsa [1900] obtained a glycuronic acid phenylhydrazine derivative melting at 160° , crystallising in yellowish needles, insoluble in water, and recrystallisable from dilute alcohol. Galacturonic acid, on the other hand, is said to yield no crystalline derivatives with phenylhydrazine [see Ehrlich, 1917].

The sugar soluble in 80 % alcohol gave two similar osazone fractions.

Other sugar tests than those described having yielded negative results, it appears that the products of hydrolysis of hemicellulose B1 consist chiefly of xylose, accompanied by a little arabinose and also a uronic acid, possibly glycuronic acid, though the evidence for this is at present incomplete. At the same time, the furfuraldehyde yield from the hemicellulose, viz. 48.4 %, is too low to be accounted for in this way, and it is possible that another sugar present has escaped detection.

Hemicellulose B2. This yielded but 3.2 % of furfuraldehyde, while uronic acids were shown to be absent. Consequently, on hydrolysis, a small amount of pentose should be obtained, the residue of the hydrolysis product being hexose. Hydrolysis was carried out in the usual way on some 2 g. of B2.

From a portion of the neutral hydrolysis liquor an osazone was prepared, the bulk of which was insoluble in hot water. This osazone was found to crystallise in sheaves of needles, resembling glucosazone. After recrystallising from 50 % alcohol it gave a melting point of 205° . Glucosazone melts at this temperature. The small amount of pentosazone formed, soluble in hot water, was not examined.

Attempts to obtain mannose hydrazone from another portion of the hydrolysis liquor failed, while the Pinoff-Selivanoff test for ketoses likewise gave a negative result.

It seems, therefore, that hemicellulose B2 is a glucosan, admixed with a little pentosan. The amount of the hemicellulose available, however, was not sufficient to allow of further test.

Hemicellulose C2. A copious white crystalline precipitate was obtained when a little of the hydrolysis liquor from hemicellulose C2 was treated in the usual way for the production of arabinose diphenylhydrazone. After two recrystallisations from 60 % alcohol this product melted at 203°, establishing the presence of arabinose.

A very small amount of cadmium xylonobromide was obtained from another portion of the hydrolysis liquor. Mucic acid could not be obtained.

An osazone was prepared, and proved to be a mixture. A portion insoluble in hot water, after purification and recrystallisation from 50 % alcohol, was found to have a crystalline form resembling that of the insoluble phenylhydrazine derivative described above as obtained from the products of hydrolysis of hemicellulose B1. It melted at 161–163°. Hence this too may be a derivative of glycuronic acid. The osazone soluble in hot water formed along with this was arabinosazone, contaminated with a little xylosazone, and melting at 155°.

Thus, wheat bran hemicellulose C2 is an araban, associated with some 8 % of uronic acid anhydride, possibly glycuronic anhydride, and contaminated with a little xylan.

DISCUSSION.

The following scheme indicates diagrammatically the method of preparation of the hemicelluloses, the products of hydrolysis being shown in brackets.

Four hemicellulose fractions with widely differing characteristics have thus been separated from wheat bran, two of them containing small amounts of uronic acid residues.

Hemicellulose B2 is of particular interest, both from its mode of separation, and from the fact that it is practically a pure hexosan. The preliminary hydrolysis described would appear to suggest that it is a glucosan. Though glucosan is not usually to be regarded as a constituent of hemicelluloses (except the amylohemicelluloses, rather different types of substance), various workers have claimed to obtain glucose from hemicellulose hydrolysis products. Thus, Schulze and Castoro [1903] obtained from certain hemicelluloses a sugar convertible to glucosazone which they regarded as probably glucose. Also, glucose has been stated to arise, along with mannose and methylpentose, when the hemicellulose-like yeast gum is hydrolysed [see Hashitani, 1927].

The results in the case of hemicellulose B1 must be considered unsatisfactory, since it would appear that a hexose has escaped detection. Xylose, arabinose, and a uronic acid were shown to be present, but the figures obtained for furfuraldehyde and carbon dioxide are so low that they indicate the presence of another constituent. The very small amounts of the individual fractions available renders the isolation of hydrolysis products a difficult problem.

On the other hand, furfuraldehyde and carbon dioxide numbers are almost completely accounted for on the assumption that this substance is a urono-araban containing some 8 % of uronic anhydride.

SUMMARY.

A general method of preparation and purification of hemicelluloses is described.

The hemicelluloses of wheat bran yield four fractions, chemically and physically distinct, two of them containing small percentages of uronic acid residues.

Preliminary hydrolyses of the four products have been carried out. Hemicellulose B2 appears to be a glucosan, whilst the other fractions are largely composed of pentose residues.

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