CXCVIII. THE HEMICELLULOSES

III. EXTRACTION AND PREPARATION

BY ARTHUR GEOFFREY NORMAN

*From the Biochemistry Section, Rothamsted Experimental Station,
Harpenden, Herts*

(Received 26 July 1937)

OF the chief cell wall substances, the hemicelluloses are probably the most difficult constituents to prepare in a satisfactory state of purity. The conditions of extraction recommended by various workers differ considerably, and the criteria of purity applied are not uniform. The observations in this paper bear on various aspects of the extraction and purification of this group of polysaccharides.

(a) Pretreatment with alcoholic sodium hydroxide

The extraction of hemicelluloses is carried out with cold or hot solutions of NaOH, in which lignin is also soluble to a considerable extent. In order to reduce the amount of lignin passing with the main extract Norris & Preece [1930] recommended a preliminary delignifying treatment with alcoholic NaOH, and this procedure has in fact been employed subsequently in a number of investigations. In an earlier paper of this series [Norman, 1935] evidence was given to show that this reagent when hot has a degradative effect on the polyuronide hemicelluloses and to some extent also on the xylan associated with the cellulose so that a considerable loss of furfuraldehyde-yielding constituents takes place and there is some chance of degradative change in those remaining. Different materials were found to vary in degree of susceptibility to this reagent, but in nearly all cases there was no doubt that as a result of the pretreatment the yield of hemicellulose would be reduced. In recent investigations of the hemicelluloses of hop and of maize cobs Angell & Norris [1936] have claimed that the alcoholic NaOH treatment did not result in any loss of hemicellulose from these materials on the grounds that the precipitate obtained on acidification of this extract failed to yield any furfuraldehyde. However, it might be pointed out that this evidence alone is not conclusive, since the degradative effect of this reagent, if any, is not necessarily similar to acid hydrolysis, and the fission products formed would not necessarily yield furfuraldehyde or even be precipitated by simple acidification. To obtain conclusive proof it must be established by analysis that the residual material after extraction is not appreciably lower

Table I. *Extraction of wheat straw with 1% NaOH in 50% alcohol*

2.5 g. portions boiled with 100 ml. for 1½ hr. Results expressed as % of original material

	Residue	Cellulose	Furfuraldehyde from cellulose	Xylan in cellulose	Total furfuraldehyde	Furfuraldehyde from polyuronides	Lignin
Untreated	—	53.5	10.35	16.1	16.52	6.2	15.1
Extracted	64.95	51.5	8.62	13.4	13.06	4.4	5.0

in furfuraldehyde constituents than before. Examples in which this was not the case are to be found in the previous paper [Norman, 1935] and an additional experiment is given in Table I.

The analyses of the residue show conclusively that with this material pre-treatment with alcoholic NaOH, although effective in delignification, did result in a considerable loss of both types of furfuraldehyde-yielding constituents. The degradative attack was such that only a part of the furfuraldehyde could be recovered from the extracts and none from the precipitate obtained on simple acidification (Table II). The straw had lost constituents yielding 3.7% furfuraldehyde, of which only about one-quarter could be found in the extract. The

Table II. *Experiments on alcoholic NaOH extracts from wheat straw*

All in duplicate. Results expressed as % of original straw						%
A.	Extract neutralized, concentrated under reduced pressure to remove alcohol. Yield of furfuraldehyde	...	...	...	...	0.84
B.	Extract acidified with excess HCl and filtered. Weight of precipitate	...	...	...	...	1.37
C.	Filtrate from B neutralized and concentrated under reduced pressure to remove alcohol. Yield of furfuraldehyde	...	...	...	...	0.92
D.	Extract neutralized, concentrated under reduced pressure to small volume, half volume concentrated HCl added. Weight of precipitate	...	...	...	...	11.89
E.	Filtrate from D. Yield of furfuraldehyde	...	...	...	...	0.53
F.	Extract brought to pH 4.0 and filtered. Weight of precipitate	...	...	...	...	3.06
G.	Filtrate from F neutralized and concentrated under reduced pressure to remove alcohol. Yield of furfuraldehyde	...	...	...	...	0.88

products produced have not been investigated since they are of no special moment. Both volatile and non-volatile acids have been found in the extract in amounts significantly greater than are obtainable from the original material by simple dilute alkali treatment. 4.8% volatile acid calculated as acetic was obtained from the wheat straw extract and non-volatile acid with a titration figure of approximately half that of the volatile.

From these experiments the conclusion must be drawn that the only satisfactory proof of the absence or magnitude of the degradative attack caused by hot alcoholic NaOH on the furfuraldehyde-yielding constituents is to be obtained by comparison of the analyses of the material before and after extraction, and not by examination of the extract.

(b) *Conditions of extraction of hemicelluloses*

Hemicellulose preparations are obtained from alkaline extracts of the material under investigation, but the precise conditions employed are variable. The strength of the alkali is not usually less than 4%. Some workers confine themselves to exhaustive cold extraction, whilst others follow a cold extraction with one at the boil. There is now a considerable weight of evidence pointing to some form of association between the polyuronide hemicelluloses and lignin, which probably amounts to actual linkage [Harris *et al.* 1934; Norman & Shrikhande, 1935]: If the existence of such a complex be recognized, preparatory work may be simpler, in that the purpose of the treatment given must be to rupture the association. This may presumably be effected with alkali, which simultaneously dissolves the hemicellulose and much of the lignin, or more easily by chlorination or oxidation of the lignin. Simple alkali extraction in the cold even if repeated many times does not usually result in complete removal of

the polyuronide hemicelluloses from mature lignified tissues such as woods. Sands & Nutter [1935] showed that if exhaustively extracted wood is then delignified by alternate treatments with chlorine water and 10% ammonium hydroxide, subsequent extraction with cold alkali will remove most of the residual hemicelluloses. Their observations have been confirmed, but their procedure is not recommended for all purposes because there is undoubtedly some loss of hemicellulose in the ammonia extracts. They also employed 10% NaOH for the final extraction, a concentration which is unnecessarily high and causes too great an attack on the β - and γ -cellulose fractions. However, their procedure in principle depends on the liberation of the residual hemicellulose by attacking and removing the lignin. The purity of the final preparation is not much improved by the solution of the lignin and oxidized lignin in ammonia, because the initial NaOH extract of most materials contains a considerable quantity of lignin. A procedure which gives an extensive removal of hemicellulose is to chlorinate the residue obtained from the initial cold alkali treatment and after washing with a little water immediately to extract again with cold alkali of the same strength. Prior to the initial treatment the material should be extracted if necessary with organic solvents, with warm water and with dilute ammonium oxalate if pectin is present. Analyses of some materials which received one treatment with 4% NaOH for 24 hr. and also a second treatment after chlorination in faintly acid solution are given in Table III.

Table III. *Extraction of hemicelluloses by cold 4% NaOH before and after chlorination*

Initial treatment 24 hr., chlorination 15 min., final treatment 24 hr.
All results expressed as % of original material

Material	*	Residue	Lignin	Cellulose	Furfur- aldehyde from cellulose	Total furfur- aldehyde	Polyuronide furfur- aldehyde
Barley straw	1	—	16.4	48.6	7.4	18.7	11.3
	2	58.6	7.5	43.9	5.0	7.0	2.0
	3	39.7	3.3	35.2	2.5	3.4	0.9
Wheat plants	1	—	12.6	38.4	6.5	12.1	5.6
	2	48.9	5.9	35.8	4.9	6.4	1.5
	3	33.6	2.9	28.2	3.0	4.1	1.1
Oak	1	—	22.3	53.9	9.2	13.4	4.2
	2	83.9	18.8	52.9	8.1	10.6	2.5
	3	75.0	15.6	51.4	7.3	8.6	1.3
Wheat straw	1	—	16.6	56.7	7.7	16.6	8.9
	2	60.8	7.3	46.0	5.8	8.9	3.1
	3	49.6	4.2	44.5	4.6	6.0	1.4
Jute	1	—	11.7	74.9	7.3	10.6	3.3
	2	86.0	10.9	69.7	5.1	7.2	2.1
	3	75.9	6.9	67.6	4.1	5.1	1.0

* 1=Untreated. 2=Initial alkali treatment only. 3=Residue from 2 chlorinated and re-extracted with alkali.

It will be seen that these two treatments taking only 48 hr. have brought most of the polyuronide hemicelluloses into solution. More can be obtained by a further treatment, but this is attended by other dangers, as will be mentioned later.

The possibility of using much more dilute alkaline solutions and carrying out the extraction at the boiling point has been examined. The assumption that hemicelluloses are unaffected by hot alkali seems generally to be made, though

without absolute proof, but it is unlikely that solutions as dilute as were employed would result in degradation. The procedure adopted was to boil for 30 min. and to alternate this treatment with brief chlorination (Table IV).

Table IV. *Extraction of hemicelluloses from oat straw by dilute alkaline solution*

Boiled for 30 min., chlorinated for 10 min.

Treatment	Residue	Lignin	Cellulose	Furfur-aldehyde from cellulose	Total furfur-aldehyde	Polyuronide furfur-aldehyde
Untreated	—	15.9	52.9	9.1	15.3	6.2
0.1 % NaOH	79.6	12.3	52.0	8.2	14.1	5.9
+ 1 chlorination	72.5	8.9	50.5	8.0	13.3	5.3
+ 2 chlorinations	68.4	6.6	50.1	7.8	12.9	5.1
0.5 % NaOH	58.5	4.4	50.7	8.5	10.2	1.7
+ 1 chlorination	51.4	1.9	45.4	7.5	8.8	1.3
+ 2 chlorinations	47.7	0.7	43.5	6.2	7.5	1.3
0.5 % Na ₂ CO ₃	77.1	11.6	52.8	8.7	13.8	5.1
+ 1 chlorination	67.3	7.6	51.0	8.0	13.1	5.1
+ 2 chlorinations	60.2	4.1	46.7	7.7	10.8	3.1
2 % Na ₂ CO ₃	70.3	8.4	52.1	8.7	12.6	3.9
+ 1 chlorination	60.5	4.3	48.3	7.3	10.8	3.5
+ 2 chlorinations	53.6	1.9	48.1	6.9	8.9	2.0
M/5 K ₂ HPO ₄	82.4	12.8	51.5	8.7	14.6	5.9
+ 1 chlorination	78.0	11.0	51.0	8.8	14.5	5.7
+ 2 chlorinations	73.5	8.9	50.8	8.3	14.0	5.7

The results indicate that removal of hemicelluloses can be achieved under conditions considerably less drastic than sometimes employed. No general method can be suggested because different materials vary considerably in behaviour to these more dilute solutions, but a procedure using first 2 % Na₂CO₃ and then, after chlorination, 0.5 % NaOH would result in the solution of the major part of the hemicelluloses.

(c) *Purity of hemicellulose extracts and preparations*

Cold alkali, in concentrations from 4 to 10 %, as commonly employed, extracts hemicelluloses of two types, as well as lignin. Not only do the encrusting polyuronide hemicelluloses go into solution but also some of the cellulosan associated with the cellulose. This dual source of the hemicelluloses has not been fully recognized and it is a strong criticism of fractionation methods that no clear separation is effected. In most cases therefore the preparations obtained have been mixtures of polyuronide hemicelluloses with non-uronide material from the cellulose. This point is discussed more fully elsewhere [Norman, 1937]. Up to a point the polyuronide hemicelluloses are more easily extracted than the cellulosan and accompanying material from the cellulose, but as the conditions of extraction become more drastic so does the proportion of the latter to the former increase. In most materials, other than softwoods, the cellulosan is xylan, and an investigation of the fractionation of an alkaline extract of isolated cellulose has showed that the major part of the xylan is precipitated in the B₁ fraction of the system developed by Norris & Preece [1930]. The hexosan simultaneously removed is not precipitated by copper and should therefore be found in the B₂ or C₂ fraction.

The improved fractionation procedure described by Angell & Norris [1936] may make it possible to distinguish more satisfactorily between the polyuronide hemicelluloses and polysaccharides having their origin in the cellulose, both of which will always be present in a simple alkaline extract if the concentration is high. Some idea of the relative proportions may be obtained from Table V in

Table V. *Composition of residues from hemicellulose extraction*

Extracted twice for 24 hr. with cold 4% NaOH, chlorinated 15 min., and re-extracted for 24 hr. with cold alkali. All results expressed as % of original material

Material	Residue	Lignin	Cellulose	Furfur- aldehyde from cellulose	Total furfur- aldehyde	Poly- uronic furfur- aldehyde	P/T*
Oat straw	—	15.9	52.9	9.1	15.3	6.2	
Residue	31.5	2.7	26.8	2.7	3.5	0.8	46
Holly wood	—	19.0	53.7	7.3	14.6	7.3	
Residue	60.6	12.1	47.5	4.0	4.9	0.9	66
Wheat straw R	—	16.0	53.5	7.7	16.4	8.7	
Residue	51.5	4.9	43.1	4.9	6.6	1.7	71
Wheat straw M	—	15.1	53.5	10.3	16.5	6.2	
Residue	41.4	2.1	38.3	3.7	3.8	0.1	48
Wheat straw S	—	15.1	54.3	9.0	16.6	7.6	
Residue	41.0	1.3	39.4	3.6	4.2	0.6	56
Wheat plants	—	12.6	38.4	6.5	12.1	5.6	
Residue	28.3	—	27.0	2.4	2.9	0.5	55

* $P/T = 100 \times \text{Polyuronic furfuraldehyde removed} / \text{Total furfuraldehyde removed}$.

which are given the analyses of the residues after exhaustive extraction with 4% NaOH in the cold as commonly employed, followed by brief chlorination and further treatment with alkali of the same strength. The ratio P/T in the final column is an expression in terms of furfuraldehyde of the contribution made by the polyuronic hemicelluloses to the total furfuraldehyde in the extract. The proportions of polyuronic hemicellulose to cellulose-derived material are of course not the same as this ratio, but the figures are sufficient to show that in most hemicellulose preparations there is a substantial contribution from the cellulose. This is equally apparent if a similar examination be made of the analyses given in Table I. Crude or unfractionated hemicellulose preparations were obtained from each of the combined extracts from holly wood, wheat straw M and wheat straw S by precipitation with acid and excess alcohol. After purification the furfuraldehyde yield of the preparations was determined, and found to be respectively 55.1, 52.5 and 51.2% on the ash- and lignin-free basis. From the data in Table V it is then possible to calculate approximately the amount of cellulose-derived xylan and hexosan present, assuming no losses. In these three preparations this amounted to:

	Holly wood	Wheat straw M	Wheat straw S
	%	%	%
Xylan	29.5	42.7	34.6
Hexosan	5.7	19.7	26.5

Too much weight cannot be attached to these figures since the experimental errors are magnified considerably, but they serve to emphasize again the fact that the alkali extract of materials such as straw may not consist predominantly of polyuronic hemicellulose. If it is desired to investigate this fraction, and if fractionation procedures do not effect a good separation, as seems to be the case

at present, other means of extraction will have to be sought. There are indications in Table IV that a brief hot extraction with 0.5% alkali has a much less drastic effect on the cellulose than extraction with cold 4% solutions, and if purity were considered more important than the completeness of extraction, such a treatment for 30 min. or less would be far better. Alternatively there is the possibility of working up the sulphite liquors obtained in the isolation of cellulose, though there might be difficulties in getting the preparations entirely free from lignin sulphonic acids. Whatever means of extraction is adopted an analysis of the residue is desirable in order that the relative proportions of the various constituents removed may be known.

There is another aspect of the purity of hemicellulose preparations which is sometimes overlooked. Extraction of any plant material with alkali removes lignin, often to a considerable extent, some of which may later be precipitated according to the conditions adopted. If the hemicellulose is carefully purified by redissolving in alcohol and precipitating with acid or acid alcohol a number of times and is thoroughly washed with alcohol, the lignin remaining in the final preparation is rarely more than 2–3%. To reduce this further is often difficult. Crude preparations may contain as much as 10–15%. Very few investigators have determined the lignin contents of their preparations. Some indeed have considered the insoluble residue remaining after simple acid hydrolysis to be an integral part of the hemicellulose molecule. For example, Sands & Gary [1933] reported 6.5% of "body X" in the hemicellulose A fraction from mesquite wood. Because of the strong probability of its presence this must be assumed to have been lignin until the opposite is proved, although there is the further possibility of formation of a small amount of insoluble matter as a result of the action of acid on pentose groups.

In order to minimize the amount of lignin to be found in the final preparation experiments have been directed along two lines: (1) the reduction of the amount of lignin in the original alkaline extract before precipitation of the hemicellulose, (2) the reduction of the amount of lignin still retained by the hemicellulose preparation precipitated in the usual manner. Of these two alternatives the latter is the more effective, since the former cannot be achieved completely without some loss of hemicellulose. The procedure which has been found most effective is to redissolve the precipitated hemicellulose in dilute alkali, either before or after fractionation and to add a small amount of dilute sodium hypochlorite followed by sufficient acetic or hydrochloric acid to liberate the chlorine. After standing 5–10 min., the minimum quantity of alcohol to effect full precipitation is added together with more acid if necessary. The precipitate is immediately filtered off and washed carefully with more alcohol of the same concentration. It is of importance that the alcohol concentration should not be higher than necessary since the lignin oxidation products seem to be less soluble in high concentrations. In practice two such treatments have been found to give excellent preparations. The expedient of first precipitating all the hemicelluloses from the extract by addition of acid and excess alcohol has been adopted followed by fractionation of the partially purified product. One chlorination may conveniently be given at this stage and the treatment repeated later if necessary on the individual fractions after separation. Preparations of low ash content have been obtained more readily by using hydrochloric acid for acidification rather than acetic acid as commonly employed.

SUMMARY

1. If hot alcoholic NaOH is to be used as a pretreatment before hemicellulose extraction, it should be established by analysis of the residue that the furfuraldehyde-yielding constituents have not been attacked. The fact that no furfuraldehyde can be obtained from the precipitate formed on acidification of the alcoholic extract is not conclusive proof that no degradative attack has occurred.

2. Extensive removal of hemicellulose material may be effected by extraction with cold 4% NaOH alternated with brief chlorinations. Such extracts may contain a high proportion of cellulose-derived polysaccharides. Brief extraction with hot, more dilute alkali has a less drastic effect on the cellulose and such extracts may consist largely of polyuronide hemicelluloses.

3. The lignin content of hemicellulose preparations should always be determined. It may be reduced by brief treatment with chlorine and thorough washing with alcohol of moderate concentration.

REFERENCES

- Angell & Norris (1936). *Biochem. J.* **30**, 2155, 2159.
Harris, Sherrard & Mitchell (1934). *J. Amer. chem. Soc.* **56**, 889.
Norman (1935). *Biochem. J.* **29**, 945.
—— (1937). *The Biochemistry of Cellulose, the Polyuronides, Lignin etc.* (Oxford.)
—— & Shrikhande (1935). *Biochem. J.* **29**, 2259.
Norris & Preece (1930). *Biochem. J.* **24**, 59.
Sands & Gary (1933). *J. biol. Chem.* **101**, 573.
—— & Nutter (1935). *J. biol. Chem.* **110**, 17.