CXIV. THE HEMICELLULOSES.

I. ALCOHOLIC SODIUM HYDROXIDE AS A PRETREATMENT TO EXTRACTION.

By ARTHUR GEOFFREY NORMAN.

From the Biochemistry Section, Rothamsted Experimental Station.

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INTRODUCTION.

THE hemicelluloses of plant materials form a group of polysaccharides, the investigation of which presents many difficulties. Neither their rôle nor their genesis is at present understood, and yet, in mature materials, they may amount to as much as 30 % of the whole, and they rank next to cellulose in quantitative importance. The name hemicellulose, due originally to Schultze [1892], is unfortunate, as it implies a close relationship to cellulose which, so far as most types of hemicellulose are concerned, does not appear to be justifiable. Hawley and Norman [1932] have discussed the occurrence and properties of hemicelluloses of plant materials and woods and differentiated them into two main groups differing in structure, distribution and function. For that type of polysaccharide intimately associated with the cellulose they have suggested the name "cellulosan". In most sources examined the cellulosan has proved to be a xylan, save in the Gymnosperms where a mannan also is found. The larger group of hemicelluloses is, however, not associated with the cellulose, but forms the incrusting material on the cell wall. Almost invariably has this type of polysaccharide been found to be a polyuronide. The two main groups of hemicelluloses, therefore, are the cellulosans and the polyuronides. In these papers it is proposed to deal more particularly with the incrusting polyuronides. Researches on the cellulosans will appear separately.

The isolation of hemicelluloses, and in particular those of the polyuronide group, in a pure condition is not easy. Extraction must be made with dilute alkali under conditions that result also in the solution of lignin and proteins, and the final preparation obtained after acidification and precipitation with alcohol or acetone is almost invariably contaminated with lignin, though this is rarely determined. The estimation of this group is still very unsatisfactory and inaccurate. Hydrolytic methods are of little use owing to the instability of uronic acids in hot mineral acids. Preece [1931, 2] produced a scheme for the evaluation of hemicelluloses in boxwood, depending on direct isolation and weighing, and Buston [1934; 1935] extended this to such materials as grasses and leaves in which the protein content is much higher. All the hemicellulose fractions obtained have to be corrected for the presence of protein.

To minimise the amount of lignin likely to pass into the preparation, Norris and Preece [1930], in isolating the polyuronides from wheat bran, carried out a pretreatment of the material with 50 % alcohol containing 1 % NaOH. Two extractions were given under a reflux for 2 hours each. This same pretreatment was also used and was then followed by further extraction with hot neutral 50 % alcohol in other preparations [Preece, 1930; 1931, 1, 2]. It was apparently assumed that because hemicelluloses are insoluble in cold 50 % alcohol under acid conditions, they will also be equally insoluble in hot 50 % alcohol under

alkaline conditions. Later, in putting forward his quantitative method for box-wood, Preece [1931, 2] recommended one treatment for $1\frac{1}{2}$ hours with boiling 50 % alcohol containing 1 % NaOH, followed by two extractions with neutral alcohol. Buston [1934], in preparing the polyuronides from cocksfoot grass, modified this treatment, giving instead a cold extraction overnight with the same concentration of alcoholic soda, and in his quantitative method he gave a similar pretreatment in the cold for 24 hours. Though the extraction with alcoholic soda was designed originally to remove lignin, it also reduces the amount of protein carried through into the subsequent alkaline extract and consequently into the hemicellulose preparation.

In some work on the delignification of straws and similar materials the effect of extraction with alcoholic soda of this and other concentrations was determined. It was found that while the removal of lignin was but partial, the material sustained heavy losses which could only be ascribed to the hemicelluloses. The results obtained caused the effect of alcoholic soda to be examined in more detail and show that its use in the heat is inadvisable prior either to preparation or estimation of hemicelluloses, and that even in the cold it is not without action on this group.

EXPERIMENTAL.

(a) *Extractions with NaOH in various alcohol concentrations.*

One gram samples of oat straw (60 mesh) were extracted in duplicate with various concentrations of alcoholic NaOH. After thorough washing with alcohol of the same concentration, containing a little acetic acid, then with 50 % alcohol to remove sodium acetate and finally with 95 % alcohol, the residue was determined. The two extractions were bulked and ground up, prior to duplicate lignin determinations, after hydrolysis with 5 % H_2SO_4 as described by Norman and Jenkins [1933]. The results are given in Table I, from which it

Table I. *Extraction of oat straw (A) with alcoholic NaOH.*

Initial lignin content 11.39 %.

Lignin contents expressed on original material.

NaOH concentration %	Alcohol concentration %	Time in hours	Temperature	Residue %	Lignin content %	Delignifi- cation %
1	90	1	B.P.	56.9	4.61	59.6
1	90	$\frac{1}{2}$	B.P.	62.9	4.74	58.4
1	90	2	60°	63.4	6.57	42.2
1	90	2	15°	70.9	8.07	29.2
0.2	90	1	B.P.	61.8	6.41	43.7
0.02	90	1	B.P.	75.8	9.84	13.6
1	60	1	B.P.	57.4	3.75	66.8
5	60	1	B.P.	57.2	3.92	65.3

is apparent that the loss in lignin forms only a small part of the total loss. Since a mature material was concerned, the three constituents, cellulose, hemicelluloses and lignin, account for about 90 % of the whole, and inasmuch as it is unlikely that true cellulose would be affected, the inference was that hemicellulose material, either polyuronide or cellulosan, or both, was extensively removed. As mentioned previously, no method for the estimation of the polyuronides is really satisfactory and accordingly no attempt at direct estimation was made, reliance being placed instead on the yield of furfuraldehyde as a measure of the hemicellulose present. The furfuraldehyde due to cellulosan may readily be determined and the difference between that and total furfuraldehyde represents that from the polyuronides.

A series of experiments was carried out on another sample of oat straw which proved to be rather less susceptible to alcoholic soda. Extractions were made with 100 ml. of a solution of 1 % NaOH in alcohol concentrations of 90 %, 70 %, 50 %, 30 % and with 1 % of NaOH in water. Each was carried out in duplicate and both hot and cold. The former were made at the boiling-point of the mixture for 1 hour and the latter at approximately 16° for 2 hours. The alcoholic soda extract in each case was acidified with H₂SO₄ and diluted so that the final volume was 600 ml. and the acid concentration 3 %. This was then hydrolysed by boiling for 2 hours, and the lignin, which remained insoluble, was filtered off, dried and weighed. The residue, after appropriate washing, was weighed, the duplicates bulked together, ground up finely and analysed. Cellulose determinations were made by the method of Norman and Jenkins [1933], furfuraldehyde determinations by the usual phloroglucide procedure and lignin determinations by treatment with 72 % H₂SO₄ for 2 hours and subsequent hydrolysis after dilution to 3 %. The results obtained are summarised in Table II, all the analyses being calculated on the original material.

Table II. *Extraction of oat straw (B) with 1 % NaOH in various alcohol concentrations.*

Results expressed as % of original material.									
	Residue	Lignin in extract	Cellulose	Furfurald. from cellulose	Xylan in cellulose	Total furfurald.	Furfurald. from polyuronides	Lignin (ash-free)	Delignification %
Original straw	—	—	49.6	8.41	13.0	16.69	8.3	14.47	—
(A) <i>Hot extraction:</i>									
1 % NaOH in alcohol									
90 %	80.45	1.99	46.8	7.96	12.4	15.59	7.6	11.33	22
70 %	68.88	7.40	45.4	8.01	12.5	15.64	7.6	6.04	58
50 %	59.99	8.57	45.2	8.17	12.7	13.07	4.9	4.99	65
30 %	52.94	7.70	44.2	7.65	11.9	8.94	1.3	4.74	67
Water	49.27	9.18	43.0	6.14	9.6	8.45	2.3	3.86	73
(B) <i>Cold extraction:</i>									
1 % NaOH in alcohol									
90 %	85.98	1.19	48.1	8.03	12.5	16.19	8.2	14.63	—
70 %	80.86	0.95	48.3	8.97	13.9	16.70	7.7	13.58	6
50 %	75.70	2.46	48.6	8.52	13.2	15.63	7.1	11.91	18
30 %	72.62	2.73	48.9	8.99	14.0	13.97	5.0	10.58	27
Water	72.51	3.15	46.5	6.91	10.7	13.36	6.5	10.73	26

Considering first the hot extractions, it is seen that there was an appreciable fall in the yield of furfuraldehyde from both polyuronides and cellulosan with even the 90 % alcoholic soda, and that with the lower concentrations of 50 % and below, the apparent loss of the former was very extensive. The 30 % solution appeared, rather surprisingly, to remove more of the polyuronides than an aqueous solution, though less of the xylan in the cellulose was extracted. The 50 % solution, as recommended by Preece [1931, 2] for the lowering of the lignin content, did in fact remove about 65 %, but this was accompanied by roughly 40 % of the polyuronides, if the furfuraldehyde yield may be taken as a direct measure, and 5 % of the xylan.

In the cold, the action of alcoholic soda was less drastic and in 90 % alcohol 1 % NaOH removed very little either of the polyuronides or the lignin. With lower concentrations there was some loss of polyuronides, and again it was noted that the 30 % alcoholic solution caused a greater loss of this constituent than did the aqueous solution. The figures for xylan in cellulose were unaccountably high in this group of extractions, and no apparent loss occurred

save in aqueous solution. Delignification was in no case very effective and did not exceed 26 %. The recovery of lignin from the extract did not appear to be quite quantitative.

From Table II as a whole it is quite clear that any treatment which brings about a considerable lowering of the lignin content also extracts appreciable quantities of polyuronide material.

The preliminary experiments given in Table I indicated that a NaOH concentration of much less than 1 % would extract considerable amounts of material from straw, and accordingly the losses with 0.1 % NaOH in various alcoholic solutions were determined for oat straw B.

Table III. *Extraction of oat straw B with 0.1 % NaOH in various alcohol concentrations for 1 hour at boiling-point.*

Alcohol %	Residue %
90	80.4
70	77.9
50	76.6
30	72.8
—	77.3

The loss with 0.1 % NaOH in 90 % alcohol was found to be almost identical with that caused by 1 % NaOH, but at lower concentrations of alcohol the more dilute alkali did not prove to be such an efficient extracting agent. The curious difference between the effect of the 30 % alcoholic and the aqueous solutions was more marked in this series, the total loss being greater in the slightly alcoholic solution. Detailed analyses were carried out on the residues from these two extractions (Table IV) and the results showed that the extra loss in alcoholic

Table IV. *Analyses of oat straw B extracted with 0.1 % NaOH in 30 % alcoholic and aqueous solutions for 1 hour at boiling-point.*

	Results expressed as % of original material.						
	Residue	Cellulose	Furfurald. from cellulose	Xylan in cellulose	Total furfurald.	Furfurald. from poly- uronides	Lignin (ash-free)
Original straw	—	49.6	8.41	13.0	16.69	8.3	14.47
0.1 % NaOH in 30 % alcohol	72.82	45.0	7.66	11.9	14.49	6.8	11.26
0.1 % NaOH aqueous	77.31	47.8	8.05	12.5	15.40	7.3	12.83

solution was due to each of the three chief extractable groups, cellulosan, lignin and polyuronides. With hot 1 % NaOH as given in Table II only the polyuronides showed a greater loss in 30 % alcohol than in water.

(b) *Extraction of various materials with 1 % NaOH in 50 % alcohol.*

Because of the apparent difference in susceptibility noted between the two samples of oat straw employed, a survey was made on a number of plant materials of widely different composition. Extractions were carried out with 1 % NaOH in 50 % alcohol for 1½ hours at the boiling-point, as recommended by Preece [1931, 2] for quantitative work, and also overnight in the cold with the same reagent, as used by Buston [1934], with the minor, and probably unimportant, difference that the time of standing was 16 hours instead of 24 hours. The materials were oak wood, jute fibre, old bean straw, mature hay, wheat plants just before

harvest, green wheat plants six weeks before harvest (1.4 % N), mature vetches (2.53 % N) and young green vetches (4.88 % N), thus giving a range from highly lignified to young green materials.

Analyses were carried out on the extracted residues as described before. The results are given in Table V.

Table V. *Extraction of various materials with 1 % NaOH in 50 % alcohol; hot extractions 1½ hours at boiling-point, cold extractions 16 hours at room temperature.*

Results expressed as % of original material.									
Material	Treatment	Residue	Cellulose	Furfurald. from cellulose	Xylan in cellulose	Total furfurald.	Furfurald. from poly- uronides	Lignin after hydrol.	Deligni- fication %
Oak	—	—	53.9	9.21	14.3	13.37	4.2	22.26	—
	Cold	88.4	51.4	8.47	13.1	12.83	4.4	19.81	11
	Hot	79.6	51.0	7.81	12.1	11.35	3.5	17.59	21
Jute	—	—	70.2	5.72	8.8	9.82	4.1	11.65	—
	Cold	89.2	68.3	5.05	7.8	8.80	3.8	8.58	26
	Hot	76.0	61.0	5.04	7.8	7.97	2.9	6.52	44
Bean straw	—	—	42.0	5.82	9.0	10.92	5.1	12.68	—
	Cold	85.1	44.5	5.77	9.0	11.49	5.7	11.70	8
	Hot	70.1	40.4	4.88	7.6	8.56	3.7	10.06	21
Wheat plants (mature)	—	—	46.7	8.31	12.9	13.27	5.0	13.79	—
	Cold	77.4	45.4	8.94	13.9	13.86	4.9	8.77	36
	Hot	66.1	41.8	7.97	12.3	12.92	4.9	3.67	73
Vetches (mature)	—	—	31.8	4.04	6.3	11.52	7.5	14.33	—
	Cold	68.9	35.3	4.10	6.4	8.74	4.6	11.03	23
	Hot	58.3	29.0	3.72	5.8	7.54	3.8	7.59	47
Hay (mature)	—	—	37.7	6.19	9.6	12.06	5.9	9.81	—
	Cold	67.6	35.9	5.29	8.2	10.81	5.5	9.64	2
	Hot	50.7	33.6	5.26	8.1	8.68	3.4	4.85	51
Wheat plants (green)	—	—	38.4	6.74	10.4	11.74	5.0	9.99	—
	Cold	57.6	38.3	7.47	11.6	11.78	4.3	6.23	38
	Hot	44.9	34.1	6.07	9.4	9.51	3.4	2.27	77
Vetches (young)	—	—	23.7	1.94	3.0	5.71	3.8	11.23	—
	Cold	58.3	27.9	2.49	3.9	5.01	2.5	7.98	29
	Hot	33.6	19.2	1.84	2.9	3.53	1.7	3.41	70

The mature materials suffered less total loss than the younger tissues, as might have been expected. In the case of the latter, much of the material removed was protein in nature. The cold treatment was not very effective in the removal of lignin, the percentage delignification only exceeding a third in one case. In some materials, though not in all, there was a distinct fall in the furfuraldehyde from polyuronides as a result of the extraction in the cold. The amount of hemicellulose removed, as judged by this figure, seemed to be larger in the case of the immature materials than in the mature; there was also a loss of xylan in some cases, though certain discrepancies occurred in these analyses. The figures obtained after the hot extraction, on the other hand, indicated in every sample an appreciable loss of polyuronide material and a fall also in the xylan associated with the cellulose. Delignification, of course, was more effective under these conditions, particularly in the less mature materials. However, in no experiment was it complete, and in oak and bean it was far from extensive.

There is no doubt that if the procedure of Preece [1931, 2] be followed, and a hot extraction with 1 % NaOH in 50 % alcohol be given, prior to the extraction of hemicelluloses, the values for that group will be low to an extent which varies with the nature of the material. It happened that Preece [1931, 2] worked with boxwood in which the hemicelluloses seemed rather more resistant to the action of alcoholic soda than those of some of the materials investigated in this

work. While the treatment in the cold adopted by Buston [1934] has not such a drastic effect, it cannot be said to be without action on the polyuronides in all cases.

(c) *Extraction of isolated hemicellulose with alcoholic NaOH.*

Hemicelluloses once isolated are known to be rather more easily soluble than when *in situ* in the plant, and for example, go slowly into solution on boiling with water and readily in dilute alkali. Despite this change in properties, it was thought to be worth while to determine the solubility of a preparation in alcoholic NaOH. A crude sample of hemicellulose A was prepared from oat straw by the usual means but omitting the pretreatment with 50 % alcoholic soda. Without attempt at purification a cream-coloured product was obtained with a furfuraldehyde yield of 37.0 % and a lignin content (determined by boiling with 3 % acid for 4 hours and weighing the residue as lignin) of 14.7 %, both on an ash-free basis. Corrected for the presence of lignin, the furfuraldehyde yield represents 43.4 %. Small samples were treated with 1 % NaOH in 50 % and 90 % alcohol, both hot and cold, the hot extractions being continued for 1½ hours and the cold for 16 hours. The residues were filtered off, weighed and ashed, and the filtrates in the case of the hot extractions were taken for furfuraldehyde determinations. The results are given in Table VI.

Table VI. *Extraction of isolated hemicellulose with alcoholic NaOH.*

Results given on ash-free basis.

1 % NaOH in alcohol	Hemicellulose taken mg.	Residue mg.	% extracted	Furfurald. on extract mg.
90 % hot	300.3	258.3	14.0	Trace
	293.6	265.1	9.8	Trace
90 % cold	299.4	300.9	—	—*
	298.6	289.8	2.9	—*
50 % hot	295.4	211.2	28.5	10.9
	303.2	220.8	27.2	9.7
50 % cold	303.9	263.8	13.2	—*
	300.4	264.4	12.0	—*

* Not determined.

The process of isolation did not produce any apparent increase in susceptibility to attack by alcoholic soda. While the 90 % solution in the cold removed little or nothing, some material went into solution in the heat. This was largely no doubt contaminating lignin since only a trace of furfuraldehyde was given by the extract. With the 50 % solution more than a quarter dissolved on boiling and about half this amount in the cold. The quantities available were too small to make possible any investigation of the nature of the material removed. If it be assumed that the hot extraction with 50 % alcoholic solution removed all the lignin from the hemicellulose, then the hemicellulosic fraction also removed had a furfuraldehyde yield of about 25 %, significantly lower than that of the parent product. This suggests the possibility of some partial fractionation or fission. These experiments are only of a preliminary nature, and this point will receive further attention.

DISCUSSION.

In his paper on the composition of boxwood, Preece [1931, 2] noted that the 50 % alcoholic soda treatments removed in addition to lignin some furfuraldehyde-yielding material which he described as a "lignosaccharide," though

he showed that it resembled the hemicelluloses in properties and composition. The chief demonstrable difference appeared to be that the "lignosaccharide" fraction was rather more resistant to hydrolysis than the main "free hemicellulose" fraction, though not very much more so than a portion of the latter. The experiments described here may be taken to support the existence of "lignosaccharides" if by that term is implied only hemicellulosic material going into solution in alcoholic soda. No evidence has however been adduced for the view that "lignosaccharides" represent an entirely new group of plant polysaccharides, and nothing in this work could be held to support such an opinion. The term seems unfortunate and likely to confuse even further the nomenclature of hemicelluloses. There does not appear to be any clear-cut fraction removable by 1 % NaOH in 50 % alcohol; the amount of material removed may be increased by heating or decreased by raising the alcohol content, and there is no indication that a different type of product is obtained in such cases. The behaviour of the polyuronides with this reagent seems to be precisely the same as that of lignin or the cellulosans, part of both of which is removable by 50 % alcohol.

The data presented make it plain that the employment of a pretreatment by boiling with 1 % NaOH in 50 % alcohol prior to a hemicellulose preparation will result in a lowering of the yield by an amount which may reach 50 %. As a preliminary to quantitative work this procedure is clearly inadmissible. In the cold its effect is less drastic and on mature materials seems to be slight. However, the effectiveness of such a cold treatment as a delignifying process is so low that it is hardly worth while carrying out. On young green materials cold alcoholic soda seems to produce some lowering of polyuronic material, and if it is desirable to give such a treatment as a means of removing much of the protein, the magnitude of the hemicellulose losses should be determined for each individual material.

If in the preparation of polyuronides purity rather than yield is the main object, there may be some justification for a pretreatment with hot alcoholic soda as a delignifying agent, though it has not been unquestionably shown that the product is wholly unchanged by this treatment. The separation of lignin and hemicellulose is at present under investigation, and preliminary results suggest that it may be better to revise the present procedure and to obtain by alkaline extraction an impure hemicellulose product contaminated with lignin, and then subsequently to remove the lignin by other means.

SUMMARY.

1. As a pretreatment to the preparation or direct estimation of hemicelluloses, an extraction of the material with 1 % NaOH in 50 % alcohol is employed as a delignifying process. The effect of this and other concentrations of alcoholic soda has been studied in this connection.

2. In the heat, 1 % NaOH in alcohol concentrations up to 90 % extracts a considerable amount of polyuronic material, the amount increasing as the alcohol concentration is decreased. In the cold, the action is less drastic in 70 and 90 % alcohol, but quite appreciable in 50 % and lower.

3. The polyuronic hemicelluloses of different materials vary in the degree of susceptibility to alcoholic soda. Those of immature tissues seem to be rather more easily removed than those of older lignified materials.

4. No indication has been obtained that the material removed by alcoholic soda represents a special group of the polyuronides.

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