CCLXVII. THE HEMICELLULOSES.

II. THE ASSOCIATION OF HEMICELLULOSES WITH LIGNIN.

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THE encrusting hemicelluloses have been presumed by most workers to exist in the cell-wall in a free condition, that is to say, not in chemical union with any other constituent. Preece [1931], in his work on boxwood, made a distinction between "free" and "combined" hemicelluloses, the former being soluble in cold 4% NaOH and the latter only going into solution on heating. He stated, however, that these terms were not to be understood literally but implied only a difference in case of extraction. Recently, Harris *et al.* [1934] have made a detailed study of the lignin from sugar maple wood and of the methylation of maple lignin *in situ* in the wood. They were unable to increase the natural methoxyl content of the lignin unless a preliminary acid hydrolysis were given. They concluded that the active "hydroxyls of lignin were not free for methylation until after hydrolysis, and that the lignin must, therefore, be attached to some of the carbohydrates, possibly the hemicellulose, in the wood cell". Further, they believed that such a combination between lignin and carbohydrate would have the effect of stabilising the lignin naturally in the enolic form, as opposed to the keto-condition in which isolated lignin is obtained. Harris *et al.* [1934] found support for this view in the observation that maple lignin *in situ* took up more chlorine than when freed by isolation. In the naturally occurring enolic form the lignin would be unsaturated and therefore more reactive. While these observations cannot be taken as proving beyond doubt the linkage of lignin with the encrusting hemicelluloses or polyuronides in the cell wall, they are sufficient to show that such a union is possible. In this paper is described some evidence, obtained by entirely different means, which points to the same conclusion.

A study has been made of the removal of lignin and encrusting hemicelluloses from various materials by means of sulphite with and without previous chlorination. Alternate exposure to chlorine and extraction with boiling sodium sulphite form the basis of the well known Cross and Bevan method for the isolation of cellulose. This procedure seems to have been devised solely with a view to the removal of lignin by reason of the formation of "lignone chloride", and its solution in hot sulphite. The concurrent removal of encrusting hemicelluloses appears to be incidental and has not been hitherto investigated. Norman and Jenkins [1933] gave the results of a few experiments on oat straw which showed that chlorination is apparently as necessary for the removal of the encrusting polyuronides as for that of lignin. Three treatments with hot sulphite solution alone effected the extraction of 57% of the lignin and a reduction of but 15% in the total furfuraldehyde yield. When, however, gaseous chlorination treatments were given alternately with three sulphite extractions, 83% of the lignin

was removed, and the furfuraldehyde yield was reduced by 40 %. Since in the sample used about 58 % of the furfuraldehyde was due to the cellulosan in the cellulose and consequently would be unaffected by the treatments, the removal of encrusting polyuronide hemicelluloses must have been practically complete. This effect of chlorination in assisting the removal of hemicelluloses had not been previously reported. The experiments described below are a further investigation of this point.

EXPERIMENTAL.

All plant materials employed were previously ground to pass a 60-mesh sieve. Extractions with sodium sulphite were carried out by boiling gently with 100 ml. solution for 20 min., 2-3 g. material being taken initially for each experiment. The residue was filtered on cloth while hot, and after the final treatment thoroughly washed with warm water. Gaseous chlorinations were given by passing a steady stream of the gas over and through the material suspended on a cloth fastened over a small Büchner funnel, suction being applied throughout. The time of contact with the gas was 10 min. in all cases. Chlorinations in solution were carried out by suspending the material in 100 ml. water and adding 5 ml. dilute sodium hypochlorite (about 3 % available Cl) and 2 ml. 20 % H_2SO_4 . After standing for 10 min. the residue was filtered off but not extensively washed before taking up in sulphite.

All determinations of total loss were made in duplicate or triplicate, the dried residues being bulked, reground and divided for duplicate determinations of furfuraldehyde yield and lignin content after hydrolysis for 1 hour with 5 % H_2SO_4 [Norman and Jenkins, 1934].

1. *Extraction of wheat straw (J. G. S.).*

The removal of lignin and encrusting polyuronides by hot sulphite solutions was studied with and without alternate exposure to chlorine gas or chlorine in solution. The results are summarised in Table I. One treatment with sulphite

Table I. *Removal of polyuronides and lignin from wheat straw.*

All results calculated on 100 g. original straw. Straw contained 51.91 % cellulose, 14.95 % lignin (after hydrolysis). Furfuraldehyde from cellulose 9.59 %.

	No. of treat- ments	Residue	Total furfur- aldehyde	Polyuronide furfur- aldehyde	Lignin (after hydrolysis)
A. 3 % sulphite alone	—	100.0	17.61	8.0	14.95
	1	83.2	17.38	7.8	10.60
	2	81.4	17.21	7.6	9.75
	3	77.8	17.02	7.4	9.39
	4	75.4	16.93	7.3	8.54
	6	72.2	15.69	6.1	7.14
B. Chlorine gas then 3 % sulphite	1	78.6	15.70	6.1	6.79
	2	73.2	14.66	5.1	3.82
	3	66.9	12.34	2.7	2.46
	4	58.9	11.00	1.4	2.04
	6	55.3	10.02	0.4	1.36
C. Chlorine in solution then 3 % sulphite	1	76.4	15.67	6.1	7.49
	2	70.5	14.05	4.5	5.95
	3	66.1	13.62	4.0	3.85
	4	58.8	10.95	1.4	2.53
	6	52.7	9.60	0.0	1.39
D. Chlorine gas then 1.5 % sulphite	1	79.3	15.46	5.9	6.61
	2	73.3	15.25	5.7	4.03
	3	69.8	14.32	4.7	2.76
	4	64.4	13.70	4.1	2.18
	6	59.3	11.95	2.4	1.60

alone removed about a third of the lignin and only a little of the polyuronides as followed by the difference in total furfuraldehyde yield and furfuraldehyde due to cellulosan. Further treatments produced a slow fall in both, but at the end of six treatments nearly half the lignin remained together with 76% of the polyuronides. Wheat lignin may, therefore, be slowly removed by neutral 3% sulphite. The polyuronides of this material, however, do not go into solution to any great extent under the same treatment. Solutions of neutral sulphite under pressure have been employed for the delignification of wood for pulps, but the results obtained have not been encouraging, as high pressures are necessary for the satisfactory removal of lignin.

By exposure to chlorine gas before each extraction with sulphite (Table I, B), the removal of lignin was, as would be expected, greatly expedited. The hemicelluloses were affected equally and rapidly came into solution also. One chlorination and sulphite extraction resulted in the removal of more lignin and as much of the polyuronide hemicelluloses as six sulphite treatments alone. The results obtained after chlorination in solution (Table I, C) were almost identical with those for gaseous chlorination. Substitution of 1.5% sulphite for 3% sulphite following treatments with chlorine gas was not significantly different in so far as the removal of lignin was concerned but resulted in a less complete extraction of the hemicelluloses. It is therefore important in cellulose determinations to use sulphite of 3%, as with a lower concentration the material might give no colour test for lignin and yet contain appreciable amounts of encrusting polyuronides unremoved.

These experiments show that the extraction of the polyuronides with sodium sulphite cannot be effected without chlorination. Further, by submitting the same material to one chlorination and then following with successive sulphite extractions without additional exposures to chlorine, the removal of the hemicelluloses was shown to be dependent on the removal of lignin. About 30 g. wheat straw were thoroughly soaked in water for some hours, after which dilute hypochlorite and acid were added to effect chlorination in the usual way for 10 min. The residue was filtered and boiled for 20 min. with 3% sulphite. After thorough washing it was dried, weighed and analysed. Small portions were subsequently given 1, 2, 3 and 5 further extractions with 3% sulphite. The results of these treatments are given in Table II. The removal of lignin by the successive sulphite treatments was slow and the loss of polyuronide hemicellulose not more rapid despite the intervention of an initial chlorination.

Table II. *Effect of one chlorination and successive sulphite extractions on the removal of polyuronides and lignin from wheat straw.*

All results calculated on 100 g. original straw.

No. of treatments*	Residue	Total furfur-aldehyde	Polyuronic furfur-aldehyde	Lignin (after hydrolysis)
—	100.0	17.61	8.0	14.95
1Cl.1SO ₃	77.0	15.16	5.6	8.26
1Cl.2SO ₃	73.0	14.94	5.4	7.62
1Cl.3SO ₃	72.3	14.78	5.2	7.43
1Cl.4SO ₃	70.6	14.59	5.0	7.16
1Cl.6SO ₃	68.1	13.76	4.2	6.46

* 1Cl.1SO₃ indicates one chlorination followed by one sulphite extraction.

The effect of extraction with sodium sulphite under pressure with and without previous chlorination was tested. Two samples of straw, 7.5 g. each, were

soaked overnight in water. To one sample sodium sulphite was added to give 250 ml. of 3 % concentration. The other was made up to 200 ml. with water and 2 ml. concentrated sodium hypochlorite were added followed by 4 ml. 20 % H_2SO_4 . After standing for 1 hour the residue was filtered off and suspended in 250 ml. 3 % sulphite solution. Both sulphite solutions were then autoclaved for 1 hour at 42 lb./sq. in. (144°). The residues were weighed and analysed as usual. The results are given in Table III.

Table III. *Effect of sulphite extraction of wheat straw under pressure with and without previous chlorination.*

All results calculated on 100 g. original straw.

Treatment	Residue	Total furfur-aldehyde	Furfur-aldehyde from polyuronide	Lignin after hydrolysis
Untreated	—	17.6	8.0	14.95
Sulphite alone	58.8	9.7	1.4	2.8
Chlorination followed by sulphite	59.0	10.4	1.6	3.2

Extraction with sulphite under pressure was clearly far more effective in the removal of lignin than prolonged boiling at 100°. Previous chlorination had no effect in increasing the extraction of lignin under these conditions and even reduced it slightly. This was possibly due to a small reduction in the concentration of the sulphite by the chlorine held in the sample.

2. *Extraction of mature meadow hay.*

The removal of lignin and polyuronide hemicelluloses from a sample of hay was studied in a similar manner. The results of analyses on this mixed material are given in Table IV. As in the case of wheat straw, a portion of the lignin went

Table IV. *Removal of polyuronides and lignin from hay.*

All results calculated on 100 g. dry material. Hay contained 44.79 % cellulose, 12.96 % lignin (after hydrolysis). Furfuraldehyde from cellulose 8.26 %.

Treatment	No. of treatments	Residue	Total furfur-aldehyde	Polyuronide furfur-aldehyde	Lignin (after hydrolysis)
A. 3 % sulphite alone	—	—	14.59	6.3	12.96
	1	72.09	13.21	4.9	9.65
	2	70.20	12.95	4.7	9.05
	3	69.17	12.64	4.4	9.00
	4	64.48	11.38	3.1	7.89
	5	64.31	11.24	3.0	7.68
B. Chlorine in solution then 3 % sulphite	1	69.46	13.07	4.8	8.44
	2	66.11	12.61	4.4	6.63
	3	60.02	11.23	3.0	4.06
	4	54.18	9.64	1.4	2.82
	5	48.35	9.06	0.8	2.26

into solution on treatment with sulphite alone and was accompanied by some of the polyuronide hemicelluloses. On further treatment with sulphite small quantities of both were removed, so that after six extractions a little over half the polyuronide material and less than half the lignin had gone. Chlorination greatly increased the rate of removal of both groups, the losses running almost parallel.

3. *Extraction of barley straw.*

Results of a similar nature were obtained in another series in which barley straw was subjected to the same extractions (Table V). Again a portion of the

lignin was readily removed by boiling with sulphite. Chlorination vastly increased the removal of polyuronide hemicelluloses as well as that of lignin.

Table V. *Removal of polyuronides and lignin from barley straw.*

All results calculated on 100 g. dry material. Barley contained 48.60 % cellulose, 16.44 % lignin (after hydrolysis). Furfuraldehyde from cellulose 7.37 %.

Treatment	No. of treatments	Residue	Total furfur-aldehyde	Polyuronic furfur-aldehyde	Lignin (after hydrolysis)
A. 3 % sulphite alone	—	—	18.69	11.3	16.44
	1	81.33	16.34	9.0	11.05
	2	79.34	15.98	8.6	10.24
	3	76.90	15.61	8.2	9.35
	4	75.40	15.00	7.6	8.87
	6	72.47	13.53	6.2	7.79
B. Chlorine in solution then 3 % sulphite	1	77.65	14.77	7.4	8.38
	2	69.42	13.75	6.4	6.03
	3	61.65	11.40	4.0	3.03
	4	59.66	10.90	3.5	2.68
	6	54.23	9.74	2.4	1.61

4. *Extraction of oak wood.*

Samples of oak, previously extracted with alcohol-benzene for the removal of resinous matter, were extracted in a similar manner.

Table VI. *Removal of polyuronides and lignin from oak.*

All results calculated on 100 g. alcohol-benzene-extracted wood. Wood contained 53.85 % cellulose, 22.26 % lignin (after hydrolysis). Furfuraldehyde from cellulose 9.21 %.

Treatment	No. of treatments	Residue	Total furfur-aldehyde	Polyuronic furfur-aldehyde	Lignin (after hydrolysis)
A. 3 % sulphite alone	—	100.0	13.37	4.2	22.26
	1	90.0	12.67	3.5	21.48
	2	88.6	12.64	3.4	21.52
	3	86.8	12.40	3.2	21.10
	4	86.4	12.10	2.9	21.25
	6	84.9	11.95	2.7	20.95
B. Chlorine in solution then 3 % sulphite	1	86.6	12.12	2.9	18.72
	2	77.3	10.65	1.4	14.25
	3	71.2	10.18	1.0	11.56
	4	62.1	9.24	0.0	7.01
	6	59.2	8.98	—	4.12

The results (Table VI) indicate certain differences between this wood and the other materials previously examined. The ratio of lignin to hemicellulose was much greater in the wood and was probably about 2, whereas in the straws the ratio was certainly less than unity. It would not be expected therefore that the removal of lignin and hemicelluloses from wood would run so closely parallel as in the other materials. Chlorination followed by sulphite extraction produced a rapid removal of both groups, but, as judged by furfuraldehyde yield, all the polyuronic hemicellulose material had gone into solution after four treatments although nearly a third of the lignin still remained. Again, contrary to the observations on straws, very little oak lignin went into solution on treatment with sulphite alone. On the other hand, about a third of the polyuronic material was removed by six successive sulphite extractions. In the case of this wood there seems to be a possibility that the association between hemicelluloses and lignin may be ruptured slowly by prolonged boiling with sulphite, without the lignin

itself coming into solution, a process presumably dependent on the ease of sulphonation of the lignin molecule. Because of the excess of lignin over hemicellulose, only a portion would be likely to be involved in such an association.

5. *Extraction of silver fir wood.*

This wood, being a gymnosperm, has a much lower pentose content than the oak, and in consequence, the furfuraldehyde yield may not be such a satisfactory indication of the encrusting hemicelluloses. The results obtained (Table VII) are similar in character to those given by other materials. As in the case of oak, very little lignin went into solution in sulphite alone.

Table VII. *Removal of polyuronides and lignin from silver fir.*

All results calculated on 100 g. wood. Wood contained 53.83 % cellulose, 29.26 % lignin (after hydrolysis). Furfuraldehyde from cellulose 2.61 %.

Treatment	No. of treatments	Residue	Total furfur-aldehyde	Polyurionide furfur-aldehyde	Lignin (after hydrolysis)
A. 3 % sulphite alone	—	100.0	5.49	2.9	29.26
	1	94.9	5.34	2.7	27.30
	2	94.4	5.34	2.7	27.83
	8	90.9	5.04	2.4	25.83
B. Chlorine in solution, then 3 % sulphite	1	93.3	5.00	2.4	24.05
	2	84.5	4.50	1.9	16.89
	3	75.1	4.15	1.5	10.41
	4	69.2	3.73	1.1	6.96
	5	66.1	3.49	0.9	1.21
	6	60.4	3.03	0.4	0.31
	8	58.0	3.19	0.6	0.12

6. *Modification of the material before extraction.*

Lignin, either isolated or *in situ*, is rapidly and extensively oxidised by a dilute solution of sodium hypochlorite, as will be shown in another communication later. Samples of two materials were treated for 16 hours at room temperature with dilute sodium hypochlorite of strength 0.75 % available chlorine, 100 ml. being used for 2.5 g., and in this way the lignin content was much reduced (Table VIII). A fall in the yield of furfuraldehyde indicated that there

Table VIII. *Removal of polyuronides after partial oxidation of lignin.*

All results expressed on the basis of 100 g. dry original material.

Material	Treatment	Residue	Total furfur-aldehyde	Polyurionide furfur-aldehyde	Lignin (after hydrolysis)
Barley straw	Untreated	100.0	18.69	11.3	16.44
	Oxidised with NaOCl	78.7	15.87	8.5	6.36
	3 % sulphite extraction after oxidation	64.3	12.90	5.5	4.20
	3 % sulphite extraction of original material	81.3	16.34	9.0	11.05
Hay	Untreated	100.0	14.59	6.3	12.96
	Oxidised with NaOCl	72.1	13.53	5.3	7.99
	3 % sulphite extraction after oxidation	62.6	12.23	4.0	6.30
	3 % sulphite extraction of original material	72.1	13.21	4.9	9.65

was some accompanying loss of hemicelluloses. Whether this was due to oxidation or to simple extraction by the slightly alkaline solution is not known, though the latter is more probable. However, one sulphite extraction following on the

oxidation process removed considerably more of the polyuronide material than if given directly on the original material. This would suggest that the removal of lignin by oxidation liberated a certain amount of hemicellulose which was then free to dissolve in the sulphite.

Experiments were made to determine whether the apparent association between hemicelluloses and lignin was affected by a pre-treatment with cold alkali. Such a treatment, of course, extracts a certain amount of both constituents, but that remaining was not appreciably more susceptible to extraction either by sulphite alone or by sulphite following on chlorination.

Aqueous extractions following chlorination were found to be nearly as effective in the removal of the hemicelluloses as sulphite treatments, but far less effective, as would be expected, in the removal of lignin.

DISCUSSION.

Sufficient evidence has been presented to show that the removal of hemicelluloses from plant materials on extraction with sulphite solution is affected by previous chlorination to almost the same degree as is that of the lignin, for which the process was especially designed. Three explanations are possible. (1) That the hemicelluloses and lignin exist in some form of combination, and that the solution of the hemicelluloses depends therefore upon the rupture of the linkage. Rupture may be brought about slowly by boiling with sulphite or rapidly by chlorination. (2) That the hemicelluloses and lignin are not combined and chlorination has a specific effect on the hemicelluloses rendering them soluble. (3) That the hemicelluloses and lignin are not combined and that the removal of hemicelluloses is mechanically and physically hindered by the presence of an insoluble layer of lignin, only removed by chlorination. Of these three possibilities the first, though not hitherto demonstrated, appears the most likely. To the second may be raised the objection that a non-reducing polysaccharide is not known to undergo any reaction upon brief treatment with a halogen. Further, when the lignin content was very much reduced by oxidation with NaOCl , one sulphite treatment extracted considerably more of the hemicellulose material than if this pre-treatment were not given. The third alternative is not in accordance with present views on the structure of the cell wall.

The theory of a combination between lignin and encrusting hemicelluloses finds further support in the changes in solubility of the latter after isolation. Whereas hemicelluloses are only removed from the tissues by agents which also will dissolve lignin and rupture the linkage, after isolation they are frequently soluble in hot water, or even slowly in cold water. It should be stated that Ritter and Kurth [1933] have shown that the polyuronide fraction of maple wood carries about one-sixth of the total methoxyl groups and all the acetyl groups present in the wood. The removal of some of these substituent groups in the ordinary process of isolation might also be a contributory factor in the observed change in solubility mentioned above.

If lignin and hemicelluloses occur in some form of combination, as this work indicates, there is the possibility of both groups existing in two conditions, attached and unattached, depending on the relative quantities of each present. Further, it implies that lignin should not be considered so inert in the plant as it is usually regarded.

SUMMARY.

The removal of polyuronide hemicelluloses from plant materials and woods is not easily effected by dilute sulphite solutions unless the material is given a previous chlorination. Some form of combination or association between lignin and this type of hemicellulose is probable, since the extraction of the latter depends on a treatment effecting the solution of the former.

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