CCCIV. THE DISTRIBUTION OF MANNAN IN SOME GYMNOSPERMS.

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MANNAN has long been known to be widely distributed in woods, particularly in the softwoods or gymnosperms. Bertrand [1899] drew attention to the fact that whereas the hardwoods or angiosperms contain much xylan and little mannan, the softwoods contain more mannan than xylan and suggested that the mannan in a softwood occupies the same place as xylan in a hardwood. Schorger [1917] showed mannan to be present in 20 different species of softwoods in amounts up to 7.6%. Later it was realized that some of the mannan of softwoods is associated with the cellulosic fraction, and mannose was obtained from wood cellulose and wood pulps by hydrolysis [Lenze *et al.* 1920]. Heuser & Dammel [1924] found that the mannan of spruce pulp passes into the β -cellulose fraction, and Hägglund & Klingstedt [1927] demonstrated the presence of mannan in specimens of wood cellulose by a consideration of the optical rotations of the cuprammonium solutions. Actual preparation of the mannan from sulphite pulp was achieved by Hess & Lüdtke [1928], who showed that its physical properties were similar to those of the mannan extracted from the ivory nut (*Phytelephas macrocarpa*).

The mannan associated with the cellulose of softwoods may be regarded as a cellulosan according to the nomenclature proposed by Hawley & Norman [1932], just as the xylan of hardwoods is so described. The xylan content of cellulose preparations is frequently determined in systems of analysis, so that a distinction may be drawn between "pentosans in cellulose" and "pentosans not in cellulose", but much less attention has been given to the mannan of softwoods. The purpose of the work described below therefore was to determine the distribution of the mannan in a few species of softwood and to examine the conditions of removal of the mannan associated with the wood cellulose.

EXPERIMENTAL.

I. *The determination of mannan.*

All methods of determination of mannan are based on the precipitation of mannosephenylhydrazone in the cold, and some modification of the procedure described by Bertrand [1899] is usually employed, such as that of Schorger [1917]. Briefly this consists of hydrolysing the wood by boiling for $3\frac{1}{2}$ hours with 5% HCl, neutralizing the extract, concentrating and precipitating the mannose by the addition of phenylhydrazine and acetic acid. Alternative methods involving extraction of mannan with alkali prior to hydrolysis have been shown to be inaccurate owing to incomplete extraction. It is not on record, however, that the quantitative aspects of the procedure recommended by Schorger [1917] have been studied, and in view of the fact that it is frequently necessary to determine mannose in low concentration this point was examined.

It was first established that the mannose produced on hydrolysis is stable to the mineral acid employed. No apparent fall in reducing value occurred when pure mannose was boiled with 5% HCl for periods up to 5 hours, the Shaffer-Somogyi micro-method being used.

The yield of hydrazone from different concentrations of mannose precipitated with (a) phenylhydrazine and acetic acid, and (b) phenylhydrazine hydrochloride and sodium acetate has been investigated, and the effect of the presence of sodium chloride examined. Some of the data from these experiments are given in Table I.

Table I. *Precipitation of hydrazone from different concentrations of mannose under varying conditions.*

Phenylhydrazine : sugar = 1 : 10. Stand at room temperature.

Conc. of mannose %	Sodium chloride added %*	Hours	Mannose recovered %†
1.44	12	6	93.4
1.20	12	6	92.4
1.20	—	6	91.8
0.62	9	6	92.4
0.60	—	6	81.5
0.60	—	2	69.0
0.56	12	6	90.0
0.37	16	6	86.0
0.30	—	2	51.9
0.30	—	6	71.7
0.30	9	6	83.9
0.15	—	2	8.0
0.15	—	6	38.0
0.075	—	4	Nil
0.075	—	6	Nil

10% phenylhydrazine hydrochloride solution containing equivalent sodium acetate.
10 ml. sugar solution used in each case.

Conc. of mannose %	ml. phenylhydrazine-HCl solution	Hours	Mannose recovered %†
2.1	5	6	94.0
2.1	10	6	95.4
2.1‡	5	6	Nil
1.5	5	6	94.7
1.2	5	6	95.3
1.2	5	24	94.9
1.2	5	6§	91.7
1.2	5	3	93.2
0.6‡	5	6	Nil
0.5	5	6	88.3
0.38	5	6	86.5
0.28	5	4§	70.5
0.28	5	4	75.2
0.13	5	6	24.0
0.06	5	6	Nil

* The significance of the concentration of NaCl added will appear later. The amounts roughly correspond to volume of 5% HCl selected for hydrolysis of mannan and final volume after concentration.

† Factor for calculation: hydrazone $\times$ 0.66 = mannose.

‡ With addition of 0.9 g. NaCl = 6% NaCl on final concentration.

§ Solution heated to 75° immediately after addition of phenylhydrazine hydrochloride, then cooled to room temperature.

Several important points emerge. Either with free phenylhydrazine or the hydrochloride the recovery of mannose is dependent on the initial concentration of sugar in the mixture and only approaches a quantitative figure when the mannose concentration is about 1% or more. At very low concentration no precipitate forms. The time of standing must be longer than the 2 hours recommended by Schorger [1917], but 6 hours appear to be sufficient if the mixture be frequently shaken. It has recently been claimed that, in low concentrations of mannose, it is necessary to allow 3 weeks for the precipitate to form [Nishida *et al.* 1936]. No advantage was gained by heating to 75° after addition of the phenylhydrazine, though the precipitated hydrazone appeared sooner. The effect of the presence of salt is difficult to understand. With phenylhydrazine in the proportions stated, the presence of salt increases the recovery of mannose so that the limit of mannose concentration for 90% recovery or better is lowered to 0.5–0.6%. Using the hydrochloride, and under conditions in which the excess of phenylhydrazine is distinctly less, the addition of salt prevents the formation of any precipitate even on standing for 24 hours.

In view of these findings, some of the figures given by Schorger [1917] for the total mannan content of wood cannot be considered reliable. If, as stated, only 10 g. of wood were used for hydrolysis, and the final precipitation was carried out in 150 ml., the recoveries of mannose must have been incomplete in many cases.

The mannan content of pulps has been determined by Klingstedt [1933], who avoided the difficulties brought about by low concentrations by hydrolysing the pulp completely with 72% sulphuric acid, boiling after dilution and concentrating to a syrup after removal of sulphuric acid. Even so the conditions of the final precipitation do not result in recoveries of mannose so nearly quantitative as obtained in this work.

There is, however, need for further investigation of the precipitation of mannose as the hydrazone in order to obtain a more sensitive method suitable for woods. It was hoped to employ the iodimetric titration devised by Ling and Nanji [1921] for the determination of small quantities of the hydrazone, but the incompleteness of the precipitation rendered this impossible. Substituted phenylhydrazine derivatives might prove more suitable in low concentration and will be tested.

II. *The distribution of mannan in wood.*

The method adopted for the determination is as follows. 10–15 g. of finely ground wood, or 35–40 g. of wet wood cellulose, are boiled with 100 or 150 ml., 5% HCl under reflux condenser for 3½ hours, the residue after filtration on cloth being thoroughly washed twice with about 300 ml. hot water. The combined extract and washings, after cooling, is neutralized with NaOH, made just acid with acetic acid and concentrated to about 25–30 ml. The concentrated solution is filtered into a small marked flask and the filter paper washed till the volume is made up to 50 ml. (or 75 ml. in some cases). After cooling 2.5–4 ml. phenylhydrazine and up to 5 ml. 50% acetic acid are added. The flask is closed and allowed to stand for 6 hours at room temperature with constant shaking. The final volume is noted, the hydrazone is filtered off on a Gooch crucible, washed with about 50 ml. cold water followed by acetone, dried and weighed. $\text{Hydrazone} \times 0.6 = \text{mannan}$ or $\times 0.66 = \text{mannose}$.

A bulky precipitate due to the salting out of phenylhydrazine acetate may form if too much acetic acid is used, and additional water may have to be added. A small amount of this precipitate has no influence on the hydrazone yield and is removed on washing with water.

In all determinations the results have been rejected if the final concentration of mannose in the solution from which the hydrazone was precipitated fell below 0.5%, and further determinations were made employing larger quantities of wood or cellulose. The period of 3½ hours' hydrolysis has been based on Schorger's observation and the knowledge that both encrusting hemicelluloses and cellulans are hydrolysed in that time. If small amounts of mannan remain unhydrolysed they cannot be detected by a second hydrolysis because they fall below the limits of quantitative precipitation. 5% H₂SO₄ has been employed for hydrolysis and the acid subsequently removed with barium carbonate. This process, though very desirable, results in losses of mannose.

To study the distribution of the mannan in wood, the total mannan and the mannan associated with the cellulose were separately determined (Table II), the cellulose preparations and determinations being made by the procedure described

Table II. *Distribution of mannan in wood.*

Wood	Expressed on wood %		Expressed on cellulose %		Expressed on wood %		
	Total mannan*	Cellulose	Mannan in cellulose*	Xylan in cellulose	Mannan in cellulose	Xylan in cellulose	Mannan not in cellulose
Sitka spruce (<i>Picea sitchensis</i>)	6.02 (0.89)	61.87	8.43 (1.53)	5.05	5.22	3.12	0.8
Canadian spruce (<i>Picea</i> sp.)	5.75 (0.98)	63.72	4.92 (0.86)	9.19	3.13	5.86	2.6
Douglas fir (<i>Pseudotsuga taxifolia</i>)	7.06 (1.17)	57.46	10.14 (1.72)	5.54	6.82	3.18	0.2
Silver fir (<i>Abies</i> sp.)	5.96 (0.63)	52.90	7.01 (0.93)	7.34	3.70	3.88	2.3
Pitch pine (<i>Pinus</i> sp.)	5.07 (0.92)	55.88	7.01 (1.21)	8.30	3.92	4.64	1.2
Louisiana Gulf cypress (<i>Taxodium distichum</i>)	2.97 (0.55)	50.55	3.84 (0.53)	7.99	1.94	4.04	1.0
African pencil cedar (<i>Juniperus procera</i>)	2.40 (0.52)	44.29	4.05 (0.65)	11.03	1.79	4.89	0.6
Redwood (<i>Sequoia</i> sp.)	2.62 (0.72)	49.08	4.77 (1.08)	11.61	2.34	5.70	0.3

* Figures in brackets give the mean concentration of mannose in the final solutions from which the hydrazone was precipitated.

by Norman & Jenkins [1933]. All results are the average of two closely agreeing determinations. In view of the fact that, as shown in Table I, completely quantitative recovery of mannose is not obtained under the conditions of precipitation employed, the figures must err slightly on the low side. The woods analysed may be taken as a representative group of softwoods. In all cases the major part of the mannan was found to be associated with the cellulose. The highest content occurred in Douglas fir, and in this wood, and in Redwood, virtually all the mannan was present as cellulosan. In the final column of Table II is given "mannan not in cellulose", obtained by difference. It is unlikely that this has any existence in fact as a true mannan, but is probably a constituent part of the encrusting hemicelluloses. As expected, no regularity was found in the proportions of mannan to xylan in the cellulose. The variation was very wide, from Douglas fir cellulose on the one hand, which contained nearly twice as much mannose as xylan, to African pencil cedar on the other, with more than twice as much xylan as mannose. The number of samples is insufficient to show whether

this may be a varietal difference, or influenced by age or tissue. Norman [1936, 1] has shown that the amount of xylan associated with the cellulose of rye grass increases with the age of the tissue. If there is any degree of constancy in the mannan/xylan ratio for particular species, this might prove a useful method of identification in certain cases. The cellulosan content of these softwood celluloses, as judged by the mannan and xylan contents combined (12–16%), is notably lower than that of hardwood celluloses in which it is often over 20%. This fact is of course represented in another way in the higher α -cellulose content of the softwood celluloses.

III. *Hydrolysis and extraction of mannan from softwood cellulose.*

The hydrolysis of wood with dilute mineral acids has been extensively studied, but few investigators have examined the behaviour of isolated celluloses. Hawley & Fleck [1927] reported that all the mannan and xylan could be easily removed from spruce cellulose by the action of dilute acid.

The purpose of the experiments described below was to study the relative behaviour of mannan and xylan to hydrolytic and extracting agents, since this has an important bearing on the form of association of the cellulosan with the true cellulose component of the wood cellulose. The wood employed was Silver fir, which seemed particularly suitable for this purpose inasmuch as the mannan and xylan contents of the cellulose are almost identical.

A large preparation of cellulose was made from this wood, and in view of the observation that drying may change the relationship between cellulose and cellulosan [Norman, 1936, 2], all treatments were carried out on the wet cellulose. The losses resulting from the different treatments were determined on small samples of cellulose under the same conditions as used for the larger amounts needed for mannan determination. The residues from these smaller quantitative experiments were later utilized for the determination of xylan by furfuraldehyde yield. In the case of the experiments on acid hydrolysis mannan was determined on the extract or the residue, whichever provided the highest concentration. It proved difficult to select such amounts as would keep the concentration above the limits necessary for maximum recovery. The results of hydrolysis with dilute acid are given in Table III.

Table III. *Hydrolysis of Silver fir cellulose with boiling dilute HCl.*

Mannan in cellulose 7.01%. Xylan in cellulose 7.34%.

Results expressed as % of oven-dry cellulose.

Treatment	Residue	Mannan in residue*	Xylan in residue	Hexosan removed
3% HCl 1 hour	84.91	4.16 (0.74)	4.78	9.68
3% HCl 3 hours	77.45	2.78 (0.44)	2.93	13.91
1% HCl 1 hour	90.09	5.29 (0.75)	5.16	6.01
1% HCl 3 hours	86.40	3.76 (0.42)	4.78	7.79
0.25% HCl 1 hour	94.94	6.62 (0.83)	5.84	3.17
0.25% HCl 3 hours	91.74	5.47 (0.67)	5.04	4.42

* Figures in brackets give the mean concentration of mannose in the final solutions from which the hydrazone was precipitated.

The removal of both mannan and xylan was far from complete even after boiling with 3% acid for 3 hours. The mannan and xylan showed little difference in behaviour excepting perhaps to the lowest concentration, 0.25%, towards which the xylan appeared to be more susceptible than the mannan. Attention is

drawn again to the last column of the table in which the amount of material removed which is not mannan or xylan, but presumably glucosan, is recorded. In every case this is greater than the combined loss of mannan and xylan.

Interesting differences in behaviour of the xylan and mannan were shown in cellulose exposed to cold and hot alkali (Table IV). The mannan appears to be

Table IV. *Treatment of Silver fir cellulose with NaOH both hot and cold.*

Mannan in cellulose, 7.01%. Xylan in cellulose, 7.34%.

Results expressed as % of oven-dry cellulose.

Treatment	Residue	Mannan in residue*	Xylan in residue	Hexosan removed
4% NaOH 2 hours (room temp.)	92.07	5.50 (0.90)	3.30	2.24
1% NaOH 2 hours (room temp.)	98.02	6.71 (0.94)	6.67	1.01
4% NaOH 20 min. (boiling)	85.43	2.30 (0.52)	4.96	7.48
1% NaOH 20 min. (boiling)	89.86	2.82 (0.56)	6.90	5.51

* Figures in brackets give the mean concentration of mannose in the final solutions from which the hydrazone was precipitated.

more resistant to extraction than xylan when cold alkali is employed, but distinctly less resistant to hot alkali. 1% NaOH at the boil for 20 min. removed 60% of the mannose and only 6% of the xylan whereas cold 4% NaOH extracted about 55% of the xylan as against 21% of the mannan. The significance of these differences is not apparent but is worthy of more extended study. In the preparation of mannan from cellulose the use of hot 1% NaOH for a short time would result in a purer extract than would be obtained with a higher concentration. The results are, of course, insufficient for generalization, but suggest that the stabilities of mannan and xylan are of the same order and the forms of association with the cellulose of a similar type.

SUMMARY.

1. The precipitation of mannose as the phenylhydrazone is not quantitative at very low concentrations. Almost complete recovery may be obtained if a certain minimum concentration is exceeded. Salt may aid precipitation if a large excess of phenylhydrazine is present.

2. Conditions suitable for the determination of mannan in woods and wood cellulose are described.

3. The major part of the mannan in softwoods is associated with the cellulose. Considerable variation is found in the proportion of mannan to xylan occurring in the cellulose.

4. Mannan may be removed from cellulose by dilute acid hydrolysis under conditions similar to those in which the xylan is extracted. At the same time there is a considerable loss of hexosan. The mannan and xylan are affected to different extents on treatment of the wood cellulose with alkali.

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