609. *The Chemistry of the Wood Cell Wall. Part III.** *The Isolation of Beech Hemicellulose A.*

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Beech hemicellulose A has been isolated from European beech wood (*Fagus sylvatica*) by a simple method in a yield of 8·2% without first delignifying the wood. The preparation was substantially free from sulphuric acid lignin and acid-soluble lignin, and had a total polysaccharide content of 91·1% (expressed as xylose). Analyses of the original extractive-free wood, the sodium hydroxide extracted wood, and the preparation of beech hemicellulose A are included.

"Hemicellulose" describes that fraction of the extractive-free wood cell wall which is soluble in dilute aqueous sodium hydroxide and is hydrolysed by hot dilute acids to reducing sugars and uronic acids. The hemicellulose complex has been subdivided further into "hemicellulose A and B etc.," and there are different definitions of these fractions. In this paper "hemicellulose A" is that fraction of the hemicellulose complex which is precipitated from the alkaline liquors by the addition of acetic acid (O'Dwyer, *Biochem. J.*, 1926, **20**, 56). For convenience, the components, pentosans, yielding furfuraldehyde have been calculated as xylans.

It has been realised that aqueous alkali dissolves part of the lignin complex as well as "hemicellulose," and the isolation of the latter in high yield from a solution containing dissolved lignin is tedious and difficult. Wise *et al.* (*Paper Trade J.*, 1946, **122**, 35) therefore

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* Part II, preceding paper.

suggested that the so-called " holocellulose " fraction of wood would be a satisfactory source of " hemicellulose " because its acid lignin content was low (about 1·5%). This suggestion has been widely adopted and the usual method of preparing hemicellulose consists in delignifying a wood sample with acidified sodium chlorite and then treating the holocellulose with aqueous alkali. This alkaline extract is treated with Benedict's solution (Chanda, Hirst, and Percival, *J.*, 1951, 1240) or Fehling's solution (Yundt, *Tech. Assoc. Pulp Paper Ind.*, 1951, **34**, 89), and a copper hemicellulose complex precipitated. The complex is decomposed by hydrochloric acid, and any residual copper carefully removed. While some authors have reported that a lignin-free preparation has been obtained by this procedure, Yamamori and Tachi (*Mem. Coll. Agric. Kyoto University*, 1950, **57**, 12) stated that their preparation from elm wood (*Ulmus Davidana* Planch var. *japonica* Nakai) contained 3—3·5% of acid lignin and that repeating this method of purification six times only decreased the acid lignin content to 2·1%.

Timell and Jahn (*Svensk Papperstidning*, 1951, **24**, 831) have proved that during the chlorite delignification of paper birch the degree of polymerisation of the polysaccharide fraction is reduced, presumably by chain-length degradation. Jayme and Hanke (*Cellulosechem.*, 1943, **21**, 127) and Bublitz (*Tech. Assoc. Pulp Paper Ind.*, 1951, **34**, 427) have shown that polysaccharides are present in the chlorite delignification liquors of spruce wood and the latter worker has identified the sugars formed on hydrolysis of these substances; xylose and uronic acids have been found. It would therefore appear inadvisable to prepare hemicelluloses for structural studies from the " holocellulose " fraction and it would be preferable, if possible, to isolate the hemicellulose by direct extraction at low temperature with aqueous sodium hydroxide of minimum strength.

Table 1 lists the percentage loss of wood substance against the time of extraction of beech sawdust, at room temperature, N-, 0·1N-, and 0·01N-aqueous sodium hydroxide being used. For future work an extraction time of 48 hours was adopted.

TABLE 1. *Percentage loss of wood substance on extraction at room temperature.*

Time, hours	2	16	23	40	48	64	88	112
N-NaOH	14·1	18·0	18·7	18·9	18·8	19·0	—	20·0
0·1N-NaOH	—	5·3	—	—	5·6	5·9	6·0	—
0·01N-NaOH	—	1·6	—	—	1·6	1·6	1·6	—

TABLE 2. *Effect of varying strengths of aqueous sodium hydroxide on extracted beech wood. Extraction time, 48 hours at room temperature (results calculated as percentages of original extractive-free wood).*

NaOH strength, N	Ratio NaOH/wood	Residue	Pentosan [a]	Acid lignin	Total loss	Pentosan [a] loss	Acid-lignin loss
—	—	100·0	25·2	24·9	—	—	—
0·01	0·004	98·4	25·2	23·5	1·6	—	1·4
0·1	0·04	94·4	25·0	22·1	5·6	0·2	2·8
0·25	0·1	90·8	23·2	21·4	9·2	2·0	3·5
0·5	0·2	87·0	20·4	21·1	13·0	4·8	3·8
1	0·4	81·2	14·8	21·6	18·8	10·4	3·3
1·5	0·6	82·1	16·3	21·4	17·9	8·9	3·5
2	0·8	84·6	18·8	—	15·4	6·4	—
3	1·2	84·7	18·5	21·9	15·3	6·7	3·0
4	1·6	84·3	—	—	15·7	—	—
5	2·0	85·9	19·2	21·2	14·1	6·0	3·7
6	2·4	85·2	—	—	14·8	—	—
7	2·8	85·4	19·2	21·2	14·6	6·0	3·7

[a] The fraction, pentosan, yielding furfuraldehyde has been calculated as xylan.

Wood samples were separately extracted for 48 hours at room temperature with varying strengths of alkaline solution. The residues were weighed and their pentosan and sulphuric acid lignin contents determined (Table 2). Increase of alkali concentration from 0·01N to N caused an increase in the amount of wood substance dissolved but the maximum was reached at the latter concentration. Further increases in alkali strength to 7N resulted in a decrease in the amount of alkali-soluble material. This variation is caused by the amount of pentosan dissolved, because the loss of sulphuric acid lignin is approximately constant above an alkali strength of 0·1N. At and below this concentration there was no significant loss of pentosan, although up to 5·6% of wood substance had been dissolved. Also, determinations of the total reducing sugar did not show any measurable loss of total polysaccharides. These observations suggested that treating beech wood with 0·1N-sodium hydroxide should remove the major part of the soluble lignin impurities without affecting the main hemicellulose fraction.

Beech wood was therefore extracted first with 0·1N-sodium hydroxide, and the wood residue was filtered off, washed until it was neutral with distilled water, and air dried. This residue was then extracted with N-sodium hydroxide, which dissolved 13·2% of wood substance. The major part of the soluble material (11·7%) was pentosan (9·7%) and uronic anhydride (2·0%).

These analyses of the extractive-free wood and the wood after extraction with sodium hydroxide enable the individual losses sustained by the various fractions to be summed, and this total approximates to the actual measured loss. Table 3 also shows that extraction of wood at room temperature with aqueous sodium hydroxide dissolves only about 38% of the total pentosan fraction. The amount of this fraction (10·4% of the wood) corresponds to the pentosan not in the Cross and Bevan cellulose fraction of the original wood (8·9%), and it is noticeable that in the determination of Cross and Bevan cellulose on the alkali-extracted wood there was a very small loss of pentosan (1·3%). The yield of carbon dioxide (calculated as uronic acid) after correction for the small amount of carbon dioxide evolved from a pure polysaccharide (McCready, Swenson, and Maclay, *Ind. Eng. Chem. Anal.*, 1946, **18**, 290) indicates that approximately one half of the original polyuronides present in the wood are alkali soluble, the remainder being retained in the wood residue.

TABLE 3. *Analyses of extractive-free beech wood and alkali-extracted wood* (*results expressed as percentages on extractive-free wood basis*).

	Extractive-free wood	After treatment with 0·1N-NaOH	After treatment with 0·1N- and then N-NaOH
Yield	—	94·4	81·2
Sulphuric acid lignin	24·9	22·1	21·6
Methoxyl in acid lignin	4·9	—	4·0
,, ,, (own basis)	20·0	—	18·7
Total methoxyl	6·2	—	4·6
Cross and Bevan cellulose	58·1	—	53·7
Pentosan [a] in Cross and Bevan cellulose	16·3	—	13·5
Pentosan [a] not in Cross and Bevan cellulose	8·9	—	1·3
Total pentosan [a]	25·2	25·0	14·8
,, corrected for uronic anhydride	23·8	—	14·1
Uronic anhydride (4 × CO_2 yield, corrected for xylan)	4·2	—	2·2
Total polysaccharide (from reducing sugar estimation)	65·5	65·4	53·1
Acid-soluble lignin (from ultra-violet spectra)	7·5	—	5·3

Sum of individual losses on extraction with 0·1N- *and* N-*sodium hydroxide.*

Sulphuric acid lignin	3·3	Total polysaccharide	12·4
Pentosan as xylan	9·7	Total loss	17·9
Uronic anhydride	2·0	Measured loss	18·8
Hexosan	0·7		
Acid-soluble lignin	2·2		

[a] Pentosan calculated as xylan.

Analysis of the sample of beech hemicellulose A prepared from the N-alkaline solution by precipitation with acetic acid gave the results shown in Table 4. Of the 11·7% of pentosan and uronic acid dissolved from wood which had been extracted with 0·1N-alkali, 7·5% was recovered as hemicellulose A. This yield is higher than that previously reported for beech wood (O'Dwyer, *loc. cit.*), owing perhaps to a better isolation technique. The product contained a greater percentage of pentosan and less acid lignin.

Although the yields of acid lignin and acid-soluble lignin are low the methoxyl content is relatively high, thus confirming the view that some of the methoxyl groups present in beech wood are associated with the polysaccharide fraction, probably the fraction which yields furfuraldehyde.

TABLE 4. *Analysis of beech hemicellulose A (results expressed as percentages on own basis).*

Methoxyl	4·5	Acid soluble lignin [b]	1·0
Pentosan [a] (uncorrected)	84·8	Total polysaccharide	91·1
,, (corrected for uronic anhydride)	81·4	Ash, as Na_2O	1·56
Uronic anhydride (4 × CO_2 yield, corrected for pentosan)	10·4	$[\alpha]_D$ ($l = 2$; $c = 0{\cdot}4715$ in N-NaOH)	−90·6°
Sulphuric acid lignin	0·9	Yield, on wood basis	8·2

[a] Pentosan calculated as xylan.

[b] Value determined by comparison of the specific absorption coefficients at 227·5 mμ and 225 mμ with those for beech acid-soluble lignin; solutions in N-NaOH being examined in a Unicam S.P. 500 spectrophotometer.

Chromatographic analyses of the acid-lignin hydrolysate of beech hemicellulose A showed the presence of xylose and an unidentified uronic acid which was not glucuronic acid. No other pentose or hexose was detected. For this reason the furfuraldehyde-yielding materials have been expressed as pentosan calculated as xylan.

EXPERIMENTAL

A sample from the batch of sawdust (20—60 mesh), prepared at the start of this series of experiments from European beech (*Fagus sylvatica*), was exhaustively extracted with alcohol-benzene (approximately 1 : 2), washed with warm water until free from solvents, and air-dried to a moisture content of about 12%. In the experiments, conducted at room temperature, to determine the optimal reaction time and strength of aqueous alkali, samples (10 g.) of this extracted sawdust were placed in 250-ml. conical flasks, alkali (100 ml.) of appropriate strength was added, and the mixture was stirred slightly to ensure complete wetting of the wood. At predetermined times the samples were filtered off on 17G2 sintered-glass filters, washed well with water until neutral, dried at 105°, and weighed.

Preparation of Bulk Sample of Beech Hemicellulose A.—Air-dried extracted wood (500 g.) and 0·1N-sodium hydroxide (4·4 l.) were set aside for 48 hours, the mixture then filtered, and the wood residue washed with distilled water (at least 20 l., or until neutral) before being air dried. Subsequent extraction with N-sodium hydroxide was only really efficient if at least 5% of wood substance had been removed, as a deep red solution, by the 0·1N-alkali. The air-dried residue was agitated with N-sodium hydroxide (4·2 l.) and the mixture set aside for 48 hours. No special precautions, such as a nitrogen atmosphere, were necessary. The alkaline solution, which was deep yellow to pale brown, was filtered, and the residue washed. The filtrate was just acidified with acetic acid, and the crude hemicellulose precipitate filtered off overnight on a double thickness of Whatman No. 1 filter paper supported by large 60° filter funnels. This method of filtration was superior to use of sintered-glass funnels or to centrifugal methods. The crude precipitate, which should be only slightly brown, was redissolved in sodium hydroxide and precipitated by the addition of an approximately equal volume of ethyl alcohol (95%). Although this procedure converts the uronic acids into sodium salts, which might be undesirable, it renders possible a separation of the hemicellulose A from the acid-soluble lignin, which is very soluble in N-sodium hydroxide and alcohol. Three repetitions of this process gave a white product (8·2%, on the original wood basis) which was analysed by the standard methods.

Examination of Sugars on Hydrolysis.—Paper chromatograms of the sulphuric acid lignin hydrolysate, butanol–ethanol–water (1 : 4 : 5) being used, indicated the presence of xylose and a uronic acid, which was not glucuronic acid.

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