RECENT ADVANCES IN METHODS OF ISOLATING AND PURIFYING HEMICELLULOSES

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It is one of the main objectives of wood chemists to study the chemical structure of the polymers cellulose, hemicellulose and lignin, the main wood constituents. Such studies must begin with the isolation and purification of these components. The isolation of natural products of low molecular weight is usually quite simple, provided there is no shortage of biological material and the percentage of the desired products is not too low. Often these products can be obtained crystalline and analytically pure. In the case of polymers, the situation is quite different. Drastic methods, which may be needed for the extraction of the product from the biological material, can result in degradation and chemical modification of the polymer. The fractionation and purification of extracted polymers is often difficult, and it is only recently that methods of more general application have been developed in this field. The present discussion will be concerned with some recent advances in the isolation and purification of the wood hemicelluloses.

A considerable part of the xylan in hardwoods may be extracted from the milled wood by alkali, while softwoods require a prior delignification before significant amounts of any polysaccharide can be extracted. Larch woods are exceptional among softwoods as the arabinogalactans, often constituting 10 per cent or more of the wood, may be extracted with cold water from the milled wood. The difference between hardwoods and softwoods in this respect is due to the higher lignification of the secondary wall in the softwood fibres, which renders the polysaccharides less accessible to extraction.

The delignification procedures usually employed are the chlorite method, first described by Jayme[1] and modified by Wise and co-workers[2], and the chlorine–ethanolamine method, devised by van Beckum and Ritter[3]. According to the former method, the wood is treated repeatedly with chlorite solutions of pH 4·7 at 60–80°. According to the latter, the wood is delignified by alternate treatments with chlorine in ice-water and ethanolamine in boiling ethanol. Both methods are oxidative, affecting not only the lignin, but also the polysaccharides to some extent. This results in a partial depolymerization (decreased molecular weight) of the cellulose and hemicelluloses, an increased number of carbonyl and carboxyl groups in these polysaccharides, and—when the degradation is severe—the presence of degraded cellulose in the hemicellulose fractions. Timell and Jahn[4] found that the degradation of polysaccharides caused during the chlorite method was more severe than that caused during the chlorine–ethanolamine method, these results being confirmed in a later investigation by Glaudemans

and Timell[5]. Softwoods, however, are more difficult to delignify by the chlorine–ethanolamine method, unless the number of treatments is high; hence softwoods are generally delignified with chlorite. Successful delignifications with chlorine–ethanolamine have, however, been reported. Occasionally it is not realized that a considerable part of the wood polysaccharides may be dissolved during the treatment with chlorite. Thus, on delignification of wood meal from spruce compression wood, more than 10 per cent of the wood polysaccharides were dissolved and could be recovered from the delignification liquors[6]. Most of this material was a

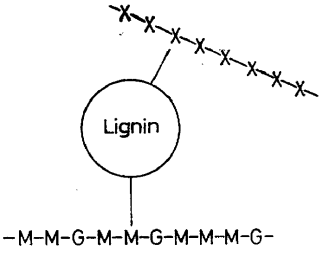


Figure 1

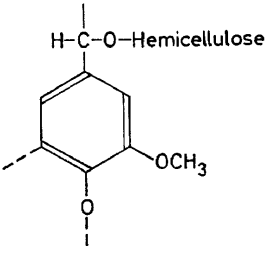


Figure 2

galactan, typical for compression wood. Xylans and glucomannans are also dissolved, however. There is an important difference between wood meal and intact wood fibres, the material being less accessible in the fibres and consequently extracted to a lower extent during the delignification.

The holocelluloses may contain 1–2 per cent lignin, most of which is dissolved, together with the hemicelluloses, during an alkaline extraction. We have observed that a fractionation of the hemicelluloses sometimes is difficult or impossible to achieve unless this lignin is removed by delignification of the hemicellulose in solution[7]. The results may be explained by assuming a lignin hemicellulose linkage, the existence of which is still a very live problem. If, for example, some xylan and glucomannan molecules are linked to the same lignin molecule, as indicated in *Figure 1*, a fractionation into pure components is of course impossible but may readily be achieved after complete delignification. Recent results of Freudenberg make the existence of lignin–hemicellulose linkages of the benzyl ether type probable (*Figure 2*). Methylenequinones are formed as intermediates in the biosynthesis of lignin and these react easily with any alcoholic groups present, including the hydroxyl groups of the hemicelluloses. (For references, see Freudenberg's contribution to this Symposium, p. 9.) Another possibility, that these linkages are glycosidic, appears less probable.

Another delignification method which appears to have some merit was devised by Poljak[8] and involves treatment with peracetic acid in aqueous sodium acetate at 70°. It has recently been investigated by Leopold[9] who found that few carboxyl groups but a great number of carbonyl groups were introduced into the holocellulose during this treatment; accordingly, he modified the method to involve a final treatment with borohydride. A holocellulose from pine wood prepared by this method was compared with a chlorite and a chlorine–ethanolamine holocellulose from the same wood.

The peracetic acid and chlorine–ethanolamine holocelluloses had somewhat similar properties and were considerably less degraded and modified than the chlorite holocellulose. It is, however, most probable that a great portion of the carbonyl groups introduced (0·2 mmole per g) are ketogroups, the reduction of which might give sugar residues which are artefacts and thus atypical for the native polysaccharides (*Figure 3*).

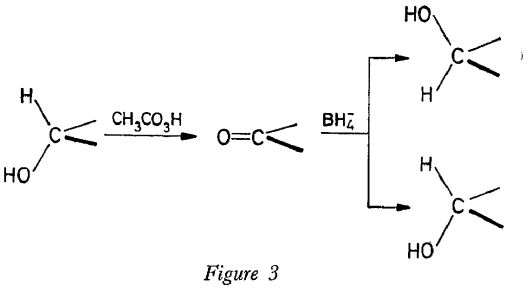


Figure 3

Hemicelluloses can be extracted from holocellulose by alkali but part of them, especially glucomannans, are exceedingly resistant to extraction. It was an important advance, therefore, when Jones and co-workers[10] devised a technique, by which these polysaccharides could be extracted. Borate forms strong complexes with cyclic α-*cis*-glycol groupings, such as are present in the mannose residues in a glucomannan (*Figure 4*), and by addition of borate to the alkali, these polysaccharides could be extracted as borate complexes. As most of the other hemicelluloses can be extracted by alkali alone, fairly pure glucomannans can thus be obtained. This method, of course, has a wide application and may be extended, not only to other biological materials and complexing agents, but, possibly, also to other types of natural polymers.

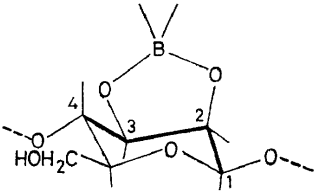


Figure 4

Even after exhaustive extraction of a holocellulose, it usually contains some percentage of hemicellulose components, which are probably inaccessible and therefore not extracted. By dissolving the whole sample, *e.g.* in copper ethylenediamine and regenerating it (thus destroying the biostructure completely), further amounts of hemicelluloses may be extracted by conventional methods.

The result of an extraction depends both on the type of base employed and its concentration; by a suitable extraction scheme, a considerable fractionation of the hemicelluloses can be achieved. These aspects are, however, considered by Kyle Ward in another paper (p. 77).

It has been known for a considerable time that wood contains *O*-acetyl groups. As such groups were also found in the derived holocellulose, it

was believed that they were linked to the hemicelluloses. When these are isolated by alkaline extraction, any ester groups present are simultanously saponified. Some years ago we demonstrated[11] that part of the hemicelluloses were extracted from the holocellulose in their native, acetylated form, by dimethylsulphoxide. From a birch holocellulose prepared by the chlorine–ethanolamine method, an almost pure xylan containing 17 per cent *O*-acetyl groups and representing about 50 per cent of the total xylan, could be isolated[12]. In a similar manner, a fraction of natively acetylated hemicelluloses could be prepared from softwood[11]. This fraction was more complex and its fractionation offered special problems which I shall discuss later. The acetylated hemicellulose components which could be isolated by this procedure, represent only a part of the total hemicelluloses. It may be assumed, however, that they are representative of the total hemicellulose fraction, and the *O*-acetyl content of the wood calculated on the basis of this assumption agrees fairly well with that actually found (*Table 1*).

Table 1. *O*-Acetyl content of some holocelluloses and isolated polysaccharides

Birch wood	Holocellulose	OAc 5·8%
	Total xylan =	35% of holocellulose
	Isolated acetylated xylan	OAc 17%
Calculated OAc content of holocellulose 17 × 0·35 = 6·0%		
Pine wood	Holocellulose	OAc 1·1%
	Total glucomannan =	20% of holocellulose
	Isolated acetylated glucomannan	OAc 6·0%
Calculated OAc content of holocellulose 6 × 0·2 = 1·2%		

The study of these products has not only theoretical interest, but has also added to our knowledge of the chemical reactions which occur during the pulping processes. The partially acetylated hemicelluloses, in analogy to the cellulose derivatives, have higher solubilities and a lower tendency to " crystallize ", than the unsubstituted products, and their physical properties are, therefore, changed to a great extent when these groups are removed, as may occur during pulping reactions. Öhrn and Croon[13] have shown that a considerable part of the *O*-acetyl groups survive a normal, acid sulphite cook. In a two-stage sulphite cook of softwoods, an improved yield of pulp is obtained which is due to reduced dissolution of the glucomannans. This " glucomannan stabilization " has been studied by Annergren *et al.*[14] who have come to the conclusion that it is probably achieved because the *O*-acetyl groups are removed in the first, neutral or alkaline step, rendering the glucomannans less accessible to a hydrolytic attack during the second, acid step.

We have seen that it is sometimes possible to obtain reasonably pure hemicellulose fractions by extraction only. It is, however, more usual that the extracts consist of mixtures of polysaccharides, which must be subjected to fractionation prior to a structural investigation. It is often difficult to decide whether or not a polysaccharide fraction is homogeneous and even the definition of homogeneity offers some difficulty. A molecular weight distribution in a homogeneous fraction must be accepted. If the different

monomer residues, *e.g.* in a glucomannan, are arranged at random and not according to a regular pattern (which is an open question but does not seem improbable), the significance of the term *homogeneous* is further reduced. Polysaccharides of the same general type but with different proportions of monomer residues have been isolated from the same wood sample. One example is the glucomannans, or, more correctly, galactoglucomannans, from softwoods, various fractions of which may differ considerably in their galactose content—from almost nil to about 10 per cent or more. There are also indications that the acidic xylans can be divided into fractions with different percentages of acidic groups, and it may be that all possible structures between some extreme limits are represented.

A fractionation should preferably be followed by as many independent analytical methods as possible. Carbohydrate composition and determination of the optical rotation are generally the first analyses performed. Electrophoresis in borate and other buffer systems, either in cells, on columns or on glass fibre sheets[15] may often reveal inhomogeneity. The last method is especially convenient as the technique is simple and the equipment quite inexpensive. Other methods, such as ultra-centrifugation studies, ultra-filtration, use of molecular sieves, infra-red absorption studies and specific precipitations with antisera, may often give further valuable information.

Precipitating agents of some selectivity are often used to purify polysaccharides. Fehling's solution has been used for the precipitation of hardwood xylans and of mannose containing polysaccharides. The latter and also some galactans are precipitated almost quantitatively by barium hydroxide[16] and several useful separations in the field of wood hemicelluloses have been reported with this agent.

Meier[17] recently made a systematic investigation of the precipitation of some polysaccharides using metallic salts as complexing agents (*Table 2*).

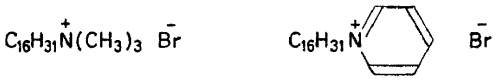
Table 2. Precipitation of some polysaccharides as metal complexes

Polysaccharide \ *Precipitant*	*Fehling's solution*	$Ba(OH)_2$ 0·03 M	$Ba(OH)_2$ 0·15 M	$Pb_2(OAc)_3OH$	$Pb(OAc)_2$
Galactomannan (guaran)	+	+	+	+	+
Glucomannan (softwood)	+	+	+	+	+
Galactan (compression wood)	−	(+)	+	+	+
Arabinoglucuronoxylan (softwood)	−	−	−	+	−
Glucuronoxylan (hardwood)	+	−	+	+	+
Acetylated glucuronoxylan (hardwood)	− → +	−	− → +	+	−
Arabinogalactan (larch)	−	−	−	−	−

He studied Fehling's solution, barium hydroxide at two concentrations, basic lead acetate and lead acetate, the latter being the only neutral precipitating agent. The galacto- and glucomannans were precipitated by all reagents. The galactan from compression wood, with 1,4-β-linked galactose residues and some uronic acid residues, was not precipitated with Fehling's solution (no *cis*-glycol groupings) and only partially with barium

hydroxide of low concentration. There are interesting differences between the xylans, an arabinoglucuronoxylan from softwood, glucuronoxylan from hardwood and the same polysaccharide, isolated under mild conditions when the *O*-acetyl groups are preserved. The deacetylated hardwood xylan is precipitated by all agents except the dilute barium hydroxide. The softwood xylan, in which the xylan chain is more heavily substituted, is precipitated only with basic lead acetate. The partially acetylated xylan is obviously deacetylated in the alkaline solutions because the precipitate is formed slowly and not immediately as with the other polysaccharides. The highly branched arabinogalactans are not precipitated with any of these agents.

One of the most important recent advances in this field is the introduction of quaternary ammonium salts, with a long alkyl chain, as precipitating agents, cetyltrimethylammonium bromide (I) or cetylpyridinium bromide (II) being the most commonly used.



(I) (II)

The method has been studied and applied by Stacey and co-workers[18] and by Scott[19]. The most extensive and systematic studies, chiefly on animal polysaccharides, have been conducted by Scott, who has also summarized the work in this area[20]. Negatively charged polyelectrolytes, *e.g.* acidic polysaccharides, combine with the base to give precipitates. The terminal ends of the long alkyl chains probably form micells (*Figure 5*), and it is significant that the longer the chains, the more efficient is the precipitating agent. It is essential therefore that the alkyl derivatives used as precipitating agents are pure and not mixtures of a homologous series, otherwise a fractionation not of the polymer but of the precipitating agent may result. Commercial products of high purity are now available. The precipitate may be dissolved by increasing the acidity, when the carboxyl ions are transformed into neutral carboxylic groups. Strongly acidic polymers (*e.g.* polysaccharide sulphate esters), those of medium acidity (carboxylic acids) and neutral polymers are easily separated, the former being precipitated also in strongly acid solutions and the latter not at all. The precipitates are dissolved when the ionic strength of the solution is increased, because of the competition between the metal ions and the organic base at the charge sites of the polyelectrolyte. Not only very different types of polysaccharides, but also closely related polysaccharides may be separated, either by gradual precipitation or by extraction of a precipitate with solutions of increasing ionic strength. The latter technique can be performed by column chromatography with gradient elution.

Another technique is to precipitate the polysaccharide mixture completely, dissolve the precipitate in a solution of sufficiently high ionic strength, and effect fractionated precipitation by simply diluting the solution. By using cetyl-pyridinium bromide as precipitating agent, the course of the precipitation can easily be followed by determining the ultraviolet adsorption of the supernatant liquid in a spectrophotometer. Finally,

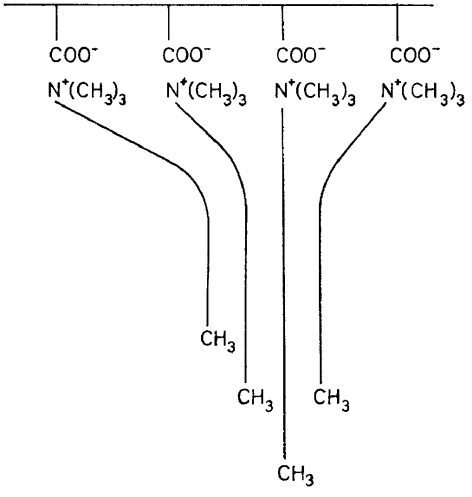


Figure 5

the precipitation of acidic polysaccharides has been developed into a method for the quantitative determination of acidic groups.

Table 3. Fractionation of 40 g (= 250 mmoles of sugar residues) arabinogalactan in aqueous 0·25 M boric acid (1000 ml)

CTA–OH* *added* (mmoles)	NaOH *added* (mmoles)	*Weight of fraction* (g)	*Components*
10	0·25	14·6	A
10	0·50	11·6	A + B
10	0·50	5·9	B
5	1	1·5	B
Residue		2·1	B

	% Arabinose	% Galactose	$[\alpha]_D^{20}$	M_w	M_G value (glass fibre sheet)
A	19	81	7°	100·000	0·76
B	21	79	10°	16·000	0·63

* Cetyltrimethylammonium hydroxide.

The method can be extended to neutral polysaccharides[18b] which give borate complexes. In order to keep the ionic strength as low as possible, it is advantageous to use boric acid and the free, quaternary base in these precipitations. We were able to separate the larch arabinogalactan into two components by this procedure[21] (*Table 3*). These fractions had similar compositions and optical rotations, but different molecular weights, immunochemical reactivities and electrophoretic mobilities in borate buffer. The old problem of the homogeneity of this wood polysaccharide has now been brought to a definite solution.

A further possibility for purifying a polysaccharide in a mixture is by selective enzymatic degradation of the other components. This technique has so far been of little value in wood chemistry because of the lack of highly pure enzymes. One example of this technique is the removal of starch from a mixture of polysaccharides from the inner bark of spruce[22].

Chromotographic fractionation of polysaccharide mixtures on different column materials such as aluminium oxide, carbon, and ion-exchange resins, has been attempted but has not proved very successful. The active surfaces of these highly porous materials are probably accessible to the polymers only to a limited extent (*Figure 6*). Furthermore, a molecular sieve effect is superimposed on the fractionation due to absorption and complicates the results. Better results may possibly be obtained by the use of pure molecular sieves which are now commercially available, such as dextrans, with a low degree of crosslinking, and which form highly swollen gels.

The introduction of a new type of ion-exchange material has changed this situation completely. When cellulose is oxidized with nitrogen dioxide, a material is obtained which is sometimes called celluronic acid or nitrogen dioxide oxocellulose. It contains a high percentage of glucuronic acid residues, and has been used for a considerable time in the pharmaceutical industry for the fractionation of biologically active proteins. Its superiority to other ion-exchange materials probably depends upon the fact that the

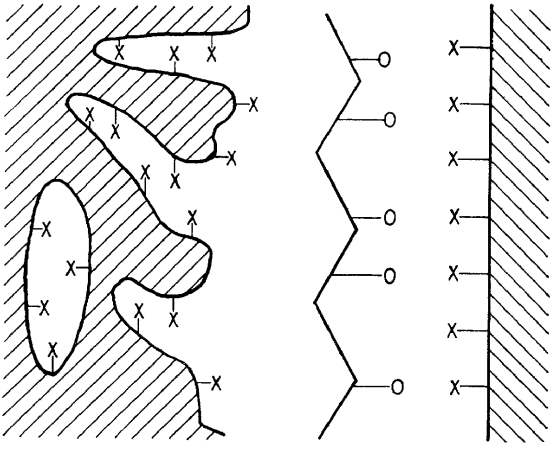


Figure 6

active groups, carboxyl functions, are situated on the surfaces of crystalline regions, and the polymers are more easily adsorbed on these surfaces than on those of the highly porous synthetic ion-exchange resins. Cellulose derivatives with all types of ionic substituents may be prepared, some are commercially available. Several successful fractionations of polysaccharides on these materials have been reported[23]. An example from our laboratory[17] is the polysaccharide mixture, referred to earlier, which was obtained on extraction of a softwood holocellulose with hot water after a dimethyl sulphoxide treatment. The mixture contained, beside the galactoglucomannan, some 15 per cent glucuronoarabinoxylan and had 4·5 per cent *O*-acetyl groups, which should have been removed by precipitation with Fehling's solution or barium hydroxide. The acidic xylan could be

adsorbed on a column of diethylaminoethylcellulose (III) in the phosphate form while the neutral galactoglucomannan was not adsorbed.

$$\text{Cellulose—}OCH_2 \cdot CH_2N(C_2H_5)_2$$

(III)

It was found that this neutral polysaccharide contained all the *O*-acetyl groups. The xylan could be eluted only by alkaline solvents. By precipitation of another portion of the original mixture with cetyltrimethylammonium bromide, a xylan devoid of *O*-acetyl groups was prepared under conditions where such groups should be stable. Fractionation of the sensitive *O*-acetylated polysaccharides was thus enabled by these modern techniques. The result—that in softwoods only the glucomannan and not the xylan is acetylated—is somewhat unexpected as the hardwood xylans contain a high percentage of *O*-acetyl groups.

The only problem discussed in this address which is unique to hemicellulose chemistry is that of delignification. All the other problems are common to polysaccharide chemistry in general and in some respects also to the chemistry of other natural polymers. Consequently the recent advances discussed are products of a joint effort by chemists working in these fields. Hence hemicellulose chemistry does not stand alone, and advances in any area of polysaccharide chemistry should be closely followed by the wood chemist.

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