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The Emission of Corrosive Vapours by Wood

SWEET-CHESTNUT (*CASTANEA SATIVA*) AND WYCH-ELM (*ULMUS GLABRAU*) *O*-ACETYL-4-*O*-METHYLGLUCURONOXYLANS EXTRACTED WITH DIMETHYL SULPHOXIDE

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1. *O*-Acetylated 4-*O*-methylglucuronoxylans were isolated from sweet chestnut and wych elm, either green or incubated at 48° and 100% relative humidity for 36 weeks. 2. The chlorine–ethanolamine method of delignification resulted in a 50% loss of *O*-acetyl groups from green wych elm compared with an 18% loss from green sweet chestnut. 3. The acid–chlorite method gave an acceptable loss of *O*-acetyl groups in three cases, but incubated sweet chestnut showed a 44·6% loss. However, it is believed that this is due to the loss of simple *O*-acetylated xylose sugars resulting from glycosidic hydrolysis, rather than removal of *O*-acetyl groups by direct hydrolysis. Assuming that this occurs in a random manner, it is unlikely to have much structural significance. 4. Dimethyl sulphoxide extraction of chestnut holocellulose and elm holocellulose, green and incubated, yielded *O*-acetyl glucuronoxylans containing 10·2, 3·8, 13·1 and 7·7% *O*-acetyl groups respectively. 5. The location of these *O*-acetyl groups was determined by Bouveng's method in which phenyl isocyanate is used as a blocking group.

Under conditions of high temperature and humidity, wood gives off a corrosive vapour, which is mainly acetic acid (Packman, 1960; Arni, Cochrane & Gray, 1965*a*,*b*). This acetic acid probably arises from the hydrolysis of bound acetyl groups known to be present in wood; there is a good correlation between loss of acetyl groups and formation of free acetic acid under these conditions.

The fact that sweet chestnut (*Castanea sativa*; 3·0% acetyl groups) and wych elm (*Ulmus glabrau*; 4·0% acetyl groups) released 66 and 13% of their bound acetate respectively under equivalent exposure conditions (Arni *et al.* 1965*a*) suggests that, in addition to removal of acetyl groups by hydrolysis, other factors may also play some part in this phenomenon.

It is believed (Hägglund, Lindberg & McPherson, 1956; Bouveng, Garegg & Lindberg, 1960; Timell, 1960; Bouveng, 1961*b*) that the *O*-acetyl groups present in hard woods are attached to the 4-*O*-methylglucuronoxylans and that only the xylose residues are *O*-acetylated. The alkaline methods normally used for extracting this type of hemicellulose from wood holocellulose remove the *O*-acetyl groups by hydrolysis, but Hägglund *et al.* (1956) showed that dimethyl sulphoxide was capable of extracting partially *O*-acetylated xylans from wood holocellulose. Garegg (1962) found that this solvent did not cause deacetylation, but a small amount of migration of *O*-acetyl groups from C-2 was observed.

The location of the position of *O*-acetyl groups in partially acetylated polysaccharides is difficult, owing to the fact that they are readily hydrolysed under alkaline conditions and, to a lesser extent, under acidic conditions. Further, *O*-acetyl groups, like *O*-acyl groups in general, may migrate (Pacsu, 1945; Sugihara, 1953; Bonner, 1959; Garegg, 1965), especially under alkaline conditions. This reaction is relatively common in carbohydrate chemistry. Bouveng (1961*b*) was the first to develop a reliable method for this purpose; it required substitution of the free hydroxyl groups with phenyl isocyanate followed by deacetylation under mild acid conditions. The regenerated hydroxyl groups were then methylated according to the method of Kuhn, Trischmann & Loew (1955) and, after the reductive removal of the phenylcarbamoyl groups, the partially methylated glucoxylan was hydrolysed to the monomeric units, which were then determined by conventional methods. When Bouveng (1961*a*) first applied his technique to several model glucose compounds, he found that there was no migration of *O*-acetyl groups during the reaction with phenyl isocyanate or of phenylcarbamoyl groups under the conditions of methylation, and that the phenylcarbamoyl groups were unaffected by mild acid hydrolysis. Garegg (1962) came to similar conclusions, although he observed a little migration of phenylcarbamoyl groups when benzyl 2-*O*-acetyl-

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and benzyl 3-*O*-acetyl-4-*O*-methyl-β-D-xylopyranoside were subjected to the series of reactions used by Bouveng (1961*b*). Leigh & Krzeminski (1966) reported a 3% loss of phenylcarbamoyl groups during deacetylation with dilute sulphuric acid when they attempted to locate the positions of the *O*-acetyl groups in secondary cellulose acetates by means of the method of Bouveng (1961*b*). The loss was small and may have resulted from C-6. de Belder & Norrman (1968) improved the method of Bouveng (1961*b*) by blocking the free hydroxyl positions with methyl vinyl ether groups.

Sweet-chestnut and wych-elm woods have been chosen for this investigation as being representative of a severely corrosive and a moderately corrosive wood respectively. By locating the positions of *O*-acetyl groups in *O*-acetylated xylans extracted from both wood species, before and after incubation at 48° and 100% relative humidity for 36 weeks, a better understanding of this phenomenon may be possible. The present paper describes such a location of *O*-acetyl groups in sweet-chestnut and wych-elm *O*-acetylated 4-*O*-methylglucuronoxylans by the method of Bouveng (1961*b*).

EXPERIMENTAL

Materials

Green wood. The outer heartwood regions of sweet chestnut and wych elm were comminuted immediately after felling (in December 1962). All samples were stored at −15° until required for use.

Incubated wood. Green sweet chestnut and wych elm were maintained at 48° and 100% relative humidity in sealed glass containers for 36 weeks. The relative humidity was obtained by inserting glass tubes containing water into the comminuted wood.

Chromatographic methods. Paper chromatography was carried out with the following solvents: *A*, ethyl acetate–pyridine–water (8:2:1, by vol.); *B*, butan-1-ol–ethanol–water (40:11:19, by vol.); *C*, ethyl acetate–acetic acid–formic acid–water (18:3:1:4, by vol.). Whatman 3MM chromatographic paper was used for the determination of methylated sugars and also for isolation of sugars, and Whatman no. 1 paper was used for determination of unsubstituted sugars. Aniline phthalate, in water-saturated butan-1-ol, was used as a spray reagent and colours were developed by heating at 105° for approx. 5 min.

Electrophoresis. The high-voltage electrophoresis apparatus was essentially that described by Gross (1959, 1961) in a commercial form (Locarte Co., London E.C.3). The paper was cooled by two stainless-steel plates through which running tap water passed, and was insulated by polyethylene sheets (1 mm. thick). A pneumatic pressure device ensured evenness of pressure and cooling, and a power supply with maximum output of 10 kv at 100 mA was incorporated in the apparatus.

Measurement of acetyl content. The acetyl content was determined by one of two methods, depending on the weight of material available. When 300–500 mg. of sample was available, the modification by Timell (1957) of the method of Whistler & Jeanes (1943) was used. For much smaller amounts of sample (10–30 mg.), the modification by Meier (1961) of the gas–liquid-chromatographic method of Spinkler & Markert (1959) was used. A Shandon Universal Gas Chromatograph fitted with a pre-heater (135°), a flame-ionization detector and a stainless-steel column (120 cm. × 6 mm. external diam.) containing polyethylene glycol 400 on Celite 545 (80–100 mesh) was used. Operating conditions were as follows: temp., 56°; carrier gas flow rate, 35 ml./min. [H_2+N_2 (75:25)]; pressure of gas, 6·5 lb./in.^{2}; air flow rate, 1 l./min.; sensitivity, × 200.

Hydrolysis of polysaccharides. Polysaccharides (approx. 100 mg.) were hydrolysed by the method of Saeman, Moore, Mitchell & Millet (1954). The neutralized hydrolysates were concentrated to approx. 1 ml. and then made up to 5 ml.

Determination of sugars in hydrolysates. Unsubstituted sugars were determined by the phenol–H_2SO_4 method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956) after they had been isolated by paper chromatography (solvent *A*). A Hilger Uvispek H700 spectrophotometer was used to measure the extinction of the solutions. Methylated sugars were separated by paper chromatography in solvent *B* and then determined by oxidation by hypoiodite (Chanda, Hirst, Jones & Percival, 1950).

Determination of uronic acids. The 4-*O*-methylglucuronic acid content of xylans was calculated from methoxyl determinations, and the uronic acid content of other hemicelluloses was determined by titration of their aqueous solutions with CO_2-free 0·01 M-NaOH after the addition of phenolphthalein. Paper-chromatography separation was carried out with solvent *C*.

Demethylation. This was carried out by the method of Bonner, Bourne & MacNally (1960).

Specific rotations. Specific rotations (equilibrium) were measured at 20 ± 1°.

Lignin. This was estimated by the method of Brauns (1952).

Determination of methoxyl groups. Micro methoxyl analysis was carried out by Alfred Bernhardt, Max Plank Institute, Mulheim (Ruhr), Germany.

Infrared spectroscopy. A Perkin–Elmer 137 apparatus was used for this work and samples were incorporated into KBr disks.

Miscellaneous. Concentration of all solutions was carried out under reduced pressure at 25–30° on a rotary film evaporator. All samples were stored at −15° when not in use. Melting points are uncorrected. Dimethyl sulphoxide was supplied by K. W. Chemicals, London W.C.1, and was distilled *in vacuo* before use.

RESULTS

Solvent extraction. Extractives were removed by allowing ethanol–water (1:1, v/v) followed by ethanol–benzene (2:1, v/v) at room temperature to flow slowly down two glass columns (160 cm. × 8 cm.) packed with comminuted wood.

Delignification. White holocellulose was prepared by the chlorine–ethanolamine method (Timell & Jahn, 1951) from green sweet chestnut and wych elm. Only trial delignifications were carried out on the latter and both woods required threefold repetition of the process. Analytical results are given in Tables 1 and 2. Both green and incubated

woods of chestnut and elm were delignified by the chlorite method (Wise, Murphy & D'Addieco, 1946), which required a 4·5hr. and a 4hr. treatment for chestnut and elm respectively. Analytical results for these are given in Tables 3, 4, 5 and 6. Table 7 gives the percentage composition of extractive-free and delignified woods.

Extraction of O-*acetylated hemicellulose.* Dimethyl sulphoxide (1l.) was added/100g. of holocellulose (weight of holocellulose used: chestnut: A, 538g., B, 189g., C, 330g.; elm: A, 508g., B, 183g., where chestnut A and elm A are the green woods delignified by the chlorite method, chestnut B and elm B are the incubated woods delignified by the chlorite method, and chestnut C is the green wood delignified by the chlorine–ethanolamine method) in a desiccator to which a vacuum was applied and released every hour for 24hr. The residue after filtration was given a second treatment with fresh solvent, which was refiltered again, and the combined extracts were treated with acidified ethanol. The resulting crude precipitate was recovered and dried in air (yields: chestnut: A, 14·6g., B, 22·3g., C, 35·1g.; elm: A, 8·1g., B, 5·5g.).

The crude xylan was purified by dissolving it in water and precipitating it with acidified ethanol, followed by recovery and drying. This procedure was repeated five times. Water-insoluble material

Table 1. *Acetyl group content of green sweet-chestnut wood, of holocellulose prepared from it by the chlorine–ethanolamine method and of its extraction products*

	Measured values (%)			Values expressed as percentages of original wood		
	Yield	Lignin	Acetyl groups	Yield	Acetyl groups	Loss of acetyl groups
Green wood			3·01		3·01	
Extractive-free wood	82·1	21·4	3·74	82·1	3·07	
Holocellulose	72·4	0·76	4·14	59·4	2·46	0·55 (18·3%)
Dimethyl sulphoxide-extracted xylan	10·4		10·30	6·3	0·65	
Hot-water-extracted polysaccharides	1·9		7·04	1·0	0·07	
Residue	74·3		2·04	44·2	0·92	

Table 2. *Trial delignification of green wych elm by the chlorine–ethanolamine method*

Lignin values are as analysed; all other values are expressed as percentages of the original wood.

	Values as analysed (%) or expressed as percentage of original wood			
Stage	Yield	Lignin	Acetyl groups	Loss of acetyl groups
Green wood			4·26	
Extractive-free wood	95·6	20·0	4·23	0·03 (0·7%)
Holocellulose no. 1	72·3	0·9	1·96	2·30 (54·0%)
Holocellulose no. 2	71·5	0·8	2·20	2·06 (48·3%)

Table 3. *Acetyl group content of green sweet-chestnut wood, of holocellulose prepared from it by the chlorite method and of its extraction products*

A hot-water extraction was not carried out on the residue remaining after dimethyl sulphoxide extraction; this was carried out on a previous occasion, when the hot-water extract was obtained in 1·2% yield (based on original wood) and contained 5·20% of acetyl groups.

	Values as analysed (%)			Values expressed as percentages of original wood		
	Yield	Lignin	Acetyl groups	Yield	Acetyl groups	Loss of acetyl groups
Green wood			3·01		3·01	
Extractive-free wood	81·8	21·4	3·74	81·8	3·06	0·00 (0·0%)
Holocellulose	82·5	3·4	4·11	67·5	2·77	0·24 (8·0%)
Dimethyl sulphoxide-extracted xylan	1·9		10·23	1·3	0·11	0·29 (9·6%)
Residue	93·9		4·11	63·4	2·61	

Table 4. *Acetyl group content of incubated sweet-chestnut wood, of holocellulose prepared from it by the chlorite method and of its extraction products*

	Values as analysed (%)			Values expressed as percentages of incubated wood*			Values expressed as percentages of original wood		
	Yield	Lignin	Acetyl groups	Yield	Acetyl groups	Loss of acetyl groups	Yield	Acetyl groups	Loss of acetyl groups
Green wood			3·01		3·01			3·01	
Incubated wood	93·2		0·74		0·74	2·27 (75·4%)	93·2	0·69	2·32 (77·1%)
Extractive-free wood	71·0	23·8	1·00	71·0	0·71	0·03 (4·1%)			
Holocellulose	71·0	2·6	0·82	50·4	0·41	0·33 (44·6%)			
Dimethyl sulphoxide-extracted xylan	3·2		3·84	1·6	0·06	0·49 (66·2%)			
'Lignin–xylan' complex	5·3	7·7	1·57	2·7	0·04				
Residue	84·9		0·35	42·8	0·15				

* With the exceptions of values for green wood and for percentage loss of acetyl groups from incubated wood.

Table 5. *Acetyl group content of green wych-elm wood, of holocellulose prepared from it by the chlorite method and of its extraction products*

	Values as analysed (%)			Values expressed as percentages of original wood		
	Yield	Lignin	Acetyl groups	Yield	Acetyl groups	Loss of acetyl groups
Green wood			4·55		4·55	
Extractive-free wood	96·1	20·0	4·62	96·1	4·44	0·11 (2·4%)
Holocellulose	84·3	3·0	4·53	81·0	3·67	0·88 (19·3%)
Dimethyl sulphoxide-extracted xylan	1·0		13·08	0·8	0·11	
Hot-water-extracted polysaccharide	0·7		5·39	0·5	0·03	1·59 (35·0%)
Residue	85·2		4·08	69·0	2·82	

Table 6. *Acetyl group content of incubated wych-elm wood, of holocellulose prepared from it by the chlorite method and of its extraction products*

	Values as analysed (%)			Values expressed as percentages of incubated wood*			Values expressed as percentages of original wood		
	Yield	Lignin	Acetyl groups	Yield	Acetyl groups	Loss of acetyl groups	Yield	Acetyl groups	Loss of acetyl groups
Green wood			4·74		4·74			4·74	
Incubated wood	96·9		2·76		2·76	1·98 (41·8%)	96·9	2·67	2·07 (43·7%)
Extractive-free wood	90·1	19·6	3·09	90·1	2·78	0·00 (0·0%)			
Holocellulose	83·9	3·5	3·12	75·6	2·36	0·40 (14·5%)			
Dimethyl sulphoxide-extracted xylan	2·0		7·66	1·5	0·12	0·16 (5·8%)			
Residue	90·0		3·65	68·0	2·48				

* With the exception of values for green wood and for percentage loss of acetyl from incubated wood.

was noticed in all cases but only that (10·0g.) from incubated chestnut xylan was retained (yields: chestnut: A, 10·0g., B, 6·2g., C, 33·4g.; elm: A, 6·2g.; B, 3·7g.). With one exception, hydrolysis of these pure products gave xylose and uronic acids. The *O*-acetylated xylan extracted from incubated wych elm contained glucose (57·9%), and it was decided not to purify the original xylan any further, to conserve the small amount of material obtained. Assuming that the glucose was not *O*-acetylated, its presence would not interfere with this investigation. Allowance is made in all the analytical results for the presence of glucose, i.e. the values are based on a pure *O*-acetylated xylan.

Table 7. *Analysis of extractive-free and holocellulose samples prepared by the chlorite method from green and incubated sweet-chestnut and wych-elm woods*

Uronic acid was not determined. It is probable that determinations of uronic acid in holocellulose give erroneously high values owing to the occurrence of oxidation reactions during delignification.

Sample		Glucose (%)	Xylose (%)	Mannose (%)	Galactose (%)	Arabinose (%)	Lignin (%)	Acetyl groups (%)
Sweet chestnut								
Green	Extractive-free	48·6	15·9	2·2	1·0	0·5	21·4	3·7
	Holocellulose	58·1	21·0	2·9	1·5	0·6	3·4	4·1
	Holocellulose*	47·9	17·3	2·4	1·2	0·5		3·4
Incubated	Extractive-free	54·2	17·2	3·4	1·1	0·1	23·8	1·0
	Holocellulose	73·3	17·5	4·8	1·0	0·1	2·6	0·8
	Holocellulose*	52·0	12·5	3·4	0·7	0·1		0·6
Wych elm								
Green	Extractive-free	50·8	15·5	1·2	1·1	0·4	20·0	4·6
	Holocellulose	60·6	19·4	2·3	1·9	0·7	3·0	4·5
	Holocellulose*	51·1	16·3	2·0	1·6	0·6		3·8
Incubated	Extractive-free	57·9	15·5	1·0	1·0	0·0	19·6	3·1
	Holocellulose	72·2	17·3	3·0	1·2	0·0	3·5	3·1
	Holocellulose*	60·6	14·5	2·5	1·0	0·0		2·6

* Values expressed as percentages of extractive-free wood.

Table 8. *Analytical results for* O-*acetyl*-4-O-*methylglucuronoxylans extracted with dimethyl sulphoxide*

Yield is expressed as percentage of original wood; the other values are as analysed.

Source of *O*-acetyl-4-*O*-methylglucuronoxylan	Values as analysed (%) or expressed as percentage of original wood		
	Yield	Acetyl groups	4-*O*-Methylglucuronic acid
Sweet chestnut			
Green	1·3	10·23	10·1
Incubated	1·6	3·84	10·7
Wych elm			
Green	0·8	13·08	14·5
Incubated	1·5	7·66	15·1

Analytical results are given in Table 8 and specific optical rotations are given in Table 9.

The water-insoluble material retained from the purification of incubated sweet-chestnut *O*-acetylated xylan contained lignin (7·7%), *O*-acetyl groups (1·57%), xylose and 4-*O*-methylglucuronic acid. For convenience this is termed a 'lignin–xylan complex', and it had $[\alpha]_D^{20}$ −53·0° (*c* 1·01% in dimethyl sulphoxide).

The residues remaining after dimethyl sulphoxide extraction of both green woods delignified by the chlorite method were further extracted twice with hot water at 98–100° for 2hr. with constant stirring. Precipitated material was obtained by pouring the filtered extracts into acidified ethanol. Purification from water gave a light-brown powder (yields: chestnut, 6·3g.; elm, 3·1g.). They were both found to be *O*-acetylated and acid hydrolysis showed that they were composed mainly of uronic acids, xylose and galactose, with smaller amounts of glucose, arabinose and an unidentified component.

Chestnut delignified by the chlorine–ethanolamine method (330·3g.) was similarly treated with dimethyl sulphoxide to give a crude *O*-acetylated xylan (43·6g.), and the pure material (34·4g.) was obtained as a white powder. It had $[\alpha]_D^{20}$ −55·8° (*c* 0·97 in water), 10·30% of acetyl groups and 10·0% of 4-*O*-methylglucuronic acid. Hot-water (98°) extraction of the resulting residue gave a product, the qualitative composition of which was found to be very similar to that of products from chlorite-delignified woods. It has 7·01% of acetyl groups and was not further used.

Reactions of O-*acetylated xylans with phenyl isocyanate.* Dry polysaccharide (weight used: chestnut: A, 5·2g., B, 5·1g.; elm: A, 4·2g., B, 2·8g.) dissolved in anhydrous *NN*-dimethylformamide (90–130ml.), was treated with phenyl isocyanate (10–12ml.) for 3hr. at 100°. The cooled solution was treated with acidified ethanol and the resulting precipitate was filtered through sintered glass, washed with ethanol and ether and then dried.

Table 9. *Specific optical rotations of* O-*acetyl*-4-O-*methylglucuronoxylans and their derivatives obtained during location of the* O-*acetyl groups by the method of Bouveng* (1961*b*)

Water was used as solvent for *O*-acetylated xylans and partially methylated glucoxylans, and tetrahydrofuran as solvent for the phenylcarbamate derivatives and methylated phenylcarbamate derivatives.

	Sweet chestnut				Wych elm			
	Green		Incubated		Green		Incubated	
Compound	$[\alpha]_D^{20}$	*c*	$[\alpha]_D^{20}$	*c*	$[\alpha]_D^{20}$	*c*	$[\alpha]_D^{20}$	*c*
O-Acetylated xylan	−53·4°	0·86	−48·9°	0·67	−43·1°	0·71	−41·3°	0·67
Phenylcarbamate derivative	−146·4	0·72	−147·4	0·74	−128·6	0·70	−118·8	0·65
Methylated phenylcarbamate	+1·3	1·12	+1·7	1·06	−1·3	1·13	−1·5	1·06
Partially methylated glucoxylan	−78·5	1·40	−65·0	0·92	−64·2	0·88	−59·6	1·52

After this reaction had been repeated, the precipitate was purified from dimethylformamide by precipitation before it was dried (yields; chestnut: A, 9·3g., B, 10·1g.; elm: A, 6·9g., B, 5·6g.). The infrared spectrum of the product showed absorption bands corresponding to the joint ester amide carbonyl at 1730cm.$^{-1}$, the amide II grouping at 1530cm.$^{-1}$ and the N–H stretch at 3300cm.$^{-1}$.

Deacetylation and methylation of xylan phenylcarbamate. Sulphuric acid (9M) was added to xylan phenylcarbamate (weight used: chestnut: A, 8·5g., B, 8·5g.; elm: A, 6·4g., B, 4·9g.), dissolved in tetrahydrofuran (150–300ml.), to give a 3% solution of sulphuric acid, to which water was added until the polysaccharide just failed to precipitate. This solution was maintained at 32° until deacetylation was complete. This was followed by removal of samples of solution, isolation of the dry polysaccharide and determination of the acetyl content by gas–liquid chromatography. When deacetylation was achieved, the material was precipitated with acidified ethanol, recovered and dried (yields: chestnut: A, 6·7g., B, 6·9g.; elm: A, 4·7g., B, 4·2g.). It was not analysed. The deacetylated product (weight used: chestnut: A, 6·6g., B, 6·8g.; elm: A, 4·5g., B, 4·1g.), dissolved in dry *NN*-dimethylformamide (90–150ml.), was methylated by the method of Kuhn *et al.* (1955) with freshly distilled methyl iodide (6–11ml.) and silver oxide (5–9g.). Methylation was taken to be complete when a treatment made no addition to the methoxyl content of the compound, which was dried after recovery (yields: chestnut: A, 3·3g., B, 3·3g.; elm: A, 2·2g., B, 2·2g.). Four to six such treatments were necessary.

Removal of N-*methyl*-N-*phenylcarbamoyl groups.* The above product (weight used: chestnut: A, 3·2g., B, 3·2g.; elm: A, 2·1g., B, 2·1g.) was dissolved in anhydrous tetrahydrofuran (250–350ml.) and lithium aluminium hydride (1·0–1·5g.) was added with constant stirring and the exclusion of moisture. It was found to be essential that the initial additions of reducing agent be in very small quantities (approx. 30mg.) to prevent gel formation; when this occurred, satisfactory reduction was never achieved. After four to six such additions had been made at 0·5hr. intervals, larger amounts could then be added. After all the reducing agent had been added, the mixture was gently stirred overnight, followed by refluxing for 1·5hr. Excess of reagent was decomposed by the careful addition of water; the mixture was neutralized with 0·33M-phosphoric acid and filtered, and the solids were washed with cold water. The combined filtrates were concentrated to a small volume (30–50ml.) before being added to acidified alcohol. The precipitated polysaccharide was purified by dissolving it in cold water, centrifuging, washing with water and recovering the methylated polysaccharide from the combined aqueous washings in the usual way. This was repeated and, as a final purification, an aqueous solution of the compound was dialysed against running tap water for 5 days. It was finally recovered as a white powder (yields: chestnut: A, 998·5mg., 6·5% ash; B, 890·7mg., 5·5% ash; elm: A, 692·3mg., 1·5% ash; B, 508·8mg., 0·7% ash). Tables 9 and 10 give specific optical rotations and analytical data for the original *O*-acetylated xylans and for the above derivatives respectively. Their infrared spectra showed the absence of aromatic absorption bands.

When dilute hydrochloric acid was added to the inorganic phosphate residue obtained after addition of phosphoric acid to reduced green wych-elm xylan phenylcarbamate it was observed that some of it was insoluble. It was also insoluble in tetrahydrofuran and cold water, but gave a hazy solution in hot water that produced an intense purple colour with Molisch reagent. Addition of acidified ethanol gave a voluminous precipitate, which was purified from hot water five times, in a manner similar to that described above. An infrared examination of this material produced rather weak absorption bands, which were, however, identical with those given by the pure, cold-water-extracted, partially methylated xylan. Analysis for methoxyl

Table 10. *Analytical results for O-acetylated glucuronoxylans and their derivatives obtained during location of the O-acetyl groups by the method of Bouveng* (1961*b*)

Since not all the phenyl isocyanate groups attached to the carboxylic acid groups are hydrolysed during deacetylation, it is not possible to obtain accurate nitrogen and methoxyl values after deacetylation (Bouveng, 1961*b*). Methoxyl values were calculated (*a*) from molar composition of *O*-methylated glucoxylan and (*b*) on the basis of the methylated glucuronoxylan phenylcarbamate.

		Acetyl (%)		Nitrogen (%)		Methoxyl (%)		
	Compound	Found	Calc.	Found	Calc.	Found	(*a*)	(*b*)
Sweet chestnut								
Green	*O*-Acetylated xylan	10·2						
	O-Acetylated xylan phenylcarbamate	4·2	4·5	6·7	7·0			
	Methylated xylan phenylcarbamate					3·7	3·8–3·9	
	Methylated glucoxylan					8·9	9·2	8·9
Incubated	*O*-Acetylated xylan	3·8						
	O-Acetylated xylan phenylcarbamate	1·4	1·5	7·0	7·2			
	Methylated xylan phenylcarbamate					1·8	1·6–1·7	
	Methylated glucoxylan					3·7	4·5	3·7
Wych elm								
Green	*O*-Acetylated xylan	13·1						
	O-Acetylated xylan phenylcarbamate	5·8	6·1	6·6	6·3			
	Methylated xylan phenylcarbamate					5·4	5·3–5·5	
	Methylated glucoxylan					10·6	12·1	10·3
Incubated	*O*-Acetylated xylan	7·7						
	O-Acetylated xylan phenylcarbamate	3·3	3·3	6·9	6·9			
	Methylated xylan phenylcarbamate					3·0	3·2–3·3	
	Methylated glucoxylan					6·6	8·1	6·6

groups gave a content of 9·3%. The material contained a considerable amount of inorganic matter, but this was inadvertently not determined.

Hydrolysis of methylated xylan and analysis of components. The reduced partially methylated polysaccharide (weight used: chestnut: A, 118·1mg., B, 96·4mg.; elm: A, 144·4mg., B, 125·7mg.) was hydrolysed with 0·25M-sulphuric acid (20ml.) on a boiling-water bath for 13hr. After cooling, the solution was neutralized with barium carbonate, filtered and washed before concentration to a small volume (approx. 50ml.). When the solution had been deionized with Amberlite IR-120 resin (H^+ form), it was filtered and the resin thoroughly washed. The aqueous filtrate was concentrated to approx. 1ml. and then diluted to 5ml. Paper-chromatographic separation in solvent *B* showed xylose, mono-*O*-methylxylose, 2,3-di-*O*-methylxylose, 4-*O*-methylglucose and in one case (chestnut A) 2,3,4-tri-*O*-methylxylose to be present. The mono-*O*-methylxylose fraction was isolated and shown by high-voltage electrophoresis in borate buffer to consist of 2-*O*- and 3-*O*-methylxylose. All the components were identified by the methods described in the next paragraph. They were quantitatively analysed (Table 11) by conventional methods, care being taken to prevent the filter paper used for electrophoretic separation of the mono-*O*-methylxylose fraction from drying, and also to avoid concentration of the eluate from this paper to dryness. 6-Deoxy-4-*O*-methyl-6-*N*-phenylamino-D-glucose was not found in the hydrolysates.

Identification of hydrolytic products. A further quantity of partially methylated glucoxylan from green wych elm was hydrolysed, and the hydrolysate, together with the remaining previous hydrolysate, was separated into its components on large sheets of chromatography paper. Four fractions were obtained.

Fraction 1. This was chromatographically identical with xylose and had $[\alpha]_D^{20}$ +19° (*c* 0·98 in water). The di-*O*-benzylidene-D-xylose derivative had m.p. and mixed m.p. 209–210°.

Fraction 2. This behaved chromatographically as 4-*O*-methyl-D-glucose. Demethylation gave glucose. The derived 4-*O*-methyl-D-glucosazone had m.p. 157° (literature m.p. 158–160°).

Fraction 3. This behaved chromatographically as 2-*O*- or 3-*O*-methylxylose, or both, and electrophoretic separation produced two components that moved the same distance as did authentic samples of these two sugars. Demethylation of both components gave xylose. Analysis of this fraction gave OMe, 18·6% (Calc. for $C_6H_{12}O_5$: OMe, 18·9%).

Fraction 4. This was chromatographically the same as 2,3-di-*O*-methylxylose. Demethylation gave xylose. The derived 2,3-di-*O*-methyl-*N*-phenyl-D-xylosylamine had m.p. and mixed m.p. 120–122°.

Table 11. *Molar composition of partially methylated glucoxylans*

Methylated xylan	Fraction	Composition (mg.)	Composition (moles/100 moles)
Sweet chestnut			
Green	Xylose	67·9	64·6
	2-Methyl-D-xylose	18·4	16·0
	3-Methyl-D-xylose	16·4	14·3
	2,3-Dimethyl-D-xylose	3·3	2·7
	2,3,4-Trimethyl-D-xylose	1·1	0·8
	4-Methyl-D-glucose	2·1	1·6
Incubated	Xylose	75·0	85·3
	2-Methyl-D-xylose	3·9	4·1
	3-Methyl-D-xylose	5·7	5·9
	2,3-Dimethyl-D-xylose	1·6	1·5
	4-Methyl-D-glucose	3·6	3·1
Wych elm			
Green	Xylose	80·3	60·5
	2-Methyl-D-xylose	17·4	12·0
	3-Methyl-D-xylose	20·3	13·9
	2,3-Dimethyl-D-xylose	12·0	7·7
	4-Methyl-D-glucose	10·1	5·9
Incubated	Xylose	34·0	71·1
	2-Methyl-D-xylose	3·3	6·3
	3-Methyl-D-xylose	4·2	9·3
	2,3-Dimethyl-D-xylose	0·7	1·3
	4-Methyl-D-glucose	7·3	11·9

DISCUSSION

Delignification studies. It had been thought that the chlorine–ethanolamine method of delignification would be preferable for this investigation, as it is less degradative than the acid–chlorite method and causes little loss of *O*-acetyl groups, as shown by Timell & Jahn (1951), Timell & Glaudemans (1957) and Timell (1960). When this method was applied to sweet chestnut, there was a loss of *O*-acetyl groups of about 18%. However, application to wych elm resulted in a loss of *O*-acetyl groups of about 50% (Table 2). Shortly after this finding had been made, Garegg (1962) reported that model *O*-acetylated xyloses suffered severe migration of *O*-acetyl groups and some deacetylation when they were heated with ethanolic 3% monoethanolamine under the conditions used during delignification. It was therefore necessary to use the acid–chlorite method of delignification.

These results show that the *O*-acetyl groups in wych elm are more labile under slightly alkaline conditions than those in sweet chestnut, which is the reverse situation to that found under acid conditions.

Table 4 shows that incubated sweet chestnut lost 44·6% of its *O*-acetyl groups during delignification by chlorite, compared with a loss of 8·0% for green sweet chestnut (Table 3). It is believed that the large loss of *O*-acetyl groups during this process is due to the loss of either monomeric or oligomeric *O*-acetylated xylose units by partial glycosidic hydrolysis of *O*-acetylated xylans owing to the acid conditions of delignification or incubation or both. Direct evidence for this is found in a comparison of the analysis of extractive-free wood and holocellulose given in Table 7, from which it was calculated that 4·6% of *O*-acetylxylan had been lost during delignification. However, it is not possible to relate this loss to *O*-acetyl-4-*O*-methylglucuronoxylan, since uronic acid determinations were not carried out. This loss amounts to 28·6% of the total *O*-acetylxylan present and, since glycosidic hydrolysis should occur in a random manner, the loss should not be very significant structurally.

A feature shown in Tables 1 and 3 arising from the dimethyl sulphoxide extraction of chestnut holocellulose prepared by the chlorite and chlorine–ethanolamine methods of delignification (lignin content 3·4 and 0·8% respectively) is that the yield of *O*-acetyl-4-*O*-methylglucuronoxylan is considerably greater in the latter case. It is difficult to see how only 3·4% of lignin could act as a physical barrier to dimethyl sulphoxide penetration, and these results may be added to the impressive weight of evidence in support of the theory of chemical combination between lignin and hemicellulose components within the cell wall (Bolker, 1963).

Table 12. *Distribution of* O-*acetyl groups in sweet-chestnut and wych-elm (green and incubated) glucuronoxylans*

Wood		Composition (moles/100 moles) Green wood	Incubated wood
Sweet chestnut	Xylose unacetylated	65·7	88·1
	Xylose *O*-acetylated at C-2	16·3	4·4
	Xylose *O*-acetylated at C-3	14·5	6·1
	Xylose *O*-acetylated at C-2 and C-3	2·7	1·6
	Xylose *O*-acetylated at C-2, C-3 and C-4	0·8	0·0
Wych elm	Xylose unacetylated	64·3	80·7
	Xylose *O*-acetylated at C-2	12·7	7·2
	Xylose *O*-acetylated at C-3	14·8	10·5
	Xylose *O*-acetylated at C-2 and C-3	8·1	1·4

Table 13. *Molar ratios of* 4-O-*methyl*-D-*glucuronic acid and* 4-O-*methyl*-D-*glucose units to xylose units found in* O-*acetylated glucuronoxylans and glucoxylans*

	Molar ratio: 4-*O*-Methyl-D-glucuronic acid units:xylose units	4-*O*-Methyl-D-glucose units: xylose units
Green sweet-chestnut xylan	1:11·6	1:62·5
Incubated sweet-chestnut xylan	1:11·8	1:30·8
Green wych-elm xylan	1:7·4	1:16·0
Incubated wych-elm xylan	1:7·6	1:7·4

'*Lignin–xylan complex*'. The insoluble material obtained from aqueous purification of incubated chestnut *O*-acetyl-4-*O*-methylglucuronoxylan, which contained 7·7% of lignin and 1·6% of *O*-acetyl groups in addition to xylose (the main component), was most unusual.

Location of O-*acetyl groups*. The distribution of *O*-acetyl groups within each of the four *O*-acetyl-4-*O*-methylglucuronoxylans, obtained by using a technique slightly modified from that developed by Bouveng (1961*a*,*b*), is shown in Table 12, which shows that most of the acetylated xylose residues are mono-*O*-acetylated, and there is no overwhelming preference for acetylation at either C-2 or C-3. These results contrast with those of Bouveng (1961*b*), who found that birch *O*-acetylglucuronoxylan was acetylated at C-2 and C-3 in a ratio of about 1:2.

Only the terminal non-reducing xylose residues occurring in green sweet-chestnut *O*-acetylxylan were slightly acetylated in all three possible positions. A similar result was reported by Bouveng *et al.* (1960), who found that most of the terminal non-reducing xylose residues were *O*-acetylated at C-2, C-3 and C-4, when a birch *O*-acetylxylan was methylated by the method of Kuhn *et al.* (1955) and then reduced with lithium aluminium hydride. However, when Bouveng (1961*b*) applied his phenyl isocyanate reaction method for locating *O*-acetyl groups to an almost identical *O*-acetylated xylan, no evidence was found to support this finding.

Bouveng (1961*b*) also found that the methoxyl content (10·2%) of his partly methylated glucoxylan was lower than that calculated (11·7–12·3%) on the basis of the methylated glucuronoxylan phenylcarbamate, the reason for which was said to be fractionation according to methoxyl contents during the cold-water extraction. Similar results (Table 10) were obtained in the present investigation, probably for the same reason.

Except in incubated wych elm, the amount of 4-*O*-methylglucose present in the glucoxylans is much less than is required by the molar ratio of xylose and 4-*O*-methylglucuronic acid residues in the original xylans, as shown in Table 13. This is probably due to the reaction between phenyl isocyanate and the carboxylic groups of the uronic acid units, to give *N*-phenylamide groups as suggested by Bouveng (1961*b*), with the result that the main part of the amide bond is apparently not hydrolysed and the corresponding residues are converted into 6-deoxy-4-*O*-methyl-6-*N*-phenylamino-D-glucose residues. The 4-*O*-methyl-D-glucose found should represent those uronic acid *N*-phenylamide bonds that were hydrolysed during deacetylation.

Hot-water-extracted O-*acetylated hemicelluloses*. These are the subject of the next paper (Cochrane, Gray & Arni, 1969).

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