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The Emission of Corrosive Vapours by Wood

HOT-WATER-EXTRACTED *O*-ACETYLATED HEMICELLULOSES FROM SWEET CHESTNUT (*CASTANEA SATIVA*) AND WYCH ELM (*ULMUS GLABRAU*) AND A DISCUSSION OF *O*-ACETYL-GROUP CHANGES OCCURRING IN THESE WOODS DURING INCUBATION AT 48° AND 100% RELATIVE HUMIDITY

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1. *O*-Acetylated polysaccharides were obtained from green wood of both sweet chestnut and wych elm by treatment of the residue remaining after dimethyl sulphoxide extraction with water at 98°. This gives a mixture of polysaccharides containing xylose, galactose, glucose and uronic acids. Analysis of these and their fractionated products suggest that only xylans in green sweet chestnut and green wych elm are *O*-acetylated. 2. The isolated *O*-acetylated xylans are not representative of the total *O*-acetylated xylans occurring in sweet chestnut and wych elm. 3. Application of the method developed by Bouveng for the location of *O*-acetyl groups to all four *O*-acetylated xylans obtained in this series of investigations by dimethyl sulphoxide extraction showed that those from sweet chestnut and wych elm, under the same conditions of incubation, lost: 74·2 and 43·4% of acetyl groups respectively, at C-2; 58·0 and 28·5% of acetyl groups respectively at C-3; 41·8 and 82·2% of acetyl groups respectively at C-2 and C-3. 4. A consideration of electronic and steric factors indicates that there does not appear to be a purely chemical reason for the difference in loss of *O*-acetyl groups between sweet chestnut and wych elm. It is suggested that the location of *O*-acetylated xylans in the wood cell walls and the presence of extractives may play some part in this difference.

Although *O*-acetyl-4-*O*-methylglucuronoxylans are the only *O*-acetylated hemicelluloses that have been isolated from hardwoods (Hägglund, Lindberg & McPherson, 1956; Timell, 1960; Bouveng, Garegg & Lindberg, 1960; Bouveng, 1961; Cochrane, Gray & Arni, 1969), it is still possible that there are others. In the preceding paper (Cochrane *et al.* 1969), the location of *O*-acetyl groups attached to *O*-acetyl-4-*O*-methylglucuronoxylans extracted from sweet chestnut (*Castanea sativa*) and wych elm (*Ulmus glabrau*) before and after they had been incubated at 48° and 100% relative humidity for 9 months was determined. A hot-water extraction of holocellulose, pre-extracted with dimethyl sulphoxide, was also described.

The present paper describes the fractionation of the *O*-acetylated mixture of hemicelluloses isolated from green sweet-chestnut and wych-elm holocellulose, pre-extracted with dimethyl sulphoxide. A possible explanation of the fact that chestnut loses more *O*-acetyl groups than elm does under equivalent incubation conditions is also given.

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EXPERIMENTAL

Material used. The *O*-acetylated mixture of hemicelluloses resulting from hot-water (98–100°) extraction of holocellulose prepared by the chlorite method from both green chestnut and elm (Wise, Murphy & D'Addieco, 1946), which had previously been extracted with dimethyl sulphoxide (Cochrane *et al.* 1969), was used as the starting material for subsequent fractionation as described below.

Analytical methods. These were described by Cochrane *et al.* (1969). Paper chromatography was carried out with the following solvents: *A*, ethyl acetate–pyridine–water (8:2:1, by vol.); *B*, ethyl acetate–acetic acid–formic acid–water (18:3:1:4, by vol.); *C*, butan-1-ol–benzene–pyridine–water (5:1:3:3, by vol.); *D*, butan-1-ol–acetone–water (4:5:1, by vol.); *E*, butan-1-ol–ethanol–water (5:1:4, by vol.). R_G and M_{Glc} or R_{Glc} represent the ratio of the distance travelled by the sugar to the distance travelled by 2,3,4,6-tetramethylglucose (G) or glucose (Glc).

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

RESULTS

Fractionation of polysaccharides extracted with hot water. The polysaccharides obtained by hot-water extraction (weight used: chestnut, 1·6g.;

Table 1. *Composition of polysaccharides extracted with hot water and their fractionated products*

All values are as analysed. Uronic acid is expressed as glucuronic acid.

	Content (%)						
	Acetyl groups	Uronic acid	Glucose	Galactose	Xylose	Arabinose	Unidentified component
Sweet chestnut							
Original polysaccharide	5·2	34·1	0·7	13·1	31·6	3·2	Present
Linear polysaccharide	9·9	37·2	0·0	12·1	41·7	0·0	Present
Branched polysaccharide	Trace	35·3	5·3	37·1	1·0	6·4	5·5
Wych elm							
Original polysaccharide	5·3	22·8	10·1	13·4	32·4	2·1	Present
Linear polysaccharide	9·4	23·8	7·2	10·9	41·3	0·0	Present
Branched polysaccharide	Trace	32·3	11·2	28·1	2·8	4·1	10·6

elm, 1·3g.) were dissolved in calcium chloride solution (sp.gr. 1·3; 150–300ml.) by the method described by Gaillard (1961). Iodine solution (3g. of iodine and 4g. of potassium iodide in 100ml. of water; 26–30ml.) was added and the solution centrifuged. The precipitate was washed with the calcium chloride solution containing 15% of the iodine–potassium iodide solution, after which it was decomposed with warm water, and excess of iodine was destroyed by the addition of sodium thiosulphate solution. The solution was concentrated to a small volume and then dialysed for 2 weeks against running tap water. The small amount of insoluble material produced at this stage was removed by filtration and the solution concentrated to a small volume before addition of acidified ethanol to precipitate the so-called linear polysaccharides (yields: chestnut, 753mg.; elm, 940mg.). Excess of iodine in the supernatant was destroyed by adding sodium thiosulphate solution and the so-called branched polysaccharides were obtained in the same way as described above (yields: chestnut, 327mg.; elm, 223mg.). Analytical results for the original polysaccharides and the fractionation products are given in Table 1.

An unidentified component was detected in the hydrolysate of all these polysaccharides on chromatography with solvents *A* and *B* and, by visual comparison of the brown-spot intensities, both branched polysaccharides were judged to have it in greatest amount.

Unidentified component. This component was examined by paper chromatography on Whatman no. 1 paper and high-voltage electrophoresis, with the following results. In solvent *C*, the component travelled just behind rhamnose and had R_{Glc} 1·63. In solvent *D*, the component travelled between rhamnose and fucose and had R_G 0·21. In solvent *E*, the component travelled just in front of 2-*O*-methylglucose and apiose. On electrophoresis in borate buffer (pH 9·4), the component travelled just behind rhamnose and had M_{Glc} 0·41 (authentic *O*-acetyl sugars produced continuous streaking). Spraying the component with 2,3,5-triphenyltetrazolium chloride solution failed to produce any colour, suggesting substitution at C-2.

Demethylation of the component produced galactose.

The above evidence suggests that the component may be 2-*O*-methylgalactose, and the quantities occurring in wych-elm and sweet-chestnut branched polysaccharides were determined by hypoiodite oxidation, giving the values shown in Table 1.

DISCUSSION

O-Acetyl polysaccharides extracted with hot water. There were two main reasons for this extraction and subsequent fractionation, namely (i) to try to find *O*-acetylated polysaccharides other than xylans in sweet chestnut and wych elm, and (ii) to determine whether *O*-acetylated xylans with a different *O*-acetyl content from those extracted with dimethyl sulphoxide could be isolated.

Table 1 shows that the aqueous extract consists mainly of xylans that are generally linear, and galactans that are normally highly branched. Although pure polysaccharides were not obtained by the fractionation technique of Gaillard (1961), considerable enrichment was achieved, with the result that the so-called branched polysaccharides contained less than 2·5% of xylan. The fact that both these fractions contained only trace amounts of *O*-acetyl groups, and that the so-called linear polysaccharides contained most of the xylan and *O*-acetyl groups originally present, implies that only the xylans are *O*-acetylated in both wood species.

It is unlikely that all of the uronic acid present is associated with xylans and this makes it difficult to calculate the acetyl content of the pure xylans. However, by calculating the molar ratios of *O*-acetyl

units to xylose units, a suitable comparison between the original and the linear polysaccharides can be made, as shown in Table 2. This shows that both original extracted polysaccharides contain xylans with similar acetyl contents, and the same is true for both linear ones. Comparison of the molar ratios in the original polysaccharides with those calculated from dimethyl sulphoxide-extracted xylans showed that the dimethyl sulphoxide-extracted xylans of wych elm contained only slightly less *O*-acetyl groups than the aqueous extract, and those extracted with dimethyl sulphoxide from sweet chestnut had a much lower acetyl-group content than those extracted with hot water. It is almost certain that some deacetylation had resulted from the hot-water treatment. When Hägglund *et al.* (1956) extracted birch holocellulose prepared by the chlorite method with dimethyl sulphoxide followed by hot water, their products had a molar ratio of acetyl groups to xylose of 1:1·06 and 1:1·26 respectively. When the same wood, delignified this time by the chlorine–ethanolamine method, was similarly treated, Bouveng *et al.* (1960) obtained products in which the molar ratios were 1:1·08 and 1:1·58 for the dimethyl sulphoxide and hot-water extracts respectively. These results are different from those obtained in the present investigation, in that they show that hot-water-extracted xylans contain less *O*-acetyl groups than dimethyl sulphoxide-extracted products.

A component, tentatively identified as 2-*O*-methylgalactose, was found in the original and fractionated polysaccharides. Both branched polysaccharides contained it in greatest amount, indicating that it may be part of the galactan series of polymers. No reference has been found for the existence of this component in wood polysaccharides, but Aspinall & McKay (1958) found 3-*O*-methylxylose in larch (*Larix decidua*) hemicellulose, and the corresponding 2-*O*-methyl ether was reported by Andrews & Hough (1956) as a constituent of plum-leaf hemicellulose.

Changes in O-*acetyl groups after incubation.* The positions at which *O*-acetyl groups are attached to 4-*O*-methylglucuronoxylans extracted from sweet chestnut and wych elm, green and incubated, were described by Cochrane *et al.* (1969) and are summarized in Table 3 together with the percentage loss of *O*-acetyl groups at the individual positions. It is assumed that no migration of *O*-acetyl groups occurred under the conditions of incubation.

The fact that delignification leads to some loss of *O*-acetyl groups explains why original wood from sweet chestnut gives a 77% loss of *O*-acetyl groups resulting from incubation, whereas delignified wood gives an 85% loss, as shown in Table 4. Assuming that the *O*-acetylated xylans extracted from green and incubated sweet chestnut are representative of the total *O*-acetylated xylans

Table 2. *Molar ratio of* O-*acetyl groups to xylose in hot-water-extracted polysaccharides and their fractionated products, and in dimethyl sulphoxide-extracted xylans*

		Molar ratio acetyl units/xylose units	
		Hot-water extract	Dimethyl sulphoxide extract
Sweet chestnut			
	Original polysaccharide	1:1·70	1:2·55
	Linear polysaccharide	1:1·18	
Wych elm			
	Original polysaccharide	1:1·69	1:1·82
	Linear polysacchaide	1:1·23	

Table 3. *Distribution of* O-*acetyl groups in glucuronoxylans from sweet chestnut and wych elm, green and incubated, and percentage loss of* O-*acetyl groups at their respective positions*

Wood		Composition (moles/100 moles) Green	Incubated	Loss of acetyl groups (%)
Sweet chestnut				
	Xylose unacetylated	65·70	88·10	
	Xylose *O*-acetylated at C-2	16·28	4·42	12·05 (74·2%)
	Xylose *O*-acetylated at C-3	14·52	6·10	8·42 (58·0%)
	Xylose *O*-acetylated at C-2 and C-3	2·73	1·59	1·14 (41·8%)
	Xylose *O*-acetylated at C-2, C-3 and C-4	0·80	0·00	0·80 (100·0%)
Wych elm				
	Xylose unacetylated	64·32	80·70	
	Xylose *O*-acetylated at C-2	12·73	7·20	5·53 (43·4%)
	Xylose *O*-acetylated at C-3	14·82	10·59	4·23 (28·5%)
	Xylose *O*-acetylated at C-2 and C-3	8·13	1·45	6·68 (82·2%)

Table 4. *Percentage loss of O-acetyl groups due to incubation*

Values for acetyl group content are expressed as percentage of original wood.

Wood		Acetyl groups or methoxyl groups: Green	Acetyl groups or methoxyl groups: Incubated	Loss of acetyl groups (%)
Sweet chestnut				
	Original wood	3·01	0·69	2·32 (77·1%)
	Holocellulose	2·77	0·41	2·36 (85·2%)
	O-Acetylated 4-*O*-methylglucuronoxylan	10·23	3·84	6·39 (62·5%)
	O-Methylated glucoxylan*	8·93	3·66	5·27 (59·0%)
Wych elm				
	Original wood	4·74	2·67	2·07 (43·7%)
	Holocellulose	3·67	2·36	1·31 (35·7%)
	O-Acetylated 4-*O*-methylglucuronoxylan	13·08	7·66	5·42 (41·4%)
	O-Methylated glucoxylan*	10·65	6·56	4·09 (38·4%)

* Methoxyl groups occupy the positions originally occupied by the *O*-acetyl groups.

present, then comparison of their *O*-acetyl-group contents should result in a loss of *O*-acetyl groups of about 85%, provided that the conditions of extraction do not cause any deacetylation (Garegg, 1962). Table 4 shows that the loss is only 62·5%, which suggests that these *O*-acetylated xylans do not represent the total *O*-acetylated hemicellulose fraction in sweet-chestnut holocellulose.

It is not clear why the holocellulose from green and incubated wych elm should only give a 35·7% loss of *O*-acetyl groups, compared with a loss of 43·7% for the original woods. By analogy with sweet chestnut, the *O*-acetylated xylans extracted from green and incubated wych elm, which show a loss of 41·4% of *O*-acetyl groups, may also be regarded as non-representative of the total *O*-acetylated xylan fraction present in wych-elm holocellulose.

It is therefore desirable to consider further the homogeneity of *O*-acetylated xylans of hardwood. Lindberg (1961) suggested that *O*-acetylxylans extracted from wood holocellulose with dimethyl sulphoxide are representative of the total *O*-acetylxylan present. However, Cochrane (1967) made calculations for other wood species from published information and from results of the current investigation and compared the results with the *O*-acetyl-group contents of the respective isolated *O*-acetylated xylans found by analysis, and found that the latter are generally appreciably lower than the former. This suggests that these hardwoods contain a series of closely related glucuronoxylans that have different *O*-acetyl-group contents, with the result that extraction may give an acidic xylan containing either more or less than the calculated average acetyl-group content, or one having the average content as obtained by Hägglund *et al.* (1956) and Bouveng *et al.* (1960).

That *O*-acetylxylans are heterogeneous within the cell wall is not too surprising, as it is known that xylan fractions from the same wood may have different uronic acid contents (Anderson, Kaster & Seeley, 1942; Hägglund *et al.* 1956). In fact, Thornber & Northcote (1961, 1962) found that those xylans that occur in the primary wall generally contain less 4-*O*-methylglucuronic acid than those found in the secondary wall.

It must therefore be considered that the *O*-acetylated glucuronoxylans isolated from sweet chestnut and wych elm, green and incubated, in the present investigation do not represent the total *O*-acetylated glucuronoxylans in these woods. There are two pieces of experimental evidence in the present investigation to support this. (i) When dimethyl sulphoxide-extracted *O*-acetylglucuronoxylans are being purified from water (Cochrane *et al.* 1969), it was observed in all cases that some of the crude hemicelluloses were insoluble, the quantity of which, for that of the incubated sweet chestnut, was appreciable (10g.). As this product was found to contain 7·7% of lignin in addition to xylose, it was termed for convenience a 'lignin–xylan complex' and had an acetyl group content of 1·6%. This was changed to a value of 1·7% when allowance for the lignin was made, and shows that a glucuronoxylan has been isolated with appreciably less *O*-acetyl group than the water-soluble material, which had an *O*-acetyl group content of 3·8%. (ii) Table 2 shows that comparison of the molar ratios of acetyl units to xylose units in hot-water-extracted polysaccharides, their fractionated products and dimethyl sulphoxide-extracted xylan, from both green sweet chestnut and wych elm, varies within each species. Chestnut showed a variation from 1:1·18 in the separated linear polysaccharide to 1:2·55 in the dimethyl sulphoxide extract, and elm from 1:1·23 to 1:1·82.

The above results all indicate that in both sweet chestnut and wych elm a series of closely related xylans exist that have different acetyl-group contents.

It is necessary to try to explain why wych elm

loses fewer *O*-acetyl groups than does sweet chestnut under the same conditions of incubation, and also to explain the large difference in loss of *O*-acetyl groups between the respective positions of both sets of *O*-acetylated glucuronoxylans. Three possibilities are considered, namely (i) difference in uronic acid content, (ii) difference in tannin content and (iii) location in the cell wall.

Difference in uronic acid content. The only real chemical difference between the *O*-acetylated glucuronoxylans isolated from both wood species, apart from their *O*-acetyl-group content, lies in their uronic acid content, which in sweet chestnut results in a molar ratio of 1:11·6–1:11·8 for 4-*O*-methylglucuronic acid to xylose, and in wych elm gives a ratio of 1:7·4–1:7·6. As uronic acids are only attached to C-2 of wood xylans, any effect they exert will only operate on the *O*-acetyl group located at C-3. Table 3 shows that elm *O*-acetylxylan loses fewer *O*-acetyl groups from C-3 than does chestnut *O*-acetylxylan. However, consideration of electronic and steric factors (Cochrane, 1967) fails to explain this difference.

Difference in tannin contents. A difference that occurs in the woods themselves is the content of aqueous-ethanol extractives, consisting mainly of tannins, which are polyphenolic. Wych elm possesses only small amounts of tannins, but sweet chestnut contains appreciable amounts and was used at one time for tanning purposes. The effect tannins could play in increasing the rate of removal of *O*-acetyl groups by hydrolysis is unknown. However, it is significant that, in previous work (Arni, Cochrane & Gray, 1965), comparison of the degree of corrosion of mild-steel washers caused by sweet chestnut from which only volatile material had been removed, and the same wood from which extractives and volatile materials had been removed, during incubation at 48° and 100% relative humidity, showed that whereas the former was completely corroded after 24hr. the latter produced virtually no corrosion. After about 2 years, the extractive-free sample had not further corroded the steel washer whereas the other washer had been rendered virtually to corroded metal filings. As the degree of corrosion is directly related to the rate of removal of *O*-acetyl groups by hydrolysis, the removal of extractives from green sweet chestnut appears to play some part in removal of *O*-acetyl groups by hydrolysis.

Location in the cell wall. One other possibility is that xylan fractions with different *O*-acetyl group contents are located in different parts of the cell wall resulting in (i) some parts of the cell wall being relatively inaccessible to hydrolytic reagents, with the result that the *O*-acetylated glucuronoxylan located there is deacetylated to a much smaller extent than the overall loss of *O*-acetyl groups within the cell wall; (ii) some parts of the cell wall being relatively accessible to hydrolytic reagents, with the result that the *O*-acetylated glucuronoxylan located there is deacetylated to a much greater extent than the overall loss of *O*-acetyl groups within the cell wall; (iii) some parts of the cell wall having moderate accessibility to hydrolytic reagents, with the result that the *O*-acetylated glucuronoxylan located there will have average deacetylation similar to the overall deacetylation within the cell wall.

If this in fact occurs, then the loss of acetyl groups during incubation given in Table 4 indicates that the *O*-acetylated glucuronoxylan isolated from sweet chestnut, when compared with the other *O*-acetylated glucuronoxylans present, will be found in that part of the cell wall corresponding to (i). Similarly, the *O*-acetylated glucuronoxylan extracted from green wych elm, when compared with the other *O*-acetylated glucuronoxylans present, will be found in that part of the cell wall corresponding to (iii). However, when the original woods are compared, most of the *O*-acetylated glucuronoxylans occurring in wych elm are located in less accessible parts of the cell wall than those present in sweet chestnut.

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