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A ^{13}C-N.M.R. STUDY OF THE ALKALINE DEGRADATION PRODUCTS OF POLYSACCHARIDES

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ABSTRACT

Thirteen products of alkaline degradation of polysaccharides have been examined by ^{13}C-n.m.r. spectroscopy. The main product of the alkaline degradation of L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan was the sodium salt of 3-deoxy-2-*C*-hydroxymethyltetronic (xyloisosaccharinic) acid. L-Arabino-(4-*O*-methyl-D-glucurono)-D-xylan and (4-*O*-methyl-D-glucurono)-D-xylan were degraded in alkaline media by the elimination of L-arabinose and 4-*O*-methyl-D-glucuronic acid residues. The first step in the elimination of the 4-*O*-methyl-D-glucuronic acid residue was the loss of MeO-4 and the formation of a 4,5-double bond. The presence of a 3-deoxyaldonic acid end-unit in the polysaccharide was also found.

INTRODUCTION

When wood is delignified, some polysaccharides are also lost[1]. The acids formed by degradation of the polysaccharide have been characterised[2] by g.l.c.–m.s. of the trimethylsilylated derivatives. Suitable standards were isolated from the mixture of products by ion-exchange chromatography[3], although methods are known which could be used for the preparation of the main types of saccharinic acids[4].

Although ^{13}C-n.m.r. spectroscopy has been used to study disaccharides in alkaline solution[5], the products of alkaline degradation of polysaccharides have not been investigated by this technique and we now report on this topic.

EXPERIMENTAL

Materials. — Compounds **1–11** (see Table I) were commercial products. The sodium salts of 3-deoxy-2-*C*-hydroxymethylpentonic acid (**12**) and 3-deoxyhexonic acid (**13**) were prepared by known procedures[4]. The sodium salt of 3-deoxy-2-*C*-hydroxymethyltetronic acid (**14**) was obtained as the water–ethanol soluble portion when the polysaccharide was precipitated after alkaline degradation of L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan (**15**) from corn cobs. This polysaccharide had

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$[\alpha]_D^{22}$ −80° (*c* 0.6, water), $\overline{M}_n$ 27,190; and contained D-xylose, 84.1%; L-arabinose, 8.3%; carboxyl, 1.0%; N, 0.1%; ash, 1.1%. The samples of degraded polysaccharide were prepared from **15** (10 g) at room temperature by storage of a solution in M NaOH (500 mL) under nitrogen for 25 days; 70% of the polysaccharide was than precipitated in water–ethanol solution (1:4–5). After storage for 2 years, 68.8% of the substrate (**16**) could be precipitated. (4-*O*-Methyl-D-glucurono)-D-xylan (**17**), prepared[6] from beach sawdust, had $[\alpha]_D^{22}$ −70° (*c* 0.5, water), $\overline{M}_n$ 18,610, and contained 19.3% of carboxyl and 2.93% of methoxyl; the neutral portion of the saccharides formed on hydrolysis contained 98% of D-xylose. The polymer **17** was degraded in 2M NaOH (1-L steel autoclave) at 100°→167° (bath) linearly during 110 min and then at 167° (bath) for 3 h. The solution was then dialysed and freeze-dried to give **18**. The samples of holocellulose and cellulose were prepared as described previously[7]. Holocellulose was degraded (→**19**) as for **17**. The cellulose sample was cooled immediately after reaching 167° (→**20**).

Methods. — ^{13}C-N.m.r. spectra were measured at 25° (100 mg per mL) in M NaOH after adding a small amount of D_2O (δ 50.15 relative to Me_4Si), using Jeol FX-60 and Bruker AM-300 spectrometers.

RESULTS AND DISCUSSION

The alkaline hydrolysis of glycosidic bonds has the greatest effect on the yield of polysaccharides during the delignification of wood. From this point of view, the peeling reaction is less important. The main products of the peeling reaction are **12** and **14** (see Table I). The competitive reaction is the stopping reaction by which the 3-deoxyaldonic acid end-unit is formed[1]. The secondary products of polysaccharide degradation formed *via* retro-aldol reactions are the salts of such acids as formic, acetic, glycolic, lactic, and hydroxybutyric. The polysaccharides with aldonic acid end-units are the products of oxidation during the delignification process[8]. This modification has the same stabilisation effect as the 3-deoxyaldonic acid end-unit. The minor degradation products of uronic acid units from (4-*O*-methyl-D-glucurono)-D-xylan are aldaric acids[9].

In order to apply ^{13}C-n.m.r. spectroscopy, the chemical shift data for suitable standards as solutions in aqueous sodium hydroxide must be known. Table I contains the chemical shift data for some sodium salts of acids which are the products of alkaline degradation of polysaccharides. The assignment of individual resonances for **1, 2, 3, 6, 7, 9**, and **10** are unambiguous. For **4, 5, 8, 11**, and **13**, the assignments of marked carbons may be interchanged. The chemical shift of the ^{13}C resonances in individual CH_2OH groups for **12** (sodium glucoisosaccharinate) was determined using the INEPT technique. In the spectrum of **13** (sodium glucometasaccharinate), the C-2 resonance was assigned on the basis of the known spectrum of sodium 3-deoxy-L-*glycero*-tetronate (C-2, 71.0 p.p.m.)[10].

The undegraded part of the polysaccharide **15** could be precipitated after

TABLE I

^{13}C-N.M.R. DATA (δ)

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C*
1	172.9						
2	183.1	27.4					
3	178.8	61.1					
4	181.1	74.1[a]	73.0[a]	72.8[a]	64.5		
5	182.6	77.1[a]	73.9[a]	76.3[a]	72.6[a]	64.8	
6	182.6	74.6	28.3	9.9			
7	181.6	47.7	66.8	23.1			
8	184.1	35.3[a]	29.6[a]	62.7			
9	183.8	69.7	21.4				
10	180.5	74.9	65.6				
11	180.6	75.1	73.0	75.1	75.1	180.6	
12	181.7	69.7[b]	39.2	78.9	67.3		67.3[c]
13	184.9	72.7[a]	38.5	71.5[a]	64.8	58.4	
14	181.7	71.7	40.8	68.9			68.9[c]
15	103.3[d]	74.4[d]	75.8[d]	77.5[d]	64.6[d]		
	102.5[e]	83.7[e]	73.6[e]	78.2[e]	64.6[e]		
	99.1[f]	73.6[f]	73.6[f]	81.5[f]	72.9[f]	178.3[f]	61.1[g]
	107.6[h]	75.0[h]	85.8[h]	71.1[h]	67.2[h]		
	110.6[i]	83.7[i]	79.7[i]	87.7[i]	63.0[i]		
16	103.5[d]	74.5[d]	76.0[d]	77.4[d]	64.7[d]		
17	103.3[d]	74.3[d]	75.7[d]	77.4[d]	64.4[d]		
	102.2[e]	84.0[e]	75.7[e]	77.8[e]	64.4[e]		
	98.6[f]	73.5[f]	73.5[f]	84.0[f]	72.9[f]	178.2[f]	61.0[g]
	—	—	38.3[j]	—	—		
18	103.2[d]	74.3[d]	75.7[d]	77.2[d]	64.5[d]		
19	103.3[d]	74.3[d]	75.7[d]	77.4[d]	64.4[d]		
	—	—	—	—	—	169.9[k]	
	104.8[l]	—	—	—	—	—	
	108.6[m]	—	—	—	—		
20	103.2[d]	74.3[d]	75.7[d]	77.2[d]	64.4[d]		
	—	—	—	146.7[n]		170.7[k]	
	109.0[m]	—	—	—	—	—	

Key: **1** Sodium formate. **2** Sodium acetate. **3** Sodium glycolate. **4** Sodium L-arabinonate. **5** Sodium D-gluconate. **6** Sodium 2-hydroxybutyrate. **7** Sodium 3-hydroxybutyrate. **8** Sodium 4-hydroxybutyrate. **9** Sodium lactate. **10** Calcium glycerate. **11** Monopotassium D-gluconate. **12** Sodium 3-deoxy-2-*C*-hydroxymethylpentonate. **13** Sodium 3-deoxyhexonate. **14** Sodium 3-deoxy-2-*C*-hydroxymethyltetronate. **15** L-Arabino-(4-*O*-methyl-D-glucurono)-D-xylan. **16** Degraded **15**[o]. **17** (4-*O*-Methyl-D-glucurono)-D-xylan. **18** Degraded **17**[o]. **19** Degraded holocellulose[o]. **20** Degraded cellulose[o].

[a]Assignments may be interchanged. [b]Isomer of **12** with the resonance for C-2 at 69.1 p.p.m. [c]Primary hydroxyl group bonded to C-2. [d]Unsubstituted β-D-xylopyranosyl residues. [e]Substituted β-D-xylopyranosyl residues. [f]D-Glucuronic acid residues. [g]MeO of (4-*O*-methyl-D-glucurono)-D-xylan. [h]D-Xylopyranosyl residues substituted at C-3 with L-arabinofuranose. [i]Resonances of L-arabinose linked to C-3 of D-xylose of the β-D-xylopyranose chain. [j]Resonance of C-3 in 3-deoxyaldonic units linked to (4-*O*-methyl-D-glucurono)-D-xylan. [k]Carbohydrate carbon in **19** after elimination of MeO-4. [l]C-1 of degraded cellulose. [m]C-1 of arabinose residue linked to β-D-xylopyranose. [n]C-4 or C-5 of hex-4-enuronic acid. [o]See Experimental.

treatment with alkali. ^{13}C-N.m.r. spectroscopy indicated the soluble degradation products to contain the salts of formic acid (172.5 p.p.m.), lactic acid (24.8, 70.9, and 184.1 p.p.m.), and a third compound, identified on the basis of the ^{13}C-n.m.r. spectrum of **12**, as the salt of xyloisosaccharinic acid (**14**): C-1, 181.7; C-2, 71.7; C-3, 38.3; C-4, 68.9; and -CH_2OH linked to C-2, 68.9 p.p.m.

The most intense peaks in the ^{13}C-n.m.r. spectrum of polysaccharide **15** were due to unsubstituted D-xylosyl residues (C-1, 103.3; C-2, 74.4; C-3, 75.8; C-4, 77.5; and C-5, 64.6 p.p.m.). On the basis of ^{13}C-n.m.r. data for model compounds and (4-*O*-methyl-D-glucurono)-D-xylan[11–13], resonances can be assigned to L-arabinose, D-glucuronic acid, and substituted D-xylose residues (Table I). A small proportion of the L-arabinofuranose residues were oxidised at C-5 (181.1 p.p.m.) during isolation of the polysaccharide. The ^{13}C-n.m.r. spectrum of the degraded polysaccharide **16** contained only resonances for D-xylose residues (Table I), thus confirming the loss of L-arabinose and D-glucuronic acid residues from the polysaccharide. This situation was also observed for L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan from different sources[14]. The fact that practically the same quantity of polysaccharide was precipitated from the samples degraded for 25 days and for 2 years confirms the presence of 3-deoxyaldonic acid end-units. The resonances for these end units were not found in the spectrum.

The (4-*O*-methyl-D-glucurono)-D-xylan (**17**) obtained from beech sawdust gave three ^{13}C signals in the anomeric region: for unsubstituted β-D-xylopyranosyl residues (103.3 p.p.m.), for β-D-xylopyranosyl residues substituted with D-glucuronic acid residues (102.2 p.p.m.), and for α-D-glucuronic acid residues (98.6 p.p.m.). These assignments and those for all the other carbons accord with published data on polysaccharides and model compounds[11,15,16] (Table I). There was also a small peak at 38.3 p.p.m.; this chemical shift is almost identical to that of C-3 of **13** (sodium 3-deoxyhexonate) and the peak is probably due to C-3 of the 3-deoxypentonic acid end-unit of **17**. The chemical shifts of the other resonances of this end unit were not detected. The degradation of **17** gave **18** which has a ^{13}C-n.m.r. spectrum almost identical to that of the degraded polysaccharide **16** (Table I).

The soluble part (**19**) obtained after degradation of holocellulose gave, after dialysis, freeze-drying, and dissolution in M NaOH, a ^{13}C-n.m.r. spectrum with the known five intense peaks of β-D-xylopyranosyl residues (Table I). The spectrum also contained one peak at 104.8 p.p.m. which could be assigned, on the basis of data for solutions of cellulose in NaOH, to the small portion of degraded cellulose[17]. The additional peak (108.6 p.p.m.) in the anomeric region confirmed the presence of a small portion of L-arabinose linked to the β-D-xylopyranose chain[13]. The peak at 169.6 p.p.m. could be due to the carboxylate group. The chemical shift of this resonance differs from that of the D-glucuronic acid residue present in **15** and **17**. The carboxylate resonance in the spectrum of **19** may originate from the residual hemicellulose portion which contains hex-4-enuronic acid residues, as shown for model compounds[18], or from oxidised positions C-6 of the cellulose degradation product. Hence, the cellulose was degraded under mild

conditions in order not to destroy the residual hemicelluloses adsorbed on this sample.

The ^{13}C-n.m.r. spectrum of **20** (Table I) contains five known peaks of β-D-xylopyranosyl residues and also two small peaks at 170.7 and 146.7 p.p.m. The latter peak belongs to the 4,5-double bond and the former to the carboxyl group. The difference in chemical shifts in the spectrum of **20** in comparison with those for the carboxyl groups of **15** and **17** is probably due to conjugation with the 4,5-double bond, as demonstrated on a model compound[18]. There were no peaks which could be assigned to degraded cellulose, and a small peak at 109.0 p.p.m. confirmed the presence of some arabinosyl groups linked to the β-D-xylopyranose chain[13].

These results indicate that the hemicellulose **15** and **17** are degraded by elimination of L-arabinose and D-glucuronic acid residues. The first step in degradation of the 4-*O*-methyl-D-glucuronate residue is the loss of MeO-4 and the formation of a 4,5-double bond. The reducing end units are transformed into 3-deoxyaldonic end units by the stopping reaction[1] and this end unit could be detected by ^{13}C-n.m.r. spectroscopy.

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