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^{13}C-N.M.R. SPECTRA OF XYLO-OLIGOSACCHARIDES AND THEIR APPLICATION TO THE ELUCIDATION OF XYLAN STRUCTURES

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ABSTRACT

^{13}C-N.m.r. spectra of thirteen xylo-oligosaccharides [a complete series of α- and β-D-xylopyranosyl derivatives of methyl β-D-xylopyranoside, β-D-xylopyranosyl derivatives of methyl 4-*O*-β-D-xylopyranosyl-D-xylopyranoside, methyl *O*-α-D-xylopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranoside, and a branched methyl β-xylotetraoside] have been interpreted. The data obtained have been used for the carbon signal assignment in the spectra of a number of red-algal xylans. ^{13}C-N.m.r. spectroscopy is shown to be a rapid and convenient method for the structural analysis of xylose-rich polysaccharides.

INTRODUCTION

^{13}C-N.m.r. spectroscopy is a powerful tool in the elucidation of polysaccharide structures, which can give the same information as much more tedious chemical-methods[1,2]. ^{13}C-N.m.r. spectra of polysaccharides may be interpreted on the basis of the spectra of simple model compounds, *e.g.*, mono-*O*-methyl derivatives of the corresponding methyl glycosides[3,4] or other carbohydrate alkyl ethers[5]. However, oligosaccharides structurally related to the polysaccharide are more valuable models. For instance, the interpretation of ^{13}C-n.m.r. spectra of various glucans has been accomplished by using data obtained from the spectra of D-glucose disaccharides[6]. The analysis of ^{13}C-n.m.r. spectra of many red-algal galactans was greatly assisted by the spectra of a series of the corresponding D- and L-galactose-containing oligosaccharides[7]. We now report on the ^{13}C-n.m.r. spectra of thirteen synthetic xylo-oligosaccharides, and the utilisation of these data for the interpretation of the ^{13}C-n.m.r. spectra of several red-algal xylans.

RESULTS AND DISCUSSION

The syntheses of the oligosaccharide derivatives **1**–**13** have been described previously[8–14]. For carbon signal assignments, the ^{13}C-n.m.r. spectra of these compounds were compared with the spectrum of methyl β-D-xylopyranoside[15], taking into account the influence of substitution at various positions in the molecule[1]. It has been assumed that, since D-xylose is a configurational analogue of D-glucose, the substitution effects at C-1–C-4 in D-xylose derivatives are similar to those in the D-glucose analogues. Therefore, for the signal assignments in the spectra of compounds studied herein, the ^{13}C-n.m.r. spectra of D-glucose disaccharides[6] have also been taken into consideration. A supplementary check of the correctness

TABLE I

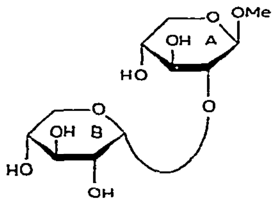
CHEMICAL SHIFTS FOR ^{13}C-N.M.R. SPECTRA OF METHYL β-D-XYLOPYRANOSIDE AND OLIGOSACCHARIDES 1–13

Substance	*Residue*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*OMe*
Methyl β-D-xylopyranoside (*cf.* ref. 15)		105.2	74.2	77.0	70.4	66.4	58.3
1	A	105.4	78.5	75.5	70.7	66.1	58.5
	B	99.1	72.7	74.2	70.7	62.6	
2	A	105.3	72.7	82.9	70.6	66.2	58.4
	B	100.1	72.9	74.3	71.0	62.7	
3	A	105.2	74.1	76.0	79.4	65.4	58.4
	B	101.5	73.0	74.4	70.7	62.9	
4	A	104.9	81.8	76.4	70.2	65.9	58.1
	B	103.7	74.7	76.8	70.4	66.3	
5	A	104.9	73.7	85.3	69.0	66.0	58.4
	B	104.8	74.6	76.9	70.4	66.4	
6	A	105.1	74.0	75.0	77.7	64.1	58.4
	B	103.1	74.0	76.9	70.4	66.5	
7	A	105.3	81.9	75.0	77.9	63.9	58.4
	B	103.6	74.4	77.2	70.8	66.8	
	C	103.9	74.8	77.2	70.8	66.8	
8	A	105.2	74.2	75.1	78.0	64.1	58.4
	B	101.8	82.0	76.5	70.3	66.2	
	C	105.5	75.1	76.8	70.6	66.5	
9	A	104.5	73.6	80.8	74.2	63.4	58.2
	B	102.4	73.5	76.6	70.4	66.3	
	C	104.2	74.2	76.6	70.4	66.3	
10	A	105.1	74.2	75.1	77.7	64.2	58.5
	B	103.0	73.8	84.9	69.0	66.2	
	C	104.8	74.7	77.0	70.5	66.5	
11	A	105.1	74.1	75.0	77.7	64.1	58.3
	B	103.0	74.1	75.0	77.7	64.3	
	C	103.1	74.0	76.9	70.4	66.5	
12	A	105.1	74.1	75.1	77.8	64.2	58.4
	B	103.3	72.6	82.6	70.6	66.1	
	C	100.1	72.8	74.3	70.9	62.7	
13	A	105.1	74.1	75.0	77.5	64.0	58.4
	B	102.4	73.6	80.6	74.3	63.7	
	C	102.5	73.6	76.6	70.4	66.3	
	D	104.0	74.1	76.6	70.4	66.3	

of the spectral interpretation was provided by a comparison of the spectra of compounds **1**–**13** (Table I).

The results obtained permit the following conclusions about the ^{13}C-n.m.r. spectra of xylo-oligosaccharides, which are important in the interpretation of the spectra of related substances. The presence of α-D-xylopyranosyl residues is revealed by chemical shifts of the C-1 and C-5 signals, which are <102 and <63 p.p.m., respectively. On the other hand, C-1 and C-5 belonging to β-D-xylopyranosyl residues resonate at >102 and >63 p.p.m., respectively. The signals at 82–85 p.p.m. correspond to C-3 of 3-*O*-substituted β-D-xylopyranosyl residues. Hence, these signals may be diagnostic for the presence of (1→3)-linkages. However, it must be emphasised that additional substituents at positions 2 or 4 will shift the C-3 signal upfield, due to the β-effect (*cf.* the spectra of **5** and **9**, Table I).

It follows from the spectra of disaccharides **1**–**6** that, independently of the position of an α- or β-D-xylopyranosyl substituent attached to the methyl β-D-xylopyranoside residue, the C-1 chemical shift of the latter (residue A) remains practically



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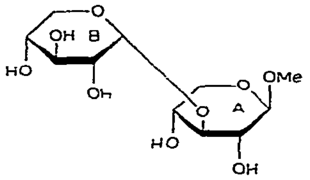

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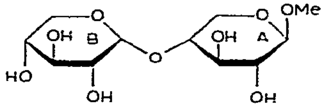

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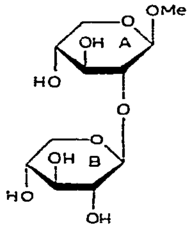

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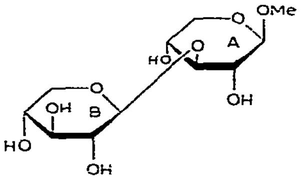

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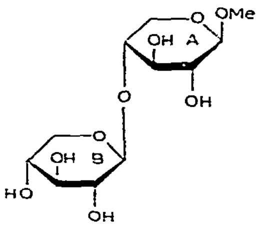

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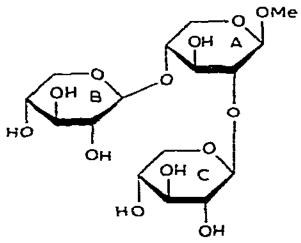

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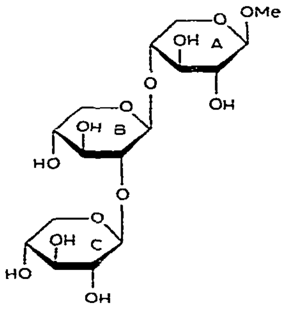

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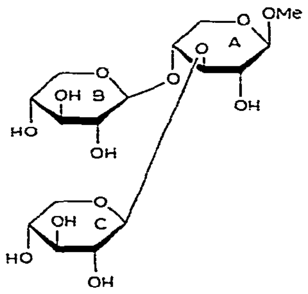

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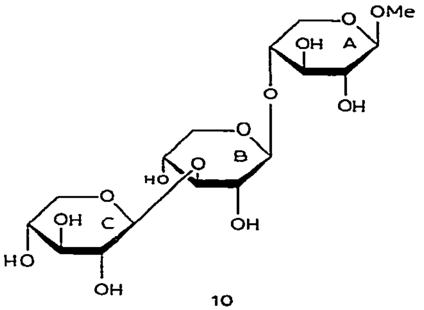

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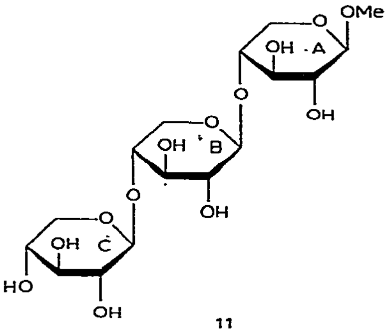

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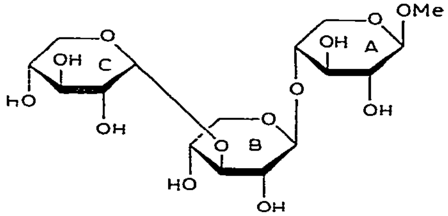

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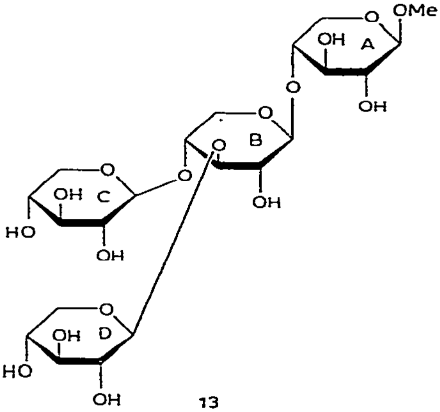

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constant. On the other hand, the position of the C-1 signal of the substituting D-xylopyranosyl group (residue B) largely depends both on the type of linkage (1→2, 1→3, or 1→4) and on the configuration of the glycosidic centre (Table II). In most cases, the introduction of a xylosyl substituent is accompanied by common α- and β-effects (*i.e.*, by a downfield shift of the α-carbon signal and by upfield shifts of the β-carbon signals, as compared with the corresponding signals in the spectrum of unsubstituted methyl β-D-xylopyranoside). However, the absolute values of the α- and β-effects depend on the position of the substituent and on the configuration of its glycosidic centre (Table II).

There are some differences in the chemical-shift values of related carbon-atom signals in the spectra of oligo- and polysaccharides, which are probably the result of unavoidable differences in the conditions of measurement of spectra for the substances of low and high molecular weight (*e.g.*, different solution viscosities). Nevertheless, the oligosaccharide spectra enabled more precise interpretation of the spectra of several red-algal xylans to be made. We have previously reported[16] the use of ^{13}C-n.m.r. spectroscopy for the structural analysis of xylan fractions obtained from the red seaweed *Rhodymenia stenogona*. Methylation-analysis and partial-hydrolysis data revealed these polysaccharides to be linear with β-(1→3) and β-(1→4) linkages between the D-xylopyranosyl residues (the latter type of linkage is preponderant).

TABLE II

SOME DIAGNOSTICALLY IMPORTANT CHARACTERISTICS OF THE ^{13}C-N.M.R. SPECTRA OF DISACCHARIDES **1–6**

Linkage	*Chemical shift of C-1 (residue B)*	*Location of resonances of residue A*		
		Chemical shift of C-atom bearing a substituent	*α-Effect*	*β-Effects*
α-(1→2)	99.1	78.7	+4.5	C-1 +0.1 C-3 −1.5
α-(1→3)	100.1	82.9	+5.8	C-2 −1.5 C-4 +0.1
α-(1→4)	101.5	79.4	+8.9	C-3 −1.0 C-5 −0.9
β-(1→2)	103.7	81.8	+7.5	C-1 −0.4 C-3 −0.6
β-(1→3)	104.8	85.3	+8.2	C-2 −0.5 C-4 −1.5
β-(1→4)	103.1	77.7	+7.2	C-3 −2.0 C-5 −2.2

The interpretation[16] of the ^{13}C-n.m.r. spectra was based mainly on the comparison of spectra of a series of xylans known to differ in the ratio of β-(1→3) and β-(1→4) linkages. There are two groups of signals in the spectra of xylans, each group containing five resonances of approximately equal intensity. These groups of signals were considered to be those of 3-*O*- and 4-*O*-substituted D-xylopyranosyl residues, and the ratio between the two types of linkage was calculated as the proportion between the sum of the integrated intensities of the resonances belonging to each group. This method of calculation was believed to give the linkage ratio more precisely than the ratio of the integrated intensities of a pair of related resonances (*e.g.*, of anomeric signals); the values obtained were confirmed by chemical analyses[16].

However, the C-1 resonance assignment made in the previous work[16] should be corrected. On the basis of the spectra of model xylo-oligosaccharides, the signal at 104.0 p.p.m. in the spectra of xylans does not belong to C-1 of a 3-*O*-substituted β-D-xylopyranosyl residue, but is characteristic of C-1 of any D-xylopyranosyl residue attached to a neighbouring ring at position 3 (the anomeric-resonance position depends exclusively upon the type of linkage with the next sugar residue and not upon the substitution of hydroxyl groups in a given residue, *cf.* Table II). Thus, the signal at 102.3 p.p.m. corresponds to C-1 of the D-xylopyranosyl residues that are β-(1→4)-linked to the neighbouring D-xylosyl residue, independent of the position of the substituent in those particular units. The revised signal-assignments in the ^{13}C-n.m.r. spectra of (1→3,1→4)-β-D-xylans are given in Table III*. The spectra of

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*The chemical shifts in Table III correspond to the spectra recorded at pD 7 or 14, since several xylans are soluble only in aqueous alkali. The differences in the resonance positions in neutral and alkaline media are probably the result of ionisation of hydroxyl groups (*cf.* ref. 17) or of polymer conformation changes (*cf.* ref. 18) in the presence of alkali.

the xylo-oligosaccharides confirmed the correctness of the C-2–C-5 signal assignments in the spectra of xylans. The previous conclusions concerning the proportion of β-(1→3) and β-(1→4) linkages in different polysaccharides, calculated on the basis of their ^{13}C-n.m.r. spectra[16], are also correct.

After complete interpretation of the ^{13}C-n.m.r. spectra of the xylans from *Rhodymenia stenogona*, the method was extended to the structural analysis of xylans from other red-algae. A series of such xylans was isolated by a differential extraction procedure from *Halosaccion glandiforme*, belonging to the same order (Rhodymeniales) as *Rhodymenia stenogona*. No chemical data on the structure of these polysaccharides have so far been reported, but, in the ^{13}C-n.m.r. spectra, the same characteristic groups of resonances were present as in the spectra of *Rhodymenia stenogona* xylans. Based on this finding, it is suggested that both xylan series are linear polysaccharides composed of β-(1→3)- and β-(1→4)-linked D-xylopyranosyl residues. The proportion of these types of linkages in different xylan fractions, calculated from

TABLE III

CHEMICAL SHIFTS FOR ^{13}C-N.M.R. SPECTRA OF (1→3,1→4)-β-D-XYLANS

β-D-Xylopyranosyl residue	*C-1*		*C-2*	*C-3*	*C-4*	*C-5*
	(1→3) linkage	*(1→4) linkage*				
3-*O*-substituted						
pD 7	104.0	102.3	74.7	84.4	68.0	65.6
pD 14	106.0	103.2	75.6	88.0	68.6	66.1
4-*O*-substituted						
pD 7	104.0	102.3	73.5	74.4	77.1	63.5
pD 14	106.0	103.2	73.4	74.9	76.8	63.9

TABLE IV

THE RATIO OF (1→3) TO (1→4) LINKAGES IN β-D-XYLANS FROM *Rhodymenia stenogona* AND *Halosaccion glandiforme*

Extraction procedure	Rhodymenia stenogona		Halosaccion glandiforme	
	Yield (%)	*(1→3):(1→4)*	*Yield (%)*	*(1→3):(1→4)*
Water, 100°	29.1	1:3.6	18.6	1:4.0
0.2M HCl, 20°	2.3	1:2.1	3.1	1:2.0
M NaOH, 20°				
water-soluble fraction	7.9	1:4.0	15.3	1:3.2
water-insoluble fraction	11.5	1:4.3	2.1	not tested
M NaOH, 100°	0.7	(1→4) only	3.2	not tested

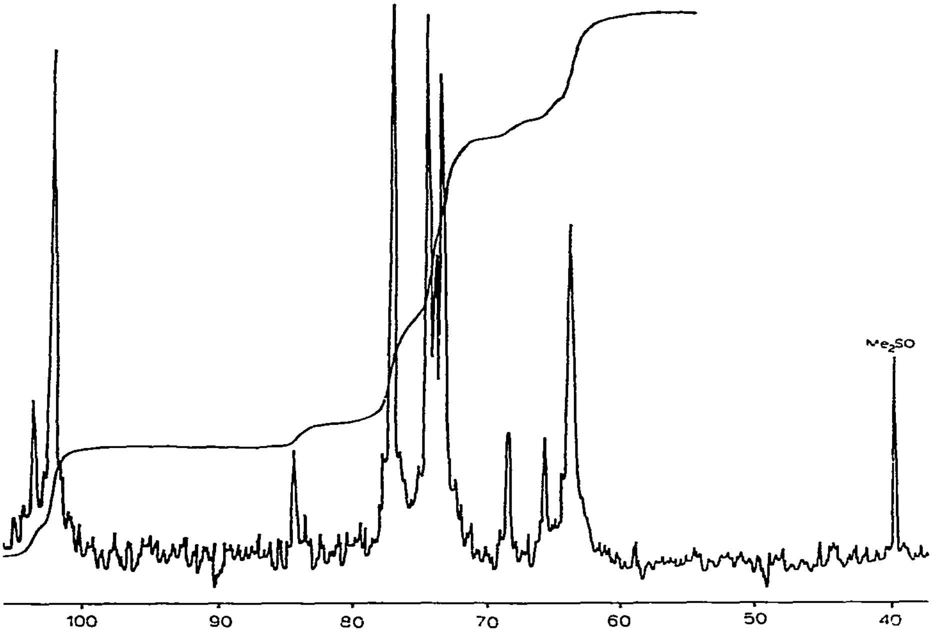

Fig. 1. The ^{13}C-n.m.r. spectrum of the xylan from *Nemalion vermiculare*; 5% solution in D_2O, 50°, 46473 accumulations.

the integrated intensities of the corresponding groups of signals in ^{13}C-n.m.r. spectra, are given in Table IV.

The ^{13}C-n.m.r. spectrum of the xylan from the red seaweed *Nemalion vermiculare*, belonging to the order Nemalionales, was also studied. Methylation analysis had shown[19,20] that the polysaccharide consists of (1→3)- and (1→4)-linked β-D-xylopyranosyl residues in the ratio ~1:4. In the ^{13}C-n.m.r. spectrum of this polysaccharide, the same pattern of signals was present as in the spectra of the xylans mentioned above. The integrated intensities of the relevant signals made it possible to correct the previous conclusion concerning the proportion of the two types of linkage, which is now found to be 1:6 (Fig. 1). In addition, the ^{13}C-n.m.r. spectrum showed that all of the D-xylose residues in the polysaccharide (as well as those in the xylans discussed above) are pyranoid (*cf.* the ^{13}C-n.m.r. spectra of methyl D-xylofuranosides[5]). Methylation analysis alone cannot distinguish 4-*O*-substituted pyranose from 5-*O*-substituted furanose residues.

Thus, ^{13}C-n.m.r. spectroscopy is of value in the structural investigation of (1→3,1→4)-β-D-xylans obtainable from different red-seaweeds. The spectral data presented may also be useful in the ^{13}C-n.m.r. spectroscopic analysis of other types of D-xylose-containing oligo- and poly-saccharides.

EXPERIMENTAL

Proton-decoupled, ^{13}C-n.m.r. spectra were recorded with a Bruker–Physik

WP-60 FT spectrometer at 15.08 MHz. Oligosaccharide samples were examined as 5–7% solutions in D_2O at 30°; chemical shifts were measured relative to internal methanol and converted into values relative to internal tetramethylsilane, according to the equation $\delta(Me_4Si) = \delta(MeOH) + 50.15$ p.p.m. Polysaccharide samples were examined at 50° as solutions in D_2O or in D_2O containing M NaOH; Me_2SO was used as the internal standard, and the observed chemical-shifts were converted into values relative to internal tetramethylsilane according to the equation $\delta(Me_4Si) = \delta(Me_2SO) + 39.41$ p.p.m.

Isolation of polysaccharides. — The algae *Rhodymenia stenogona* and *Halosaccion glandiforme* were dried, milled, extracted with methanol (Soxhlet), and air-dried. The resulting material was stirred several times with water for 6 h at 100°, the galactan sulphate was precipitated from the combined aqueous extract by using cetyltrimethylammonium bromide, and the neutral, water-soluble xylan was obtained from the solution after dialysis by precipitation with 4 vol. of ethanol. The residue after water extraction was stirred 3 times with 0.2M hydrochloric acid for 3 days at 20°, and the combined extracts were neutralised with sodium hydrogencarbonate, concentrated, and treated with 4 vol. of ethanol to give the acid-soluble xylan. Then the residue was stirred 3 times with M sodium hydroxide for 3 days at 20° and the combined extracts were neutralised with acetic acid; the precipitated material gave the fraction of water-insoluble xylan, and treatment of the solution with ethanol afforded the fraction of alkali-extracted, water-soluble xylan. The residue after alkaline extraction was stirred 3 times with M sodium hydroxide for 6 h at 100°, and the combined extract was neutralised, concentrated, and dialysed to give the water-insoluble, hot alkali-extracted xylan. The yields of fractions and proportions of $(1\rightarrow3)$- and $(1\rightarrow4)$-β-D linkages in these xylans, calculated from the spectral data, are given in Table IV. The isolation of xylan from *Nemalion vermiculare* has been described previously[20].

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