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ANALYSIS OF BROMINE-OXIDISED DEXTRAN BY ^{13}C-N.M.R. SPECTROSCOPY

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ABSTRACT

Dextran T 10, elaborated by *Leuconostoc mesenteroides* NRRL B-512, was oxidised with aqueous bromine at pH 7.0. The resulting oxodextran and its methoximated derivative were analysed by ^{13}C-n.m.r. spectroscopy. The total amount of keto groups and their positions were established. Assignments of the ^{13}C signals were made by referring to spectra of the corresponding methyl glucosiduloses and an oxodextran having most of the carbonyl groups at position 3 of the glycopyranosyl residues. In accordance with the mechanism for bromine oxidation of mono- and di-saccharides, the glucopyranosyl residues of dextran were oxidised mainly at C-2 and C-4. Over-oxidation resulted in a small proportion of acidic, ring-cleavage products.

INTRODUCTION

Carbonyl groups introduced into polysaccharides by oxidation allow further modification of the glycosyl residues. For example, amino groups can be introduced *via* reductive amination[1], epimerisation can be achieved *via* reduction[1], and deoxy and branched-chain glycosyl residues can be generated[2,3].

The selective oxidation of specific, secondary hydroxyl groups in carbohydrates usually requires partially protected derivatives. However, studies of the bromine oxidation of glycopyranosides[4–6] have shown that hydroxyl, methoxyl, and glycosyl groups in *syn*-diaxial relation to an axial hydrogen prevent oxidation at the latter position. Thus, the aglycon group of an α-D anomer in the $^4C_1$ conformation protects position 3, the axial HO-4 in galactopyranosides protects position 2, and the axial HO-2 in mannopyranosides protects position 4. In order to establish whether similar effects operate in polysaccharides, the oxidation of dextran with bromine has been investigated.

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Keto-glycosyl residues are rapidly degraded in alkali and are also acid-sensitive. Analysis of oxoglycosides of low molecular weight can be performed by converting them into more-stable *O*-methyloximes, which are relatively stable in alkali and, for example, can be trimethylsilylated. Such derivatised *O*-methyloximes can be characterised by ^{1}H-n.m.r. spectroscopy and by g.l.c.–m.s. Other methods must be applied for the analysis of the distribution of carbonyl groups in oxidised polysaccharides. In the present publication, this has been done by ^{13}C-n.m.r. spectroscopy. By using the gated, decoupling technique to suppress the n.O.e., in combination with a sufficiently long, pulse repetition time to achieve complete relaxation, ^{13}C-n.m.r. signals can be integrated properly[7].

RESULTS AND DISCUSSION

Dextran **1** (T 10; elaborated by *Leuconostoc mesenteroides* NRRL B-512), which is a (1→6)-linked α-D-glucan having side chains (5%) linked to O-3 of the D-glucosyl residues, was oxidised with aqueous bromine at room temperature and pH 7.0, to give the modified polymer **3**: ~1 mol of bromine per glycosyl residue was used and the oxidant was consumed within 7 h. Treatment of **3** with methoxylamine hydrochloride gave the more-stable *O*-methyloxime **5**.

In order to assign the signals in the ^{13}C-n.m.r. spectra of the polymers **3** and **5**, an oxodextran (**2**) having most of the carbonyl groups at position 3 (see below), the methyl glycosides of α-D-*arabino*-hexopyranosidulose^{+} (**6**), α-D-*ribo*-hexopyranosid-3-ulose^{+} (**9**), and α-D-*xylo*-hexopyranosid-4-ulose^{+} (**12**), and their respective *O*-methyloxime derivatives (**4**, **8**, **11**, and **14**) were used as reference compounds. In aqueous solution, the 2-ulose **6** is almost completely converted into the hydrated form (**7**) and the 4-ulose **12** is partly converted into **13**, whereas the 3-ulose **9** exists almost exclusively in the keto form. Assignments (Table I) of the signals in the ^{13}C-n.m.r. spectra of the methyl glycosiduloses and their *O*-methyloximes were made by increment-calculation and decoupling techniques. The oxodextran **2**, reported to have

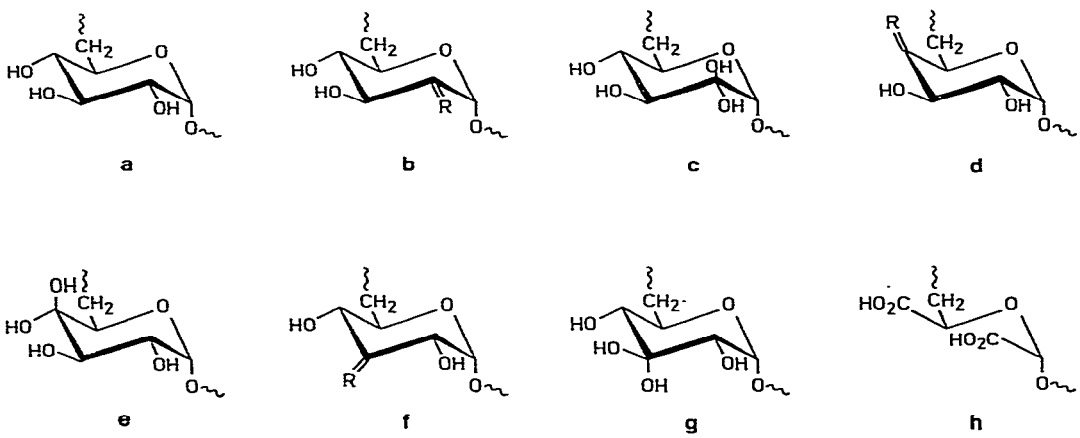

TABLE I

^{13}C-N.M.R. DATA FOR OXIDISED METHYL GLYCOSIDES **7**, **9**, **12**, AND **13**, AND METHOXIMATED DERIVATIVES **8**, **11**, AND **14**

Atom	*Chemical shifts (p.p.m.)*							
	7	**8**	**9**	**11**[a]		**12**[b,d]	**13**[b]	**14**[d]
C-1	101.56	91.57	101.95	98.92	99.91	99.51	99.42	97.06
C-2	93.43	154.12	74.71[c]	68.32[c]	67.05[c]	72.36	70.49	70.24
C-3	74.31	70.94	207.24	155.21	154.37	75.73	73.56	71.51
C-4	69.02	72.46[c]	74.52[c]	64.60[c]	70.93[c]	204.94	94.05	154.72
C-5	72.38	72.27[c]	71.92	73.62	74.35	74.03	72.69	70.24
C-6	61.24	60.90	60.91	61.00	61.00	59.08	59.42	60.85
OCH_3	55.19	55.01	55.49	55.52	55.52	56.05	55.33	56.51
$NOCH_3$	—	62.32	—	63.30	63.30	—	—	62.34

[a]Equilibrium mixture of *E* and *Z* forms. [b]Equilibrium mixture: 35% of **12** and 65% of **13**. [c]Pairwise uncertain assignment. [d]Compound adopts boat or skew conformation.

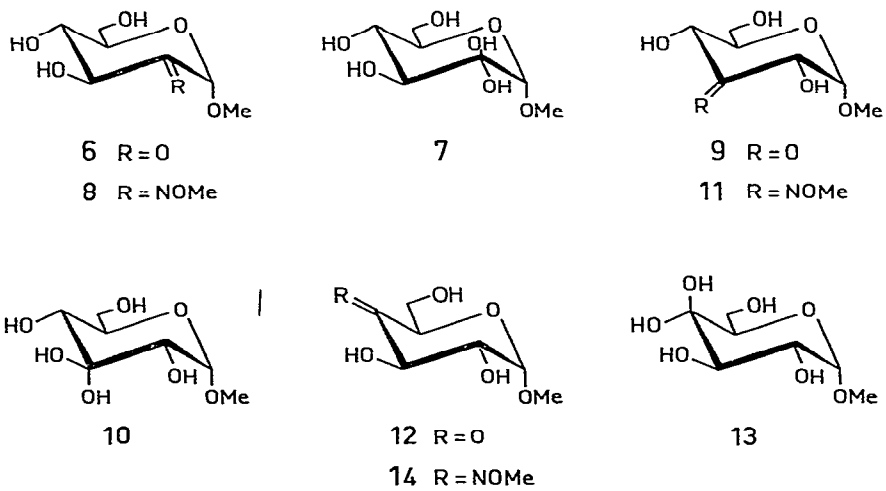

Fig. 1. Sub-units in dextran (**1**), oxodextrans (**2** and **3**), and the *O*-methyloxime derivatives (**4** and **5**); R = O or NOMe.

most of the keto groups at position 3, was prepared by oxidising (with dimethyl sulfoxide–acetic anhydride[8]) dextran having positions 2 and 4 blocked by phenylboronic acid. Reduction of **2** and then hydrolysis gave glucose, allose, and small proportions of mannose and galactose, indicating that some oxidation had also occurred at C-2 and C-4 of the D-glucopyranosyl residues.

The ^{13}C-n.m.r. data for **2**–**5** are presented in Table II. The assignments were made by careful integration and by comparison with the data in Table I. Signals

TABLE II

^{13}C-N.M.R. DATA FOR COMPOUNDS 1–5[a]

Atom	**1**[b]		**2**			**3**			**4**[f]			**5**		
	δ ($^1J_{C,H}$, Hz)	*Integral*[c]	*δ*	*Integral*[c]	*Sub-unit*[d]	*δ* ($^1J_{C,H}$, Hz)	*Integral*	*Sub-unit*	*δ*	*Integral*	*Sub-unit*[d]	*δ* ($^1J_{C,H}$, Hz)	*Integral*	*Sub-unit*[d]
C-1	97.84(171.8)	100.0	100.30	14.5	e,f	103.76(~166)	~3.4	b	98.45	8.1	f	98.41(174.0)	13.1	d,f
			97.91	81.9	a	100.08(172.0)	19.4	c,f	97.93	81.4	a	97.88(170.0)	46.8	a,h
			96.44	3.6	g	97.83(173.0)	77.2	a,d,e,h	97.38	2.9	f	97.22(n.d.)	~2.0	f
									96.09	1.5	b	95.93(170.0)	23.0	d,b
									90.05	6.1	b	89.99(175.0)	15.1	b
C-2	71.57(142.4)	100.3	207.19	9.6[e]	b	207.23	6.3	b	155.31*	0.3	b	154.69*	21.7	b
			93.45	6.4	c	93.39	18.0	c	153.36	1.7	b	153.39*	19.6	b
			71.62	101.4	a	71.58(142.0)	~61.0	a	71.64	n.d.	a	71.59(142.0)	68.6	a
						176.82* }	18.1	h				176.80*	14.7	h
						176.74* }		h						
C-3	73.58(142.0)	92.4	207.19	[e]	f	207.23	[e]	f	154.23*	0.6	f	155.03*	[e]	f
			93.84	3.5	g	93.79	19.9	g	154.97	2.1	f	73.56 } (149.0)	58.3	a
			73.61	101.1	a	73.55(144.0)	~80.0	a	73.62	n.d.	a	73.42 }		
C-4	70.33(143.6)	103.9	70.40	112.4	a	204.22	6.1	d	70.43	n.d.	a	154.90*	[e]	d
						93.79	[e]	e				153.39*	[e]	d
						70.34(144.0)	~69.0	a				70.35(148.0)	79.4	a
						176.35*	[e]	h				174.20*	6.9	h
						174.00*	6.3	h						
C-5	69.71(145.8)	117.9	69.76	117.3	a	69.73(145.0)	~65.6	a	69.78	n.d.	a	69.75(144.0)	69.1	a
C-6	65.71(144.7)	104.9	65.76	98.3	a	65.76(146.0)	63.3	a	65.78	n.d.	a	65.75(145.0)	70.2	a
OMe	—		—			—			62.46 }	22.0	b,f	62.43 } (146.0)	47.0	b,d,f
									62.37 }		b,f	62.31 }		

[a]The integral is obtained from inverse-gated-decoupled spectra, unless otherwise stated. The sum over all C-1 signals is normalised to 100; *designates uncertain assignments. [b]Assignments taken from ref. 12. [c]Integral obtained from a spectrum with 90° pulse width and a pulse repetition time of 0.475 s. [d]See Fig. 1; missing sub-unit carbon atoms are not assigned. [e]Overlapping signals; their total integrals are specified only once in this list. [f]Measured with a 45° pulse width and a pulse repetition time of 0.475 s. [g]*E* and *Z* forms are not assigned specifically.

arising from the side chains are not considered, as they are too small to interfere with the interpretation of the spectra. For the methoximated compounds and sub-units, which often showed separate signals from the (*E*)- and (*Z*)-isomers, the C-1 shifts were particularly useful. The sub-units **b**, **d**, and **f** of **2** and **3** (Fig. 1) were assumed to parallel the respective methyl glycosiduloses (**6**, **12**, and **9**) as to the position of the hydration equilibrium. By using this assumption and by comparison with the relevant shifts for **7**, **9**, **12**, and **13**, the signals from the oxidised carbon atoms of **2** were identified. Hence, it was concluded that **2** is oxidised only at C-2 and C-3, in the ratio 0.7:1. Since this finding must also apply to **4**, it was possible to identify the C-1 signals in the spectrum of **4** by reference to the C-1 shifts for **8**, **11**, and **14**.

The absence of units in **2** oxidised at C-4 indicates that the reduction of **2** is accompanied by some isomerisation **f** → **d**, since a trace of galactose was obtained in the sugar analysis of reduced **2**. The ratio between the integrated signals for C-1 of **4b** and **4f** [both (*E*)- and (*Z*)-forms], respectively, and for C-1 of the **4a** units reveals a total degree of oxidation of 19%. A comparison of the integrated signals for the *O*-methyl groups of methoximes with the integrated signals for all anomeric carbon atoms indicates a total degree of oxidation of 22%. This minor difference demonstrates the accuracy of the integration method.

Assignments of the signals in the spectrum of oxodextran **3** were made by a comparison with the spectra of **2**, **7**, **9**, **12**, and **13**. The **3h** units, which result from ring cleavage, gave signals for carboxyl carbon atoms at δ 174.00–176.82 and for C-1 at δ 97.83 (Table II). A comparison of the integrated signals for the carbonyl and hydrated carbonyl carbons with those for all anomeric carbon atoms shows that 50% of the glycopyranosyl residues in **3** are oxidised. In the sugar analysis of reduced **3**, a minor amount of allose was detected in addition to the expected glucose, mannose,

TABLE III

PROPORTIONS (%) OF THE SUB-UNITS IN COMPOUNDS **2–5**

Sub-unit	**2**	**3**	**4**	**5**
a	80.0	37.5	82.5	36.0
b	1.5	3.5	6.0	15.0
c	6.5	18.0	1.5	4.0
Total C-2 oxidation	8.0	21.5	7.5	19.0
f	8.0	3.0	3.0	2.0
g	3.5	1.0	8.0	5.5
Total C-3 oxidation	11.5	4.0	11.0	7.5
d	—	6.0	—	7.5
e	—	19.0	—	19.5
Total C-4 oxidation		25.0		27.0
Acidic, ring-cleavage products (**h**)	—	12.0	—	11.0

and galactose. If **f** and **g** units are present, the signal for C-3 of **3f** should appear at δ ~207.2, that for C-3 of **3g** at δ ~93.8, and that for C-1 of **3f** at δ ~ 100.3. The data in Table II show that these signals are overlapped by signals from **3b** and **3d**. Further, the signal from C-1 of **3g** is hidden under the shoulder of the signal from C-1 of the **3a** units. In comparison with the signal from C-1 of **3b**, the carbonyl signal from C-2 of **3b** at δ 207.23 has a higher value than expected. This observation, together with the results from a sugar analysis of reduced **3**, indicates the presence of 3.0% of **3f**. The ^{13}C-n.m.r. spectrum of **2** reveals that the proportion between **f** and **g** units in oxidised dextrans is 2.3. Hence, 1.2% of **3g** is expected in **3**. Thus, **3** contains 25.0% of units oxidised at C-4, 4.0% oxidised at C-3, and 21.5% oxidised at C-2 (Table III). Comparison of the integrated signal for carboxyl carbon atoms at δ 175 with those for all anomeric carbon atoms indicates the presence of 12.0% of units resulting from ring cleavage. This is in agreement with the value (11.0%) obtained by titration.

Finally, a comparison of the spectra of the oxodextran *O*-methyloximes **4** and **5** revealed the C-1 signals in the spectrum of **5**. A comparison of the integrated signals for the anomeric carbon atoms implies that units oxidised at C-2 (**5b**), C-3 (**5f**), and C-4 (**5d**) are present in the relative proportions 2.3 : 1 : 3.4, which, in comparison with the results given above, yields a higher value for units oxidised at C-3. The total amount of methoximated glycosylulose residues, as measured from elemental analysis of **4**, was 43%.

As expected[4-6], the D-glucopyranosyl residues of dextran are oxidised with bromine mainly at C-2 and C-4. The small proportion of D-glucosyl-3-ulose residues found most probably originates from isomerisation of the 2- and 4-ulose units. Taking the integrated signal for all anomeric carbon atoms as a reference, the proportions of methoximated glycosylulose residues can be calculated as 53, 47, and 41% from the integrated signals for anomeric carbon atoms of oxidised units, N-OMe carbon atoms, and C=N carbon atoms, respectively. The rather low value of the integrated signal for the C=N carbon atoms is most probably caused by their long relaxation-times. If further oxidation occurs on some glycosylulose residues, the pyranoid rings are cleaved and dicarboxylic acids (**3h**, Fig. 1) are formed. Assuming that oxidation occurs quantitatively, the molar consumption of bromine (y) is given by:

$$y = X(\text{C-2}_{ox}) + X(\text{C-4}_{ox}) + 4X(\text{cleavage}),$$

where X(i) represents the molar amount of the corresponding sub-unit (i). If the values from the ^{13}C-n.m.r. spectrum of **3** are inserted in this equation, a calculated consumption of 0.98 mol of bromine per mol of glucopyranosyl residue is obtained. This is in agreement with the actual amount consumed (0.97 mol).

EXPERIMENTAL

General methods. — Solutions were concentrated at reduced pressure below 40°. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. G.l.c. was performed with a Packard 427 instrument, fitted with a flame-ionisation detector.

Separations were performed on a glass column (240 × 0.15 cm) containing 3% of OV 225 on Gas Chrom Q (100–120 mesh). N.m.r. spectra (^1H and ^{13}C) were recorded on a Bruker WH 270 instrument at 298 K with a total data memory 32 k and spectral width 1724.1 Hz. The sample concentrations were 25% in D_2O, and acetone was used as internal standard (δ 30.5). T_1-values were determined from inversion–recovery experiments, using an exponential fit. Integrations were performed on inverse gated-experiments, using a 12° flip angle ($\tau_{90°}$ = 28.2 μs) and a pulse repetition time[7] of 1.2 s.

Preparation of oxidised methyl glycosides. — Methyl α-D-manno- and -galacto-pyranoside were oxidised with bromine, as described in ref. 4, to yield methyl α-D-*arabino*-hexopyranosidulose (**6**) and methyl α-D-*xylo*-hexopyranosid-4-ulose (**12**). Methyl α-D-*ribo*-hexopyranosid-3-ulose (**9**) was prepared as described in ref. 9. The methoximes (**8**, **11**, and **14**) of **6**, **9**, and **12** were prepared as described in ref. 4. The n.m.r. data of **6**, **9**, and **12**, the hydrated forms (**7** and **13**) of **6** and **12**, and the methoximes **8**, **11**, and **14** are given in Table I.

Bromine oxidation of dextran. — Dextran (T 10; elaborated by *Leuconostoc mesenteroides* NRRL B-512) (5 g, 31 mmol of D-glucosyl residues) was dissolved in water (250 ml). An aqueous solution (0.1M, 300 ml) of bromine was added and the volume was adjusted to 900 ml. The mixture was kept at room temperature, and the pH was kept constant at 7.0 by addition of 0.5M sodium hydroxide using a Methrom E 300 B pH meter. When the oxidant had been consumed (after 7 h), the pH was adjusted to 5 and the mixture was concentrated to 250 ml. One portion (150 ml) was withdrawn, dialysed against distilled water, and freeze-dried, yielding the oxidised dextran **3** (see Table II for n.m.r. data). To a second portion (50 ml) was added methoxylamine hydrochloride (1.0 g), and the pH was adjusted to 4.0. The solution was stirred at 50° for 2.5 h, dialysed against distilled water, and freeze-dried, yielding the methoximated dextran **5** (see Table II for n.m.r. data) (Found: C, 44.04; H, 4.81; N, 3.43%).

A third portion (50 ml) was reduced with sodium borohydride (0.5 g), acidified (acetic acid), dialysed against distilled water, and freeze-dried. Sugar analysis[10] revealed the presence of glucose, galactose, mannose, and allose in the proportions 90:4:9:1. A sample (100 mg) of reduced **3** was treated with Dowex 50W (H^+) resin. On titration with 0.1M sodium hydroxide[11], the polymer was found to contain 1.29 mequiv. of carboxylic functions/g.

Dimethyl sulfoxide–acetic anhydride oxidation of dextran 2,4-phenylboronate. — Oxidation was performed as described by de Belder *et al.*[8], to give the oxidised dextran **2**. Part of **2** was treated with methoxylamine, as described above, to yield the methoximated dextran **4**. Another part of **2** was reduced with sodium borohydride as described above. The relative proportions of glucose, galactose, allose, and mannose in the sugar analysis of reduced **4** were 33:2:9:1.

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