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Preliminary communication

An apparent $^4C_1 \rightarrow {}^1C_4$ conformational transition in periodate-oxidised alginate, induced by changes in pH and ionic strength. the anomeric effect in glycuronans

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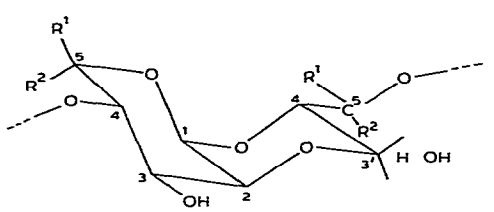
When sodium alginate is oxidised in aqueous periodate at pH ~4 and low ionic strength, only 0 44 mol of periodate is consumed per non-terminal, hexuronic acid residue[1] This result is obtained regardless of the relative proportion of β-D-mannopyranuronic and α-L-gulopyranuronic acid residues in the chains (ManA/GulA ratio)[1] It corresponds closely to the value calculated[1,2] on the assumption that oxidation of any residue immediately protects the two adjacent, unoxidised residues from subsequent oxidation For protection of only one adjacent residue the calculated oxidation-limit is 0 64 mol per hexuronic acid residue[1,3]

Methylation analysis[1] and supporting evidence[1,4] have established that the protective mechanism involves the formation of 6-membered hemiacetal rings (**1** and **2**) between the aldehyde groups of oxidised hexuronic acid residues and the closest hydroxyl groups on unoxidised residues adjacent to them in the chains

It will be noticed that the aldehyde group originating from C-3 of the oxidised residue could, in principle, form a hemiacetal with HO-2 of an unoxidised neighbour, either when the latter is in its $^4C_1$ (**1**) or $^1C_4$ conformation (**3**) On the other hand, the aldehyde group originating from C-2 of the oxidised residue could form a hemiacetal with HO-3 of an unoxidised neighbour only when the latter is in its $^4C_1$ conformation (**2**), in the $^1C_4$ conformation (**4**), O-3 and O-4 are trans-diaxial, and too far apart to allow ring-closure

For long sequences of contiguous β-D-mannopyranuronic acid residues, it is not surprising that both kinds of hemiacetal (**1** and **2**, $R^1 = CO_2^-$, $R^2 = H$) are formed, because the $^4C_1$ conformation is predicted by free-energy calculations[5], and confirmed by X-ray fibre analysis[6] and n m r spectroscopy[7,8] On the other hand, the same methods all indicate that the α-L-gulopyranuronic acid residues in alginates exist mainly in the $^1C_4$ conformation[5,7–9], even though the expected free-energy difference between the two conformers is, in this case, much smaller[5] Evidently, therefore, under the conditions of periodate oxidation used earlier (25mM sodium metaperiodate at 20°, either unbuffered or in the presence of sodium-salt buffers of ionic strength not exceeding 0 2), formation of **2** ($R^1 = H$, $R^2 = CO_2^-$) with unoxidised α-L-gulopyranuronic acid residues entails a $^1C_4 \rightarrow {}^4C_1$ transition, at the expense of some conformational energy



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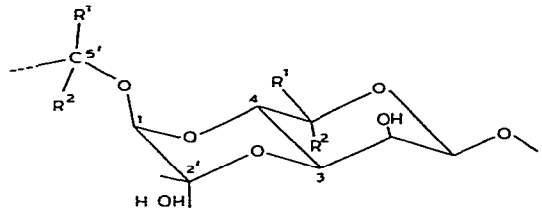

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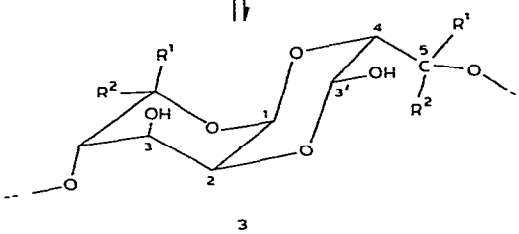

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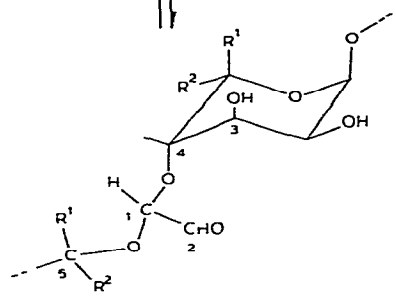

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We now report that, when these periodate oxidations were repeated under the same conditions[1], but in the presence of sodium chloride at a concentration of 2 5–5 0M, the final oxidation-limit increased above the theoretical value for bilateral protection (0 44 mol), and the extent of the increase was clearly correlated with the proportion of α-L-gulopyranuronic acid residues in the sample For an alginate having a very low content of L-guluronic acid (ManA/GulA = 13 5, hereinafter referred to as "mannuronan"), the increase was negligible, whereas for an alginate having ManA/GulA = 0 38, the final oxidation-limit in 2 5M sodium chloride was 0 62 mol With a fragment of alginate (hereinafter referred to as "guluronan") having $\overline{d\,p}_n$ ~15 and composed almost entirely of α-L-gulopyranuronic acid residues, there was, in 2 5M sodium chloride, an initial, rapid uptake of 0 64 mol of periodate per non-terminal residue, followed by a much slower consumption of further periodate, these results were obtained after correction for end-group consumption and over-oxidation, as described elsewhere[10]

Conversion of the alginates from sodium salts into propylene glycol esters did not eliminate the salt effect, but enhanced it Even in the absence of added salt, the propylene glycol ester of an alginate having ManA/GulA = 0 38 showed an oxidation-limit of 0 60 mol , and this increased to 0.70 mol in 2 5M sodium chloride Lowering the pH had a similar effect, because of the insolubility of alginates at low pH, this was shown by first oxidising them to their "normal" limit of 0 44 mol at pH ~4 and low ionic strength, adding various mixtures of acid and salt, and then measuring the increase in periodate uptake

Since opening a hemiacetal ring eliminates the chirality of the aldehydic carbon atom, it was expected that addition of salt or acid to a solution of periodate-oxidised guluronan would have a large effect upon its optical rotation, which would not be shown

by periodate-oxidised mannuronan This was indeed observed, and it indicates a simple method for comparing the effects of different salts upon ring-opening

Evidence that it is mainly* the hemiacetal 2 (R^1 = H, R^2 = CO_2^-) that is opened by salt, while the other hemiacetal (3, R^1 = H, R^2 = CO_2^-) opens to a comparatively small extent*, was obtained by selective oxidation with aqueous bromine Earlier work[4] had shown that, when sodium alginate is oxidised to its "normal" limit of 0 44 mol and then treated with aqueous bromine at pH ~4 5, half of the hemiacetal rings are quantitatively converted into lactones, while the other half are very resistant to oxidation ** The easily oxidised hemiacetal must have been 1 (or 3), because acid hydrolysis of the product gave only traces of glyoxylic acid, detected colorimetrically[12,13] Guluronan was therefore oxidised, first with periodate at low ionic strength to the 0 44 mol limit, and then with aqueous bromine as before[4], but in the presence of 2 5M sodium chloride The amount of rapidly consumed bromine (measured titrimetrically[4]) was now twice that observed previously[4], and the equivalent weight of the isolated product (determined by titration with cetylpyridinium chloride[14]) indicated that rather more than half of the aldehyde groups had been converted into free carboxylic acid, while the remainder gave lactones as before The product yielded approximately† the expected amount of glyoxylic acid after acid hydrolysis

Direct evidence that the ring-opening of 2 (R^1 = H, R^2 = CO_2^-) is accompanied by the expected reconversion of the unoxidised α-L-gulopyranuronic acid residue into its energetically preferred $^1C_4$ conformer (4, R^1 = H, R^2 = CO_2^-) is lacking, because the n m r spectra have not yet been interpreted However, indirect evidence was obtained, when it was observed that the pK_a of the oxidised guluronan in its free-acid form was considerably higher than that of the same material after reduction with borohydride, but decreased to about the same value as that of the reduced material when sodium chloride was added to both solutions Since equatorial carboxyl groups generally have a lower pK_a than axial carboxyl groups (because the carboxylate anion is more easily stabilised by solvation when it is equatorial[15]), this result supports the idea of a $^4C_1 \rightarrow {}^1C_4$ transition, induced by salt

It remains to be decided whether the $^4C_1 \rightarrow {}^1C_4$ transition is related to ring-opening as cause is to effect, or *vice-versa* This dilemma led us, several years ago, to investigate the possibility that inorganic ions, in aqueous solution may change the magnitude of the anomeric effect[16,17] The results[18] clearly indicated that they do, and may be interpreted[19] in this case, as a selective salting-out[20] of the conformer in which O-1 is equatorial We therefore suggest that the driving force for the transition was the anomeric effect, which was increased in magnitude by sodium chloride The effects of esterification and low pH in

*These expressions refer to the positions of the equilibria that are very rapidly established between the aldehydic and cyclic hemiacetal forms after oxidation of any residue[2,3]

**The work of Barker *et al* [11] suggests that 2 should be resistant in its closed-ring form In the intermediate hypobromite ester, the bulky bromine atom has to become antiperiplanar with the anomeric hydrogen atom (to eliminate HBr) and in 2, but not in 1, it would encounter syn-axial interactions in both configurations of the hemiacetal carbon atom

†The colorimetric method[12,13] is not very accurate, because the colour fades rapidly

promoting the transition may be seen as examples of the reverse anomeric effect[21], or the enhancement in the magnitude of the anomeric effect that is known to be brought about by electron-withdrawing substituents at C-5 of a pyranose ring[16]

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REFERENCES

1 T J Painter and B Larsen, *Acta Chem Scand*, 24 (1970) 813–833
2 T J Painter and B Larsen, *Acta Chem Scand*, 24 (1970) 2366–2378
3 T J Painter and B Larsen, *Acta Chem Scand*, 24 (1970) 2724–2736
4 M F Ishak and T J Painter, *Acta Chem Scand*, 27 (1973) 1321–1327
5 S J Angyal, *Aust J Chem*, 21 (1968) 2737–2760
6 E D T Atkins, W Mackie, I A. Nieduszynski, K D Parker, and E E Smolko, *Biopolymers*, 12 (1973) 1865–1878
7 A Penman and G R Sanderson, *Carbohydr Res*, 25 (1972) 273–282
8 H Grasdalen, B Larsen, and O Smidsrǿd, *Carbohydr Res*, 56 (1977) C11–C15
9 E D T Atkins, W Mackie, I A Nieduszynski, K D Parker, and E E Smolko, *Biopolymers*, 12 (1973) 1879–1887
10 T J Painter, *Carbohydr Res*, 55 (1977) 95–103
11 I R L Barker, W G Overend, and C W Rees, *J Chem Soc*, (1964) 3254–3262
12 M Paget and R Berger, *Bull Biol Pharm*, (1938) 70–72, *Chem Abstr*, 32 (1938) 4908
13 M. J Clancy and W J Whelan, *Chem Ind (London)*, (1959) 673–675
14 J E Scott, *Methods Biochem Anal*, 8 (1960) 145–197
15 R D Stolow, *J Am. Chem Soc*, 81 (1959) 5806–5811
16 R U Lemieux, in P De Mayo (Ed), *Molecular Rearrangements*, Vol 2, Interscience, New York, 1964, p 740
17 J F Stoddart, *Stereochemistry of Carbohydrates*, Wiley–Interscience, New York, 1971, pp 72–87.
18 T J. Painter *Acta Chem Scand*, 27 (1973) 2463–2484, 3839–3860
19 T J Painter, *J Chem Soc, Perkin Trans 2*, (1976) 215–228
20 F A Long and W F McDevit, *Chem Rev*, 51 (1952) 119–169
21 C B Anderson and D T Sepp, *J Org Chem*, 33 (1968) 3272–3276