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Transient Hemiacetal Structures Formed during the Periodate Oxidation of Xylan

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When a homopolymeric, β-1,4-linked xylan, having a number-average degree of polymerisation of 42, was oxidised in 25 mM sodium metaperiodate at 20°, the second-order rate-constant decreased rapidly as reaction progressed, and became constant at about 2 % of its initial value after the consumption of 0.60 mole of periodate per anhydropentose unit.

At this stage, Barry degradation indicated the absence of contiguous, unoxidised xylose residues in the chains, and methylation showed that the hydroxyl groups of the remaining, unoxidised residues were largely involved in hemiacetal structures.

Reduction of the xylan with sodium borohydride at any stage in its oxidation restored the second-order rate-constant to approximately its initial value.

Xylopentaose and xylotetraose also showed large decreases in rate with increasing degree of oxidation, whereas xylotriose, xylobiose and xylose obeyed second-order kinetics throughout.

It was concluded that deviation from second-order kinetics was specifically associated with the presence of contiguous, non-terminal xylose residues in the chains, and was caused by the formation of 6-membered hemiacetal rings between the aldehyde groups of oxidised xylose residues and the closest hydroxyl groups on neighbouring, unoxidised xylose residues. The kinetic data indicated that these hemiacetal structures were present in equilibrium with the free aldehydic forms, the position of the equilibrium lying about 85 % on the side of the former.

The anomalously low consumption of periodate by alginate (0.45 – 0.55 mole per anhydrohexuronic acid residue)[1] has been traced[2,3] to the formation of stable, 6-membered hemiacetal rings between the aldehyde groups of oxidised hexuronic-acid residues and the closest hydroxyl groups on adjacent, unoxidised residues in the chains. This behaviour could not be attributed to some unique influence of the carboxyl groups in alginate, and it was suggested that it should occur generally in 1,4-linked polysaccharides.[3] This idea is compatible with the known fact that most polysaccharides will ultimately consume sufficient periodate to cleave all the *vic*-diol groups initially

present, if it is assumed that the inter-residue hemiacetals exist in equilibrium with the corresponding, open-chain aldehydic forms. Oxidation could then proceed beyond the stage[3] of maximum possible hemiacetal formation, but at a rate which is diminished to an extent determined by the position of the equilibrium.

The β-1,4-linked xylans are particularly interesting model substrates in this connection, because they resemble alginate in lacking a hydroxyl group at position 6. Such a hydroxyl group might be expected to diminish any tendency for inter-residue hemiacetal formation, by competitively forming intra-residue hemiacetal structures.[4-6] Furthermore, xylans of this type should not be susceptible to overoxidation,[7] and should therefore permit an unequivocal interpretation of any consumption of periodate taking place after the stage of maximum possible hemiacetal formation[3] has been reached.

Native, β-1,4-linked xylans are reported[8,9] to consume about 1 mole of periodate per anhydropentose unit, but only after prolonged oxidation in unusually concentrated periodate (0.25 M). When xylan is oxidised under more usual analytical conditions, for example in 0.025 M periodate at 20°, the reaction curve bears a striking resemblance to that given[1,3] by sodium alginate. Such a curve was, for example, published recently by Zitko and Bishop[10] in connection with a comparative study of the oxidative efficacies of periodate and lead tetra-acetate. It showed a well-defined oxidation "limit" after the consumption of 0.48 mol of periodate per anhydropentose unit, and differed relevantly from the curve for alginate only in that oxidation continued at a measurable rate after this limiting condition was reached.

These facts cannot be readily attributed to the insolubility of xylan in neutral, aqueous media, because its solubility increases upon oxidation.[8,9] Moreover, Zitko and Bishop[10] observed the same, clear oxidation "limit" when oxidation was carried out, in dimethyl sulphoxide, with lead tetra-acetate; the only visible effect of the good solvent was to enhance the rate at which the limiting condition was reached.

In the work now described, the oxidation was studied of a homopolymeric fragment of maize-cob xylan, having a number-average degree of polymerisation of 42. This material dissolved quite freely in water, but gave a turbid, and, therefore, possibly not a true solution. The data were therefore supplemented and confirmed by studies of the oxidation of water-soluble oligosaccharides derived from the xylan.

THEORY

In the previous investigation of sodium alginate,[2,3] it was found that the anomalous oxidation-limit could be predicted quantitatively on the assumption that oxidation is initially random, and that hemiacetal formation, leading to the protection of the two nearest neighbours of the oxidised units from subsequent attack, ensues as a relatively rapid process. The theoretical oxidation-limit, corresponding to the degree of oxidation at which every unoxidised unit has at least one oxidised unit as a nearest neighbour, was calculated by a synthetic method, consisting in a direct simulation of the assumed process in the digital computer.

In the present work, it is again useful to know the degree of oxidation corresponding to maximum possible hemiacetal formation, when the latter process is relatively rapid and largely complete. Since these oxidation-limits are probably of rather general significance, the opportunity is taken of describing the derivation of a simple recursive formula, permitting the accurate calculation of the oxidation-limit for a chain of any length.

Imposing the condition of random attack, a population of chains, all N units in length, is divided into N equal subgroups, each composed of chains that suffered their first oxidative attack in the same position relative to the terminal units. For convenience, it may be assumed that the two terminal units in any chain are distinguishable, and the units are numbered consecutively, beginning with one of these.

In all chains that were initially attacked on the first unit, the second unit will be protected, so that the mean degree of oxidation for the first pair of units in this subgroup is 1/2. The mean degree of oxidation of the remaining sequences of $(N-2)$ contiguous units is clearly identical with that (D_{N-2}) of a complete population of chains, $(N-2)$ units in length. The overall oxidation-limit of this subgroup can then be written:

$$(d_N)_1 = (2/N)(1/2) + [(N-2)/N]\ D_{N-2}$$

In the subgroup whose chains were first attacked on the second unit, the first and third units will be protected, so that the mean degree of oxidation of this first triplet is 1/3, while that of the remaining $(N-3)$ units can be written as D_{N-3}. The overall oxidation-limit of this subgroup is then:

$$(d_N)_2 = (3/N)(1/3) + [(N-3)/N]D_{N-3}$$

Proceeding along the chains in this manner, it is found that the oxidation-limit of the nth subgroup can be written:

$$(d_N)_n = (3/N)(1/3) + [(n-2)/N]D_{n-2} + [(N-n-1)/N]D_{N-n-1}$$

where $1<n<N$.

The last subgroup, however, must be considered separately like the first, and its oxidation-limit, $(d_N)_N$, is clearly equal to $(d_N)_1$.

The oxidation-limit of the entire population is now given by the arithmetic mean of those of the individual subgroups, each being weighted equally:

$$D_N = (1/N)^2[N + 2(N-2)D_{N-2} + \sum_{n=2}^{N-1}[(n-2)D_{n-2} + (N-n-1)D_{N-n-1}]]$$

By priming this expression with the values of D_1 and D_2 (1 and 1/2, respectively), the degree of oxidation for chains of any desired length can be arrived at in a stepwise manner. This is accomplished with particular ease in the digital computer, and a selection of values is given in Table 1. Also given in Table 1 are the corresponding values, $(D_N)_{\text{corr.}}$, calculated on the assumption that the two terminal residues of each chain do not participate in inter-residue hemiacetal formation, and that each consumes two moles of periodate in an independent manner. These quantities are given by the formula:

$$(D_N)_{\text{corr.}} = (1/N)[4 + (N-2)D_{N-2}]$$

Table 1. Theoretical oxidation-limits, expressed as moles of periodate consumed per monosaccharide unit, and calculated on the assumption of instantaneous and complete protection of the nearest neighbours of oxidised units from subsequent oxidation. D_N is the limit for an N-mer, assuming participation of all units and only one oxidisable site in each. $(D_N)_{corr.}$ is the limit for an N-mer, assuming non-participation of terminal units, two oxidisable sites in each terminal unit, and one oxidisable site in non-terminal units.

N	D_N	$(D_N)_{corr.}$	N	D_N	$(D_N)_{corr.}$
2	0.500	2.000	15	0.452	0.661
3	0.556	1.667	20	0.447	0.604
4	0.500	1.250	25	0.444	0.569
5	0.493	1.133	30	0.442	0.547
6	0.481	1.000	40	0.440	0.518
7	0.475	0.924	42	0.439	0.514
8	0.469	0.861	50	0.438	0.501
9	0.465	0.814	75	0.436	0.478
10	0.462	0.775	100	0.435	0.467

EXPERIMENTAL

The experimental conditions were mostly the same as those used in the previous study of sodium alginate,[3] and only significant variations are reported here.

Materials and methods. Crude xylan, containing about 10 % of arabinose and traces of uronic acids, was prepared from maize (corn) cobs as described by Adams [11] and the oligoxylosides were prepared from it essentially as described by Wolfrom and Franks.[12]

The xylan dispersed in water as a milky colloid, and homopolymeric material was prepared from it by incubating a 1 % (w/v) suspension (500 ml) with enzyme (500 mg) at 37° for 4 days. The enzyme was a commercial pectinase (Th. Schuchardt AG, München, Germany), showing high L-arabinofuranosidase activity [13] and relatively weak xylanase activity.[14] The enzymic digest was dialysed exhaustively against water, and the non-diffusible polysaccharidic material was precipitated by addition of an equal volume of ethanolic potassium acetate (2 % w/v). The precipitate was collected by centrifugation, washed with 50 % (v/v) aqueous ethanol, dispersed in water (200 ml), dialysed thoroughly, and then freeze-dried.

The protein-free product (yield, 2 g), which dispersed freely upon warming in water to give a stable, slightly turbid solution, had a number-average degree of polymerisation (reducing end-group assay [15]) of 42. After complete acid-hydrolysis, xylose was the only product detectable on chromatograms and, by the phenol method,[16] the material analysed as 100.5 % xylose.

Both analytical and preparative oxidations were normally carried out at 20° in unbuffered, 25 mM sodium metaperiodate, with a substrate concentration of about 7.5 mM with respect to *vic*-diol groups. However, in the experiments with borohydride-reduced, partially oxidised xylans, the concentrations were decreased in accordance with the amount of oxidation that the substrate had already undergone (see text).

In preparative oxidations, reaction was stopped at the desired time by addition of an excess of ethane-1,2-diol, and, after concentration of the reaction mixture to a convenient volume by distillation under diminished pressure, followed by exhaustive dialysis against water, the substrate was isolated by freeze-drying. The borohydride-reduced materials were also isolated, after dialysis, by freeze-drying, and their ash contents (usually less than 1 %) and moisture contents (usually about 5 %) were determined and used to correct the data obtained with them. The freeze-dried, partially oxidised materials all dissolved freely upon warming in water, to give stable, slightly turbid solutions.

In connection with the very protracted oxidations that were carried out on the xylan, possible sources of error were investigated. Provided that light was excluded and the glassware was very clean, the 25 mM periodate showed no significant spontaneous de-

composition over the relevant time-period. The 0.01 M thiosulphate, however, decomposed spontaneously at the rate of about 1 % in 24 h, and it was therefore freshly prepared and standardised every day. The most serious source of error arose from the inadvertent admission into the reaction mixture of dust and reducing contaminants, through the repeated opening of the reaction vessel and the introduction of pipettes for the withdrawal of samples. This was largely overcome by carrying out the reaction in a number of different reaction flasks simultaneously, and taking only a limited number of samples from each in turn. The pipettes were also repeatedly cleaned during the experiment.

In the preparation of partially oxidised xylopentaose, the reaction was terminated at the desired time, and the substrate simultaneously reduced, by the direct addition of an excess of sodium borohydride to the reaction mixture. After 5 h at room temperature, the solution was then brought to pH 6 by the dropwise addition of 2 N acetic acid, and deionised by percolation through a mixture of Dowex 50 (H^+ form) and Dowex 1 (acetate form) resins. The solution was then concentrated to dryness, and boric acid not removed by the resin was removed by the repeated distillation of added methanol. After further oxidation of the isolated material, formaldehyde was determined by the chromotropic acid method.[17]

Methylation of partially oxidised xylan. Partially oxidised xylan (270 mg), having a degree of oxidation of 0.57, was dissolved by warming in a mixture of freshly distilled dimethylformamide (10 ml) and dimethyl sulphoxide (5 ml). To the cooled solution, freshly precipitated, dry silver oxide (5 g) and methyl iodide (10 ml) were added, and the mixture was shaken mechanically in the dark for 5 days at room temperature. It was then filtered, and the residue was washed with chloroform (150 ml). The filtrate and washings were combined and kept at 0° overnight. The precipitate was removed by filtration, and the filtrate washed by shaking, first with sodium thiosulphate (0.1 M in 2 M sodium chloride), then with sodium cyanide (5 % w/v in 2 M sodium chloride), and finally with 2 M sodium chloride. After drying over anhydrous sodium sulphate, removal of the solvent yielded 168 mg of pale yellow solid. (Found: OCH_3 21.6. Calc. for $[(C_5H_6O_3)(OCH_3)_2\cdot(C_5H_6O_4)]_n$: OCH_3 21.4 %; see text).

The product showed no significant absorption in the hydroxyl region of the infrared, and its methoxyl content was unchanged after further methylation with methyl iodide and silver oxide. It did, however, show a weak carbonyl-stretching absorption at 1730 cm^{-1}, which was not removed by further methylation.

The methylated product (40 mg) was hydrolysed, first with formic acid and then with 0.5 N sulphuric acid, as described earlier [3] for methylated, limit-oxidised alginate. The hydrolysate (27 mg) had a methoxyl content of 2.6 %.

An unmethylated sample of partially oxidised xylan was similarly hydrolysed, and both hydrolysates were examined by thin-layer chromatography on Eastman-Kodak silica-gel coated plastic sheets, irrigated with ethyl acetate-acetic acid-water (9:2:2 v/v), followed by spraying with aniline hydrogen phthalate. Free, unsubstituted xylose and glycerose in similar proportions were detected as major components in both hydrolysates. The hydrolysate of the methylated material contained in addition a small amount of a component migrating at the speed of a mono-*O*-methyl xylose, and a possible, minute trace of di-*O*-methyl xylose. Glyoxal streaked in the solvent system, but its presence in both hydrolysates was evident from its pungent odour.

RESULTS

Fig. 1 (curve A) shows the consumption of periodate by the homopolymeric xylan as a function of time. The changes in the second-order rate-constant were determined in the conventional way, by plotting $[1/(a-b)]\ \ln\ [(b/a)(a-x)/(b-x)]$ against time, and measuring the slopes of tangents throughout. In this formula, a and b represent the initial concentrations of periodate and *vic*-diol groups, respectively, and $x=b\alpha$, where α is the degree of oxidation, expressed as a fraction of the theoretical maximum (1.04 mole of periodate per anhydroxylose unit).

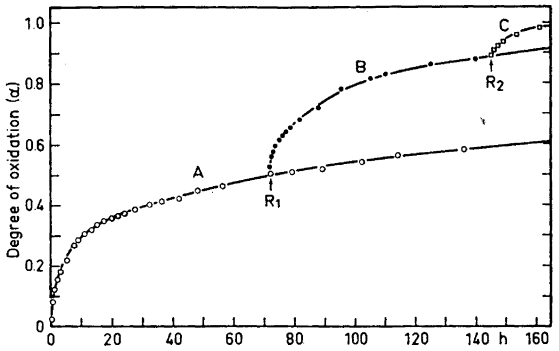

Fig. 1. Oxidation of homopolymeric xylan fragment in 25 mM periodate at 20°. The degree of oxidation (α) is expressed as a fraction of the theoretical maximum. Curve A: unmodified xylan fragment. Curve B: partially oxidised xylan after reduction at point R_1. Curve C: partially oxidised xylan after reduction at point R_2.

In Fig. 2 (curve A), the second-order rate-constants so determined are plotted against α, and are seen to approach a constant value when α is approximately 0.55 – 0.60. The indicated limits of error were those entailed in measuring the slopes of tangents, and are of the order of ±10 % throughout. It may be concluded that the final rate is between 1.7 and 2.5 % of the initial rate.

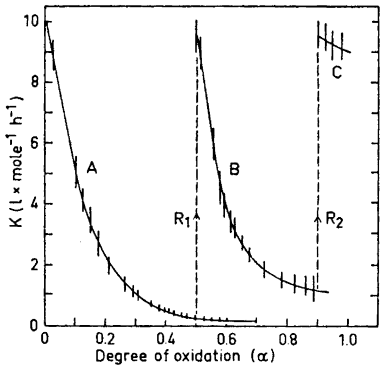

Fig. 2. Data from Fig. 1, plotted as second-order rate-constants (k) against degree of oxidation (α).

Fig. 1 (curve B) shows the oxidation of the product obtained when the xylan is reduced with borohydride after having already consumed 0.5 mole of periodate per xylose residue. In this experiment, the initial concentration of periodate was 22 mM and that of the unoxidised xylose residues was 3.7 mM, so that Fig. 1 gives an accurate impression of the increase in rate brought about by reduction.

Fig. 2 (curve B) shows that the rate is, in fact, restored to its initial value by reduction with borohydride, although the limits of error are rather large

(about ±20 %). The rate then decreases again, tending this time to about 8–16 % of the initial value. The greater error in this case arises because, at high degrees of oxidation, the calculated value of the rate-constant is highly sensitive to small errors in measuring the concentration of substrate.

A second reduction with borohydride when the degree of oxidation reaches about 0.9 permits completion of the oxidation at a rate close to the initial rate (Figs. 1 and 2, curves C). When the first reduction with borohydride is carried out after the degree of oxidation has been allowed to reach 0.6, there is no significant decrease in rate during the second phase of the oxidation, which proceeds to completion in good accordance with second-order kinetics.

Although it is not readily adapted for accurate quantitative work, the Barry degradation[18] provided a convenient means of studying the action-pattern of periodate on xylan. At a degree of oxidation of 0.35, the homologous series of oligoxyloside phenylosazones was readily detected by thin-

Table 2. Specific optical rotations of some xylans and their oxidised derivatives at 20–25°.

Material	Periodate consumed (mole/unit)	Further treatment	$[\alpha]_D$	Solvent	Remarks
Esparto grass xylan	None	None	−92°	0.5 N NaOH	From Chanda *et al.*[8]
Maize cob xylan	None	None	−103°	0.5 N NaOH	From Ehrenthal *et al.*[9]
Homopolymeric fragment	None	None	−103°	0.5 N NaOH	Aqueous solutions were too turbid to measure
Homopolymeric fragment	0.57	None	−34°	Dimethyl sulphoxide	Too turbid in water; degradation in alkali
Homopolymeric fragment	0.57	Methylation	−31°	Chloroform	Becomes −35° after correction for methoxyl content
Homopolymeric fragment	0.57	Reduction	−65°	0.5 N NaOH	Too turbid in water
Homopolymeric fragment	0.57	Reduction	−70°	Dimethyl sulphoxide	
Homopolymeric fragment	0.75	None	+28°	Water	Possible presence of inter-residue hemialdals
Homopolymeric fragment	0.75	Reduction	−41°	Water	
Maize cob xylan	1.05	None	+69°	Water	From Ehrenthal *et al.*[9] Value after reduction not reported

layer chromatography [3] in the product. As the degree of oxidation approached 0.5, however, the pattern of products rapidly simplified, the phenylosazones of xylose and xylobiose becoming the only significant components (apart from glycerose phenylosazone and glyoxal bisphenylhydrazone). After the consumption of 0.6 mole of oxidant, xylose phenylosazone was the only significant product apart from the smaller fragments.

Methylation of the xylan after oxidation to a degree of oxidation of 0.6 (without reduction) was carried out by treatment of a homogeneous solution of the material in a mixture of dimethyl formamide and dimethyl sulphoxide with methyl iodide and silver oxide. Methylation proceeded without depolymerisation, and the ease with which it was accomplished suggested that the hydroxyl groups undergoing methylation were more acidic than those in an unoxidised polysaccharide. The methoxyl content of the product was slightly higher than that expected for the introduction of two methoxyl groups for every unoxidised xylose residue, assuming that the two terminal xylose residues were both fully oxidised (see below), and infra-red spectroscopy indicated the virtual absence of free hydroxyl groups in the material.

Hydrolysis of the methylated product under conditions that are known [19] to bring about very little demethylation of alcoholic hydroxyl groups caused it to lose most of its original methoxyl content, and chromatography of the hydrolysate indicated that it consisted mainly of free, unsubstituted xylose, glycerose, and glyoxal.

Since methylation was carried out in a non-aqueous medium which could be expected [6] to promote hemiacetal formation, and since methylation could perhaps be expected to drive the equilibrium towards the hemiacetal form by preferentially substituting the hemiacetal hydroxyl groups, evidence for the extent of any change in the number of asymmetric centres caused in these ways was sought by measurements of optical rotation. The results, together with some relevant data taken from the earlier literature, are summarised in Table 2.

The consumption of periodate by xylose, xylobiose, xylotriose, xylotetraose, and xylopentaose is shown in Fig. 3, with some of the data for xylan included for comparison. To facilitate comparison, the consumption of oxidant is in this figure expressed as mole of periodate per xylose residue. The theoretical oxidation-limits, assuming complete Malapradian oxidation, are therefore 3.0, 2.0, 1.66, 1.50, and 1.40 for the mono- and oligo-saccharides, respectively.

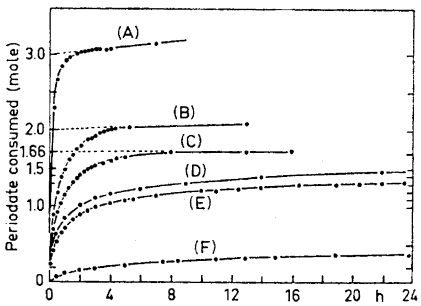

Fig. 3. Oxidation in 25 mM periodate at 20° of: (A), xylose; (B), xylobiose; (C), xylotriose; (D), xylotetraose; (E), xylopentaose; and (F), xylan. The periodate consumption is expressed as mole per xylose residue.

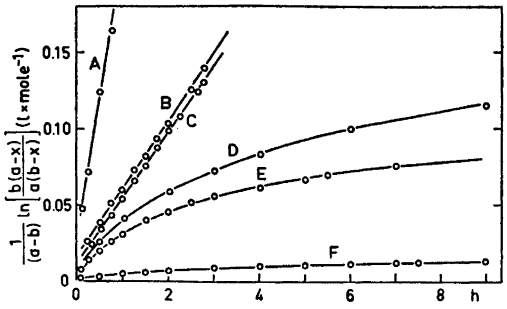

Fig. 4. Data from Fig. 3, plotted according to second-order kinetics.

In Fig. 4, these data are plotted according to second-order kinetics. Xylose, xylobiose, and xylotriose are thereby shown to conform closely to the second-order rate law,* while xylotetraose and xylopentaose deviate sharply from it. In Fig. 5, the second-order rate-constants for xylotetraose and xylopentaose are plotted as a function of the degree of oxidation (α). In both Figs. 4 and 5, the data are calculated with the concentration (*b*) of substrate ex-

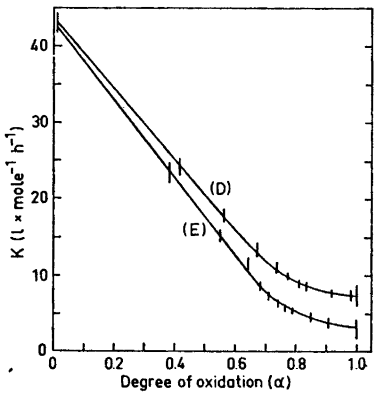

Fig. 5. Data from Fig. 3 for xylotetraose and xylopentaose (D and E, respectively), plotted as second-order rate-constants (*k*) against degree of oxidation (α).

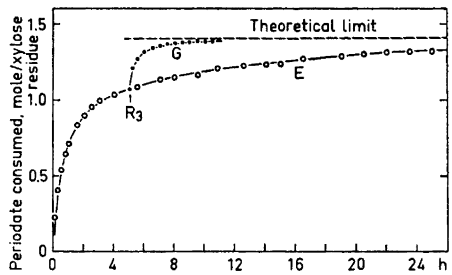

Fig. 6. Oxidation in 25 mM periodate at 20° of xylopentaose (curve E) and partially oxidised xylopentaose, after reduction at point R_3 (curve G).

* *Note added in proof*: This is true for the measured range of experimental points, as shown in Fig. 4. It will be noticed, however, that none of the curves passes through the origin. In fact, the intercept on the ordinate axis corresponds fairly closely in every case to a very rapid, initial uptake of 1 mole of periodate for every mole of total substrate. In the light of existing knowledge [see, for example, Warsi, S. A. and Whelan, W. J. *Chem. and Ind.* (1958) 71, and references therein cited], it is very probable that this is due to an initial, selective cleavage of the reducing terminal xylose residues between C(1) and C(2). Our failure to notice the significance of the intercepts when the manuscript was prepared does not materially affect the validity of the conclusions that we have drawn.

pressed as *vic*-diol groups, and the degree of oxidation (α) is expressed as a fraction of the theoretical maximum.

Fig. 6 shows the effect upon the rate of oxidation of xylopentaose of reduction with borohydride after the consumption of 1.1 mole of periodate per xylose residue. The concentrations of reactants were, again, such that the two curves may be compared directly. After completion of the oxidation of the borohydride-reduced material, which proceeded according to second-order kinetics, the yield of formaldehyde was 0.12 mole per mole of total substrate, showing that the two terminal units were almost fully oxidised at the time when borohydride reduction was carried out.

DISCUSSION

The principal effect of the limited solubility of xylan in water appears to consist in a general decrease in the overall rate of oxidation. Whereas this can be expected to have a modifying influence on the kinetics, it is clear that the very large deviation from second-order kinetics is primarily a fundamental property of the β-1,4-linked xylan chain, and is specifically associated with the presence of contiguous, non-terminal xylopyranose residues. This implies a nearest-neighbour, auto-inhibitory mechanism. Since this inhibitory effect is irreversibly removed by reduction with borohydride, it must entail some kind of interaction between the aldehyde groups of oxidised residues and the hydroxyl groups of adjacent, unoxidised residues.

The methylation data indicate that this interaction consists, simply, in hemiacetal formation. It is very unlikely that the hemiacetals were formed during methylation, because the optical rotation of the methylated, partially oxidised xylan was closely similar to that of the unmethylated material in dimethyl sulphoxide, especially after correction for the methoxyl content of the former (Table 2). On the other hand, reduction of the partially oxidised xylan with borohydride brought about a substantial change in the optical rotation, as measured in dimethyl sulphoxide, which strongly suggests that the hemiacetal structures were already present in the material, at least when dissolved in this solvent.

It is possible, and indeed likely,[6] that the position of the equilibrium between the free aldehydic and hemiacetal forms is not the same in dimethyl sulphoxide as it is in water, and unfortunately, aqueous solutions of the material used for methylation were too turbid to permit measurement of their optical rotation. However, as the degree of oxidation increases, the polymer becomes more soluble, and in a more highly oxidised sample, a large decrease in specific rotation occurring upon reduction with borohydride was clearly demonstrable with water as the solvent.

When alginate is oxidised with periodate under conditions that avoid overoxidation [1,3] and depolymerisation,[3] reaction ceases completely when the degree of oxidation reaches 0.45, and the material gives no reaction with Schiff's reagent at any stage in its oxidation.[2,3] In contrast, the oxidation of xylan proceeds ultimately to completion, without the possibility of over-oxidation, and the material reacts strongly with Schiff's reagent throughout. In the case of xylan, therefore, hemiacetal formation cannot bring about

complete protection of any unit from future oxidation, and a fraction of the liberated aldehyde groups must be free at any time.

The question then arises as to whether an equilibrium is rapidly established between a liberated aldehyde group and its inter-residue, hemiacetal form, or whether the hemiacetal formation is a slow reaction. With alginate, it was readily demonstrated that the hemiacetal formation was rapid compared to the rate of oxidation, because the same oxidation-limit was reached when the rate of oxidation was varied between wide limits, and was identical with that calculated on the assumption that protection of the nearest neighbours of oxidised units was instantaneous and complete. With xylan, it is more difficult to reach such a clear conclusion, but nevertheless the difference between the rates of oxidation of "free" and "inhibited" units is sufficiently large that, if the inhibition is rapid, a reasonably close agreement should be expected between the theoretical oxidation-limits in Table 1 and the degree of oxidation at which the rate-constant tends to a constant value (Fig. 2, curve A; and Fig. 5)

For a chain of 42 units with fully oxidised end-groups, Table 1 indicates an expected oxidation-limit of 0.514 mole of periodate per monomeric unit, corresponding to a degree of oxidation of 0.495. For xylotetraose, the theoretical limit is 1.25 mole of periodate per xylose residue, corresponding to a degree of oxidation of 0.835, and for xylopentaose, the calculated limit is 1.13 mole of periodate per xylose residue, corresponding to a degree of oxidation of 0.809. Since these theoretical figures are clearly lower limits, and must be expected to increase as the degree of inhibition decreases, the agreement with the data in Figs. 2 and 5 is quite good.

A very rough estimate of the position of the equilibrium (that is, the degree of inhibition) can be made from the data in Fig. 2. The Barry degradations showed that, when the degree of oxidation reached 0.6, virtually all the unoxidised units had oxidised units as their nearest neighbours, and at this stage, the rate of oxidation was about 1/40th to 1/60th of the initial rate. Since both hydroxyl groups on these unoxidised units were subject to hemiacetal formation, the square root of this fraction (about 1/6 to 1/8) is the geometric mean value for the fraction of each hemiacetal that exists at any time in the open-chain aldehydic form.

After reduction with borohydride at a stage in the oxidation when the unoxidised units are present as singlets and doublets only, further oxidation (curve B) can give rise only to singly protected units, and the final rate in this case, although not known accurately, is indeed of the order of 1/6th to 1/8th of the initial rate.

A rigorous, computer-based analysis of the kinetics of auto-inhibitory reactions of the present type is now in progress in this laboratory, and will, it is hoped, provide much more accurate and detailed information about the position of the equilibrium and the relative speed with which it is established.

The experiment illustrated in Fig. 6, in which xylopentaose was reduced with borohydride at an intermediate stage in its oxidation, and then oxidised again, was carried out to establish that the behaviour of xylan is also shown by a substrate whose solubility in water is beyond doubt. The reduction was carried out when it was expected that maximum possible hemiacetal forma-

tion should have been established (Table 1). The low yield of formaldehyde after the second oxidation proved that the increase in rate caused by reduction was not due to further oxidation of incompletely oxidised end-units, nor to any cleavage of the chain taking place upon borohydride reduction.

The close obedience to second-order kinetics shown by xylose, xylobiose, and xylotriose, and then the sudden change in proceeding up the homologous series to xylotetraose (Fig. 4), are impressive but surprising. In general, it should not be expected that even a monosaccharide ought to obey second-order kinetics, and the behaviour of xylose, xylobiose, and xylotriose should not be accepted as typical for mono-, di-, and tri-saccharides generally.

Possibly it is important to distinguish sharply between monomeric units containing only one reactive site, and those containing more than one. In the latter, the fact of having opened the ring at the first attack might, in one sense, be expected to increase the rate of the next attack, because open-chain compounds such as alditols are well known to oxidise much more rapidly than cyclic polyols. On the other hand, intra-residue hemiacetal formation may take place after the first attack,[6] and decrease the rate of the next. With the present substrates, it must be supposed that these two opposite effects were mutually compensatory under the conditions of the experiment.

The apparent inability of terminal xylose residues to participate significantly in inter-residue hemiacetal formation can perhaps also be understood in terms of competitive reactions. Not only are they able, after the first attack, to form intra-residue hemiacetals in competition with inter-residue forms, but also, after the second attack, they would be able to form a six-membered, intra-residue hemialdal, thus retaining a permanent competitive influence, and permitting a more independent oxidation of their nearest neighbour.

Xylotetraose is the first member of the homologous series that can form an inter-residue hemiacetal without competition from other forms. In proceeding up to xylopentaose, still another transition is encountered, because this is the first member in which two non-terminal units can co-operate to inhibit the oxidation of another, the combined effect of double hemiacetal formation being vastly greater than the separate contributions of each (Figs. 4 and 5).

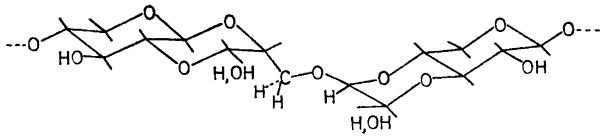

Fig. 7. An oxidised site in a xylan chain, showing single protection of the two nearest neighbours.

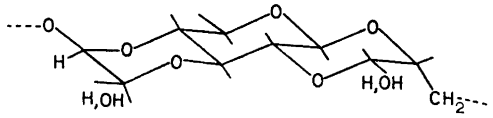

Fig. 8. A xylose residue, doubly protected by two oxidised neighbours.

In summary, all the present data seem to be explicable in terms of the rapid establishment of equilibria, and they suggest the importance of the positions of the equilibria, the presence or absence of competitive forms, and possibilities for co-operative (double) inhibition of oxidation. The proposed hemiacetal structures are depicted in Figs. 7 and 8, with chair conformations arbitrarily assigned to them.

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