Carbohydrate Research, 89 (1981) 73–82
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

PERIODATE OXIDATION OF METHYL GLYCOPYRANOSIDES RATE COEFFICIENTS AND RELATIVE STABILITIES OF INTERMEDIATE HEMIACETALS

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(Received May 15th, 1980, accepted for publication, June 2nd, 1980)

ABSTRACT

For oxidation in aqueous sodium metaperiodate at 20° rate coefficients describing the decay of intact glycoside (k_G) and the consumption of periodate (k_P) were measured for the methyl α- and β-glycopyranosides of D-glucose, D-galactose D-mannose, D-xylose, and D-(or L-)arabinose, and for methyl α-L-rhamnopyranoside For glycosides containing a *cis*-1,2-diol group, k_G was four to sixteen times larger than for those containing only *trans*-1,2-diol groups For anomeric pairs, $(k_G)_\alpha/(k_G)_\beta$ varied from 0 6 to 1 7 The kinetics showed that the singly oxidised intermediates existed partly in an unreactive form, which was inferred to be a cyclic hemiacetal For hexopyranosides, but not pentopyranosides, the stabilities of the hemiacetals, as indicated by the limiting values of k_P, were much greater with α than with β anomers From a consideration of possible structures and conformations of the hemiacetals it was inferred that this result was a manifestation of the anomeric effect

INTRODUCTION

Although the methyl glycopyranosides of the common aldohexoses and aldopentoses were among the first carbohydrate derivatives to be studied by periodate oxidation[1], measurements of the rate of reaction have been restricted to 2-*O*-, 4-*O*-, and 4,6-di-*O*-substituted derivatives that contain only one oxidisable site[2] We have investigated the more-complex kinetic behaviour of unsubstituted glycopyranosides, to provide a background for such developments as the selective Smith-degradation of terminal groups in heteroglycans[3,4], and *in situ* modification of membrane-bound antigens on whole cells[5,6]

We now report kinetic data for eleven common methyl glycopyranosides in unbuffered sodium metaperiodate The results indicate the extent of variations in reactivity that can arise from differences in configuration, especially at the anomeric centre, and illustrate the role of intermediate hemiacetals in determining overall reaction rates

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0008-6215/81/0000–0000/S 02 50,

THEORY

The periodate oxidation of an alicyclic *vic*-triol is a competitive, consecutive, second-order reaction[7], with the added complication that the intermediates formed after the first oxidative attack may exist either in a reactive, acyclic form, or an unreactive, cyclic, hemiacetal form[8-10] The complete reaction scheme is shown in Fig 1 González has provided a theoretical analysis of this system which, in principle, could be used to evaluate all of the eight rate-constants, but more than one solution is possible with easily accessible data[11]

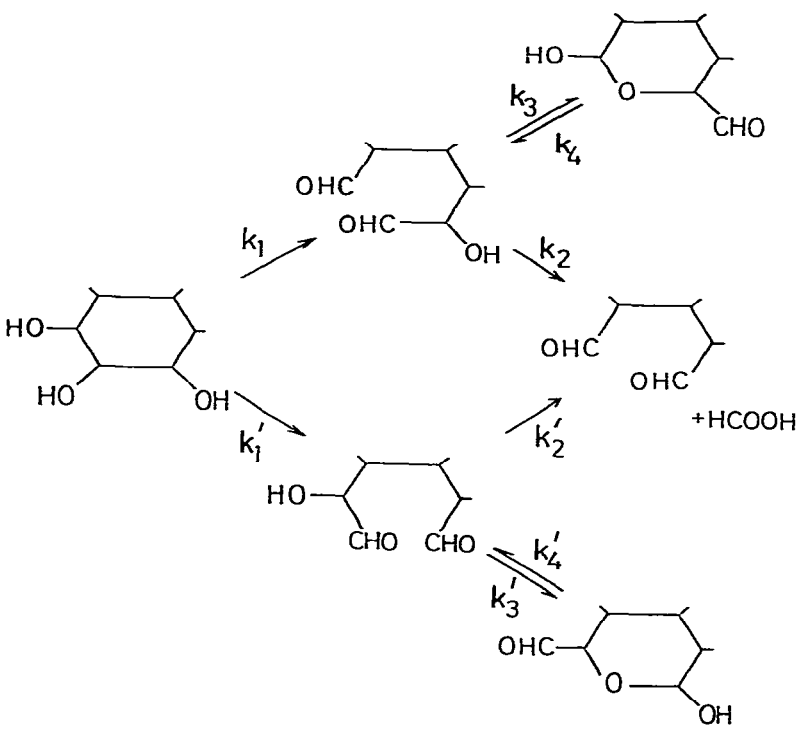

Fig 1 General scheme for periodate oxidation of an alicyclic *vic*-triol Only the kinetically relevant steps are shown, and the formation of additional hemialdal structures, as well as hydrates of the aldehydes, is expected

The following, simplified discussion shows how a maximum of useful information can be obtained from the two quantities that are easy to measure accurately These are the molar proportions of periodate consumed (P_t) and formic acid released (F_t) at any time, t, where $P_\infty = 2\,0$ and $F_\infty = 1\,0$ Together with the initial concentrations of periodate (P) and glycoside (G), these data permit calculation of the concentrations at any time of (*i*) periodate P $-$ G P_t (*ii*) unreacted glycoside, G$(1 - P_t + F_t)$, (*iii*) singly oxidised intermediate(s), G$(P_t - 2F_t)$, and (*iv*) doubly oxidised glycoside, G F_t

Expressions (*i*) and (*ii*) furnish a rate constant, k_G, describing the decay of intact pyranose rings, namely,

$$\frac{d(1 - P_t + F_t)}{dt} = -2k_G(1 - P_t + F_t)(P - G\,P_t),$$

whence

$$\frac{1}{2(P - G)} \ln \left[\frac{P - G\,P_t}{P(1 - P_t + F_t)}\right] = k_G\,t \qquad (1)$$

A plot of the left-hand side of Eq *1* against *t* should give a straight line of slope k_G. This is a true rate *constant*, identical with $\frac{1}{2}(k_1 + k'_1)$ in Fig. 1[4].

With expressions (*i*) (*ii*), and (*iii*), it is possible to define a second-order rate coefficient, k_P, describing the rate of consumption of periodate at any time

$$\frac{d(P - G\,P_t)}{dt} = -k_P\,G(P - G\,P_t)[2(1 - P_t + F_t) + (P_t - 2F_t)]$$

$$= -k_P\,G(P - G\,P_t)[2 - P_t] \qquad (2)$$

whence,

$$\frac{1}{(P - 2G)} \ln \left[\frac{2(P - G\,P_t)}{P(2 - P_t)}\right] = k_P\,t \qquad (3)$$

A plot of the left-hand side of Eq *3* against *t* will, in general, be a curve whose slope at any time is k_P. The initial slope, $(k_P)_0$, and the final, limiting slope, $(k_P)_{lim}$ are of special interest, because they provide information about k_2/k_3 (and/or k'_2/k'_3) and k_3/k_4 (and/or k'_3/k'_4), respectively. This can be seen by expressing the rate of consumption of periodate in terms of k_G and another second-order rate coefficient, k_S, which describes the decay of the singly oxidised intermediates

$$\frac{d(P - G\,P_t)}{dt} = -2k_G\,G(P - G\,P_t)(1 - P_t + F_t)$$

$$-k_S\,G(P - G\,P_t)(P_t - 2F_t) \qquad (4)$$

Combining Eq *2* and Eq *4* gives

$$k_P = \frac{2k_G(1 - P_t + F_t) + k_S(P_t - 2F_t)}{(2 - P_t)} \qquad (5)$$

It is evident from Eq *5* that $(k_P)_0$ should normally be identical with k_G, but an exception occurs[7] when $k_S \gg k_G$. The rate of formation of doubly oxidised product, represented by $k_S(P_t - 2F_t)$ in Eq *5*, then becomes almost identical with the rate of decay of intact glycoside, represented by $2k_G(1 - P_t + F_t)$, and P_t is only slightly greater than $2F_t$. Imposition of these conditions on Eq *5* gives $k_P = 2k_G$ as $k_S/k_G \to \infty$.

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*In a preliminary communication[4], values of the sum $(k_1 + k'_1)$ were tabulated. The present definition of k_G facilitates comparison with k_P.

It is well known that acyclic carbohydrates consume periodate much faster than pyranoid rings, and, indeed, it was found (Table I) that for all glycopyranosides studied $(k_P)_o/(k_G)$ had a value between 1 and 2 This implies that k_2 and k_3 (or k'_2 and k'_3) in Fig 1 are generally rather similar in magnitude, and both very much larger than k_1 (or k'_1) The fraction of molecules that are doubly oxidised, without existing at any time as an unreactive hemiacetal is thus $[(k_P)_o/(k_G) - 1]$

TABLE I

OXIDATION OF METHYL GLYCOPYRANOSIDES (2 5MM) IN 12 5MM SODIUM METAPERIODATE AT 20°

Methyl glycopyranoside	k_G[a]	$(k_P)_o$[b]	$(k_P)_o/(k_G) - 1$	$(P_t)_{break}$	$(k_P)_{lim}$[b]	$(P_t - 2F_t)_{max}$
α-D-Gluco-	0 67	1 02 ±0 1	0 52 ±0 05	~1 55	0 23 ±0 01	0 47
β-D-Gluco-	0 39	0 82 ±0 08	1 05 ±0 10	~1 85	0 78 ±0 02	0 10
α-D-Galacto-	4 88	6 05 ±1 0	0 23 ±0 20	~1 45	0 21 ±0 01	0 71
β-D-Galacto-	6 18	10 9 ±1 5	0 77 ±0 25	>1 95	Uncertain[c]	0 44
α-D-Manno-	3 15	4 30 ±0 3	0 38 ±0 10	~1 35	0 11 ±0 005	0 68
β-D-Manno-	5 15	8 30 ±0 8	0 64 ±0 06	~1 75	0 63 ±0 03	0 33
α-L-Rhamno-	3 03	3 60 ±0 2	0 23 ±0 06	~1 20	0 09 ±0 005	0 75
α-D-Xylo-	0 50	1 07 ±0 03	1 14 ±0 06	None	1 45 ±0 15	0 15
β-D-Xylo-	0 49	0 85 ±0 04	0 80 ±0 09	None	1 25 ±0 10	0 23
α-D-Arabino-	6 50	12 0 ±0 8	0 85 ±0 12	~1 90	1 33 ±0 03	0 31
β-L-Arabino-	6 00	11 2 ±0 7	0 87 ±0 11	~1 80	2 31 ±0 05	0 30

[a]In litres per dermimole per min [b]In litres per *vic*-diol group per min [c]See text for discussion of limits of error

At some intermediate stage in the reaction, all intact glycoside molecules have reacted, and P_t becomes equal to $(1 + F_t)$, giving $k_P = k_S$ from Eq 5 At this point, k_P does not necessarily become constant, because there may be two singly oxidised intermediates which decay at different rates In most cases, k_P did in fact become constant, giving well-defined values for $(k_P)_{lim}$ These values are clearly determined (Fig 1) by an equilibrium constant, corresponding to k_3/k_4 (or k'_3/k'_4), together with k_2 (or k'_2) They therefore reflect the stabilities of the hemiacetals For groups of hexopyranosides, or pentopyranosides, cleaved in the same position, and especially for anomeric pairs of glycopyranosides, the values of $(k_P)_{lim}$ should provide a direct comparison of these stabilities, because the values of k_2 (or k'_2) are expected to be closely similar (see Discussion)

EXPERIMENTAL

Materials — Methyl α-D-arabinopyranoside was a gift from the late Professor J K N Jones Methyl β-D-mannopyranoside was kindly donated by Professor P J Garegg The other glycosides were purchased from Koch–Light Laboratories, Ltd , Colnbrook, England Where necessary, samples were recrystallised from ethanol

until they consumed 2 00 ±0 04 mol of periodate and liberated 1 00 ±0 02 mol of formic acid All samples were dried *in vacuo* over phosphorus pentaoxide at 77° for 6 h before use

Methods — Glassware was cleaned with aqueous chromic acid and 'Decon 90' laboratory detergent Stock solutions (0 25M) of sodium metaperiodate were prepared in subdued light, and kept in flasks covered with aluminium foil for a maximum of 5 days Solutions of 10mM sodium thiosulphate, 5mM sodium hydroxide, and 60% (w/v) aqueous potassium iodide were freshly prepared every day

The reaction mixtures were prepared by rapidly mixing equal volumes of sodium metaperiodate (25mM) and glycoside (5mM) solutions, previously brought to 20° in a large, shallow, thermostatic water-bath Rapid mixing at 20° was facilitated by using wide-necked conical flasks and magnetic stirrers with the driving mechanism under the water-bath The temperature of the laboratory was not normally more than 22° Periodate-containing solutions were protected from light by covering the flasks with aluminium foil, and all pipetting and mixing operations were performed in subdued light

Periodate was assayed by transferring portions (10 ml) of reaction mixture to ice-cold mixtures of sodium phosphate buffer (0 5M, pH 7 0 20 ml) and aqueous potassium iodide (60% w/v 3 ml), followed by rapid titration with 10mM sodium thiosulphate, with starch as indicator The exact procedure was determined by the delivery times of the pipettes in relation to the time of reaction For short reaction times, the portion (10 ml) of reaction mixture was first transferred to a 100-ml conical flask at 20° and reaction was stopped at the required time by very rapid addition of buffered potassium iodide from another flask with vigorous swirling

Formic acid was assayed by mixing portions (10 ml 50%, v/v) of reaction mixture with aqueous ethane-1,2-diol (3 ml), followed after 10 min, by titration with 5mM sodium hydroxide, with Methyl Red as indicator For short times of reaction, a procedure similar to that described above was used

Blank solutions of 12 5mM sodium metaperiodate were prepared and titrated similarly in connection with every experiment The blank titrations were normally performed within 10 min of the test assay

RESULTS

A complete tabular and graphical presentation of the results is given in a thesis[12], which is available upon request In Figs 2–5, a selection of results is given which illustrates the scope of variation from one glycoside to another In the top diagrams, the unprocessed data for P_t and F_t are shown, together with calculated values of $(P_t - 2F_t)$, the mole fraction of singly oxidised intermediates In the central diagrams, the results for k_G are plotted according to Eq *1*, and in the bottom diagrams, the results for k_P are plotted according to Eq *3*

In most cases, the plots for k_P showed a fairly well-defined break after P_t became equal to $(1 + F_t)$, and k_P was thereafter constant at $(k_P)_{lim}$ The value of

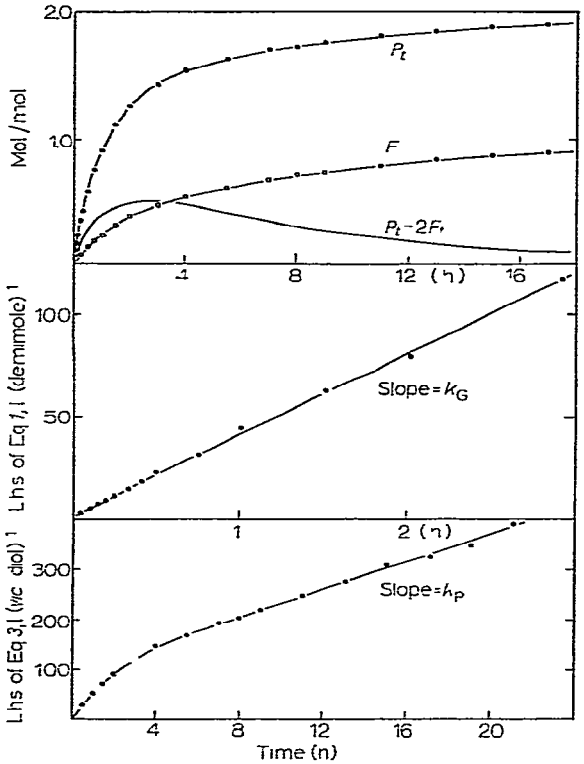


Fig 2 Methyl α-D-glucopyranoside

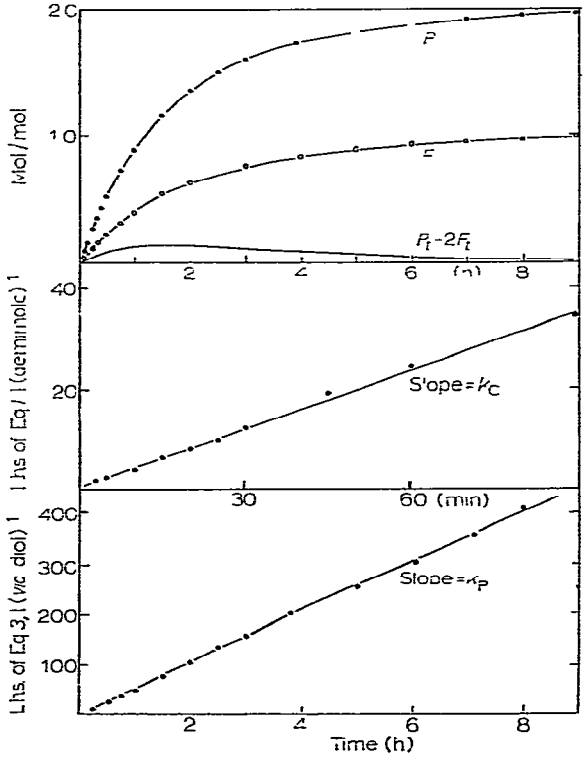


Fig 3 Methyl β-D-glucopyranoside

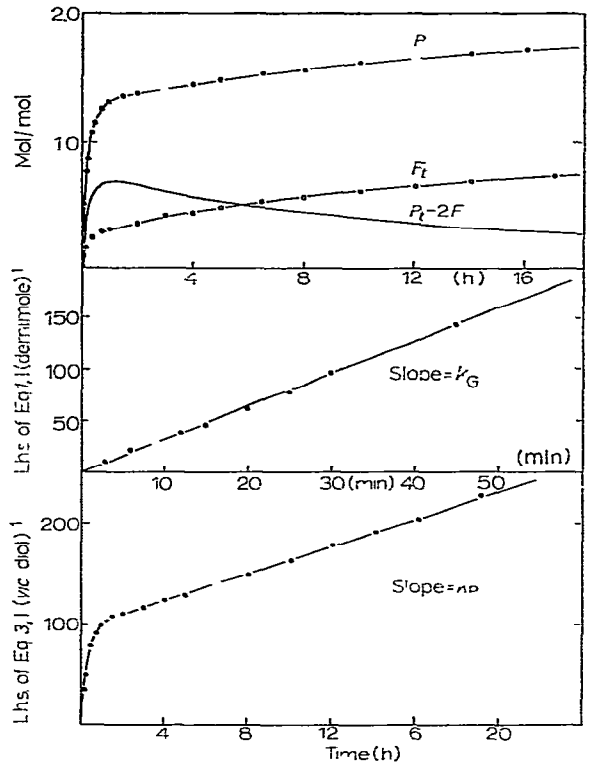


Fig 4 Methyl α-D-mannopyranoside

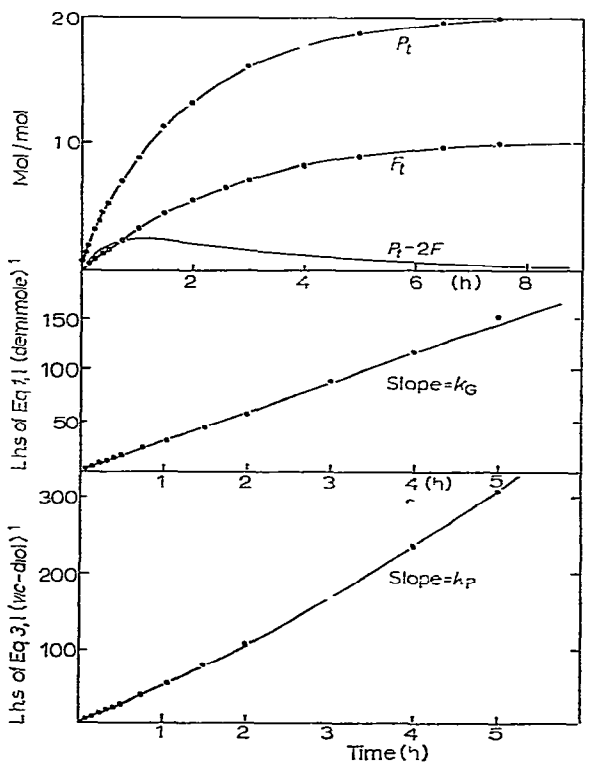


Fig 5 Methyl β-D-xylopyranoside

P_t when this occurred $(P_t)_{break}$, was generally greater than unity by an amount closely similar to $[(k_P)_0/(k_G) - 1]$, the fraction of molecules passing directly to the fully oxidised state, without forming an unreactive hemiacetal A summary of all these quantities for the eleven glycosides is presented in Table I Also included are values for $(P_t - 2F_t)_{max}$, the maximum yield of singly oxidised intermediates, since these provide an additional indication of the stabilities of the unreactive hemiacetal forms

Some comments about accuracy are needed In most cases, the values for k_G were provided by good straight-line plots of Eq *1* through 9 or 10 points and should therefore be particularly reliable The considerable limits of error indicated for $(k_P)_0$ are a consequence of the difficulty in drawing tangents, accurately, at the origin when the slope is changing rapidly This was particularly difficult with the faster-reacting glycosides because it was not practically possible to take readings more frequently than every 3 min The values for $(k_P)_{lim}$ also became unreliable when $(P_t)_{break}$ was high With methyl β-D-galactopyranoside, for example the relevant readings represent the last 2 5% of the reaction and since the presence of only 1% of an impurity could make the results meaningless they are not reported

DISCUSSION

For practical purposes the values of k_G should be particularly useful They readily permit calculation of the time required for virtually complete (say 99%) oxidation, at 20°, with any given initial concentrations of reactants They are the most relevant quantities for use in connection with selective Smith-degradations[3,4] and *in situ* modification of whole cells[5,6] because for these purposes, it is unnecessary that both oxidisable sites be cleaved Caution is nonetheless needed in extrapolating these results to heteroglycans and glycoconjugates The results obtained so far[4,10,11,13] indicate that the methyl glycopyranosides are good models for terminal groups when these are linked to the rest of the glycan chain *via* position 6 of a hexose residue

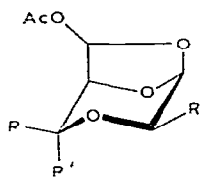
As regards the remainder of the reaction, it is clear that the scheme shown in Fig 1 is usually fully applicable in the sense that k_2 (or k'_2) is usually of the same order of magnitude as k_3 (or k'_3) This finding means that the formation of unreactive hemiacetals by singly oxidised intermediates usually has a major influence upon the overall kinetic pattern In some cases, however k_2 (or k'_2) exceeds k_3 (or k'_3) to such an extent that the uptake of periodate approximates to that special case of a simple, consecutive, second-order reaction[7] in which $k_S \gg k_G$, and k_P remains almost constant throughout at a value of $\sim 2k_G$

This raises the question of what happens when the initial concentration of periodate is increased above 12 5mM Since k_3 and k'_3 are first-order rate-constants, it would be expected that all oxidations of glycopyranosides should approach the special case just mentioned as the concentration of periodate increases This, however, is not so Preliminary observations indicate that k_3 and k'_3 are profoundly sensitive to the presence of all salts, including both periodate and inert buffer salts,

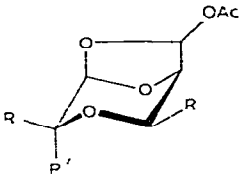
and that, in some cases, they increase with increasing periodate concentration This may be most simply interpreted as a large, primary salt effect but the possibility of complex formation between periodate and the unreactive hemiacetals has not been eliminated

The results in Table I show that, without exception, the singly oxidised intermediates derived from the α anomers of hexopyranosides react more slowly than those derived from the corresponding β anomers It is hard to interpret this as indicating a lower reactivity of the acyclic forms, because the aldehyde group is symmetrical and there is no evident way in which the chirality at C-1 could affect the formation of a cyclic ester[14] with periodate It is therefore more likely that the intermediates derived from α anomers form more-stable hemiacetals

It is established[8,9] that at least after *O*-acetylation, the singly oxidised product from methyl β-L-arabinopyranoside has the structure **1** (R = H), in which the axial orientation of the methoxyl group is expected to be stabilised by an electronic effect similar to the anomeric effect[15] By similar methods[8,9], we have confirmed[16] that all the singly oxidised intermediates from all the glycopyranosides named in Table I yield upon acetylation structures **1**, **2**, **3**, or **4**, where R = CH_2OAc for products from hexopyranosides and R = H for products from pentopyranosides The product from methyl α-L-rhamnopyranoside is the mirror image of **3**, with R = CH_3



1 R′ = H R′ = OMe
2 R′ = OMe R′ = H



3 R′ = H R′ = OMe
4 R′ = OMe R = H

Although it is not clear whether similar, bicyclic structures are formed in aqueous solution, it is certain that the 1,4-dioxane rings must be formed because the hemiacetals do not react with periodate The steric bulk of the hydroxymethyl group in the products from hexopyranosides and of the methyl group in the product from the rhamnoside, would impose a strong preference for the chair conformation of the 1 4-dioxane rings in which these groups are equatorial It turns out that, in this conformation, the methoxyl group is always axial in products derived from α anomers and equatorial in products derived from β anomers

This finding provides a rationalisation of the results in terms of the anomeric effect It presumes, of course, that the acyclic forms of the products from β anomers are free to take up a *gauche,gauche* conformation about the bonds adjoining O-1, C-1, and O-5 without severe steric clashes, and this appears from models to be the case With pentopyranosides, the anomeric configuration does not markedly affect

the stabilities of the hemiacetal intermediates, probably because the 1,4-dioxane rings are more free to accommodate the anomeric effect, by taking up the appropriate chair conformation

A more detailed account of the structures and conformations of the hemiacetals will be presented elsewhere

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