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The kinetics of periodate oxidation of carbohydrates: a calorimetric approach

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Abstract

A calorimetric approach is described for analysing the kinetics of periodate oxidation on a series of monosaccharidic substrates. Rate constants at several temperatures were calculated from the calorimetric decay curves that are proportional to the rate of conversion. Arrhenius plots provided the activation parameters for the various carbohydrates and a linear correlation was found between the values of enthalpy and entropy of activation. The dependence of the values of kinetic rates on stereochemistry is interpreted in terms of conformational probability of the reactive state. The suitability of the calorimetric method to track the kinetic process of slow reactions is emphasised, in particular its ability to monitor, directly and continuously, the course of the reaction.

Keywords: Periodate oxidation; Kinetics; Isothermal calorimetry; Conformation

1. Introduction

The periodate oxidation reaction of carbohydrates is widely known and even very recently it has been investigated from the point of view of both the kinetic modelling [1] and the structure of the substrate [2–5]. Besides its routine use as a tool for elucidating the structural features of polysaccharides [6], its use as a systematic approach to quantify kinetics parameters and to explain the hidden protection of vicinal residues is mainly ascribable to the work of Painter and co-workers [7,8]. A summary of relevant results is now given, not only for the purpose of reviewing the fundamental accepted knowledge, but specifically to provide the background for the discussion of the results presented here.

The reaction involves the action of a periodate ion on a molecule with one or more vicinal diols, and implies the oxidation of a reaction site with the breakage of the C–C bond and the subsequent formation of two aldehydic groups. The mechanism of the reaction, based on experimental evidence [9,10], can be depicted in the following way: in the first step, one of the I–O bonds of the periodate attacks one of the two hydroxyl groups of the vicinal diol; this reaction should be pH-independent and therefore could be catalysed by both acids and bases. The second step is the formation of the planar cyclic ester [10], the rate of which must depend on the acidity of the oxygen of the –OH groups and their relative positions.

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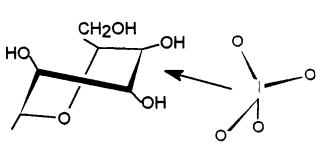
When a diol is part of a cyclic structure, its conformational freedom is restricted and the relative configuration of the –OH groups has a considerable importance on the rate of reaction [11]. For example, in a six-membered ring, the ester can be formed only if the chair conformation is puckered or flattened, depending on the isomer form. For the *cis*-diol isomer, the ester formation implies a flattening of the cycle towards its conversion into one of the half-chair forms. On the contrary, for a *trans*-diol, planarity can only be partially obtained through a further distortion of the chair towards a more puckered form (Scheme 1).

For glycosidic compounds, the oxidation process can be complicated by the presence of two or more sites, the stereochemistry of all substituents having a relevant influence on the kinetics. For the most simple case of a vicinal triol within a cyclic structure, the reaction follows a competitive and consecutive second-order kinetic [7]: the first oxidation step gives two reactive acyclic structures, each one with two aldehydic groups and one –OH group, and in equilibrium with an unreactive cyclic hemiacetal form. In the second step, the oxidation proceeds on the reactive acyclic structures. The time-evolution of the reaction can be followed in several ways, depending on the species monitored: i.e., by the variation of the triol concentration, and of the periodate, or of the formic acid that is formed during the reaction. Several rate constants, for the two sites in competition with each other, can be defined. A systematic analysis of the change in concentration of the several reactants and reaction products was carried out by Aalmo and Painter [7] and it can be summarised as saying that the amount of singly oxidised substrate firstly increases, reaches a maximum and then decreases; formic acid is formed as soon as the singly oxidised product is attacked for the second time.



Scheme 1.

A complication occurs with homopolysaccharidic or heteropolysaccharidic chains for which an oxidation limit is found, defined as the number of moles of periodate anions consumed for each mole of monosaccharidic monomer. In fact, the reaction leads to an oxidised form that, in compatibility with the structure of the monomer, may give hemiacetalic forms of two types. Intra-residue hemiacetals are formed by condensation of an aldehydic group with a hydroxyl group inside the same monomeric unit. Inter-residue hemiacetals are obtained by condensation between a –CHO group and an –OH group belonging to two different residues. This reaction protects the –OH group [12,13] belonging to non-oxidised residues adjacent to oxidised residues, thus lowering the oxidation limit compared with theoretical values. The lack of –OH on C-6, as in (1→4)-polyuronates in general or in C-6 derivatives, does not permit the formation of intra-residue hemiacetals, but only inter-residue ones. This situation is described for alginates, polygalacturonates and others [14].

Thus, the evaluation of concentration changes for the purpose of characterising the kinetics of the periodate attack is not only experimentally tiring, but also complicated if several sites are present simultaneously. The calorimetric method presented here is simply another way of following the reaction 'on-line' by measuring the heat evolved as a consequence of the reaction. The method does not introduce any specific advantage over other instrumental methods for concentration determination, but it offers a number of benefits, such as a high sensitivity (due to fact that the heat involved in a bond rupture is very high, of the order of 100 kcal mol^{-1}), and its insensitivity to physical characteristics or to whether the sample is transparent or not (which may rule out optical methods). Our long-standing experience in calorimetry has, therefore, suggested this technique be used for a comparative study of the reaction rates of several carbohydrate substrates towards periodate oxidation. The method is applied here to simple monosaccharides, with a variety of stereochemistry, and is currently used in our laboratory for the determination of kinetic

rates of some polysaccharidic systems. Once standardised, the same approach may also be used to deconvolute other time-dependent calorimetric signals, such as those obtained following the conformational transition of ionic polysaccharides, e.g., the ion-induced ordered structure of carrageenan [15] and of the capsular polysaccharide EPS-TA1 [16].

2. Experimental

Samples.—All the carbohydrate substrates were obtained from Sigma Chemical Co., St Louis (USA), and used without any further purification. Sodium periodate and sodium perchlorate were supplied by Carlo Erba (Italy). The sample of carboxymethylamylose DS = 0.3 was prepared in the laboratory of Professor D.A. Brant (University of California, Irvine). All substrates were used in aq soln, freshly prepared from mother solutions always stored in the dark.

Calorimeter.—A batch-type isothermal microcalorimeter LKB 10700-2, equipped with twin gold cells, was used. The power unit of the system was modified in order to expand the temperature control up to 50 °C. The temperature, once set, was checked directly inside the thermostatted air bath. One mL each of reactant and substrate were equilibrated inside the calorimeter for at least 6 h. The output signal of the thermopiles, amplified by a Keithley 150B microammeter, was connected to a PC through a Picolog® A/D data acquisition interface. The capability of the digital acquisition was limited to 1 datapoint/s as an average over 10 points. The time scale was set to zero at the mixing of the two solutions. Mathematical analysis of the calorimeter data was carried out using the ORIGIN graphic package. All measurements were carried out with an excess of substrate (concentration of periodate, C_A^0, equal to 6.25×10^{-4} M and substrate concentration, C_B^0, equal to 6.25×10^{-2} M) to keep the reaction within the conditions of a pseudo-first order reaction rate ($C_A^0 \ll C_A^0$). These conditions simplify the treatment of the calorimetric data, since the assumption is made that the conversion rate of periodate is directly proportional to the heat evolution measured by the calorimeter (see below). Most of the measurements were carried out at 25 °C; other measurements in the range from 15 to 45 °C were used to construct the Arrhenius plots.

3. The calorimetric approach to kinetic rate

Theoretical treatment of kinetic data in a heat-conduction isothermal calorimeter.—The use of calorimetric techniques to study reaction kinetics implies a knowledge of the 'dynamic behaviour' of the calorimeter itself. The thermal power — the quantity related to the intensity of the process under investigation — can be either accumulated inside the calorimetric cells or exchanged with the surroundings. The balance of the thermal power depends, therefore, not only on the process, but also on the calorimeter in use. The well-known Tian equation (1) [17] describes the heat balance in a calorimetric system:

$$C\,\mathrm{d}T = \mathrm{d}H - k\Delta T\,\mathrm{d}t \quad (1)$$

where C is the heat capacity of the sample, ΔT the temperature difference between the reference and the sample cell, k the thermal conductivity of the sample cell and $\mathrm{d}H$ the heat evolved by the reaction in the time $\mathrm{d}t$.

In several calorimeters the differences in temperature are minimised and therefore an approximation is made and it is assumed that the temperature in the calorimeter cells remains constant. Thus, as a first approximation, the response of a calorimeter is given by Eq. (2):

$$W(t) = K_c\left[S(t) + \tau\frac{\mathrm{d}S}{\mathrm{d}t}\right] \quad (2)$$

where $W(t)$ is the 'real' power curve (in an ideal calorimeter), $S(t)$ the recorded signal, K_c the calorimetric constant (watts/volts) and τ the characteristic time constant of the calorimeter. The equation clearly shows that the heat generated during the reaction is distorted by the thermal lag of the calorimeter. The kinetic phenomenon under study gives a heat-input $W(t)$ that is being transformed into another output function of time, the calorimetric curve, $S(t)$.

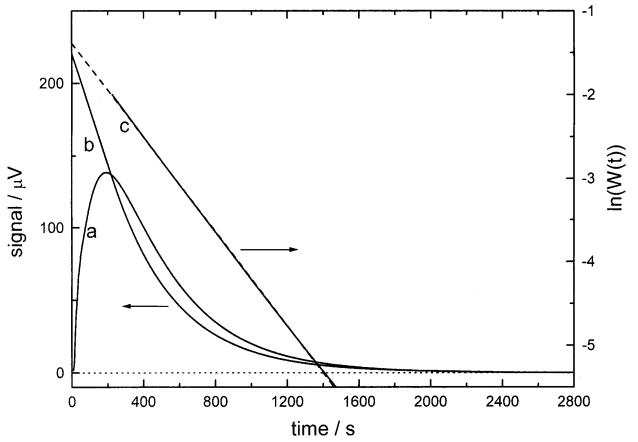


Fig. 1. Typical calorimetric curve (a) recorded for the reaction of IO_4^- ($c = 6.25 \times 10^{-4}$ mol L^{-1}) with methyl xyloside ($c = 6.25\ 10^{-2}$ mol L^{-1}) at 35 °C in water. The reconstructed curve (b) by taking into account the dynamic response of the calorimeter, and its logarithmic function (c) are also reported (dash-line is the linear fit for the pseudo first-order reaction).

Input and output are related to each other through Eq. (3):

$$W(t) = h(t)S(t) \tag{3}$$

where $h(t)$ is a transformation matrix containing the terms (4):

$$h(t) = \sum_i a_i \exp\left(\frac{-t}{\tau_i}\right) \tag{4}$$

where the quantities τ_i are the dynamic constants of the 'real' calorimeter. In principle, time constants of orders greater than unity should be used, but for 'slow' reactions it is often acceptable to use only a constant of the first order, since the values of the successive orders decrease very sharply.

In this work, an average time constant of the calorimeter has been determined by fitting the decay of the calorimeter response to a 'fast' chemical reaction, the mixing of a dilute aqueous solution of NaOH with an aqueous solution of HCl. Furthermore, the evaluation of the calorimetric time constant was also checked by means of electric calibration, as the heater is located in the central part of the calorimetric cell. Two slightly different values were obtained for the two procedures and only the 'chemical calibration' was considered appropriate. The value of τ for the calorimeter used in this work is 79.4 ± 10.12 s at 25 °C and decreases with increasing temperature.

Reaction kinetics and data treatment.—In a batch calorimeter with an extremely fast response, a first-order (or a pseudo-first-order) reaction, which takes place at a moderate rate and with an enthalpy change ΔH_R, is measured by the extent of reaction $\xi(t)$ at time t (after mixing the reactant solutions to the final volume V) simply related to the partial area $Q(t)$ by Eq. (5):

$$\xi(t) = \frac{Q(t)}{VC_A^0 \Delta H_R} \tag{5}$$

where C_A^0 is the initial concentration of the reactant A at $t = 0$ (for a pseudo-first-order process, it is the initial concentration of the reactant in marked defect) and $Q(t)$ is the heat associated with the reaction.

The rate of reaction is then given by Eq. (6):

$$\frac{d\xi(t)}{dt} = \frac{W(t)}{VC_A^0 \Delta H_R} \tag{6}$$

where $W(t)$ is the power at time t. Integrating (7):

$$W(t) = VC_A^0 \Delta H_R k_1' \exp^{-k_1' t} \tag{7}$$

whence (8):

$$\ln W(t) = \ln W_1(0) - k_1' t \tag{8}$$

where $k_1' t$ is the rate constant, $W_1(0) = VC_A^0 \Delta H_R k_1'$ and $k_2 = k_1'/\Delta C$ ($\Delta C = C_B^0 - C_A^0$).

A typical data treatment of the calorimetric output, therefore, follows these steps: (i) the recorded thermogram is treated according to Eq. (2); a reconstructed signal (see Fig. 1) is obtained which corresponds to that of an ideal calorimeter with no time lag; (ii) the curve is then treated according to Eq. (8) in order to evaluate k_2 from the slope of the curve of $-\ln(W(t))$ versus t. From the intercept, $\ln W_1(0)$, of the same graph and from k_2, the enthalpic parameter of the reaction can be determined.

4. Results and discussion

All the measurements were carried out with an excess of substrate in order to consider the reaction as a pseudo first-order one to simplify the data treatment. The excess of substrate allows the reaction to leave most of the sub-

strate (up to 98%) unoxidised at the end of the reaction. This condition has been chosen to give reproducible results for the determination of k_2, irrespective of the type of site present in the oxidisable substrate. From the calorimetric curve (see typical plot in Fig. 1) the linearisation of the 'reconstructed' power $W(t)$ gives, according to Eq. (8), the value of k_2 and that of ΔH_R, the total enthalpy of oxidation (60.2 ± 0.5 kcal mol^{-1} as an average over all measurements). The experimental data at 25 °C are reported in Table 1 (which also gives the activation data discussed below). A substantial agreement or a similar trend is found between the k_2 values of Table 1 with those reported in literature (k_2 of Ref. [11] and $(k_P)_0$ of Ref. [7]).

Comparison between k_2^{25} *for monosaccharides and their stereochemistry*.—The common characteristic of all monomers used in this study is the presence of a methoxyl group instead of a hydroxyl one on the anomeric carbon. The reason for using methyl glycoside is twofold: (i) the methoxylation at C-1 eliminates the possibility of attack on the site C-1—C-2; (ii) by blocking the anomeric form, the oxidation involves only the ring form of the sugar and, in this way, it is possible to study the influence of the anomeric effect at C-1. However, most sugar monomers have two oxidisable sites and therefore some overlapping of the two reactions is obtained, unless the difference between the two rates hides the slower reaction. In any case, major variations in the values of the rate constants arise from the different relative positions of the hydroxyl groups of the reactive site.

These features can be identified for the several monosaccharides used in Scheme 2.

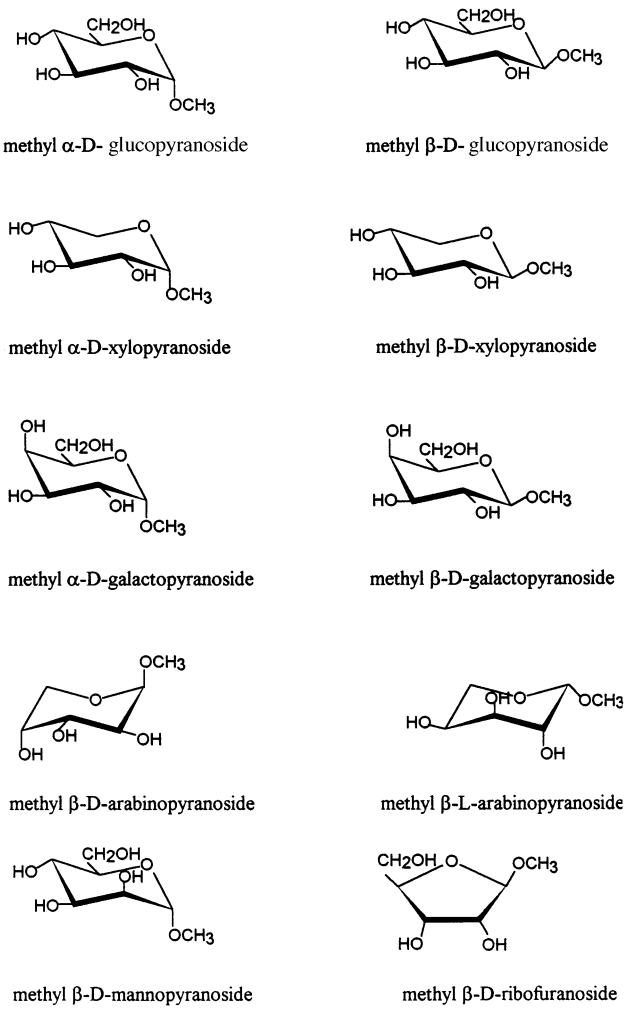
Glucosides and xylosides have all three –OH groups in the equatorial position. A reaction site is given by the hydroxyl groups in gauche relationship at C-3—C-4; the other site is at C-2—C-3. Rate constants are therefore similar and are of the order of 10^{-2} L mol^{-1} s^{-1}. Due to the fact that the reaction implies the formation of a cyclic ester of periodate with the hydroxyl groups in an eclipsed position, the rotation of 60° about the C—C bond must lead to an unfavourable deformation of the chair $^4C_1$.

The rate constant increases approximately by a factor of 5 for the two galactopyranosides. The stereochemistry of these two saccharides is such that the –OH groups at C-2 and C-3 are both equatorial and in *trans* relationship while the –OH at C-4 is axial and in *cis* disposition with the –OH at C-3. Between the two sites of reaction, the favoured one appears to be that involving C-3—C-4. The rotation of 60°, to give the eclipsed position, requires an energy much lower than that necessary for the rotation around the C-2—C-3 bond. As the energy of the rotation is lower, the breakage of this bond is faster (k_2 higher) than the one for the C-2—C-3 bond, as well as that for other similar configurations (i.e., glycosides and xylosides).

An analogous situation to methyl galactosides is seen in methyl mannoside that has the –OH at C-2 and C-3 in axial and equatorial positions, respectively. Although the position of the site on the ring is different, the

Table 1
Kinetic data (k_2^{25}, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) for the initial stage of periodate oxidation on some carbohydrates

Compound	k_2^{25} (L mol^{-1} s^{-1})	$\Delta H^{\ddagger}$ (kcal mol^{-1})	$-\Delta S^{\ddagger}$ (cal mol K^{-1})
Methyl α-D-glucopyranoside	3.99×10^{-2}	10.7	28.8
Methyl β-D-glucopyranoside	2.96×10^{-2}	11.0	28.3
Methyl α-D-xylopyranoside	3.44×10^{-2}	8.92	35.1
Methyl β-D-xylopyranoside	2.71×10^{-2}	8.38	37.3
Methyl α-D-galactopyranoside	1.62×10^{-1}	16.6	6.2
Methyl β-D-galactopyranoside	1.92×10^{-1}	16.7	5.6
Methyl β-D-arabinopyranoside	2.91×10^{-1}	13.4	16.2
Methyl β-L-arabinopyranoside	2.81×10^{-1}	12.2	21.6
Methyl α-D-mannopyranoside	1.39×10^{-1}	9.85	29.2
Methyl β-D-ribofuranoside	28.6	–	–
CMA DS 0.3, $I = 0.21$	4.42×10^{-2}	7.82	38.2



Scheme 2.

configuration corresponds to the favourite one for oxidation. Therefore, the values of the rate constant of the two classes of compound (galactosides and mannosides) confirm their similarity relative to oxidation, irrespective of the –OH positions in the ring.

Another category of pyranosides is given by methyl β-D-arabinopyranoside and methyl β-L-arabinopyranoside. No difference is found for the two enantiomeric forms. The conformation of these substrates is such that the –OH at C-2, C-3 and C-4 are equatorial, equatorial and axial, (or axial, axial and equatorial), respectively. The –OH at C-3 and C-4 are in *cis* disposition for both monosaccharides. The reaction, therefore, will be equally fast at C-3—C-4, as for galactosides. The rate constants are similar to the values found for the other substrate in which one site is more favourable than the other (of the order of 2–3 10^{-1} L mol^{-1} s^{-1}).

The results of all the above nine monomers can be grouped together and compared with those obtained for methyl β-ribofuranoside. The latter has only two vicinal –OH groups and therefore only one site to be attacked. The two –OH groups are in *cis* disposition and the eclipsed isomer is the most favourite of all the other conformations. Therefore, the high value of the kinetic rate is due to this peculiar aspect which makes the ribofuranoside much more reactive than the other classes of compounds.

It is worth emphasising that, when there are two oxidisable sites, the two reactions are competitive. However, if one C–C bond is more reactive than the other, the overall rate constant mainly reflects the velocity of the site with a less conformational distortion. Instead, if the two sites have the same stereochemistry, the rate constants are comparable and therefore both sites are similarly active for the reaction. The result is an apparent increase in site concentration.

Some further complications arise for the kinetics involving *vic*-triols, as in the present case [7]. The presence of a second attack (consecutive reaction) may affect the rate measured — as it has been noted by a referee — unless masked by the values of the heats and kinetic rates of the two reactions, as it appears from the linearity of the data shown in Fig. 1 (line c).

When sugars differ from each other by characteristics that do not imply the relative position of the –OH groups, only minor differences in the rate constants are detected and these are possibly related to structural differences and to solvation effects due to the structural differences [18]. In particular, when comparing different anomers of the same monosaccharide, the rate constant of the α anomer is higher than the β anomer for glucopyranoside and xyloside while that of the β anomer of galactoside is higher than that of the corresponding α form. In the first two

cases, the $-OCH_3$ group in α speeds up the reaction, while the contrary is seen for the galactopyranoside; the effect of the $-OCH_3$ can be attributed to the interaction between the group and the –OH present in the sugar.

In order to extend the investigation to a carbohydrate chain, a sample of carboxymethylamylose (CMA) with DS = 0.3 was used. The reaction was carried out in the presence of salt (ionic strength 0.21 M), in order to screen all the charges of the polyelectrolyte whose average number of modified units is about 1:3. The value of the rate constant (see Table 1) shows a surprisingly apparent insensitivity of the reaction rate to the presence, or absence of the monomer in a chain. In fact, since the mechanism of oxidation implies a distortion of the ring, one should expect the reaction to be more hampered if the monomer is inserted into a chain. However, the constancy of the reaction rates means that the probability of thermal fluctuations of the ring geometry towards the planar conformation at O-2—O-3 is not a function of chain substituents at C-1 and C-4. This assumption surely needs to be verified by direct experimental investigation.

Temperature dependence and energy of activation.—The reaction kinetics were measured as a function of temperature, over the range from 15 to 45 °C. Despite the limited temperature variation, the kinetic rates changed substantially and allowed the construction of Arrhenius plots (Fig. 2), according to general empirical equations [19], which give the activation energy E_a:

$$\ln k_2 = \ln A + E_a/RT \quad (9)$$

From the values of the activation energy and rate constants at 25 °C, the kinetic parameters of the enthalpy and entropy of activation, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, were obtained through the following equations:

$$E_a = RT^2\left(\frac{d \ln k_2}{dT}\right) \quad (10)$$

For a monomolecular reaction:

$$E_a = \Delta H^{\ddagger} + RT \quad (11)$$

and:

$$-\Delta S^{\ddagger} = \frac{-RT \ln K^{\ddagger} - \Delta H^{\ddagger}}{T} \quad (12)$$

where:

$$K^{\ddagger} = \frac{k_2 h}{K_B T} = k_2 \ 1.61 \times 10^{-13} \quad (13)$$

The values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are reported for each substrate in Table 1. They show a variation of between 7 and 17 kcal mol^{-1} for $\Delta H^{\ddagger}$ and between -5 and -38 cal mol^{-1} K^{-1} for $\Delta S^{\ddagger}$. The trends of these values do not seem to be related to any structural feature of the glycosidic compounds, while the negative value of the entropy of activation must be ascribed to the decrease in the degree of freedom of the activated state. In addition, the effect of hydration can be also hypothesised to differentiate the compounds.

In the literature, a series of similar reactions is characterised by correlation between the kinetic and static (thermodynamic) parameters. These correlations are often empirical, but they do provide a test as to whether the experimental data refer to homologue situations or not. A scrutiny of the two series of values, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, reported in Table 1 reveals that a correlation exists; i.e., the less negative $\Delta S^{\ddagger}$ the higher $\Delta H^{\ddagger}$. In particular, the plot reported in Fig. 3 shows that the enthalpy of activation correlates very well with the en-

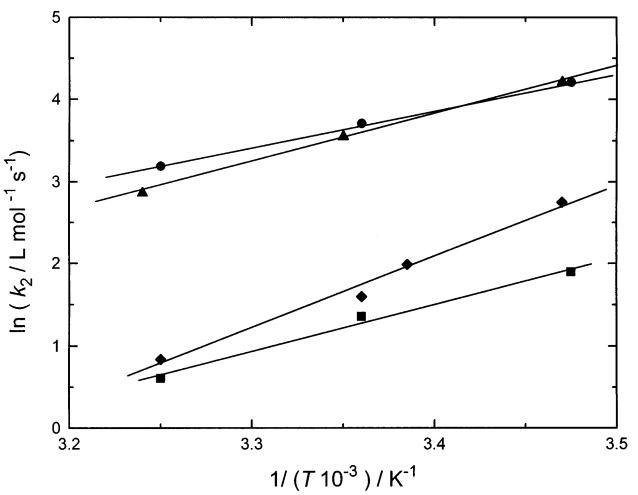


Fig. 2. Arrhenius plot of the rate constants for methyl β-glycosides: methyl β-D-glucopyranoside (▲); methyl β-L-arabinopyranoside (■); methyl β-D-xylopyranoside (●); methyl β-D-galactopyranoside (◆).

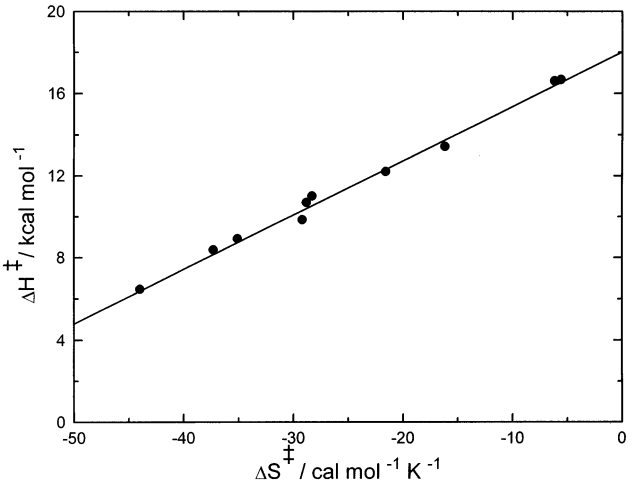
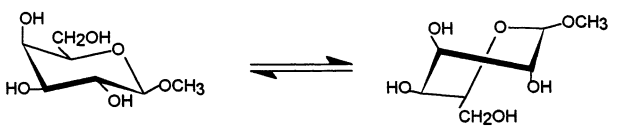


Fig. 3. Correlation between the enthalpy and entropy of activation (calculated according to Eqs. (11) and (12) for the periodate reaction at 25 °C. Substrates are listed in Table 1.



Scheme 3.

tropy of activation, the data showing an alignment on a straight line with a slope of 264 K. The linearity confirms that the mechanism of reaction for the kinetic rates considered here is the same, although this may seem obvious on the basis of the periodate oxidation reaction, that is, the oxidation involves a vicinal diol on a sugar ring.

A further conclusion can be drawn from these experimental findings. The whole kinetic process involves the same activation state, which is believed to correspond to the planar orientation of the vicinal diol, bridged with the periodate ion in a cyclic ester. Therefore, one would expect the reaction rates to be essentially a function of the probability (i.e., the conformational energy) of the quasi-planar form of the sugar as in Scheme 3.

If the above statement is true, it immediately follows that the kinetic rates of the periodate oxidation are proportional to the statistical weight of the quasi-planar conformational forms of the sugars. This means that the kinetic values really 'rate' the actual concentration of molecules which are in a suitable conformation for the reaction. Therefore, by using the simple Boltzmann expression (14):

$$p_a/p_b = \exp(-\Delta G^{\ddagger}_a/RT)/\exp(-\Delta G^{\ddagger}_b/RT) = k_a/k_b \quad (14)$$

where p_i is the probability of finding a molecule in the given conformation i, and k_i corresponds to the reaction rate of that conformation, by rounding off the kinetic rates of Table 1, the following statistical weights can be assigned, relatively to the ribose molecule (which has the highest rate):

1. 0.0007–0.0014 for glycosides, xylosides, CM-amylose
2. 0.0050–0.0087 for galactosides, mannosides and arabinosides
3. 1 for β-D-riboside

On the assumption that the most stable conformation of riboside corresponds to a planar vicinal diol, then an estimate of about $+4.2$ kcal mol^{-1} can be assigned to the conformational state obtained by distortion of the ring, to reach the quasi-planar form for the vicinal diol of the other sugars. This value is indeed extremely interesting in view of the fact that conformational statistics of carbohydrate molecules usually take into account states with energy ranging from 0 to $+5$ kcal mol^{-1}. If the assumptions behind the calculations reported here are acceptable, then molecular dynamic studies on these carbohydrate molecules could easily give the 'theoretical' population of the 'excited' states. One must be aware, however, that the high energy of these conformational states implies, within the framework of the ergodic theorem, that calculations have to be run for more than 1 ms, in order to catch about 0.7 ps of existence of the above states. Thus, kinetic studies can be very helpful in providing information for low populated states that are involved in the reaction.

5. Conclusions

Two main conclusions can be drawn from the results reported here: the first concerns the assessment of the calorimetric method for kinetic studies, the second refers to the possibility of determining the statistical weight of a reactive conformational state.

The calorimetric method can be safely used to determine kinetic parameters, provided that care is taken over correcting of the dynamic response of the calorimeter. In particular, given the recent progress of data acquisition, the kinetic data can be averaged by using a high number of data points and therefore a good degree of accuracy of measurements is obtained. In the absence of a predetermined reaction order, a useful mechanistic approach to the determination of both thermodynamic and kinetic parameters has recently been proposed by Beezer and co-workers [20]. We should like to emphasise that their method is not limited to infinitely slow kinetics (as described in their report), but that it can be extended with the proper dynamic correction to 'faster' reactions. The objective of the present investigation, however, was not to study the mechanism or the reaction order of periodate oxidation, which had already been examined in great detail. One of the outstanding aspects of our results is the fact that the determinations are able to disclose, quantitatively, the course of the reaction with a reactant concentration in the ratio 1:100. The signal was still intense enough to be able to work with 10–50 times more dilute a system.

Provided that the compounds can be grouped into a class of similarly reactive species, the reaction rates can be used to determine the probability of the conformational form that is involved in the activation state. It is, therefore, claimed that kinetic rate of periodate oxidation is capable of detecting the existence of a fraction of conformational states down to 0.07%. To the authors' best knowledge, no other experimental method is able to detect down to such a low fraction of conformational states.

An extension of these approaches to other glycans is underway with the intention of providing new insights into this 'old' analytical tool.

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