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Kinetics of the Reaction Between 3-Deoxyhexosulose and Nitrite Ion

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ABSTRACT

The reaction between 3-deoxyhexosulose (DH) and NO_2^- *at pH 5·5 and 55°C proceeds with 1:1 stoichiometry. The kinetics show it to be of first order with respect to DH,* NO_2^-, H^+ *and components of acetate buffer. No specific ion effects could be observed for* Na^+, K^+, Cs^+, NO_3^-, Cl^-, ClO_4^- *and* SO_4^{2-} *and the rate of reaction is unaffected by change in ionic strength. These observations are explained by rate-determining formation of a reaction intermediate with composition* $DH\text{-}NO^+$ *and the role of acetate buffer is suggested to be that of an acid-base catalyst.*

INTRODUCTION

Compounds with activated methylene groups (CH_2 adjacent to a carbonyl group) are known to react with nitrosating species to form C-nitroso products which subsequently rearrange to oximes. Such activated methylene compounds are formed widely in carbohydrate degradation reactions and perhaps the best known example is 3-deoxyhexosulose (DH) which is the product of the degradation of glucose catalysed by acid or assisted by amines as in the Maillard reaction. DH is expected to be formed widely in foods; it is a precursor of 5-hydroxymethylfurfural which is found in many thermally processed foods and in some foods there is indirect evidence for the formation of DH from its conversion to 3,4-dideoxy-4-sulphohexosulose in the presence of sulphur dioxide (Wedzicha, 1987). DH and

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compounds derived from it are thought to be important intermediates in the formation of colour in the Maillard reaction.

Nonenzymic browning is possible in all nitrite-treated foods. The reported occurrence of nitrosamines in beer (Spiegelhalder *et al.*, 1979; Goff & Fine, 1979) and the suggestion of the presence of nitrosating species at the time of malting, where extensive nonenzymic browning takes place, adds further interest to a study of potential nitrosation reactions involving DH. Carbohydrate degradation products should also form when media for microbiological assays are autoclaved; the reactivity of such heated media towards nitrite ion is also of interest.

It has already been demonstrated that 3-deoxyosuloses react with nitrite ion at pH 4·7 and 7·0 (Wedzicha *et al.*, 1982). The purpose of this investigation is to report the kinetics of the reaction between DH and nitrite ion and to propose a mechanism.

EXPERIMENTAL

Preparation and purity of 3-deoxyhexosulose

3-Deoxyhexosulose was prepared according to the method of Madson & Feather (1981) by forming the bis(benzoylhydrazone) of DH from glucose in a single step. This intermediate gave on microanalysis: C = 57·8%; H = 5·8%; N = 13·5%, calculated for $C_{20}H_{22}O_5N_4$: C = 57·5%; H = 5·8%; N = 13·5%, and was converted to DH by a transhydrazonisation reaction with benzaldehyde. The only difference between our procedure and that reported by Madson & Feather (1981) is the use of Amberlite IRA93 resin in the OH^- form in place of IRN-78 resin in the carbonate form to remove acidic impurities from DH. On microanalysis the sample of DH gave: C = 38·5%; H = 6·7%; N < 0·3% (the limit of detection), calculated for $C_6H_{10}O_5.(1{\cdot}5H_2O)$: C = 38·5%; H = 6·8%; N = nil. The homogeneity of the product was demonstrated by thin-layer chromatography on silica $60F_{254}$ plates with water-saturated butanol and ethanol:0·880 ammonia solution:water (4:2:1) solvents. The spots were revealed by spraying with *p*-anisidine hydrochloride solution (6% w/v in ethanol) and heating plates at 120°C for 6–8 min.

For comparison, a sample of DH was prepared using the browning reaction of glucose with butylamine as described by Kato (1962). Microanalysis showed the composition to be $C_6H_{10}O_5.(3H_2O)$. Samples of DH prepared by the two methods were compared by tlc on silica as described above. The samples were also converted to 2,4-dinitrophenylhydrazones (Wedzicha & McWeeny, 1974) and analysed by tlc on silica $60F_{254}$ using butanol:0·880 ammonia solution (4:1) as solvent.

Standard solutions of DH were prepared by weight. Since DH is hygroscopic, solutions were standardised by conversion of DH to the metasaccharinic acid with limewater and titrating the acid produced, as described by Anet (1961) and modified by Wedzicha & Kaban (1986).

General procedure used for following the reaction

Kinetic measurements were carried out at 55·0 ± 0·1°C. Reaction mixtures were prepared in acetate buffer ($[CH_3COOH] + [CH_3COO^-] =$ 0·11–4·12 M, pH 3·7–5·7) and contained $NaNO_2$ (0·0925–0·365 M) and DH (0·049–0·391 M). When the concentration of $NaNO_2$ was varied, ionic strength was maintained constant by the addition of $NaNO_3$. The effect of ionic strength on initial rate was investigated by preparing reaction mixtures containing added $NaNO_3$ (0–3 M) and its effect on the response of the pH electrode was determined by measuring the pH of a solution of HCl (0·01 M) whose ionic strength had been adjusted, also, with $NaNO_3$. pH measurements were made using a combined glass–calomel electrode connected to a Jenway PHM6 digital pH meter and the apparent pH values of reaction mixtures were corrected as described by Wedzicha & Goddard (1988). The possibility of a specific kinetic effect due to buffer was investigated by varying buffer concentration ($[\text{buffer}] = [CH_3COOH] + [CH_3COO^-] =$ 0·11–2·36 M) and the role of the individual species investigated by means of experiments at constant acetic acid concentration (0·154 M) and variable acetate ion concentration (0·1–1·0 M). The specific ion kinetic effect was also measured for ClO_4^-, Cl^-, SO_4^{2-}, K^+, Cs^+ (0·5 M).

The progress of the reaction was followed by measurement of residual nitrite ion. Samples were withdrawn at timed intervals and analysed by the spectrophotometric method of Vogel (1978) using sulphanilamide for reaction with nitrous acid and coupling the diazotised product with *N*-(1-naphthyl)-ethylenediamine hydrochloride. Initial rates were obtained by drawing tangents to concentration–time graphs, at zero time.

RESULTS

Purity of 3-deoxyhexosulose

The sample of DH prepared by the method of Madson & Feather (1981) showed a single spot when subjected to tlc developed with water-saturated butanol or ethanol:water:0·880 ammonia solution with R_f 0·44 and 0·64, respectively. The sample of DH prepared by the method of Kato (1962) was less pure, showing in each case two additional spots: R_f 0·64 and 0·29 in water-saturated butanol and R_f 0·72 and 0·41 in ethanol:water:0·880 ammonia solution. Anet (1961) obtained similar results for this preparation and suggests that the two additional spots correspond to the *cis* and *trans* isomers of 3,4-dideoxyhexosulos-3-ene, formed by the dehydration of DH. Solutions in water of DH prepared according to Madson & Feather (1981), which had been allowed to stand for 24 h, showed the same impurities as found in the preparation according to Kato (1962). The method of Madson and Feather also allows several grams of DH to be prepared in high yield (36% based on the weight of glucose used) in contrast to yields of the order of 0·5% by the method of Kato. The former method was, therefore, used for all kinetic work described here and the purity of freshly prepared DH was of the order of 97% when determined as the metasaccharinic acid.

Stoichiometry of the reaction

The stoichiometry of the reaction was determined by measuring, after the reaction had gone to completion, the change in concentration of nitrite ion in mixtures containing initially an excess of $NaNO_2$. The initial concentrations of reactants and the amounts of nitrite ion consumed in the reaction are shown in Table 1, from which it is clear that the stoichiometry is close to 1:1.

Measurement of hydrogen ion concentration

The emf, E, of the glass–calomel electrode assembly is related to the activity, a_{H^+}, of H^+ by:

$$E = E' - (RT/F)\ln a_{H^+} \tag{1}$$

where R is the gas constant, T is the absolute temperature, F is the Faraday constant and E' is a constant which is the sum of the standard electrode

TABLE 1

Stoichiometry of the Reaction between DH and Nitrite Ion. Initial Conditions: $pa_{H^+} = 5{\cdot}62$ [buffer] = 0·59 M, 55°C

Initial [DH] (M)	*Initial [NO_2^-]* (M)	*Change in [NO_2^-]* (M)	*Stoichiometry* (mol NO_2^-/mol DH)
0·0490	0·1800	0·0562	1·15
0·1013	0·1800	0·0993	0·98
0·1967	0·3500	0·1840	0·94
0·1967	0·4720	0·2270	1·15
0·1967	0·4920	0·2380	1·21
0·1967	0·5900	0·2130	1·08

potential of the cell, the liquid junction potential and other contributions to the emf which do not arise from the cell reaction (e.g. the asymmetry potential of the glass electrode). The value of E' depends on ionic strength and, in principle, it is possible to measure pa_{H^+} (i.e. $-\log a_{H^+}$) reliably, in any real system if the variation of E' with ionic strength can be estimated. It can be shown that the difference in apparent pH (ΔpH_{app}) of a 0·01 M solution of HCl and that of the same solution whose ionic strength had been adjusted by the addition of a salt, is given by (Wedzicha & Goddard, 1988):

$$\Delta pH(app) - 0{\cdot}043 = \Delta E'' + \log y_{H^+} \qquad (2)$$

where y_{H^+} is the activity coefficient in terms of concentration of hydrogen ion in the salt-containing medium, E'' is the value of E' in the equivalent number of pH units (i.e. $E'F/2{\cdot}303RT$) and 0·043 is $-\log y_{H^+}$ for 0·01 M HCl. In this work, ionic strength was adjusted using $NaNO_3$ and y_{H^+} can be estimated from tables of mean ionic activity coefficients of $H^+NO_3^-$ at various concentrations (Robinson & Stokes, 1965). Hence measurement of the apparent pH of a solution of 0·01 M HCl at various ionic strengths and taking into account the value of $\log y_{H^+}$ allows E'' to be estimated and used as a correction for other pH measurements provided that E'' is independent of pH. This is a reasonable approximation. The results of measurements give:

$$E'' = -0{\cdot}111\,I - 0{\cdot}065 \qquad (3)$$

where I is the ionic strength, for $I = 0{\cdot}20\text{–}2{\cdot}74$ M. The true value of pa_{H^+} may be found from the apparent pH-meter reading since

$$pa_{H^+} = pH(app) - E'' \qquad (4)$$

The correction is strictly applicable only to systems in which the major anionic species in NO_3^- and should apply well to NO_2^-. Unavoidably, the simplest buffer system which may be used to maintain pH at approximately pH 5·5, chosen as a reasonable pH for many food system models, is acetic acid/acetate ion. The correction described may not be carried out experimentally with acetate ion since it is a salt of a weak acid and will alter the acid-base behaviour of the system. The correction using NO_3^- to control ionic strength should, therefore, be regarded as an approximation.

Kinetics of the initial reaction

Measurement of the initial rate of reaction as a function of the concentrations of species normally present in reaction mixtures showed the rate to be of first order with respect to each component over an 8-fold

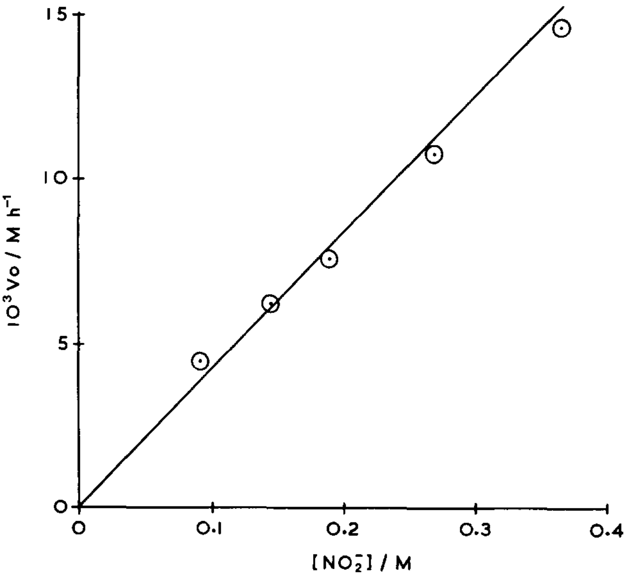


Fig. 1. Graph of initial rate (V_0) versus concentration of nitrite ion. Initial conditions: [DH] = 0·201 M, pa_{H^+} = 5·58, [buffer] = 0·59 M, 55°C. The ionic strength was maintained by adding $NaNO_3$ such that $[NaNO_2] + [NaNO_3]$ = 0·36 M.

variation of $[NO_2^-]$, 4-fold variation of [DH], 25-fold variation of [buffer] and 100-fold variation of $[H^+]$. Graphs of initial rate versus concentration for NO_2^- and DH are shown, respectively, in Figs 1 and 2. These are seen to be good straight lines passing through the origin. The effect of hydrogen ion concentration on initial rate is shown as a plot of log (initial rate) versus pa_{H^+} in Fig. 3, where the actual slope of the line, which gives directly the order with respect to H^+, is 0·99. The results for the effect of buffer concentration on initial rate are shown in Fig. 4. In these experiments pH was found to vary from run to run in the range pH 5·36–5·67 and account of this is taken by plotting (initial rate)/a_{H^+} versus [buffer]; that is, using the result that the reaction is of first order with respect to H^+ to compensate for the variation. This graph shows the initial rate to obey the following rate equation:

$$(\text{initial rate})/a_{H^+} = 4700\ [\text{buffer}]\ \text{h}^{-1} \tag{5}$$

and the fact that the graph passes through the origin implies that the observed reaction does not proceed at a significant rate, on the timescale of the measurements reported here, unless sufficient buffer is present.

If the rate law for the buffer-catalysed reaction is taken to be:

$$\text{initial rate} = k\,[\text{DH}]\,[NO_2^-]\,[H^+]\,[\text{buffer}]\ \text{M}\,\text{h}^{-1} \tag{6}$$

where all concentrations are initial concentrations, the values of k calculated

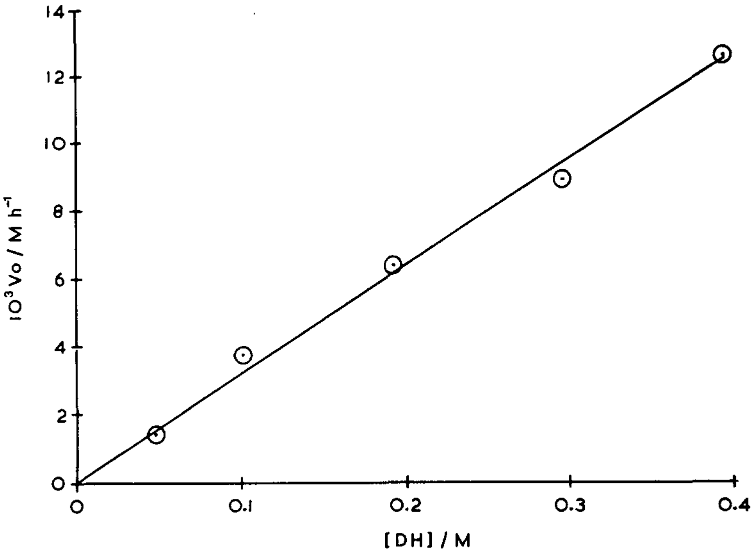


Fig. 2. Graph of initial rate (V_0) versus concentration of DH. Initial conditions: [NO_2^-] = 0·180 M, pa_{H^+} = 5·58, [buffer] = 0·59 M, 55°C.

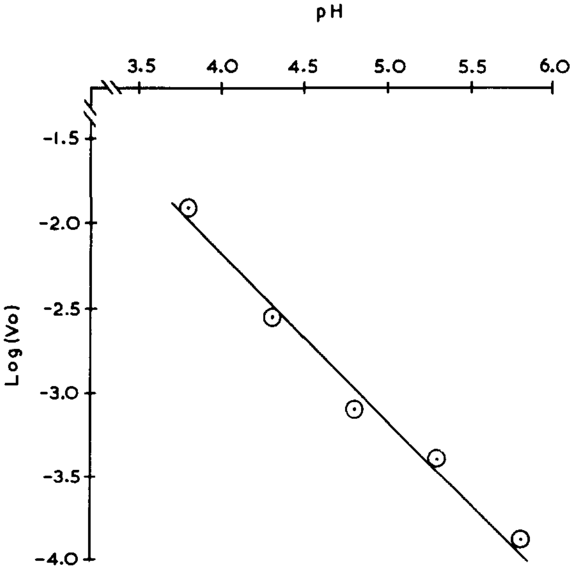


Fig. 3. Graph of log (initial rate) versus pa_{H^+}. Initial conditions: [DH] = 0·196 M, [NO_2^-] = 0·20 M, [buffer] = 0·73–4·12 M, 55°C. The pH was varied by changing the concentration of acetic acid. V_0 denotes initial rate.

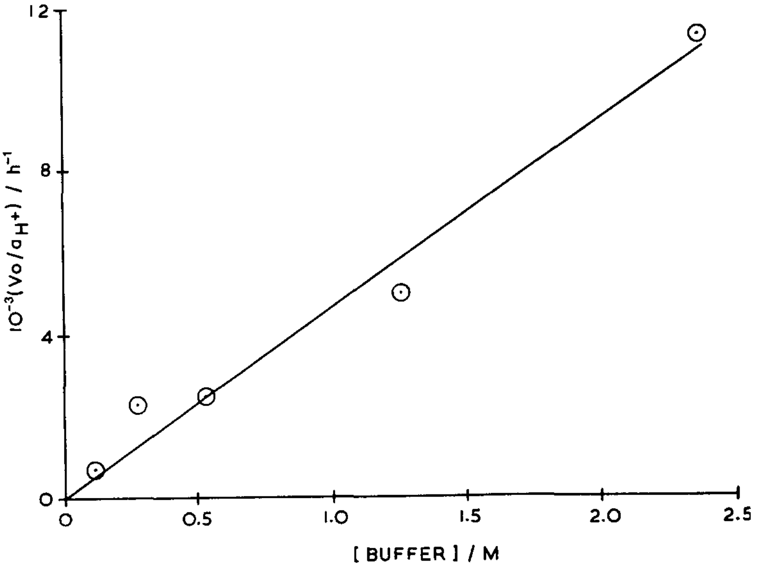


Fig. 4. Graph of (initial rate)/a_{H^+} versus [buffer] ([buffer] = [CH_3COO^-] + [CH_3COOH]). Initial conditions: [NO_2^-] = 0·196 M, [DH] = 0·180 M, 55°C. V_0 denotes initial rate.

from the first order rate constants obtained from the graphs in Figs 1, 2 and 4, and a graph of initial rate versus a_{H^+}, are, respectively, 1·41, 1·47, 1·44 and 1·42 × 10^5 M^{-3} h^{-1}. Thus, all the kinetic experiments are consistent with each other despite differences in ionic strength between sets of kinetic runs. With the exception of runs used to deduce the effect of buffer, the ionic strength of reaction mixtures was in the range 0·7–0·9 M, over which the values of activity coefficients are relatively insensitive to change in ionic strength and, therefore, any kinetic effect due to ionic strength will be small. In the case of kinetic runs in which buffer concentration was varied, the ionic strength varied in the range 0·28–2·40 M and the result shown in Fig. 4 could well include a contribution from the effect of ionic strength on rate. However, no kinetic effect was observed when $NaNO_3$ was added to reaction mixtures to vary the ionic strength in the range 0·1–3·2 M. This result also suggests that Na^+ and NO_3^- do not show specific ion effects. When the initial rate was measured for mixtures containing 0·5M salt solutions, the relative rate constants (k for $NaNO_3$ = 1) were, respectively, 0·93, 1·27, 0·95, 0·80 and 1·43 for KNO_3, $CsNO_3$, NaCl, Na_2SO_4 and $NaClO_4$. It may be concluded that anions and cations in general have little effect on the kinetics of the reaction.

The results for the kinetic effect of buffer provide no indication of which component of the buffer is the catalyst. Most of the experiments were carried out over a narrow range of pa_{H^+} and the ratio, [CH_3COO^-]/CH_3COOH] is

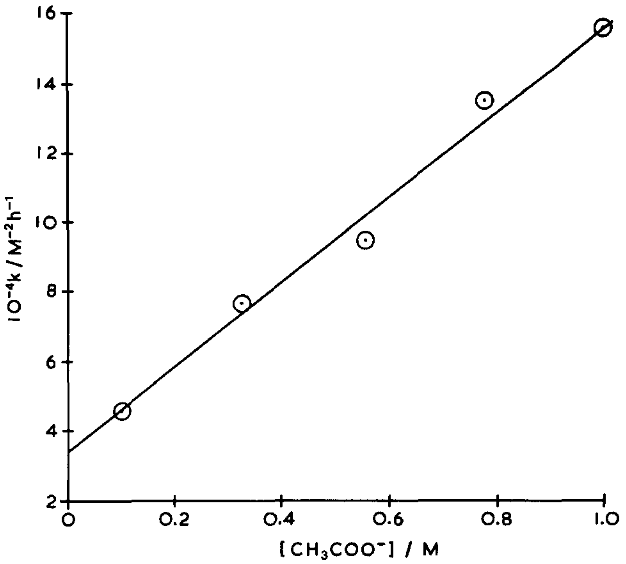


Fig. 5. Graph of third order rate constant (k = (initial rate)/[DH]$[NO_2^-]a_{H^+}$) versus $[CH_3COO^-]$ at constant $[CH_3COOH]$. Initial conditions: [DH] = 0·18–0·20 M, $[NO_2^-]$ = 0·20 M, $[CH_3COOH]$ = 0·154 M, pa_{H^+} = 5·16–6·18, 55°C.

relatively constant throughout the measurements plotted in Fig. 4. The kinetic effect of acetate ion at constant acetic acid concentration is shown in Fig. 5 as a graph of (initial rate)/([DH]$[NO_2^-]a_{H^+}$) and is consistent with:

$$k = (3{\cdot}4 + 12{\cdot}1\,[CH_3COO^-]) \times 10^4\,M^{-2}\,h^{-1} \qquad (7)$$

It is seen, therefore, that acetate ion exerts a specific catalytic effect. The deduction from Fig. 4 that the reaction does not appear to proceed in the absence of buffer, and the overall linearity of the graph, suggest that the constant term in eqn (7) should be an acetic acid-catalysed step and it is likely that it will be of first order with respect to $[CH_3COOH]$. If this were the case, the value of the rate constant for catalysis by the acid could be estimated as $3{\cdot}4 \times 10^4/0{\cdot}154 = 22{\cdot}1 \times 10^4\,M^{-3}\,h^{-1}$ which is approximately twice the value of the constant for catalysis by acetate ion. In most experiments the initial rate was measured at *c.* pH 5·5 because it was difficult to measure the relatively fast rates at lower pH. The concentration of acetic acid was, therefore, small compared to that of acetate ion and the dominant reaction was the acetate catalysed process. In order to study the acid catalysed reaction it is necessary to carry out experiments at constant acetate ion concentration but variable acetic acid concentration, under conditions where the acid catalysed reaction is more important. It was found that the precision of the measurements at the lower pH required was insufficient to resolve the effect of acetic acid from the large effect of pH.

During the preparation of DH it was observed that solutions of the product were unstable if left for 24 h at room temperature. The timescale over which kinetic measurements were made was, in many cases, similar. Any decomposition of DH would be accelerated at 55°C and it is interesting to consider if such changes in DH exert any kinetic effect on the reaction with nitrite ion. For this reason buffered solutions of DH were allowed to stand at 55°C for 24 h before being used to make up reaction mixtures. Subsequent experiments involved initial rate measurements at variable $[NO_2^-]$ (0·041–0·40 M) and [DH] (0·065–0·34 M). The overall quality of the data was similar to that plotted in Figs 1 and 2, and gave, by eqn (6), $k = 1{\cdot}42 \times 10^5\ \mathrm{M}^{-3}\,\mathrm{h}^{-1}$, which is indistinguishable from the values given above.

Kinetics of the overall reaction

In all kinetic experiments carried out here the concentrations of DH and NO_2^- were comparable and, therefore, both changed during the course of a kinetic run. It is expected, therefore, that the reaction will obey pseudo-second order kinetics during any run at constant pH. Integration of the second order rate equation, with the stoichiometry of the reaction taken as 1:1, gives,

$$\{1/([DH]-[NO_2^-])\}\ln\{[NO_2^-] \times ([DH]-[NO_2^-]+[NO_2^-]_t)/[DH][NO_2^-]_t\} = k_2 t \quad (8)$$

where k_2 is a second order rate constant and subscript t denotes concentrations at time t. Otherwise, all concentrations are initial concentrations. Examples of two second order plots according to this equation are shown in Figs 6 and 7, the runs chosen to illustrate, respectively, the behaviour of the fastest reaction followed (half-life 6 min) and one on the longest time scale followed (half-life 11·4 h). The graphs show that second order behaviour is followed convincingly beyond the initial stage of reaction and second order rate constants are, respectively, 29·0 and 0·27 $\mathrm{M}^{-1}\,\mathrm{h}^{-1}$. Rate constants calculated from the corresponding initial rates are, respectively, 19·2 and 0·18 $\mathrm{M}^{-1}\,\mathrm{h}^{-1}$ indicating that the overall reaction appears to proceed more rapidly than suggested by the initial rate. This effect could be attributed to a slow decomposition of DH to form a more reactive intermediate which would give rise to an apparently faster reaction over a long period of time. This is, however, inconsistent with the observation that the rate of reaction of preheated solutions of DH is the same as for fresh solutions. Any spontaneous changes in DH alone do not appear to affect the rate of reaction on the timescales investigated here. On the other hand, it was observed that mixtures of DH and nitrite ion undergo browning on the

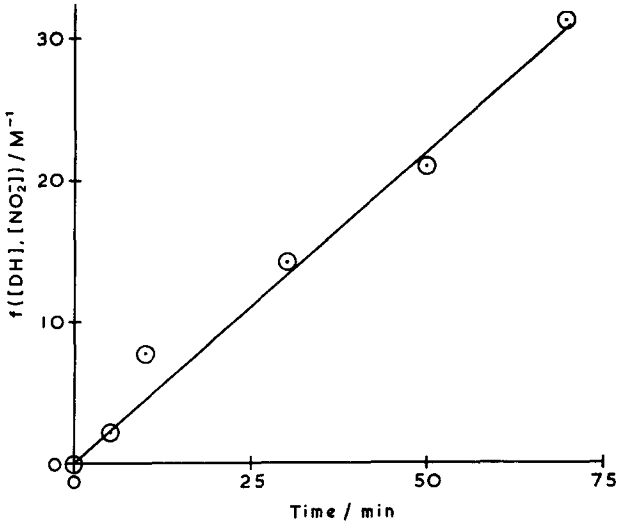


Fig. 6. Second order plot. Initial conditions: [DH] = 0·196 M, [NaNO$_2$] = 0·208 M, pa_{H^+} = 3·81, [buffer] = 4·12 M, 55°C. Slope = 0·48 M^{-1} min^{-1}. f([DH], [NO$_2^-$]) represents the left hand side of eqn (8).

timescale of reactions such as that shown in Fig. 7. No browning was evident in control experiments containing DH but no nitrite ion. It is possible, therefore, that the experiment involving preheating of DH does not allow for a more complicated situation which exists in the presence of nitrite. A change in mechanism at long reaction times cannot be ruled out. It is interesting to note that the ratio of rate constants obtained from second

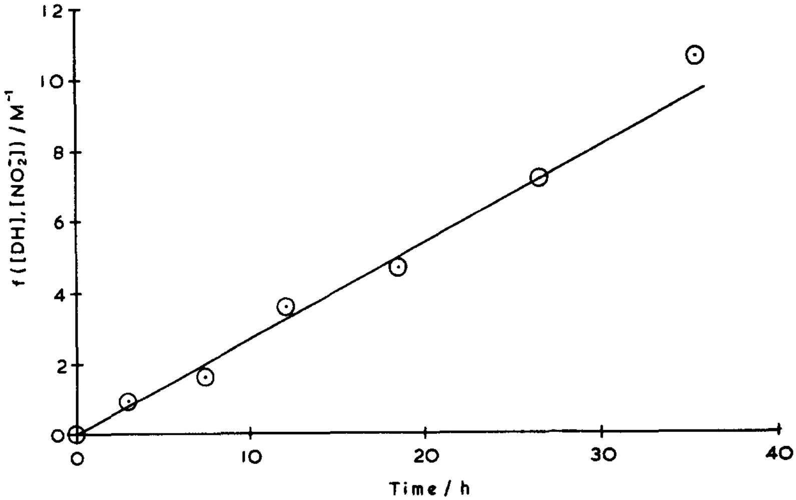


Fig. 7. Second order plot. Initial conditions: [DH] = 0·391 M, [NaNO$_2$] = 0·180 M, pa_{H^+} = 5·58, [buffer] = 0·59 M, 55°C. Slope = 0·27 M^{-1} h^{-1}. f([DH], [NO$_2^-$]) represents the left hand side of eqn (8).

order plots (Figs 6 and 7) to those calculated from initial rates is 1·5:1 irrespective of whether the reaction timescale is that of minutes or hours. This again points to differences in mechanism between the initial reaction and the overall reaction.

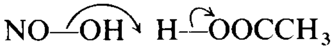
DISCUSSION

The kinetic data suggest that one mole of each of NO_2^-, H^+ and DH are involved in making up the rate determining step of the initial reaction. The reaction is catalysed by components of acetate buffer but is relatively unaffected by Cl^-, ClO_4^-, SO_4^{2-}, K^+ or Cs^+. The function of the buffer could therefore be that of a general acid-base catalyst or the result of some specific interaction of buffer components with nitrite ion, some species derived from it or the kinetically significant intermediate. The small kinetic effect of the other ions suggests that the reaction intermediate is not stabilised through ion pair formation and it is possible that its charge is small (e.g. singly charged).

First order behaviour with respect to nitrite and hydrogen ions suggests that the reactive species is derived from HNO_2 and the most likely reactant is, therefore, NO^+. This is formed by the dissociation of the acid according to the following stoichiometric equation:

$$HNO_2 \rightleftharpoons NO^+ + OH^- \tag{9}$$

In acid solution the reaction takes place through protonation of HNO_2 with elimination of water. In neutral solution NO^+ reacts as NO—NO_2 and, under these conditions, nitrosation reactions are of second order with respect to nitrite ion. In this investigation, first order behaviour with respect to nitrite ion at pH 5·5 requires the participation of some other species, *X*, to enhance the formation of NO^+ or to stabilise it as *X*—NO. A possible explanation for the role of acetate buffer is that acetic acid could act as a general acid catalyst for the formation of NO^+ by donating a hydrogen ion to the OH^- ion; that is,

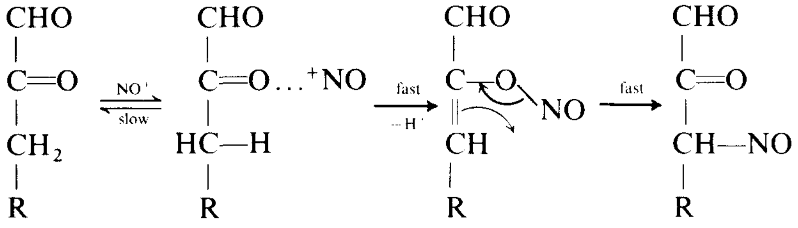


$$NO\text{—}OH \quad H\text{—}OOCCH_3$$

It is envisaged, therefore, that the overall reaction for the formation of reaction intermediate could be:

$$H^+ + NO_2^- + DH + CH_3COOH \rightleftharpoons DH\text{—}NO^+ + CH_3COO^- + H_2O \tag{10}$$

where DH—NO^+ is the kinetically significant reaction intermediate. Singer & Vamplew (1957) suggest that C-nitrosation of activated methylene compounds proceeds by way of attack by NO^+ on the carbonyl oxygen in a slow step followed by a rapid elimination of H^+ and rearrangement to complete the reaction. A possible sequence of events leading to the formation and reaction of the intermediate in the case of DH is, therefore,



(DH) (DH—NO^+)

where $R = (CHOH)_2CH_2OH$.

The involvement of acetate ion as a catalyst could be reconciled by stabilisation of NO^+ as the ion pair, $CH_3COO^-NO^+$, which would then react with DH to form DH—NO^+,

$$CH_3COO^-NO^+ + DH \rightleftharpoons DH\text{—}NO^+ + CH_3COO^- \qquad (11)$$

and the overall reaction for formation of the kinetically significant intermediate would be,

$$H^+ + NO_2^- + CH_3COO^- + DH \rightleftharpoons DH\text{—}NO^+ + CH_3COO^- + OH^- \qquad (12)$$

The C-nitroso-compound formed after the elimination of H^+ from DH—NO^+ would rapidly rearrange to an oxime. The stoichiometry of the overall reaction is 1:1, DH:NO_2^- and it is reasonable to suggest that the most likely initial product is the C-nitroso-compound although no product has yet been identified.

According to the Brønsted theory, the rate constant, k, for the reaction shown in eqn (10), for a real system, is given by:

$$k = k_0(y_{H^+}\, y_{NO_2^-})/(y_{DHNO^+}\, y_{CH_3COO^-}) \qquad (13)$$

where k_0 is the rate constant when the activity coefficients, denoted by y, are unity. If the activity coefficient of an ion in water is given by (Robinson & Stokes, 1965),

$$-\log y = Az^2F(I) \qquad (14)$$

where A is a constant, z is the charge on the ion and $F(I)$ is an appropriate function of the ionic strength, it is evident that, for the reaction shown in eqn (10), $k = k_0$; that is, the rate constant will be independent of ionic strength. Similarly, the mechanism shown in eqn (12) involves only singly charged ions and the same number of such species is present on either side of the equation. Application of the Brønsted formulation as in eqn (13) shows that this process is also independent of ionic strength. This is found to be the case over the wide range of ionic strengths studied (0·1–3·2 M). The kinetic and stoichiometric data are, therefore, consistent with the formation of DH—NO^+ as a kinetically significant intermediate through acid and base catalysed mechanisms involving components of the buffer as suggested in eqns (10)–(12).

CONCLUSION

The kinetics of the reaction of DH with nitrite ion are consistent with C-nitrosation by NO^+ and an important feature is the dependence on catalysis by acetate ion and acetic acid. Foods contain numerous weak acids and bases also capable of acid/base catalysis and the results reported here should now provide an incentive to develop methods for the detection of the reaction products in appropriate food systems.

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