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Kinetics of the Reaction Between 3-Deoxyhexosulose and Sulphur(IV) Oxospecies in the Presence of Glycine

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ABSTRACT

The kinetics of the reaction between 3-deoxyhexosulose (DH) and sulphur (IV) oxospecies (S(IV)) in the presence of glycine (0·5M) at pH 5·5 and 55°C are reported. The initial reaction is of first order with respect to DH ($k = 0{\cdot}0164\,h^{-1}$) and of zero order with respect to S(IV). The presence of glycine increases the rate of reaction whilst S(IV) acts as an inhibitor through formation of the hydroxysulphonate of DH. A kinetically derived value for the dissociation constant of the hydroxysulphonate in the presence of glycine is 0·004M. Possible mechanisms and the application of the results to the S(IV)-inhibited Maillard reaction are considered.

INTRODUCTION

From the point of view of colour formation, the most important intermediates in Maillard browning are 3-deoxyosuloses and 3,4-dideoxyosulos-3-enes (McWeeny *et al.*, 1974). In the case of a typical Maillard reaction, that between glucose and glycine, the intermediates are, respectively, 3-deoxyhexosulose (DH) and 3,4-dideoxyhexosulos-3-ene (DDH). The success of sulphur(IV) oxospecies (S(IV)) in inhibiting Maillard browning lies partly in its ability to react irreversibly with these intermediates. Thus, during the inhibition of glucose/glycine browning, reaction of DH with S(IV) leads to 3,4-dideoxy-4-sulphohexosulose (DSH). It is likely that this reaction proceeds by way of DDH.

A kinetic model for the reversible and irreversible binding of S(IV) in

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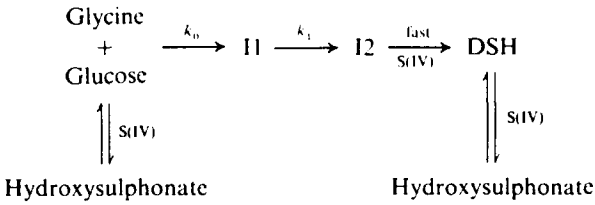

Fig. 1. Kinetic model for the S(IV)-inhibited Maillard reaction of glucose and glycine (Wedzicha, 1984*a*).

the system glucose/glycine/S(IV) suggests the scheme shown in Fig. 1 for initial conditions: [glucose] = 1·0M, [glycine] = 0·5M, [S(IV)] = 0·039M, pH 5·5 and 55°C where k_0 and k_1 are zero and first-order rate constants, respectively ($k_0 = 3{\cdot}2 \times 10^{-5}$M h^{-1} and $k_1 = 5{\cdot}5 \times 10^{-3}$ h^{-1}) and I1 and I2 are unspecified intermediates (Wedzicha, 1984*a*). A significant feature of this mechanism is that the intermediates do not interact reversibly with S(IV); the previous view of the inhibition of Maillard browning by S(IV) was one which involved a large contribution from reversible addition of S(IV) to reactive carbonylic intermediates to form hydroxysulphonate adducts as well as irreversible reaction to form DSH (McWeeny *et al.*, 1974).

The purpose of this study is to consider relevant aspects of the kinetics of the reaction of DH with S(IV) and, in so doing, to suggest the relationship between DH and intermediates I1 and I2.

EXPERIMENTAL

Preparation and purity of 3-deoxyhexosulose

3-Deoxyhexosulose was prepared according to the method of Madson & Feather (1981) involving direct conversion of glucose to the bisbenzoylhydrazone of DH followed by decomposition of the derivative. The bisbenzoylhydrazone was obtained in 43% yield and, on microanalysis, gave C: 58·9%, H: 5·6%, N: 13.1%. Calc for $C_{20}H_{22}N_4O_5$ C: 60·3%, H: 5·6%, N: 14·1%. The conversion of the derivative to DH involved the use of benzaldehyde in a transhydrazonisation reaction followed by treatment of the crude product with a mixed ion-exchange resin (Amberlite IR-120 H^+ form and Amberlite IR-45 OH^- form). This treatment was repeated until the nitrogen content of the DH had fallen to well below 1%. The product was obtained in 82% yield based on the amount of bisbenzoylhydrazone derivative used and, on microanalysis, gave C: 44·5%, H: 7·2%. Calc for

$C_6H_{10}O_5$ C: 44·4%, H: 6·2%. The nitrogen content was in the range 0·3–0·8%.

The homogeneity of DH was demonstrated by means of thin-layer chromatography on silica gel 60 F_{254} plates with ethyl acetate:methanol: water, 5:1:1 as eluting solvent. Components were revealed under ultraviolet light when the sample was found to run as a single spot, R_f 0·20. The identity of the DH was checked by converting a sample back to the bisbenzoylhydrazone derivative and subjecting this to ^1H nmr. To prepare the derivative, DH (0·12 g) and benzoylhydrazine (0·16 g) in water (10 ml) were heated for 5 min on a boiling water bath. The solid which separated was recrystallised from ethanol. The ^1H nmr spectrum was recorded at 60 MHz from a sample dissolved in $CDCl_3$:DMSO-d_6, 4:1. All signals which were observed were assigned to the derivative of DH or solvent as follows: δ12·2, 11·95 two singlets 1H (2 NH protons); δ8·35 singlet 1H (C_1–H); δ7·95 multiplet 4H (*ortho*-H in aromatic ring); δ7·4 multiplet 6H (aromatic ring); δ6·1 broad singlet 1H (primary OH); δ5·3 broad singlet 2H (secondary OH); δ4·2 multiplet 2H (C_6–H); δ3·7 multiplet 2H (C_3–H); δ3·0–3·5 two doublets and multiplet 2H (C_4–H and C_5–H). A peak at δ2·58 was attributed to DMSO and a broad peak at *ca.* δ3 was due to residual water. Peaks at δ1·1–1·3 were due to traces of ethanol in the sample.

Since DH is hygroscopic, its purity was determined immediately before the preparation of stock solutions. Analysis was carried out by conversion of DH to the corresponding metasaccharinic acid followed by determination of the acid formed. The procedure was based on that used by Anet (1961). A sample of DH (1 g) was dissolved in oxygen-free limewater (0·02M $Ca(OH)_2$, 300 ml) and the solution maintained at 25°C. Aliquots (25 ml) were withdrawn at timed intervals and run into an excess of washed Amberlite IR-120 H^+ form ion-exchange resin (20 g). The resin was filtered off, washed with water (3 × 10 ml) and the filtrate and washings combined before diluting to 100 ml. Aliquots of the solution were titrated using 0·01M NaOH. The yield of metasaccharinic acid reached a constant value after 5 days and the purity of the DH was typically 95%.

General procedure used for following the reaction

The reaction of DH with S(IV) was followed under conditions appropriate to the model Maillard system which formed the basis for earlier studies (McWeeny *et al.*, 1969; Wedzicha, 1984*a*). Reaction mixtures contained DH (0·0095–0·0477M), S(IV) (0·0097–0·0492M) and glycine (0–0·5M). All solutions were prepared in acetate buffer (0·05M), pH 5·5. To start the reaction, reactant solutions were mixed at room temperature and aliquots

(5 ml) of the mixture immediately transferred to glass ampoules of 7 ml capacity. Air in the headspace was displaced with nitrogen, the ampoules sealed and heated in a water bath at 55·0 ± 0·1°C. Ampoules were withdrawn periodically for analysis. The concentration of S(IV) was determined spectrophotometrically by the method of Humphrey *et al.* (1970). Aliquots (1 or 2 ml) of reaction mixture were diluted to 100 ml. The colour reaction involved mixing an aliquot (10 ml) of this solution with a solution of 5,5′-dithiobis(2-nitrobenzoic acid), DTNB reagent, (25 ml, 0·001M in pH 8·0 phosphate buffer) and making up to 100 ml. The absorbance of the resulting solution was measured at 412 nm after 5 min and the concentration of S(IV) calculated from calibration data prepared using iodimetrically standardised S(IV) solutions. All kinetic experiments were carried out in duplicate.

In order to confirm that the reaction between DH and S(IV) had proceeded to give DSH, the reaction mixture (1 ml) was added to perchloric acid (60%, 1 ml) and mixed with a solution of 2,4-dinitrophenylhydrazine hydrochloride (80 mg) in perchloric acid (30%, 2 ml). The precipitated product was filtered, washed with decreasing concentrations of perchloric acid and, finally, water. The solid was dissolved in pyridine and subjected to thin-layer chromatography on silica gel 60 with butanol: 0·880 ammonia solution, 4:1 as solvent. Chromatograms of partly reacted mixtures showed two purple spots: R_f 0·34–0·38, attributable to the bis(2,4-dinitrophenylhydrazone) of DSH and R_f 0·75–0·83, due to the derivative of DH.

Concentrations of free and reversibly bound S(IV) in reaction mixtures were determined iodimetrically as described by McWeeny *et al.* (1969).

RESULTS AND DISCUSSION

Preparation and purity of 3-deoxyhexosulose

There are two published methods for the preparation of DH. The earliest (Kato, 1962) involves the Maillard reaction of glucose with butylamine followed by chromatographic isolation of the intermediate. The yield of DH is of the order of 0·5% based on the amount of glucose used and the required scale of the separation procedures renders it tedious to produce gram-quantities of DH. The reaction of benzoylhydrazine with glucose to give the bisbenzoylhydrazone of DH was first reported by El Khadem *et al.* (1971) and an improved method published by Madson & Feather (1981) was applied here to produce 20 g of DH in 35% yield based on the amount of glucose used. No chromatographic purification of the product was deemed necessary. The reaction between this product and S(IV) in the

presence or absence of glycine led to the formation of DSH as the only product detectable by thin-layer chromatography of the 2,4-dinitrophenylhydrazine derivatives.

Preliminary kinetic studies

The spectrophotometric determination of S(IV) by means of DTNB has previously been used to follow the fate of S(IV) in kinetic studies (Wedzicha & McWeeny, 1974). Under mildly alkaline conditions (pH 8·0) used for the analysis, hydroxysulphonate adducts are relatively labile and the reaction of S(IV) with DTNB is quantitative. It is expected that measurement of S(IV) by this method gives the concentration of total (i.e. free + reversibly bound) S(IV). This was confirmed by analysis of a reaction mixture (initial conditions: [DH] = 0·0239M, [S(IV)] = 0·0250M, [glycine] = 0·5M, pH 5·5, 55°C) after 120 h of reaction, for total S(IV) by iodimetry and spectrophotometry. Duplicate iodimetric determinations gave residual total [S(IV)] = 0·0134, 0·0131M whilst the determination using DTNB reagent gave [S(IV)] = 0·0131, 0·0133M. Analysis of the reaction mixture for free S(IV) showed that only some 15% of this amount was, in fact, free, thereby demonstrating the efficiency with which hydroxysulphonate adducts are decomposed during the spectrophotometric analysis.

Concentration/time data for S(IV) in duplicate runs were generally reproducible to within ±2% and mean values were used for analysis of data. For runs in which the concentrations of DH and S(IV) were similar the data appeared to conform to overall first-order kinetics. Assuming simple behaviour, the following two rate equations could describe the observed reaction:

$$-\frac{d[S(IV)]}{dt} = k[DH] \tag{1}$$

or:

$$-\frac{d[S(IV)]}{dt} = k[S(IV)] \tag{2}$$

It is difficult to imagine how the reaction could be of zero order with respect to DH and rate law (1) was therefore tested. The time-dependent concentration of DH was calculated from the initial conditions and the time-dependent concentration of S(IV) assuming that the stoichiometry of the irreversible reaction between DH and S(IV) is 1:1. From this assumption it also follows that:

$$-\frac{d[S(IV)]}{dt} = -\frac{d[DH]}{dt}$$

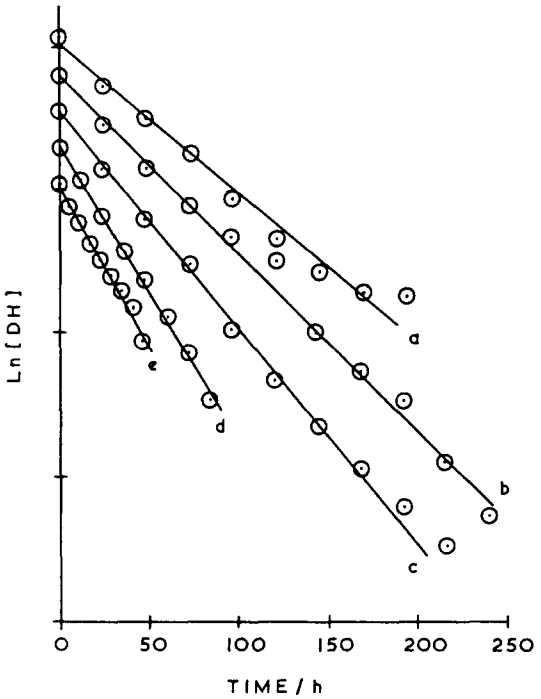

Fig. 2. First-order plots for the reaction of DH with S(IV). For all runs [S(IV)] = 0·0243M, [glycine] = 0·5M, pH 5·5 and 55°C. The concentration of DH was variable as follows: (a) 0·0095M, (b) 0·0191M, (c) 0·0286M, (d) 0·0382M, (e) 0·0477M. Each division on the Ln[DH] axis represents one unit. Successive runs displaced for clarity.

Representative first-order plots are shown in Figs 2 and 3 for runs at variable [DH] (0·0095–0·0477M) and variable [S(IV)] (0·0097–0·0492M), respectively. The adherence to first-order behaviour within individual runs is encouraging and first-order rate constants are summarised in Table 1. The values of these constants for runs at [glycine] = 0·5M are comparable with the value of 0·0055 h^{-1} deduced earlier for the reaction of a kinetically important but unknown intermediate in the S(IV)-inhibited Maillard reaction with S(IV) (Wedzicha, 1984*a*). This suggests that the intermediate in question, intermediate II in Fig. 1, could be DH or some other reaction intermediate which is derived from DH.

The rate constant varies linearly with concentration of glycine, as illustrated in Fig. 4, and the involvement of glycine in the reaction can be described by the following rate equation:

$$-10^3\frac{d[S(IV)]}{dt} = 1{\cdot}7 + 7{\cdot}0[\text{glycine}]$$

The reaction appears to consist of two parallel processes, one of which

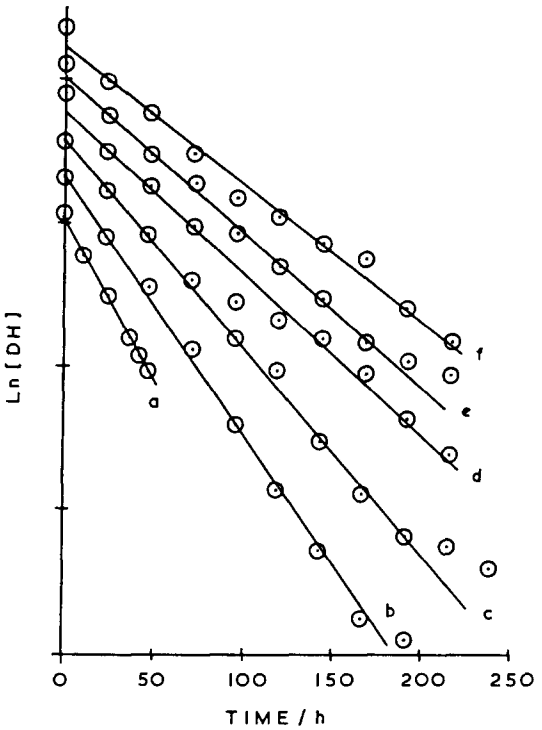

Fig. 3. First-order plots for the reaction of DH with S(IV). For all runs [DH] = 0·0239M, [glycine] = 0·5M, pH 5·5 and 55°C. The concentration of S(IV) was variable as follows: (a) 0·0097M, (b) 0·0194M, (c) 0·0243M, (d) 0·0295M, (e) 0·0395M, (f) 0·0492M. Each division on the Ln[DH] axis represents one unit. Successive runs displaced for clarity.

involves the amino acid in the rate determining step. In mixtures containing 0·5M glycine, the concentration of glycine is large compared with that of other reactants and the concentration of glycine is not expected to change significantly during the course of a kinetic run.

The main difficulty posed by the results shown in Table 1 is the variation of rate constant with [DH] and [S(IV)] suggesting that the kinetic data do not comply fully with the proposed rate equation. The dependence of rate constant on concentration is relatively small: a fivefold increase in [DH] leads to approximately a twofold increase in k whilst a fivefold increase in [S(IV)] leads to a reduction in k by a factor of 2·5. The reduction in k with increase in [S(IV)] suggests an involvement of hydroxysulphonate adducts reducing the amount of available DH as [S(IV)] is increased. The simplest model which allows for the formation of the hydroxysulphonate of DH is shown in Fig. 5 where I is a kinetically significant intermediate, k is a rate constant and K_1 and K_2 are equilibrium constants for the dissociation of the respective hydroxysulphonates. DSH is known to form

TABLE 1
First Order Rate Constants as a Function of [DH], [S(IV)] and [Glycine]

Initial concentrations			10^3k
[DH] (M)	*[S(IV)]* (M)	*[Glycine]* (M)	(h^{-1})
0·0239	0·0245	0	1·8
0·0239	0·0246	0·1	2·4
0·0239	0·0246	0·2	3·3
0·0239	0·0243	0·3	3·7
0·0239	0·0244	0·4	4·5
0·0239	0·0234	0·5	5·1
0·0239	0·0097	0·5	9·4
0·0239	0·0194	0·5	7·1
0·0239	0·0243	0·5	5·3
0·0239	0·0295	0·5	4·5
0·0239	0·0395	0·5	4·1
0·0239	0·0492	0·5	3·8
0·0095	0·0243	0·5	4·0
0·0191	0·0243	0·5	5·0
0·0286	0·0243	0·5	5·7
0·0382	0·0241	0·5	8·2
0·0477	0·0246	0·5	8·6

a monohydroxysulphonate with a dissociation constant of 0·0044M under the conditions of the kinetic experiments reported here (Wedzicha *et al.*, 1985). The values of the remaining constants are as yet unknown.

Initial reaction

The analysis of kinetic data which follows is based on the assumption that the kinetically significant reactant is free DH. In the first instance, to avoid complications in the calculation of $[DH]_{free}$ by the formation of the hydroxysulphonate of DSH, it is advantageous to attempt to fit initial rates to a rate equation involving initial concentrations of reactants. The occurrence of mono- and dihydroxysulphonates of α-dicarbonyl compounds has been discussed in detail elsewhere (Wedzicha, 1984*b*). It is reasonable to expect that DH forms the monoadduct preferentially. The main difficulty rests with determining the value of the dissociation constant of this hydroxysulphonate since irreversible addition of S(IV) to DH begins to take place as soon as the reactants are mixed. Measurement after 1 h of free and reversibly bound S(IV) in mixtures initially containing

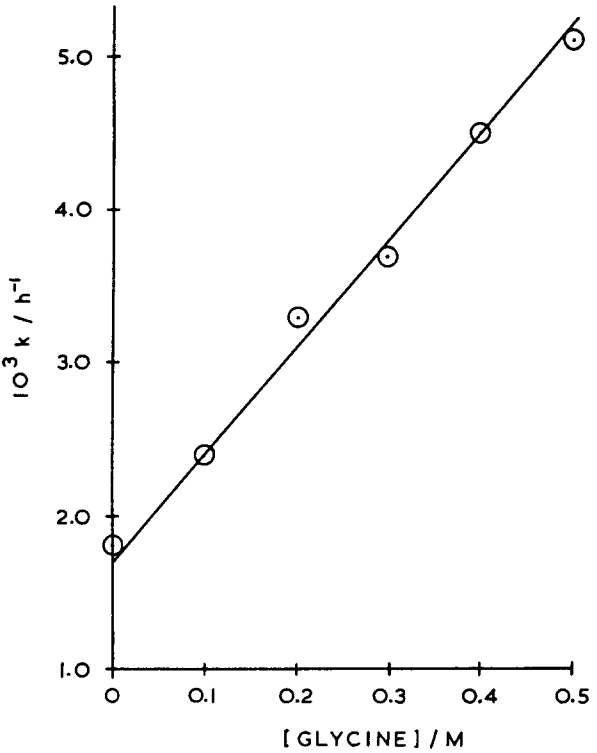

Fig. 4. Effect of [glycine] on first-order rate constant.

[DH] = 0·0239M, [S(IV)] = 0·0249M and [glycine] = 0·5M suggests a value in the region of 0·006M but it is unlikely that sufficient time has been allowed for equilibration and the true value is probably smaller. A kinetically derived value was obtained by applying the law of mass action to concentrations of S(IV) and DH shown in Table 1 for experiments in which [glycine] = 0·5M to calculate the apparent initial $[DH]_{free}$ given different values of dissociation constant. The results were examined for any simple correlation with initial rates of reaction. The best result was the linear relationship shown in Fig. 6 when the dissociation constant was

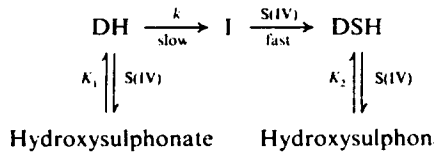

Fig. 5. Kinetic model for the reaction of DH with S(IV) involving the formation of hydroxysulphonates of DH and DSH.

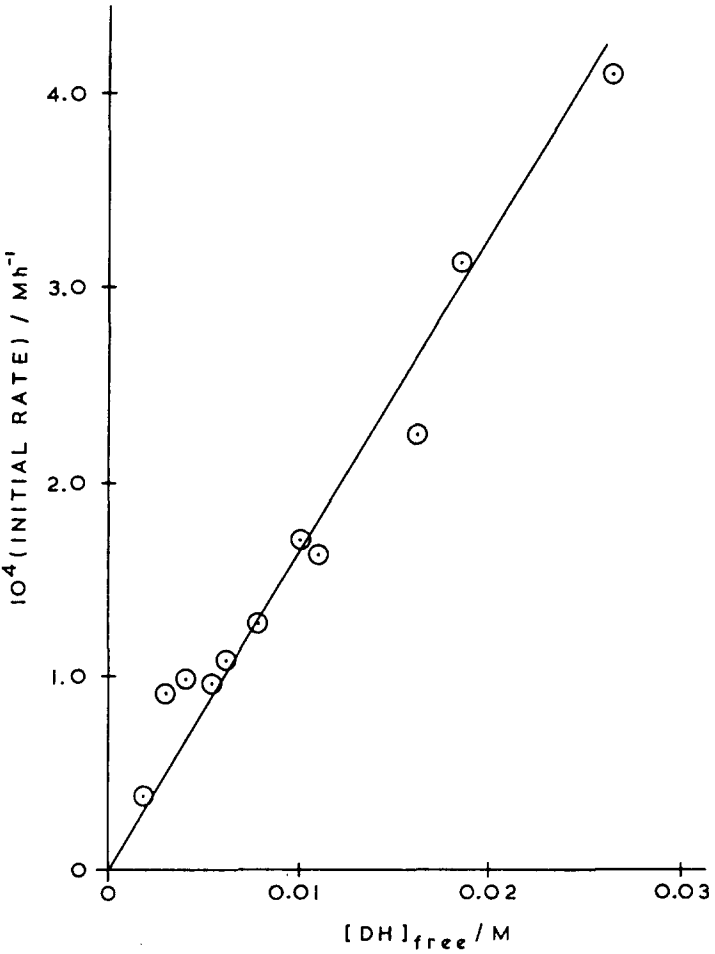

Fig. 6. Relationship between initial rate and $[DH]_{free}$ for kinetic experiments shown in Table 1 with [glycine] = 0·5M.

0·004M. The corresponding rate equation, which is seen to apply over a thirteenfold variation of $[DH]_{free}$ is, therefore:

$$-\frac{d[S(IV)]}{dt} = 0{\cdot}0164[DH]_{free}$$

It is interesting to note that the value of the dissociation constant derived above is similar to that for the hydroxysulphonate of DSH. In most of the kinetic runs the initial rate describes the progress of the reaction for at least 24 h but the value of the dissociation constant could still be a time-averaged value if the formation of hydroxysulphonate was slow on this time-scale.

Overall reaction

Calculation of the amount of free DH present in reaction mixtures once some DSH has been formed can be carried out using the approach of Wedzicha & Chishya (1983). If, at any instant, the total amounts of DH, DSH and S(IV) present are a, b and s, respectively, and at equilibrium the amounts of the respective hydroxysulphonates are x and y, then application of the law of mass action to each equilibrium gives the following equations:

for the dissociation of DH-hydroxysulphonate:

$$x^2 - (a + s - y + K_1)x + as - ay = 0 \tag{3}$$

for the dissociation of DSH-hydroxysulphonate:

$$y^2 - (b + s - x + K_2)y + bs - bx = 0 \tag{4}$$

By initially setting x and y to zero and successively evaluating these quantities from eqns (3) and (4), iteration leads to gradually improving values which converge to the equilibrium concentrations.

In order to test whether the amount of free S(IV) in reaction mixtures is dependent only on the concentrations of DH and DSH, predicted values using $K_1 = 0{\cdot}004$M and $K_2 = 0{\cdot}0044$M for a kinetic run with initial concentrations [DH] = 0·0239M, [S(IV)] = 0·0250M and [glycine] = 0·5M are compared with measured values in Table 2. Reasonable agreement between observed and predicted values is found after 24 h. The observed zero-time measurement is expected to be high due to incomplete equilibration. The remaining data are, however, inconsistent. Some improvement may be found by reducing the value of K_2 to 0·001M but there is no justification for this since there is no reason to doubt the reliability of the published

TABLE 2

Comparison of Observed and Predicted Values of $[S(IV)]_{free}$. Initial Conditions: [DH] = 0·0239M, [S(IV)] = 0·0250M, [Glycine] = 0·5M, pH 5·5 and 55°C

Time (*h*)	*Observed*		*Predicted*
	Total [S(IV)] (M)	*Free [S(IV)]* (M)	*Free [S(IV)]* (M)
0	0·0249	0·0099	0·0086
24	0·0214	0·0062	0·0066
48	0·0188	0·0041	0·0053
72	0·0168	0·0025	0·0044
96	0·0148	0·0027	0·0036
120	0·0133	0·0018	0·0031

TABLE 3

Comparison of Observed and Predicted Values of $[S(IV)]_{total}$. Initial Conditions: [DH] = 0·0239M, [S(IV)] = 0·0295M, [Glycine] = 0·5M, pH 5·5 and 55°C

Time (h)	*Observed Total [S(IV)]* (M)	*Predicted Total [S(IV)]* (M)
0	0·029 5	0·029 5
24	0·026 1	0·027 1
48	0·024 1	0·024 6
72	0·022 3	0·022 0
96	0·019 0	0·019 3
120	0·018 4	0·016 7
144	0·017 8	0·014 2
168	0·016 6	0·012 0
192	0·015 4	0·010 3
216	0·014 5	0·009 0

value. The concentration of total S(IV) during a kinetic run may be predicted by numerical integration of the following equation:

$$-\frac{d[S(IV)]}{dt} = 0{\cdot}0164(a - x)$$

An example of such an integration using $K_1 = 0{\cdot}004$M and $K_2 = 0{\cdot}0044$M is shown in Table 3. In the early stages of the reaction, up to 96 h, the

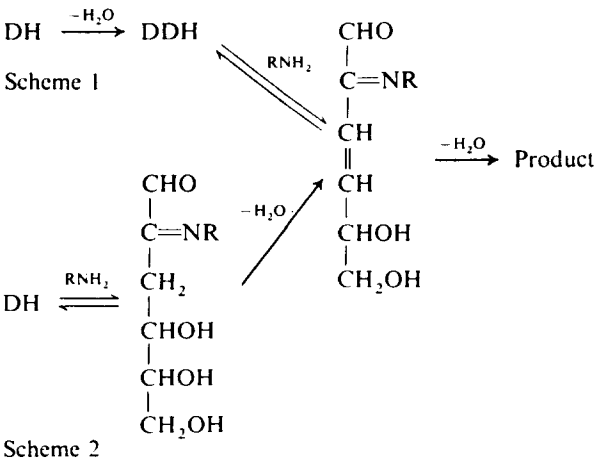

Fig. 7. Possible reaction mechanisms for the formation of *N*-substituted pyrroles from DH and amines.

predictions are excellent but at longer times the reaction appears to proceed more slowly than expected. These data are consistent with a conversion of DH to a form which is less reactive towards S(IV) with respect to irreversible combination but is more reactive as far as reversible combination is concerned. These additional complications introduce too many variables for a meaningful analysis.

Mechanism of reaction

The reaction of DH with S(IV) proceeds by a rate-determining transformation of DH which does not involve S(IV) but which is speeded up in the presence of glycine. A possible involvement of the amino acid can be discussed by reference to the well known reaction of DH with amines to form *N*-substituted pyrroles (Kato & Fujimaki, 1970). Two possible schemes for this reaction are shown in Fig. 7. Considering first the common

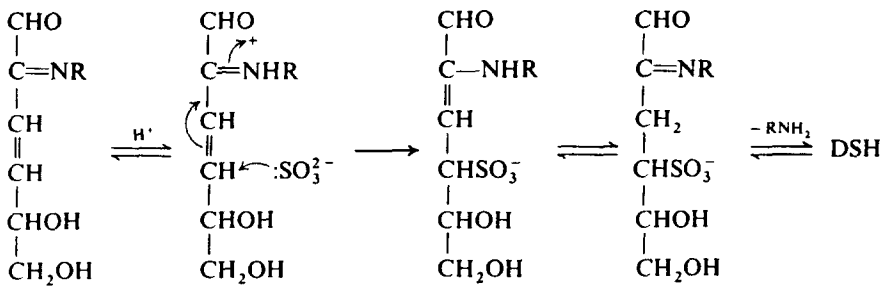

Fig. 8. Possible involvement of glycine (shown as RNH_2) in the reaction of DH with S(IV).

intermediate, i.e. the Sciff's base of DDH, the nitrogen atom could well render this intermediate significantly more reactive than DDH towards nucleophilic attack by S(IV) in position 4, as suggested in Fig. 8. Alternatively, if scheme 2 is more important, then formation of the unsaturated intermediate could be the result of amine-assisted dehydration of DH. Both possibilities are consistent with the observed kinetics.

CONCLUSION

The kinetic data presented in this paper support some aspects of the kinetic model for the S(IV)-inhibited Maillard reaction (Wedzicha, 1984*a*) but appear to disprove others. The magnitude of the apparent first-order rate constant obtained from the preliminary treatment of data is similar to the

expected value if the kinetically significant intermediate in the loss of S(IV) in the system glucose/glycine/S(IV) is DH. A rate law, which is of first order with respect to intermediate, and of zero order with respect to S(IV), was also predicted. On the other hand, in the reaction studied here a very significant amount of unreacted S(IV) was found to be reversibly bound to DH. The lack of any such binding was a distinct feature of the model of the S(IV)-inhibited Maillard reaction. The introduction of this reversible binding into the model renders it a poor description of the kinetic data. In view of the already successful prediction of the dissociation constant of the hydroxysulphonate of DSH by use of the model (Wedzicha *et al.*, 1985) one is prompted to ask whether the DH sample, prepared for the kinetic studies, is identical to the compound which is an intermediate in the Maillard reaction. The work is continuing on these lines.

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REFERENCES

Anet, E. F. L. J. (1961). Degradation of carbohydrates. II. The action of acid and alkali on 3-deoxyhexosones. *Aust. J. Chem.*, **14**, 295–301.

El Khadem, H., Horton, D., Meshreki, H. & Nashed, M. A. (1971). New route for the synthesis of 3-deoxyaldos-2-uloses. *Carbohydr. Res.*, **17**, 183–92.

Humphrey, R. E., Ward, M. H. & Hinze, W. (1970). Spectrophotometric determination of sulphite with 4,4'-dithiopyridine and 5,5'-dithiobis(2-nitrobenzoic acid). *Anal. Chem.*, **42**, 698–702.

Kato, H. (1962). Chemical studies on amino–carbonyl reaction. Part I. Isolation of 3-deoxypentosone and 3-deoxyhexosones formed by browning degradation of *N*-glycosides, *Agric. Biol. Chem.*, **26**, 187–92.

Kato, H. & Fujimaki, M. (1970). Formation of 1-alkyl-5-(hydroxymethyl)pyrrole-2-aldehydes in the browning reaction between hexoses and alkylamines. *Agric. Biol. Chem.*, **34**, 1071–7.

Madson, M. A. & Feather, M. S. (1981). An improved preparation of 3-deoxy-D-*erythro*-hexos-2-ulose via the bis(benzoylhydrazone) and some related constitutional studies. *Carbohydr. Res.*, **94**, 183–91.

McWeeny, D. J., Biltcliffe, D. O., Powell, R. C. T. & Spark, A. A. (1969). The Maillard reaction and its inhibition by sulphite. *J. Food Sci.*, **34**, 641–3.

McWeeny, D. J., Knowles, M. E. & Hearne, J. F. (1974). The chemistry of non-enzymic browning and its control by sulphites. *J. Sci. Food Agric.*, **25**, 735–46.

Wedzicha, B. L. (1984*a*). A kinetic model for the sulphite-inhibited Maillard reaction. *Food Chem.*, **14**, 173–87.

Wedzicha, B. L. (1984*b*). *Chemistry of sulphur dioxide in foods*, Elsevier Applied Science Publishers, London, 214–7.

Wedzicha, B. L. & Chishya, B. E. (1983). The stability constant for the interaction between bovine serum albumin and magnesium *N*-phenyl-8-aminonaphthalenesulphonate. *Food Chem.*, **10**, 170–93.

Wedzicha, B. L. & McWeeny, D. J. (1974). Non-enzymic browning reactions of ascorbic acid and their inhibition. The production of 3-deoxy-4-sulphopentosulose in mixtures of ascorbic acid, glycine and bisulphite ion. *J. Sci. Food Agric.*, **25**, 577–87.

Wedzicha, B. L., Kaban, J. & Aldous, D. G. (1985). The dissociation constant of the hydroxysulphonate of 3,4-dideoxy-4-sulphohexosulose in the sulphite-inhibited Maillard reaction of glucose and glycine. *Food Chem.*, **17**, 125–9.