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Research Article

Dextrose Adduct Formation in Aqueous Teicoplanin Solutions

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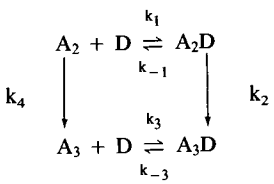
The interaction of Teicoplanin, a glycopeptide antibiotic, with dextrose in aqueous solution has been investigated. The equilibrium concentrations of the adduct formed by the interaction of the dextrose aldehyde and the Teicoplanin amino group is shown to be directly related to the concentration of the dextrose and is thought to be inversely proportional to the hydrogen ion concentration. It was also found that phosphate ion catalyzed the reverse reaction. Approximately 30 days were required to reach equilibrium a 4°C, while equilibrium was established in about 7 days at room temperature. The reaction is reversed with dilution and the rate of the reverse reaction is two to three times faster than expected when phosphate ion is present at 0.05 *M*. From the temperature dependence of the rate constants, the activation energies for the various reactions were determined to be in the range 67–80 kjoules (16–19 kcal)/mol.

KEY WORDS: kinetics; rate constants; antibiotics; adduct formation; equilibrium.

INTRODUCTION

The stability of Teicoplanin in aqueous solution in the presence of dextrose has been investigated at 4°C and room temperature (~24°C). Teicoplanin, a glycopeptide antibiotic, produced in a fermentation process, is comprised of six major species (Fig. 1). Five of the species are designated A_2, while the sixth species is designated A_3 and is formed by the hydrolysis of the A_2 species (1). Teicoplanin is an antibiotic related to Vancomycin structurally and the A_2 species are due to five different N-acyl-substituted β-D-glucosamines in place of a proton at one of the phenolic sites. The hydrolysis of the N-acyl group results in the species designated A_3. Scheme I shows these equilibria between the Teicoplanin species and dextrose, where D represents the dextrose and A_2D and A_3D are the respective dextrose adducts.



Scheme I

The purpose of this study was to elucidate these interactions in the presence of 5% dextrose and determine the effect of dilution, which could occur during administration, upon the equilibria.

EXPERIMENTAL

Dextrose (45 mg/ml) solutions of Teicoplanin (6.58 and 6.79 mg/ml) were prepared under aseptic conditions, and aliquots filled into sterilized autosampler vials. These vials were stored at either 4°C or room temperature (~24°C) and removed periodically for analysis. Samples equilibrated at room temperature for 2 days were diluted (1/200) with either 0.05 *M* phosphate buffer, pH 7.3, or the buffer with 1.25 mg/ml dextrose. The phosphate buffer was used in these dilution experiments to maintain the original pH. A standard reference solution was prepared by dissolving the contents of one standard vial in 3.0 ml of water. At each time interval, both samples and standard were chromatographed using the previously determined stability-indicating HPLC method with the following conditions.

Column:	25 cm × 4.6-mm (ID) 5-μm-Hypersil ODS	
Mobile phase:	A:	0.025 *M*, pH 6, phosphate buffer in 10/90 acetonitrile water
	B:	0.025 *M*, pH 6, phosphate buffer in 70/30 acetonitrile water
	Program:	0 to 40% B in 44 min, then 40 to 60% in 50 min, then 60 to 100% B in 55 min
Flow rate:	2 ml/min	
Detector:	UV at 254 nm	
Sample size:	10 μl containing about 80 μg of Teicoplanin—conc. solutions 200 μL containing about 8 μg of Teicoplanin—1/200 dilutions	

The HPLC instruments used in this study were Waters Model 510 pumps with a Model 680 controller, Model 710B

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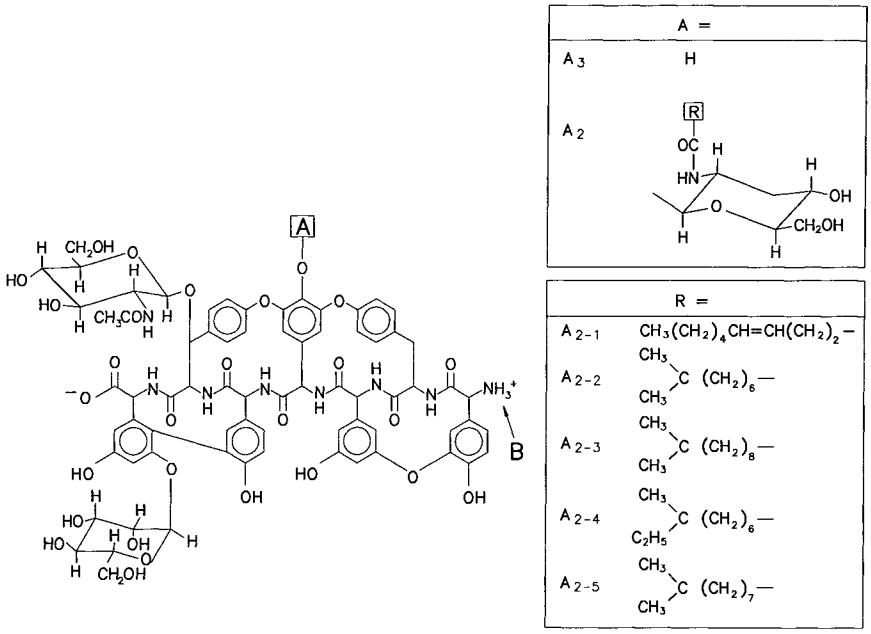


Fig. 1. Teicoplanin structure.

WISP autosampler, LDC SpectroMonitor III detector, Kipp and Zonen BD41 recorder, and Computer Automated Laboratory System (CALS) for data acquisition and analysis.

RESULTS AND DISCUSSION

Figure 2 shows representative chromatograms of Teicoplanin solutions with and without dextrose. The dextrose products of the reaction are indicated on the curves. The areas acquired from these curves for the individual peaks were used to determine the forward and reverse rate constants for the reactions of the dextrose with both the A_2 species and the A_3 species. It was assumed that the dextrose adducts had the same response factors as the original species. Examination of the original chromatographic data indicated that the hydrolysis reaction $A_2 \rightarrow A_3$ did not occur at a rate sufficient to be observed during this investigation (10 days at room temperature and 29 days at 4°C). Because the reaction is very slow, it was not possible to determine values for the rate constants for the hydrolysis reaction (k_2 and k_4).

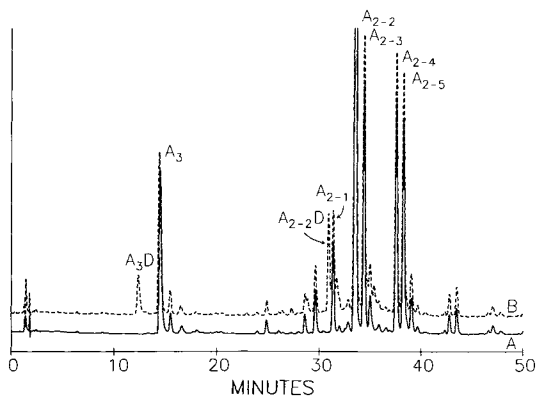


Fig. 2. HPLC chromatograms of Teicoplanin and Teicoplanin with dextrose adducts. (A) Teicoplanin; (B) Teicoplanin with dextrose adduct.

The experimental data were reported as areas and were converted to fractions (normalized) using the following relationships:

$$f_{\Sigma A2} = \frac{\Sigma A_2}{\Sigma A_2 + \Sigma A_2 D}$$

$$f_{\Sigma A2D} = 1.0 - f_{\Sigma A2}$$

$$f_{A3} = \frac{A_3}{A_3 + A_3 D}$$

$$f_{A3D} = 1.0 - f_{A3}$$

These normalized data, given in Table I and in Figs. 3 and 4, are plotted as the sum of all the A_2 species (ΣA_2 or $\Sigma A_2 D$) or the A_3 species (A_3 or $A_3 D$). It can be seen in these curves that the A_3 species appears to react more rapidly with the dextrose than the A_2 species react with dextrose. These data were analyzed using first-order kinetic expressions for reactions which form an equilibrium. Although the forward reaction would actually be a second-order reaction, the concentration of the dextrose was significantly greater than that of the Teicoplanin and therefore could be considered to remain constant during the course of the reaction. Under these conditions, the forward reaction rate can be considered as a pseudo first-order reaction. From the curves shown in Figs. 3 and 4, it can be seen that the reaction reaches equilibrium in approximately 30 days at 4°C and approximately 7 days at room temperature. The percentages of adduct species present at equilibrium are ~15% for the $\Sigma A_2 D$ species, $\Sigma A_2 D/(\Sigma A_2 + \Sigma A_2 D)$, and ~33% for the $A_3 D$ species, $A_3 D/(A_3 + A_3 D)$, at both 4°C and RT. As discussed below, these equilibrium concentrations are dependent upon the concentration of the dextrose.

The effect of diluting Teicoplanin solutions containing

Table I. Normalized Experimental Data

t (days)	ΣA_2	ΣA_2D	A_3	A_3D
	Temperature = RT, Study A			
0	1.000	0.000	1.000	0.000
1	0.912	0.088	0.852	0.148
2	0.869	0.131	0.766	0.233
3	0.862	0.138	0.712	0.287
4	0.855	0.145	0.688	0.312
7	0.852	0.148	0.705	0.295
9	0.852	0.148	0.714	0.286
10	0.854	0.146	0.715	0.285
	Temperature = 4°C, Study A			
0	1.000	0.000	1.000	0.000
1	0.960	0.040	0.932	0.068
2	0.947	0.053	0.905	0.095
3	0.931	0.069	0.863	0.137
4	0.933	0.067	0.872	0.128
7	0.895	0.105	0.803	0.197
9	0.885	0.115	0.786	0.214
10	0.876	0.124	0.769	0.231
11	0.866	0.134	0.751	0.249
14	0.862	0.138	0.732	0.268
16	0.847	0.153	0.706	0.294
29	0.818	0.182	0.677	0.323
	Temperature = RT, Study B			
0	1.000	0.000	1.000	0.000
1	0.894	0.106	0.792	0.208
2	0.862	0.138	0.733	0.267
3	0.849	0.151	0.683	0.317
4	0.840	0.160	0.673	0.327
7	0.840	0.160	0.657	0.343
	Equilibration for 2 days and then diluted 1/200			
	Diluted with 0.05 M phosphate buffer (pH 7.3) Final dextrose concentration, 0.23 mg/ml			
0.146	0.910	0.090	0.890	0.110
0.250	0.915	0.085	0.878	0.122
0.346	0.914	0.086	0.922	0.078
0.438	0.930	0.070	0.932	0.068
0.542	0.943	0.057	0.940	0.060
0.625	0.953	0.047	0.948	0.052
1.179	0.967	0.033	0.969	0.031
	Diluted with 0.05 M phosphate buffer (pH 7.3) containing 1.25 mg/ml dextrose Final dextrose concentration, 1.48 mg/ml			
0.154	0.902	0.098	0.856	0.144
0.254	0.911	0.089	0.861	0.139
0.350	0.922	0.078	0.862	0.138
0.438	0.927	0.073	0.932	0.068
0.538	0.934	0.066	0.940	0.060
0.633	0.944	0.056	0.948	0.052
1.183	0.953	0.047	0.986	0.014
2.025	0.959	0.041		
5.154	0.986	0.014		

45 mg/ml dextrose was also studied and the data are given in Table I and plotted in Figs. 5 and 6. After equilibrating for 2 days and then diluting with either phosphate buffer (pH 7.3) or phosphate buffer with 1.25 mg/ml dextrose, the concentration of adducts decreased.

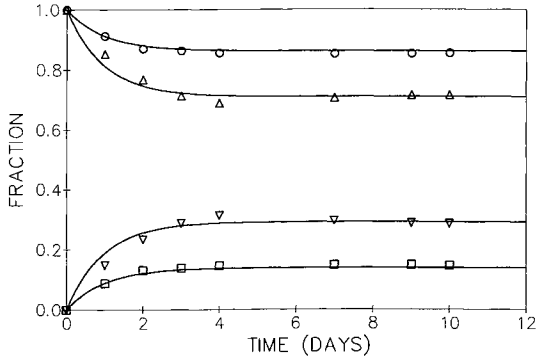


Fig. 3. Reaction of Teicoplanin with dextrose at room temperature. (○) ΣA_2; (□) ΣA_2D; (△) A_3; (▽) A_3D.

Theoretical

Expressions for the change in concentration of the individual species with respect to time can be obtained by considering the equilibria in the scheme given before. These expressions are as follows:

$$d[\Sigma A_2]/dt = -k_1^*[\Sigma A_2][D] - k_4[\Sigma A_2] + k_{-1}^*[\Sigma A_2D] \quad (1)$$

$$d[A_3]/dt = -k_3^*[A_3][D] + k_{-3}^*[A_3D] + k_4[\Sigma A_2] \quad (2)$$

$$d[\Sigma A_2D]/dt = -k_{-1}^*[\Sigma A_2D] - k_2[\Sigma A_2D] + k_1^*[\Sigma A_2][D] \quad (3)$$

$$d[A_3D]/dt = -k_{-3}^*[A_3D] + k_2[\Sigma A_2D] + k_3^*[A_3][D] \quad (4)$$

The rate constants with the asterisk superscripts are complex constants which are described later. These equations assume that within the accuracy of the experiment, it would not be possible to observe differences in the rate constants for the individual A_2 species and therefore the sum of the concentrations of all A_2 and A_2D species (ΣA_2 and ΣA_2D) can be used in the rate expressions. To solve these equations, the terms containing k_2 and k_4 can be removed from the expressions because their magnitudes are very small compared to the remaining terms. Assuming that the dextrose concentration remains constant during the course of the reaction, the following derivations can be made to give the concentrations of the individual species as a function of the rate constants, initial concentrations and time.

[ΣA_2] Species

At equilibrium Eq. (1) = 0 and therefore

$$k_{-1}^* = ([\Sigma A_2]_e[D]f_0k_1^*)/[\Sigma A_2D]_e \quad (5)$$

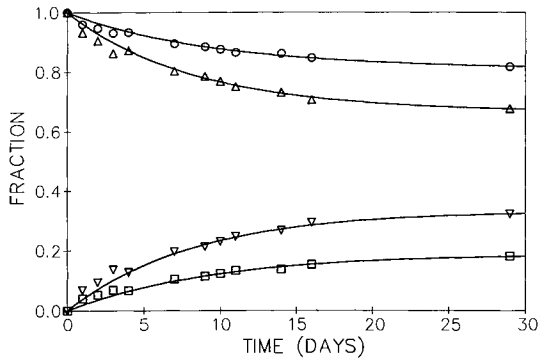


Fig. 4. Reaction of Teicoplanin with dextrose at 4°C. (○) ΣA_2; (□) ΣA_2D; (△) A_3; (▽) A_3D.

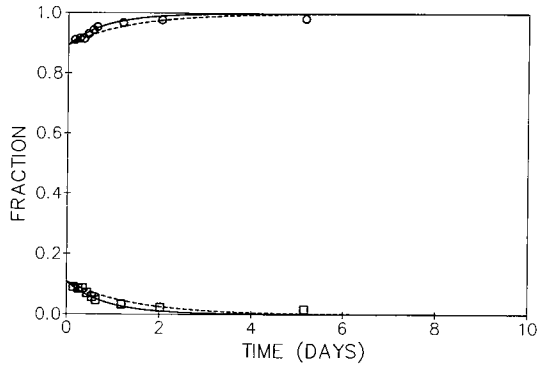


Fig. 5. Effect of dilution of Teicoplanin/dextrose solution upon ΣA_2 species. (○) ΣA_2; (□) ΣA_2D; (——) calculated curve including phosphate ion catalysis; (----) calculated curve without phosphate ion catalysis.

The quantity f_0 in this equation is the fraction of A_2 molecules for which the $-NH_2$ group (labeled B in Fig. 1) is not protonated and the subscript e refers to equilibrium concentrations. As discussed later, the dextrose probably interacts with the $-NH_2$ group in the Teicoplanin, and when this group is protonated the adduct will not be formed.

Since the hydrolysis reaction is very slow, over the course of this study, the concentration $[\Sigma A_2D]$ can be determined according to the mass balance equation:

$$[\Sigma A_2D] = [\Sigma A_2]_0 + [\Sigma A_2D]_0 - [\Sigma A_2] \quad (6)$$

Substituting Eq. (5) and Eq. (6) into Eq. (1) gives

$$d[\Sigma A_2]/dt = -[\Sigma A_2][D]f_0k_1^* + [\Sigma A_2]_e[D]f_0k_1^*([\Sigma A_2]_0 + [\Sigma A_2D]_0 - [\Sigma A_2])/[\Sigma A_2D]_e \quad (7)$$

Equation (7) can be rearranged to Eq. (8):

$$d[\Sigma A_2]/dt = [\Sigma A_2]_e[D]f_0k_1^*([\Sigma A_2]_0 + [\Sigma A_2D]_0)/[\Sigma A_2D]_e - [\Sigma A_2][D]f_0k_1^*([\Sigma A_2]_e + [\Sigma A_2D]_e)/[\Sigma A_2D]_e \quad (8)$$

This differential equation can be put in the form

$$dy + [p(x)y - q(x)]dx = 0 \quad (9)$$

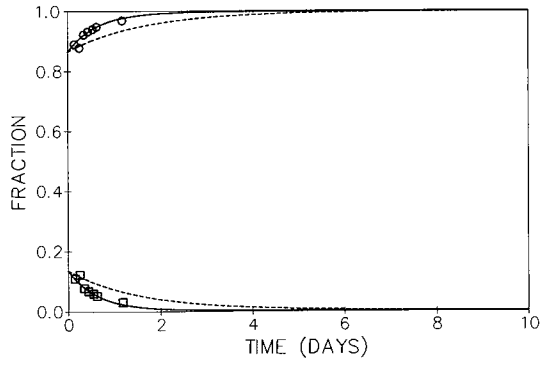


Fig. 6. Effect of dilution of Teicoplanin/dextrose solution upon A_3 species. (○) A_3; (□) A_3D; (——) calculated curve including phosphate ion catalysis; (----) calculated curve without phosphate ion catalysis.

A solution to Eq. (9) can be obtained as follows (2). Let

$$v = e^{\int p(x)dx} \quad (10)$$

Then

$$y = v^{-1}\int q(x)vdx + Cv^{-1} \quad (11)$$

where C is the constant of integration.

Using this procedure, Eq. (8) becomes

$$[\Sigma A_2] = [\Sigma A_2]_e + ([\Sigma A_2]_0 - [\Sigma A_2]_e)e^{-t[D]f_0k_1^*([\Sigma A_2]_e + [\Sigma A_2D]_e)/[\Sigma A_2D]_e} \quad (12)$$

The value for k_{-1} was obtained from the equilibrium concentrations and the forward rate constant according to Eq. (5).

Knowing the values for k_1^* and k_{-1}^* it is possible to determine the concentration of ΣA_2 and ΣA_2D as a function of time without having knowledge of the equilibrium concentrations. The equation required to make this calculation can be obtained by substituting Eq. (6) into Eq. (1) and rearranging into the form of Eq. (9). This is then solved as above, giving Eq. (13).

$$[\Sigma A_2] = \frac{k_{-1}^*([\Sigma A_2]_0 + [\Sigma A_2D]_0)}{[D]f_0k_1^* + k_{-1}^*} + \frac{([D]f_0k_1^*[\Sigma A_2]_0 - k_{-1}^*[\Sigma A_2D]_0)e^{-([D]f_0k_1^* + k_{-1}^*)t}}{[D]f_0k_1^* + k_{-1}^*} \quad (13)$$

In order to calculate the concentration of ΣA_2 as a function of time, all that is needed are the initial concentrations $[\Sigma A_2]_0$ and $[\Sigma A_2D]_0$, the concentration of dextrose, [D], the fraction of the species which have the $-NH_2$ not protonated, f_0, and values for the rate constants k^* and k_{-1}^*. The concentration of ΣA_2D can be obtained according to Eq. (6).

[A_3] Species

Using the same approach as that used for the ΣA_2 species, the equation obtained for the determination of k_3 is the same as Eq. (12) except that the A_3 and A_3D species are substituted for the ΣA_2 and ΣA_2D species and k_3 for k_1, i.e.,

$$[A_3] = [A_3]_e + ([A_3]_0 - [A_3]_e)e^{-t[D]f_0k_3^*([A_3]_e + [A_3D]_e)/[A_3D]_e} \quad (14)$$

The value for k_{-3}^* can be obtained by substituting into Eq. (15).

$$k_{-3}^* = \frac{[A_3]_e[D]f_0k_3^*}{[A_3D]_e} \quad (15)$$

The concentration of the A_3D species can be determined using Eq. (16).

$$[A_3D] = [A_3]_0 + [A_3D]_0 - [A_3] \quad (16)$$

Similar to the ΣA_2 and ΣA_2D species equilibria, Eq. (17) can be obtained which relates the concentration of the A_3 species to the rate constants k_3^* and k_{-3}^*, the initial concentrations $[A_3]_0$ and $[A_3D]_0$, and the time t.

$$[A_3] = \frac{k^*_{-3}([A_3]_0 + [A_3D]_0)}{[D]f_0k^*_3 + k^*_{-3}} + \frac{([D]f_0k^*_3[A_3]_0 - k^*_{-3}[A_3D]_0)e^{-([D]f_0k^*_3 + k^*_{-3})t}}{[D]f_0k^*_3 + k^*_{-3}} \quad (17)$$

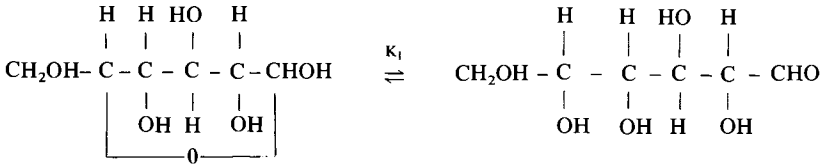
In the above equations, the reverse rate constants are written as k^*_{-1} and k^*_{-3} rather than k_{-1} and k_3. The reason for this was that additional observations indicated the reverse reaction occurred much more rapidly than would be predicted by the above rate constants when the pH of solutions containing the dextrose adduct were decreased. It would therefore appear that the reverse rate constants are actually second-order rate constants and the observed rate constants should be expressed as functions of the pH, i.e.,

$$k^*_{-1} = k_{-1} + k^{H}_{-1}[H] \simeq k^{H}_{-1}[H] \quad (18)$$

$$k^*_{-3} = k_{-3} + k^{H}_{-3}[H] \simeq k^{H}_{-3}[H] \quad (19)$$

The approximate relationships on the right-hand side of Eqs. (18) and (19) were used in these calculations because all of the solutions had the same pH and therefore the actual pH dependency could not be determined.

Dextrose exists in the aldehyde form at approximately the 0.024% (mol) level (3) at neutral pH with the equilibria shown in Scheme II.



Hemiacetal Form ⇌ Aldehyde Form

Scheme II

The formation of the aldehyde form is both acid and base catalyzed (4), and therefore at lower or higher pH the rate of formation would be greatly accelerated. The aldehyde form has some base character and therefore can be protonated (4,5). Let D-CHO represent the aldehyde form.

$$\text{D—CHO} + H^+ \overset{K_2}{\rightleftharpoons} \text{D–CHO} \cdots H^+$$

Interaction of the dextrose with Teicoplanin can then be explained as a reaction between the protonated form of the aldehyde and the nonprotonated amine species of the Teicoplanin, B in the Teicoplanin structure. This reaction would form a Schiff base where the rate-controlling step would be the formation of a carbinolamine followed by rapid conversion to imine.

Slow:

$$\text{D—CHO} \cdots H^+ + \text{T—NH}_2 \overset{K_3}{\rightleftharpoons} \text{D—CH(OH)—NH}_2^+\text{T}$$

Rapid:

$$\text{D—CH(OH)—NH}_2^+\text{T} \overset{K_4}{\rightleftharpoons} \text{D—CH=N–T} + H_2O + H^+$$

The forward rate expression for the interaction between dextrose and Teicoplanin will then be given by

$$\text{rate} = [\text{D—CHO} \cdots H^+][\text{T—NH}_2]k_f \quad (20)$$

where k_f is the forward rate constant from the equilibrium given by K_3.

The following substitution can be made for [D–CHO · · · H^+] by considering the equilibrium expressions for K_1 and K_2

$$[\text{D—CHO} \cdots H^+] = [K_1/(1 + K_1 + K_1K_2[H]))]K_2[D][H] \quad (21)$$

where [D] is the total dextrose concentration

Substituting into the rate equation, Eq. (20),

$$\text{rate} = [K_1/(1 + K_1 + K_1K_2[H]))]K_2[D][H][\text{T—NH}_2]k_f \quad (22)$$

or approximately,

$$\text{rate} = [\text{T—NH}_2][D][H](K_1K_2k_f) \quad (23)$$

This last relationship will hold when K_1 and $K_1K_2[H]$ are $\ll 1$.

Since K_1 is small (~0.024% aldehyde) and the pH is neutral under the conditions of this study, the approximate rate expression should describe the rate of formation of the dextrose adducts.

Comparing the expressions for the adduct formation in Eqs. (1)–(4) with Eq. (23), it can be seen that

$$k^*_1 = K_1K_2[H]k_1 \quad (24)$$

and

$$k^*_3 = K_1K_2[H]k_3 \quad (25)$$

Exact values for the constants K_1 and K_2 are not known and therefore the above expressions can be written

$$k^*_1 = k'_1[H] \quad (26)$$

and

$$k^*_3 = k'_3[H] \quad (27)$$

where

$$k'_1 = K_1K_2k_1 \quad \text{and} \quad k'_3 = K_1K_2k_3$$

The curves in Figs. 3 and 4 were obtained using Eq. (12) for the ΣA_2 species and Eq. (14) for the A_3 species. It can be seen that there is good agreement between the observed values and the calculated values. In Table II the values calcu-

Table II. Calculated Rate Constants

Rate Constant	T = RT Value	T = 4°C Value
k_1^*	0.936	0.132
k_{-1}^H	1.71×10^7	1.65×10^6
k_3^*	2.02	0.267
k_{-3}^H	1.45×10^7	1.57×10^6
k_1'	1.87×10^7	2.63×10^6
k_3'	4.03×10^7	5.33×10^6
$k_{-1}^{PO_4}$	9.57	
$k_1^{PO_4}$	12.0	
$k_{-3}^{PO_4}$	18.7	
$k_3^{PO_4}$	59.7	

Units for rate constants

Unit	Order	Rate constant	
Day^{-1}	1	k_{-1}^*	k_{-3}^*
$mol/L^{-1} \times day^{-1}$	2	k_1^*	k_3^*
		k_{-1}^H	k_{-3}^H
		k_1	k_3
		$k_{-1}^{PO_4}$	$k_{-3}^{PO_4}$
$mol/L^{-2} \times day^{-1}$	3	k_1'	k_3'
		$k_1^{PO_4}$	$k_3^{PO_4}$

lated for the rate constants are given. Knowing the values of the rate constants at two temperatures, the Arrhenius equation can be used to determine the activation energies and preexponential factors for the reactions as well as the rate constants at other temperatures. The integrated form of the Arrhenius equation is given by Eq. (28).

$$k = Ae^{-E_a/RT} \tag{28}$$

where

A = preexponential factor
E_a = activation energy
R = gas law constant
T = temperature

Using Eq. (28), the values for the activation energy and preexponential factor were determined for both the A_2 reaction and the A_3 reaction with dextrose. These results are given in Table III.

Table III. Activation Energies for Rate Constants

Rate Constant	E_a[a]	E_a[b]	A[c]
k_1^*	64.1	15.3	1.60×10^{11}
k_1'	64.1	15.3	3.40×10^{18}
k_{-1}^H	76.3	18.2	4.09×10^{20}
k_3^*	66.2	15.8	7.98×10^{11}
k_3'	66.2	15.8	9.98×10^{20}
k_{-3}^H	72.7	17.4	7.98×10^{19}

[a] kjoule/mol.
[b] kcal/mol.
[c] Units of rate constant.

Dilution and Catalytic Effects

As previously mentioned, the rate at which the adduct concentration decreased upon dilution with phosphate ion-containing solutions was much greater than expected. It was concluded that there was a catalytic effect upon the reaction related to the phosphate ion and the data were used to determine the respective catalytic rate constants. The catalytic effect due to the phosphate ion has been reported specifically for the hydrolytic cleavage of the amide linkage in chloramphenicol (6), where it was determined that the HPO_4^{2-} species catalyzed the reaction.

An equation similar to Eqs. (12) and (14) can be derived which relates the observed rate constant to the concentration of the adduct species. This equation for the A_3D species is

$$\ln([A_3D] - [A_3D]_e) = \ln([A_3D]_0 - [A_3D]_e) - k_{-3}^r \left(\frac{[A_3D]_e + [A_3]_e}{[A_3]_e}\right) t \tag{29}$$

The superscript r refers to the catalyzed reverse reaction. Since a catalyst changes only the rate of a reaction and not the final equilibrium concentrations of the species, the equilibrium values can be determined using Eq. (13) for $[\Sigma A_2]_e$ and Eq. (17) for $[A_3]_e$. These values can be subtracted from the total concentrations of the A_2 or A_3 species to obtain the equilibrium concentrations of the adduct species. Values for k_{-3}^r can then be obtained according to Eq. (29). This rate constant is related to k_{-3}^H and the phosphate catalyst rate constant $k_{-3}^{PO_4}$ according to Eq. 30:

$$k_{-3}^r = [H]k_{-3}^H + [PO_4]k_{-3}^{PO_4} \tag{30}$$

The forward rate constant will also be affected by the phosphate ions and the corresponding forward rate constant will be given by Eq. (31):

$$k_3^f = f_0[D]\{[H]k_3' + [PO_4]k_3^{PO_4}\} \tag{31}$$

where the superscript f refers to the catalyzed forward reaction.

At equilibrium the following relationship must be satisfied because the equilibrium concentrations are not affected by the phosphate ions:

$$K_e = \frac{k_3^f}{k_{-3}^r} = \frac{f_0[D]k_3}{k_{-3}^H} = \frac{f_0[D]\{[H]k_3' + [PO_4]k_3^{PO_4}\}}{[H]k_{-3}^H + [PO_4]k_{-3}^{PO_4}} \tag{32}$$

It is therefore possible to determine $k_3^{PO_4}$ by rearranging Eq. (32):

$$k_3^{PO_4} = \frac{k_3'k_{-3}^{PO_4}}{k_{-3}^H} \tag{33}$$

The concentration of $[A_3]$ as a function of time can be calculated using the values of k_{-3}^r and k_3^f from Eqs. (30) and (31) in Eq. (34).

$$[A_3] = \frac{k_{-3}^r([A_3]_0 + [A_3D]_0)}{k_3^f + k_{-3}^r} + \frac{(k_3^f[A_3]_0 - k_{-3}^r[A_3D]_0)e^{-(k_3^f + k_{-3}^r)t}}{k_3^f + k_{-3}^r} \tag{34}$$

Equation (34) is identical to Eq. (17) with the appropriate substitutions made for the rate constants.

Equations for the ΣA_2 species can be derived in a similar manner. The equations obtained are the same as those in Eqs. (29)–(34) but with the appropriate changes made in the species and rate constants.

The curves in Figs. 5 and 6 were calculated using the rate constants given in Table II. It can be seen that the inclusion of the phosphate catalytic rate constants result in a good correlation between the experimental data and the calculated values.

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