

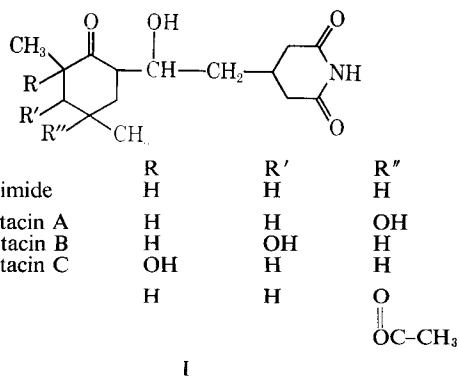
# Catalysis of Streptovitacin A Dehydration: Kinetics and Mechanisms

ROBERT E. NOTARI and STEPHEN M. CAIOLA\*

**Abstract** □ The dehydration of streptovitacin A (StA) is compared to that of cycloheximide (CY) in this paper. Acidic dehydration of CY produces the  $\alpha,\beta$ -unsaturated ketone, anhydrocycloheximide. Under similar conditions StA dehydration yields an analogous product, anhydrostreptovitacin A (AnS), which undergoes further dehydration to form a phenolic derivative. The ratios of the rate constants at the temperatures studied are ( $k_{CY}/k_{StA}$ ): 7.5, 40°; 6.2, 50°; 5.7, 60°; and 4.8, 70°. Dehydration of StA in alkaline buffered solutions gives rise to AnS with no evidence for the formation of the phenolic product. The apparent first-order rate constant,  $k$ , for the formation of AnS defined as  $k = k_{H^+}[H^+] + k_{OH^-}[OH^-] + k_{HPO_4^-}[HPO_4^-] + k_a$ . Log  $k$  versus pH profiles constructed from 40 to 70° show a minimum at pH 4 where StA is more stable than CY. Mechanisms for the catalysis by  $H^+$ ,  $OH^-$  and  $HPO_4^-$  are postulated.

**Keyphrases** □ Streptovitacin A dehydration—catalysis □ Kinetics, mechanism—streptovitacin A catalysis □ Cycloheximide, streptovitacin A hydrolysis—comparison □ pKa—streptovitacin A acid-catalyzed dehydration products □ UV spectrophotometry—analysis

Glutarimide antibiotics related to cycloheximide (CY) (*Ia*) exhibit significant differences in biological activity due to relatively minor chemical or stereochemical variations. For example, streptovitacin A (StA) (*Ib*) and E-73 (*Ic*) were both reported to have approximately 200 times the antitumor activity of CY (*I*). This increase in biological activity was attributed to the additional oxy-group in the cyclohexanone ring. However, a comparison of antifungal activity shows that CY is 100 times more potent than StA (*2*) and 5 times more potent than E-73 (*3*).



The kinetics and mechanisms of CY transformations have previously been reported (*4, 5*). A major transformation of CY is its dehydration to the  $\alpha,\beta$ -unsaturated ketone, anhydrocycloheximide. In the present study, the dehydration of StA was chosen for comparison to that of CY for two reasons. The small difference in chemical structure (5-OH versus 5-H) results in a dramatic difference in biological activity as previously mentioned. Secondly, Herr (*6*) has re-

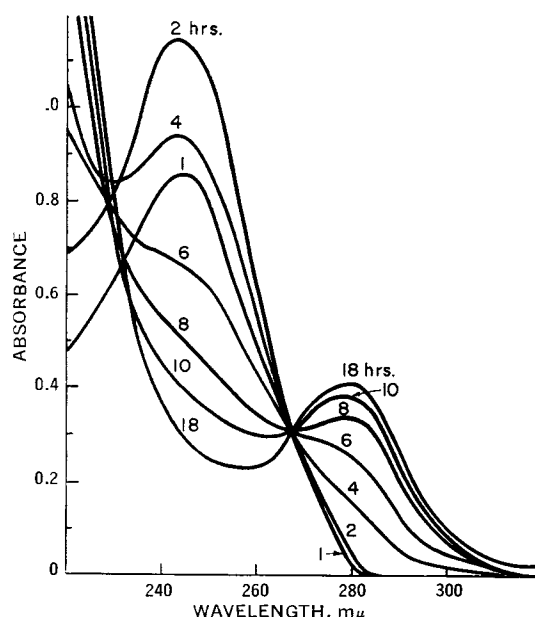
ported that acid-catalyzed dehydration of StA yields a phenolic product rather than the  $\alpha,\beta$ -unsaturated ketone as in the CY case.

This paper compares the rates and mechanisms of StA dehydration as a function of pH, temperature, and buffer, with that previously reported for CY dehydration (*4, 5*).

## EXPERIMENTAL

**Spectral Transformations of StA in Acidic Solutions**—One-milliliter aliquots of an aqueous stock solution of StA were mixed with 100 ml. of HCl solutions previously equilibrated in constant-temperature baths. Samples representing various stages of the reaction were removed, cooled, and their UV absorption spectra determined on a spectrophotometer (Cary model 15). Typical results are illustrated in Fig. 1 where the wavelengths of maximum absorbance for the reaction products are shown to be 242 and 282  $m\mu$ . Additional samples were removed as a function of time, cooled, and the absorbances read on a spectrophotometer (Beckman DU) at each of these wavelengths. The two types of data collected in these studies are illustrated in Figs. 2 and 3. Experimental conditions resulting in the type of plots shown in Fig. 2 are given in Table I and those in Fig. 3 are in Table II.

**pKa Determination of the StA Acid-Catalyzed Dehydration Products**—A  $1.0 \times 10^{-3}$  M solution of StA was allowed to react in 1 N HCl at 70°. Aliquots of the reaction were removed at the end of 1 hr. and 25 hr. and treated in the following manner. Samples were diluted with phosphate buffer or NaOH to obtain the desired pH while maintaining a constant concentration of reaction mixture. Immediately after dilution, the UV spectrum was recorded, the absorbance at 242 and/or 297  $m\mu$  was determined, and the pH was read. Throughout a 3-hr. period following each dilution, the UV spectra were again obtained in order to check for any spectral changes.



**Figure 1**—UV spectra for  $2.5 \times 10^{-4}$  M StA in 1.0 N HCl, 70°, at various time intervals.

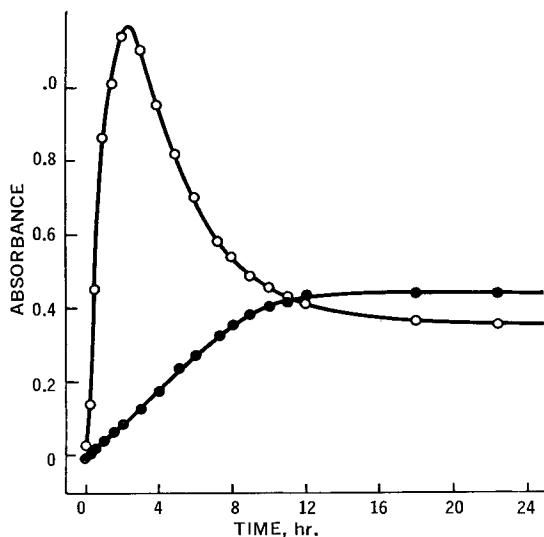


Figure 2—Absorbance at 242  $m\mu$   $\circ$  and 282  $m\mu$   $\bullet$  as a function of the reaction time at 70° for  $2.5 \times 10^{-4}$  M StA in 1.0 N HCl.

The apparent molar absorptivity of the final acid-catalyzed dehydration product was determined as follows. Aliquots of the 25-hr. reaction mixture, prepared for the above pKa determination, were diluted to appropriate concentrations and adjusted to a pH greater than 13 with 1 N NaOH. The 297  $m\mu$  absorption of each sample was immediately determined. The resulting Beer's law plot is discussed under results.

**Spectral Transformations of StA in Buffer Solutions**—Standard buffer solutions were prepared, adjusted to constant ionic strength with NaCl, and equilibrated in constant temperature baths. Aliquots of an aqueous StA solution were then added. Samples were removed throughout the reaction and their UV absorption spectra determined. Reaction rates were established by sampling such solutions as a function of time and determining the absorbance at the 245  $m\mu$  maximum on a spectrophotometer (Beckman DU). Results were similar to that illustrated in Fig. 4 for all buffer studies. The experimental conditions are given in Table III.

## RESULTS

**Spectral Transformations of StA in Acidic Solutions**—Under the experimental conditions listed in Table II, StA was found to undergo dehydration which resulted in the gradual increase of an UV absorption maximum at 242  $m\mu$ . The determination of the absorbance at 242  $m\mu$  on reactions sampled as a function of time gave plots similar to that shown in Fig. 3. Apparent first-order rate constants,  $k$ , were calculated from these data using the equation,

$$\ln(A_m - A_t) = -kt + \ln A_m \quad (\text{Eq. 1})$$

where  $A_m$  is the maximum absorbance value and  $A_t$  is the absorbance at any time,  $t$ . Typical plots based on Eq. 1 are shown in Fig. 5. Figure 6 shows the dependency of the apparent first-order rate constants on the hydrogen-ion activity,  $[H^+]$ , in accordance with:

$$k = k_{H^+}[H^+] \quad (\text{Eq. 2})$$

where  $[H^+]$  was calculated from the experimental concentrations

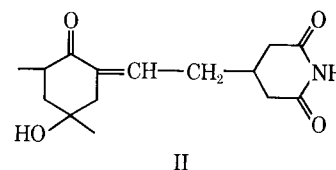
Table I—Experimental Conditions and Apparent First-Order Rate Constants,  $k_1$ , Based on Initial Rates  $(dS/dt)_0$ , of Streptovitamin A Dehydration in 0.95 N at 70°C

$10^3[S]_0^a$	$10^6, dS/dt_0^b$	$10^3 k_1, \text{min.}^{-1}$
1.00	9.65	9.65
0.75	6.85	9.13
0.50	4.83	9.66
0.25	2.32	9.28
0.10	0.935	9.35

<sup>a</sup> Initial concentration of StA. <sup>b</sup> Based on UV absorbance data for the formation of the  $\alpha, \beta$ -unsaturated ketone dehydration product.

and the literature values for activity coefficients (7). A plot of  $\log k$  versus pH (Fig. 7) based on the logarithmic transformation of Eq. 2 has the expected slope of minus one. The values of the catalytic constants,  $k_{H^+}$  in  $\text{l. mole}^{-1} \text{min.}^{-1}$ , calculated from the slopes of the lines for the various temperatures in Fig. 6 are  $2.28 \times 10^{-2}$ , 70°;  $1.00 \times 10^{-2}$ , 60°;  $3.79 \times 10^{-3}$ , 50°; and  $1.32 \times 10^{-3}$ , 40°. An Arrhenius plot of these catalytic constants is linear with a heat of activation,  $\Delta H_a$ , value equal to 20.3 kcal./mole.

Garrett and Notari (4, 5) have reported on the dehydration of CY to yield a 245  $m\mu$  absorption band attributed to the  $\alpha, \beta$ -unsaturated ketone in anhydrocycloheximide. The first dehydration product of StA, with its absorption maximum at 242  $m\mu$  (Fig. 1) is analogous to the CY case and may be named on that basis as anhydrostreptovitamin A (II). The absorptivity of anhydrostrepto-



vitamin A would be expected to be similar to that of anhydrocycloheximide (5-H replaces 5-OH). The final yields of anhydrostreptovitamin A, AS, in the acidic dehydration studies listed in Table II were calculated on that basis from

$$\%AS = 100 A_m/aS_0 \quad (\text{Eq. 3})$$

where  $A_m$  is defined in Eq. 1,  $a = 8250$  is the absorptivity of anhydrocycloheximide in acid at 245  $m\mu$  (4) and  $S_0$  is the initial concentration of StA. The percent yield of anhydrostreptovitamin A for all cases in Table II was found to be 60%.

Initial rate studies were run for a variety of StA concentrations given in Table I. The concentration of StA,  $S$ , remaining at time,  $t$ , was calculated from,

$$S = S_0 - A_t/a \quad (\text{Eq. 4})$$

where  $S_0$ ,  $A_t$ , and  $a$ , have been previously defined. The initial rates of dehydration of StA,  $(dS/dt)_0$ , calculated from the slopes  $m_0$ , of the initial 10% of the reactions are given in Table I. A plot of  $S_0$  versus  $m_0$  is linear in agreement with the relationship,

$$m_0 = (dS/dt)_0 = k_1 S_0 \quad (\text{Eq. 5})$$

In addition a plot of  $\log m_0$  versus  $\log S_0$  is linear with a slope of one, further supporting a first-order reaction in StA. Equation 5 may be

Table II—Experimental Conditions and Apparent First-Order Rate Constants,  $k$  in  $\text{min.}^{-1}$  for the Acid-Catalyzed Dehydration of Streptovitamin A<sup>b</sup>

[HCl]	70°		[HCl]	60°		50°		40°	
	$A_{H^+}$	$10^3 k$		$A_{H^+}$	$10^3 k$	$A_{H^+}$	$10^3 k$	$A_{H^+}$	$10^3 k$
0.050	0.040	0.720	0.248	0.182	1.75	0.184	0.603	0.186	0.218
0.074	0.059	1.06	0.496	0.359	3.70	0.363	1.20	0.371	0.432
0.099	0.077	1.51	0.745	0.545	5.57	0.555	2.08	0.565	0.704
0.149	0.112	2.36	0.994	0.747	8.39	0.767	3.22	0.782	1.00

<sup>a</sup> Based on UV absorbance data for the formation of the  $\alpha, \beta$ -unsaturated ketone dehydration product. <sup>b</sup> The initial concentration of StA was  $2 \times 10^{-4}$  M.

**Table III**—Experimental Conditions and Apparent First-Order Rate Constants,<sup>a</sup> *k*, for the Dehydration of StA<sup>b</sup>

Obs. pH	Buffer Composition			10 <sup>3</sup> <i>k</i> , min. <sup>-1</sup>		
	[NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O]	[Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O]	[NaCl]	70°	60°	50°
5.74	0.150	0.015	0.005	1.03	—	—
±0.05	0.110	0.011	0.057	0.850	—	—
	0.070	0.007	0.109	0.625	—	—
	0.030	0.003	0.161	0.423	—	—
6.73	0.050	0.050	0.000	4.23	1.52	0.552
±0.06	0.035	0.035	0.060	3.32	—	—
	0.030	0.030	0.080	—	1.04	0.372
	0.020	0.020	0.120	2.66	—	—
	0.005	0.005	0.180	1.57	0.526	0.152
7.20	0.020	0.058	0.006	7.50	2.40	0.858
±0.07	0.014	0.041	0.064	6.91	—	—
	0.011	0.032	0.093	—	1.71	0.732
	0.008	0.023	0.122	5.08	—	—
	0.002	0.006	0.181	3.99	1.19	0.449
7.73	0.006	0.060	0.014	15.2	—	—
±0.08	0.004	0.040	0.076	13.4	—	—
	0.002	0.020	0.138	11.6	—	—
	0.001	0.010	0.169	10.7	—	—
8.09	0.0004	0.016	0.194	28.5	—	—
8.21	0.001	0.040	0.121	32.8	—	—
8.27	0.0015	0.060	0.060	39.7	—	—
8.31	0.002	0.080	0.000	50.5	—	—
	[H <sub>2</sub> BO <sub>3</sub> ]	[NaH <sub>2</sub> BO <sub>3</sub> ]	[NaCl]			
7.91	0.072	0.008	0.172	19.5	—	—
7.98	0.018	0.002	0.178	18.8	—	—
8.68	0.001	0.001	0.179	93.0	—	—
9.20	0.130	0.130	0.050	274.	—	—

<sup>a</sup> Based on UV absorbance due to formation of the  $\alpha,\beta$ -unsaturated ketone. <sup>b</sup> The initial concentration of StA was  $2 \times 10^{-4}$  M and the ionic strength was adjusted to 0.2.

solved for the apparent first-order rate constant by solving the expression  $m_0/S_0 = k_1$ . Results of these calculations are given in Table I.

Since the apparent first-order constants,  $k_1$ , are calculated from initial rates, they represent the rate constant for the forward reaction in the equilibrium,



The overall first-order rate constant for achieving this equilibrium can be expressed as,

$$k = k_1 + k_{-1} \quad (\text{Eq. 7})$$

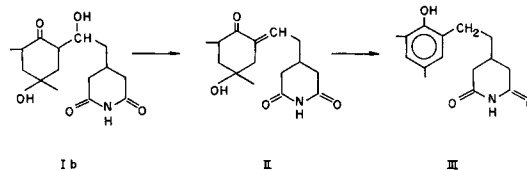
The percent yield of anhydrostreptovitamin A can be expressed in terms of these rate constants as,

$$\%AS = 100 k_1/k \quad (\text{Eq. 8})$$

The mean value for  $k_1$  in 0.95 N HCl at 70° from Table I is  $9.41 \times 10^{-3}$  (min.<sup>-1</sup>). The value for  $k$  under these conditions was calculated from Eq. 2 and is  $16 \times 10^{-3}$  (min.<sup>-1</sup>). Solving Eq. 8 with

these values for  $k_1$  and  $k$  gives the yield of anhydrostreptovitamin A as 59%. This agrees with the value calculated previously from the spectral data using Eq. 3.

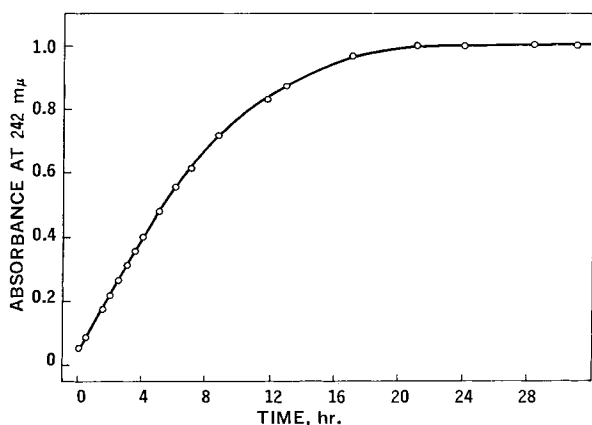
**pKa Determination of the StA Acid-Catalyzed Dehydration Products**—When reaction conditions were stronger than those in Table II, relative to HCl concentration and temperature, the 242 m $\mu$  absorbance first increased and then decreased giving rise to a new maximum at 282 m $\mu$ . Figure 1 shows the presence of an isobestic point associated with this spectral change indicating a 1:1 transformation between the chromophores. Plots of the 242 and 282 m $\mu$  absorbance as a function of reaction time, such as those illustrated in Fig. 2, were typical of an  $A \rightarrow B \rightarrow C$  reaction. The absorbance at 242 m $\mu$  was attributed to the  $\alpha,\beta$ -unsaturated ketone in anhydrostreptovitamin A (II) and the 282 m $\mu$  maximum was attributed to the phenol in III (6) which is shown in Scheme I. In



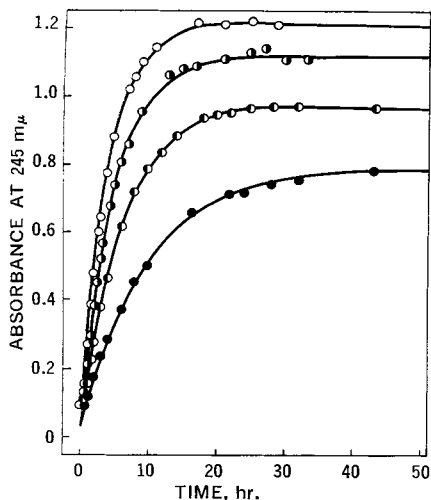
order to further verify this assignment, a spectrophotometric pKa determination was done on the reaction mixture described in Fig. 2 after 1 hr. of reaction and after 25 hr. when only the phenolic product is present.

As expected for  $\alpha,\beta$ -unsaturated ketone, the 242 m $\mu$  absorption maximum after 1 hr. of reaction did not demonstrate any pKa. The absorbance was essentially constant for the dilutions in buffers of pH 1 to 9.5. At higher pH, the absorbance was found to increase slightly and then decrease with time indicating formation and subsequent loss of the chromophore rather than any pKa function.

The spectra of the reaction mixture after 25 hr. showed a maximum near 210 and 282 m $\mu$  corresponding to the two maxima for an undissociated phenolic compound (8). An absorbance maximum at 280 m $\mu$  has been reported for III (3). As the pH was increased, both maxima exhibited bathochromic shifts to 242 and 297 m $\mu$ , respectively. These maxima correspond to the absorbance maxima for phenoxide (8). The pKa was determined by plotting the



**Figure 3**—Increase in absorbance at 242 m $\mu$  due to formation of  $\alpha,\beta$ -unsaturated ketone from dehydration of  $2 \times 10^{-4}$  M StA in 0.745 N HCl at 50°.



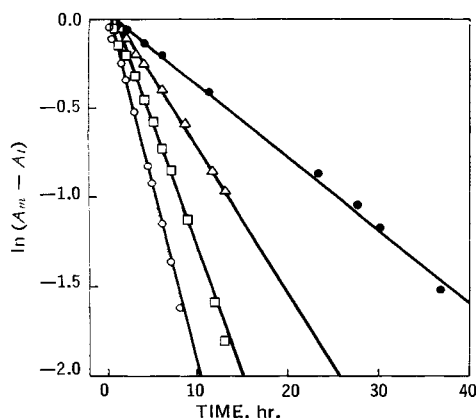
**Figure 4**—Increase in 245 m $\mu$  absorbance from dehydration of  $2 \times 10^{-4}$  M StA in pH 6.74 buffers ( $\mu = 0.2$ ) at 70°. The curves are for equimolar  $[H_2PO_4^-]$  and  $[HPO_4^{2-}]$  where the concentration, M, of each species is:  $\circ$ , 0.050;  $\odot$ , 0.035;  $\bullet$ , 0.040, and  $\bullet$ , 0.005.

absorbance at 242 or 297 m $\mu$  versus pH as shown in Fig. 8. The pKa from these plots is 10.8 which agrees with the value for 2,4,6-trimethylphenol at 25° (8).

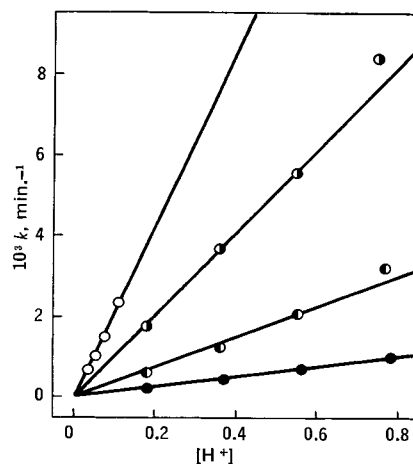
A Beer's law plot of the phenoxide product was made by determining the absorbance at 297 m $\mu$  for various dilutions of the 25-hr. reaction mixture (described above) at pH > 13. The plot of absorbance versus concentration was made by assuming all of the StA had been converted to its phenolic derivative within the 25 hr. It can be seen in Fig. 1 that the absorbance at 242 m $\mu$  after 18 hr. is due to the primary absorbance band for phenol at 210 m $\mu$ . Figure 2 shows that the absorbance due to the phenol is constant after 20 hr. The absorptivity of the phenoxide was calculated from the Beer's law plot to be 2610 which agrees favorably with that of phenoxide which is 2512 (8).

The lack of any pKa associated with the absorption band due to the first dehydration product and the pKa of 10.8 and absorptivity of 2610 associated with the absorption band of the final dehydration product both support Scheme I as the HCl-catalyzed reaction pathway.

**Spectral Transformations of StA in Buffer Solutions**—In phosphate and borate buffer solutions such as those listed in Table III, StA gave rise to an UV spectrum with absorbance maximum at 245 m $\mu$ . The absorbance at 245 m $\mu$  increased as a function of time approaching a constant value. Typical plots are shown in Fig. 4. There was no indication of further dehydration to form the phenolic product which was formed in the HCl-catalyzed dehydration studies. The final absorbance values,  $A_m$ , were found to be constant over long periods of time and in one case no change was observed for



**Figure 5**—First-order plots for the increase in absorbance at 242 m $\mu$ ,  $A_t$ , from dehydration of  $2 \times 10^{-4}$  M StA in HCl at 50°.  $[HCl] = \bullet$ , 0.25 M;  $\triangle$ , 0.50 M;  $\square$ , 0.75 M;  $\circ$ , 1.00 M.



**Figure 6**—Apparent first-order rate constants for StA dehydration,  $k$ , versus hydrogen-ion activity,  $[H^+]$  at temperatures of  $\circ$ , 70°;  $\odot$ , 60°;  $\bullet$ , 50°, and  $\bullet$ , 40°.

$30 \times t_{1/2}$ . First-order plots were obtained using Eq. 1 and were generally linear for more than 80% of the reaction. Apparent first-order rate constants,  $k$ , were calculated from the slopes of these plots and the results are given in Table III. In the phosphate buffer system, this rate constant may be defined

$$k = k_{H_2PO_4^-} [H_2PO_4^-] + k_{HPO_4^{2-}} [HPO_4^{2-}] + k_0 \quad (\text{Eq. 9})$$

where

$$k_0 = k_{H^+} [H^+] + k_{OH^-} [OH^-] + k_s \quad (\text{Eq. 10})$$

Equation 9 can be written

$$k = (k_{H_2PO_4^-} R + k_{HPO_4^{2-}}) [HPO_4^{2-}] + k_0 \quad (\text{Eq. 11})$$

where  $R = [H_2PO_4^-]/[HPO_4^{2-}]$  is constant at a given pH. A plot of  $k$  versus  $[HPO_4^{2-}]$  (see Fig. 9) will be linear with slope,  $m$ , defined

$$m = k_{H_2PO_4^-} R + k_{HPO_4^{2-}} \quad (\text{Eq. 12})$$

and intercept  $k_0$ . Since Eq. 12 has two unknowns, a knowledge of  $m$  and  $R$  at two or more pH values will allow calculation of the catalytic rate constants,  $k_{H_2PO_4^-}$  and  $k_{HPO_4^{2-}}$ . Using the data at pH 5.74, 6.73, 7.20, and 7.73 from Table III, the values for the catalytic constants,  $k_{HPO_4^{2-}}$  (l./mole min.), at the various temperatures are  $6.2 \times 10^{-2}$ , 70°;  $2.4 \times 10^{-2}$ , 60°; and  $8.8 \times 10^{-3}$ , 50°. No significant catalytic effect could be assigned to the  $H_2PO_4^-$  component of the buffer.

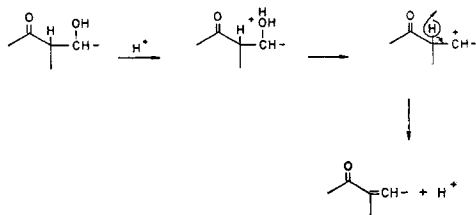
The intercepts of plots such as Fig. 9 are defined by Eq. 10. These can be corrected for the contribution of  $[H^+]$  using the values for  $k_{H^+}$  given previously in this paper. The catalytic constant for  $OH^-$ ,  $k_{OH^-}$ , was calculated from the corrected intercepts (equal to  $k_{OH^-} [OH^-] + k_s$ ) where the  $[OH^-]$  was calculated from observed pH and the literature values for the pKw of  $H_2O$  at the various temperatures (7). The values for  $10^{-3} k_{OH^-}$  (l./mole min.) at the various temperatures are 1.35, 70°; 0.713, 60°; and 0.370, 50°. The values for the rate constants,  $k$ , in the phosphate solutions at pH 8.09, 8.21, 8.27, 8.31 (see Table III) can be adequately described (within 3, 18, 14, and 0.6%, respectively) using Eq. 9 and the previously calculated values for the constants. This series could not be used to calculate  $k_{HPO_4^{2-}}$  because of the variation in pH between the buffer solutions and the fact that 90% of the value for an observed first-order rate constant at this pH is due to hydroxide-catalyzed dehydration. A number of borate buffer solutions were evaluated and the results for a few are listed in Table III. The contribution of the buffer components in the borate systems studied was negligible in comparison to that of the  $[OH^-]$ . An Arrhenius plot for  $k_{HPO_4^{2-}}$  gives a  $\Delta H_a$  of 21.6 kcal./mole and for  $k_{OH^-}$  a  $\Delta H_a$  of 15.1 kcal./mole.

## DISCUSSION

**Dehydration of StA in Acidic Solutions**—Although StA (Ib) differs structurally from CY (Ia) only by a hydroxyl group in the

5-position,<sup>1</sup> its acid-catalyzed dehydration differs in reaction rate and in final product from that previously reported for CY (4). StA undergoes a stepwise dehydration according to Scheme I. Loss of the first mole of water yields the  $\alpha,\beta$ -unsaturated ketone, anhydrostreptovitamin A (II), which has an UV absorption maximum at 242  $m\mu$  in aqueous acid. Anhydrostreptovitamin A undergoes further acid-catalyzed dehydration to yield the final phenolic product (III) which has an UV absorption maximum at 282  $m\mu$ . The phenolic product (III) has been previously prepared by Herr (6) by the acidic dehydration of StA. The initial dehydration product, anhydrostreptovitamin A (II), has not been previously reported. However the acid-catalyzed dehydration of CY (Ia) results in formation of its corresponding  $\alpha,\beta$ -unsaturated ketone, anhydrocycloheximide (4).

The product of StA acidic dehydration, then, is dependent upon the conditions. Under conditions of relatively strong HCl and/or temperature two moles of H<sub>2</sub>O are lost and the phenolic compound (III) is formed. Under moderate conditions, as in Table II, results (Fig. 2) are typical of consecutive reactions which are in this case  $Ib \rightarrow II \rightarrow III$ . The dehydration product in this scheme is dependent on time. Initially there is predominantly II present as a product but finally there is only III. By proper control of temperature and HCl concentration, it was possible to study the dehydration of StA to anhydrostreptovitamin A (II) without any interference from the subsequent dehydration to the phenolic product (III). Typical conditions allowing calculation of the first-order rate constants for the dehydration of  $Ib$  to II are given in Table II. These rate constants can be compared to those reported for the dehydration of CY to its corresponding  $\alpha,\beta$ -unsaturated ketone. StA, like CY, undergoes specific H ion-catalyzed dehydration. This catalysis, which is typical of the dehydration following an aldol condensation (9), is illustrated in Scheme II.



Scheme II

Although both are subject to H ion catalyzed dehydration, StA is more stable to dehydration than CY. A comparison of the catalytic constants,  $k_{H^+}$ , calculated from Eq. 2 with those previously reported for CY (4) shows that the ratio,  $k_{CY}/k_{StA}$  at the tempera-

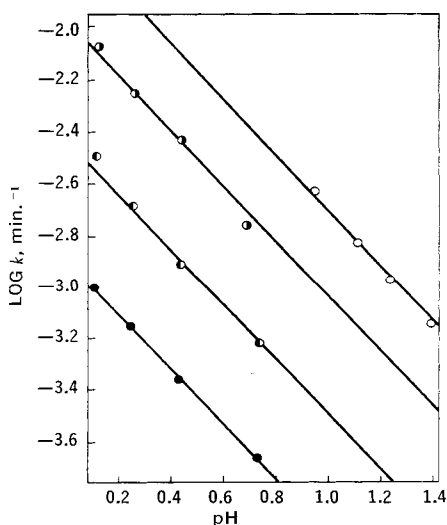


Figure 7—Log  $k$  versus  $pH$  for dehydration of StA at 70°, ○; 60°, □; 50°, △, and 40°, ●.

<sup>1</sup> Streptovitamin A can be named 3-[2-(5-hydroxy-3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide.

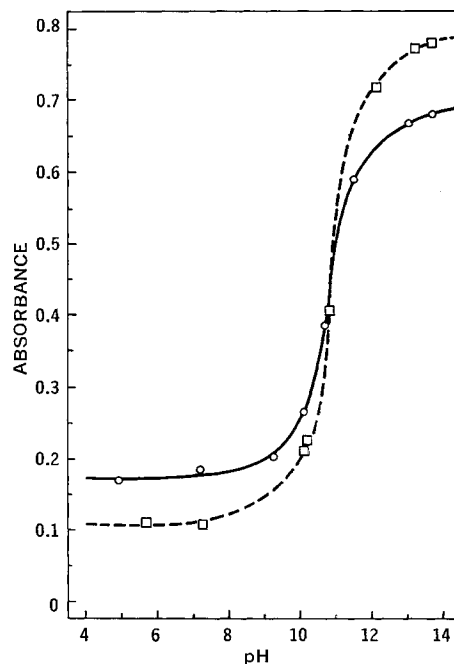


Figure 8—Absorbance at 297  $m\mu$  (□) and 242  $m\mu$  (○) versus  $pH$  for the reaction product from StA in 1.0 N HCl at 70° for 25 hr.

tures studied is 7.5, 40°; 6.2, 50°; 5.7, 60°; and 4.8, 70°. One possible explanation for the increase in stability which is observed with the replacement of the 5-H in cycloheximide (Ia) by the 5-OH in StA (Ib) lies in the fact that the additional OH presents an additional site for protonation. Protonation at the 5-OH position would make protonation of the exocyclic OH more difficult. Since H<sup>+</sup> catalyzed dehydration, as depicted in Scheme II, proceeds *via* protonation of the side chain hydroxyl, a decrease in the ease of this protonation would be reflected by a decrease value in the  $k_{H^+}$ . The StA would be thus expected to be more acid stable than CY on that basis. This type of phenomena has been observed in other systems. For example, the rate of *N*-glycosyl cleavage of nu-

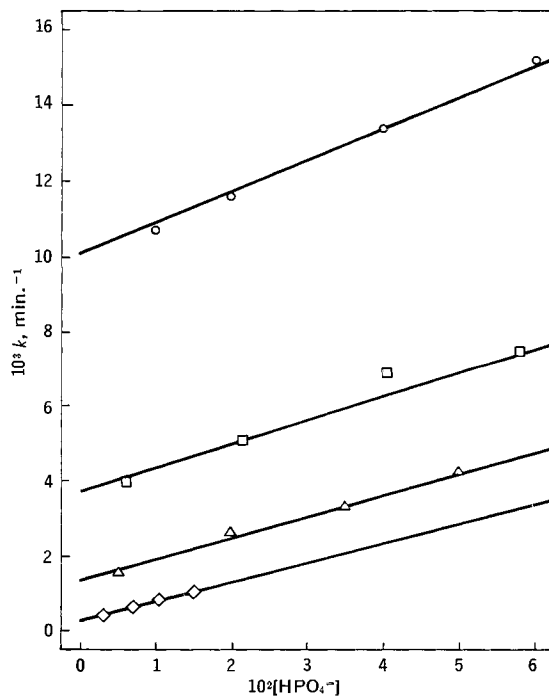
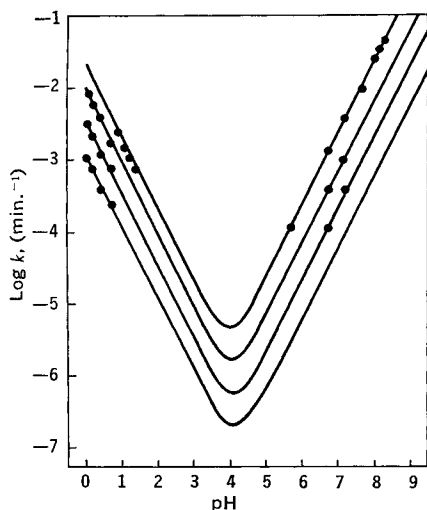


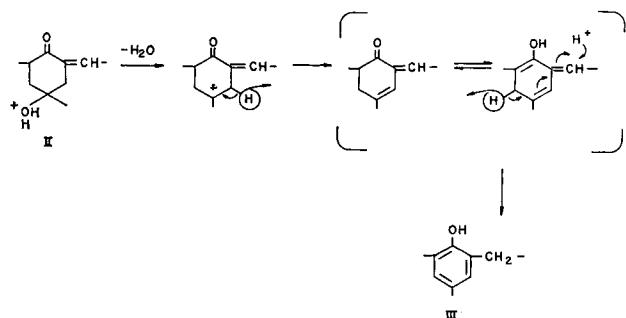
Figure 9—Apparent first-order rate constants for StA dehydration at 70°,  $\mu = 0.2$  as a function of phosphate concentration at  $pH$  5.74 ◇, 6.73 △, 7.20 □, and 7.73 ○.



**Figure 10**—Log  $k$  versus  $pH$  for StA dehydration at 70, 60, 50, and 40°. The lines are calculated from  $k = k_{H^+}[H^+] + k_{OH^-}[OH^-]$  and the points are experimental.

cleosides in aqueous acid is decreased as the number of hydroxyls is increased from 1 to 2 to 3 (10).

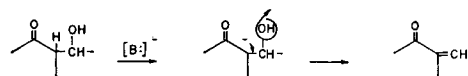
The additional OH group in StA can also lead to the formation of a new dehydration product as compared to the CY case where there is no OH on the cyclohexyl ring (see Ia versus Ib). Under conditions of sufficiently high HCl concentration and temperature, the anhydrostreptovitamin A (II) undergoes further dehydration forming the phenolic product, III. Typical experimental conditions yielding such results are given in Table I. This dehydration does not occur in alkaline buffer solutions (discussed in the next section) and is too slow to observe in dilute HCl such as the case shown in Fig. 3 where there is no appreciable decrease in the final absorbance,  $A_m$ , during 10 hr. The dehydration of the  $\alpha,\beta$ -unsaturated product, anhydrostreptovitamin A (II), to form the phenolic compound, III, is thus dependent upon H-ion concentration. A mechanism for the H-ion catalyzed dehydration of anhydrostreptovitamin A is illustrated in Scheme III.



**Scheme III**

**Dehydration of StA in Buffer Solutions**—Dehydration of StA in alkaline phosphate and borate buffer solutions yielded the  $\alpha,\beta$ -unsaturated ketone anhydrostreptovitamin A (II). This is analogous

to the dehydration of CY which resulted in formation of anhydrocycloheximide in acetate buffer solutions (5). Both reactions were subject to specific hydroxyl ion and general-base catalysis. StA was not studied in the acetic acid-acetate buffer system, as was CY (5), because the increased stability of StA made its reaction time too long. However, while CY dehydration was shown to be general-base catalyzed by acetate ions (5), StA dehydration was catalyzed by the basic component of the phosphate system,  $HPO_4^{2-}$ . The base-catalyzed dehydration of CY or StA is illustrated by Scheme IV.



**Scheme IV**

A log versus pH profile was prepared for StA dehydration to anhydrostreptovitamin A at several temperatures by summing the contributions from  $H^+$  and  $OH^-$ . Results, shown in Fig. 10, indicate that the reaction is at its minimum rate at pH 4.0. As would be expected, this is approximately the same as the pH of maximum stability of CY which was reported as 4.4 (5). The slight shift in pH of maximum stability, from 4.4 to 4.0 can be explained by the increased stability of StA to H-ion catalyzed dehydration as discussed earlier. Comparison of the H-ion catalytic constants,  $k_{H^+}$ , to the OH-ion catalytic constants,  $k_{OH^-}$ , for dehydration of StA to anhydrostreptovitamin A shows that OH-ion is roughly  $10^6$  more effective as a catalyst than is H-ion. This indicates that the mechanism illustrated in Scheme IV is much more effective in the dehydration of the aldol than is the mechanism shown in Scheme II. This also indicates that excessive base would be of primary concern to one synthesizing aldols of this type. The catalytic constant for  $HPO_4^{2-}$ ,  $k_{HPO_4^{2-}}$ , is in fact about 2.5 times larger than  $k_{H^+}$ .

## REFERENCES

- (1) K. V. Rao, *Antibiot. Chemotherapy*, **12**, 123(1962).
- (2) W. T. Sokolski, N. J. Eilers, and G. M. Savage, *Antibiot. Ann.*, 1959, p. 551.
- (3) K. V. Rao, *J. Am. Chem. Soc.*, **82**, 1129(1960).
- (4) E. R. Garrett and R. E. Notari, *J. Org. Chem.*, **31**, 425 (1966).
- (5) E. R. Garrett and R. E. Notari, *J. Pharm. Sci.*, **54**, 209(1965).
- (6) R. R. Herr, *J. Am. Chem. Soc.*, **81**, 2595(1959).
- (7) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd ed., Reinhold, New York, N. Y., 1958.
- (8) Y. Yukawa, "Handbook of Organic Structural Analysis," W. A. Benjamin, New York, N. Y., 1965.
- (9) E. R. Alexander, "Principles of Ionic Organic Reactions," Wiley, New York, N. Y., 1960, p. 176.
- (10) E. R. Garrett, J. K. Seydel, and A. J. Sharpen, *J. Org. Chem.*, **31**, 2219(1966).

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