Table III—Comparison of Calculated and Experimental Values of Z

Experimental	Calculated
0.822	0.826
1.117	1.213
1.391	1.426
1.607	1.684
1.808	1.872
2.025	1.988
2.055	2.069
2.132	2.184

obtained along with those reported in the literature are given in Table II.

It can be seen that there is wide discrepancy in the reported stability constant for SnF^+. The reported value (4) was determined by regression analysis. In a review article, Bond (12) cautioned against applying statistical analysis of complexation data without appropriate weighing of the data relative to the known chemistry of the system. Using Leden's method, Bond calculated the following stability constants from the data of Hall and Slater (4): $\beta_1 = 3 \times 10^4$, $\beta_2 = 1.5 \times 10^8$, and $\beta_3 = 2 \times 10^{10}$. Thus, the new β_1 is in closer agreement with the value reported by Bond and Taylor (5), but they expressed uncertainty in their reported value because of curvature in their Leden plot.

The agreement in reported values of β_3 is satisfactory considering the differences in experimental methods, computational analyses, and ionic strengths. The β_2 values reported range over two orders of magnitude, with the one determined in this work occupying an intermediate position. If the values are transformed to association constants for the sequential reaction shown in Scheme III, then the respective constants for the current work would be 4×10^3, 2.75×10^3, and 9.09×10^1.

$$SnF_{i-1}^{(3-i)} + F^- \rightleftharpoons SnF_i^{(2-i)} \qquad i = 1, 2, 3$$

Scheme III

The respective constants of Bond and Taylor (5) would be 1.2×10^4, 4×10^2, and 6.02×10^2. It can be seen that the latter values are not sequentially in order, which is physically unrealistic.

To assess whether stability constants are satisfactory, it has been suggested that a point-by-point comparison be made between experimental and calculated values (12). Such a comparison is shown in Table III. The stability constants reported in this work are quite satisfactory by this criterion and give a much better fit than if other reported sets of β are used.

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ACKNOWLEDGMENTS AND ADDRESSES

Received July 22, 1974, from the *College of Pharmacy, University of Minnesota, Minneapolis, MN 55455*

Accepted for publication September 11, 1974.

Supported in part by Training Grant 5 T01 GM02064 and Research Grant DE03688 of the National Institutes of Health, U.S. Public Health Service.

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Dehydration of Tetracycline

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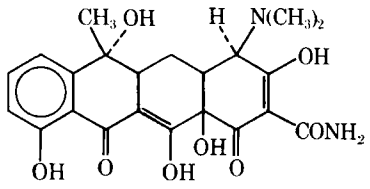
Abstract □ The dehydration of tetracycline at the C-5a–C-6 position as a function of acidity was investigated at various temperatures. The rate was first order with respect to tetracycline and with respect to $[H^+]$. Rate constants and an activation energy are reported. Tetracycline was unstable in dilute acid.

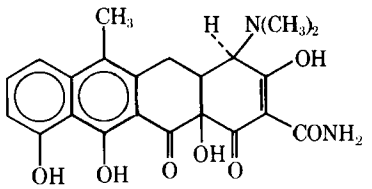
Keyphrases □ Tetracycline—dehydration kinetics, effect of acidity at various temperatures □ Dehydration kinetics—tetracycline, effects of acidity at various temperatures □ Stability—tetracycline, dehydration, effects of acidity at various temperatures

Tetracyclines have been subjected to numerous reactions to aid in elucidating their structures; one reaction involves dehydration at the C-5a–C-6 position in the presence of warm mineral acids (1–4). When tetracycline [4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide] (I) is dehydrated, anhydrotetracycline [4-(dimethylamino)-1,4,4a,5,12,12a-hexahydro-3,10,11,12a-tetrahydroxy-6-methyl-1,12-dioxo-2-naphthacenecarboxamide] (II) is formed; II has aromatic character in both the C- and D-rings.

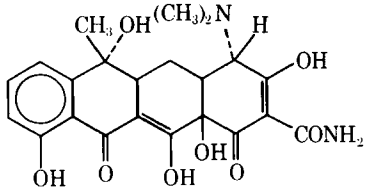
The benzylic C-6 position of I has a tertiary hydroxyl, which is *trans* to the adjacent C-5aH, thereby making the hydroxyl very acid labile and anhydro formation an easy process. Recently, the ease of anhydro formation has been used in the analysis of tetracyclines. Generally, I and its main degradation products [II, 4-epitetracycline (III), and 4-epianhydrotetracycline (IV)] can be separated by TLC, treat-



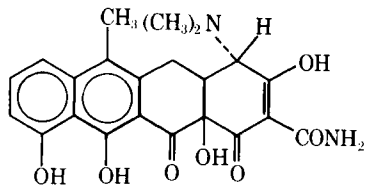
I



II



III



IV

ed with hydrochloric acid to ensure that each constituent exists as the corresponding anhydro compound, and determined spectrophotometrically (5–8).

Reports of the time and conditions required for quantitative conversion to the anhydro compound seem to vary: 2 *N* HCl and heating at 100° for 5 min (5), 5 *N* HCl (time and temperature were not mentioned) (8), and 2 *N* HCl and heating "over hot water" for 5 min (6).

In 1968, Clive (9) compiled some very approximate half-lives (determined by changes in bioactivity) of various tetracyclines degrading to the anhydro species at various temperatures and acid strengths. Aside from the original reports of anhydro formation, little has been reported characterizing the reaction. Further characterization of anhydro formation would be useful for quantitative conversion and pharmaceutical studies since IV is generally believed to be responsible for a "reversible Fanconi-type syndrome," which has been observed in patients who have taken degraded tetracyclines, resulting in lengthy syndrome treatment (sometimes in excess of 1 year) (6, 10).

EXPERIMENTAL

I-HCl was obtained from a commercial source. Compound II was prepared by a method similar to that reported by McCormick *et al.* (11); 4.0 g of I-HCl was dissolved in 2-propanol–methanol–hydrochloric acid (4:1:2), heated to 70° for at least 0.5 hr, cooled, and filtered. Recrystallization from methanol–hydrochloric acid (30:1) yielded yellow needle-like crystals, which were dried under vacuum for 2 days, yielding 1.91 g of crystals, mp 219–222° dec. The product was found to be pure by TLC. The TLC method of analysis used acid-washed Kieselguhr MN slurried with 5% edetic acid (EDTA) at pH 7.5, as reported by Fernandez *et al.* (6).

Spectra and absorbances of kinetic reactions were obtained on a

Table I—Observed Rate Constants for II Formation at Various [H$^+$] Values and Temperatures and 1.00 *M* Ionic Strength[a]

Temperature	[H$^+$]	$10^4\ k_{obs}$, sec^{-1}
30°	0.250	0.212
	0.375	0.322
	0.500	0.452
	0.625	0.565
	0.750	0.675
	0.825	0.790
	1.00	0.903
35°	0.125	0.214
	0.250	0.430
	0.375	0.622
	0.500	0.862
	0.625	1.07
	0.750	1.30
	0.875	1.52
	1.00	1.72
40°	0.125	0.418
	0.375	1.20
	0.500	1.61
	0.875	2.76
	1.00	3.14
45°	0.125	0.862
	0.250	1.60
	0.375	2.40
	0.500	3.21
	0.625	4.11
	0.750	4.77
	0.875	5.69
	1.00	6.84
50°	0.125	1.57
	0.375	4.65
	0.500	6.38
	0.625	7.61
	0.750	9.43
	0.875	10.8
	1.00	12.0

[a] Monitored at 432 nm, $[I]_0 = 1 \times 10^{-4}$ *M*.

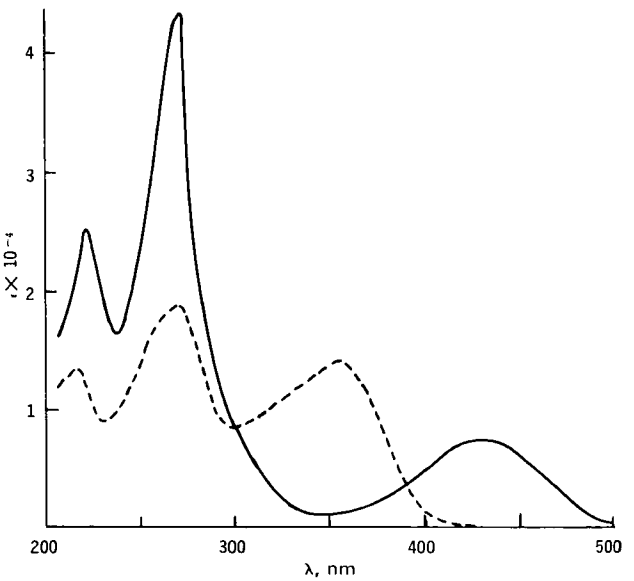


Figure 1—*UV–visible spectra of tetracycline* (- - -) *and anhydrotetracycline* (——) *(both in 0.03* N *HCl).*

Table II—k Values for II at Various Temperatures

Temperature	10^4 k, M^{-1} sec^{-1}, $\pm SD$ (Estimated)
30°	0.924 ± 0.008
35°	1.74 ± 0.014
37°	2.28 (calculated value)
40°	3.12 ± 0.02
45°	6.43 ± 0.09
50°	12.0 ± 0.25

spectrophotometer[1] equipped with a thermostated cell holder and using matched silica cells (10.0 mm). Aliquots of 1.25 M HCl were used to acidify the tetracycline solutions, and 1.00 M ionic strength was maintained using aliquots of 1.25 M KCl.

RESULTS

The UV–visible spectra of I and II in 0.03 N HCl are shown in Fig. 1. It is apparent that the kinetics of anhydro formation may be followed at either 360 or 432 nm. The agreement of the observed rate constants, k_{obs}, obtained at both wavelengths was well within experimental error. Apparent first-order kinetics fit the data with respect to I over more than 80% of the reaction:

$$\text{rate} = k_{obs}[\text{I}] \qquad \text{(Eq. 1)}$$

for a specific $[H^+]$.

When the initial concentration of I was increased by a factor of 10, the fit was still excellent and agreed, within experimental error, with the data at the lower concentration. The k_{obs} values obtained from 30 to 50° at various $[H^+]$ values are given in Table I. The k_{obs} of Eq. 1 contains a dependence on $[H^+]$. A plot of k_{obs} *versus* $[H^+]$ yielded a straight line and zero intercept at each temperature studied. This finding is consistent with the following overall rate law:

$$\text{rate} = k[\text{I}][H^+] \qquad \text{(Eq. 2)}$$

Table II lists the overall rate constants, k, obtained between 30 and 50°, as well as the estimated rate constant at 37°.

An Arrhenius plot of the data yielded an activation energy, Ea, for anhydro formation of 25.1 kcal/mole (SD 0.6).

DISCUSSION

The reported data at constant ionic strength suggest that the reaction is fairly simple: attack of H^+ on the tetracycline species in solution. Under the conditions reported here, the tetracycline is protonated at the C-4 nitrogen. The dehydrated reaction mixtures were analyzed for evidence of epimerization at the C-4 position, which is a significant degradation reaction in less acidic solutions (12). TLC was used, and no epimerization products were detected.

The data also suggest that I is not as stable in slightly acidic solutions as might be desired. Calculations with the results reported here indicate that 10% of a I solution in 0.1 M HCl (1 M ionic strength) would degrade to II in a little more than 3 hr.

Extrapolation of the results to stronger hydrochloric acid concentrations indicates that conversion to II is not as rapid as was previously assumed for quantitative determinations of I (7, 8, 10). The use of both 2 N HCl at elevated temperature and 5 N HCl near room temperature has been reported. The data presented here suggest that heating is required to attain quantitative conversions to II even in strong acid solutions.

[1] Coleman 124.

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ACKNOWLEDGMENTS AND ADDRESSES

Received April 1, 1974, from the *Department of Chemistry, State University of New York, State University College at Brockport, Brockport, NY 14420, and the ‡Department of Chemistry, University of Iowa, Iowa City, IA 52240

Accepted for publication August 19, 1974.

Samples of tetracycline hydrochloride were donated by Chas. Pfizer and Co. Appreciation is also expressed to Shell Oil Co. and Monsanto Chemical Co. for support in the form of fellowships.

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