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CONCERNING THE STRUCTURAL ASSIGNMENT OF THE SECOND AND THIRD ACIDITY CONSTANTS OF THE TETRACYCLINE ANTIBIOTICS

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DURING the course of our investigations of the tetracycline antibiotics, it became necessary to determine the acidity constants (pKa values) of some tetracycline derivatives. We feel that our results require a reversal of the assignment of the second and third acidity constants proposed by Stephens et al.[1] The hydrochloride salts of the tetracycline antibiotics contain three acid groups. These are shown in I as the tricarbonylmethane system A, the ammonium cation B, and the phenolic diketone moiety C.[1] Table 1 lists the thermodynamic pKa values of three tetracycline hydrochlorides.[1]

From I and the data in Table 1, it may be seen that these molecules are sufficiently similar so that ionization information obtained from one or more of them will be generally applicable to all.

In considering the assignment of the acidity constants in any molecule, the complete ionization scheme should be examined. This is shown in Figure I for the tetracyclines, wherein A, B, and C are the moieties shown

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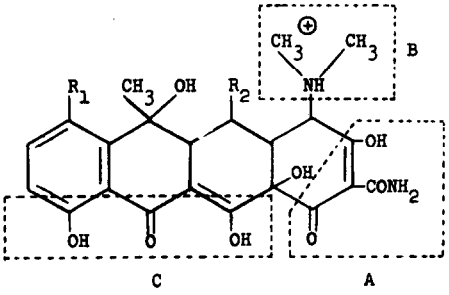
[1] C.R. Stephens, K. Murai, K.J. Brunings, and R.B. Woodward, J. Amer. Chem. Soc. 78, 4155 (1956). It should be noted that in footnote 1 of this publication, reference was made to the possibility of an incorrect assignment of pKa_2 and pKa_3.



I

Ia - Tetracycline: $R_1=R_2=H$

Ib - Chlortetracycline: $R_1=Cl$, $R_2=H$

Ic - Oxytetracycline: $R_1=H$, $R_2=OH$

TABLE 1

Thermodynamic pKa Values of Various Tetracyclines in Aqueous Solution at 25°[1]

	pKa_1	pKa_2	pKa_3
Tetracycline (Ia)	3.30	7.68	9.69
Chlortetracycline (Ib)	3.30	7.44	9.27
Oxytetracycline (Ic)	3.27	7.32	9.11

in I. The superscripts refer to the charge on each group, in accord with the accepted practice for representing the microionization constants of polyfunctional acids.[2] Thus, $A^oC^oB^+$ denotes the protonated tetracycline molecule.

[2] John T. Edsall and Jeffries Wyman, "Biophysical Chemistry," Vol. I, Academic Press Inc., New York, N. Y., (1958), p. 495.

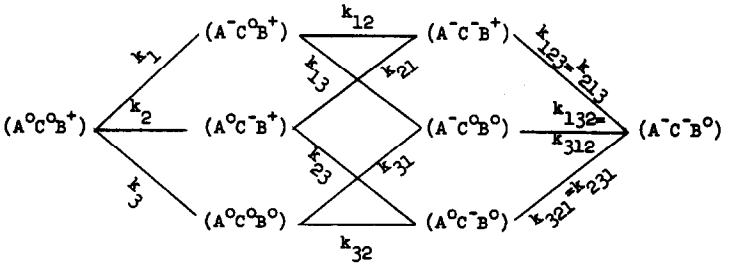


Figure 1. Complete Ionization Scheme for Tetracycline Antibiotics

The microscopic dissociation constants (k) are mathematically related to the macroscopic values (Ka) in numerous ways.[2] For our discussion, however, the most important ones are

$$Ka_1Ka_2Ka_3 = k_1k_{12}k_{123} = k_1k_{13}k_{312} \qquad (1)$$

$$\frac{1}{Ka_3} = \frac{1}{k_{123}} + \frac{1}{k_{132}} \qquad (2)$$

$$k_{12}k_{123} = k_{13}k_{132} \qquad (3)$$

$$Ka_2 = k_{12} + k_{13} \qquad (4)$$

Because Ka_1, which has been assigned to the tricarbonylmethane system,[1] differs from the other values by a factor of at least 10^4, it may be considered in the ionization scheme shown in Figure 1, as essentially equal to k_1. The concern of this discussion, therefore, is whether Ka_2 is primarily related to the ionization step represented by k_{12} or that represented by k_{13}, and correspondingly, whether Ka_3 is primarily related to the ionization represented by k_{123} or that represented by k_{132}.

Our pKa values were determined potentiometrically by a modification of

the method of Parke and Davis,[3] and are listed in Table 2. Although these are not thermodynamic values, it is felt that they are sufficiently accurate for comparative purposes. As an indication of the accuracy, we have included our results for tetracycline (Ia) and chlortetracycline (Ib).

TABLE 2

Apparent pKa Values of Tetracycline Derivatives

in Aqueous Solution at 25°

	pKa_1	pKa_2	pKa_3
Tetracycline HCl (Ia)	3.33	7.75	9.61
Chlortetracycline HCl (Ib)	3.27	7.36	9.22
Tetracycline Methiodide (II)	3.56	7.80	-
Desimethylaminotetracycline (III)	5.97[4]	8.56	-

Since quaternization of the dimethylamino group destroys acidic center B, the assignment of pK_1 and pKa_2 of tetracycline methiodide (II) to the tricarbonylmethane and phenolic diketone systems respectively seems justified; in terms of Figure I, Ka_1 corresponds to k_1 and Ka_2 to k_{12} of the methiodide. Here also, ionization described by Ka_1 must occur within the tricarbonylmethane system (A). Since the electronic character of the quaternized dimethylamino moiety should not differ markedly from that of the corresponding protonated function, its influence on the acid groups A and C should be approximately the same as in Ia. This is especially true of the spatially more remote phenolic diketone system. Because Ia and II are electronically and structurally analogous in the microionization step

[3] T.V. Parke and W.W. Davis, Anal. Chem. 26, 642 (1954).

[4] Cf. pKa_1 5.95 of desimethylaminooxytetracycline.[1]

$A^-C^oB^+ \longrightarrow A^-C^-B^+$, then Ka_2 of II is approximately equal to k_{12} of Ia. That k_{12} of Ia is the primary contributor to Ka_2 of Ia follows from the numerical similarity of the Ka_2 values for Ia and II, and equation 4. Equation 1 then requires Ka_3 of Ia to correspond to k_{123}.

Additional evidence for this conclusion arises from desimethylamino-tetracycline (III), the acidity constants of which would be expected to decrease relative to those of Ia as a result of removing the positive charge associated with the protonated amino function. For this reason the assignment of Ka_3 of Ia to the phenolic diketone system (C) is excluded, since a change in pKa value of this acid center from 9.61 (in Ia) to 8.56 (in III) represents an <u>increase</u> in ionization. We conclude that Ka_1 and Ka_2 of III are approximately equal to k_{31} and k_{132}, respectively, of Ia.[5]

An unqualified assignment of a macroionization constant to a particular functional group is an oversimplification since macro constants may be a composite of two (or even more) micro constants (equation 4). The relative contributions of k_{13} and k_{123} to Ka_2 and Ka_3 of Ia have been estimated by employing equations 2 and 3, and the assignments suggested herein (Ka_2 of II $\cong$ k_{12} of Ia; Ka_2 of III $\cong$ k_{123} of Ia); pk_{123} is then found to be approximately 9.6 and pk_{13} 8.8. From equation 4 it may be seen that k_{12} contributes ten times as much to Ka_2 as k_{13} does, and it is in this sense that we associate pKa_2 with ionization of the phenolic diketone system. By a similar argument k_{123} may be shown to be the major contributor to Ka_3.

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[5] A dimethylamino group at C-4 would have a somewhat different effect from that of a hydrogen atom. However, in general, the literature indicates this effect to be small even when the amino group is α to the acid function. (Reference 2, pps. 485-487, compare pk_a for glycine to pKa for acetic acid).

Although these arguments apply only to tetracycline, it is felt that the similarities in structure and ionization constants make our conclusions generally applicable to other tetracycline group antibiotics. At a future date we intend to present additional data, including spectral evidence, bearing on this matter.