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CIRCULAR DICHROISM AND SOLUTION CONFORMATION OF THE TETRACYCLINE ANTIBIOTICS.

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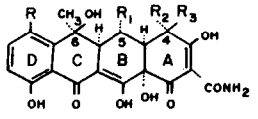
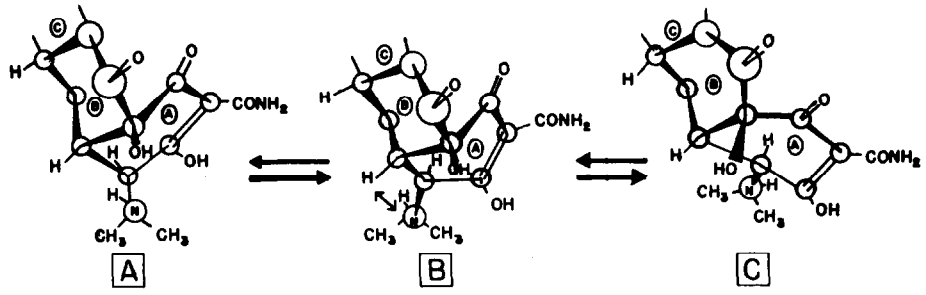
It is axiomatic that in circular dichroism studies, knowledge of the absolute configuration allows determination of the conformation, and vice versa (1). The tetracycline antibiotics are unusually complex from a conformational standpoint, but choice of appropriate conditions allows one to obtain a great deal of useful information from the CD spectra. Hopefully, this information should be useful in understanding structure-activity relationships and physico-chemical properties.



I R = Cl, R_1 = R_3 = H, R_2 = $N(CH_3)_2$
II R = R_1 = R_3 = H, R_2 = $N(CH_3)_2$
III R = R_3 = H, R_1 = OH, R_2 = $N(CH_3)_2$
IV R = R_1 = R_2 = H, R_3 = $N(CH_3)_2$

The absolute configuration of chlortetracycline (CTC-I) has been assigned (2) and the relative configuration-conformation has been established by X-ray measurements (3). It was predicted that conformation B should persist in solution under a wide range of conditions and this was confirmed for tetracycline (TC-II) by NMR measurements in organic solvents, although aqueous conditions were precluded by poor solubility (4). Conformation B, then, is a reasonable approximation of the TC molecule. This form, intermediate between extreme forms A and C, is apparently



A ⟷ B ⟷ C

preferred in acid conditions because of the strong repulsion between the solvated 12a-OH and the protonated dimethylamine functions. We have measured the CD spectra of numerous tetracycline derivatives in 0.03N HCl solution to avoid epimerization at C-4 (5) and to generate the same

tautomeric state as far as possible (Figure 1). All of the natural, bioactive, tetracyclines give spectra that are remarkably similar in shape and intensity leading to the reasonable conclusion that they all belong to the same stereochemical family, and that they very probably possess the same overall conformation in these conditions. Using TC as an example, all of the UV bands are optically active (even though the 360 nm band is relatively weak). The BCD chromophore is responsible for the bands at ca. 360, 320, 285, and 225 nm, while the ring A chromophore contributes the 262 nm band. The composite nature of the 275 nm UV band of the tetracyclines, long suspected (5), is strongly confirmed by these measurements as the 262 and 285 nm CD bands are intense and of opposite sign (Figure 1). The 262 nm band occurs at an appropriate wavelength for the π to π^* transition of a 2-acetamido-1,3-dioxo function (6). The π to π^* transition of conjugated systems is known from other studies to be sensitive to chirality (7). As a test of our assignment, we felt that deprotonation of the C-4 amino function by raising the pH should now favor conformation A over B for the OH and NMe_2 functions should become hydrogen-bonded and the formerly destabilizing steric and electronic repulsions between the two groups should be largely overcome. This change in conformation should alter the chirality of the A ring chromophore and be visible in the CD spectrum. In fact, the 262 nm band undergoes a striking change in intensity as the pH rises above 7 where deprotonation is expected, and the effect becomes more pronounced at higher pH's (Figure 2). Acidification immediately restores the original spectrum. The spectrum of TC in base resembles that of 4-epiTC in acid (Figure 3), after correction for the expected bathochromic effect of base on the BCD chromophore. No dipolar 1,3-diaxial interaction is present in the 4-epiTC series so conformation A should be favored over B regardless of the pH. In fact, the spectrum of 4-epiTC undergoes a much smaller change in the 262 nm region in going from acid to base (Figure 3). These observations strongly suggest that TC and 4-epiTC, only the former an active antibiotic, possess the same general conformation in alkaline but not in acid solutions. This helps rationalize the well known tendency of the tetracyclines to epimerize most efficiently at pH 3-5 (5). In addition, the Noseworthy (8) process for converting 4-epiTC's back to the natural epimers by use of Ca ion in alkaline solutions is easily understood in these terms.

Chelation between Ca ion and the 12a-OH and 4-NMe_2 functions of TC should help stabilize the molecule in conformation A by fixing the N atom in the axial conformation. Strong evidence for this can be seen in CD measurements (Figures 2 & 3). Ca ion promotes and exaggerates the

tension changes referred to above in the 262 nm region of TC much more than with 4-epiTC, with the effect becoming apparent at pH values where deprotonation is occurring. Ca ion is much more efficient at this than pH alone. It is also apparent from the spectra that Ca ion is binding with the BCD chromophore at the same time. The binding sites of various metal ions with the tetracyclines have been studied intensively with various conflicting views emerging (9). We feel that CD measurements provide fresh insight into this problem and provide clear evidence for two potential binding sites depending upon the pH of the solution. The biochemical and microbiological implications of these findings are especially intriguing. These conclusions do not agree in detail with other studies involving other metal ions, particularly transition element ions, and using quite different measurement techniques. The conflict is more apparent than real, in our opinion, and will be dealt with in detail in a full paper in preparation. Several obvious extensions of this work are in progress, such as the effect of other ions, analytical applications and kinetic studies, and the detailed results will be published shortly in another place.

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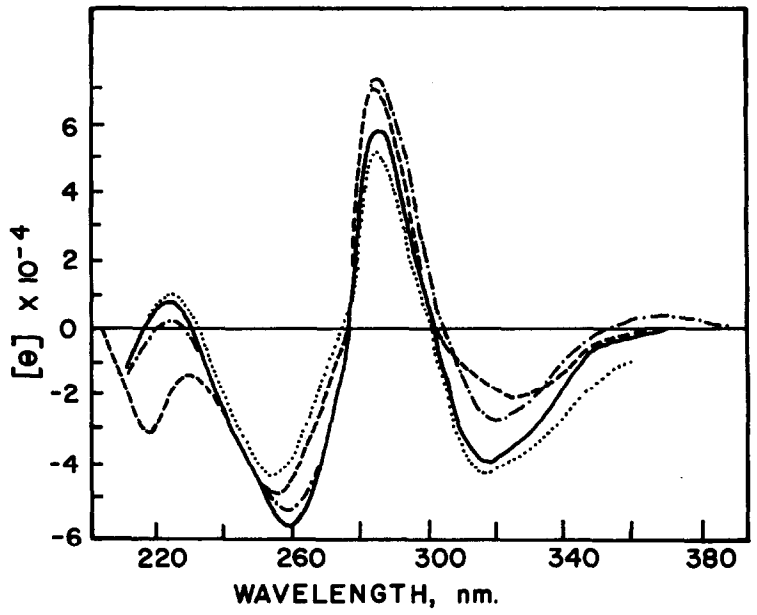

Figure 1. Circular dichroism spectra of several tetracyclines in 0.03 N HCl:
(——), Tetracycline HCl
(----), 6-demethyl-6-deoxytetracycline HCl
(····), α-6-demethyltetracycline HCl
(-·-·), Oxytetracycline HCl

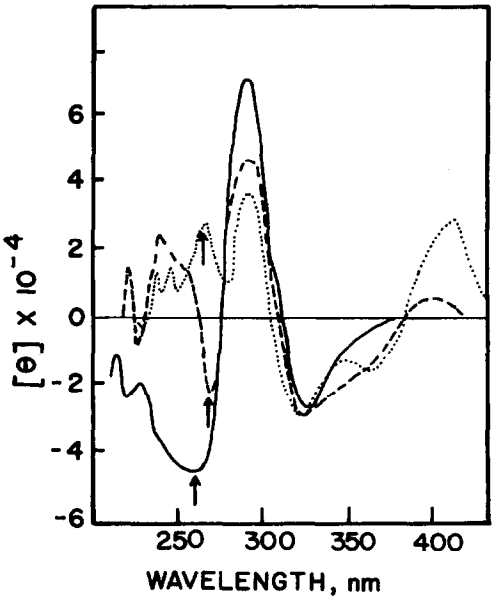

Figure 2. The circular dichroism spectra of 6-demethyl-6-deoxytetracycline:
(——), with and without calcium chloride at pH 3.28
(----), without calcium chloride at pH 11.23
(····), with calcium chloride at pH 11.19

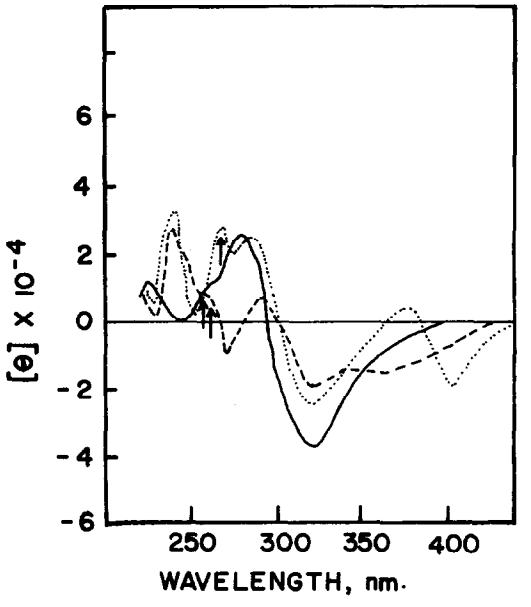

Figure 3. The circular dichroism spectra of 4-epitetracycline ammonium salt:
(——), with and without calcium chloride at pH 3.40
(----), without calcium chloride at pH 11.11
(····), with calcium chloride at pH 11.18