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Structural Studies of Tetracyclines. Crystal and Molecular Structures of Anhydrotetracycline Hydrobromide Monohydrate and 6-Demethyl-7-chlorotetracycline Hydrochloride Trihydrate[1]

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Abstract: The crystal and molecular structures of two tetracyclines, anhydrotetracycline hydrobromide (I) and 6-demethyl-7-chlorotetracycline hydrochloride (II) have been determined by x-ray diffraction techniques. Aromatization of the C ring of tetracyclines gives I but also destroys any useful therapeutic value. In contrast II has excellent antibacterial properties. The crystals of I monohydrate are orthorhombic, space group $P2_12_12_1$ with unit cell dimensions of $a = 19.562$ (2), $b = 16.586$ (2), and $c = 6.796$ (2) Å. Crystals of II as the trihydrate are monoclinic, space group $P2_1$, with cell dimensions of $a = 9.219$ (1), $b = 11.634$ (1), $c = 11.240$ (1) Å; $\beta = 102.39$ (4)°. Although the conformation of I is similar to that of II, significant differences are observed. In addition, various bond lengths are affected by the aromatization of the C ring. These differences may account for the different antibacterial spectrum of the two compounds. The conformation of II, except for the orientation of the amide group, is virtually identical with the conformations found in all therapeutically useful tetracyclines. The amide group in II has the same orientation as in tetracycline free base but is rotated 180° relative to that found in the other derivatives. The difference in hydration and crystal packing of II relative to the other tetracyclines suggests that the conformation of II is very stable and is most likely the conformation found at the site of biological activity.

Tetracyclines (Figure 1) are a group of hydronaphthacenes,[3] some of which are important antibiotics. When we initiated our program, in spite of the importance of these drugs,[6] there were x-ray crystal structures for only two active tetracyclines.[7,8] However, a comparison of both therapeutically active and inactive drugs is necessary to elucidate the conformational requirements for biological activity. The resulting knowledge of the possible conformations for active tetracyclines is required for an understanding of the mechanism of biological action on a molecular level.

Aromatization of the C ring of tetracyclines occurs easily by removal of a molecule of water to give anhydrotetracyclines.[9] The anhydro derivatives have only minimal activity against staphylococcus aureus and are not of any therapeutic significance.[10] We undertook a crystal structure determination of anhydrotetracycline hydrobromide, henceforth ANTC·HBr, to study the changes in the molecule which resulted from aromatizing the C ring.

In contrast to ANTC, 7-chlorotetracycline hydrochloride, henceforth 7-CLTC·HCl, 5-hydroxytetracycline hydrochloride, henceforth 5-HTC·HCl, and 6-demethyl-7-chlorotetracycline hydrochloride, henceforth 6-DM-7-CLTC·HCl, are widely used antibiotics. Crystals of 7-CLTC·HCl and 5-HTC·HCl[7] were found to be isomorphous and the two cations had identical conformations. The question was raised as to whether the observed conformations were a result of crystal packing forces. Therefore, when we found that 6-DM-7-CLTC·HCl crystallized in a completely different arrangement and with a different degree of hydration, we decided to determine the crystal structure. If the conformations of the cations in 7-CLTC·HCl and 5-HTC·HCl were a result of crystal packing forces, then we might expect a different conformation in 6-DM-7-CLTC·HCl. The present report provides a detailed description of our structural studies of ANTC·HBr and 6-DM-7-CLTC·HCl, together with a comparison of these results with recently reported structural studies of other tetracyclines.[11-15]

Experimental Section

Yellow, acicular crystals of both compounds were grown from saturated methanol solutions by slow evaporation of the solvent. The pH of the solutions had been adjusted to ~1.5 with either HBr or HCl to prevent epimerization. Preliminary Weissenberg and precession photographs indicated that ANTC·HBr crystallized in the orthorhombic space group $P2_12_12_1$ (D_2^4) and that 6-DM-7-CLTC·HCl was monoclinic with space group $P2_1$ (C_2^2) or $P2_1/m$ (C_{2h}^2). The latter seemed unlikely since with 2 molecules of 6-DM-7-CLTC·HCl per unit cell the molecule would be required to have a mirror plane of symmetry. The subsequent intensity statistics were consistent with the choice of $P2_1$ (C_2^2).

Small, approximately equidimensional crystals were used for the measurement of cell constants and intensity data using a General Electric XRD-6 diffractometer. The crystal sizes and other pertinent data are summarized in Table 1. The cell dimensions were obtained by a least-squares procedure from the 2θ values of Cu Kβ ($\lambda = 1.39217$ Å) peaks. The intensity measurements were made using previously described techniques[16] to a 2θ limit of 135° (Cu Kα radiation) in both cases. Only those measurements in which the intensity was greater than or equal to 1.2 times the appropriate background were considered

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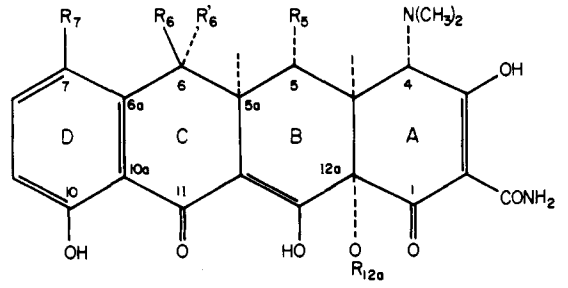

R_7	R'_6	R_6	R_5	R_{12a}	Derivative
H	CH_3	OH	H	H	TC
CL	CH_3	OH	H	H	7-CLTC
H	CH_3	OH	OH	H	5-HTC
CL	H	OH	H	H	6-DM-7-CLTC
H	CH_3	H	OH	H	6-DH-5-HTC
H	CH_2		OH	H	6-ME-5-HTC
$N(CH_3)_2$	H		H	H	7-DMATC
H	CH_3	OH	$OCOCH_3$	$COCH_3$	5,12a-DA-5-HTC

Figure 1. The chemical structure, atomic numbering, and abbreviations for various tetracycline derivatives. The molecule is shown in the correct absolute configuration. The abbreviations are TC, tetracycline; 7-CLTC, 7-chlorotetracycline; 5-HTC, 5-hydroxytetracycline; 6-DM-7-CLTC, 6-demethyl-7-chlorotetracycline; 6-DH-5-HTC, 6-deoxy-5-hydroxytetracycline; 6-ME-5-HTC, 6-methylene-5-hydroxytetracycline; 7-DMATC, 7-dimethylaminotetracycline; and 5,12a-DA-5-HTC, 5,12a-diacetyl-5-hydroxytetracycline.

reliable and used in the analysis. Absorption corrections were considered unnecessary, and the data were reduced to a set of structure amplitudes on an arbitrary scale in the usual manner.

Structure Determination and Refinement. The structure of ANTC·HBr was determined using the heavy-atom method with successive Fourier syntheses. With all the nonhydrogen atoms located, the usual residual $R = \Sigma||F(\text{obsd}) - F(\text{calcd})||/\Sigma F(\text{obsd})$ was 0.19. Six full-matrix least-squares cycles with individual isotropic thermal parameters reduced R to 0.13. The choice between O and N in the amide group was made by refining both possibilities. In one case the individual thermal parameters (4.17 and 3.96) were reasonable while in the other orientation, the values were widely different (5.50 and 2.89). The assignment was also consistent with the Fourier peak heights. Six least-squares cycles using anisotropic thermal parameters with the block approximation reduced R to 0.069. A difference Fourier synthesis indicated reasonable positions for the majority of the hydrogen atoms. The main problem involved the amide group and whether O(am) or O(3) was the site for protonation. Small peaks could be found which were consistent with either possibility. The peak near O(3), called H(6), was chosen and used in the subsequent calculations. The hydrogen atom contributions were included in the structure factor calculations but their parameters were not varied. Two additional least-squares cycles reduced R to 0.054 and the refinement was terminated. The indicated shifts in the last cycle were all $<1/6$ of the corresponding esd. The final parameters in Tables II and III are available.[17]

The structure determination of 6-DM-7-CLTC·HCl presented a somewhat more difficult problem. The positions of the two chlorine atoms were deduced from the sharpened Patterson function. However, a Fourier synthesis based on phases determined by the two chlorine atoms provided very little additional information. An E map was then calculated after a tangent formula refinement utilizing the phases calculated from the two chlorine atoms. The tetracycline skeleton was now clearly evident. A Fourier synthesis was used to locate the remaining atoms at which point the R value was 0.21. The identification of the oxygen and nitrogen atoms in the amide group was made using the behavior of the isotropic thermal parameters during least-squares refinement. With all the atoms correctly assigned, three full-matrix least-squares cycles with individual isotropic thermal parameters reduced R to 0.11. Two additional cycles with only the two chlorine atoms having anisotropic thermal parameters lowered R to 0.079. All subsequent least-squares calculations used the block approximation to the full matrix. Three cycles with all atoms having anisotropic thermal parameters lowered R to 0.057 and a difference Fourier synthesis revealed the positions of all the hydrogen atoms. Two least-squares cycles with fixed hydrogen atom contributions and then four cycles in which the hydrogen atom parameters were also varied produced a final R of 0.039. The average shifts in all parameters were $<1/5$ of an esd during the final cycle. The final atomic parameters for all the atoms are available in Tables IV and V.[17]

Table I. Crystal Data for Anhydrotetracycline Hydrobromide Monohydrate (I) and 6-Demethyl-7-chlorotetracycline Hydrochloride Trihydrate (II)

	I	II
a, Å	19.562 (2)	9.219 (1)
b, Å	16.586 (2)	11.634 (1)
c, Å	6.796 (2)	11.240 (1)
β, degree		102.39 (4)
Volume, Å^3	2205.0	1177.3
Formula	$C_{22}H_{25}O_8N_2Br$	$C_{21}H_{28}O_{11}N_2Cl_2$
Mol wt	525.36	555.36
Space group	$P2_12_12_1$	$P2_1$
Z	4	2
D_M, g cm^{-3}	1.58	1.52
D_C, g cm^{-3}	1.582	1.566
Size of crystal, mm	0.08 × 0.07 × 0.05	0.19 × 0.16 × 0.12
μ (cm^{-1})	33.0	31.3
No. intensity measurements	6613	6964
No. unique reflections	2296	2234
No. reflections used in structure analysis	2165	2211

The weighting scheme for both refinements was $w = (F(\text{obsd})/F(\text{min}))^2$ if $F(\text{obsd}) < F(\text{min})$; $w = 1$ if $F(\text{min}) \leq F(\text{obsd}) \leq 2F(\text{min})$; and $w = (2F(\text{min})/F(\text{obsd}))^2$ if $F(\text{obsd}) > 2F(\text{min})$. For ANTC·HBr $F(\text{min})$ was 10.8; for 6-DM-7-CLTC·HCl $F(\text{min})$ was 10.0. The correction for anomalous scattering for Br was made[18] and the scattering factors were from a usual source.[19] In the case of 6-DM-7-CLTC·HCl the scattering factors were from several different compilations.[20]

Results and Discussion

The cations ANTC·H$^+$ and 6-DM-7-CLTC·H$^+$ are shown in Figures 2 and 3, respectively. Figure 2 illustrates the fact that the C ring in ANTC·H$^+$ is planar, a result of the aromatization. This planarity can also by appreciated by considering the dihedral angles given in Table VI. The dihedral angles for the C ring cluster around 0° in ANTC·H$^+$ (as required for a planar hexagon), while in the other tetracyclines the angles are relatively large (up to ±50°). The planarity of the C ring in ANTC·H$^+$ causes concomitant changes in the conformations of the A and B rings, although the overall conformations of ANTC·H$^+$ and 6-DM-7-CLTC·H$^+$ are surprisingly similar. The conformation of 6-DM-7-CLTC·H$^+$ is virtually the same as that found in TC$^\pm$ and in all other reported structures of biologically active tetracyclines.[21,22] In contrast vastly different conformations were found for 5-HTC[11] and 5,12a-DA-5-HTC[15] whose biological activities are not known.[23] The biological implications of the conformational differences will be discussed below.

The orientation of the amide group on C(2) appears to be different in ANTC·H$^+$ and in 6-DM-7-CLTC·H$^+$. In both 6-DM-7-CLTC·H$^+$ and TC$^\pm$ the amide oxygen is cis to O(1), while in ANTC·H$^+$ the arrangement is trans. The fact that the orientation of the amide group is not fixed is somewhat surprising. There are intramolecular hydrogen bonds between the amide group and both ring oxygen atoms (vide infra) so that the barrier to rotation might be expected to be high.

The distances and angles in ANTC·H$^+$ and 6-DM-7-

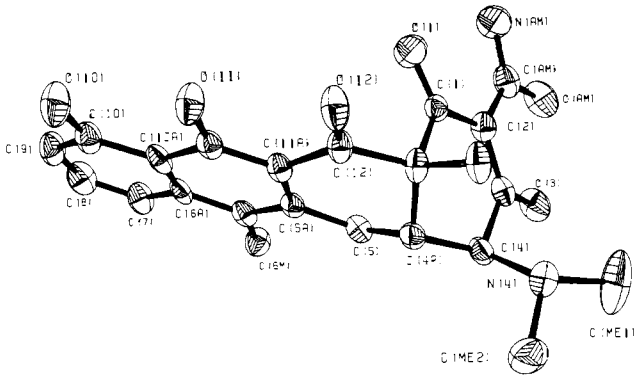

Figure 2. An ORTEP drawing of the anhydrotetracyclinium cation showing the thermal ellipsoids and atomic numbering. The standard numbering for tetracyclines has been used. The atom labels C(3), C(6), C(11), C(12a), and O(12a) were omitted for clarity.

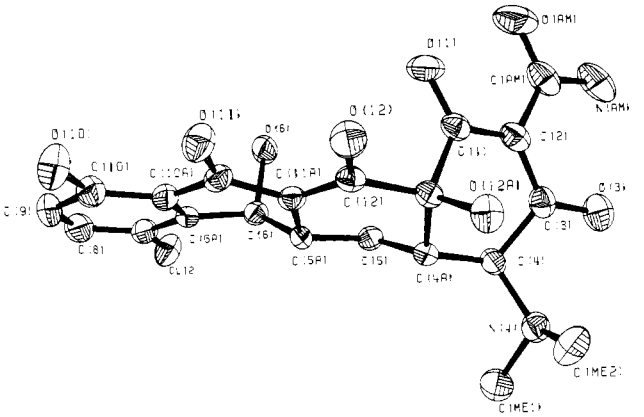

Figure 3. An ORTEP drawing of the 6-demethyl-7-chlorotetracyclinium cation showing the thermal ellipsoids. The atomic numbering is identical with that used in Figure 1. The atom labels C(7), C(11), and C(12a) were omitted for clarity.

Table VI. A Comparison of the Torsion Angles in Anhydrotetracycline Hydrobromide (ANTC·HBr), Anhydrous 5-Hydroxytetracycline (A-5-HTC), 5,12a-Diacetyloxytetracycline (5,12a-DA-5-HTC), 6-Demethyl-7-chlorotetracycline Hydrochloride (6-DM-7-CLTC·HCl), and Tetracycline Free Base (TC±). The Angles Were Calculated Using the Program GEOM from the Cambridge Crystallographic Data Center and the Published Parameters for A-5-HTC, 5,12a-DA-5-HTC, and TC±

Atoms	ANTC·HBr	A-5-HTC	5,12a-DA-5-HTC	6-DM-7-ClTC·HCl	TC±
C(1)-C(2)-C(3)-C(4)	−4.5	−9.4	−3.7	26.1	34.1
C(2)-C(3)-C(4)-C(4a)	24.4	2.0	−17.5	−19.2	−30.2
C(3)-C(4)-C(4a)-C(12a)	−50.2	29.3	46.9	−20.5	−12.1
C(4)-C(4a)-C(12a)-C(1)	58.2	−53.2	−59.8	52.2	49.4
C(4a)-C(12a)-C(1)-C(2)	−38.7	47.5	39.6	−48.2	−48.4
C(12a)-C(1)-C(2)-C(3)	11.3	−16.4	−7.0	8.3	5.4
O(12a)-C(12a)-C(1)-C(2)	82.0	169.0	167.4	69.3	72.6
O(1)-C(1)-C(2)-C(am)	5.2	−16.9	−13.1	5.3	4.5
C(1)-C(2)-C(am)-O(am)	−177.5	−177.1	−171.3	−6.3	8.6
C(3)-C(4)-N(4)-C(Me_1)	−46.0	−84.3	−84.7	−159.5	−159.0
C(3)-C(4)-N(4)-C(Me_2)	−171.6	44.9	47.9	72.8	73.3
C(11a)-C(12)-C(12a)-C(1)	86.5	152.8	168.6	98.1	102.3
C(12)-C(12a)-C(1)-C(2)	−161.0	−72.7	−80.6	−169.8	−169.5
C(5a)-C(5)-C(4a)-C(4)	−171.8	−79.0	−85.8	174.1	173.3
C(5)-C(4a)-C(4)-C(3)	71.0	153.8	169.8	101.6	110.5
C(4a)-C(5)-C(5a)-C(11a)	18.0	−6.6	6.7	43.5	46.1
C(5)-C(5a)-C(11a)-C(12)	5.7	−22.8	−25.4	−15.3	−17.6
C(5a)-C(11a)-C(12)-C(12a)	4.4	10.0	−1.8	5.1	5.2
C(11a)-C(12)-C(12a)-C(4a)	−37.2	31.4	44.9	−23.1	−20.3
C(12)-C(12a)-C(4a)-C(5)	59.0	−58.3	−60.1	50.0	47.8
C(12a)-C(4a)-C(5)-C(5a)	−50.6	46.4	35.4	−62.3	−63.8
C(6a)-C(6)-C(5a)-C(5)	179.6	−171.8	174.8	176.5	−179.0
C(10a)-C(11)-C(11a)-C(12)	174.1	174.0	−168.0	178.2	177.8
C(5a)-C(6)-C(6a)-C(10a)	−1.9	28.9	44.2	41.2	36.5
C(6)-C(6a)-C(10a)-C(11)	3.1	−6.2	−6.0	−6.2	−3.2
C(6a)-C(10a)-C(11)-C(11a)	−0.5	−2.6	−19.6	−13.7	−13.0
C(10a)-C(11)-C(11a)-C(5a)	−3.3	−12.9	3.4	−3.7	−7.5
C(11)-C(11a)-C(5a)-C(6)	4.6	35.4	35.5	38.5	41.6
C(11a)-C(5a)-C(6)-C(6a)	−1.9	−41.6	−57.2	−55.6	−53.8
C(6a)-C(7)-C(8)-C(9)	0.1	0.4	2.2	−0.6	−0.1
C(7)-C(8)-C(9)-C(10)	−0.4	−1.2	0.5	1.3	0.7
C(8)-C(9)-C(10)-C(10a)	2.5	2.0	−3.0	−2.6	−1.0
C(9)-C(10)-C(10a)-C(6a)	−4.4	−1.4	2.9	3.1	0.9
C(10)-C(10a)-C(6a)-C(7)	4.1	−0.2	−0.1	−2.4	−0.3
C(10a)-C(6a)-C(7)-C(8)	−2.0	1.1	−2.3	1.2	−0.1
N(4)-C(4)-C(4a)-C(5)	−165.2	−80.4	−61.1	−128.5	−119.2

CLTC·H^+ are given in Tables VII and VIII. The distances found in some other tetracycline structures are included in Table VII for comparison. Many of the differences in the bond distances between ANTC·H^+ and the other derivatives can be related to the changes in the C ring. In ANTC·H^+ the aromatization of the C ring requires that C(11)-O(11) be a single bond rather than the double bond found in the other tetracyclines. Similarly, in the B ring C(12)-O(12) is usually a single bond and C(11a)-C(12) is a double bond but in ANTC·H^+ the reverse is true (C(12)-O(12) is the double bond) as a result of the changes in the C ring. However, in the A ring the dimensions in ANTC·H^+, with the exception of the C(1)-O(1) and C(3)-O(3) distances, are not significantly different from those found in the other protonated tetracyclines. One point of difference between ANTC·H^+ and 6-DM-7-CLTC and TC± involves the orientation of the amide

Table VII. A Comparison of the Bond Distances in Anhydrotetracycline Hydrobromide (ANTC·HBr), 6-Demethyl-7-chlorotetracycline Hydrochloride (6-DM-7-CLTC·HCl), Tetracycline Free Base (TC±), Anhydrous Oxytetracycline (A-5-HTC), and 6-Deoxy-5-oxytetracycline Hydrochloride (6-DH-5-HTC·HCl)

Bond	ANTC·HBr	6-DM-7-CLTC·HCl	TC±	A-5-HTC	6-DH-5-HTC·HCl
		Ring A			
C(1)-C(2)	1.436 (10)	1.429 (5)	1.434 (4)	1.438 (3)	1.417 (3)[a]
C(2)-C(3)	1.409 (11)	1.417 (5)	1.437 (4)	1.391 (3)	1.442 (4)[a]
C(3)-C(4)	1.532 (9)	1.528 (6)	1.534 (4)	1.523 (3)	1.530 (3)
C(4)-C(4a)	1.541 (9)	1.539 (5)	1.538 (4)	1.546 (3)	1.542 (3)
C(4a)-C(12a)	1.524 (10)	1.526 (5)	1.542 (4)	1.532 (3)	1.529 (3)
C(12a)-C(1)	1.539 (9)	1.539 (5)	1.559 (4)	1.527 (3)	1.549 (3)
C(1)-O(1)	1.223 (10)	1.241 (5)	1.237 (3)	1.233 (3)	1.266 (3)[a]
C(3)-O(3)	1.255 (9)	1.224 (5)	1.237 (3)	1.304 (3)	1.230 (3)[a]
C(2)-C(am)	1.442 (10)	1.437 (6)	1.466 (4)	1.473 (3)	1.448 (3)
C(am)-N(am)	1.299 (12)	1.310 (6)	1.341 (4)	1.324 (4)	1.312 (5)
C(am)-O(am)	1.295 (10)	1.310 (6)	1.262 (3)	1.274 (3)	1.310 (4)
C(4)-N(4)	1.523 (9)	1.505 (5)	1.497 (4)	1.471 (3)	1.513 (3)
N(4)-C(Me_1)	1.493 (12)	1.480 (6)	1.493 (4)	1.468 (4)	1.494 (4)
N(4)-C(Me_2)	1.489 (11)	1.488 (6)	1.492 (4)	1.462 (4)	1.495 (4)
C(12a)-O(12a)	1.423 (7)	1.414 (4)	1.430 (4)	1.424 (3)	1.424 (3)
		Ring B			
C(4a)-C(5)	1.538 (10)	1.537 (5)	1.532 (4)	1.542 (3)	1.545 (3)
C(5)-C(5a)	1.498 (10)	1.518 (5)	1.527 (4)	1.563 (3)	1.542 (3)
C(5a)-C(11a)	1.454 (10)	1.502 (5)	1.519 (4)	1.516 (3)	1.514 (3)
C(11a)-C(12)	1.440 (9)	1.353 (5)	1.370 (4)	1.365 (3)	1.368 (3)
C(12)-C(12a)	1.548 (9)	1.516 (5)	1.522 (4)	1.520 (3)	1.512 (3)
C(12)-O(12)	1.224 (9)	1.330 (4)	1.333 (3)	1.337 (3)	1.332 (3)
		Ring C			
C(5a)-C(6)	1.370 (9)	1.513 (5)	1.548 (4)	1.533 (3)	1.546 (3)
C(6)-C(6a)	1.447 (10)	1.516 (5)	1.533 (4)	1.538 (3)	1.524 (3)
C(10a)-C(11)	1.443 (9)	1.468 (5)	1.475 (4)	1.453 (4)	1.462 (3)
C(11)-C(11a)	1.393 (9)	1.452 (5)	1.439 (4)	1.451 (4)	1.448 (3)
C(11)-O(11)	1.313 (9)	1.256 (5)	1.269 (3)	1.270 (3)	1.275 (3)
C(6)-O(6)		1.437 (5)	1.449 (3)	1.444 (3)	
C(6)-C(6M)	1.517 (11)		1.520 (4)	1.530 (4)	1.528 (4)
		Ring D			
C(6a)-C(7)	1.413 (10)	1.371 (5)	1.387 (4)	1.383 (4)	1.390 (4)
C(7)-C(8)	1.366 (12)	1.393 (6)	1.405 (4)	1.400 (4)	1.402 (4)
C(8)-C(9)	1.379 (13)	1.356 (7)	1.381 (4)	1.381 (4)	1.382 (4)
C(9)-C(10)	1.374 (10)	1.380 (6)	1.388 (4)	1.397 (4)	1.392 (4)
C(10)-C(10a)	1.439 (10)	1.405 (5)	1.411 (4)	1.416 (4)	1.412 (4)
C(10a)-C(6a)	1.404 (10)	1.411 (5)	1.413 (4)	1.421 (4)	1.414 (3)
C(10)-O(10)	1.336 (10)	1.350 (5)	1.359 (4)	1.355 (4)	1.356 (4)
C(7)-Cl(2)		1.761 (4)			

[a] There are two tautomeric forms in the crystal which differ in the orientation of the amide group. We have used the average values of the distances in the two tautomers except for the C(1)-C(2), C(2)-C(3), C(1)-O(1), and C(3)-O(3) bonds which appear to differ in the two forms.

group and the C(1)-O(1) and C(3)-O(3) bonds. As has been observed by us[21] and others,[11,14] the tetracycline free bases *normally* exist as zwitterions. In the formation of salts the oxygen atom of the amide group is protonated rather than either O(1) or O(3). The protonation of the oxygen atom of the amide group results in the C(am)-O(am) and C(am)-N(am) bond distances becoming approximately equal.[24] In TC± or the *high-temperature*[25] anhydrous forms 5-HTC and 5,12a-DA-5-HTC, where there is no proton on O(am), the C(am)-O(am) and C(am)-N(am) bond lengths are significantly different. The situation in ANTC·H^+ is not clear-cut since peaks close to both O(3) and O(am) were found in the difference Fourier. The dimensions of the hydrogen bonds involving O(am) (see Table IX) are very close to those expected for a symmetrical hydrogen bond so that in the solid state both possibilities (protonation of O(am) or O(1) or O(3)) may exist. Disorder in the amide group of ANTC·H^+ seems unlikely since the isotropic refinement was consistent with an ordered model. Furthermore, the C(3)-O(3) bond in ANTC·H^+ is longer than the C(1)-O(1) bond which is consistent with the proton being either on O(3) or in the immediate vicinity, i.e., on O(am) hydrogen-bonded to O(3). In both 6-DM-7-CLTC·H^+ and 6-DH-5-HTC the C-O bond nearest O(am) is longer than the C-O bond nearest N(am) side, in agreement with a strong intramolecular hydrogen bond between O(am) and the corresponding ring oxygen. In 6-DM-7-CLTC·H^+, although the proton was assigned to O(am), a small peak near O(1) was also observed in the difference Fourier synthesis. The refined thermal parameter for H(8) which is bonded to O(am) was somewhat larger than those of the other ring protons which again suggests some disordering of the proton between the two possible sites.

The crystal packing in both ANTC·HBr and 6-DM-7-CLTC·HCl involves extensive hydrogen bonding between the cations, anions, and lattice water. A packing diagram for 6-DM-7-CLTC·HCl·$3H_2O$ is given in Figure 4. The dimensions of the various intra- and intermolecular hydrogen bonds are summarized in Table IX.

The question of the preferred conformation for biologically active tetracyclines has been a matter of much speculation and

Table VIII. Bond Angles (Degrees)[a] in Anhydrotetracycline Hydrobromide (ANTC·HBr) and 6-Demethyl-7-chlorotetracycline Hydrochloride (6-DM-7-CLTC·HCl)

Angle	ANTC · HBr	6-DM-7-CLTC · HCl
O(1)-C(1)-C(2)	125.1	123.6
O(1)-C(1)-C(12a)	118.5	120.1
C(2)-C(1)-C(12a)	116.4	116.2
C(1)-C(2)-C(3)	122.0	121.0
C(1)-C(2)-C(am)	120.8	117.4
C(3)-C(2)-C(am)	117.1	121.6
C(2)-C(3)-C(4)	121.6	117.5
C(2)-C(3)-O(3)	123.1	125.2
C(4)-C(3)-O(3)	115.3	116.9
C(3)-C(4)-C(4a)	111.4	117.1
C(3)-C(4)-N(4)	110.7	108.6
C(4a)-C(4)-N(4)	111.0	115.5
C(4)-C(4a)-C(5)	110.3	110.3
C(4)-C(4a)-C(12a)	109.9	111.9
C(5)-C(4a)-C(12a)	109.8	109.5
C(4a)-C(5)-C(5a)	114.0	111.4
C(5)-C(5a)-C(6)	121.0	113.4
C(5)-C(5a)-C(11a)	119.9	112.9
C(6)-C(5a)-C(11a)	119.1	110.5
C(5a)-C(6)-C(6a)	120.5	110.5
C(5a)-C(6)-O(6)		112.7
C(5a)-C(6)-C(6M)	120.6	
C(6a)-C(6)-O(6)		103.9
C(6a)-C(6)-C(6M)	118.9	
C(6)-C(6a)-C(10a)	120.7	118.3
C(7)-C(6a)-C(10a)	119.3	118.8
C(6)-C(6a)-C(7)	120.1	122.6
C(6a)-C(7)-C(8)	119.4	121.4
C(6a)-C(7)-Cl(2)		121.0
C(8)-C(7)-Cl(2)		117.6
C(7)-C(8)-C(9)	122.5	119.9
C(8)-C(9)-C(10)	120.0	120.6
C(9)-C(10)-O(10)	120.3	117.5
C(9)-C(10)-C(10a)	119.4	120.2
O(10)-C(10)-C(10a)	120.3	122.3
C(10)-C(10a)-C(6a)	119.3	119.0
C(10)-C(10a)-C(11)	121.8	121.2
C(6a)-C(10a)-C(11)	118.9	119.8
C(10a)-C(11)-O(11)	119.2	119.8
C(10a)-C(11)-C(11a)	119.8	118.9
O(11)-C(11)-C(11a)	121.0	121.2
C(11)-C(11a)-C(5a)	121.0	118.3
C(11)-C(11a)-C(12)	118.3	119.4
C(5a)-C(11a)-C(12)	120.7	122.3
C(11a)-C(12)-O(12)	124.0	124.6
C(11a)-C(12)-C(12a)	118.3	123.3
O(12)-C(12)-C(12a)	117.6	112.0
C(12)-C(12a)-C(4a)	109.8	110.8
C(12)-C(12a)-C(1)	109.7	108.7
C(12)-C(12a)-O(12a)	110.2	112.1
C(4a)-C(12a)-O(12a)	112.3	108.6
C(1)-C(12a)-O(12a)	102.5	106.4
C(2)-C(am)-O(am)	119.8	119.4
C(2)-C(am)-N(am)	121.5	121.9
O(am)-C(am)-N(am)	118.7	118.7
C(4)-N(4)-C(Me_1)	112.7	112.2
C(4)-N(4)-C(Me_2)	112.7	115.6
C(Me_1)-N(4)-C(Me_2)	110.3	110.3

[a] The estimated standard deviations are 0.6° for ANTC·HBr and 0.3° for DMTC·HCl.

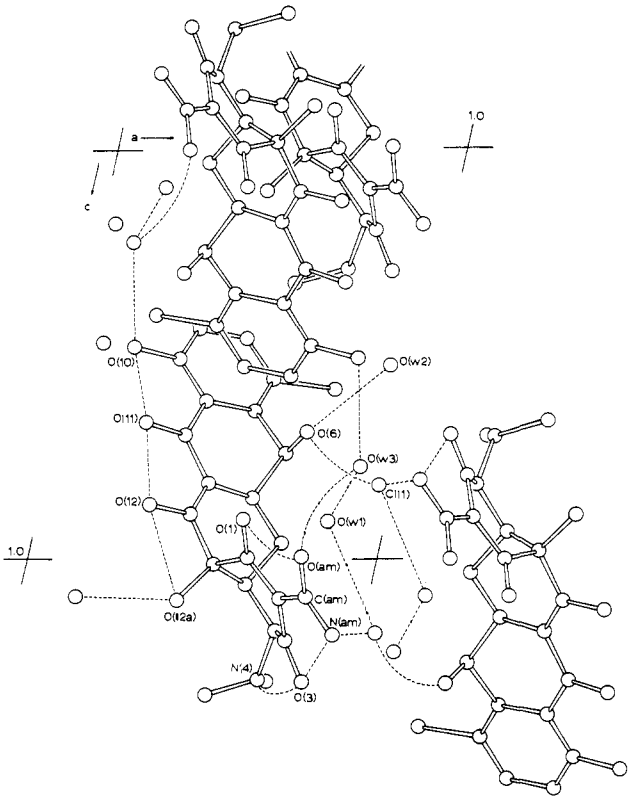

Figure 4. A crystal packing diagram for 6-demethyl-7-chlorotetracycline hydrochloride trihydrate. The broken lines indicate hydrogen bonds and the lines are curved in several places to avoid ambiguities.

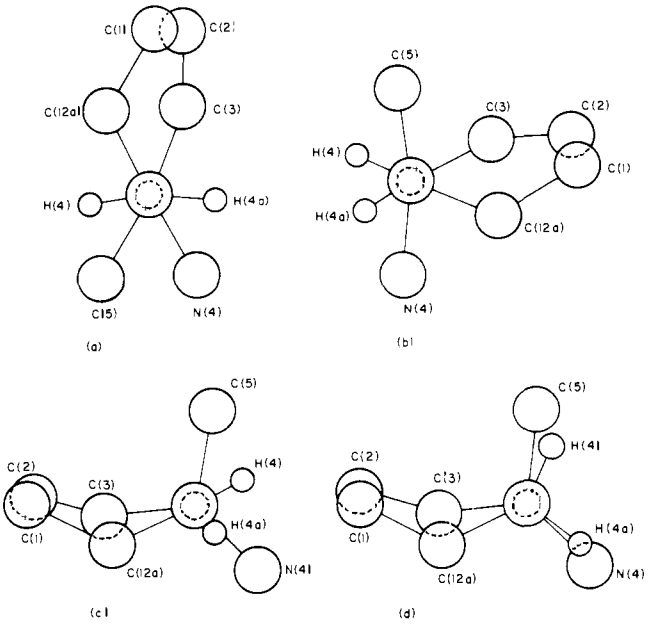

Figure 5. Projections down the C(4a)-C(4) bond in four different tetracyclines: (a) 5,12a-diacetyloxytetracycline; (b) anhydrotetracycline hydrobromide; (c) oxytetracycline hydrochloride; (d) 6-demethyl-7-chlorotetracycline hydrochloride.

discussion[22] and several points can be made at this time. The tetracyclines appear to exist in essentially two different conformations as represented by 6-DM-7-CLTC·H^+, $TC^{\pm}$, and the other protonated species vs. the anhydrous forms shown by 5,12a-DA-5-HTC and 5-HTC. However, the relevance of the anhydrous form to the biological activity of these drugs is obscure. In the preparation of both 5-HTC and 5,12a-DA-5-HTC, the hydrated materials are heated at elevated temperatures (60–100 °C) for long periods.[25] Although the anhydrous materials should be more lipid soluble, the question

Table IX. Probable Hydrogen Bonds

D-H · · · A[a]	Position of A	D-H, Å	H · · · A, Å	D · · · A, Å	D-H · · · A, deg
		In Anhydrotetracycline Hydrobromide Monohydrate			
N(am)-H(1) · · · Br	$-x-\frac{1}{2}, 1+y, \frac{1}{2}+z$	0.79	2.72	3.371 (8)	141
N(am)-H(2) · · · O(1)	x, y, z	0.81	1.98	2.673 (8)	143
N(4)-H(3) · · · O(12a)	x, y, z	0.80	2.15	2.775 (8)	136
N(4)-H(3) · · · O(w)	$-x-1, y-\frac{1}{2}, -z-\frac{1}{2}$	0.80	2.41	3.039 (8)	137
O(10)-H(4) · · · O(11)	x, y, z	0.66	2.20	2.557 (7)	116
O(12a)-H(5) · · · Br	$x-\frac{1}{2}, -\frac{1}{2}-y, -z$	0.74	2.48	3.152 (4)	153
O(11)-H(6) · · · O(12)	x, y, z	0.92	1.70	2.506 (7)	145
O(3)-H(7) · · · O(am)	x, y, z	1.00	1.68	2.435 (8)	130
O(w)-H(24) · · · Br	x, y, z	0.86	2.43	3.286 (7)	173
O(w)-H(25) · · · Br	$-x-\frac{1}{2}, -y, z-\frac{1}{2}$	0.89	2.50	3.343 (6)	158
		In 6-Demethyl-7-chlorotetracycline Hydrochloride			
N(am)-H(1) · · · O(3)	x, y, z	0.89 (4)	2.02 (4)	2.711 (5)	134 (3)
N(am)-H(2) · · · Cl(1)	$x-1, y-1, z-1$	0.89 (6)	2.53 (6)	3.407 (4)	171 (5)
N(4)-H(3) · · · O(3)	x, y, z	0.84 (4)	2.23 (5)	2.689 (5)	114 (4)
O(10)-H(4) · · · O(11)	x, y, z	0.97 (6)	1.88 (5)	2.595 (4)	129 (5)
O(12a)-H(5) · · · $O(w_1)$	$x, y, z-1$	0.79 (4)	2.00 (4)	2.731 (4)	152 (4)
O(12a)-H(5) · · · O(12)	x, y, z	0.79 (4)	2.25 (4)	2.661 (4)	113 (4)
O(12)-H(6) · · · O(11)	x, y, z	0.88 (4)	1.79 (5)	2.544 (4)	143 (4)
O(6)-H(7) · · · Cl(1)	$-x-1, y-\frac{1}{2}, -z-1$	0.82 (4)	2.24 (4)	3.041 (3)	165 (4)
O(am)-H(8) · · · O(1)	x, y, z	1.36 (9)	1.20 (9)	2.461 (5)	148 (5)
$O(w_1)$-H(23) · · · Cl(1)	$-x, y-\frac{1}{2}, -z$	0.84 (5)	2.38 (5)	3.215 (3)	176 (5)
$O(w_1)$-H(24) · · · $O(w_3)$	x, y, z	0.96 (5)	2.06 (5)	3.009 (5)	169 (4)
$O(w_2)$-H(25) · · · O(6)	x, y, z	0.84 (6)	2.04 (6)	2.875 (4)	172 (6)
$O(w_2)$-H(26) · · · O(3)	$x, y, z-1$	0.95 (6)	2.65 (5)	3.107 (5)	110 (4)
$O(w_3)$-H(28) · · · O(10)	x, y, z	0.92 (5)	2.03 (5)	2.933 (4)	168 (5)
$O(w_3)$-H(27) · · · O(am)	$-x+1, y-\frac{1}{2}, -z+1$	0.81 (6)	2.44 (6)	2.904 (5)	118 (6)

[a] Donor-hydrogen · · · acceptor. D-H at x, y, z.

should be posed as to whether these forms can be obtained at room temperature in a reasonable length of time. The relatively rapid conversion of the anhydrous forms to the "normal" conformation suggests that these high temperature modifications are probably of little biological significance.

If the conformation found in 6-DM-7-CLTC·H^+ and related tetracyclines is the conformation required for useful therapeutic activity, then why is the closely related ANTC·H^+ species inactive? This is not an easy question to answer since there are small changes in bond lengths as well as in the conformation. Furthermore, although the biological activity of the tetracyclines involves the 30*S* moiety of the ribosomal A site, the exact mechanism is not known.[26] However, since the tetracyclines inhibit the binding of aminoacyl-tRNA to the A site, the drugs are presumably bound to the ribosome in some way. Since the dimethylamino group is essential to activity, the binding probably involves an interaction with N(4). Therefore, we have focused our attention on N(4) and the relative orientation of this group with respect to the rest of the molecule. The differences in the orientation of N(4) can be appreciated by considering the views down the C(4a)-C(4) bond given in Figure 5. The views in 5a and 5b are for the 5,12a-DA-5-HTC and ANTC·H^+, respectively, while 5c and 5d illustrate the situation in 5-HTC·H^+ and 6-DM-7-CLTC·H^+, respectively. We see that the orientation of N(4) relative to the A ring and the rest of the molecule is quite different in ANTC·H^+ and 5,12a-DA-5-HTC compared with the other two drugs. The net result is a vastly different steric requirement for interactions involving N(4) in the two conformations. The fact that epimerization of the tetracycline which dramatically decreases the therapeutic value involves N(4) lends support to the above observations.

In conclusion we suggest that the conformation found in 6-DM-7-CLTC·H^+ is the preferred conformation for biological activity and that the anhydrous forms are only of academic interest. Furthermore, relatively small changes in conformation can yield startling change in the important N(4) site and subsequently alter activity.

Acknowledgment. This investigation was supported in part by NIH Research Grant AI 11825 from the National Institute of Allergy and Infectious Diseases. We thank the Center for Instructional and Research Computing Activities, University of Florida, for a grant of computer time and Lederle Laboratories for a gift of 6-demethyl-7-chlorotetracycline hydrochloride.

Supplementary Material Available: Structure factor amplitudes for 6-demethyl-7-chlorotetracycline hydrochloride trihydrate and anhydrotetracycline hydrobromide monohydrate and final parameters for all atoms (32 pages). Ordering information is given on any current masthead page.

References and Notes

(1) These studies were initiated in 1968 when all three authors were at the University of Waterloo but were completed by M.M. and G.J.P. at the University of Florida.
(2) Address correspondence to this author at the University of Florida.
(3) The abbreviations given for the tetracyclines in ref 4 and 5 are inconsistent and somewhat confusing. The abbreviations used in this paper are given in Figure 1 and are an attempt to be systematic and consistent in the abbreviations. The conversion is as follows: 7-CLTC is CTC[4] or CITC;[5] 5-HTC is OTC[4] or OXY;[5] 6-DM-7-CLTC is DMCT;[4] 6-DH-5-HTC is DOOTC[4] or DOXY;[5] 6-ME-5-HTC is MOTC;[4] 7-DMATC is MITC[4]; and 5,12a-DA-5-HTC is DAOXY.[5]
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(22) Discussions of the conformations of tetracyclines can be found in virtually all papers on tetracyclines. The discussion in ref 13 is one of the latest and gives the pertinent references to other work.
(23) In neither ref 11 or 15 is there any mention of the biological activity of the solid used in the x-ray studies. This omission is not completely unreasonable since both forms revert to the hydrated species when dissolved in water which makes an assessment of biological activity impossible.
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Structural Studies of Tetracyclines. Crystal and Molecular Structure of Tetracycline–Urea Tetrahydrate

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Abstract: The first structural study of a tetracycline adduct has been carried out by x-ray diffraction techniques. The crystals of the tetracycline-urea tetrahydrate complex are orthohombic. The space group is $P2_12_12_1$ and the unit cell dimensions are $a = 12.228$ (3), $b = 12.884$ (3), and $c = 16.663$ (4) Å. There are four molecules of tetracycline-urea tetrahydrate per unit cell. The structure was solved by direct methods and refined by least-squares techniques to a final unweighted residual of 0.043 for the 2395 reflections used in the analysis. The hydrogen atoms were all located in a difference Fourier synthesis and refined with isotropic thermal parameters. The urea molecule is hydrogen bonded to O(1) and O amide of the A ring. The tetracycline molecule exists as a zwitterion in the adduct. The conformation and zwitterionic character of the tetracycline moiety are virtually identical with that found in both the free bases and protonated species of therapeutically active tetracyclines. Interaction with the zwitterionic species and the proper conformation both appear to be important requirements for biological activity in tetracyclines.

Introduction

The tetracyclines are an important class of widely used antibiotics.[1,2] Their mode of action involves the inhibition of protein synthesis by interference with the binding of aminoacyl-tRNA to the ribosome,[3] but the precise molecular mechanism remains obscure. The binding of the tetracycline molecule to an appropriate site on the ribosome could prevent the attachment of the aminoacyl-tRNA. Consequently, the manner in which the tetracycline molecule interacts with other molecules is important for the development of a model for the binding to ribosomes.

The solubilities of tetracyclines can be increased by various anions and neutral molecules, indicating complex formation.[4,5] However, in the case of urea an insoluble adduct is formed with tetracycline but under the same conditions, not with either 7-chlorotetracycline or 5-hydroxytetracycline.[6] To elucidate this puzzling difference in these three closely related drugs, we initiated a crystal structure study of the tetracycline-urea adduct.

Experimental Section

Light yellow octahedral crystals were formed from a urea-tetracycline solution. Preliminary Weissenberg and precession photographs indicated that the crystals were orthorhombic with the space group $P2_12_12_1$. An approximately equidimensional crystal, 0.15 mm on edge, was used for the measurement of the cell constants and intensity data. The cell dimensions obtained from a least-squares fit of 15 2θ values for Cu K_β peak ($\lambda = 1.39217$ Å) were $a = 12.228$ (3), $b = 12.884$ (3), and $c = 16.663$ (4) Å. The cell volume is 2625.2 Å^3. The density calculated for four molecules of tetracycline-urea tetrahydrate, $C_{23}H_{36}N_4O_{13}$, fw 576.56, is 1.459 g cm^{-3}, in good agreement with the value of 1.45 g cm^{-3} determined by flotation. The adduct had previously been reported to be a trihydrate.[6]

The intensity data were measured using previously described techniques.[7] All the reflections in one octant of reciprocal space to a limit of $2\theta \leq 135°$ were measured first and then one half of the hemisphere was measured. The intensities were corrected for a small (maximum 3%) variation in the four standard reflections and then equivalent reflections were averaged. Of the 2682 reflections in the octant, 2395 had an intensity >1.2 times the appropriate background and were considered reliable and used in the analysis. The value of μ for Cu Kα radiation is only 10.4 cm^{-1} and no corrections for absorption were necessary.

Structure Determination and Refinement. The structure was eventually solved by direct methods when we obtained a copy of MULTAN.[8] The correct E map indicated the positions of the tetracycline molecule and many of the other atoms. However, only the tetracycline group was used in calculating a Fourier synthesis which

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