Chemical–Structural Properties of Tetracycline Derivatives. 9. 7-Chlorotetracycline Derivatives with Modified Stereochemistry

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Abstract: High-resolution crystal structure analyses have been carried out for two derivatives of 7-chlorotetracycline: 5a-epi-7-chlorotetracycline hydrochloride, $C_{22}H_{23}ClN_2O_8 \cdot HCl$, and 5a,11a-dehydro-7-chlorotetracycline, $C_{22}H_{21}ClN_2O_8$. The former crystallized with space-group symmetry $P2_1$, $Z = 2$, and $a = 8.608$ (1) Å, $b = 11.849$ (2) Å, $c = 11.125$ (2) Å, and $\beta = 108.16$ (1)°; crystals of the latter derivative displayed space-group symmetry $P2_12_12_1$ with $Z = 4$ and $a = 16.942$ (2), $b = 11.618$ (1), and $c = 10.606$ (1) Å. Lattice parameters for both derivatives are for crystals at ~120 K. The conventional R value is 0.047 for each structure determination. The circular dichroism spectra for the dehydro-7-chlorotetracycline free base were found to be solvent dependent in a manner indicative of an equilibrium between several molecular species.

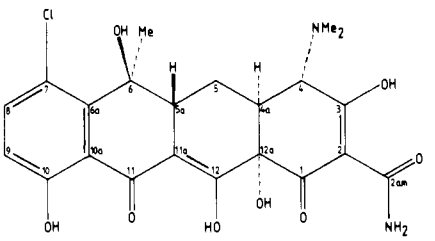
Previous reports from this laboratory have presented structural data for naturally occurring,[1-3] chemically modified,[4,5] or totally synthetic[6,7] tetracyclines. The data were obtained from crystals grown under various conditions and were most relevant to the properties of the A ring under the influence of either solvent character[1,3] or chemical modification.[6] Analysis of the observed molecular structures led to the proposal that two free-base species, a zwitterion and a nonionized form, were of biological importance. A solvent-dependent equilibrium between these molecular species has been demonstrated recently for oxytetracycline free base.[8] To a lesser extent we have also probed the stereochemical properties of the tetracyclines by confirming the absolute configuration[4] that had been derived earlier by chemical means[9] and by investigating the properties of 4-epioxytetracycline free base.[5]

In this and the accompanying paper, we extend our investigation to tetracyclines with dramatically altered structure and/or stereochemistry in the BCD ring system. This report describes crystal structures for 5a-epi-7-chlorotetracycline hydrochloride, 5a-epi-7-ClTC·HCl, a tetracycline belonging to a class that displays significant in vitro antibacterial activity[10] but disappointing in vivo activity, and for 5a,11a-dehydro-7-chlorotetracycline free base, 5a,11a-DH-7-ClTC. The latter tetracycline displays essentially no antibacterial activity[11] but is of interest because of uncertainties in its chemical structure.[12] The possibility that the tautomeric form of the BCD chromophore of 5a,11a-DH-7-ClTC might be solvent dependent caused us to measure the circular dichroism spectra of this derivative under different solvent conditions. The molecular structures revealed by the crystal structure determination and the solvent-dependent nature of the circular dichroism spectra of the dehydrotetracycline provide further insight into the developing ellucidation of the interrelationships among chemical structure, configuration, conformation, and biological activity of the tetracyclines.

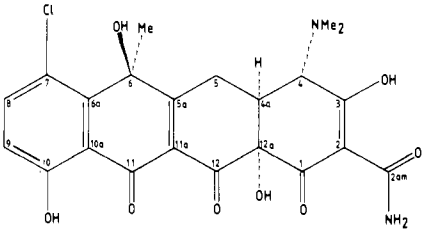
Table I. Data for the Characterization of the Crystals of 5a-epi-7-ClTC·HCl and 5a,11a-DH-7-ClTC and Their Respective Data Sets

	5a-epi-7-ClTC·HCl	5a,11a-DH-7-ClTC
space group	$P2_1$	$P2_12_12_1$
lattice parameters		
a, Å	8.608 (1)	16.942 (2)
b, Å	11.849 (2)	11.618 (1)
c, Å	11.125 (2)	10.606 (1)
β, deg	108.16 (1)	
no. of reflections in lattice parameter refinement[13]	67	42
$2\theta_{min}$, deg	30	40
$2\theta_{max}$, deg	43	48
	Intensity Data	
$2\theta_{max}$, deg	80	85
reflections measd	6894	8136
reflections obsd[a]	4017	5332
formula of the asymmetric unit	$C_{22}H_{23}ClN_2O_8 \cdot HCl$	$C_{22}H_{21}ClN_2O_8$

[a] Number of reflections classified as observed.



5a-epi-7-ClTC



5a,11a-DH-7-ClTC

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Table II. Characterization of the Refinement of the Crystallographic Model

	5a-epi-7-ClTC·HCl	5a,11a-DH-7-ClTC
no. of contributing reflections	5649	7036
no. of variables	410	382
R	0.047	0.047
R_w	0.052	0.059
weighting scheme[a]		
a	0.0	0.2
b	1.0	1.0
c	1.25×10^{-2}	1.0×10^{-2}
d	1.00×10^{-3}	1.6×10^{-3}
e	1.0×10^{-4}	4.0×10^{-7}
p	3	4
f_1	0.88	1.7
f_2	1.0	2.0
σ[b]	0.990	0.940

[a] $1/w = (a + b\sigma^2(F) + c|F| + d|F|^2 + e|F|p)(1/f)$, where $f = f_1$ = scale factor for weighting observed data and $f = f_2$ = scale factor for weighting less-thans. [b] Standard deviation of an observation of unit weight.

Experimental Section

Crystals of 5a-epi-7-ClTC·HCl were obtained from the research group of the late Professor H. Muxfeldt, as was a sample of 5a,11a-DH-7-ClTC free base. The latter was subsequently crystallized from benzene by slow evaporation at room temperature. All quantitative crystallographic measurements were made with a Syntex PĪ autodiffractometer (monochromatized Mo Kα radiation, λ = 0.71069 Å) equipped with a low-temperature device (Syntex LT-1) which maintained the crystals at ca. 120 K. Crystallographic data, including space group, lattice parameters, data set resolution, number of unique data collected, number of data classified as observed under the criterion $I \geq 3\sigma(I)$, and the content of the asymmetric unit, are displayed in Table I.

Intensity data were collected in an ω-scan mode for which the scan range was 0.75°; the scan speed varied as a function of maximum peak intensity from 2.0 to 24.0° min^{-1}. Background was counted on each side of the reflection center ($\Delta\omega$ = 1.0°) for half the total scan time. Three reference reflections were measured periodically to test for instrument and crystal stability. Data were corrected for minor deviations therein and for Lorentz and polarization effects.

Circular dichroism (CD) spectra were measured with a Jasco J-500 spectropolarimeter for freshly prepared solutions of recrystallized 5a,11a-DH-7-ClTC free base. Samples in $CHCl_3$, THF–H_2O, and EtOH–H_2O were prepared with commercial, analytical reagent grade solvents used without further purification; unbuffered distilled–demineralized water was used for the aqueous component. Base-line determinations were made for each solvent system and automatically subtracted from solution spectra with a Jasco DP-500 data processor.

Crystal Structure Determination and Refinement

The initial structural models for 5a-epi-7-ClTC·HCl and 5a,11a-DH-7-ClTC were determined by direct methods and developed by difference Fourier and least-squares refinement. Indications of minor disorder in the orientation of the amide group of 5a-epi-7-ClTC·HCl were found in difference Fourier maps in the later stages of refinement (R = 5.1%). The population of molecules with the oxygen atom of the amide group on the O(3) side of the tricarbonylmethane system was estimated to be ca. 85%. The credibility of the less populous orientation was tested by least-squares refinement of its O, N, and C atoms (labeled 2″ in parameter lists) with isotropic temperature factors.

All crystallographically appropriate atomic coordinates, anisotropic temperature factors for Cl, O, N, and C atoms (other than those indicated above) and isotropic temperature factors for H atoms were refined[13] by block-diagonal least-squares techniques; a detailed block structure is available as supplementary material.[14] The weighting schemes employed and the results of the refinement are summarized in Table II. Fractional atomic coordinates for the Cl, O, N, and C atoms of 5a-epi-7-ClTC·HCl and 5a,11a-DH-7-ClTC free base are presented in Table III; their temperature factors, coordinates, and temperature factors for H atoms and calculated and observed structure factors are available as supplementary material.

Discussion

The crystal structures reported here present tetracycline derivatives in conformations that display intriguing similarities and differences with those observed for medicinally important analogues. To facilitate analysis, we present stereoscopic projections[15] of the cationic entity, 5a-epi-7-ClTC(+), the nonionized free base, 5a,11a-DH-7-ClTC(0), the zwitterionic tetracycline free base, TC(±), and the nonionized oxytetracycline free base, OTC(0), in analogous orientations in Figure 1. The latter two examples are typical of the two conformations associated with the medicinally important tetracycline free bases.[1,3,8] The conformation of TC(±) is that of the free base in aqueous-rich solvents and of the cation of medicinally important tetracyclines in aqueous solution[16] and in nonaqueous solvents such as Me_2SO,[3,17] whereas that of OTC(0) is the conformation of the free base in nonaqueous solvents.

It is obvious from Figure 1 that the conformation of 5a-epi-7-ClTC(+) differs markedly from that of TC(±) but less so from that of OTC(0). A more quantitative appraisal of the differences and similarities in these conformations can be obtained from a comparison of the dihedral angles in Table IV. It is clear from the table that the dihedral angles associated with the A ring and the A,B-ring juncture of 5a-epi-7-ClTC(+) conform with those of OTC(0). This fact may correlate with the observation that 5a-epi-7-ClTC displays considerable in vitro antibacterial activity but little in vivo activity.[10] We interpret the observed conformation of 5a-epi-7-ClTC(+) as evidence that the equilibrium between the zwitterionic and nonionized forms of the free base of this derivative differs from that of the medicinally important tetracyclines. The circular dichroism curve for 5a-epi-7-ClTC in aqueous solution at low pH[18] is sufficiently similar to that of OTC free base in solutions with high ethanol content[8] to indicate that the conformation observed here is that in solution as well. Further support is given by NMR spectroscopy[20] of the hydrochloride in Me_2SO-d_6 and D_2O solutions which indicates that H(4) and H(4a) are trans diaxial (Me_2SO-d_6, $J_{4,4a}$ = 11.3 Hz; D_2O, $J_{4,4a}$ = 10 Hz; in both spectra the $N(CH_3)_2$ protons form one singlet). We suspect that the zwitterion of 5a-epi-7-ClTC free base, if formed, also displays this conformation instead of that adopted by the in vivo active tetracyclines. Evidence from the 6-thiatetracyclines[19] indicates that the lack of in vivo activity of the 5a-epi-TC's is not simply a result of increased lipophilicity.

The observed conformation of 5a-epi-7-ClTC(+) is consistent with the conformational properties that we have described for other tetracyclines. In the first paper of this series[1] the differences in lipophilicities of α- and β-6-deoxyoxytetracycline were attributed to differences in internal energy of the respective nonionized free-base molecules resulting from steric interactions between the 6-β substituent and the H atom at position 4. The implications of the conformation of 5a-epi-7-ClTC(+) with respect to this interpretation merit examination, particularly since the bonding geometries and conformations of the BCD chromophores of 5a-epi-7-ClTC(+), TC(±), and OTC(0) are very similar. In contrast thereto, the curvature of the "backbone" of the B,C ring system, i.e., the conformation of the sequence of atoms (C(6), C(5a), C(5), and C(4a), is reversed in 5a-epi-7-ClTC(+) compared with that in TC(±) and OTC(0). The result of this conformational difference is that the 6-β substituent in 5a-epi-7-ClTC(+) is nearly equatorial with respect to the C ring, whereas in TC(±) and

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Table III. Fractional Atomic Coordinates for C, N, O, and Cl Atoms with Estimated Deviations

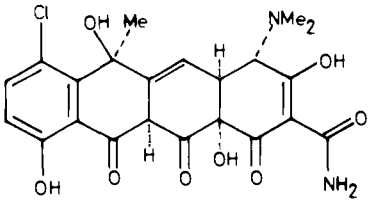
atom	x	y	z	PP[a]
	5a-epi-7-ClTC(+)			
Cl(1)	0.83128 (9)	0.03150	0.58533 (7)	
C(1)	0.6034 (3)	0.5652 (2)	0.3810 (2)	
O(1)	0.5162 (3)	0.6426 (2)	0.3231 (2)	
C(2)	0.7433 (3)	0.5828 (2)	0.4907 (2)	
C(2am)	0.8053 (4)	0.6942 (3)	0.5350 (3)	0.85
N(2am)	0.7384 (4)	0.7848 (2)	0.4692 (3)	0.85
O(2am)	0.9280 (3)	0.7075 (2)	0.6372 (3)	0.85
C(2″)	0.762 (2)	0.715 (1)	0.504 (1)	0.15
O(2″)	0.673 (2)	0.783 (1)	0.419 (1)	0.15
N(2″)	0.879 (2)	0.747 (1)	0.611 (1)	0.15
O(3)	0.9445 (3)	0.5088 (2)	0.6679 (2)	
C(3)	0.8281 (3)	0.4931 (2)	0.5677 (2)	
C(4)	0.7802 (3)	0.3712 (2)	0.5286 (2)	
N(4)	0.8194 (3)	0.2942 (2)	0.6434 (2)	
C(42m)	0.9976 (4)	0.2683 (3)	0.7005 (3)	
C(41m)	0.7472 (3)	0.3330 (3)	0.7432 (2)	
C(4a)	0.6000 (3)	0.3597 (2)	0.4472 (2)	
C(5)	0.5530 (3)	0.2411 (2)	0.3922 (2)	
C(5a)	0.6532 (3)	0.2043 (2)	0.3072 (2)	
C(6)	0.5799 (3)	0.0993 (2)	0.2264 (2)	
O(6)	0.5727 (3)	0.0108 (2)	0.3122 (2)	
C(6m)	0.4048 (3)	0.1165 (3)	0.1382 (3)	
C(6a)	0.6892 (3)	0.0665 (2)	0.1449 (2)	
C(7)	0.6928 (3)	−0.0420 (2)	0.0980 (2)	
Cl(7)	0.57279 (9)	−0.15401 (6)	0.12141 (7)	
C(8)	0.7931 (4)	−0.0693 (2)	0.0240 (2)	
C(9)	0.8912 (3)	0.0110 (3)	−0.0051 (2)	
C(10)	0.8864 (3)	0.1212 (2)	0.0351 (2)	
O(10)	0.9810 (3)	0.1977 (2)	0.0007 (2)	
C(10a)	0.7841 (3)	0.1500 (2)	0.1090 (2)	
C(11)	0.7715 (3)	0.2698 (2)	0.1392 (2)	
O(11)	0.8300 (2)	0.3462 (2)	0.0861 (2)	
C(11a)	0.6867 (3)	0.2990 (2)	0.2290 (2)	
C(12)	0.6531 (3)	0.4097 (2)	0.2455 (2)	
O(12)	0.6944 (3)	0.4956 (2)	0.1843 (2)	
C(12a)	0.5600 (3)	0.4433 (2)	0.3351 (2)	
O(12a)	0.3929 (2)	0.4351 (2)	0.2622 (2)	
	5a,11a-DH-7-ClTC(0)			
C(1)	0.92782 (8)	0.4321 (1)	0.7909 (2)	
O(1)	0.97265 (7)	0.4868 (1)	0.8609 (1)	
C(2)	0.95155 (8)	0.3450 (1)	0.7028 (2)	
C(2am)	1.03413 (9)	0.3145 (1)	0.6902 (2)	
N(2am)	1.08890 (9)	0.3669 (1)	0.7575 (2)	
C(3)	0.89534 (8)	0.2821 (1)	0.6314 (2)	
O(2am)	1.05508 (7)	0.2341 (1)	0.6131 (1)	
O(3)	0.91706 (7)	0.2045 (1)	0.5530 (1)	
C(4)	0.80654 (8)	0.3046 (1)	0.6443 (2)	
N(4)	0.75662 (8)	0.2054 (1)	0.6175 (1)	
C(41m)	0.7783 (1)	0.0987 (2)	0.6818 (2)	
C(42m)	0.7406 (1)	0.1863 (2)	0.4836 (2)	
C(4a)	0.78694 (8)	0.3562 (1)	0.7757 (1)	
C(5)	0.70008 (9)	0.3899 (1)	0.7878 (2)	
C(5a)	0.67825 (8)	0.5052 (1)	0.7326 (1)	
C(6)	0.59015 (9)	0.5293 (1)	0.7226 (2)	
C(6m)	0.5598 (1)	0.5549 (2)	0.8574 (2)	
O(6)	0.55930 (9)	0.4249 (1)	0.6709 (2)	
C(6a)	0.56915 (8)	0.6331 (1)	0.6400 (1)	
C(7)	0.49267 (9)	0.6554 (1)	0.5998 (2)	
Cl(7)	0.41222 (2)	0.56720 (4)	0.63549 (6)	
C(8)	0.4750 (1)	0.7501 (2)	0.5232 (2)	
C(9)	0.5330 (1)	0.8279 (2)	0.4891 (2)	
C(10)	0.60928 (9)	0.8130 (1)	0.5348 (2)	
O(10)	0.66298 (8)	0.8940 (1)	0.5043 (1)	
C(10a)	0.62829 (8)	0.7148 (1)	0.6084 (1)	
C(11)	0.71032 (8)	0.6972 (1)	0.6478 (1)	
O(11)	0.75924 (7)	0.7761 (1)	0.6390 (1)	
C(11a)	0.73292 (8)	0.5841 (1)	0.6982 (1)	
C(12)	0.81847 (8)	0.5530 (1)	0.7019 (1)	
O(12)	0.86638 (6)	0.5935 (1)	0.6296 (1)	
C(12a)	0.83980 (8)	0.4612 (1)	0.8001 (1)	
O(12a)	0.82320 (7)	0.5078 (1)	0.9219 (1)	

[a] PP is the population parameter. Where not otherwise indicated, PP = 1.

OTC(0) it is more nearly axial. Thus in 5a-epi-7-ClTC(+) there is little steric interaction between the A and C rings, whereas in most tetracyclines with the natural configuration at C(5a) there is considerable potential for such interaction. This interaction most likely directly influences the equilibrium between the non-ionized and zwitterionic free bases of in vivo active tetracyclines.

The observed antibacterial activity of the parent 7-ClTC (in vivo and in vitro) and of 5a-epi-7-ClTC (in vitro) but lack of activity of the DH-7-ClTC implies that changes in the structure of the BCD chromophore resulting from dehydrogenation are responsible for deactivation. The chemical structure of DH-7-ClTC is complicated by potential tautomerism in the BCD chromophore (Chart I).

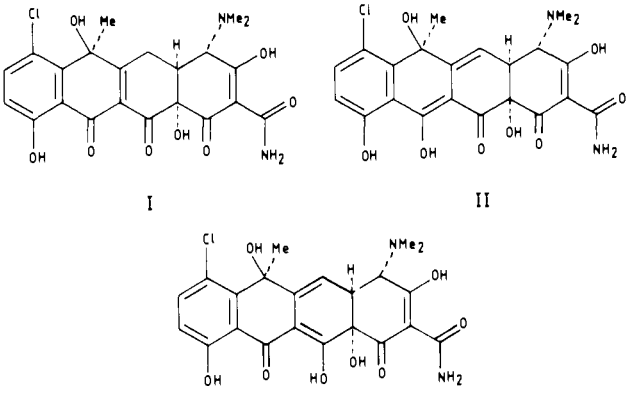
Schach von Wittenau et al.[12] have reported isolation of two tautomers of the free base, one from water and the other from chloroform. The latter displays a λ_{max} at 5.83 μm in its IR spectrum. A "ketonic tautomer" such as IV with the double bond between atoms C(5) and C(5a) and a tetrahedral carbon atom separating the ketone groups at positions C(11) and C(12) was suggested as an attractive alternative structure. NMR evidence



IV

for DH-7-ClTC in deuteriotetrahydrofuran was inconsistent with

Chart I



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this assignment, though the IR spectrum of the same solution displayed the ketonic absorption at 5.82 μm. The IR spectrum of DH-7-ClTC crystallized from benzene displays a strong absorption at 5.83 μm, which is evidence that the tautomer in question is that presented by this crystal structure determination. Both the bond distances and the positions of the H atoms demonstrate the presence of 5a,11a-DH-7-ClTC(0), tautomer I in Chart I. It is also noteworthy that the C(12)–O(12) bond distance in 5a,11a-DH-7-ClTC(0), 1.213 (2) Å, is the shortest of any carbonyl group in the molecule. This carbonyl group in the present tautomer is devoid of hydrogen bonds (which tend to lengthen the C–O distance and increase the λ_{max} of the IR absorption). The other tautomeric forms in Chart I are expected to have intra-

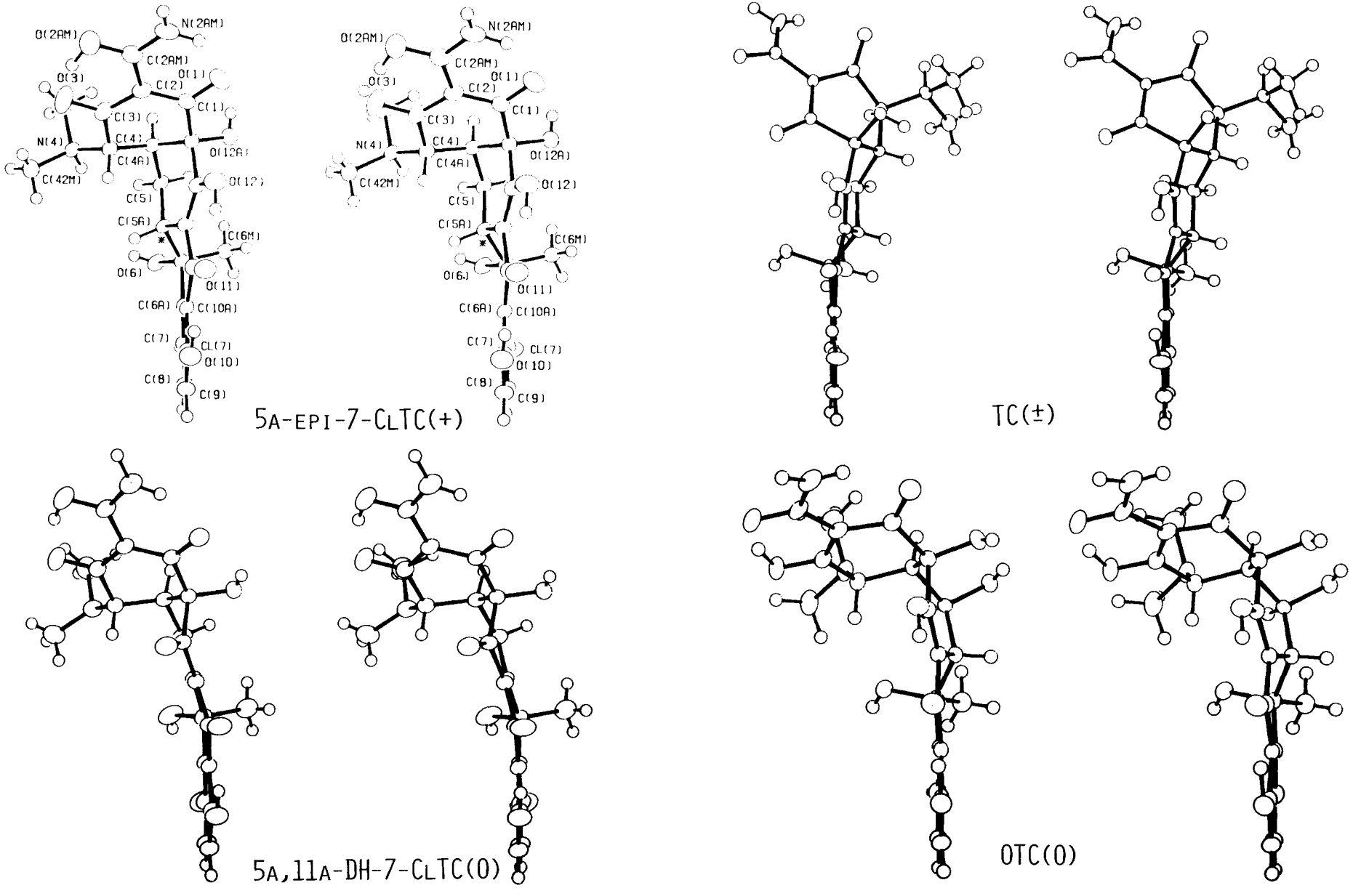

Figure 1. Stereoscopic projections[15] of cationic 5a-epi-7-chlorotetracycline, 5a-epi-7-ClTC(+), nonionized 5a,11a-dehydro-7-chlorotetracycline free base, 5a,11a-DH-7-ClTC(0), zwitterionic tetracycline free base,[1] TC(±), and nonionized oxytetracycline free base,[3] OTC(0). The atom-labeling scheme is presented in the projection of the cation; the carbon atom with reversed stereochemistry to the natural configuration is marked with an asterisk.

Table IV. Selected Dihedral Angles with Estimated Standard Deviations (Deg)

	TC(±)	5a-epi-7-ClTC(+)	5a,11a-DH-7-ClTC(0)	OTC(0)
C(12a)-C(1)-C(2)-C(3)	5.4 (3)	−9.0 (5)	−3.7 (2)	−16.9 (3)
C(1)-C(2)-C(3)-C(4)	34.1 (3)	5.2 (5)	0.4 (2)	−3.1 (4)
C(2)-C(3)-C(4)-C(4a)	−30.2 (3)	−27.0 (4)	−25.4 (2)	−4.3 (4)
C(3)-C(4)-C(4a)-C(12a)	−12.1 (3)	51.6 (3)	52.4 (1)	31.1 (3)
C(4)-C(4a)-C(12a)-C(1)	−49.4 (3)	−55.0 (3)	−56.9 (2)	−50.0 (3)
C(4a)-C(12a)-C(1)-C(2)	−48.4 (3)	34.1 (4)	32.7 (2)	43.2 (3)
C(4)-C(4a)-C(12a)-C(12)	169.9 (2)	66.8 (3)	64.1 (1)	70.1 (3)
C(5)-C(4a)-C(12a)-C(1)	−72.7 (3)	−179.9 (2)	179.3 (1)	−175.9 (2)
C(11a)-C(12)-C(12a)-C(4a)	−20.3 (3)	32.4 (3)	54.6 (2)	30.4 (3)
C(12)-C(12a)-C(4a)-C(5)	47.7 (3)	−58.2 (3)	−59.7 (2)	−55.6 (3)
C(12a)-C(4a)-C(5)-C(5a)	−63.8 (3)	62.8 (2)	40.3 (2)	46.4 (3)
C(4a)-C(5)-C(5a)-C(11a)	46.1 (3)	−37.5 (2)	−13.0 (2)	−9.8 (3)
C(5)-C(5a)-C(11a)-C(12)	−17.6 (3)	9.3 (3)	6.4 (2)	−18.1 (3)
C(5a)-C(11a)-C(12)-C(12a)	5.2 (4)	−7.4 (3)	−28.2 (2)	7.4 (4)
C(10a)-C(11)-C(11a)-C(5a)	−7.5 (4)	14.1 (3)	−13.9 (2)	−16.4 (4)
C(11)-C(11a)-C(5a)-C(6)	41.6 (3)	−47.3 (3)	−0.5 (2)	41.7 (3)
C(11a)-C(5a)-C(6)-C(6a)	−53.8 (3)	51.9 (2)	15.4 (2)	−44.0 (3)
C(5a)-C(6)-C(6a)-C(10a)	36.5 (3)	−27.1 (3)	−16.7 (2)	24.4 (3)
C(6)-C(6a)-C(10a)-C(11)	−3.2 (4)	−4.9 (3)	3.5 (2)	1.2 (4)
C(6a)-C(10a)-C(11)-C(11a)	−13.0 (4)	12.8 (3)	12.1 (2)	−6.2 (4)
C(9)-C(10)-C(10a)-C(6a)	0.8 (4)	1.5 (3)	−2.4 (2)	1.6 (4)
C(10)-C(10a)-C(6a)-C(7)	−0.3 (4)	−4.0 (3)	−1.9 (2)	0.5 (4)
C(10a)-C(6a)-C(7)-C(8)	−0.0 (4)	3.4 (3)	4.4 (2)	−1.6 (4)
C(6a)-C(7)-C(8)-C(9)	−0.2 (4)	−0.3 (4)	−2.7 (3)	0.6 (5)
C(7)-C(8)-C(9)-C(10)	0.7 (5)	−2.4 (4)	−1.8 (3)	1.5 (5)
C(8)-C(9)-C(10)-C(10a)	−1.0 (4)	1.8 (3)	4.3 (2)	−2.5 (4)
O(12a)-C(12a)-C(1)-C(2)	72.6 (3)	157.3 (3)	155.1 (1)	161.6 (2)
O(1)-C(1)-C(2)-C(2am)[a]	4.5 (4)	−7.1 (5)	−0.3 (2)	−15.9 (4)
C(1)-C(2)-C(2am)-N(2am)	−169.6 (3)	−4.0 (5)	−0.8 (2)	2.4 (4)
C(3)-C(2)-C(2am)-O(2am)	−170.4 (2)	−0.3 (5)	1.8 (2)	0.4 (4)
C(2am)-C(2)-C(3)-O(3)	23.7 (4)	1.9 (5)	−3.5 (2)	0.6 (4)
O(3)-C(3)-C(4)-N(4)	25.1 (3)	28.4 (4)	28.6 (2)	50.0 (3)
C(3)-C(4)-N(4)-C(42m)	−159.0 (2)	−74.8 (3)	−82.7 (2)	−83.9 (3)
C(4a)-C(4)-N(4)-C(41m)	−60.7 (3)	−72.6 (3)	−76.6 (2)	−83.7 (3)
C(5)-C(5a)-C(6)-C(6m)	56.6 (3)	59.6 (3)	74.2 (2)	63.2 (3)
O(6)-C(6)-C(6a)-C(7)	96.8 (3)	38.2 (3)	50.7 (2)	83.6 (3)
C(6)-C(6a)-C(7)-Cl(7)		1.4 (3)	−1.3 (2)	
O(10)-C(10)-C(10a)-C(11)	−0.5 (4)	5.4 (3)	−2.8 (3)	−3.2 (4)
C(10)-C(10a)-C(11)-O(11)	−13.3 (4)	10.1 (3)	13.4 (2)	−2.8 (4)
O(11)-C(11)-C(11a)-C(12)	−1.7 (4)	7.6 (3)	−18.2 (2)	−6.6 (4)
C(11)-C(11a)-C(12)-O(12)	0.5 (4)	−0.5 (3)	−26.8 (2)	0.8 (4)
O(12)-C(12)-C(12a)-O(12a)	35.9 (3)	92.5 (2)	119.4 (2)	93.8 (3)

[a] Dihedral angles involving the second orientation of the amide moiety of 5a-epi-7-ClTC(+) are as follows: O(1)-C(1)-C(2)-C(2″) = −5.6 (7), C(1)-C(2)-C(2″)-O(2″) = −6.9 (16), C(3)-C(2)-C(2″)-N(2″) = −1.6 (17), C(2″)-C(2)-C(3)-O(3) = −1.1 (9)°. The amide moiety is estimated to be in the second orientation in ca. 15% of the molecules in the crystal.

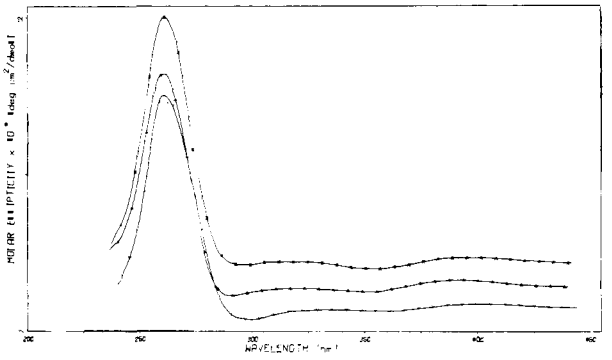

Figure 2. Circular dichroism spectra for dehydro-7-chlorotetracycline free base in three nonaqueous solvents: $CHCl_3$, THF, and 95% ethanol. The ordinate has been shifted for clarity.

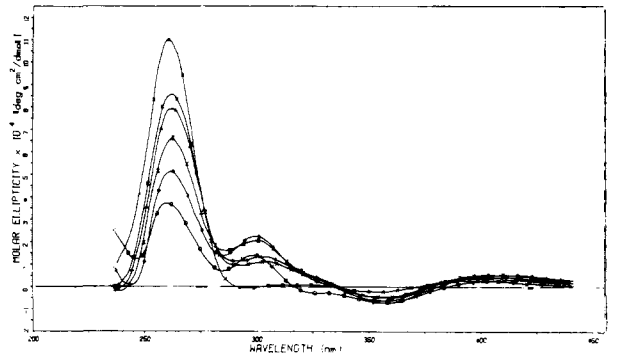

Figure 3. Solvent-dependent circular dichroism spectra for dehydro-7-chlorotetracycline in the EtOH–H_2O system. The curves with decreasing maxima at ~260 nm correspond, respectively, to solvent compositions with the following percent EtOH: 95, 85, 83, 71, 47, 0.

molecularly hydrogen-bonded carbonyl groups.

It is logical to assume that the tautomer obtained from aqueous solution (which displays no resolvable absorption below 6 μm[12]) is either tautomer II or III. This suggestion is compatible with our earlier results which have demonstrated the solvent-dependent change in tautomeric form of the A ring of the medicinally important tetracyclines.[8] It appears probable that DH-7-ClTC undergoes two tautomeric changes in structure when going from an aqueous solution to a nonaqueous environment, one involving the A ring (zwitterionic to nonionized) and the other involving the BCD chromophore. The solvent-dependent nature of the circular dichroism spectra for DH-7-ClTC free base supports this contention.

The CD spectra for 5a,11a-DH-7-ClTC(0) dissolved in $CHCl_3$, THF, and 95% EtOH are presented in Figure 2 with the ordinate shifted to facilitate comparison. As pointed out above the IR spectra indicate that DH-7-ClTC is in the ketonic tautomeric form in $CHCl_3$ and THF; by inference from the CD spectra the same tautomeric form predominates in 95% ethanol as well. Earlier

Table V. Bond Distances with Estimated Standard Deviations (Å)

	5a-epi-7-ClTC(+)	5a,11a-DH-7-ClTC(0)
C(1)–C(12a)	1.540 (4)	1.529 (2)
C(1)–O(1)	1.232 (3)	1.239 (2)
C(1)–C(2)	1.438 (3)	1.436 (2)
C(2)–C(2am)[a]	1.451 (4)	1.450 (2)
C(2)–C(3)	1.416 (4)	1.419 (2)
C(2am)–O(2am)	1.298 (4)	1.294 (2)
C(2am)–N(2am)	1.326 (4)	1.321 (2)
C(3)–O(3)	1.259 (3)	1.279 (2)
C(3)–C(4)	1.527 (4)	1.535 (2)
C(4)–N(4)	1.519 (3)	1.456 (2)
C(4)–C(4a)	1.539 (3)	1.554 (2)
N(4)–C(41m)	1.498 (4)	1.463 (2)
N(4)–C(42m)	1.503 (4)	1.463 (3)
C(4a)–C(5)	1.537 (4)	1.530 (2)
C(4a)–C(12a)	1.544 (4)	1.535 (2)
C(5)–C(5a)	1.529 (4)	1.507 (2)
C(5a)–C(6)	1.549 (3)	1.523 (2)
C(5a)–C(11a)	1.501 (4)	1.356 (2)
C(6)–C(6m)	1.535 (3)	1.548 (3)
C(6)–O(6)	1.433 (3)	1.429 (2)
C(6)–C(6a)	1.546 (4)	1.532 (2)
C(6a)–C(7)	1.392 (4)	1.390 (2)
C(6a)–C(10a)	1.418 (4)	1.420 (2)
C(7)–Cl(7)	1.750 (3)	1.746 (2)
C(7)–C(8)	1.404 (5)	1.401 (3)
C(8)–C(9)	1.377 (4)	1.381 (3)
C(9)–C(10)	1.386 (4)	1.395 (2)
C(10)–O(10)	1.351 (4)	1.348 (2)
C(10)–C(10a)	1.421 (4)	1.418 (2)
C(10a)–C(11)	1.471 (4)	1.464 (2)
C(11)–O(11)	1.268 (4)	1.242 (2)
C(11)–C(11a)	1.451 (4)	1.470 (2)
C(11a)–C(12)	1.368 (4)	1.493 (2)
C(12)–O(12)	1.334 (4)	1.213 (2)
C(12)–C(12a)	1.513 (4)	1.536 (2)
C(12a)–O(12a)	1.417 (3)	1.429 (2)

[a] Bond distances to atoms of the amide group in the second orientation in 5a-epi-7-ClTC(+) are as follows: C(2)–C(2″) = 1.581 (13), C(2″)–O(2″) = 1.291 (16), C(2″)–N(2″) = 1.351 (17) Å. The amide group is estimated to be present in this orientation in ca. 15% of the molecules in the crystal.

studies with OTC free base in EtOH–H_2O[8] imply that the A ring should be in the nonionized-enolic form in these solvent systems, so we conclude that the spectra in Figure 2 are representative of 5a,11a-DH-7-ClTC(0). Figures 3 and 4 display the CD spectra for DH-7-ClTC in mixtures of EtOH–H_2O and THF–H_2O, respectively. There is a noteworthy lack of isosbestic points in both sets of spectra, which we take as evidence that more than two molecular species are present in solution. We consider it probable that there is an equilibrium between as many as six free-base species, describable as I(0), I(±), II(0), II(±), III(0), and III(±), where the symbols in parentheses represent the molecular ionization state of the A ring and the Roman numerals correspond to the tautomers in Chart I. Furthermore, from the first two pK_a

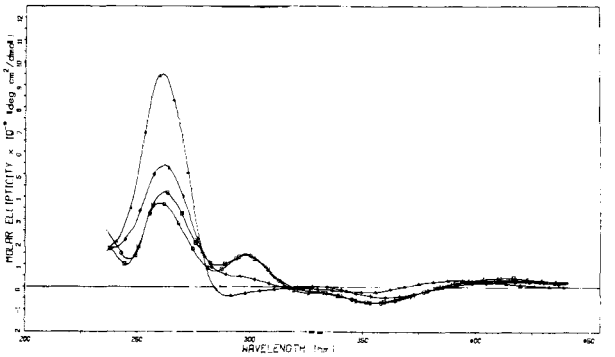

Figure 4. Solvent dependent circular dichroism spectra for dehydro-7-chlorotetracycline in the THF–H_2O system. The curves with decreasing maxima at ~260 nm correspond, respectively, to the following percent THF: 100, 50, 20, and 0.

values[12] for DH-7-ClTC, ca. 3.3 and 5.0, it can be calculated that about one-fifth of the neutral compound from the crystalline sample reproportionates to a cation and a monovalent anion in solution. The spectra for DH-7-ClTC in aqueous solution are most likely representative of the zwitterionic forms. The reported IR spectra for material isolated from aqueous solution may result from a mixture of II(±) and III(±).

Species III is very likely to be the tautomeric form of 6-epi-DH-7-ClTC that is responsible for its antibacterial activity.[11] This tautomeric form presents the di-β-hydroxy ketone structure that is common to all medicinally important tetracyclines. The presence of the additional double bond in the B ring may be expected to have only a minor influence on the conformation of the remainder of the chromophore. The fact that the 6-epimer shows substantial antibacterial activity, both in vivo and in vitro, while the parent is essentially inactive, implies that a different conformer is favored in the equilibrium of the 6-epimer. Once again, we attribute this effect to different steric interaction between the β substituents at C(6) and the A ring. Further evidence that these interactions are important to the properties of the tetracyclines is presented by the 6-thiatetracyclines.[19]

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Supplementary Material Available: Details of the matrix blocking, anisotropic temperature factors for the C, N, O, and Cl atoms, fractional atomic coordinates and isotropic temperature factors for the H atoms, bond angles between the C, N, and O atoms, and calculated and observed structure factors (69 pages). Ordering information is given on any current masthead page.