Chemical–Structural Properties of Tetracycline Derivatives. 1. Molecular Structure and Conformation of the Free Base Derivatives

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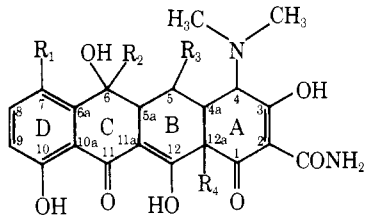
Abstract: The crystal structures of tetracycline hexahydrate, oxytetracycline dihydrate, and anhydrous oxytetracycline have been determined. Tetracycline hexahydrate crystallizes with space-group symmetry $P2_12_12_1$ with a = 12.056 (1), b = 21.417 (3), and c = 9.512 (1) Å and Z = 4; oxytetracycline dihydrate displays space-group symmetry $P2_12_12$ with a = 11.985 (4), b = 15.820 (5), and c = 11.487 (3) Å and Z = 4; anhydrous oxytetracycline displays space-group symmetry $P2_12_12_1$ for which a = 10.297 (1), b = 10.770 (2), and c = 18.369 (3) Å and Z = 4. All crystallographic data, including the lattice parameters, were determined with a crystal maintained at approximately −150 °C. The anhydrous oxytetracycline crystals contain un-ionized molecules which display extensive intramolecular hydrogen bonding and present a molecular species suitable for lipid phase solubility. The hydrate crystals are composed of zwitterionic molecules with both charge centers associated with the A ring. The orientation of the A-ring amide substituents in the two zwitterionic structures differs by a rotation of 171.3° about the C_2–C_{2am} bond, that joining the amide group to the A ring. The resultant tautomeric forms of the A ring display indications of differences in the distribution of the negative charge over the tricarbonylmethane system.

There are three acid dissociation constants normally associated with the tetracycline antibiotics,[1] for which pK_a values of approximately 3.3, 7.7, and 9.7 have been reported;[1] a fourth acid dissociation constant, with a pK_a of 10.7, has also been reported.[2]



	R_1	R_2	R_3	R_4	
1a	H	CH_3	H	OH	tetracycline
1b	H	CH_3	OH	OH	oxytetracycline
1c	H	CH_3	$OCOCH_3$	$OCOCH_3$	5,12a-diacetyloxy-tetracycline
1d	Cl	CH_3	H	OH	7-chlorotetracycline
1e	Cl	H	H	OH	7-chloro-6-demethyl-tetracycline

Although there is some disagreement as to the appropriate assignment of the various acid groupings to the respective macroscopic dissociation constants,[1-3] it is highly probable that the free base plays a significant role in the biological activity of the tetracycline derivatives. Kunin and Finland[4] have reported that the tetracycline antibiotics exhibit their optimum antimicrobial activity in the pH range 5.5–6.0. Colaizzi and Klink[5] have emonstrated, for several tetracycline derivatives, that the zwitterionic form of the free base is the predominant molecular species in this pH range and that the maximum lipid solubility also occurs in this pH range. They have proposed that the zwitterion is the lipid-soluble form and have further speculated as to the mechanism of lipid phase solubility. An intramolecular interaction between the positively charged dimethylammonium group and the negatively charged tricarbonylmethane system was proposed to produce effective charge cancellation and thus facilitate its solubility in the nonpolar lipid medium, or as an alternative explanation for the lipid solubility of the zwitterion, the formation of an ion pair complex between the tetracycline derivative and adjunctive substances such as glucosamine was proposed.

Relatively little is known about the conformation and bonding geometry of the zwitterionic tetracycline derivatives. Solution studies in nonaqueous solvents led Schach von Wittenau and Blackwood[6] to propose a principal conformation for several free base derivatives that differs from that found by x-ray diffraction studies for various fully protonated biologically active tetracycline derivatives: **1b**,[7] **1d**,[8] and **1e**.[9] Crystalline 5,12a-diacetyloxytetracycline (**1c**) has been shown[10] to display the conformation proposed by Schach von Wittenau and Blackwood; however, the crystals are composed of nonzwitterionic molecules. The NMR spectrum of tetracycline in pyridine was reported[6] to correspond to that expected from the conformation observed in the fully protonated crystalline salts as do the results of the circular dichroism studies in dilute acidic solutions,[11] though the latter observations are most likely assignable to the fully protonated molecule. Further CD studies[12] have indicated that all the biologically active tetracycline derivatives display similar conformations in the physiologically important pH regions and also that, relative to the fully protonated species, they undergo a subtle conformational change due to the formation of a hydrogen bond between the 12a-hydroxyl group and the dimethylamino group in neutral or slightly alkaline solutions.

In an effort to further elucidate the structural properties of the free base forms of the tetracycline derivatives, attempts were made to crystallize tetracycline, oxytetracycline, and 7-chlorotetracycline free bases under various solvent conditions. Suitable crystals of three crystalline modifications of the free bases were obtained and their crystal structure analyses were undertaken. This report details the results of these analyses.

Experimental Section

The free base form of each of the three tetracycline derivatives investigated was precipitated from an aqueous acetone solution of the respective hydrochloride salt by addition of excess sodium acetate. In the case of oxytetracycline, the precipitate contained high-quality single crystals of the free base dihydrate, one of which was utilized for the crystal structure analysis. The hydrated precipitates were subsequently dried by distillation of the water azeotrope from a toluene solution to give the anhydrous free bases. High-quality crystals of anhydrous oxytetracycline free base were obtained by slow evaporation of warm toluene or benzene solutions; attempts to recrystallize an-

hydrous tetracycline or 7-chlorotetracycline have repeatedly produced multicrystal arrays of extremely thin platelets. Rehydration and recrystallization of tetracycline and oxytetracycline were readily accomplished with several mixed solvents, such as methanol–water, ethanol–water, butanol–water, dioxane–water, and acetone–water over a broad range of relative water content; high-quality crystals of hydrated 7-chlorotetracycline have not yet been obtained.

All crystallographic data were measured with graphite crystal monochromated Mo Kα radiation on a Syntex P$\bar{1}$ autodiffractometer equipped with a low-temperature device (Syntex LT-1) operating at approximately −150 °C. Tetracycline hexahydrate (TC·6H_2O) crystallizes with space-group symmetry $P2_12_12_1$ for which lattice parameters a = 12.056 (1), b = 21.417 (3), and c = 9.512 (1) Å were obtained from least-squares refinement[13] with the automatically centered 2θ values for 44 reflections in the angular range $25.0 < 2\theta < 38.0°$. Oxytetracycline dihydrate (OTC·2H_2O) displays space-group symmetry $P2_12_12$ for which refined lattice parameters a = 11.985 (4), b = 15.820 (5), and c = 11.487 (3) Å were obtained with 43 centered reflections in the angular range $27.5 < 2\theta < 40.0°$; similarly, 30 reflections in the angular range $27.5 < 2\theta < 41.0°$ contributed to the refinement of the lattice parameters of anhydrous oxytetracycline (OTC), a = 10.297 (1), b = 10.770 (2), and c = 18.369 (3) Å, for which the space-group symmetry is $P2_12_12_1$.

Diffraction intensities were measured in an ω scan mode to a resolution of $\sin\theta/\lambda < 0.807$ Å^{-1} for TC·6H_2O and to $\sin\theta/\lambda < 0.906$ Å^{-1} for the two oxytetracycline crystals. The scan rate was allowed to vary as a function of maximum peak intensity and background intensities were measured on each side of the reflection for one-half the scan time; three reference reflections were periodically measured for each crystal. A 0.08 × 0.20 × 0.25 mm parallelepiped crystal of TC·6H_2O was employed for the measurement of 5950 unique data; each reflection was scanned through 1.0° employing a scan rate in the range 1.0–24.0° min^{-1}. The reference reflections, monitored after each 63 reflections were measured, remained constant to within an average deviation of 2.5% of their respective intensities. To reduce the quantity of liquid nitrogen consumed in the course of data collection for the OTC·2H_2O and OTC crystals, the scan range was reduced to 0.6°, the minimum scan rate was increased to 2.0° min^{-1}, and the reference reflections were monitored after each 129 data were measured. In this manner, a 0.15 × 0.15 × 0.20 mm rectangular prismatic crystal of OTC·2H_2O was used for the collection of 7377 unique data; a total of 6935 unique data were measured from a 0.17 × 0.17 × 0.20 mm hexagonal prismatic crystal of OTC. The average deviation in the intensities of the reference reflections was 3.1 and 2.6% for the respective oxytetracycline crystals. Those data for which the measured intensity was greater than twice the estimated standard deviation were subsequently classified as objectively observed; 4137 data for TC·6H_2O, 4397 data for OTC·2H_2O, and 4587 data for OTC were so classified. Lorentz and polarization corrections were applied to the data; absorption corrections were not considered necessary.

Structure Determination and Refinement. The initial structural models were determined by direct methods. All hydrogen atoms of the tetracycline and oxytetracycline molecules were located in difference Fourier maps, as were the hydrogen atoms of the water molecules in TC·6H_2O; attempts to locate the hydrogen atoms of the water molecules in OTC·2H_2O were not successful. The respective space-group enantiomorphs were selected to conform with the absolute configuration assigned to oxytetracycline.[14]

The appropriate fractional atomic coordinates and anisotropic temperature factors for all carbon, nitrogen, and oxygen atoms were refined; fractional atomic coordinates and isotropic temperature factors were refined for the hydrogen atoms. Refinement was effected by variable block least-squares techniques employing blocks constructed such that the scale factor and isotropic extinction coefficient, g, were refined in one block and the remaining blocks contained all parameters associated with one carbon, nitrogen, or oxygen atom and any hydrogen atoms bonded to it. In addition to the data classified as observed, reflections classified as unobserved for which the calculated structure factor was greater than the cut-off value were used in the refinement. In this manner 5089 reflections contributed to the refinement of 489 variables in the TC·6H_2O structure to give a standard residual R value of 0.057 and an empirically weighted R_w of 0.061.[15] The estimated standard deviation of an observation of unit weight, σ, is 1.15. Similarly, 5931 reflections contributed to the refinement of 415 variables associated with the OTC·2H_2O structure to give R = 0.072, R_w = 0.082, and σ = 1.25; 6060 reflections contributed to the refinement of 396 variables to give R = 0.060, R_w = 0.070, and σ = 1.11 for the OTC crystal structure.

Results and Discussion

Stereoscopic projections[16] of the molecular entities of the tetracycline derivatives, drawn from the refined atomic coordinates[15] by application of identical orienting vectors to facilitate comparison, are presented in Figure 1. Carbon, nitrogen, and oxygen atoms are presented with ellipsoid representations consistent with their refined temperature factors (75% probability level); hydrogen atoms are depicted with uniform isotropic thermal parameters. Isotropic extinction corrections proved to be minor; the refined extinction coefficients are 0.5 (7) × 10^{-5}, 8.0 (8) × 10^{-5}, and 10.3 (6) × 10^{-5}, respectively, for the TC·6H_2O, OTC·2H_2O, and OTC crystals. Bond distances between nonhydrogen atoms are presented in Table I; a selected set of dihedral angles[17] which fully characterize the tetracycline ring system[10] and the orientation of the substituent amide and amine groups are displayed in Table II.

The molecular entities in the hydrated and anhydrous crystals are different in conformation and molecular structure. The hydrated crystals are composed of zwitterionic molecules, TC$^{\pm}$ and OTC$^{\pm}$, which display a general conformation similar to that of fully protonated, biologically active tetracycline derivatives, represented in Table II by OTC·HCl,[7] whereas the anhydrous oxytetracycline crystals contain un-ionized molecules, OTC, with a conformation similar to that reported for crystalline 5,12a-diacetyloxytetracycline,[10] DAOTC.

The observation of the oxytetracycline free base molecule in the zwitterionic and nonionized forms, the relationship between the chemical structure and conformation, and the conditions under which the two crystalline forms were obtained, together with the reported solution studies in aqueous[5,12] and nonaqueous solvents,[6] provide an indication of the role played by the two forms of the free base in the biological activity of the tetracycline derivatives. The conclusion drawn by Colaizzi and Klink[5] that the zwitterion is the important form of the tetracycline derivatives in aqueous solutions in the pH range of their optimum antimicrobial activity is supported by the ease with which the crystalline hydrates were obtained from a variety of aqueous–organic solvents; however, the zwitterion does not appear to be the lipid-soluble species for the uncomplexed free base. The observed conformation provides little indication of an interaction between the positive and negative portions of the zwitterion and, furthermore, there is considerable intermolecular hydrogen bonding (see Table III) in the crystalline hydrates indicating that it is a highly polar species. On the other hand, the nonionized molecule presents a molecular structure of considerably reduced polarity in a conformation that displays extensive intramolecular hydrogen bonding. As can be seen from an examination of Table III, the only hydrogen atoms suitable for hydrogen bonding that are not intramolecularly hydrogen bonded are the 6-hydroxyl hydrogen atom and one hydrogen atom of the amide NH_2 group. Of particular interest is the intramolecular hydrogen bonding displayed by the 5- and 12a-hydroxyl groups, both of which are strongly intermolecularly hydrogen bonded in the zwitterionic form. In contrast, the extensive intramolecular hydrogen bonding displayed by the conformation of the un-ionized form further reduces the polarity of the molecule. Another indication of reduced polarity displayed by the nonionized molecule is provided by the dimethylamino group which is not involved in any form of hydrogen bonding. This is in contrast with the dimethylammonium group of the zwitterionic form which is both inter- and intramolecularly hydrogen bonded, though the latter hydrogen bond displays rather unfavorable geometry. Thus the chemical structure and conformation of the nonionized molecule combine to present an entity more

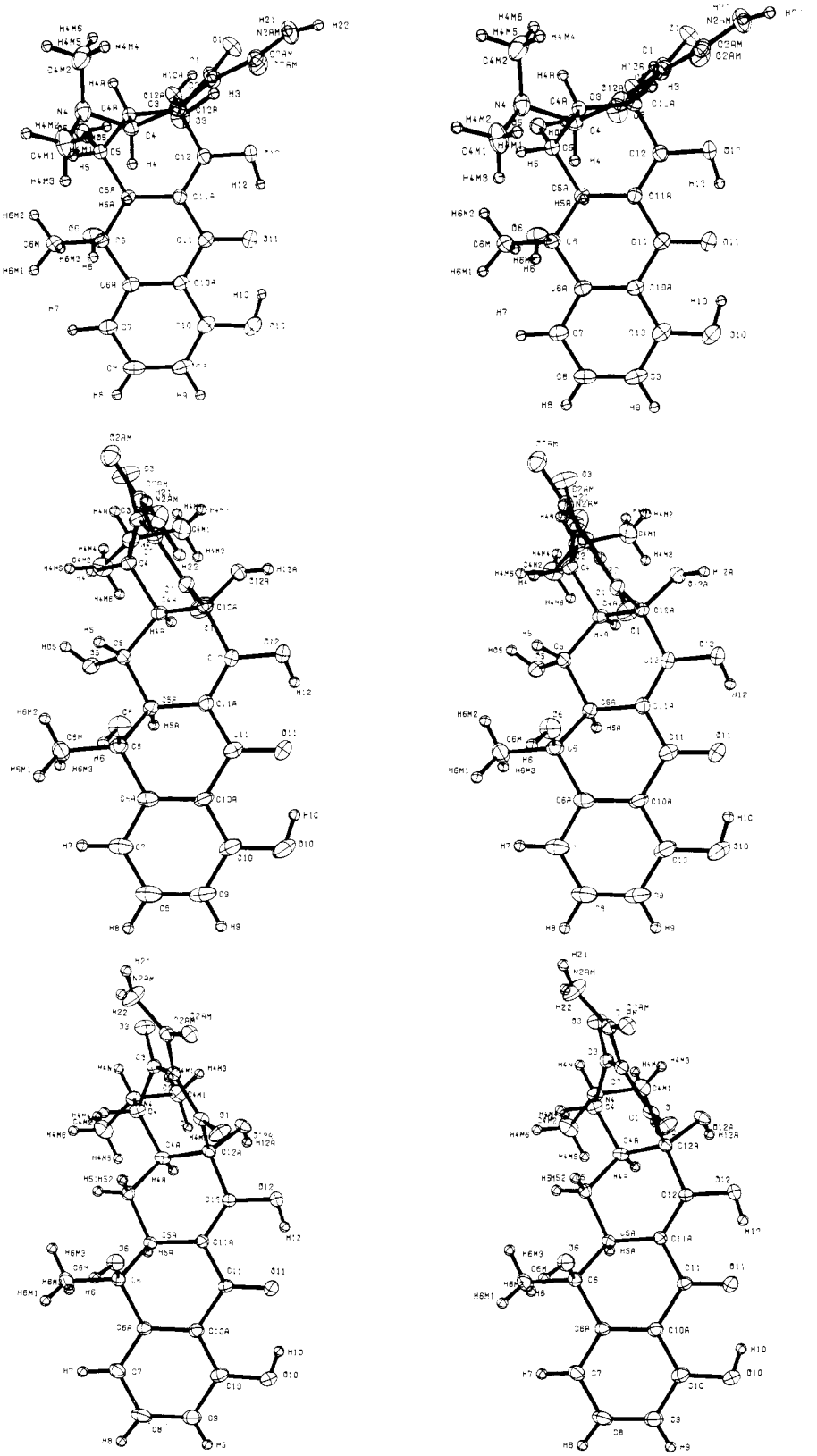


Figure 1. Stereoscopic projections for the free base tetracycline derivatives. The carbon, nitrogen, and oxygen atoms are depicted with thermal elipsoids representative of the 75% probability level for the refined anisotropic temperature factors. Hydrogen atoms are depicted with uniform isotropic temperature factors. Presented are the molecular conformations for **1a** (top), nonionized oxytetracycline; **1b** (middle), zwitterionic oxytetracycline; and **1c** (bottom), zwitterionic tetracycline free bases.

suitable for lipid membrane solubility than that of the zwitterion.

Blackwood and English[19] have tabulated a series of tetracycline derivatives in order of increasing lipophilicity. Among those tabulated are three oxytetracycline derivatives for which the relative lipophilicity merits comment here. Of the three, oxytetracycline, α-6-deoxyoxytetracycline, and β-6-deoxyoxytetracycline, the parent compound is least lipophilic. Substitution of a hydrogen atom for the 6-hydroxyl group, the only hydroxyl substituent not intramolecularly hydrogen

Table I. Bond Distances (Å) to Carbon, Nitrogen, and Oxygen Atoms for the Free Base Derivatives with Estimated Standard Deviations[a]

Bond	TC±	OTC±	OTC	DAOTC[b]
C_1-O_1	1.237 (3)	1.250 (4)	1.233 (3)	1.229 (5)
C_1-C_2	1.434 (4)	1.419 (4)	1.438 (3)	1.434 (5)
C_1-C_{12a}	1.559 (4)	1.552 (4)	1.527 (3)	1.531 (5)
C_2-C_3	1.437 (4)	1.441 (4)	1.391 (3)	1.397 (6)
C_2-C_{2am}	1.466 (4)	1.467 (4)	1.473 (3)	1.468 (6)
C_{2am}-O_{2am}	1.262 (3)	1.248 (4)	1.274 (3)	1.274 (5)
C_{2am}-N_{2am}	1.341 (4)	1.347 (5)	1.324 (4)	1.335 (6)
C_3-O_3	1.237 (3)	1.233 (4)	1.304 (3)	1.291 (5)
C_3-C_4	1.534 (4)	1.550 (4)	1.523 (3)	1.535 (6)
C_4-C_{4a}	1.538 (4)	1.545 (4)	1.546 (3)	1.558 (5)
C_4-N_4	1.497 (4)	1.508 (4)	1.471 (3)	1.460 (5)
N_4-C_{4m1}	1.493 (4)	1.496 (5)	1.468 (4)	1.471 (6)
N_4-C_{4m2}	1.492 (4)	1.501 (5)	1.462 (4)	1.475 (6)
C_{4a}-C_5	1.532 (4)	1.542 (4)	1.542 (3)	1.519 (5)
C_{4a}-C_{12a}	1.542 (4)	1.531 (4)	1.532 (3)	1.538 (5)
C_5-C_{5a}	1.527 (4)	1.540 (4)	1.563 (3)	1.563 (5)
C_5-O_5		1.426 (4)	1.435 (3)	1.479 (4)
C_{5a}-C_6	1.548 (4)	1.552 (4)	1.533 (3)	1.541 (6)
C_{5a}-C_{11a}	1.519 (4)	1.503 (4)	1.516 (3)	1.521 (5)
C_6-C_{6a}	1.533 (4)	1.530 (5)	1.538 (3)	1.527 (6)
C_6-O_6	1.449 (3)	1.452 (4)	1.444 (3)	1.436 (5)
C_6-C_{6m}	1.520 (4)	1.519 (5)	1.530 (4)	1.525 (6)
C_{6a}-C_7	1.387 (4)	1.392 (4)	1.383 (4)	1.389 (6)
C_{6a}-C_{10a}	1.413 (4)	1.415 (5)	1.421 (4)	1.425 (6)
C_7-C_8	1.405 (4)	1.412 (6)	1.400 (4)	1.405 (6)
C_8-C_9	1.381 (4)	1.369 (6)	1.381 (4)	1.379 (7)
C_9-C_{10}	1.388 (4)	1.392 (6)	1.397 (4)	1.398 (7)
C_{10}-C_{10a}	1.411 (4)	1.415 (5)	1.416 (4)	1.399 (5)
C_{10}-O_{10}	1.359 (4)	1.374 (5)	1.355 (4)	1.352 (6)
C_{10a}-C_{11}	1.475 (4)	1.460 (5)	1.453 (4)	1.464 (6)
C_{11}-C_{11a}	1.439 (4)	1.435 (5)	1.451 (4)	1.449 (5)
C_{11}-O_{11}	1.269 (3)	1.286 (4)	1.270 (3)	1.271 (5)
C_{11a}-C_{12}	1.370 (4)	1.359 (5)	1.365 (3)	1.342 (5)
C_{12}-C_{12a}	1.522 (4)	1.507 (4)	1.520 (3)	1.520 (5)
C_{12}-O_{12}	1.333 (3)	1.334 (4)	1.337 (3)	1.337 (5)
C_{12a}-O_{12a}	1.430 (4)	1.423 (4)	1.424 (3)	1.443 (4)

[a] The numbers enclosed in parentheses are the estimated standard deviations in the least significant digit. [b] See ref 10.

bonded in the conformation observed for nonionized oxytetracycline free base, would be expected to increase the lipophilicity of the derivative. As indicated, this is the case; however, there is a large difference with lipophilicity of the two forms of 6-deoxytetracycline. The large increase in the lipophilicity of α-6-deoxyoxytetracycline is consistent with maintaining the observed conformation and reducing the polarity of the substituents. The 6-epimer, β-6-deoxyoxytetracycline, shows only a modest increase in lipophilicity relative to the parent compound. The chemical configuration of this form requires that the methyl group occupy the site normally occupied by the 6-hydroxyl group. The oxygen atom of this group is only 3.224 Å from the tetrahedral carbon atom C_4. Consequently, substitution of the bulkier methyl group in this site requires a significant change in the molecular conformation in the direction of that of the zwitterion, which reduces the intramolecular hydrogen bonding between the hydroxyl groups at C_5 and C_{12a} and thus increases the polarity of the molecule. Such an increase in polarity is also consistent with the reported lipophilicity of β-6-deoxyoxytetracycline.

The relatively short interatomic distances between atom C_4 and atoms C_6 and O_6 in this conformation may also at least partially explain the biological inactivity of the 4-epitetracycline derivatives. The hydrogen atom at C_4 is only 2.40 Å from atom O_6 and 3.25 Å from atom C_6. The nitrogen atom of the dimethylamine group must occupy this site in the 4-epitetracycline derivatives. In view of the bulky character of this group, it is sterically impossible for the 4-epi derivatives to adopt this conformation for the nonzwitterionic molecule.

Mitscher et al.[20] have stated that derivatives not possessing the 5-hydroxyl group do not adopt the conformation displayed by oxytetracycline in the anhydrous crystals and have attributed the observation of this conformation for 5,12a-diacetyloxytetracycline to steric effects associated with the 5-acetyl group. The authors cited crystallographic evidence[7,8,21,22] and NMR evidence[6] in support of this conclusion. The crystallographic evidence is drawn only from examples of fully protonated derivatives which, in view of our observations concerning the occurrence of this conformation, is probably not relevant to the question. The NMR spectrum of tetracycline in pyridine may be that of the zwitterionic form resulting from the presence of a small amount of water in the solution either from the solvent or from the tetracycline used to prepare the sample. For example, tetracycline hexahydrate may contain adequate water of crystallization to favor the zwitterionic form of the molecule in solution even when this is the only source of water in a nonaqueous solvent system. In addition to their conformation, the one feature that both crystalline examples of anhydrous oxytetracycline free base derivatives have in common is the nonionized character of the A-ring tricarbonylmethane system (Figure 1). It has been long recognized[23] that the extent of enolization in such a system is greatly effected by the nature of the solvent and that water tends to favor the keto form, which in this case is the form displayed by the zwitterion. This keto-enol tautomerism is a feature common to all biologically active tetracycline derivatives. As indicated earlier, a second crystalline modification of tetracycline free base has been obtained in this laboratory. The high degree of disorder displayed by these apparently anhydrous crystals[24] may be indicative of relatively little intermolecular hydrogen bonding. Since reduced intermolecular hydrogen bonding is an attribute of the conformation displayed by nonionized oxytetracycline, it seems advisable to maintain the question of the conformation of the other anhydrous and thus probably enolic free base derivatives open for further investigation.

The correlation between the conformation of nonionized oxytetracycline and the reported lipophilicities of its derivatives indicates that this form may play an important role in nearly anhydrous regions of biological systems; however, it is also clear that the zwitterion must play an important role as well. The chemical structure and conformation of the zwitterions observed in this investigation are most likely representative of the free base tetracycline derivatives in the blood stream and in the aqueous rich regions of microbial cells.

The chemical structure of the zwitterion is such that both charge centers are associated with the A ring. The effect of the charge distribution on the bonding geometry of this portion of the molecule can be clearly demonstrated by a comparison with the average bond distances from the crystalline nonionized derivatives (Figure 2, top), For example, the protonated amine group at C_4 displays average C-N bond distances that are 0.03 Å longer than those of the nonionized derivatives. Unlike the positive charge, which is localized at the protonated dimethylamine group, the negative charge is distributed over the tricarbonylmethane system. This system also displays systematic differences in bond lengths between the ionized and nonionized forms; however, there are also significant differences between the two zwitterionic derivatives. The latter differences appear to be correlated with the orientation of the amide group at C_2 and thus to reflect geometric tautomerism[26] of the A ring. The orientation of the amide group is the same for the two oxytetracycline structures; consequently, these two structures may be expected to display the same tautomeric form of the tricarbonylmethane system and differences in their bonding geometry should primarily reflect the differences in

Table II. Dihedral Angles (Deg) for the Free Base Tetracycline Derivatives

Atoms	TC±	OTC±	OTC	DAOTC[a]	OTC·HCl[b]
$C_{12}C_{12a}C_1C_2$	−169.5	−174.9	−72.7	−80.6	−174.7
$C_{12a}C_1C_2C_3$	5.4	9.4	−16.4	−7.0	19.2
$C_1C_2C_3C_4$	34.1	31.3	−9.1	−3.7	17.0
$C_2C_3C_4C_{4a}$	−30.2	−28.3	2.0	−17.5	−17.0
$C_3C_4C_{4a}C_5$	110.5	109.2	153.8	169.8	106.0
$C_4C_{4a}C_{12a}C_1$	49.4	52.8	−53.2	−59.8	49.1
$C_{11}C_{11a}C_{12}C_{12a}$	179.7	−170.8	−177.0	169.3	−178.5
$C_{11a}C_{12}C_{12a}C_1$	102.3	89.0	152.8	168.6	99.2
$C_{12}C_{12a}C_{4a}C_5$	47.7	50.1	−58.3	−60.1	48.7
$C_4C_{4a}C_5C_{5a}$	173.3	−172.3	−79.0	−85.8	170.9
$C_{4a}C_5C_{5a}C_6$	171.5	155.0	123.0	133.3	162.5
$C_5C_{5a}C_{11a}C_{12}$	−17.6	−6.7	−22.8	−25.4	−13.2
$C_{10}C_{10a}C_{11}C_{11a}$	167.1	166.2	−179.7	157.4	166.4
$C_{10a}C_{11}C_{11a}C_{12}$	177.8	172.1	173.9	−168.0	178.8
$C_{11}C_{11a}C_{5a}C_6$	41.6	44.3	35.3	35.5	43.0
$C_5C_{5a}C_6C_{6a}$	−179.0	172.7	−171.8	174.8	175.8
$C_{5a}C_6C_{6a}C_7$	−146.6	−142.9	−159.9	−138.9	−135.1
$C_6C_{6a}C_{10a}C_{11}$	−3.2	−4.8	−6.1	6.0	1.8
$C_8C_9C_{10}C_{10a}$	−1.0	−0.6	2.2	−3.0	−7.7
$C_9C_{10}C_{10a}C_{11}$	−179.3	−176.8	−175.7	−174.2	−176.9
$C_{10}C_{10a}C_{6a}C_7$	−0.3	−1.5	−0.4	0.1	−11.4
$C_6C_{6a}C_7C_8$	−176.9	−178.0	−170.0	−179.3	−178.1
$C_{6a}C_7C_8C_9$	−0.1	1.1	−0.7	2.2	−2.0
$C_7C_8C_9C_{10}$	0.6	−1.6	−3.2	0.5	6.7
$C_1C_2C_{2am}O_{2am}$	8.6	179.9	−177.3		171.1
$C_2C_3C_4N_4$	−163.3	−161.1	−122.7		−147.4
$C_3C_4N_4C_{4m1}$	73.2	76.7	−84.1		62.2
$C_3C_4N_4C_{4m2}$	−159.0	−154.6	44.8		−171.1

[a] See ref 10. [b] See ref 7 and 18.

charge distribution. Particularly noteworthy, for comparison with both examples of the zwitterion, are the differences within the sets of bond distances (C_1–O_1, C_3–O_3) and (C_1–C_2, C_2–C_3) for the nonionized molecule, 0.067 and 0.042 Å, respectively, and also the intermediate C_{2am}–O_{2am} bond distance of 1.274 Å. Zwitterionic oxytetracycline displays equivalent C_1–O_1 and C_{2am}–O_{2am} bond distances which are midway between those found for the nonionized molecule; the C_3–O_3 bond distance in OTC± is nearly identical with the C_1–O_1 bond distance for the nonionized molecules, that of a carbonyl group. The C_2–C_3 bond in the zwitterion, rather than the C_1–C_2 bond, is the appropriate length for a single bond in a conjugated double bond system;[10] furthermore, the C_1–C_2 bond in OTC± is significantly shortened indicating localized double bond character. Thus, while the orientation of the amide group is the same in the two oxytetracycline structures, the nature of the keto–enol tautomerism differs as a result of the distribution of the negative charge in the A ring. It seems to be appropriate to characterize the tricarbonylmethane system of crystalline zwitterionic oxytetracycline as a *trans*-ketonate group consisting of atoms O_1, C_1, C_2, C_{2am}, and O_{2am} in conjugation with a carbonyl group at C_3. This *trans*-ketonate group displays a strong hydrogen bond between atom O_1 and a hydrogen atom of the amide group (Table III). This hydrogen bond is significantly stronger than the other intramolecular hydrogen bond involving the A-ring substituents, that between the dimethylammonium group and the carbonyl group at C_3. The latter hydrogen bond is representative of the unfavorable geometry displayed by five-atom (including the hydrogen atom) rings[27] though it appears to be strong enough to influence the orientation of the dimethylammonium group.

The crystalline tetracycline zwitterion displays the amide group in an orientation differing by a rotation about the C_2–C_{2am} bond through 171.3° from that displayed by OTC±. The observed A-ring bonding geometry displays systematic differences from those described above. Crystalline TC± displays equivalence in the (C_1–O_1, C_3–O_3) and (C_1–C_2, C_2–C_3) bond pairs (Figure 2, bottom); the bond distance in the amide carbonyl is only slightly shorter than that of the nonionized molecule. Furthermore, there is no evidence of localized double bond character in the TC± A ring; thus the charge appears to be more evenly distributed through the entire tricarbonylmethane system. This conclusion is also supported by the intramolecular hydrogen bonding. As can be seen from an examination of Table III, this structure displays no particularly strong intramolecular hydrogen bonding involving the A-ring substituents; there is nearly equivalent hydrogen bonding between the carbonyl group at C_3 and the hydrogen atoms of the amide group and of the dimethylammonium group even though the former hydrogen bonding displays the more favorable six-atom ring geometry.

Each of the structures reported here displays intermolecular hydrogen bonding between symmetry-related tetracycline molecules (Table III). The nonionized oxytetracycline molecule displays only two such bonds of rather modest strength. The oxygen atom of the amide group is hydrogen bonded to atom H_6 of the C_6 hydroxyl group and the oxygen atom of the C_5 hydroxyl group is bonded to the amide hydrogen atom not involved in intramolecular hydrogen bonding. These two intermolecular hydrogen bonds and the intramolecular hydrogen bonding described above involve all polar hydrogen atoms of the un-ionized molecule in hydrogen bonding. Both structures containing the zwitterionic form of the tetracycline derivatives display hydrogen bonding between the amide oxygen atom and hydroxyl groups of symmetry-related molecules. The oxytetracycline zwitterion utilizes the hydrogen atom of the 5-hydroxyl group to form a strong hydrogen bond with the amide carbonyl group; the oxygen atom of this hydroxyl group is intermolecularly hydrogen bonded to a 12a-hydroxyl group of another symmetry-related molecule. The amide carbonyl group of TC± hydrogen bonds to the 6- and 12a-hydroxyl groups of different molecular ions. Both crystalline zwitterionic deriv-

Table III. Interatomic Distances (Å) between Atoms Involved in Hydrogen Bonding

a. Nonionized Oxytetracycline (OTC), Intramolecular			
O_1-H_{12a}	2.14	O_1-O_{12a}	2.609
O_1-H_{21}	2.10	O_1-N_{2am}	2.763
O_{2am}-H_3	1.39	O_{2am}-O_3	2.429
O_{12a}-H_{O5}	2.25	O_{12a}-O_5	2.857
O_{11}-H_{10}	1.67	O_{11}-O_{10}	2.491
O_{11}-H_{12}	1.62	O_{11}-O_{12}	2.552
Intermolecular			
O_{2am}-H_6	2.14	O_{2am}-O_6	2.818
O_5-H_{22}	2.05	O_5-N_{2am}	2.953
b. Zwitterionic Oxytetracycline (OTC±), Intramolecular			
O_1-H_{22}	1.80	O_1-N_{2am}	2.636
O_3-H_{4N}	2.22	O_3-N_4	2.617
O_{11}-H_{10}	1.68	O_{11}-O_{10}	2.538
O_{11}-H_{12}	1.84	$O_{11}O_{12}$	2.505
O_{12}-H_{12a}	2.28	O_{12}-O_{12a}	2.638
Intermolecular			
O_{2am}-H_{O5}	1.77	O_{2am}-O_5	2.614
O_5-H_{12a}	1.74	O_5-O_{12a}	2.675
O_6-H_{w1}[a]		O_6-O_{w1}	2.842
O_{10}-H_{w2}[a]		O_{10}-O_{w2}	2.854
O_{w2}-H_{4N}	2.17	O_{w2}-N_4	2.886
O_{w2}-H_6	2.00	O_{w2}-O_6	2.791
O_{w2}-$H_{w2'}$[a]		O_{w2}-$O_{w2'}$	2.758
c. Zwitterionic Tetracycline (TC±), Intramolecular			
O_3-H_{22}	2.20	O_3-N_{2am}	2.731
O_3-H_{4n}	2.29	O_3-N_4	2.647
O_{11}-H_{10}	1.77	O_{11}-O_{10}	2.553
O_{11}-H_{12}	1.76	O_{11}-O_{12}	2.495
Intermolecular			
O_1-H_{w52}	2.00	O_1-O_{w5}	2.845
O_{2am}-H_{12a}	1.93	O_{2am}-O_{12a}	2.732
O_{2am}-H_6	2.08	O_{2am}-O_6	2.790
O_6-H_{w11}	1.96	O_6-O_{w1}	2.771
O_{10}-H_{w21}	2.36	O_{10}-O_{w2}	2.992
O_{12}-H_{w42}	2.30	O_{12}-O_{w4}	2.903
O_{w2}-H_{4N}	1.77	O_{w2}-N_4	2.708
O_{w1}-H_{w41}	1.94	O_{w1}-O_{w4}	2.755
O_{w1}-H_{w22}	1.94	O_{w1}-O_{w2}	2.745
O_{w3}-H_{w51}	1.93	O_{w3}-O_{w5}	2.709
O_{w4}-H_{w31}	2.07	O_{w4}-O_{w3}	2.896
O_{w4}-H_{w61}	2.18	O_{w4}-O_{w6}	2.858
O_{w5}-H_{w12}	1.80	O_{w5}-O_{w1}	2.700
O_{w5}-H_{w62}	2.15	O_{w5}-O_{w6}	2.884
O_{w6}-H_{w32}	2.06	O_{w6}-O_{w3}	2.772

[a] The hydrogen atoms of the water molecules in oxytetracycline dihydrate were not located in the difference Fourier maps. They are included here only as an indication that the interaction is through water-hydrogen atoms.

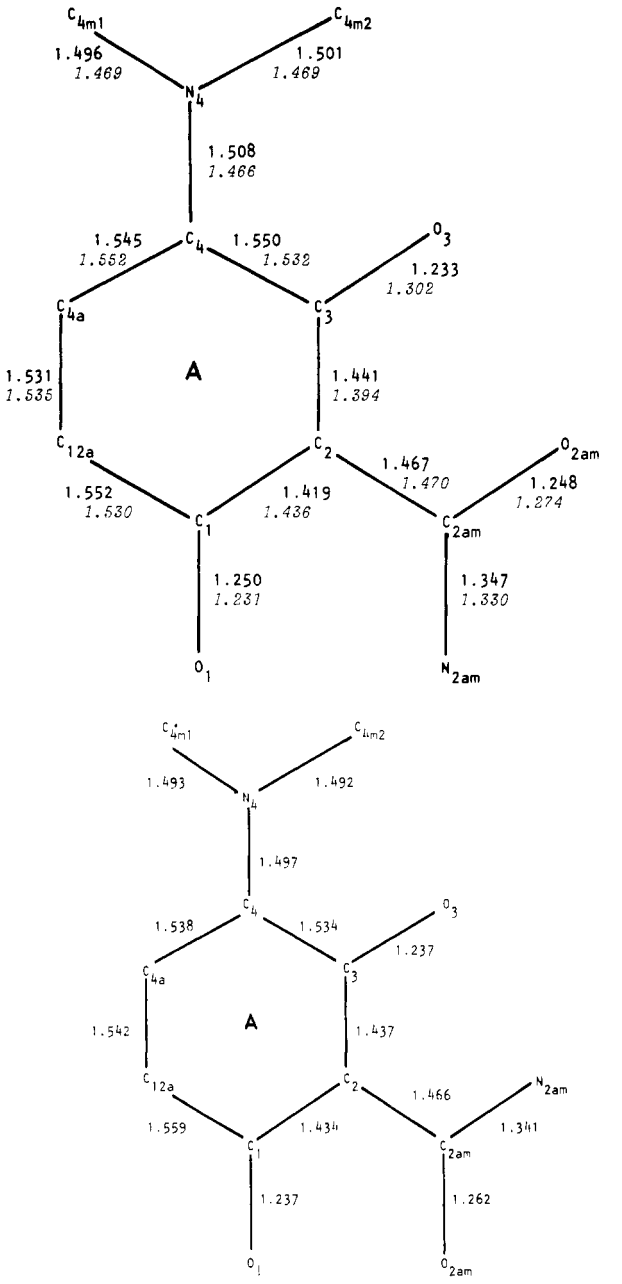


Figure 2. A-ring bond distances. The observed bond distances for zwitterionic oxytetracycline free base (normal type) and the average bond distances from nonionized oxytetracycline and diacetyloxytetracycline free bases (italicized) are presented in **2a** (top). Similarly, **2b** (bottom) displays the bond distances for zwitterionic tetracycline free base.

atives display hydrogen bonding between the hydrogen atom of their dimethylammonium group and a water molecule. Thus, this hydrogen atom, which is also intramolecularly hydrogen bonded to the carbonyl oxygen atom at C_3, displays two hydrogen bonds. Water molecules are also involved in hydrogen bonding with the 6- and 10-hydroxyl groups in both zwitterionic structures; tetracycline hexahydrate, which does not display the weak H_{12a}-O_{12} intramolecular hydrogen bond present in the oxytetracycline dihydrate crystals, displays a hydrogen bond of similar strength between the 12-hydroxyl oxygen atom and a water molecule. As might be expected, the tetracycline hexahydrate crystals present considerably more hydrogen bonding between water molecules than the oxytetracycline dihydrate crystals.

In summary, the free base derivatives of the tetracycline antibiotics have been shown to display very different conformations in the zwitterionic and nonionized forms. Furthermore, the two forms show conformational integrity even when the substituents, crystal symmetry, and intermolecular hydrogen bonding differ. The zwitterionic form, which appears to be the predominant form when even small concentrations of water are present, clearly plays a significant role in the activity of these antibiotics in aqueous rich environments. The nonionized molecule presents a probable species for the lipid phase solubility of these derivatives as a molecular entity. The qualitative correlation between the observed conformation of this species and the lipophilicity of the oxytetracycline derivatives provides substantiating evidence that this chemical structure and conformation are suitable for lipid phase transport without the necessity of ion pair formation.

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Supplementary Material Available: fractional atomic coordinates, thermal parameters, bond angles between carbon, nitrogen, and oxygen atoms, and tabulated observed and calculated structure factor amplitudes (110 pages). Ordering information is given on any current masthead page.

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(24) The disordered crystals of tetracycline free base have been obtained by slow evaporation of toluene solutions from which water had been removed by distillation. Definitive assignment of the space group and accurate determination of the lattice parameters have been severely hindered by the disorder; however, an analysis of Buerger precession photographs allows tentative assignment of the crystals to space group $P2_12_12_1$ with a = 12.7, b = 16.3, and c = 25.1 Å. The resultant unit cell volume, 5200 Å, is reasonably consistent with Z = 8.
Two crystalline modifications have been reported for tetracycline, 7-chlorotetracycline, and oxytetracycline free bases.[25] One modification of each derivative was recrystallized from distilled water and the other from hot anhydrous methanol. The analytically determined water content reported for these modifications differs from that found here in the crystal structure analyses, except for the oxytetracycline dihydrate which corresponds to that crystallized from distilled water. Attempts are underway in this laboratory to obtain suitable crystals of the second modification (those obtained from anhydrous methanol) in an effort to further clarify the conformational characteristics of the enolic tetracycline free base derivatives.
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Chemical–Structural Properties of Tetracycline Derivatives. 2. Coordination and Conformational Aspects of Oxytetracycline Metal Ion Complexation

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Abstract: The shift in the long wavelength absorption maximum (ca. 365 nm) as a function of pH has been determined for oxytetracycline (OTC) in the presence and absence of metal-chelating agents. Crystal structures have also been determined for a mercuric chloride complex of oxytetracycline and for a dipotassium salt of OTC. The former crystallizes with space-group symmetry $P2_12_12_1$ for which a = 11.377 (1), b = 17.277 (2), and c = 12.731 (2) Å; the formula per asymmetric unit is $(C_{22}H_{24}N_2O_9)HgCl_2 \cdot 2H_2O$. The dipotassium salt crystallizes with space group symmetry $C222_1$ for which a = 13.472 (2), b = 23.724 (6), and c = 19.643 (4) Å and the formula per asymmetric unit is $K_2(C_{22}H_{22}N_2O_9) \cdot 2H_2O \cdot 2CH_3OH$. All crystallographic data were measured at reduced temperature, ca. −150 °C. The mercuric chloride complex, which displays metal–oxygen coordination involving only A-ring coordination sites of zwitterionic oxytetracycline, has been shown by the uv spectral measurements to be atypical of the metal complexes of oxytetracycline. The dipotassium salt, which displays the oxytetracycline moiety in a new conformation, displays two coordination sites which appear to be suitable model sites for the typical oxytetracycline–metal complexes. Both coordination sites utilize both the BCD and A-ring chromophores, one through atoms O_1, O_{12}, and O_{11} and the other through atoms O_1, O_{12a}, and O_{12}. The former coordination site presents the most favorable interaction between metal atoms and the negative charge centers of the ligand.

The chemical structures and conformations of examples of the free base forms of the tetracycline derivatives were described in the preceding report and some of the structural differences were discussed in terms of their implications concerning the biological activity of these antibiotics.

The tetracycline antibiotics are also known to complex with