der Universitaet Stuttgart, for making this research possible.

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.

References and Notes

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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

Kurt H. Jogun and John J. Stezowski*

Contribution from the Institut fuer Organische Chemie, Biochemie und Isotopenforschung der Universitaet, 7000 Stuttgart 80, Pfaffenwaldring 55, Federal Republic of Germany.
Received May 1, 1975

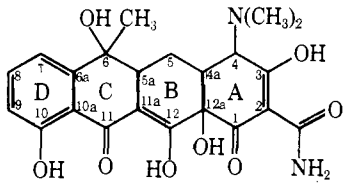
Abstract: The shift in the long wavelength absorption maximum (ca. 365 nm) as a function of pH has been determined for oxytetracycline (OTC) in the presence and absence of metal-chelating agents. Crystal structures have also been determined for a mercuric chloride complex of oxytetracycline and for a 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

a variety of metal chelating agents to form what have been characterized as MHL and ML complexes,[1] where HL and L have been used to characterize the ligand as the monovalent and divalent anion, respectively. The formation of 1:2 complexes of the ML_2 type has also been reported.[2,3] While the role of metal ion coordination in the activity of the tetracycline antibiotics is not known, it has been demonstrated that complex formation increases the stability of the various tetracycline derivatives,[4-7] though in some cases it reduces their ability to enter the blood stream[8] or eliminates their biological activity.[2]

The biologically active tetracycline derivatives display several functional groups (1) that are capable of serving as the



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ligand site for complex formation. The bathochromic shift in the long-wavelength uv absorption spectrum resulting from complex formation of the tetracycline derivatives and model compounds led Conover[9] to propose that complex formation occurs through oxygen coordination to the BCD chromophore. This conclusion is supported by Mitscher et al.[10,11] as a result of analysis of extensive CD spectral measurements for a series of tetracycline derivatives and metal ions. Another viewpoint is expressed by Baker and Brown,[12] who have proposed A-ring coordination through oxygen atoms of the tricarbonylmethane chromophore as the likely site based upon their analysis of reflectance ir spectra. This proposal has recently been supported by Williamson and Everett[13] as the result of extensive NMR measurements of a series of para- and diamagnetic metal ion complexes with tetracycline in dimethyl sulfoxide solutions. Chelation involving both the BCD and A-ring chromophores has also been proposed.[14,15]

In an effort to bring a new perspective to this problem we have undertaken an examination of the metal complex geometry from a crystallographic viewpoint. We have encountered numerous difficulties in obtaining crystals suitable for single-crystal structural analysis; however, we have succeeded in obtaining suitable crystals of a mercuric chloride complex and of a dipotassium salt of oxytetracycline. In an effort to better understand the significance of the crystal structure analysis we have also measured the bathochromic shift in the uv spectra for oxytetracycline as a function of pH in the presence and absence of metal chelating agents. Our interpretation of the results of these analyses is the topic of this report.

Experimental Section

Solutions for the uv spectrophotometric measurements were prepared in the following manner not more than 15 min before the spectra were determined. Aqueous 0.1 M KCl[16] was used as the electrolytic solvent for all solutions including the 1.0×10^{-3} M NaOH solution used to adjust pH. The final concentrations employed were 9.05×10^{-5} M for oxytetracycline hydrochloride and 0.01 M for the appropriate divalent metal salt. The pH was determined with a standardized combined glass reference electrode[17] for solutions maintained under a nitrogen atmosphere to avoid CO_2 contamination from the air. Each absorption spectrum was measured three times with 1.000-cm quartz cells maintained at 23.2 ± 0.5 °C.

The crystalline mercuric chloride complex of oxytetracycline was prepared from a 50% aqueous–methanol solution containing oxytetracycline free base and excess $HgCl_2$. Suitable crystals for a crystal structure analysis were obtained within 2 days from a solution maintained at approximately 8 °C.

The x-ray diffraction symmetry and systematic absences were uniquely assignable to space group $P2_12_12_1$. Crystallographic data were obtained from a $0.025 \times 0.10 \times 0.40$ mm crystal with a Syntex $P\bar{1}$ autodiffractometer equipped with a low-temperature device (Syntex LT-1) operating at approximately −150 °C. Monochromatized Mo $K\alpha$ radiation was used for all measurements. The lattice parameters, a = 11.377 (1), b = 17.277 (2), and c = 12.731 (2) Å, were determined from a least-squares refinement[18] of the automatically centered 2θ values obtained for 57 reflections within the angular range $25° < 2\theta < 36.5°$; there is one formula unit, $(C_{22}H_{24}N_2O_9)$ $HgCl_2 \cdot 2H_2O$, per asymmetric unit.

Diffraction intensities were measured in an ω-scan mode for which the scan rate was allowed to vary, as a function of maximum peak intensity, from 2.0 to 24.0° min^{-1}; a scan of 1.0° was measured and background intensity was measured on each side of the reflection for one-half the total scan time. Three reference reflections, which were monitored after each 63 data were collected, remained constant to within 2% of their initial intensities. Of the 3998 unique data measured, $\sin\theta/\lambda < 0.70$ $Å^{-1}$, 3227 were classified as subjectively observed, $I > 2\sigma(I)$. Lorentz, polarization, and absorption corrections, for which the range of the calculated absorption coefficients was 1.17–2.03, were applied to the data.

The crystalline dipotassium salt was prepared by the addition of concentrated methanolic KOH to oxytetracycline dissolved in acetone. The solution was also maintained at 8 °C and allowed to evaporate slowly. After several days a crystalline product was obtained. The crystalline material decomposed within 8 h at room temperature through an apparent initial loss of solvent molecules, as evidenced by crystal cracking, and a subsequent change in color from light yellow to dark brown.

As with the mercuric chloride complex, all crystallographic data were measured with a crystal, in this case enclosed in a thin-walled capillary tube, cooled to approximately −150 °C. The crystals display space-group symmetry $C222_1$ for which refined lattice parameters, a = 13.472 (2), b = 23.724 (6), and c = 19.643 (4) Å, were obtained from automatically centered 2θ values for 44 reflections within the angular range $22.0° < 2\theta < 35.4°$; there is one formula unit, $K_2C_{22}H_{22}N_2O_9 \cdot 2H_2O \cdot 2CH_3OH$, per asymmetric unit.

Diffraction intensities, for a $0.20 \times 0.10 \times 0.25$ mm parallelepiped crystal, were measured as described above with the exception that the scan range was reduced to 0.80° and the three reference reflections were monitored after each 129 data were measured; they remained constant to within 3% of their initial intensities and there was no indication of crystal decomposition; of the 3084 unique data measured, $\sin\theta/\lambda < 0.60$, 2028 were classified as observed $[I > 3\sigma(I)]$. Lorentz and polarization corrections were applied to the data; absorption corrections were not considered necessary (μ = 3.6 cm^{-1}).

Structure Determination and Refinement. The structural model for the mercuric chloride complex was obtained by the heavy atom technique; the space-group enantiomorph was selected to conform with the absolute configuration assigned to oxytetracycline.[19] Because of the dominant character of the Hg atom contribution to the diffracted intensities, no effort was made to locate the hydrogen atoms.

The fractional atomic coordinates and anisotropic temperature factors for all atoms of the structural model were refined by block-diagonal least-squares techniques employing the parameters of one atom in each block. In addition to the observed data, those data classified as unobserved for which the calculated structure factor was greater than the cut-off value were also used in the refinement. In this manner, 3698 data contributed to the refinement of 343 parameters to produce a conventional R factor of 0.048 and an empirically weighted R_w of 0.056; the estimated standard deviation of an observation of unit weight is 1.29.

The initial structural model for the dipotassium salt of oxytetracycline was obtained by direct methods and the space-group enantiomorph was selected as above. Because of unusually high thermal motion in some parts of the structure, not all of the hydrogen atoms were located by difference Fourier techniques. Those that have been located were included with isotropic temperature factors in the refinement. All appropriate fractional atomic coordinates and anisotropic temperature factors for the potassium, carbon, nitrogen, and oxygen atoms have been refined. Refinement was affected by application of block-diagonal least-squares techniques with the blocks constructed such that the scale factor was refined in one block and the remaining blocks contained the variables of one potassium, carbon, nitrogen, or oxygen atom and any hydrogen atom bonded to it. A total of 2566 reflections contributed to the refinement of 372 parameters

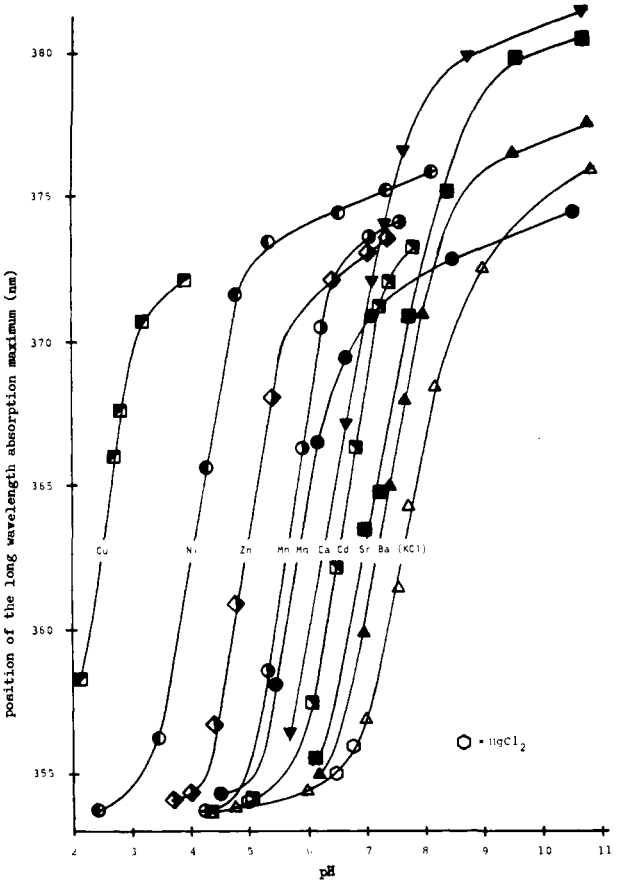


Figure 1. Position of the long wavelength absorption maximum vs. pH for oxytetracycline in the presence of various divalent metal cations.

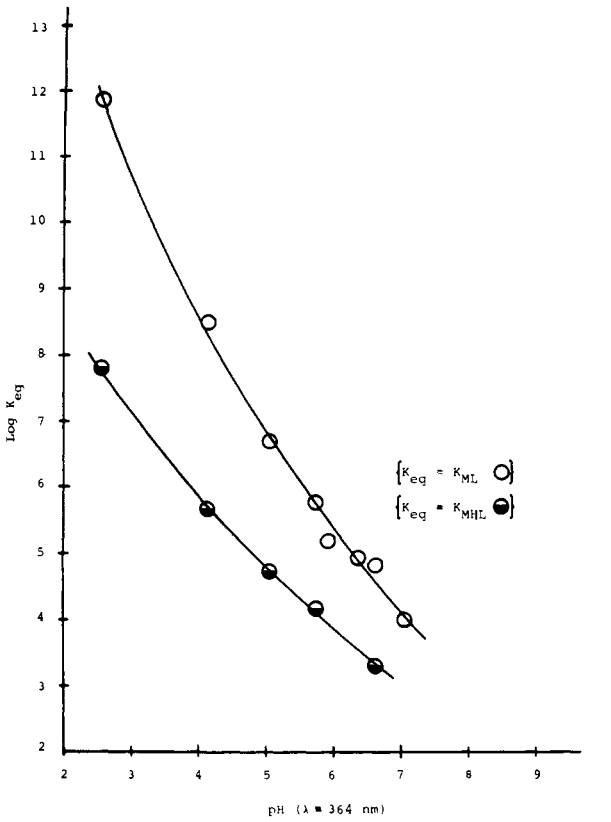


Figure 2. Correlation between the pH value at which the long wavelength absorption maximum is 364 nm and log K_{MHL} or log K_{ML}.[1]

to give $R = 0.080$, $R_w = 0.097$ and an estimated standard deviation of an observation of unit weight equal to 1.61.[20]

Results and Discussion

The position of the long-wavelength absorption maximum of the oxytetracycline molecule in the presence of various divalent metal cations, along with that for oxytetracycline in the 0.1 M KCl electrolyte, is presented graphically as a function of pH in Figure 1. This absorption has been demonstrated to be associated with the BCD chromophore[9] of the tetracycline derivatives. The pH region in which the bathochromic shift for the uncomplexed molecule lies indicates that the initial shift corresponds to an increase in the concentration of the monovalent oxytetracycline anion,[21] which we believe provides additional evidence that the principal dissociation site for the second proton in the overall deprotonation scheme for the tetracycline derivatives is from the BCD chromophore.[22] The continuation of the spectral shift, generally with a more modest slope, may be taken as an indication that the dissociation of a second proton from the BCD chromophore contributes significantly to the acid-base dissociation behavior at higher pH[23] and thus a microspecies with a doubly dissociated BCD chromophore may contribute significantly to the concentration of the divalent anion.

In the presence of alkaline earth and divalent transition metal cations, the shift in the long-wavelength absorption maxima occurs at lower pH. The pH regions in which the shifts occur are correlatable with the reported stability constants of the MHL and ML complexes[1] (Figure 2). Consequently, as indicated by Conover,[9] the increased acidity of the BCD chromophore appears to be directly related to complex formation.

The crystal structure analysis of the mercuric chloride complex with oxytetracycline was initially undertaken in the hope that it would provide a typical example for the divalent metal complexes of the tetracycline derivatives. Unfortunately, as indicated by the lack of displacement in the long-wavelength absorption maxima curve displayed in Figure 1, the interaction of $HgCl_2$ with oxytetracycline in solution was found to be atypical in that there was no indication of increased acidity in the BCD chromophore of the ligand and also in that the long-wavelength absorption spectra were found to be time dependent above pH 7. Thus the complex observed in the crystalline state may not be considered to be representative of the complexes formed by the transition metal and alkaline earth cations. However, an examination of the observed complex, in conjunction with the anomalies in the solution chemistry and the crystal structure of the dipotassium salt, stands to contribute to our understanding of the chemistry of the tetracycline antibiotics.

Electronically neutral mercuric chloride is complexed with zwitterionic oxytetracycline to give a complex which may be formulated as an MH_2L complex, whereas most 1:1 transition metal and alkaline earth metal complexes have been characterized as either MHL or ML complexes.[1] Such characterization is generally compatible with our interpretation of the spectral shift curves presented here and the observed bond distances in this crystal structure[24] (Tables I and II). The ligand in the observed complex utilized oxygen atoms of the tricarbonylmethane moiety of the A ring, Figure 3 (top), to form rather weak, though not insignificant, covalent bonds with the central mercury atom. The Hg-O interatomic distances are considerably longer than those between mercury and the chlorine atoms; however, they are significantly shorter than the sum of the van der Waals radii for the mercury and oxygen atoms, 3.13 Å,[25] and are comparable with those reported for the crystalline bis(2-imidazolidinone)mercury(II) chloride complex.[26]

As can be readily seen from a comparison of the bond distances and dihedral angles (Tables I and III) in the complexed

Table I. Bond Distances (Å) to Carbon, Nitrogen, and Oxygen Atoms for the Chelated Oxytetracycline Molecular Ions

Bond	$OTC^{\pm}$	OTC^{2-}
C_1-O_1	1.235 (10)[a]	1.261 (11)
C_1-C_2	1.415 (12)	1.428 (13)
C_1-C_{12a}	1.571 (11)	1.552 (13)
C_2-C_3	1.426 (11)	1.413 (12)
C_2-C_{2am}	1.471 (12)	1.482 (13)
C_{2am}-O_{2am}	1.277 (11)	1.263 (11)
C_{2am}-N_{2am}	1.338 (12)	1.351 (13)
C_3-O_3	1.236 (10)	1.270 (11)
C_3-C_4	1.565 (12)	1.537 (13)
C_4-C_{4a}	1.521 (12)	1.520 (13)
C_4-N_4	1.491 (11)	1.488 (13)
N_4-C_{4m1}	1.504 (12)	1.521 (14)
N_4-C_{4m2}	1.492 (12)	1.484 (16)
C_{4a}-C_5	1.555 (12)	1.538 (13)
C_{4a}-C_{12a}	1.546 (11)	1.506 (12)
C_5-C_{5a}	1.548 (12)	1.507 (14)
C_5-O_5	1.414 (10)	1.446 (11)
C_{5a}-C_6	1.553 (12)	1.543 (17)
C_{5a}-C_{11a}	1.515 (12)	1.536 (14)
C_6-C_{6a}	1.541 (12)	1.532 (19)
C_6-O_6	1.450 (11)	1.464 (16)
C_6-C_{6m}	1.506 (13)	1.552 (19)
C_{6a}-C_7	1.377 (13)	1.393 (24)
C_{6a}-C_{10a}	1.424 (12)	1.394 (18)
C_7-C_8	1.421 (13)	1.416 (32)
C_8-C_9	1.390 (14)	1.424 (32)
C_9-C_{10}	1.430 (14)	1.397 (20)
C_{10}-C_{10a}	1.399 (13)	1.440 (16)
C_{10}-O_{10}	1.345 (13)	1.328 (14)
C_{10a}-C_{11}	1.442 (12)	1.462 (15)
C_{11}-C_{11a}	1.467 (12)	1.415 (13)
C_{11}-O_{11}	1.254 (10)	1.312 (10)
C_{11a}-C_{12}	1.355 (12)	1.422 (13)
C_{12}-C_{12a}	1.521 (12)	1.536 (13)
C_{12}-O_{12}	1.338 (12)	1.258 (10)
C_{12a}-O_{12a}	1.429 (10)	1.447 (11)

[a] The numbers enclosed in parentheses are the estimated standard deviations in the least significant digits.

zwitterionic oxytetracycline with those reported for the crystalline zwitterionic free base molecules in the preceding paper,[27] the ligand in general, and the BCD chromophore in particular, is virtually unaltered by the formation of the observed complex. This is consistent with our observations concerning the uv absorption spectra below pH 7 and provides evidence that complex formation utilizing only the tricarbonylmethane moiety of the A ring will not account for the increased acidity of the BCD chromophore displayed by the other complexes investigated.

In contrast to the mercuric chloride complex, the dipotassium salt of oxytetracycline provides several indications of metal ion interaction with the BCD chromophore. The crystals contain three symmetry-independent potassium ions, two of which lie on a crystallographic twofold symmetry axis and all of which interact with both the A-ring tricarbonylmethane and BCD chromophores. The potassium ions are distributed in a planar array in a manner that allows them to be sandwiched between two symmetry-related oxytetracycline molecular anions (Figure 3, bottom). The postassium ion, K_3, which occupies a general crystallographic position is coordinated (Table II) to atoms O_1 and O_{2am} of the tricarbonylmethane moiety of one molecule and to atom O_3 of a symmetry-related molecule in a manner similar to that displayed by the mercuric chloride complex, Table II and Figure 3 (top). This potassium ion is also coordinated to atoms O_{11} and O_{12} of still another symmetry-related oxytetracycline molecular anion. The two

Table II. Environmental Parameters for the Hg(II) Atom and K^+ Ions

	Distances to Hg, Å		
Hg-Cl_1	2.289 (3)[a]	Hg-O_{2am}	2.654 (6)
Hg-Cl_2	2.273 (4)	Hg-O_{w2}	3.025 (6)
Hg-O_1	2.796 (4)	Hg-O_3	3.218 (7)
	Angles, Deg		
Cl_1HgCl_2	169.1 (4)	Cl_1HgO_3	85.5 (6)
Cl_1HgO_1	89.4 (6)	Cl_1HgO_{2am}	97.4 (6)
Cl_1HgO_{w2}	84.8 (6)	Cl_2HgO_1	99.0 (6)
Cl_2HgO_{2am}	92.7 (6)	Cl_2HgO_{w2}	91.6 (6)
Cl_2HgO_3	83.9 (6)	O_1HgO_{2am}	60.6 (7)
O_1HgO_{w2}	147.9 (7)	O_1HgO_3	152.6 (7)
$O_{2am}HgO_{w2}$	88.9 (7)	$O_{2am}HgO_3$	146.8 (7)
$O_{w2}HgO_3$	58.4 (7)		
	Distances to K_1, Å		
K_1-O_1	3.134 (10)	K_1-O_{12a}	2.840 (10)
K_1-O_{12}	2.679 (9)	K_1-O_{m2}	2.666 (10)
	Angles, Deg		
$O_1K_1O_{12a}$	59.6 (8)	$O_1'K_1O_{12a}$[b]	125.8 (9)
$O_1K_1O_{12}$	60.5 (8)	$O_1'K_1O_{12}$	70.7 (8)
$O_1K_1O_{m2}$	142.4 (10)	$O_1'K_1O_{m2}$	72.9 (10)
$O_1K_1O_1'$	104.8 (9)	$O_{12a}K_1O_{12a}'$	172.6 (9)
$O_{12a}K_1O_{12}$	56.2 (8)	$O_{12a}'K_1O_{12}$	130.0 (9)
$O_{12a}K_1O_{m2}$	90.6 (9)	$O_{12a}'K_1O_{m2}$	86.4 (9)
$O_{12}K_1O_{m2}$	84.5 (10)	$O_{12}'K_1O_{m2}$	130.2 (10)
$O_{12}K_1O_{12}'$	95.3 (9)	$O_{m2}K_1O_{m2}'$	131.8 (12)
	Distances to K_2, Å		
K_2-O_1	3.069 (10)	K_2-O_{12}	2.752 (10)
K_2-O_{11}	2.792 (9)	K_2-O_{m1}	2.722 (10)
	Angles, Deg		
$O_1K_2O_{12}$	60.7 (8)	$O_1'K_2O_{12}$	70.9 (8)
$O_1K_2O_{11}$	105.1 (9)	$O_1'K_2O_{11}$	88.6 (8)
$O_1K_2O_{m1}$	79.5 (8)	$O_1'K_2O_{m1}$	169.9 (10)
$O_1K_2O_1'$	108.0 (9)	$O_{12}K_2O_{12}'$	92.0 (8)
$O_{12}K_2O_{11}$	58.0 (8)	$O_{12}'K_2O_{11}$	143.9 (9)
$O_{12}K_2O_{m1}$	108.4 (10)	$O_{12}'K_2O_{m1}$	129.2 (10)
$O_{11}K_2O_{m1}$	81.5 (8)	$O_{11}'K_2O_{m1}$	82.8 (8)
$O_{11}K_2O_{11}'$	156.9 (10)	$O_{m1}K_2O_{m1}'$	94.0 (9)
	Distances to K_3, Å		
K_3-O_1	2.644 (9)	K_3-O_3	2.759 (9)
K_3-O_{2am}	2.778 (9)	K_3-O_{12}	2.619 (9)
K_3-O_{11}	2.901 (10)	K_3-O_{w1}	3.373 (11)
K_3-O_{w2}	2.693 (9)	K_3-O_{m2}	3.252 (10)
	Angles, Deg		
$O_1K_3O_3$	117.9 (9)	$O_1K_3O_{2am}$	60.2 (8)
$O_1K_3O_{12}$	80.0 (8)	$O_1K_3O_{11}$	95.1 (8)
$O_1K_3O_{w1}$	66.4 (9)	$O_1K_3O_{w2}$	132.0 (10)
$O_1K_3O_{m2}$	71.1 (10)	$O_3K_3O_{2am}$	81.1 (9)
$O_3K_3O_{12}$	121.5 (9)	$O_3K_3O_{11}$	64.9 (8)
$O_3K_3O_{w1}$	51.5 (9)	$O_3K_3O_{w2}$	96.9 (10)
$O_3K_3O_{m2}$	161.6 (10)	$O_{2am}K_3O_{12}$	140.2 (10)
$O_{2am}K_3O_{11}$	121.8 (9)	$O_{2am}K_3O_{w1}$	52.8 (9)
$O_{2am}K_3O_{w2}$	97.4 (10)	$O_{2am}K_3O_{m2}$	91.2 (10)
$O_{12}K_3O_{11}$	58.0 (8)	$O_{12}K_3O_{w1}$	113.2 (9)
$O_{12}K_3O_{w2}$	110.2 (9)	$O_{12}K_3O_{m2}$	74.6 (9)
$O_{11}K_3O_{w1}$	69.3 (9)	$O_{11}K_3O_{w2}$	130.8 (10)
$O_{11}K_3O_{m2}$	132.4 (10)	$O_{w1}K_3O_{w2}$	135.4 (10)
$O_{w1}K_3O_{m2}$	134.1 (10)	$O_{w2}K_3O_{m2}$	67.4 (9)
	Interatomic Distances within Methanol Groups, Å		
C_{m1}-O_{m1}	1.449 (18)	C_{m2}-O_{m2}	1.610 (27)

[a] The numbers enclosed in parentheses are the estimated standard deviations in the least significant digits. [b] Atoms designated with the sign (') are related to their subscripted counterparts by twofold crystallographic symmetry.

potassium ions on the crystallographic twofold axis occupy coordination sites which may be somewhat more representative of the ligand-metal ion interactions in solutions. Each of these

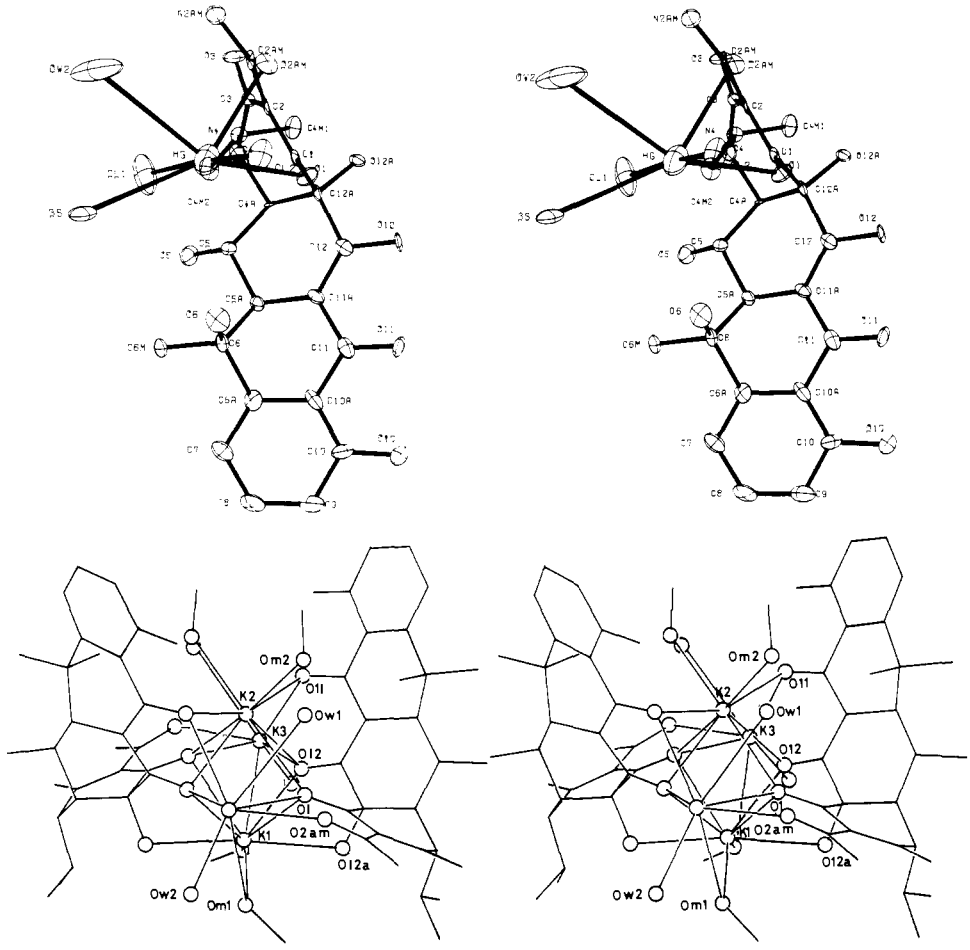


Figure 3. Stereoscopic projections displaying oxytetracycline metal atom interactions. Top: zwitterionic oxytetracycline mercuric chloride. Bottom: oxytetracycline dipotassium salt.

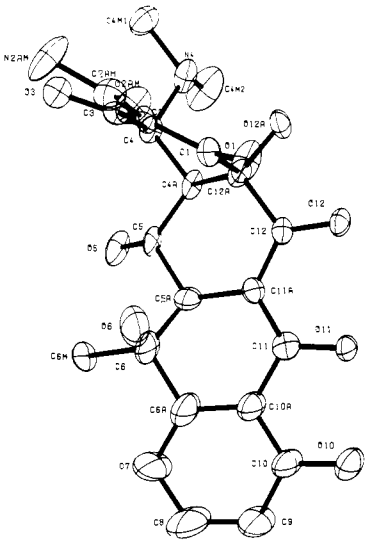
Table III. Dihedral Angles (Deg) for Chelated Oxytetracycline Molecular Ions

Atoms	$OTC^{\pm}$	OTC^{2-}
$C_{12}C_{12a}C_1C_2$	−176.3	−150.8
$C_{12a}C_1C_2C_3$	14.8	3.2
$C_1C_2C_3C_4$	26.5	−9.2
$C_2C_3C_4C_{4a}$	−29.0	36.5
$C_3C_4C_{4a}C_5$	112.4	65.9
$C_4C_{4a}C_{12a}C_1$	49.0	54.4
$C_{11}C_{11a}C_{12}C_{12a}$	−179.3	−168.2
$C_{11a}C_{12}C_{12a}C_1$	98.2	89.5
$C_{12}C_{12a}C_{4a}C_5$	49.1	55.6
$C_4C_{4a}C_5C_{5a}$	−178.3	−175.4
$C_{4a}C_5C_{5a}C_6$	158.0	154.9
$C_5C_{5a}C_{11a}C_{12}$	−7.3	−9.1
$C_{10}C_{10a}C_{11}C_{11a}$	165.3	169.2
$C_{10a}C_{11}C_{11a}C_{12}$	174.3	170.1
$C_{11}C_{11a}C_{5a}C_6$	47.2	45.7
$C_5C_{5a}C_6C_{6a}$	172.0	175.0
$C_{5a}C_6C_{6a}C_7$	−141.1	−152.7
$C_6C_{6a}C_{10a}C_{11}$	−0.2	−3.8
$C_8C_9C_{10}C_{10a}$	−5.6	−6.4
$C_9C_{10}C_{10a}C_{11}$	−177.0	−178.2
$C_{10}C_{10a}C_{6a}C_7$	−2.4	6.4
$C_6C_{6a}C_7C_8$	179.4	−179.0
$C_{6a}C_7C_8C_9$	1.9	4.5
$C_7C_8C_9C_{10}$	1.9	9.6
$C_1C_2C_{2am}O_{2am}$	3.1	−4.4
$C_2C_3C_4N_4$	−162.8	−86.0
$C_3C_4N_4C_{4m1}$	77.8	−53.1
$C_3C_4N_4C_{4m2}$	−155.8	−169.1

ions interacts with only two oxytetracycline anions, those related by the twofold axis containing the cations. Potassium ion K_1 is coordinated to oxygen atoms O_1, O_{12}, and O_{12a} from each of these anions while ion K_2 is similarly coordinated to atoms O_1, O_{12}, and O_{11} of the same pa of molecular anions. The intramolecular interaction, through metal ion coordination, between the BCD and A-ring chromophores appears to be greatest for the coordination site occupied by potassium ion K_2; however, either this site or that occupied by ion K_1 appears to present suitable model sites for the ML or ML_2 complexes.

The strongly basic conditions under which the dipotassium salt was crystallized clearly do not fall within the physiological pH range; however, the pronounced shift in acidity displayed by the ligand in the complexed form (Figure 1) indicates that the divalent anion may play a role in the biological activity of the tetracyclines. It therefore seems appropriate to examine the molecular anion in some detail.

The bonding geometry of the crystalline divalent molecular anion displays several variations in comparison with the free base forms or the fully protonated examples.[28] Unfortunately, the high thermal motion displayed by portions of the molecular anion, even though the crystal was cooled to ca. −150 °C, has resulted in rather poor estimated precision in individual bond distances. This rather limits the analysis of the bonding geometry; however, there are sufficient systematic differences in those regions of the molecular anion displaying low thermal motion[29] to allow some insight to be gained as to the influence of the relatively high negative charge on the bonding geometry in the chromophoric groups.



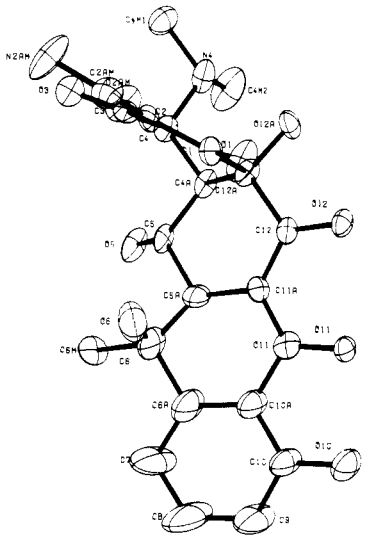


Figure 4. Stereoscopic projection of the oxytetracycline anion.

Table IV. Distances (Å) between Hydrogen Bonded[a] C, N, and O Atoms in the Zwitterionic Oxytetracycline Mercuric Chloride Complex and in the Dipotassium Salt

I. Zwitterionic Oxytetracycline Mercuric Chloride, Intramolecular			
O_3–N_4	2.58 (1)[b]	O_3–N_{2am}	2.66 (1)
O_{11}–O_{10}	2.60 (1)	O_{11}–O_{12}	2.49 (1)
Intermolecular			
O_1–O_{w1}	2.75 (1)	O_{2am}–O_{12a}	2.78 (1)
N_4–O_{w2}	2.89 (1)	O_6–O_{w1}	2.81 (1)
O_{10}–O_{w1}	2.96 (1)		
II. Oxytetracycline Dipotassium Salt, Intramolecular			
N_4–O_{12a}	2.65 (1)	O_{10}–O_{11}	2.49 (1)
Intermolecular			
O_{2am}–O_{w1}[a]	2.78 (1)	O_{2am}–O_5	2.79 (1)
O_3–O_{w1}[a]	2.72 (1)	O_6–O_{m1}	2.72 (2)
O_6–O_{w2}	2.83 (2)		

[a] The pairs of atoms designated with this symbol share adjacent coordinations sites with the same potassium ion and thus may or may not be hydrogen bonded. [b] The numbers enclosed in parentheses are the estimated standard deviations in the least significant digits.

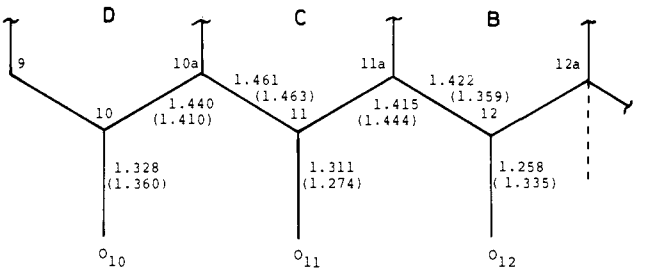
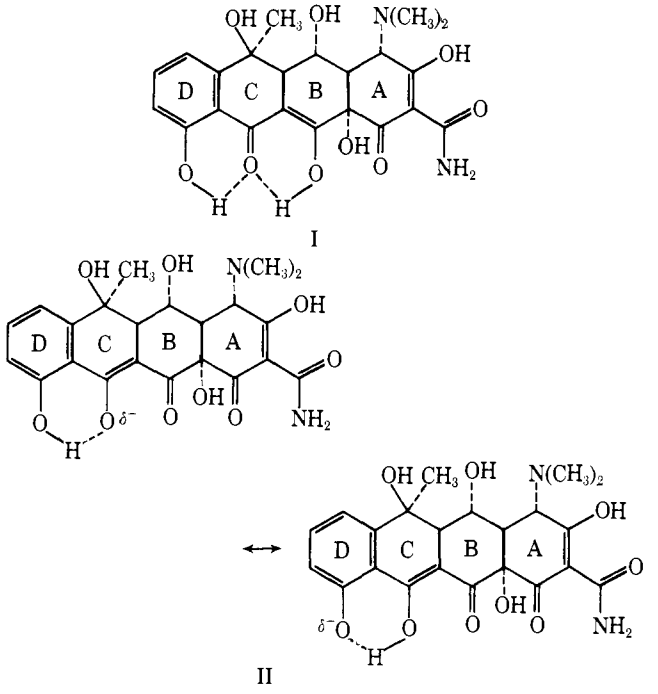


Figure 5. The average bond distances for the nonionized BCD chromophore (in parentheses) as determined from the crystal structure analyses of free base tetracycline derivatives[27] and the bond distances for the BCD chromophore of the divalent anion.

The A-ring tricarbonylmethane system displays an amide group orientation and bonding geometry which resemble that displayed by zwitterionic tetracycline. Those differences which do appear are consistent with the effects of increased negative charge. For example, there is near equivalence in the bond pairs (C_1–O_1, C_3–O_3) and (C_1–C_2, C_2–C_3); however, in comparison with the tetracycline zwitterion, the C–C distances are somewhat shorter and the C–O distances are longer, which brings the latter into equivalence with the analogous bond distances in the amide groups for both structures. This equivalency in bond distances is indicative of uniform charge distribution throughout the nearly planar tricarbonylmethane chromophore; the estimated standard deviation of the least-squares plane fit to atoms C_{12a}, C_1, O_1, C_2, C_{2am}, O_{2am}, N_{2am}, C_3, and O_3 is 0.067 Å. It was demonstrated in the preceding report that the bonding geometry of the dimethylamine substituent differs significantly between the zwitterionic and nonionized free base molecules. In the divalent anion, this group shows rather high thermal motion (Figure 4) which almost certainly accounts for the internal differences in the observed C–N bond distances (Table I). Thus it seems probable that the bonding geometry within this group resembles that in the zwitterionic forms and that the strong hydrogen bond between the nitrogen atom and the hydroxyl group at C_{12a} (Table IV) compensates for the deprotonation.

We believe the shift in the long-wavelength absorption maximum as a function of pH indicates that the BCD chromophore is greatly affected by loss of the second proton in the overall deprotonation scheme for oxytetracycline and that differences in the observed bond distances, relative to the free base forms, should be observed. Fortunately, as a result of the extensive interaction of this portion of the molecule with the potassium ions in the crystalline salt, the sequence of atoms O_{10}, C_{10}, C_{10a}, C_{11}, O_{11}, C_{11a}, C_{12}, O_{12} displays nearly isotropic thermal parameters. The resultant bond distances, though not as precise as might be desired, can be expected to be reasonably valid for comparison with those reported for the free base derivatives. Such a comparison is facilitated by examination of Figure 5, which displays the average bond distances for the free base derivatives and those observed here. As expected, there are striking differences in the bond distances presented; furthermore, these differences reflect a change in the tautomeric form of the BCD chromophore as well as the change in its ionization state. When fully protonated, the BCD chromophore has displayed tautomeric form I in Chart I. This tautomer is clearly favored by strong intramolecular hydrogen bonding between the carbonyl group at C_{11} and the β-hydroxyl groups at C_{10} and C_{12}. In contrast, the negatively charged BCD chromophore displays the tautomeric forms displayed in II of Chart I. The C–O distance appropriate for a carbonyl group is that displayed at C_{12}, while the nearly equivalent C–O distances at C_{10} and C_{11} are significantly shorter than those observed for the hydroxyl groups in the nonionized chromophore. There are also significant changes in the C–C bond

Chart I. BCD Chromophore Tautomers[a]



[a] Tautomer I is that observed for the nonionized chromophore. Tautomer II is that observed in the crystalline dipotassium salt of oxytetracycline.

distances within the chromophore, particularly for the C_{11}-C_{11a} and C_{11a}-C_{12} bonds. The resultant bonding geometry within the sequence of atoms C_{11}, C_{11a}, C_{12}, O_{12}, C_{12a} is very similar to that of the A-ring chromophore sequence C_3, C_2, C_1, O_1, C_{12a} (see Figure 4). This similarity provides further indication that the coordination sites occupied by cations K_1 and K_2 are particularly suitable for covalent interaction between metal ions and the charge centers of the ligand in ML and ML_2 complexes.

The equilibrium represented in II of Chart I may provide a partial explanation of the highly anisotropic temperature factors of the D-ring carbon atoms (see Figure 4) of the divalent anion. The formal difference in the two tautomers represented is the position of the bonded hydrogen atom associated with the C and D ring C-O moieties. It is clear, from the O-H distances observed for the nonionized chromophores,[27] that a very modest change in the position of this hydrogen atom would be associated with the alternate bonding sites. Thus it is easily conceivable that a dynamic equilibrium of these tautomers occurs in the crystalline state. A modest conformational change in the CD ring juncture associated with the transition between the tautomeric forms would contribute to the apparent high thermal motion observed in the cooled crystal.

The oxytetracycline divalent anion displays a conformation (Figure 4) not previously observed in the crystalline state; thus it presents the third emphatically different conformation for the biologically active tetracycline derivatives to be encountered in crystal structure analyses. The dihedral angles presented in Table III characterize the conformation in a quantitative manner consistent with those reported previously.[27,30] It also seems appropriate at this time to compare these conformations in a more qualitative, though more readily visualized manner. This may be accomplished by comparing the conformations of the various rings in terms of either a planar conformation, which suitably characterizes the D ring for the three conformations, an envelope, or a half-chair conformation. The latter possibilities serve to characterize the remaining rings. The selection of the conformational model for each ring has been made on the basis of ring geometry as reflected by a comparison of the intra-ring dihedral angles in the manner utilized in the characterization of the conformation of steroid ring systems.[31] Table V presents a summary of the deviations from ideal symmetry, ΔC_s or ΔC_2, for the best fit to the envelope and/or half-chair conformations, respectively. The C rings of the oxytetracycline derivatives display nearly identical conformations, that of a half-chair for which the twofold symmetry axis relating the dihedral angles passes through the C_{5a}-C_6 and C_{10a}-C_{11} bonds.

Table V. Deviations from Ideal Ring Symmetry for the Envelope (ΔC_s) and/or the Half-Chair (ΔC_2) Conformation as Displayed by the Symmetry of Intra-ring Dihedral Angles (Deg)[c]

Ring	OTC^+	$OTC^{\pm}$	OTC	OTC^{2-}
A	ΔC_s 1.4	ΔC_s 3.4	ΔC_s 9.1	ΔC_s 6.9
	C_{12a},C_3[a]	C_{12a},C_3	C_{12a},C_3	C_{4a},C_2
B	ΔC_2 7.9		ΔC_2 16.0	
	[(C_{11a}-C_{12}) (C_{5a}-C_6)][b]		[(C_{4a}-C_{12a}) (C_5-C_{5a})]	
	ΔC_s 14.9	ΔC_s 3.6	ΔC_s 17.4	ΔC_s 4,9
	C_{4a},C_{11a}	C_{4a},C_{11a}	C_{4a},C_{11a}	C_{12a},C_{5a}
C	ΔC_2 3.3	ΔC_2 4.7	ΔC_2 6.8	ΔC_2 5.0
	[(C_6-C_{5a}) (C_{10a}-C_{11})]	[(C_6-C_{5a}) (C_{10a}-C_{11})]	[(C_6-C_{5a}) (C_{10a}-C_{11})]	[(C_6-C_{5a}) (C_{10a}-C_{11})]

[a] The mirror plane relating symmetry equivalent intra-ring dihedral angles of an ideal envelope conformation passes through the indicated atoms. [b] The twofold axis relating the symmetry equivalent intra-ring dihedral angles of an ideal half-chair conformation passes through the midpoint of the bond between the indicated atoms. [c] See ref 31.

Minor differences in the conformations displayed by the cation and the zwitterionic free base molecule, as well as the larger differences in the three highly different conformations, emerge in the analysis of the conformations displayed by the B rings. The B ring of the cation (OTC^+) displays a conformation more nearly that of a half-chair, while the zwitterionic free base ($OTC^{\pm}$) displays an envelope conformation in which atom C_{4a} is displaced in the direction of its hydrogen atom from the plane of the remaining five ring atoms. The nonionized molecule (OTC) presents a B-ring conformation that is midway between the envelope and half-chair forms. If one selects the envelope conformation to characterize this ring, atom C_{4a} is displaced in the opposite direction from the appropriate plane as it is in the zwitterionic molecule. The divalent anion (OTC^{2-}) displays an envelope conformation with atom C_{12a}, rather than atom C_{4a}, in the conformationally unique position and displaced toward atom C_1 from the usual plane.

The cation and zwitterionic free base display similar A-ring conformations which may be characterized as an envelope form with atom C_{12a} displaced in the direction of its hydroxyl group from the appropriate five-atom plane. This ring may be similarly characterized for the nonionized molecule; however, once again, the displacement of the unique atom is inverted relative to that of the zwitterionic free base. The divalent anion also displays an envelope conformation for the A ring but in this case atom C_{4a} is the unique atom and is displaced in the direction of atom C_5.

Thus if one characterizes the A and B rings in each of the three definitively different conformations as envelope forms, the free base conformations display the same atoms in the unique positions; however, their displacement from the appropriate planes is in the opposite direction for the two conformations. In contrast, the divalent anion conformation has interchanged the unique atoms of the A and B rings in comparison with the other conformations. These observations lead one to speculate as to the existence of a fourth conformation related to that of the divalent anion in the manner in which the

zwitterionic free base and the nonionized molecule are related, by inversion of the displacement of the conformationally unique atoms. Examination of a molecular model reveals an open conformation similar to that of the zwitterionic derivatives but with the fold about the A–B ring juncture in the opposite direction.

Observations in our laboratory concerning the chemical structure and conformation adopted by oxytetracycline under varying pH conditions in the presence and absence of metal chelating agents have lead us to considerably different conclusions than those reported by Mitscher et al.[11] based upon their analysis of extensive CD measurements made under similar conditions. While we are in general agreement with their conclusion that the biologically active tetracycline derivatives display essentially the same conformation in aqueous solutions in the pH range associated with physiological activity, we do not concur with their conclusion that under rather alkaline conditions oxytetracycline displays the conformation proposed by Schach von Wittenau and Blackwood.[32] The preceding report[27] describes the conditions under which we believe this conformation may be expected to occur. The conformation we have observed in the crystalline dipotassium salt appears not to have been considered by Mitscher et al. The observed conformation displays some of the attributes they assign to their proposed conformation for the non-5-hydroxylated tetracycline derivatives in alkaline solution or in metal ion complexes. Because of inadaquate characterization of their proposed conformation, it is difficult to make a definitive comparison; however, the following points are indicated. The drawing presented displays an open conformation in which there is a hydrogen bond between the dimethylamine group and the 12a-hydroxyl group similar to that displayed by the crystalline divalent oxytetracycline anion. The general conformation depicted for the A ring also seems similar to that reported here. Thus the differences in the conformation of the anionic (or complexed) 5-hydroxylated derivatives from their nonhydroxylated analogues appear to arise from a change in the conformation of the B ring. The conformation proposed by Mitscher et al. for the nonhydroxylated derivatives appears to display eclipsed C_5–O_5 and C_6–C_{6m} bonds. As a consequence of the resultant peri interaction between the 6-methyl and 5-hydroxyl groups, the authors felt this conformation was unsuitable for oxytetracycline derivatives. The conformation we report displays a $C_{4a}C_5C_{5a}C_6$ dihedral angle of 154.9° which may be compared with the 180° dihedral angle required for the eclipsed conformation.[33] Thus the observed conformation reduces the close nonbonded contact between the peri groups. Examination of a molecular model indicates that the eclipsed bond geometry and the open conformation proposed by Mitscher et al. are achieved when the B ring adopts the half-chair form with the twofold symmetry axis passing through the C_{11a}–C_{12} and C_5–C_{4a} bonds. As described above, the oxytetracycline divalent anion displays the B ring in an envelope conformation and thus presents a more sharply folded molecular conformation than that proposed for the non-5-hydroxylated derivatives. We feel that the similarities in the A-ring conformation observed for the divalent oxytetracycline anion and that proposed by Mitscher et al. for the nonhydroxylated derivatives are consistent with the conformational integrity displayed by the fully protonated and zwitterionic derivatives and that these conformational models may well provide suitable models for the ligand conformations in the various metal ion complexes involving one metal ion. Furthermore, our interpretation of the conformation proposed by Mitscher et al. for non-5-hydroxylated tetracycline derivatives presents a similar orientation of the O_1, O_{12a}, O_{12}, and O_{11} oxygen atoms to that observed for the oxytetracycline divalent anion. Thus we feel that a strong case has been built for metal ion coordination to both the BCD and A-ring chromophores in which the principal coordination site might be expected to utilize ligand atoms O_1, O_{12}, and O_{11}.

A second cation may coordinate to the BCD chromophore, most likely through atoms O_{10} and O_{11}, or to the N atom of the dimethylamine group (Scheme I). The long-wavelength uv

Scheme I[a]

$$(--0)(M^{2+})_2 + H^+ \quad (K_N)$$

$$M^{2+} + (--+)M^{2+}$$

$$(-2-+)(M^{2+})_2 + H^+ \quad (K_O)$$

$$K_N = \frac{[(--0)(M^{2+})_2][H^+]}{[(--+)M^{2+}][M^{2+}]} \qquad K_O = \frac{[(-2-+)(M^{2+})_2][H^+]}{[(--+)M^{2+}][M^{2+}]}$$

[a] The symbols in parentheses indicate the ionization state of the A ring, the BCD chromophore, and the dimethylamine group, respectively.

absorption maximum should display a bathochromic shift only when the coordination involves the BCD chromophore. In comparison with excess K^+ ion (which may be considered to present little coordination effect), Ca^{2+} and Sr^{2+} display a much stronger bathochromic shift indicating that $K_O > K_N$. In contrast, the curve for the Mg ion displays a very different behavior in the pH region where coordination of a second cation is expected (Figure 1), which we feel is consistent with Mg coordination to the nitrogen of the dimethylamine group ($K_N > K_O$). Furthermore, it is generally accepted that Mg displays a greater affinity for nitrogen coordination than Ca or Sr.[34] Definitive clarification of the coordination of the tetracycline derivatives in the presence of excess Ca or Mg ions can be accomplished by additional crystallographic investigations and thus we are attempting to obtain suitable crystalline material with which to continue this investigation.

It is appropriate at this time to briefly describe the hydrogen bonding displayed by these structures. The intra- and intermolecular hydrogen bonding exhibited by the zwitterionic ligand of the mercuric chloride complex shows many similarities to that of the uncomplexed zwitterionic examples[27] (Table IV). The intramolecular hydrogen bonding is virtually identical and striking similarities are observed concerning the hydrogen bonding between the tetracycline moieties and water molecules. All three zwitterionic examples display hydrogen bonds between water molecules and the dimethylamine group, the C_6-hydroxyl group, and the phenolic hydroxyl group at C_{10}. Intermolecular hydrogen bonding between complexed zwitterionic molecules occurs through the amide oxygen atom and the 12a-hydroxyl group of a symmetry-related molecule.

Because of the extensive interaction between the potassium ions and the various carbonyl groups of the divalent anion, there is relatively little hydrogen bonding in the crystalline divalent anion salt. The intramolecular hydrogen bonding is limited to that described above for the dimethylamine group and the phenolic hydroxyl group. The C_6-hydroxyl group hydrogen bonds with water molecule w2 and a methanol molecule, m1, thus bridging the coordination spheres of potassium ion K_2 and K_3. The remaining hydroxyl group, that at C_5, intermolecularly hydrogen bonds to the oxygen atom of the amide group of a symmetry-related molecule. Oxygen atoms O_{2am} and O_3 are within hydrogen bonding distannance to atom O_{w1}; however, it should be noted that all three are part of the coordination sphere of ion K_3 and consequently may or may not be hydrogen bonded. The second methanol molecule, which interacts with two potassium ions, shows no indication of hydrogen bonding.

The various bond distances and angles associated with the metal atom coordination have been presented in Table II to allow the interested reader to examine the coordination ge-

ometry of the formally six-coordinate Hg atom and eight-coordinate potassium ions. This aspect of the crystal structure will not be elaborated upon here.

Supplementary Material Available: fractional atomic coordinates, thermal parameters, bond angles between carbon, nitrogen, and oxygen atoms, and tabulated observed and calculated structure factor amplitudes (53 pages). Ordering information is given on any current masthead page.

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(17) The following equipment was used in the course of the spectral measurements: glass-reference electrode type EA 121 from Metrohm Ag., Flawyhl, Switzerland, pH meter Model DIGI 610(E) from WTW GmbH, Weilheim (Obb), Federal Republic of Germany, and a Cary 14 recording spectrophotometer from Applied Physics Corp., Monrovia, Calif.
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Ultraviolet and γ-Ray-Induced Free-Radical Reactions of Nucleic Acid Constituents. Selectivity of Some Reactions for Purines. Suppression of the Reactivity of Pyrimidines

A. A. Frimer,[1a] A. Havron,[1b] D. Leonov,[1c] J. Sperling, and D. Elad*

Contribution from the Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel.

Received December 22, 1975

Abstract: The photochemical reactions of purine and pyrimidine bases, nucleosides, nucleotides, and polynucleotides with 2-propanol, employing di-*tert*-butyl peroxide as initiator, were found to be selective for purines. The selectivity was shown to result from the suppression of the reactivity of the pyrimidines due to the presence of the purines. This was evidenced by comparison of the quantum yields for product formation, when the bases were allowed to react separately, to those in mixtures. In equimolar mixtures of pyrimidines and purines, the pyrimidine reactivity was suppressed, whereas that of the purine remained unchanged. The concentration and temperature dependence and the effect of anions on the degree of suppression of pyrimidine's reactivity in the presence of purines, as well as the correlation with changes in the osmotic coefficients upon dilution of mixtures, suggest that heteroassociation in the form of base-stacking is responsible for the effect. The thermally initiated reactions exhibit the same effect, thus indicating the general scope of the phenomenon.

The multiplicity of products formed in irradiated DNA complicates the chemical identification of products and further interferes with the correlation between a given photoproduct and the accompanying biological effect. The induction of selective photochemical modifications of specific moieties in the nucleic acid can serve as a most powerful tool for this correlation. The pyrimidine bases have been regarded as the light-sensitive sites in nucleic acids; accordingly, until recently, the study of the photochemistry of nucleic acid constituents concentrated mainly on the reactions of pyrimidines.[2] The major