would be of value only if exceptionally good data were available in a high data–parameter ratio. Nevertheless, the principal features of the molecular vibration are clear. The most rigid section of the ring is centered in the ester group at C_1; the thermal parameters at O_1, C_1, and C_2 are less than 4 Å^2, the lowest values in the structure. This observation supports the suggestion[4] that C_2 behaves like a bridgehead carbon atom in a relatively rigid ring. This proposal was advanced to explain the difficulty of deuterium exchange at C_2; the rigidity of the ring was indicated by the temperature independence of the nmr spectrum of the molecule.

The thermal parameters of the groups on either side of the ester group are also relatively low. In fact, all ring atoms in the sequence C_6, C_5, . . . , C_{12}, C_{11} have values of B less than 5 Å^2. The parameters increase markedly at C_9 and C_{10} and, not unexpectedly, are even larger at the pendant atoms O_{9a} and C_{8a}. In a very rigid organic structure, atomic thermal parameters are about 2.0–3.5 Å^2, and in a very soft structure, values of B range from 6 to 12 Å^2 and even higher. Unfortunately, it is rarely possible to distinguish the effects of intramolecular vibrations and group oscillations from overall rigid-body libration of the molecule. In the present case, the evidence for moderate ring strain and the absence of strong intermolecular interactions indicates that the ring is fairly rigid and that it librates with a maximum amplitude of oscillation at O_{9a} and C_{8a}.

Correction of bond lengths for apparent foreshortening due to molecular vibration scarcely seems warranted in the present case. Typically, the corrections are about 0.01 Å; even if the effect is somehow curiously focused at C_{10}–C_{11}, it is doubtful that it would exceed 0.02 Å at this point. This would still leave this bond inexplicably short. Moreover, since all other bonds in that region of the molecule appear to be of normal length, large libration effects are not indicated.

Acknowledgment. The authors wish to gratefully acknowledge support from the National Institutes of Health (Grants No. GM-14832 and AI-07626, and to J. J. S., Fellowship No. 5-F2AI-43,217-02), from the NSF (GP-13351), and from the Materials Science Center, Cornell University. We thank Professor H. Muxfeldt for helpful discussions.

Crystal and Molecular Structure of 5,12a-Diacetyloxytetracycline

R. B. Von Dreele and R. E. Hughes*

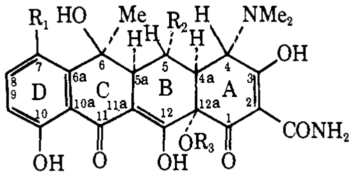
Contribution from the Department of Chemistry, Cornell University, Ithaca, New York 14850. *Received April 3, 1971*

Abstract: A crystal structure analysis of 5,12a-diacetyloxytetracycline has revealed a conformation of the tetracycline ring structure that differs markedly from the one observed in 5-hydroxytetracycline and in 7-chlorotetracycline. The principal difference between the two conformations involves a drastic twist of 108.9° about C_{4a}–C_{12a} and associated rotations about all contiguous bonds. The detailed geometries at C_4, C_{4a}, C_5, C_{5a}, and C_{12a} are consistent with the interpretation of recent nmr studies of oxytetracyclines in nonaqueous solvents. The structure was solved by direct methods analysis of data from a crystal with space group $P2_12_12_1$ and $a = 18.896 \pm 0.010$, $b = 14.229 \pm 0.007$, $c = 9.406 \pm 0.006$ Å, $Z = 4$, and density, $\rho_{calcd} = 1.43$ g cm^{-3}. An anisotropic least-squares refinement converged to a conventional residual of $R = 0.076$ for 3124 independent reflections recorded with Mo $K\alpha$ radiation on an automatic four-circle diffractometer.

The chemistry of tetracycline derivatives has been reviewed at length in a recent report.[1] The range of conformations accessible to the basic four-ring system and the relative stabilities of different conformers are matters of considerable importance in the formulation and interpretations of detailed reaction mechanisms. Although nmr[2] studies of tetracycline and oxytetracycline derivatives in solution suggest that more than one conformation exists, the crystal structures of two different derivatives were found to be virtually identical; a single molecular conformation emerged from the analyses of the isomorphous hydrochloride salt structures of 7-chlorotetracycline[3,4a,b] (Aureomycin[4c]) (**1**) and 5-hydroxytetracycline[5,6a] (Terramycin[6b]) (**2**) and, as well, from circular dichroism studies[7] in dilute aqueous solutions. Since the unit cell was clearly different for crystals of the free base, 5,12a-diacetyloxytetracycline[8] (**3**), the present study was undertaken with the expectation that a different molecular packing and **a** new molecular conformation would be revealed. Moreover, a large number of high quality diffraction data were accessible and this provided an opportunity to establish the mo-

(1) D. L. J. Clive, *Quart. Rev., Chem. Soc.*, **22**, 435 (1968).
(2) M. Schach von Wittenau and R. K. Blackwood, *J. Org. Chem.*, **31**, 613 (1966).
(3) J. Donohue, J. D. Dunitz, K. N. Trueblood, and M. S. Webster, *J. Amer. Chem. Soc.*, **85**, 851 (1963).
(4) (a) S. Hirokawa, Y. Okaya, F. M. Lovell, and R. Pepinsky, *Acta Crystallogr.*, **12**, 811 (1959); (b) *Z. Krist.*, **112**, 439 (1959); (c) Registered trademark, American Cyanamid Co.
(5) H. Cid-Dresdner, *Z. Kristallogr., Kristallgeometrie, Kristallphys. Kristallchem.*, **121**, 170 (1965).
(6) Y. Takeuchi and M. J. Buerger, *Proc. Nat. Acad. Sci., U. S.*, **46**, 1366 (1960); (b) Registered trademark, C. F. Pfizer, Inc.
(7) L. A. Mitscher, A. C. Bonacci, and T. D. Sokolski, *Antimicrob. Ag. Chemother.*, 78 (1968).
(8) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings, and R. B. Woodward, *J. Amer. Chem. Soc.*, **75**, 5455 (1953).

lecular parameters with considerable precision for this moderately complex structure.



1 2 3

$R_1 = Cl$ $R_1 = H$ $R_1 = H$
$R_2 = H$ $R_2 = OH$ $R_2 = OCOCH_3$
$R_3 = H$ $R_3 = H$ $R_3 = COCH_3$

Experimental Section

The 5,12a-diacetyloxytetracycline,[9] $C_{26}H_{28}O_{11}N_2$, was prepared by acylation of 5-hydroxytetracycline (Chas. Pfizer and Co.) with acetic anhydride and was crystallized as well-formed tabular prisms by evaporation of a 2-propanol solution. X-Ray diffraction photographs displayed orthorhombic symmetry with systematic extinctions $h00$ for $h = 2n + 1$, $0k0$ for $k = 2n + 1$, and $00l$ for $l = 2n + 1$, and uniquely conformed to the space group $P2_12_12_1$. A total of 32 reflections within the angular range $30° \leq 2\theta \leq 42°$ for Mo Kα radiation were automatically centered on a Picker FACS-I four-circle diffractometer; a least-squares refinement of the angular settings yielded the lattice parameters $a = 18.896 \pm 0.010$ Å, $b = 14.229 \pm 0.007$ Å, and $c = 9.406 \pm 0.006$ Å which for $Z = 4$ gives $\rho_{calcd} = 1.430$ g/cm³ ($\rho_{calcd} = 1.44$ g/cm³).

The diffraction intensities were measured on a 0.55 mm × 0.32 mm × 0.12 mm crystal using Zr filtered Mo Kα radiation at a take-off angle of 3.5° with the diffractometer operating in the θ–2θ scan mode. The scans, with a systematic allowance for dispersion, were taken at 1°/min over 1.35–1.70° with 20-sec background counts at each end of the scan. Of the 3292 independent reflections investigated ($\sin\theta/\lambda \leq 0.6486$) a total of 3124 were retained as objectively observed with $|F_o| > 0.675\sigma_F$; $\sigma_F = 0.02 \cdot |F_o| + (C + k^2B)^{1/2}/(2|F_o|L_p)$, wherein C is the total count in a scan and k is the ratio of scanning time to the time for the total background count B. Periodic monitoring of three reflections showed a maximum of 5% random variation in intensity over a 10-day period. Corrections were applied for Lorentz and polarization effects but absorption and extinction effects proved to be negligible. An average thermal parameter (2.61 Å²) and a scale factor (1.78), required for the calculation of normalized structure factors $|E_{hkl}|$, were obtained from a Wilson analysis.[10]

Structure Determination and Refinement. A starting set (Table I) of 11 phase angles was developed for the initial phase determination. The first five reflections were those with the highest $|E|$ values in a set of 18 $0kl$ ($k, l = 2n$) with consistent Σ_2 interactions.[11] The next three, being linearly independent reflections, were arbitrarily assigned[12] phases to specify the origin. The last three reflections were assigned combinations of the phases $\pm\pi/4$ and $\pm3\pi/4$ in a computerized[13] multiple-solution calculation of phases for 232 additional reflections. The resulting set with the highest average consistency index[14] yielded 56 phases with consistency indices greater than 0.5. These were used in a calculation of modified Σ^2 interactions,[15] $\varphi_{\vec{h}} = \Sigma|E_{\vec{k}}E_{\vec{h}-\vec{k}}|(\varphi_{\vec{k}} + \varphi_{\vec{h}-\vec{k}})/\Sigma\varphi|E_{\vec{k}}E_{\vec{h}-\vec{k}}|$, in a set of 301 reflections. This yielded 184 phases determined with consistency indices greater than 0.35. Three subsequent iterations of tangent refinement[16] yielded a set of 283 phases with an average consistency index of 0.466. In a Fourier synthesis utilizing these phases, 12 maxima were identified as a fragment of the molecule containing the A ring. The vector distribution from these 12 atoms reflected some of the prominent features of the Patterson synthesis but a Fourier synthesis ($\sin\theta/\lambda = 0.43$) phased with them ($R = 0.60$) contained no other maxima. At this point, a procedure based upon one suggested by Karle[17] was used for the introduction of partial structure information into the tangent refinement of phases. The process is summarized in Table II; the full structure was developed from the final 35 atom positions by difference Fourier synthesis. Reexamination of the E synthesis based upon the first set of 283 phases revealed that 30 of the 39 highest maxima corresponded to atomic positions.

Table I. Starting Set for Phase Determination

h	k	l	E	Phase
0	0	8	4.57	0
0	4	4	2.52	0
0	6	4	2.43	π
0	8	4	1.98	0
0	4	2	1.76	0
7	5	0	3.00	$\pi/2$
1	0	7	2.29	$\pi/2$
0	1	8	2.90	$\pi/2$
8	8	5	2.82	$\pm\pi/4, \pm3\pi/4$
7	9	4	2.92	$\pm\pi/4, \pm3\pi/4$
4	1	4	2.71	$\pm\pi/4, \pm3\pi/4$

Table II. Summary of Structure Development by Tangent Refinement[a]

	Input		Output		
Stage	Atoms	Reflections	Phases	Atoms	R^d
I	b	301	283	12	0.60
II	12[c]	300	286	25	0.47
III	24[c]	360	356	35	0.38

[a] Three iterations were applied at each stage with rejection criteria set at $C_{min} = 0.10$ and, also,[15] $\alpha_{min} = 2.00$. [b] Expansion of 11 reflection starting sets. [c] All atoms as point carbon atoms with $f = 6$ and $B = 0$. [d] All atoms as carbon atoms with $B = 2.75$ Å².

The model was refined with isotropic thermal parameters by full-matrix, least-squares[18] analysis with each reflection assigned a weight $w = 1/\sigma_F^2$ and with atomic scattering factors for C⁰, N⁰, and O⁰ calculated by Cromer and Mann.[19] At convergence the standard residual was $R = 0.114$ and the weighted residual, $R_w = (\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2)^{1/2}$, was 0.111. A difference Fourier synthesis based on these results allowed objective placement of nearly all the hydrogen atoms. The parameters for the nonhydrogen atoms were again refined by full-matrix, least-squares to yield $R = 0.101$ and $R_w = 0.104$. Six final cycles of refinement of the model with anisotropic thermal motion by block-diagonal least squares converged with $R = 0.076$ and $R_w = 0.076$. The total number of independent parameters was 352; all hydrogen atoms were included with fixed parameters and a fixed value of $B = 4$ Å². The estimated error in a reflection of unit weight was 0.759 for the final refinement.[20]

Results

Final atomic coordinates and thermal parameters for 5,12a-diacetyloxytetracycline are presented in Tables III, IV, and V along with the estimated standard deviations derived from the least-squares analysis.

(9) We are indebted to H. Muxfeldt for a sample of this material.
(10) A. J. C. Wilson, *Nature (London)*, **150**, 151 (1942).
(11) H. Hauptman and J. Karle, "Solution of the Phase Problem. I. The Centrosymmetric Crystal," A.C.A. Monograph No. 3, Polycrystal Book Service, New York, N. Y., 1953.
(12) H. Hauptman and J. Karle, *Acta Crystallogr.*, **9**, 45 (1956).
(13) C. C. Tsai, Ph.D. Thesis, Indiana University, 1968.
(14) M. G. B. Drew, D. H. Templeton, and A. Zalkin, *Acta Crystallogr., Sect. B*, **25**, 261 (1969).
(15) J. Karle and I. L. Karle, *ibid.*, **21**, 849 (1966).
(16) J. Karle and H. Hauptman, *ibid.*, **9**, 635 (1956).
(17) J. Karle, *ibid.*, *Sect. B*, **24**, 182 (1968).
(18) W. R. Busing and H. A. Levy, ORF-LS, A Fortran Crystallographic Least-Squares Program, ORNL-TM-305, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1962.
(19) D. T. Cromer and J. B. Mann, *Acta Crystallogr., Sect. A*, **24**, 321 (1968).
(20) A table of observed and calculated structure amplitudes from the final refinement has been deposited as Document No. NAPS-01557 with the ASIS National Auxiliary Publications Service, c/o CCM Information Corp., 909 3rd Ave., New York, N. Y. 10002. A copy may be secured by citing the document number and by remitting $5.00 for photocopies or $2.00 for microfiche. Advance payment is required. Make checks or money order payable to ASIS–NAPS.

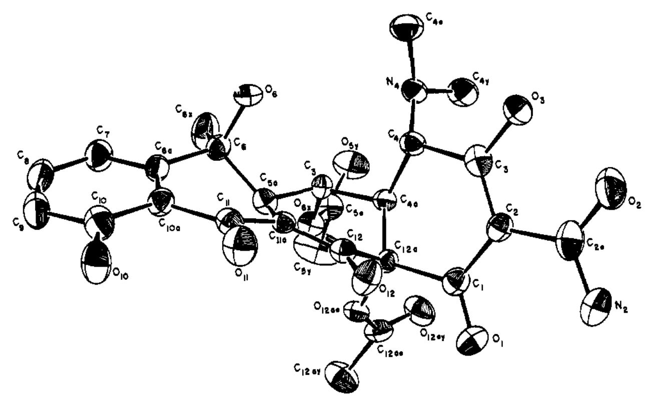


Figure 1. A perspective representation of the structure of 5,12a-diacetyloxytetracycline.

The perspective view shown in Figure 1 displays the essential configurational and conformational features of the molecule. Each atom is represented by an ellipsoid consistent with the anisotropic thermal parameters in Table IV. The six chiral centers are (R)-C_4, (R)-C_{4a}, (S)-C_5, (R)-C_{5a}, (S)-C_6, and (R)-C_{12a}. Since the absolute configuration at C_6 has been determined,[21] the molecule is shown in the correct enantiomorphic form.

Table III. Atomic Fractional Coordinates[a]

Atom	10^4x	10^4y	10^4z
C_1	4893 (2)	2845 (3)	1049 (4)
C_2	4175 (2)	3171 (3)	1062 (4)
C_3	3861 (2)	3544 (2)	2282 (4)
C_4	4231 (2)	3590 (2)	3732 (4)
C_{4a}	4877 (2)	2910 (2)	3771 (4)
C_5	5320 (2)	3083 (2)	5092 (4)
C_{5a}	5893 (2)	3872 (3)	4982 (4)
C_6	5889 (2)	4546 (3)	6264 (4)
C_{6a}	6510 (2)	5224 (3)	6105 (5)
C_7	6934 (2)	5494 (3)	7241 (5)
C_8	7492 (2)	6130 (3)	7018 (6)
C_9	7622 (2)	6526 (3)	5705 (6)
C_{10}	7178 (2)	6287 (3)	4572 (5)
C_{10a}	6635 (2)	5628 (3)	4742 (4)
C_{11}	6217 (2)	5318 (3)	3517 (4)
C_{11a}	5855 (2)	4422 (2)	3597 (4)
C_{12}	5580 (2)	4053 (2)	2405 (4)
C_{12a}	5314 (2)	3045 (2)	2407 (4)
O_1	5186 (2)	2437 (2)	60 (3)
C_{2x}	3767 (2)	3213 (3)	−264 (5)
N_2	4035 (2)	2856 (3)	−1460 (4)
O_2	3162 (2)	3608 (2)	−291 (4)
O_3	3228 (1)	3886 (2)	2268 (3)
N_4	3777 (2)	3435 (2)	4966 (4)
C_{4x}	3303 (2)	2613 (3)	4886 (5)
C_{4y}	3407 (2)	4284 (3)	5462 (5)
O_{5x}	5739 (1)	2228 (2)	5393 (3)
C_{5x}	5442 (2)	1541 (3)	6145 (5)
O_{5y}	4859 (2)	1566 (2)	6645 (4)
C_{5y}	5927 (4)	698 (4)	6232 (8)
O_6	5235 (2)	5056 (2)	6152 (3)
C_{6x}	5920 (3)	4005 (4)	7663 (5)
O_{10}	7313 (2)	6701 (2)	3306 (4)
O_{11}	6233 (2)	5782 (2)	2362 (3)
O_{12}	5554 (2)	4486 (2)	1145 (3)
O_{12ax}	5968 (1)	2520 (2)	2422 (3)
C_{12ax}	5957 (2)	1557 (3)	2443 (4)
O_{12ay}	5434 (1)	1103 (2)	2485 (4)
C_{12ay}	6704 (2)	1214 (3)	2452 (6)

[a] The numbers in parentheses are estimated standard deviations in the last significant figure.

Bond lengths and bond angles within the molecule are systematically recorded in Table VI. The carefully selected set of dihedral angles listed in Table VII fully characterizes all of the conformational features of the molecule; a similar set, calculated from the data given for 5-hydroxytetracycline,[5] is provided for comparison.

Discussion

The A Ring. Apart from the dihedral angles (see explanation, Table VII) which determine the relative orientations of the A and B rings (*vide infra*), the most interesting structural parameters in the A ring are found in the sequence C_1–C_2–C_3 and its associated pendant groups. All other bonds in the ring, C_3–C_4, C_4–C_{4a}, C_{4a}–C_{12a}, and C_{12a}–C_1, have lengths and angles that lie well within the normal range for C–C single bonds.

The bond lengths and angles at C_1–C_2 (1.434 Å) and C_2–C_3 (1.397 Å) are characteristic of an sp^2 conjugated system (*cf.* 1.397 Å in benzene[22]). Neither these results nor those reported earlier[3,5] are compatible with the formal assignment of a double bond at C_2–C_3. Atom C_1 and the three atoms directly bonded to it form a plane with an average out-of-plane distance of ±0.001 Å. Similarly, for C_2, C_3, C_4, O_3 and C_1, C_2, C_3, C_{2x}, the average deviations from planarity are ±0.0007 Å and ±0.004 Å, respectively. The slight nonplanarity of the bonded sequence O_1–C_1–C_2–C_3–O_3 (±0.027 Å) reflects the constraints imposed upon this partially conjugated system by the ring on one side and by the strong hydrogen bonding interactions between $O_1 \cdots N_2$ and $O_2 \cdots O_3$ on the other side.

The amide group at C_2 appears to be partially conjugated into the $O_1 \cdots O_3$ sequence. The C–N distance (1.335 Å) is normal[23] (*cf.* 1.33 Å in succinamide[24]) but the carbonyl bond length (1.274 Å) is distinctly elongated and the C_{2x}–C_2 distance (1.468 Å) has the typical length of a single bond in a conjugated double bond system. As would be expected, the enolic sequence O_2–C_{2x}–C_2–C_3–O_3 is moderately planar (±0.018 Å); the sequence O_1–C_1–C_2–C_{2x}–N_2 is somewhat less planar with an average out-of-plane distance of ±0.031 Å.

In the enolic system the C_3–O_3 bond length (1.291 Å) is normal (*cf.* 1.287 Å in acetylacetone[25]) and the $O_2 \cdots O_3$ contact distance (2.443(5) Å) is on the short end of the normal range (*cf.* 2.44 Å in hydrogen maleate[26] and 2.55 Å in hexafluoroacetylacetone[27]). This indicates strong hydrogen bonding between O_2 and O_3 with an O_3–H_3 distance of 0.98 Å, an O_2–H_3 distance of 1.55 Å, and an O_3–H_3–O_2 angle of 150°.

(21) V. N. Dobrynin, A. I. Gurevitch, M. C. Karapetyan, M. N. Kolosov, and M. M. Shemyakin, *Tetrahedron Lett.*, 901 (1962).
(22) A. Langseth and B. P. Stoicheff, *Can. J. Phys.*, **34**, 350 (1956).
(23) Since C_{2x}–N_2 is 1.335 Å and C_{2x}–O_2 is 1.274 Å in the present structure, the curious results reported[3,5] for the other tetracyclines (C_{2x}–O_2 at 1.31–1.32 Å and C_{2x}–N_2 at 1.28–1.30 Å) are not supported. In view of the considerably greater accuracy inherent in the present analysis, it is probably not useful to contemplate detailed chemical or structural interpretation of those results.
(24) D. R. Davies and R. A. Pasternak, *Acta Crystallogr.*, **9**, 334 (1956).
(25) A. L. Andreassen and S. H. Bauer, to be published.
(26) S. W. Peterson and H. A. Levy, *J. Chem. Phys.*, **29**, 948 (1958).
(27) A. L. Andreassen, D. Zebelman, and S. H. Bauer, *J. Amer. Chem. Soc.*, **93**, 1148 (1971).

Table IV. Thermal Parameters for 5,12a-Diacetyloxytetracycline[a]

Atom	Anisotropic parameters, Å^2						
	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}	B_{iso}[b]
C_1	2.7 (1)	2.2 (1)	2.0 (1)	−0.4 (1)	0.1 (1)	0.0 (1)	2.2
C_2	2.5 (2)	2.1 (1)	2.2 (2)	−0.3 (1)	−0.1 (1)	−0.1 (1)	2.3
C_3	2.4 (1)	1.7 (1)	3.1 (2)	−0.3 (1)	−0.5 (1)	0.5 (1)	2.3
C_4	2.0 (1)	2.1 (1)	2.5 (2)	−0.1 (1)	0.3 (1)	−0.2 (1)	2.1
C_{4a}	1.9 (1)	1.9 (1)	2.2 (1)	0.0 (1)	0.4 (1)	−0.2 (1)	2.0
C_5	2.4 (1)	2.2 (1)	2.0 (1)	0.2 (1)	−0.0 (1)	0.3 (1)	2.1
C_{5a}	2.0 (1)	2.3 (1)	2.1 (1)	−0.1 (1)	−0.0 (1)	0.4 (1)	2.1
C_6	2.9 (2)	3.3 (2)	1.9 (2)	−0.3 (1)	−0.1 (1)	−0.4 (1)	2.6
C_{6a}	2.5 (2)	2.5 (2)	3.4 (2)	0.2 (1)	−0.5 (2)	−0.5 (1)	2.7
C_7	3.4 (2)	3.6 (2)	3.7 (2)	−0.0 (2)	−1.0 (2)	−0.5 (2)	3.5
C_8	3.2 (2)	3.1 (2)	5.5 (3)	0.2 (2)	−1.5 (2)	−1.6 (2)	3.4
C_9	2.7 (2)	2.7 (2)	5.3 (3)	−0.3 (2)	−1.0 (2)	−0.5 (2)	3.3
C_{10}	2.7 (2)	2.0 (2)	4.6 (2)	0.1 (1)	−0.1 (2)	−0.1 (2)	2.9
C_{10a}	2.4 (1)	2.0 (1)	2.6 (2)	0.0 (1)	−0.1 (1)	−0.4 (1)	2.3
C_{11}	1.9 (1)	2.2 (1)	2.8 (2)	0.2 (1)	0.1 (1)	0.0 (1)	2.3
C_{11a}	1.9 (1)	1.9 (1)	2.0 (1)	−0.0 (1)	0.1 (1)	0.0 (1)	1.9
C_{12}	2.1 (1)	1.9 (1)	2.2 (2)	0.1 (1)	0.3 (1)	0.1 (1)	2.1
C_{12a}	2.1 (1)	2.2 (1)	1.7 (1)	−0.3 (1)	0.1 (1)	−0.1 (1)	2.0
O_1	3.7 (1)	4.3 (1)	2.4 (1)	−0.0 (1)	0.1 (1)	−0.6 (1)	3.3
C_{2x}	3.6 (2)	2.4 (2)	3.3 (2)	−0.7 (1)	−1.0 (2)	0.3 (2)	2.9
N_2	4.4 (2)	5.2 (2)	2.9 (2)	−0.0 (2)	−0.9 (2)	−0.5 (2)	3.9
O_2	3.6 (1)	4.6 (1)	3.9 (2)	0.2 (1)	−1.4 (1)	0.2 (1)	3.8
O_3	2.6 (1)	3.5 (1)	3.5 (1)	0.7 (1)	−0.5 (1)	−0.0 (1)	3.1
N_4	2.1 (1)	2.9 (1)	2.7 (1)	−0.3 (1)	0.5 (1)	−0.1 (1)	2.5
C_{4x}	3.2 (2)	3.8 (2)	3.7 (2)	−0.7 (2)	0.8 (2)	0.2 (2)	3.5
C_{4y}	3.3 (2)	3.9 (2)	4.0 (2)	0.3 (2)	1.1 (2)	−0.9 (2)	3.5
O_{5x}	2.9 (1)	2.3 (1)	2.7 (1)	0.3 (1)	0.4 (1)	0.9 (1)	2.5
C_{5x}	3.8 (2)	2.6 (2)	3.4 (2)	0.2 (2)	0.5 (2)	0.6 (2)	3.2
O_{5y}	4.4 (2)	3.8 (1)	5.1 (2)	−0.1 (1)	1.7 (1)	1.5 (1)	4.0
C_{5y}	7.7 (5)	5.0 (3)	9.2 (4)	2.4 (3)	3.8 (4)	4.6 (3)	5.3
O_6	2.7 (1)	4.0 (1)	4.1 (2)	0.2 (1)	0.6 (1)	−1.7 (1)	3.3
C_{6x}	5.3 (2)	6.0 (3)	2.1 (2)	−2.3 (2)	−0.4 (2)	0.3 (2)	3.8
O_{10}	4.2 (1)	2.8 (1)	4.7 (2)	−1.4 (1)	−0.2 (1)	0.3 (1)	3.6
O_{11}	3.6 (1)	2.4 (1)	3.3 (1)	−0.9 (1)	−0.2 (1)	0.8 (1)	2.9
O_{12}	4.0 (1)	2.7 (1)	1.8 (1)	−0.7 (1)	−0.2 (1)	0.5 (1)	2.6
O_{12ax}	2.2 (1)	2.0 (1)	2.6 (1)	0.0 (1)	0.6 (1)	−0.0 (1)	2.2
C_{12ax}	3.0 (2)	2.3 (1)	2.8 (2)	0.0 (1)	0.7 (2)	−0.2 (1)	2.6
O_{12ay}	3.1 (1)	2.8 (1)	5.6 (2)	−0.6 (1)	0.6 (1)	−0.5 (1)	3.6
C_{12ay}	3.5 (2)	3.0 (2)	6.8 (3)	0.7 (2)	0.9 (2)	0.3 (2)	4.1

[a] Numbers in parentheses are estimated standard deviations in the last significant figure. The relation between B_{ij} in Å^2 and the dimensionless β_{ij} used during refinement is $B_{ij} = 4\beta_{ij}/a_i^*a_j^*$. [b] Isotropic thermal parameter calculated from $B_{iso} = 4[V^2\det(\beta_{ij})]^{1/3}$.

The interaction of the amide group with the rather normal carbonyl C_1–O_1 (1.229 Å) is moderately strong; the hydrogen bond distance between O_1 and N_2 is 2.669 Å with an N–H distance of 1.14 Å, an O–H distance of 1.67 Å, and an N–H–O angle of 142°.

The B Ring. A careful examination of the data in Table VI reveals bonding parameters at atoms C_{4a}, C_5, C_{5a}, and C_{12a} that lie in the normal range for a constrained ring system. The acetoxy groups at C_5 and C_{12a} are also normal and exhibit nearly identical geometries. The five atoms C_{12a}, O_{12ax}, C_{12ax}, C_{12ay}, O_{12ay} are remarkably coplanar (±0.004 Å), but the corresponding group at C_5 has a larger average out-of-plane distance (±0.020 Å).

The bond at C_{11a}–C_{12} is 1.352(5) Å which is precisely the value for a localized double bond. Appropriately, the bond angles at C_{11a} are very close to 120°; at C_{12} the C_{11a}–C_{12}–O_{12} bond angle is opened somewhat to 125.1° probably as a result of the interaction of O_{12} with O_{11}.

Moreover, the other bonds at C_{11a} and C_{12} seem to be characteristic of a conjugated system. Thus, the bond C_{12}–O_{12}(H) is 1.337(5) Å, which is intermediate between a normal C–O(H) (1.43 Å) and a normal carbonyl bond (1.22 Å). Similarly, in the C ring the C_{11a}–C_{11} bond is 1.449(5) Å, which is intermediate between a single C–C bond (1.54 Å) and a double bond (1.335 Å).

The C Ring. The partially conjugated system starting at O_{12} appears to extend across the C ring through C_{11a}, C_{11}, O_{11}, and C_{10a} and on into the D ring. The carbonyl bond C_{11}–O_{11} is somewhat extended (1.271 Å) and the C_{10a}–C_{11} bond is of intermediate length at 1.464(6) Å. All bond angles at C_{11a}, C_{11}, and C_{10a} are very close to 120° but there is considerable puckering in the entire sequence as can be seen from the dihedral angles C_{10}–C_{10a}–C_{11}–C_{11a}, C_{10a}–C_{11}–C_{11a}–C_{12}, and C_{11}–C_{11a}–C_{12}–C_{12a} which are 157.4, −168.0, and 169.3°, respectively.

The carbonyl oxygen O_{11} is involved in two intramolecular hydrogen bonds with the protons on O_{10} and O_{11} at distances of 2.581(4) and 2.521(4) Å, respectively; the indicated hydrogen atom positions correspond to moderately bent hydrogen bonds. All other bonding in the ring is normal.

The D Ring. All C–C bonds in this aromatic ring average 1.399(13) Å, a value identical with that found in benzene. The C_{10}–O_{10} bond length is 1.352(6) Å which is the same value reported for resorcinol.[28] All

(28) G. E. Bacon and N. A. Curry, *Proc. Roy. Soc., Ser. A*, **235**, 552 (1956).

Table V. Hydrogen Atomic Parameters[a]

Atom	Bound to	10^3x	10^3y	10^3z	Bond, Å
$H2_1$	N_2	460	260	−125	1.14
$H2_2$	N_2	380	285	−250	1.08
H3	O_3	315	400	125	0.98
H4	C_4	440	425	385	1.00
$H4x_1$	C_{4x}	295	275	420	0.95
$H4x_2$	C_{4x}	305	265	590	1.07
$H4x_3$	C_{4x}	360	205	475	0.99
$H4y_1$	C_{4y}	320	410	635	0.96
$H4y_2$	C_{4y}	300	450	500	0.94
$H4y_3$	C_{4y}	360	490	535	0.95
H4a	C_{4a}	475	220	385	1.04
H5	C_5	500	320	610	1.14
$H5y_1$	C_{5y}	640	80	575	1.01
$H5y_2$	C_{5y}	575	0	655	1.09
$H5y_3$	C_{5y}	600	60	750	1.21
H5a	C_{5a}	635	350	515	1.02
H6	O_6	520	580	675	1.20
$H6x_1$	C_{6x}	560	345	800	1.04
$H6x_2$	C_{6x}	640	395	800	0.96
$H6x_3$	C_{6x}	580	460	850	1.18
H7	C_7	680	520	840	1.20
H8	C_8	780	655	780	1.11
H9	C_9	800	700	550	1.00
H10	O_{10}	720	640	250	0.90
H12	O_{12}	585	505	125	0.98
$H12a_1$	C_{12ay}	685	160	325	0.97
$H12a_2$	C_{12ay}	685	55	275	1.02
$H12a_3$	C_{12ay}	680	140	150	0.95

[a] Reasonable estimates of the error in the fractional coordinates and the bond lengths are ~0.005 and ~0.1 Å, respectively.

bond angles are close to 120° and all atoms lie within an average distance of 0.012 Å of a mean plane.

The Molecular Conformation. The tetracycline ring system in the present structure exhibits a conformation that differs markedly from the one observed in the hydrochloride salt structures of 7-chlorotetracycline[3,4] and 5-hydroxytetracycline.[5,6] As can be seen from the stereoscopic drawings in Figure 2 and Figure 3 and from the listing of dihedral angles in Table VII, the major differences between the two conformations appear in the A and B rings. As would be expected, the aromatic D rings are virtually identical and the partially conjugated sequence that extends from O_{12} to C_{10a} apparently stabilizes the conformation of the C ring.

The transition between the two conformers involves a drastic twist of 108.9° about the bond C_{4a}–C_{12a} at the juncture of the A and B rings. This is necessarily accompanied by similar rotations around all four contiguous ring bonds; in the A ring these are C_{4a}–C_4 (95.8°) and C_{12a}–C_1 (94.1°), and in the B ring they are C_{12a}–C_{12} (87.9°) and C_{4a}–C_5 (103.3°). The associated changes that occur elsewhere in the two rings, while nontrivial, are very much smaller. In the A ring this reflects the rigidity of the amide stabilized sequence from O_1 to O_3. The relatively rigid geometry of the C ring imparts stability to C_5 and C_{12} in the B ring.

This new molecular conformation does not appear to result from any strong or highly directional intermolecular forces in the crystal, although the absence of long range ionic forces in the present structure represents a significant change in the molecular environment. The shortest intermolecular hydrogen bond contacts are about 3.0 Å from O_6 and N_2 to O_{5y} and all other contacts are equal to or greater than the sums of the appropriate van der Waals radii.

Table VI. Bond Distances and Angles for 5,12a-Diacetyloxytetracycline[a]

Atom	Bond	Dist, Å	Angle, deg
C_1	C_1–C_2	1.434 (5)	C_2–C_1–O_1, 125.8 (4)
	C_1–C_{12a}	1.531 (5)	C_2–C_1–C_{12a}, 115.1 (3)
	C_1–O_1	1.229 (5)	O_1–C_1–C_{12a}, 119.1 (3)
C_2	C_2–C_3	1.397 (6)	C_1–C_2–C_3, 122.2 (3)
	C_2–C_{2x}	1.468 (6)	C_1–C_2–C_{2x}, 120.2 (3)
			C_3–C_2–C_{2x}, 117.3 (3)
C_3	C_3–C_4	1.535 (6)	C_2–C_3–C_4, 123.5 (3)
	C_3–O_3	1.291 (5)	C_2–C_3–O_3, 122.0 (4)
			C_4–C_3–O_3, 114.5 (3)
C_4	C_4–C_{4a}	1.558 (5)	C_3–C_4–C_{4a}, 110.6 (3)
	C_4–N_4	1.460 (5)	C_3–C_4–N_4, 115.6 (3)
			C_{4a}–C_4–N_4, 110.4 (3)
C_{4a}	C_{4a}–C_5	1.519 (5)	C_4–C_{4a}–C_5, 110.5 (3)
	C_{4a}–C_{12a}	1.538 (5)	C_4–C_{4a}–C_{12a}, 108.9 (3)
			C_5–C_{4a}–C_{12a}, 111.4 (3)
C_5	C_5–C_{5a}	1.563 (5)	C_{4a}–C_5–C_{5a}, 116.4 (3)
	C_5–O_{5x}	1.479 (4)	C_{4a}–C_5–O_{5x}, 108.6 (3)
			C_{5a}–C_5–O_{5x}, 103.5 (3)
C_{5a}	C_{5a}–C_6	1.541 (6)	C_5–C_{5a}–C_6, 113.0 (3)
	C_{5a}–C_{11a}	1.521 (5)	C_5–C_{5a}–C_{11a}, 113.2 (3)
			C_6–C_{5a}–C_{11a}, 110.5 (3)
C_6	C_6–C_{6a}	1.527 (6)	C_{5a}–C_6–C_{6a}, 108.2 (3)
	C_6–C_{6x}	1.525 (6)	C_{5a}–C_6–C_{6x}, 111.2 (4)
	C_6–O_6	1.436 (5)	C_{5a}–C_6–O_6, 105.1 (3)
			C_{6a}–C_6–C_{6x}, 112.0 (4)
			C_{6a}–C_6–O_6, 109.5 (3)
			C_{6x}–C_6–O_6, 110.5 (3)
C_{6a}	C_{6a}–C_7	1.389 (6)	C_6–C_{6a}–C_7,, 122.9 (4)
	C_{6a}–C_{10a}	1.425 (6)	C_6–C_{6a}–C_{10a}, 118.1 (4)
			C_7–C_{6a}–C_{10a}, 119.0 (4)
C_7	C_7–C_8	1.405 (6)	C_{6a}–C_7–C_8, 119.7 (4)
C_8	C_8–C_9	1.379 (7)	C_7–C_8–C_9, 122.0 (4)
C_9	C_9–C_{10}	1.398 (7)	C_8–C_9–C_{10}, 118.5 (4)
C_{10}	C_{10}–C_{10a}	1.399 (5)	C_9–C_{10}–C_{10a}, 121.0 (4)
	C_{10}–O_{10}	1.352 (6)	C_9–C_{10}–O_{10}, 116.9 (4)
			C_{10a}–C_{10}–O_{10}, 122.1 (4)
C_{10a}	C_{10a}–C_{11}	1.464 (6)	C_{10}–C_{10a}–C_{11}, 120.5 (4)
			C_{10}–C_{10a}–C_{6a}, 119.7 (4)
			C_{11}–C_{10a}–C_{6a}, 119.8 (3)
C_{11}	C_{11}–C_{11a}	1.449 (5)	C_{10a}–C_{11}–C_{11a}, 118.6 (3)
	C_{11}–O_{11}	1.271 (5)	C_{10a}–C_{11}–O_{11}, 120.2 (3)
			C_{11a}–C_{11}–O_{11}, 120.8 (3)
C_{11a}	C_{11a}–C_{12}	1.342 (5)	C_{11}–C_{11a}–C_{12}, 118.9 (3)
			C_{11}–C_{11a}–C_{5a}, 118.4 (3)
			C_{12}–C_{11a}–C_{5a}, 122.1 (3)
C_{12}	C_{12}–C_{12a}	1.520 (5)	C_{11a}–C_{12}–C_{12a}, 119.7 (3)
	C_{12}–O_{12}	1.337 (5)	C_{11a}–C_{12}–O_{12}, 125.1 (3)
			C_{12a}–C_{12}–O_{12}, 115.1 (3)
C_{12a}	C_{12a}–O_{12ax}	1.443 (4)	C_{12}–C_{12a}–O_{12ax}, 101.9 (3)
			C_{12}–C_{12a}–C_1, 110.2 (3)
			C_{12}–C_{12a}–C_{4a}, 107.3 (3)
			C_1–C_{12a}–C_{4a}, 113.2 (3)
			C_1–C_{12a}–O_{12ax}, 110.9 (3)
			C_{4a}–C_{12a}–O_{12ax}, 112.8 (3)
C_{2x}	C_{2x}–O_2	1.274 (5)	C_2–C_{2x}–O_2, 120.5 (4)
	C_{2x}–N_2	1.335 (6)	C_2–C_{2x}–N_2, 120.0 (4)
			O_2–C_{2x}–N_2, 119.5 (4)
N_4	N_4–C_{4x}	1.471 (6)	C_4–N_4–C_{4x}, 114.0 (3)
	N_4–C_{4y}	1.475 (6)	C_4–N_4–C_{4y}, 115.9 (3)
			C_{4x}–N_4–C_{4y}, 112.3 (3)
O_{5x}	O_{5x}–C_{5x}	1.331 (5)	C_5–O_{5x}–C_{5x}, 118.7 (3)
C_{5x}	C_{5x}–O_{5y}	1.199 (6)	O_{5x}–C_{5x}–O_{5y}, 125.0 (4)
	C_{5x}–C_{5y}	1.511 (8)	O_{5x}–C_{5x}–C_{5y}, 110.8 (4)
			O_{5y}–C_{5x}–C_{5y}, 124.1 (4)
O_{12ax}	O_{12ax}–C_{12ax}	1.370 (4)	C_{12a}–O_{12ax}–C_{12ax}, 120.4 (3)
C_{12ax}	C_{12ax}–O_{12ay}	1.182 (5)	O_{12ax}–C_{12ax}–O_{12ay}, 124.0 (4)
	C_{12ax}–C_{12ay}	1.492 (6)	O_{12ax}–C_{12ax}–C_{12ay}, 108.3 (3)
			O_{12ay}–C_{12ax}–C_{12ay}, 127.7 (4)

[a] Numbers in parentheses are estimated standard deviations in last significant figure.

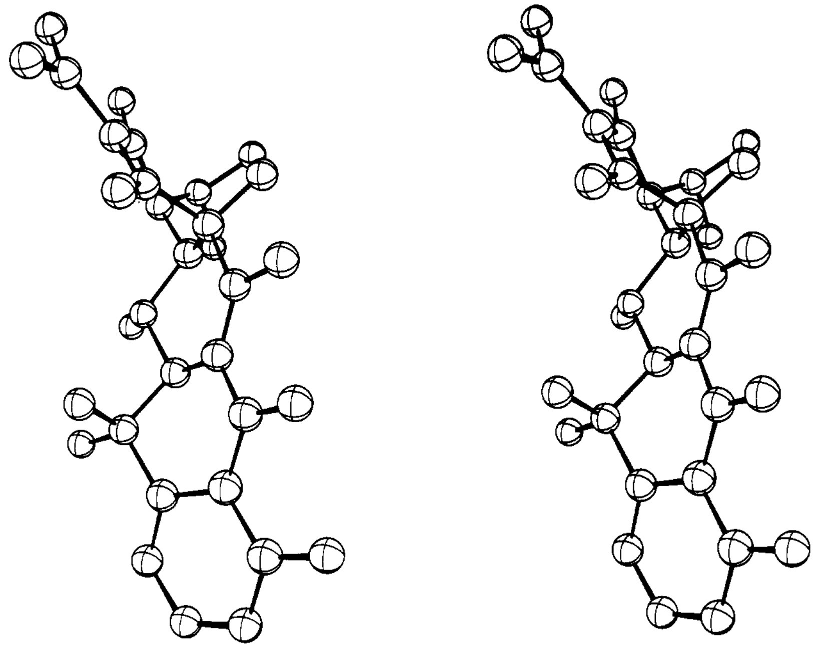


Figure 2. A stereoscopic representation of 5-hydroxytetracycline.[5]

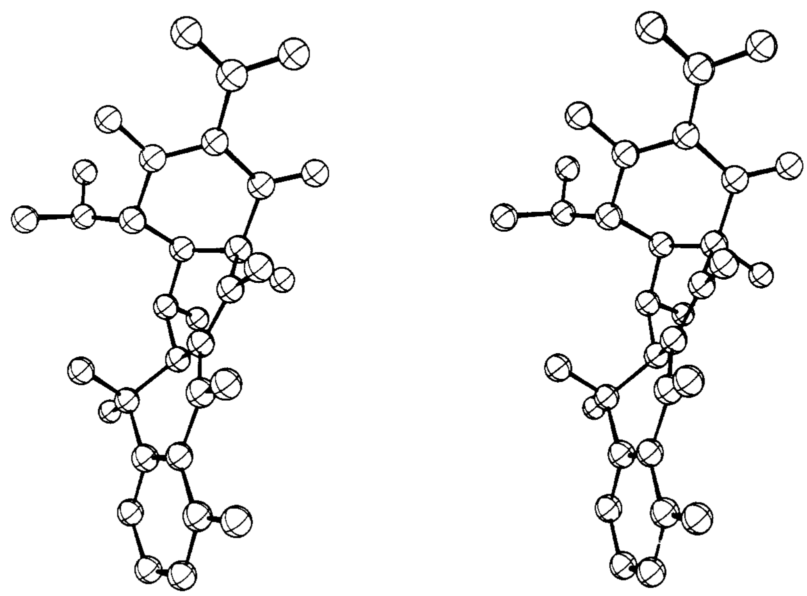


Figure 3. A stereoscopic representation of the oxytetracycline ring system in 5,12a-diacetyloxytetracycline.

The molecule is rigid; typical root mean square displacements for carbon atoms on the ring system are 0.16 Å and the motion is nearly isotropic. Even the substituent groups show displacements of only about 0.15–0.30 Å, except for the terminal atoms of the acetyl groups which, not unexpectedly, range from 0.20 to 0.50 Å. Some anisotropy appears in the thermal parameters for the D ring, but it appears to reflect a slight concerted motion of that end of the molecule parallel to the C_8–C_9 bond. Parenthetically, it is worth noting that because of this motion the isotropic refinement produced a bond length for C_8–C_9 which was fully 0.04 Å shorter than that which resulted from the full anisotropic refinement.

Since there are relatively few stable conformations accessible to a molecule with such a highly constrained ring system, it is appropriate to consider the merits of this new conformation as a model for the molecule in solution. In fact, several features of the model are in excellent accord with the results of some recent nmr studies[2] of several oxytetracyclines (including the present one) in various nonaqueous solvents.

The nmr spectra of the protons on C_4, C_{4a}, C_5, and C_{5a} should provide a measure of the dihedral angles around the respective bonds. Thus, the apparent coupling constants of 9–13 cps reported for the bond C_4–C_{4a} are consistent with a trans conformation[2] across that bond; this condition is met in the present structure with a dihedral angle of 169.8°, but in the other structural model[3,5] this angle is strikingly different (74.0°). Similarly, in the present case, the dihedral angle for the bond C_{4a}–C_5 (85.8°) is consistent with

Table VII. Selected Dihedral Angles[a] in the Tetracycline Ring System

	Atoms	5-Hydroxytetra-cycline[b] HCl, deg	5,12a-Diacetyl-oxytetracycline,[c] deg
Ring A	$C_{12}-C_{12a}-C_1-C_2$	−174.7	−80.6
	$C_{12a}-C_1-C_2-C_3$	19.2	−7.0
	$C_1-C_2-C_3-C_4$	17.1	−3.7
	$C_2-C_3-C_4-C_{4a}$	−17.0	−17.5
	$C_3-C_4-C_{4a}-C_5$	74.0	169.8
	$C_4-C_{4a}-C_{12a}-C_1$	49.1	−59.8
Ring B	$C_{11}-C_{11a}-C_{12}-C_{12a}$	−178.5	169.3
	$C_{11a}-C_{12}-C_{12a}-C_1$	80.7	168.6
	$C_{12}-C_{12a}C_{4a}-C_5$	48.7	−60.1
	$C_4-C_{4a}-C_5-C_{5a}$	170.9	−85.8
	$C_{4a}-C_5-C_{5a}-C_6$	162.5	133.3
	$C_5-C_{5a}-C_{11a}-C_{12}$	−13.2	−25.4
Ring C	$C_{10}-C_{10a}-C_{11}-C_{11a}$	166.5	157.4
	$C_{10a}-C_{11}-C_{11a}-C_{12}$	178.9	−168.0
	$C_{11}-C_{11a}-C_{5a}-C_6$	43.0	35.5
	$C_5-C_{5a}-C_6-C_{6a}$	175.8	174.8
	$C_{5a}-C_6-C_{6a}-C_7$	−135.1	−138.9
	$C_6-C_{6a}-C_{10a}-C_{11}$	1.8	6.0
Ring D	$C_8-C_9-C_{10}-C_{10a}$	7.7	−3.0
	$C_9-C_{10}-C_{10a}-C_{11}$	177.0	−174.2
	$C_{10}-C_{10a}-C_{6a}-C_7$	11.4	0.1
	$C_6-C_{6a}-C_7-C_8$	−178.1	−179.3
	$C_{6a}-C_7-C_8-C_9$	2.0	2.2
	$C_7-C_8-C_9-C_{10}$	−2.7	0.5

[a] An arbitrary but self-consistent set defined for the sequence a–b–c–d as the positive clockwise rotation from a to d in the projection of the array down the line b–c. [b] The angles reported here have been calculated from the coordinates provided in ref 5, with an estimated error given as ~0.01 Å. [c] The estimated error for the coordinates from which these angles were calculated is ~0.004 Å.

the small (0–2 cps) apparent coupling constants observed.[2] Moreover, the nearly eclipsed conformation about C_5-C_{5a} (133.3°) is in accord with the relatively small apparent coupling constants[2] (<4 cps) across this bond.

In contrast to these results on the oxytetracycline systems it should be noted[2] that the nmr results on tetracycline in nonaqueous solvents are more nearly in accord with the previously reported[3,5] molecular conformation.

Circular dichroism (CD) spectra[7] of dilute solutions of variously substituted tetracyclines and oxytetracyclines in aqueous HCl (0.03 *N*) all have the same general shape and intensity. Moreover, the spectra are insensitive to the removal of asymmetry at C_5 and C_6, two of the six asymmetric centers of the molecule. This suggests[7] that the spectra primarily reflect the detailed twisting or chirality of the chromophores and that these substances all belong to the same stereochemical family and possess the same conformation.

The CD band at 262 nm was assigned to the $\pi \rightarrow \pi^*$ transition of the highly enolized β-tricarbonyl chromophore of the A ring; the rest of the spectrum was attributed to the BCD chromophore that includes the D ring and the partially conjugated system between O_{10} and O_{12}. The interaction with these chromophores was probed with epi substitution at C_4 and at C_5 and it was concluded[7] that the molecular conformation was close to that reported in the hydrochloride salt structures.[3,5]

In the present conformation, the chirality of the twisting of both chromophores is reversed with respect to the other conformation. This is reflected, in the A ring, in a reversal of the signs of the dihedral angles at C_1-C_2 and C_2-C_3 and, in the BCD chromophore, by a similar reversal in the signs of the dihedral angles at $C_{11}-C_{11a}$ and $C_{11a}-C_{12}$. If the present conformation were to be retained, either in aqueous or nonaqueous solutions, the CD spectrum would be significantly affected. This question must be held for future elaboration after completion of an nmr and CD study.

Acknowledgment. The authors gratefully acknowledge support from the National Institutes of Health (Grant No. GM-14832-03 and AI-07626-05), from the NSF (GP-13351), and from the Materials Science Center, Cornell University.