Chemical–Structural Properties of Tetracycline Derivatives. 10. The 6-Thiatetracyclines

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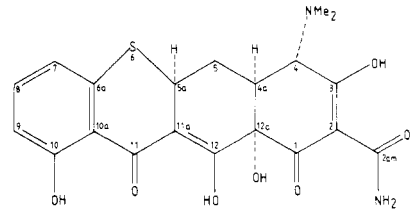
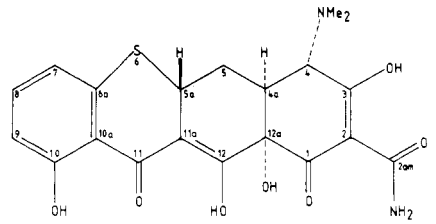
Abstract: Crystal structure determinations for three tetracycline derivatives, 6-thiatetracycline, 6-STC, 5a-epi-6-thiatetracycline, 5a-epi-6-STC, and 11a-hydroxy-12a-dehydroxy-6-thiatetracycline, 11a-OH-12a-DOH-6-STC, have been carried out with data from cooled crystals ($T \sim 120$ K). Their respective space groups, lattice parameters (120 K), and the resultant conventional residuals are as follows: $P2_1/c$, a = 5.5360 (3) Å, b = 19.538 (2) Å, c = 17.540 (2) Å, β = 99.93 (1)°, R = 0.043; $P\bar{1}$, a = 8.934 (1) Å, b = 10.420 (2) Å, c = 12.726 (2) Å, α = 94.73 (1)°, β = 97.68 (1)°, γ = 104.95 (1)°, R = 0.053; $P2_1/c$, a = 12.049 (1) Å, b = 10.698 (1) Å, c = 17.240 (2) Å, β = 93.46 (1)°, R = 0.050. Synthetic pathways for 5a-epi-6-STC and 11a-OH-12a-DOH-6-STC are described. Partition coefficients for 6-STC and 5a-epi-6-STC in the $CHCl_3$–H_2O system were determined and indicate that the differences in their antibacterial activity are not the result of lipid absorption of the latter derivative.

The accompanying report[1] demonstrates that changes in stereochemistry or in the structure of the BCD chromophore of the tetracyclines give rise to chemical and antibacterial behavior that can be interpreted at the molecular level in terms of conformational properties and tautomerism. An understanding of the interrelationships among chemical structure, conformation, configuration, and antibacterial activity of the tetracyclines is emerging from the comparison of the structural studies with the empirically determined structure–activity relationships reviewed by Blackwood and English[2] and Dürkheimer.[3] To date these interrelationships have been attributable to the interactions of three components of the tetracycline's molecular structure: the A ring, with its tricarbonylmethane chromophore and dimethylamino group, the BCD chromophore, and the nonchromophoric portions of the B, C, and D rings.

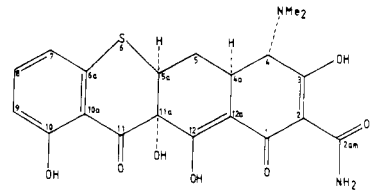
To further probe the structural properties of tetracycline derivatives, we have determined crystal structures for three totally synthetic 6-thiatetracyclines, 6-STC's. The 6-STC's differ markedly from the usual tetracyclines in that a heteroatom, sulfur, has been incorporated into the C ring at the position that normally carries methyl and hydroxyl substituents. We communicated earlier[4] that nonionized 6-thiatetracycline free base, 6-STC(0), displays a conformation very similar to that of nonionized oxytetracycline, OTC(0),[5,6] i.e., to that which we have attributed to the free base of all medicinally important tetracyclines in nonaqueous environments. It was also pointed out that the bonding geometry of the BCD chromophore was remarkably similar to that reported from high-precision crystal structure determinations for medicinally important tetracyclines.[5-7] We now present the detailed crystal structure analyses for 6-STC(0), 5a-epi-6-STC(0), and 11a-hydroxy-12a-dehydroxy-6-thiatetracycline, 11a-OH-12a-DOH-6-STC(0). The parent of this subset of tetracyclines, 6-STC, displays broad-spectrum antibacterial activity[8] both in vivo and in vitro; the 5a-epimer displays in vitro activity similar to that of the parent but lacks significant in vivo activity. The derivative with the most drastically altered structure, 11a-OH-12a-DOH-6-STC, is not active against bacteria.



6-STC



5a-epi-6-STC



11a-OH-12a-DOH-6-STC

Experimental Section

The thiatetracyclines were prepared as racemic mixtures and characterized spectroscopically in the laboratories of E. Merck. The preparation of 6-STC has been communicated.[9]

Preparation of 5a-epi-6-STC was accomplished by dissolving 6-STC in a 1:1 mixture of dimethylformamide and piperidine. The solution was warmed for 2 h and then stirred with acidified (HCl) water. The product was extracted into chloroform which was subsequently washed, dried, and evaporated to dryness. The solid residue was dissolved in dichloromethane, methanol was added, and the dichloromethane was distilled off to give a yellow crystalline sample of 5a-epi-6-STC: mp 221 °C; UV (EtOH) λ_{max} (ϵ) 353 (9950), 252 (20800) nm; UV (0.1 M methanolic borate) λ_{max} (ϵ) 374 (14250), 251 (23100) nm; ^{1}H NMR (90 MHz, Me_2SO-d_6) δ 2.46 (NMe_2, 6 H, s), 3.56 (H(4), d, J = 12 Hz), 4.52 (H(5a), dd, J = 11 Hz), 6.76–7.50 (3 aromatic H, m), 9.04, 9.29 ($CONH_2$, 2 s), 11.53 (phenolic OH, s); MS, m/e P^+ 432.

The hydroxylation step in the preparation of 6-STC[9] gives rise to an 11a-hydroxyl byproduct in ca. 15% yield. It was separated from the main product, the 12a-hydroxyl intermediate, by column chromatography over

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Table I. Crystal Data for 6-STC, 5a-epi-6-STC, and 11a-OH-12a-DOH-6-STC

	6-STC	5a-epi-6-STC	11a-OH-12a-DOH-6-STC
crystallization solvent	CH_2Cl_2	C_3H_7NO (DMF)	C_3H_6O (acetone)
space group	$P2_1/c$	$P\bar{1}$	$P2_1/c$
lattice parameters[a]			
a, Å	5.5360 (3)	8.934 (1)	12.049 (1)
b, Å	19.538 (2)	10.420 (2)	10.698 (1)
c, Å	17.540 (2)	12.726 (2)	17.240 (2)
α, deg	90	94.73 (1)	90
β, deg	99.93 (1)	97.68 (1)	93.46 (1)
γ, deg	90	104.95 (1)	90
no. of contributing 2θ's[b]	45	33	37
2θ range, deg	35–48	29–40	30–40
formula per asymmetric unit	$C_{20}H_{20}N_2O_7S$	$C_{20}H_{20}N_2O_7S \cdot C_3H_7NO$	$C_{20}H_{20}N_2O_7S \cdot C_3H_6O$
	Intensity Data		
no. of unique data	13450	6567	9751
no. of obsd[c] data	8559	3267	5081
$[(\sin\theta)/\lambda]_{max}$, Å^{-1}	0.951	0.704	0.807
R	0.043	0.053	0.050
R_w	0.055	0.064	0.059
Σ[d]	1.012	1.017	1.046
no. of contributing reflections	11246	5134	7679
no. of variables	351	466	411

[a] $T \sim 120$ K. [b] Used to refine lattice parameters. [c] Classification criterion: $I \geq 3\sigma(I)$. [d] Estimated standard deviation of an observation of unit weight.

silica gel. Further handling of the 11a-OH intermediate to give 11a-OH-12a-DOH-6-STC was carried out in an analogous manner to the preparation of 6-STC. The physical data are as follows: mp 168–170 °C (from acetone, petroleum ether); UV (EtOH) λ_{max} (ϵ) 377 sh (6900), 315 (14 450), 252 (21 500) nm; UV (0.1 M methanolic borate) λ_{max} (ϵ) 337 (19 900), 244 (22 000) nm; ^{1}H NMR (100 MHz, Me_2SO-d_6) δ 2.50 (NMe_2, 6 H, s), 3.25 (H(4), d, J = 12 Hz), 3.68 (H(5a), d, J = 12 Hz), 6.58–7.45 (3 aromatic H, m), 8.83, 9.19 ($CONH_2$, 2 s); MS m/e P^+ 432.

Partition coefficients for 6-STC and 5a-epi-6-STC between aqueous and chloroform phases were determined spectrophotometrically.

Crystal Structure Determinations

The solvents from which crystals were obtained, space groups, lattice parameters, formulas per asymmetric unit, data set resolution, and the number of reflections classified as observed under the criterion $I \geq 3\sigma(I)$ are among the data presented in Table I. All quantitative crystallographic data were collected with a Syntex $P\bar{1}$ autodiffractometer (monochromatized Mo Kα radiation, λ = 0.710 69 Å) equipped with a low-temperature device (Syntex LT-1) which maintained the crystal at ca. 120 K.

The tabulated lattice parameters resulted from least-squares refinement[10] with the indicated number of automatically centered 2θ values in the angular range tabulated. Intensities were measured in an ω-scan mode for which the scan range was 0.75°; the scan rate varied between 2.0 and 24.0° min^{-1} as a function of maximum peak intensity. Background was measured on each side of the reflection center ($\Delta\omega$ = 1.0°) for half the scan time. Three reference reflections were measured periodically to check for crystal and instrumental stability; the data were corrected for minor variations therein and for Lorentz and polarization effects.

The initial structural models were determined[10,11] by direct methods and developed by difference Fourier and least-squares techniques. Twofold disorder in the position of the DMF molecule of solvation was encountered in the crystal of 5a-epi-6-STC. The occupancy factors for the two contributors were set at 0.77 and 0.23 in accordance with the peak heights of the fully resolved atoms. All hydrogen atoms, with the exception of some of those associated with the disordered DMF molecule, were located by difference Fourier techniques.

Least-squares refinement was effected by the variable-block technique. Most blocks consisted of the parameters (fractional atomic coordinates and anisotropic temperature factors) of one C, N, O, or S atom and those of any H atom (fractional atomic coordinates and isotropic temperature factors) bonded to it. The parameters of atoms O(2am), O(3), and H(3) were included in a single block to increase the reliability of the refinement of the H-atom parameters. All parameters associated with the disordered DMF molecule were refined in a single block. Those H atoms that had been located for the minor component of the DMF molecule were included in the structure factor calculation but were not refined; the coordinates of the N atom with occupancy 0.23 could not be refined because of the close proximity of the other N atom. In addition to the observed data, those unobserved data for which the calculated intensity was greater than the $3\sigma(I)$ cutoff value were included in the refinement. The residuals from each structure determination are presented in Table I, as are the number of reflections contributing to each refinement. The refined atomic coordinates for C, N, O, and S atoms are presented in Table II; bond distances and dihedral angles are contained in Tables III and IV, respectively.

Discussion

As expected, in view of the solvents from which the crystals were obtained, each of the 6-STC derivatives is present as the nonionized form of the free base. The conformation of each is illustrated in a stereoscopic projection[12] in Figure 1; comparable orienting vectors were used to prepare these drawings and those in Figure 1 of the accompanying paper.[1]

The structural data from 6-STC(0) and 5a-epi-6-STC(0) serve to underline the conformational integrity displayed by the tetracyclines. The crystal structure analysis demonstrates that 6-STC can adopt one of the two chemical structures for the free base, in its expected conformation, that we feel are necessary to support in vivo antibacterial activity. The question of the existence of the zwitterionic form in the expected conformation has not yet been unequivocally answered but there is indirect evidence that it can be adopted by 6-STC. The ^{1}H NMR spectrum of 6-STC·HCl[9] displays a narrow doublet (J = 2 Hz) for the H(4) signal. The small coupling constant is characteristic of the conformation associated with the zwitterion which is also that of the fully protonated medicinally important tetracyclines.[5,13] The near identity in the conformations of 5a-epi-7-ClTC(+) and 5a-epi-6-STC(0) leads us to believe that, under physiological conditions, the zwitterion is either not formed or adopts a conformation different from that typical of the medicinally important tetracyclines.

To probe the possibility that the lack of in vivo activity of the 5a-epitetracyclines described above is the result of lipid absorption, we examined the partition behavior of 5a-epi-6-STC and 6-STC in the $CHCl_3$–H_2O system (Table V). The partition coefficients indicate comparable lipophilicity for the two derivatives under

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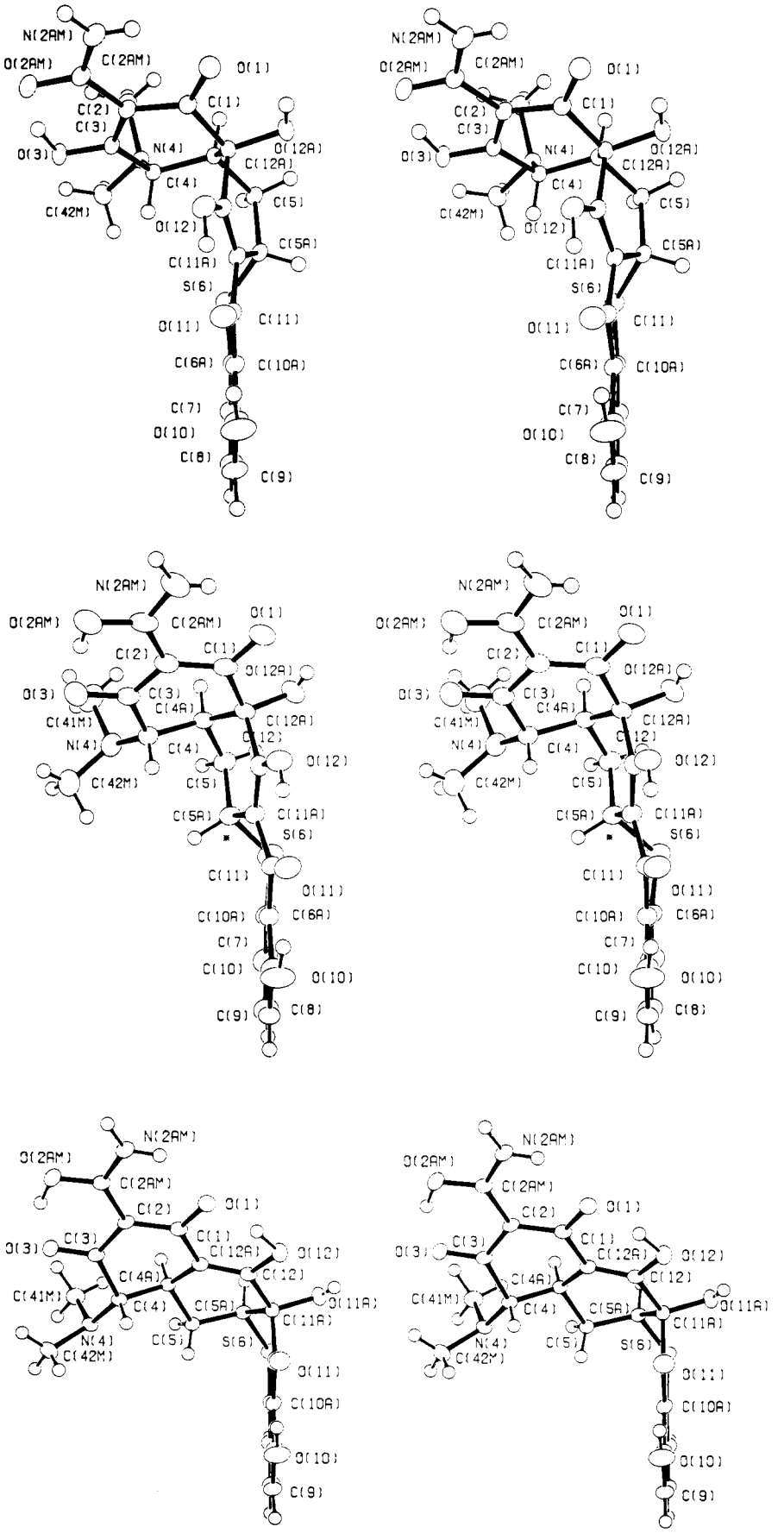

Figure 1. Stereoscopic projections[11] for the thiatetracycline derivatives: top, 6-STC(0); middle, 5a-epi-6-STC(0); bottom, 11a-OH-12a-DOH-6-STC(0). The molecules are projected with comparable orienting vectors used to plot the molecules of 5a-epi-7-ClTC(+) and 5a,11a-DH-7-ClTC(0).[1] Atom labels are presented for each molecule.

Table II. Fractional Atomic Coordinates for C, N, O, and S Atoms with Estimated Standard Deviations

atom	x	y	z	PP[a]
		6-STC(0)		
S(6)	0.43037 (5)	0.46276 (1)	0.40068 (1)	
C(1)	0.8256 (2)	0.19100 (5)	0.41377 (5)	
O(1)	0.9654 (2)	0.15157 (4)	0.38749 (5)	
C(2)	0.6510 (2)	0.17172 (5)	0.46195 (5)	
C(2am)	0.5915 (2)	0.09988 (5)	0.47415 (6)	
N(2am)	0.7139 (2)	0.05054 (5)	0.44600 (6)	
O(2am)	0.4192 (2)	0.08602 (4)	0.51168 (5)	
O(3)	0.3560 (1)	0.20672 (4)	0.53565 (5)	
C(3)	0.5314 (2)	0.22202 (5)	0.49758 (5)	
C(4)	0.6026 (2)	0.29752 (4)	0.50006 (5)	
N(4)	0.6498 (2)	0.32497 (4)	0.57930 (5)	
C(41m)	0.7983 (2)	0.27992 (6)	0.63533 (6)	
C(42m)	0.4329 (2)	0.34930 (6)	0.60793 (6)	
C(4a)	0.8287 (2)	0.31189 (5)	0.46218 (5)	
C(5)	0.8465 (2)	0.38665 (5)	0.43821 (6)	
C(5a)	0.6563 (2)	0.40788 (5)	0.36668 (5)	
C(6a)	0.2266 (2)	0.47663 (5)	0.31362 (6)	
C(7)	0.0830 (2)	0.53568 (6)	0.30942 (7)	
C(8)	−0.0928 (2)	0.54763 (6)	0.24371 (8)	
C(9)	−0.1281 (2)	0.50199 (7)	0.18261 (8)	
C(10)	0.0122 (2)	0.44229 (6)	0.18636 (6)	
O(10)	−0.0328 (2)	0.39926 (6)	0.12555 (6)	
C(10a)	0.1950 (2)	0.42860 (5)	0.25220 (5)	
C(11)	0.3274 (2)	0.36347 (5)	0.25729 (5)	
O(11)	0.2571 (2)	0.31719 (5)	0.20756 (5)	
C(11a)	0.5333 (2)	0.34971 (5)	0.31874 (5)	
C(12)	0.6178 (2)	0.28413 (5)	0.32910 (5)	
O(12)	0.5244 (2)	0.23103 (4)	0.28610 (5)	
C(12a)	0.8339 (2)	0.26660 (5)	0.39162 (5)	
O(12a)	1.0535 (1)	0.27846 (4)	0.36189 (5)	
		5a-*epi*-6-STC(0)		
S(6)	0.2897 (1)	0.47681 (8)	0.05116 (7)	
C(1)	0.7200 (4)	0.9242 (3)	0.3585 (2)	
O(1)	0.8565 (3)	0.9271 (2)	0.3976 (2)	
C(2)	0.6391 (4)	1.0197 (3)	0.3891 (3)	
C(2am)	0.7084 (4)	1.1234 (3)	0.4788 (3)	
N(2am)	0.8478 (4)	1.1405 (4)	0.5335 (3)	
O(2am)	0.6276 (3)	1.2046 (3)	0.5078 (2)	
O(3)	0.4174 (3)	1.1020 (3)	0.3601 (2)	
C(3)	0.4905 (4)	1.0186 (3)	0.3325 (3)	
C(4)	0.4101 (3)	0.9146 (3)	0.2364 (2)	
N(4)	0.3066 (3)	0.9568 (3)	0.1557 (2)	
C(41m)	0.3739 (6)	1.0838 (4)	0.1178 (4)	
C(42m)	0.1509 (4)	0.9496 (4)	0.1835 (3)	
C(4a)	0.5290 (3)	0.8570 (3)	0.1845 (2)	
C(5)	0.4476 (4)	0.7392 (3)	0.0983 (3)	
C(5a)	0.3395 (4)	0.6275 (3)	0.1461 (2)	
C(6a)	0.1605 (4)	0.3739 (3)	0.1220 (3)	
C(7)	0.0309 (4)	0.2792 (3)	0.0636 (3)	
C(8)	−0.0684 (4)	0.1911 (4)	0.1171 (3)	
C(9)	−0.0387 (4)	0.1940 (3)	0.2257 (3)	
C(10)	0.0932 (4)	0.2878 (3)	0.2857 (3)	
O(10)	0.1178 (3)	0.2809 (3)	0.3912 (2)	
C(10a)	0.1929 (4)	0.3828 (3)	0.2353 (3)	
C(11)	0.3312 (4)	0.4824 (3)	0.2987 (2)	
O(11)	0.3777 (3)	0.4650 (2)	0.3930 (2)	
C(11a)	0.4105 (3)	0.6000 (3)	0.2536 (2)	
C(12)	0.5424 (3)	0.6882 (3)	0.3118 (2)	
O(12)	0.6042 (3)	0.6725 (2)	0.4092 (2)	
C(12a)	0.6353 (3)	0.8105 (3)	0.2677 (2)	
O(12a)	0.7526 (3)	0.7705 (3)	0.2174 (2)	
C(1s1)	0.0794 (6)	0.4443 (4)	0.7601 (4)	0.77
O(s1)	−0.0375 (9)	0.3547 (8)	0.7129 (7)	0.77
N(s1)	0.1817 (6)	0.5280 (5)	0.7135 (6)	0.77
C(2s1)	0.3176 (6)	0.6271 (5)	0.7751 (5)	0.77
C(3s1)	0.1616 (7)	0.5251 (6)	0.5987 (4)	0.77
C(1s2)	0.060 (2)	0.415 (1)	0.649 (1)	0.23
O(s2)	−0.007 (3)	0.333 (3)	0.695 (2)	0.23
N(s2)	0.1797	0.5205	0.6956	0.23
C(2s2)	0.260 (2)	0.618 (2)	0.632 (1)	0.23
C(3s2)	0.232 (3)	0.537 (1)	0.811 (1)	0.23
		11a-OH-12a-DOH-6-STC(0)		
C(1)	0.1529 (2)	0.3775 (2)	0.1003 (1)	
O(1)	0.2154 (1)	0.4189 (1)	0.15649 (7)	
C(2)	0.0535 (1)	0.3073 (2)	0.11257 (9)	
C(2am)	0.0149 (2)	0.2907 (2)	0.1898 (1)	
N(2am)	0.0652 (1)	0.3362 (2)	0.25290 (9)	
O(2am)	−0.0762 (1)	0.2261 (1)	0.19851 (8)	
O(3)	−0.0974 (1)	0.1908 (1)	0.05801 (7)	
C(3)	−0.0066 (1)	0.2471 (2)	0.0494 (1)	
C(4)	0.0425 (1)	0.2459 (2)	−0.03059 (9)	
N(4)	−0.0323 (1)	0.2034 (1)	−0.09564 (8)	
C(41m)	−0.1342 (2)	0.2776 (2)	−0.1100 (1)	
C(42m)	−0.0566 (2)	0.0686 (2)	−0.0928 (1)	
C(4a)	0.0927 (1)	0.3760 (2)	−0.0446 (1)	
C(5)	0.1394 (2)	0.3891 (2)	−0.1248 (1)	
C(5a)	0.2149 (1)	0.5080 (2)	−0.1276 (1)	
S(6)	0.25359 (4)	0.53844 (4)	−0.22502 (2)	
C(6a)	0.3379 (2)	0.4082 (2)	−0.2408 (1)	
C(7)	0.3476 (2)	0.3729 (2)	−0.3178 (1)	
C(8)	0.4137 (2)	0.2710 (2)	−0.3353 (1)	
C(9)	0.4696 (2)	0.2035 (2)	−0.2775 (1)	
C(10)	0.4630 (2)	0.2384 (2)	−0.2002 (1)	
O(10)	0.5217 (1)	0.1700 (1)	−0.1459 (1)	
C(10a)	0.3972 (1)	0.3428 (2)	−0.1797 (1)	
C(11)	0.3916 (1)	0.3761 (2)	−0.0978 (1)	
O(11)	0.4480 (1)	0.3206 (1)	−0.04581 (8)	
C(11a)	0.3178 (1)	0.4863 (2)	−0.0727 (1)	
O(11a)	0.3824 (1)	0.5971 (1)	−0.07379 (8)	
C(12)	0.2791 (1)	0.4596 (2)	0.0080 (1)	
O(12)	0.3523 (1)	0.4954 (1)	0.06504 (7)	
C(12a)	0.1800 (2)	0.4043 (2)	0.0204 (1)	
C(1ac)	0.6447 (2)	0.0486 (3)	0.0412 (2)	
C(2ac)	0.7035 (2)	0.0090 (2)	0.1166 (1)	
O(2ac)	0.7664 (1)	−0.0798 (2)	0.1203 (1)	
C(3ac)	0.6808 (2)	0.0839 (2)	0.1864 (2)	

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

physiological conditions; both are among the most lipophilic of tetracyclines.[14] It, therefore, appears that lipid absorption is not responsible for the observation that 5a-epi-6-STC (and 5a-epi-7-ClTC) is in vitro active but in vivo inactive.

Combined, the lipophilicity data, the empirical antibacterial activity data, and the conformation data may provide some insight into the mechanism of activity of the tetracyclines. Since 7-ClTC and 6-STC and their 5a-epimers display comparable in vitro activity, it must be concluded that each is capable of entering the bacterial cell and interrupting protein synthesis.[15] It is therefore probable that the mechanism of cell transport and binding to the ribosome for 5a-epi-6-STC (and 5a-epi-7-ClTC) is the same as that for the parent. The lack of in vivo activity for the 5a-epimers implies that they do not reach the bacteria in sufficient concentration to be effective. In view of the lipophilicity data for the thiatetracyclines, the explanation of this phenomena most likely lies in the transport of the antibiotic in the bloodstream. The tetracyclines are known to bind to serum proteins which results in a reduction in biological potency.[17] The binding of oxytetracycline and demecyclocycline to bovine serum albumin has been reported to be hydrophobic.[18] We therefore suggest that the tetracycline is bound in the conformation of the nonionized free base and that the lack of significant in vivo activity of the 5a-

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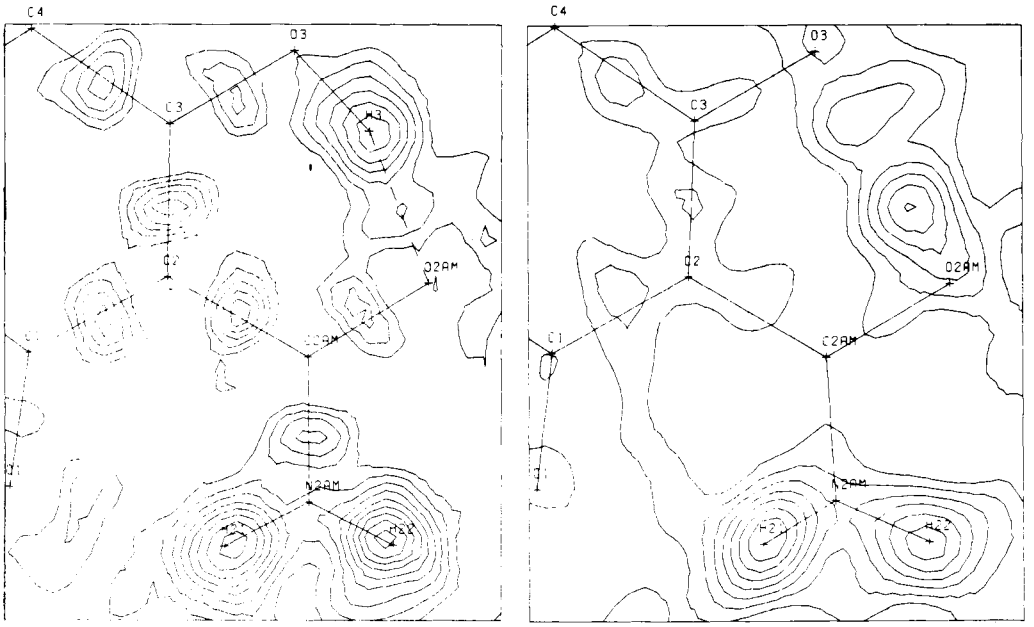

Figure 2. Difference electron density plots for the tricarbonylmethane region of the A ring. Hydrogen atoms were not included in the structure factor calculation for either 6-STC(0) (left) or 5a-epi-6-STC(0) (right). Partial occupancy of the enol hydrogen atom is indicated in both plots.

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

	6-STC(0)	5a-epi-6-STC(0)	11a-OH-12a-DOH-6-STC(0)
C(1)-C(12a)	1.530 (1)	1.546 (4)	1.464 (2)
C(1)-O(1)	1.236 (1)	1.246 (4)	1.270 (2)
C(1)-C(2)	1.440 (2)	1.430 (5)	1.440 (3)
C(2)-C(2am)	1.466 (1)	1.455 (4)	1.448 (2)
C(2)-C(3)	1.392 (1)	1.420 (5)	1.425 (2)
C(2am)-O(2am)	1.278 (2)	1.306 (5)	1.314 (2)
C(2am)-N(2am)	1.322 (2)	1.304 (5)	1.307 (2)
C(3)-O(3)	1.304 (1)	1.268 (5)	1.265 (2)
C(3)-C(4)	1.526 (1)	1.527 (4)	1.534 (2)
C(4)-N(4)	1.471 (1)	1.461 (4)	1.468 (2)
C(4)-C(4a)	1.540 (1)	1.542 (5)	1.543 (3)
N(4)-C(41m)	1.462 (1)	1.454 (5)	1.470 (3)
N(4)-C(42m)	1.459 (2)	1.466 (5)	1.473 (3)
C(4a)-C(5)	1.528 (1)	1.530 (4)	1.531 (2)
C(4a)-C(12a)	1.526 (1)	1.521 (4)	1.521 (2)
C(5)-C(5a)	1.549 (1)	1.534 (4)	1.523 (3)
C(5a)-S(6)	1.824 (1)	1.818 (3)	1.809 (2)
C(5a)-C(11a)	1.505 (1)	1.511 (4)	1.524 (2)
S(6)-C(6a)	1.756 (1)	1.759 (4)	1.755 (2)
C(6a)-C(7)	1.396 (2)	1.388 (4)	1.392 (3)
C(6a)-C(10a)	1.417 (1)	1.423 (5)	1.421 (3)
C(7)-C(8)	1.394 (2)	1.392 (6)	1.393 (3)
C(8)-C(9)	1.382 (2)	1.368 (6)	1.373 (3)
C(9)-C(10)	1.397 (2)	1.403 (4)	1.391 (3)
C(10)-O(10)	1.347 (2)	1.342 (5)	1.353 (3)
C(10)-C(10a)	1.423 (1)	1.410 (5)	1.426 (3)
C(10a)-C(11)	1.464 (1)	1.480 (4)	1.462 (3)
C(11)-O(11)	1.270 (1)	1.260 (4)	1.243 (2)
C(11)-C(11a)	1.452 (1)	1.452 (4)	1.554 (3)
C(11a)-C(12)	1.365 (1)	1.370 (4)	1.520 (2)
C(11a)-O(11a)			1.419 (2)
C(12)-O(12)	1.333 (1)	1.329 (4)	1.337 (2)
C(12)-C(12a)	1.516 (1)	1.525 (4)	1.361 (3)
C(12a)-O(12a)	1.422 (1)	1.428 (4)	

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

	6-STC(0)	5a-epi-6-STC(0)	11a-OH-12a-DOH-6-STC(0)
C(12a)-C(1)-C(2)-C(3)	−11.6 (1)	−5.3 (5)	10.6 (3)
C(1)-C(2)-C(3)-C(4)	−10.5 (1)	0.2 (5)	6.5 (2)
C(2)-C(3)-C(4)-C(4a)	−2.6 (1)	−23.0 (4)	−41.3 (2)
C(3)-C(4)-C(4a)-C(12a)	36.3 (1)	50.5 (3)	57.9 (2)
C(4)-C(4a)-C(12a)-C(1)	−56.6 (1)	−55.7 (3)	−45.1 (2)
C(4a)-C(12a)-C(1)-C(2)	44.9 (1)	33.5 (4)	10.2 (2)
C(4)-C(4a)-C(12a)-C(12)	65.0 (1)	68.4 (2)	138.8 (2)
C(5)-C(4a)-C(12a)-C(1)	178.0 (1)	−179.2 (3)	−170.9 (2)
C(11a)-C(12)-C(12a)-C(4a)	34.8 (1)	27.6 (4)	−6.7 (2)
C(12)-C(12a)-C(4a)-C(5)	−60.4 (1)	−55.1 (3)	13.0 (2)
C(12a)-C(4a)-C(5)-C(5a)	52.3 (1)	64.3 (4)	−40.9 (2)
C(4a)-C(5)-C(5a)-C(11a)	−16.4 (1)	−42.9 (4)	65.1 (2)
C(5)-C(5a)-C(11a)-C(12)	−12.1 (1)	14.4 (5)	−55.5 (2)
C(5a)-C(11a)-C(12)-C(12a)	2.4 (2)	−6.8 (5)	27.5 (2)
C(10a)-C(11)-C(11a)-C(5a)	−15.7 (1)	6.8 (5)	31.2 (2)
C(11)-C(11a)-C(5a)-S(6)	48.4 (1)	−49.0 (4)	−61.3 (2)
C(11a)-C(5a)-S(6)-C(6a)	−49.7 (1)	58.8 (2)	56.4 (1)
C(5a)-S(6)-C(6a)-C(10a)	28.1 (1)	−38.6 (3)	−27.5 (2)
S(6)-C(6a)-C(10a)-C(11)	0.9 (2)	3.0 (5)	1.4 (3)
C(6a)-C(10a)-C(11)-C(11a)	−12.3 (2)	19.4 (5)	0.7 (3)
C(9)-C(10)-C(10a)-C(6a)	0.9 (2)	−3.9 (5)	0.8 (3)
C(10)-C(10a)-C(6a)-C(7)	−0.2 (2)	3.0 (5)	−2.1 (3)
C(10a)-C(6a)-C(7)-C(8)	−0.3 (2)	−0.3 (6)	1.5 (3)
C(6a)-C(7)-C(8)-C(9)	0.1 (2)	−1.5 (6)	0.4 (3)
C(7)-C(8)-C(9)-C(10)	0.6 (2)	0.6 (6)	−1.7 (3)
C(8)-C(9)-C(10)-C(10a)	−1.1 (2)	2.3 (6)	1.0 (3)
O(12a)-C(12a)-C(1)-C(2)	165.3 (1)	152.7 (3)	
O(11a)-C(11a)-C(12)-C(12a)			146.8 (2)
O(1)-C(1)-C(2)-C(2am)	−10.8 (1)	−6.3 (5)	5.8 (3)
C(1)-C(2)-C(2am)-N(2am)	5.8 (1)	3.1 (5)	−0.1 (3)
C(3)-C(2)-C(2am)-O(2am)	5.5 (1)	4.5 (5)	−3.0 (3)
C(2am)-C(2)-C(3)-O(3)	−5.6 (1)	−1.9 (5)	7.0 (3)
O(3)-C(3)-C(4)-N(4)	50.5 (1)	30.1 (4)	13.4 (2)
C(3)-C(4)-N(4)-C(41m)	44.4 (1)	50.7 (4)	60.1 (2)
C(4a)-C(4)-N(4)-C(42m)	148.6 (1)	151.5 (3)	164.5 (2)
O(10)-C(10)-C(10a)-C(11)	−4.6 (2)	−0.9 (6)	−0.8 (3)
C(10)-C(10a)-C(11)-O(11)	−8.3 (2)	14.6 (5)	4.4 (3)
O(11)-C(11)-C(11a)-C(12)	−11.1 (1)	4.0 (5)	−31.8 (2)
C(11)-C(11a)-C(12)-O(12)	0.3 (2)	0.2 (5)	85.4 (2)
O(12)-C(12)-C(12a)-O(12a)	94.0 (1)	86.8 (3)	
O(11)-C(11)-C(11a)-O(11a)			89.4 (2)

epimers may be the result of their propensity to adopt this conformation.

The chemical structure of 11a-OH-12a-DOH-6-STC is very different from that of the antibacterially active derivatives, particularly with respect to the structure of the chromophores. It is not surprising that the conformation of its nonionized free base differs markedly from those of 6-STC(0) and 5a-epi-6-STC(0). The bonding geometry of 11a-OH-12a-DOH-6-STC(0) provides an interesting opportunity to examine the effects of hydrogen bonding on the carbonyl groups of both the tricarbonylmethane system and the BCD chromophore.

Table V. Partition Coefficients for 6-STC and 5a-epi-6-STC in the $CHCl_3$-H_2O System

6-STC		5a-epi-6-STC	
pH	K_D[a]	pH	K_D[a]
7	28.0	7	30.8
6	>50[b]	6	>50
5	>50	5	12.0

[a] K_D = (concentration in $CHCl_3$)/(concentration in H_2O). [b] The lipophilicity of the thiatetracycline was so high that accurate determination of the concentration in the aqueous phase was not practical.

Table VI. Intramolecular Hydrogen Bonding in the Thiatetracyclines[a]

	6-STC(0)	5a-epi-6-STC(0)	11a-OH-12a-DOH-6-STC(0)
O(2am)-H(3)	1.48 (3)	0.79 (7)	0.98 (3)
O(3)-H(3)	0.99 (3)	1.65 (6)	1.55 (3)
O(2am)-H(3)-O(3)	158 (3)	171 (9)	150 (3)
O(2am) · · · O(3)	2.431 (1)	2.427 (3)	2.450 (2)
O(10)-H(10)	0.86 (3)	0.82 (5)	0.84 (3)
O(11)-H(10)	1.74 (3)	1.89 (5)	1.82 (3)
O(10)-H(10)-O(11)	152 (3)	144 (5)	146 (3)
O(10) · · · O(11)	2.534 (1)	2.598 (4)	2.559 (2)
O(12)-H(12)	0.87 (2)	0.72 (5)	
O(11)-H(12)	1.68 (2)	1.85 (5)	
O(11)-H(10)-O(12)	152 (2)	155 (5)	
O(11) · · · O(12)	2.494 (1)	2.523 (3)	
O(12)-H(12)			0.97 (3)
O(1)-H(12)			1.56 (3)
O(12)-H(12)-O(1)			158 (3)
O(12) · · · O(1)			2.488 (2)
N(2am)-H(21)	0.86 (2)	0.77 (5)	0.92 (3)
O(1)-H(21)	2.03 (2)	2.12 (4)	1.97 (2)
O(1)-H(21)-N(2am)	137 (2)	136 (4)	133 (2)
O(1) · · · N(2am)	2.717 (1)	2.726 (5)	2.681 (2)

[a] Distances in angstroms, angles in degrees.

The enol moiety of the tricarbonylmethane system is of considerable interest not only because it sustains a central role in the equilibrium between the two forms of the free base but also because it presents an unusually short intramolecular hydrogen bond. We have reported examples where the hydrogen atom was found to be primarily associated with atom O(3)[4-6] and others in which it was localized primarily on atom O(2am).[1,16] Emphasis should be placed on the word primarily, since in an unusually short hydrogen bond the hydrogen atom is expected to bind to either oxygen atom with nearly equivalent bond energy. Difference electron density plots[19] for the appropriate region of the A ring are presented in Figure 2 for 6-STC(0) and 5a-epi-6-STC(0). These plots provide indications of partial, but not equivalent, occupancy for the hydrogen atom on each oxygen of the enol. Comparison of the appropriate C–O bond distances in Table III supports the validity of the plotted electron density. The intramolecular hydrogen-bonding geometry of the three thiatetracyclines is presented in Table VI. It is clear from Tables III and VI that the extension of the A-ring chromophore in 11a-OH-12a-DOH-6-STC(0) has not altered the hydrogen-bonding character of the A-ring enol significantly.

The extension of the A-ring chromophore in the 11a-hydroxyl derivative results in the carbonyl group at C(1) being part of an enolic β-diketone in which this group serves as an acceptor in two intramolecular hydrogen bonds. The observed carbonyl C–O bond distance closely resembles that at C(11) in the tetracyclines with the usual BCD chromophore.[7] In 11a-OH-12a-DOH-6-STC(0), the carbonyl group at C(11) also reflects the change in chemical structure. It is shortened significantly when compared with the usual value[7] but is very similar to that in 5a,11a-DH-7-ClTC(0)[1] in which the carbonyl group serves as an acceptor in only one hydrogen bond, that from the phenolic hydroxyl group. These observations and the general trend in bond distances from the high-resolution crystal structure determinations demonstrate that the tetracyclines display a high degree of integrity in their bonding geometry.

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Supplementary Material Available: Anisotropic temperature factors for the C, N, O, and S atoms, fractional atomic coordinates and isotropic temperature factors for the H atoms, bond angles between C, N, and O atoms, and calculated and observed structure factors for each structure (111 pages). Ordering information is given on any current masthead page.

(19) Program JIMPLAN, an oblique-plane Fourier plotting program in which the slant-plane Fourier transform routine of van de Waal has been incorporated by Hansen.

Synthesis of β-Lactams from Substituted Hydroxamic Acids

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Abstract: An efficient biomimetic β-lactam synthesis has been developed on the basis of cyclization of substituted β-hydroxyhydroxamic acids. The method is experimentally simple and, by appropriate choice of amino acid starting material, allows complete control of the stereochemistry at all positions of the β-lactam. The method is also compatible with the incorporation of sensitive peripheral functionality required for potential elaboration to biologically useful β-lactam derivatives. The key to the process is the low N–H pK of the intermediate *O*-alkylhydroxamic acid which facilitates diethylazodicarboxylate–triphenylphosphine (DEAD/Ph_3P) or $Ph_3P/CCl_4/Et_3N$ mediated N–C_4 bond closure to *N*-alkoxy-2-azetidinones. The sequential reduction of the latter by H_2/Pd–C followed by N–O cleavage with $TiCl_3$ leads to N-unsubstituted β-lactams.

The β-lactam antibiotics are the most widely used antimicrobial agents. However, the bacterial development of β-lactamase enzymes[1] which render some of the antibiotics ineffective has prompted a persistent search for modified antibiotic forms. While

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