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Structural Studies of Tetracyclines.[1] Crystal and Molecular Structure of Tetracycline Methyl Betaine Pentahydrate

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Abstract: The crystal structure of tetracycline methyl betaine pentahydrate was determined by x-ray diffraction techniques. Crystals of the betaine are orthorhombic, with space group $P2_12_12_1$ and unit cell dimensions of $a = 9.608$ (2), $b = 12.143$ (2), and $c = 21.036$ (4) Å. The unit cell and space group are surprisingly similar to those of tetracycline hexahydrate. The structure was solved by direct methods and refined by least-squares procedures to a final unweighted residual of 0.036 for the 1791 reflections used in the analysis. The conformation of tetracycline methyl betaine is virtually identical with that found for tetracycline (in the hexahydrate), as well as in the biologically active amide-protonated tetracyclines. Consequently, the loss of antibacterial activity with quaternization is not due to conformational changes. The differences in the biological activity of tetracycline are explicable on the basis of the loss of the hydrogen bonding potential of the amino group. Therefore, models for the mechanism of inhibition of protein synthesis by tetracyclines will most likely require hydrogen bonding to the dimethylamino group and the zwitterionic form of the drug.

Tetracyclines are widely used antibiotics. Their antimicrobial action appears to be related to the inhibition of protein synthesis by interference with the binding of aminoacyl-t-RNA to the ribosome.[5] However, on a molecular level the nature of the mechanism is not known. In fact, the conformation and state of the tetracycline molecule (i.e., neutral, protonated, or zwitterionic) at the active site has been the subject of much speculation. Our previous studies[2,3] suggested that the conformation found for both the active protonated and zwitterionic species was most likely the conformation at the active site. The next step in developing a molecular model for the mode of action of tetracyclines was to ascertain the relative importance and role of the various functional groups in the molecule.

There are a number of chemical modifications of tetracyclines which occasionally improve the therapeutic usefulness of these drugs but the majority of the chemical changes destroy the antimicrobial properties.[4] One such destructive modification is the quaternization of the dimethylamino group. Both tetracycline methyl iodide and tetracycline methyl betaine have relatively little useful antibacterial activity.[6] The structure of one of these two compounds was deemed essential for determining whether a conformational change was responsible for the loss of biological activity. We decided to study the methyl betaine derivative since the molecular dimensions determined in this equal-atom compound would be more precise than from the iodide salt.

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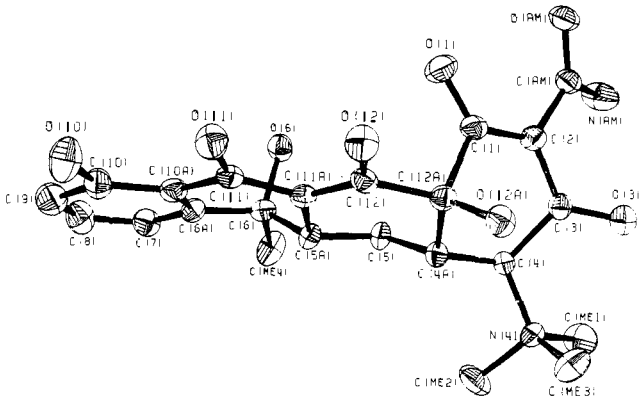

Figure 1. An ORTEP drawing of tetracycline methyl betaine. The orientation is identical with that used for the tetracycline moiety in our previous studies.[2,3] The ellipsoids are at 50% probability. The atomic numbering of the tetracycline framework is that usually used for tetracyclines.

Experimental Section

Recrystallization of tetracycline methyl iodide from water gives beautiful yellow crystals of the tetracycline methyl betaine. When the betaine is placed in the melting point apparatus at 110 °C, the solid pops at 125 °C, turns brown, and decomposes by 164 °C. The literature value[6] of 180–186 °C is for tetracycline methyl betaine with a molecule of methanol of solvation while our material corresponds to a pentahydrate.

Preliminary Weissenberg and precession photographs indicated that the crystals were orthorhombic with the space group $P2_12_12_1$. The unit cell dimensions and space group are very similar to those reported for tetracycline hexahydrate.[7,8]

Crystal Data. $C_{23}H_{26}N_2O_8 \cdot 5H_2O$, mol wt 548.54, $a = 9.608$ (2), $b = 12.143$ (2), $c = 21.036$ (4) Å, $V = 2454.3$ (8) Å^3, space group $P2_12_12_1$, $Z = 4$, ρ(calcd) = 1.484 g cm^{-3}, ρ(obsd) = 1.50 g cm^{-3}, μ(Cu Kα radiation) = 10.5 cm^{-1}, crystal size 0.14 × 0.16 × 0.17 mm.

Collection of X-Ray Diffraction Data. The intensity data were measured with a Syntex P$\bar{1}$ diffraction using Ni-filtered Cu Kα radiation. The procedure has been described.[9] The 1791 reflections (out of 1935 with $2\theta \leq 115°$) with $I \geq 2\sigma(I)$ were considered reliable and used in the analysis.

Solution and Refinement of the Structure. The structure was solved in a relatively straighforward manner using MULTAN.[10] A Fourier synthesis calculated using only the tetracycline molecule for phasing established the presence of only five water molecules. The assignment of O(6), C(Me4), O(am), and N(am) was made using the least-squares refinement of the thermal parameters and was consistent with the peak heights and bond distances. Three full matrix least-squares cycles with individual isotropic thermal parameters and all atoms correctly identified reduced R, the usual residual, to 0.092. Six block-diagonal least-squares cycles with anisotropic thermal parameters reduced R to 0.061. A difference Fourier synthesis at this stage revealed the positions of 30 of the 36 hydrogen atoms. The hydrogen atom contributions were included but not varied for six additional least-squares cycles which further reduced R to 0.042 and a goodness of fit of 1.0. Five additional cycles in which all the parameters were varied reduced R to 0.036 (the GOF was 0.88) and the refinement was terminated. The final positional parameters of the nonhydrogen atoms are given in Table I and the anisotropic thermal parameters are available.[11] A final difference Fourier synthesis gave reasonable positions for the remaining hydrogen atoms (Table II) but was otherwise featureless.

The quantity minimized in the least-squares calculations was $\Sigma w(|F_o| - |F_c|)^2$, where $w = (F_o/6.0)^2$ if $F_o < 6.0$, $w = 1$ if $6.0 \leq F_o \leq 24.0$, and $w = (24.0/F_o)^2$ if $F_o > 24.0$. The scattering factors for the nonhydrogen atoms were from ref 12 while those for H were from ref 13. Tables of observed and calculated structure amplitudes are available.[11]

Results and Discussion

An ORTEP drawing of the tetracycline methyl betaine molecule is given in Figure 1. The conformation, bond distances, and bond angles are virtually identical with those reported for tetracycline hexahydrate,[7,8] tetracycline urea, and various protonated tetracycline derivatives.[14,15] The similarities in the conformations can be seen by comparing Figure 1 with Figure 2 in ref 2 and with Figure 1 in ref 3. The three figures were drawn using the same orientation and scale to facilitate comparisions. The dihedral angles for tetracycline methyl betaine given in Table II can also be compared with those reported for other tetracycline compounds.[2] If we exclude the angles involving the trimethylamino group, the average difference between the corresponding 34 dihedral angles in Table III and those reported for the tetracycline free base[16] is 1.9°. The largest difference between the dihedral angles for the two compounds is 10.8° for the C1-C2-Cam-Oam group which probably reflects the small differences in the crystal packing between the two structures.

A comparison of the bond distances in tetracycline methyl betaine (Table IV) with those in tetracycline hexahydrate reveals an average difference of only 0.009 Å for the 36 common bond lengths. There are only three significant bond length differences between the two molecules. The C4-N4 bond length increases from 1.497 (4) Å in the free base to 1.532 (5) Å in the betaine. This increase is not surprising since the molecule changes from a protonated dimethylamino in the free base to a formally positive trimethylamino group in the betaine. There is also a significant increase in the N4–C(methyl) distances in the betaine relative to those in the free base. The third significant difference involves the C1-C2 bond length which

Table I. Final Positional Parameters (×10^4) for Tetracycline Methyl Betaine[a]

Atom	x	y	z
C(1)	−1393 (4)	1846 (3)	−2485 (2)
O(1)	−2179 (3)	2190 (2)	−2059 (1)
C(2)	−1159 (4)	2337 (3)	−3080 (2)
C(am)	−1757 (4)	3411 (3)	−3244 (2)
O(am)	−2182 (3)	4077 (2)	−2825 (1)
N(am)	−1918 (4)	3670 (3)	−3855 (2)
C(3)	−263 (4)	1786 (3)	−3534 (2)
O(3)	464 (3)	2276 (2)	−3926 (1)
C(4)	−400 (4)	518 (3)	−3566 (2)
N(4)	776 (3)	−48 (3)	−3934 (1)
C(Me1)	816 (5)	328 (4)	−4624 (2)
C(Me2)	507 (5)	−1276 (3)	−3968 (2)
C(Me3)	2170 (4)	142 (4)	−3631 (2)
C(4a)	−727 (3)	−35 (3)	−2916 (2)
C(5)	−2194 (4)	−522 (3)	−2922 (2)
C(5a)	−2487 (4)	−1230 (3)	−2326 (2)
C(6)	−4015 (4)	−1578 (3)	−2279 (2)
O(6)	−4864 (3)	−593 (2)	−2244 (1)
C(Me4)	−4423 (5)	−2233 (4)	−2861 (2)
C(6a)	−4215 (4)	−2224 (3)	−1657 (2)
C(7)	−5157 (4)	−3089 (4)	−1621 (2)
C(8)	−5402 (5)	−3613 (4)	−1041 (2)
C(9)	−4731 (4)	−3291 (4)	−501 (2)
C(10)	−3784 (4)	−2427 (3)	−524 (2)
O(10)	−3122 (3)	−2136 (2)	21 (1)
C(10a)	−3514 (4)	−1889 (3)	−1104 (2)
C(11)	−2478 (4)	−1006 (3)	−1130 (2)
O(11)	−2007 (3)	−605 (2)	−615 (1)
C(11a)	−2019 (4)	−600 (3)	−1735 (2)
C(12)	−1094 (4)	242 (3)	−1758 (2)
O(12)	−564 (3)	710 (2)	−1233 (1)
C(12a)	−555 (4)	764 (3)	−2361 (2)
O(12a)	863 (2)	1110 (2)	−2291 (1)
O(w1)	−2893 (4)	−1289 (3)	−4638 (2)
O(w2)	−796 (4)	−3769 (3)	−5243 (2)
O(w3)	−1676 (3)	−1865 (3)	−5838 (1)
O(w4)	−2104 (3)	−3308 (3)	−4061 (2)
O(w5)	−4475 (4)	−4524 (3)	−3915 (1)

[a] The estimated standard deviations are in parentheses.

Table II. Final Positional Parameters ($\times 10^3$) and the Isotropic Thermal Parameters ($Å^2$) for the Hydrogen Atoms in Tetracycline Methyl Betaine

Atoms	*x*	*y*	*z*	*B*	Bonded to	Distance
H(1)	−258 (6)	422 (5)	−392 (3)	8 (2)	N(am)	0.93 (6)
H(2)	−178 (5)	317 (4)	−424 (2)	5 (1)	N(am)	1.02 (5)
H(4)	−125 (4)	45 (3)	−385 (2)	1.4 (7)	C(4)	1.01 (3)
H(1M1)	−20 (6)	16 (5)	−480 (3)	8 (2)	C(Me1)	1.07 (6)
H(2M1)[a]	164	−10	−484		C(Me1)	1.05
H(3M1)	100 (6)	122 (5)	−466 (3)	8 (2)	C(Me1)	1.11 (6)
H(1M2)	−56 (6)	−138 (4)	−422 (2)	16 (1)	C(Me2)	1.17 (6)
H(2M2)	125 (5)	−155 (4)	−434 (2)	5 (4)	C(Me2)	1.12 (5)
H(3M2)	62 (6)	−157 (4)	−354 (2)	6 (1)	C(Me2)	0.98 (5)
H(1M3)	296 (5)	−18 (4)	−391 (2)	5 (1)	C(Me3)	1.04 (5)
H(2M3)	213 (6)	−35 (4)	−318 (2)	6 (1)	C(Me3)	1.13 (5)
H(3M3)	228 (5)	96 (4)	−355 (2)	5 (1)	C(Me3)	1.01 (5)
H(4a)	−4 (4)	−61 (3)	−286 (2)	1.6 (7)	C(4a)	0.97 (4)
H(15)	−287 (4)	4 (3)	−296 (2)	2.4 (8)	C(5)	0.95 (5)
H(25)	−228 (4)	−102 (3)	−327 (2)	1.5 (7)	C(5)	0.96 (4)
H(5a)	−201 (4)	−184 (3)	−236 (2)	1.9 (7)	C(5a)	0.90 (4)
H(6)	−583 (5)	−80 (4)	−231 (2)	5 (1)	O(6)	0.97 (5)
H(1M4)	−541 (4)	−242 (3)	−287 (2)	2.2 (8)	C(Me4)	0.98 (4)
H(2M4)	−420 (5)	−185 (4)	−322 (2)	5 (1)	C(Me4)	0.91 (4)
H(3M4)	−390 (5)	−295 (4)	−290 (2)	4 (1)	C(Me4)	1.01 (4)
H(7)	−564 (4)	−332 (3)	−195 (2)	2.6 (9)	C(7)	0.88 (4)
H(8)	−612 (6)	−427 (4)	−98 (2)	6 (1)	C(8)	1.06 (5)
H(9)	−492 (4)	−370 (3)	−9 (2)	3.1 (9)	C(9)	1.01 (4)
H(10)	−251 (5)	−149 (4)	−11 (2)	6 (1)	O(10)	1.02 (5)
H(12)	−102 (6)	31 (4)	−86 (2)	6 (1)	O(12)	1.03 (5)
H(13)	133 (4)	55 (4)	−222 (2)	3.3 (9)	O(12a)	0.82 (4)
H(1W1)[a]	−272	−189	−433		O(W1)	0.99
H(2W1)[a]	−281	−132	−504		O(W1)	0.85
H(1W2)[a]	−101	−353	−487		O(W2)	0.86
H(2W2)[a]	36	−384	−528		O(W2)	0.80
H(1W3)[a]	−103	−334	−551		O(W3)	0.92
H(2W3)	−219 (6)	−193 (5)	−622 (3)	8 (2)	O(W3)	0.95 (6)
H(1W4)	−139 (6)	−344 (5)	−381 (3)	8 (2)	O(W4)	0.89 (6)
H(2W4)	−279 (8)	−374 (6)	−394 (3)	10 (2)	O(W4)	0.88 (7)
H(1W5)	−461 (5)	−474 (4)	−354 (2)	5 (1)	O(W5)	0.85 (5)
H(2W5)	−517 (6)	−402 (5)	−404 (3)	8 (2)	O(W5)	0.95 (6)

Table III. Dihedral Angles (deg) in Tetracycline Methyl Betaine

Atoms	Angle	Atoms	Angle
C1-C2-C3-C4	39.1	C11a-C12-C12a-C4a	−21.9
C2-C3-C4-C4a	−34.2	C12-C12a-C4a-C5	47.9
C3-C4-C4a-C12a	−11.5	C12a-C4a-C5-C5a	−63.0
C4-C4a-C12a-C1	51.7	C6a-C6-C5a-C5	−178.4
C4a-C12a-C1-C2	−50.3	C10a-C11-C11a-C12	177.9
C12a-C1-C2-C3	3.9	C5a-C6-C6a-C10a	37.5
O12a-C12a-C1-C2	70.3	C6-C6a-C10a-C11	−6.8
O1-C1-C2-Cam	5.7	C6a-C10a-C11-C11a	−9.7
C1-C2-Cam-Oam	19.4	C10a-C11-C11a-C5a	−7.9
C3-C4-N4-CMe1	−61.2	C11-C11a-C5a-C6	39.9
C3-C4-N4-CMe2	−176.9	C11a-C5a-C6-C6a	−52.3
C3-C4-N4-CMe3	61.8		
C11a-C12-C12a-C1	98.9	C6a-C7-C8-C9	0.0
C12-C12a-C1-C2	−170.6	C7-C8-C9-C10	0.0
C5a-C5-C4a-C4	172.5	C8-C9-C10-C10a	−0.2
C5-C4a-C4-C3	112.1	C9-C10-C10a-C6a	0.5
C4a-C5-C5a-C11a	46.0	C10-C10a-C6a-C7	−0.5
C5-C5a-C11a-C12	−18.9	C10a-C6a-C7-C8	0.2
C5a-C11a-C12-C12a	7.5	N4-C4-C4a-C5	−115.1

Table IV. Bond Distances (Å) for Tetracycline Methyl Betaine

Atoms	Distance	Atoms	Distance
C(1)-O(1)	1.244 (4)	C(5a)-C(6)	1.541 (5)
C(1)-C(2)	1.404 (5)	C(6)-C(6a)	1.537 (5)
C(1)-C(12a)	1.563 (5)	C(6)-O(6)	1.449 (5)
C(2)-C(3)	1.450 (5)	C(6)-C(Me4)	1.512 (6)
C(2)-C(am)	1.466 (5)	C(6a)-C(10a)	1.405 (5)
C(am)-O(am)	1.264 (4)	C(6a)-C(7)	1.388 (6)
C(am)-N(am)	1.332 (5)	C(7)-C(8)	1.397 (6)
C(3)-C(4)	1.548 (5)	C(8)-C(9)	1.363 (6)
C(3)-O(3)	1.234 (4)	C(9)-C(10)	1.390 (6)
C(4)-C(4a)	1.555 (5)	C(10)-C(10a)	1.407 (5)
C(4)-N(4)	1.532 (5)	C(10)-O(10)	1.358 (5)
N(4)-C(Me1)	1.522 (5)	C(10a)-C(11)	1.464 (5)
N(4)-C(Me2)	1.515 (5)	C(11)-O(11)	1.271 (4)
N(4)-C(Me3)	1.500 (5)	C(11)-C(11a)	1.435 (5)
C(4a)-C(12a)	1.527 (5)	C(11a)-C(12)	1.356 (5)
C(4a)-C(5)	1.529 (5)	C(12)-C(12a)	1.510 (5)
C(5)-C(5a)	1.528 (5)	C(12)-O(12)	1.343 (4)
C(5a)-C(11a)	1.510 (5)	C(12a)-O(12a)	1.429 (4)

is 1.404 (5) Å in the betaine, 1.434 (4) Å in the low-temperature study of the free base,[8] and 1.426 (5) Å in the tetracycline urea complex.[3] The shortening of the C1-C2 bond distance in the betaine is accompanied by a possibly significant increase in the C2-C3 bond length when compared to either the free base or urea complex. Apparently, the delocalization of the negative charge over the C1-C2-C3-O1-O3 system is not as uniform as in the free base. An explanation may be found in the differences between the hydrogen bonding patterns in the three structures. In both tetracycline hexahydrate and tetracycline urea tetrahydrate there is a moderately strong hydrogen bond from N4-H to O3 which, of course, is no longer present in the betaine (the hydrogen bonding dimensions are in Table V and are discussed in more detail below). The sen-

Table V. Hydrogen Bonds in Tetracycline Methyl Betaine Pentahydrate

D-H···A	Position of A	D···A	H···A	D-H···A
N(am)-H(1)···O(w5)	$x, y-1, z$	3.295 (5)	2.38 (5)	170 (5)
N(am)-H(2)···O(3)	x, y, z	2.851 (5)	2.50 (5)	99 (3)
N(am)-H(2)···O(10)	$\frac{1}{2}-x, -y, \frac{1}{2}+z$	3.011 (4)	2.00 (5)	170 (4)
O(6)-H(6)···O(am)	$1-x, \frac{1}{2}+y, \frac{1}{2}-z$	2.871 (4)	1.94 (5)	160 (4)
O(10)-H(10)···O(11)	x, y, z	2.529 (4)	1.60 (5)	151 (4)
O(12)-H(12)···O(11)	x, y, z	2.482 (4)	1.54 (5)	149 (5)
O(12a)-H(13)···O(am)	$-x, \frac{1}{2}+y, \frac{1}{2}-z$	2.774 (4)	1.97 (4)	168 (4)
O(w1)-H(1w1)···O(w4)	x, y, z	2.838 (5)	1.91	156
O(w1)-H(2w1)···O(w3)	x, y, z	2.870 (4)	2.11	149
O(w2)-H(1w2)···O(w4)	x, y, z	2.842 (5)	2.02	160
O(w2)-H(2w2)···O(w1)	$-\frac{1}{2}+x, \frac{1}{2}-y, 1-z$	2.801 (5)	1.70	170
O(w3)-H(1w3)···O(w2)	x, y, z	2.762 (5)	1.86	170
O(w3)-H(2w3)···O(1)	$\frac{1}{2}-x, -y, \frac{1}{2}+z$	2.821 (4)	1.89 (6)	167 (5)
O(w4)-H(1w4)···O(12)	$-x, \frac{1}{2}+y, \frac{1}{2}-z$	2.894 (4)	2.14 (6)	142 (5)
O(w4)-H(1w4)···O(12a)	$-x, \frac{1}{2}+y, \frac{1}{2}-z$	3.167 (4)	2.43 (6)	141 (5)
O(w4)-H(2w4)···O(w5)	x, y, z	2.732 (5)	1.88 (7)	162 (7)
O(w5)-H(1w5)···O(6)	$1-x, \frac{1}{2}+y, \frac{1}{2}-z$	2.836 (4)	2.01 (5)	165 (5)
O(w5)-H(2w5)···O(w3)	$\frac{1}{2}+x, \frac{1}{2}-y, 1-z$	2.754 (5)	1.82 (6)	170 (5)

sitivity of the A-ring dimensions to the orientation of the amide group and the hydrogen bonding patterns has been discussed previously.[15]

In summary we see that the tetracycline methyl betaine molecule has a conformation and dimensions which are virtually identical with those found in the active tetracyclines. The lack of a useful antimicrobial spectrum must be related in some way to the presence of the trimethylamino group on N4. There are several possible explanations for the relative inactivity of the betaine, such as lipid solubility, poor transport to the active site, instability due to the loss of trimethylamine,[6] and subsequent changes in conformation, or the lack of a proton on N4. The latter may be particularly important if the tetracycline is to bind to the ribosome. Although the exact molecular mechanism for the inhibition of protein synthesis is not known, the dimethylamino group is essential for useful activity. The removal of the dimethylamino group could alter at least two important properties of the tetracycline moiety: the molecular conformation and the ability to form a zwitterion. Since the zwitterion is the predominant species at the physiological pH, it is reasonable to assume that the zwitterion is the active species. The formation of the zwitterion involves a transfer of the proton from the A-ring oxygen atom O1 or O3 to the dimethylamino nitrogen N4. Consequently, N4 is now capable of hydrogen bonding to an appropriate receptor site. However, in the betaine not only is the hydrogen bonding prevented because of the lack of the N4 proton, but the methyl group would introduce steric repulsion with a potential hydrogen bonding site. On the other hand, if the function of the dimethylamino group is only to stabilize the conformation and zwitterion, then the lack of activity in the betaine may be due to other causes. Experiments are in progress to attempt to elucidate the role of the dimethylamino group on the biological activity of tetracyclines.

A surprising fact is that the tetracycline methyl betaine pentahydrate is virtually isomorphous with tetracycline hexahydrate. The two compounds are both orthorhombic, with space group $P2_12_12_1$ and very similar cell constants. However, the near isomorphism and the difference in the degree of hydration are understandable by considering the hydrogen bonding patterns in the two compounds. In the free base one of the water molecules (Ow1 in ref 7 or Ow2 in ref 8) is linked to the dimethylamino group via an N-H···O hydrogen bond but is only weakly hydrogen bonded via the water hydrogens to other acceptors. The formation of the betaine destroys the hydrogen bonding potential of the dimethylamino group, and the corresponding water molecule cannot be stabilized in the crystal lattice. The minor role which the water molecule plays in the crystal packing and the bulk provided by the added methyl group produce a nearly isomorphous structure. The five water molecules in the betaine correspond to the remaining five waters in the free base. The hydrogen bonds involving the five water molecules in the betaine are very similar to the ones formed in the free base.

Conclusions

Tetracycline methyl betaine has virtually the same conformation and dimensions as tetracycline free base. Consequently, the fact that tetracycline methyl betaine is relatively inactive as an antibacterial agent cannot be explained on the basis of conformational changes. The disappearance of a suitable hydrogen bonding site on the amino nitrogen as a result of quaternization of the dimethylamino group is the most probable explanation for the change in the antibacterial properties. The difference in the degree of hydration between tetracycline methyl betaine and tetracycline is a direct result of the quaternization. Therefore, a model for the molecular mechanism of protein inhibition by tetracyclines will undoubtedly require both a hydrogen bonding interaction with the dimethylamino group and the zwitterionic form of the drug.

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Supplementary Material Available: Table of anisotropic thermal parameters and of observed and calculated structure amplitudes (13 pages). Ordering information is given on any current masthead page.

References and Notes

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Conformational Analysis of 8-Azanucleosides. Crystal and Molecular Structure of 8-Azatubercidin Monohydrate, a Nucleoside Analogue Exhibiting the "High Anti" Conformation

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Abstract: The crystal structure of 8-azatubercidin monohydrate, $C_{10}N_5O_4H_{14}\cdot H_2O$, a cytotoxic analogue of tubercidin, has been determined. The unit cell constants are $a = 7.458$ (2) Å, $b = 9.744$ (3) Å, $c = 17.267$ (5) Å, with $Z = 4$ and space group $P2_12_12_1$. The structure was solved by direct methods and refined by least squares to an R value of 0.040 using the intensities of 1112 reflections with $I > 1.50\sigma$. The ribose exhibits a nearly symmetrical C(1′)-exo-C(2′)-endo pucker (^2_1T), and the conformation about the exocyclic C(4′)-C(5′) bond is trans ($\psi = 179.5°$). The glycosyl conformation is in the "high-anti" range ($\chi = 102.4°$). There are close intramolecular contacts between the base ring nitrogen N(8) and the ribose C(2′) and H(2′) atoms of 2.81 and 2.49 Å, respectively. All available sites on the ribose and pyrazolopyrimidine base rings, except N(8), are involved in intermolecular hydrogen bonds. These include an N(6)-H(6)···O(1′) hydrogen bond of 2.99 Å involving the ribose ring oxygen atom. The screw-related bases exhibit a head-to-tail stacking with partial overlap of the rings and a minimum interplanar separation of 3.6 Å.

Adenine is outstanding among the common nucleic acid bases as precursor to a wide range of biologically important substances. In addition to its role as a component of DNA and RNA, it is essential in the varied functions of energy metabolism (ATP), hormonal control (cAMP), and antibiotic action. It is likely that adenine was of central evolutionary importance in the primordial development of biochemical systems. Among the antibiotic derivatives of adenosine (II) is the series of compounds generated by substitution of nitrogen for carbon or vice versa at one or more sites within the imidazole ring. 4-Amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (I) is one such compound. Because of its similarity to tubercidin, it is hereafter referred to as 8-azatubercidin. To facilitate comparison with other purine systems, we adopt the purine numbering throughout this work. The structure and conformation of similar compounds have also been the subject of much interest in our own and other laboratories. Structural studies of formycin monohydrate (VI),[2] tubercidin (III),[3] toyocamycin (IV),[4] formycin hydrobromide,[5] oxoformycin B, formycin B,[6] 2-methylformycin,[7] and 8-azaadenosine (VII)[8] have been published.

The conformational properties of such compounds include a number of noteworthy features. Outstanding among these is the tendency to assume the "high-anti"[2,8] glycosyl conformation (χ) in the solid state. The high-anti conformation, although unusual, is allowed in normal nucleosides and has been observed in the crystal structures of guanosine and inosine.[9,10] The preference for the high χ values in 8-azapurine and 6-azapyrimidine nucleoside analogues is influenced by the presence of the unprotonated and electronegative nitrogen atom N(8) or N(6) as will be discussed below in greater detail.

The antibiotic action of 8-azatubercidin (II)[11] appears analogous to that of adenosine[12] in its inhibition of uridine biosynthesis. In contrast to the 8-deaza analogues tubercidin (III) and sangivamycin (V), 8-azatubercidin (II), 8-azaadenosine (VII), and formycin (VI) are substrates for adenosine deaminase[11,13] although they bind less effectively to the enzyme than adenosine itself. The x-ray crystallographic analysis of 8-azatubercidin was undertaken to elucidate the effect of the pyrazolopyrimidine ring on the overall molecular conformation.

Experimental Section

Intensity Data Collection. A sample of 8-azatubercidin ($C_{10}N_5O_4H_{14}$) provided by Dr. Sidney Hecht was crystallized by slow evaporation from a solution of 50% aqueous ethanol. The crystals formed parallelepipeds elongated in the a direction and preliminary photographic work showed that they belong to the orthorhombic space group $P2_12_12_1$. Unit cell dimensions were measured by a least-squares refinement based on the goniostat angles θ, χ, and ϕ of 10 accurately centered reflections with $30° \leq 2\theta \leq 60°$. They are $a = 7.458$ (2) Å, $b = 9.744$ (3) Å, and $c = 17.267$ (5) Å. There are four molecules in the unit cell and the calculated density is 1.517 g cm^{-3}. Intensity data were collected on a Picker FACS-I diffractometer with Cu Kα radiation using a crystal measuring 0.5 mm × 0.15 mm × 0.15 mm mounted with the a axis coincident with the ϕ axis of the goniostat. Reflections (1231) were measured to a 2θ limit of 127° using a θ-2θ scan technique at scan rate of 2° min^{-1} with 10-s background counts taken at each scan limit. Of these reflections, 1119 had intensities greater than 1.5σ and were considered observed. Three moderately strong reflections were monitored at regular intervals during the course of data collection and displayed no significant change in intensity. The data were corrected for Lorentz and polarization effects and an empirical absorption correction based on the variation of the 400 reflection at $\chi = 90°$ as a function of ϕ (maximum variation, 7% on intensity) was also applied.

Structure Determination and Refinement. The structure was solved by direct methods with the program MULTAN.[14] The correct solution had a figure of merit of 1.08 and a residual $R(E)$ of 21.4%. A Fourier

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