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Structural Studies of Tetracyclines. Crystal and Molecular Structure of Tetracycline–Urea Tetrahydrate

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Abstract: The first structural study of a tetracycline adduct has been carried out by x-ray diffraction techniques. The crystals of the tetracycline–urea tetrahydrate complex are orthohombic. The space group is $P2_12_12_1$ and the unit cell dimensions are $a = 12.228$ (3), $b = 12.884$ (3), and $c = 16.663$ (4) Å. There are four molecules of tetracycline–urea tetrahydrate per unit cell. The structure was solved by direct methods and refined by least-squares techniques to a final unweighted residual of 0.043 for the 2395 reflections used in the analysis. The hydrogen atoms were all located in a difference Fourier synthesis and refined with isotropic thermal parameters. The urea molecule is hydrogen bonded to O(1) and O amide of the A ring. The tetracycline molecule exists as a zwitterion in the adduct. The conformation and zwitterionic character of the tetracycline moiety are virtually identical with that found in both the free bases and protonated species of therapeutically active tetracyclines. Interaction with the zwitterionic species and the proper conformation both appear to be important requirements for biological activity in tetracyclines.

Introduction

The tetracyclines are an important class of widely used antibiotics.[1,2] Their mode of action involves the inhibition of protein synthesis by interference with the binding of aminoacyl-tRNA to the ribosome,[3] but the precise molecular mechanism remains obscure. The binding of the tetracycline molecule to an appropriate site on the ribosome could prevent the attachment of the aminoacyl-tRNA. Consequently, the manner in which the tetracycline molecule interacts with other molecules is important for the development of a model for the binding to ribosomes.

The solubilities of tetracyclines can be increased by various anions and neutral molecules, indicating complex formation.[4,5] However, in the case of urea an insoluble adduct is formed with tetracycline but under the same conditions, not with either 7-chlorotetracycline or 5-hydroxytetracycline.[6] To elucidate this puzzling difference in these three closely related drugs, we initiated a crystal structure study of the tetracycline–urea adduct.

Experimental Section

Light yellow octahedral crystals were formed from a urea–tetracycline solution. Preliminary Weissenberg and precession photographs indicated that the crystals were orthorhombic with the space group $P2_12_12_1$. An approximately equidimensional crystal, 0.15 mm on edge, was used for the measurement of the cell constants and intensity data. The cell dimensions obtained from a least-squares fit of 15 2θ values for Cu K_β peak ($\lambda = 1.39217$ Å) were $a = 12.228$ (3), $b = 12.884$ (3), and $c = 16.663$ (4) Å. The cell volume is 2625.2 Å^3. The density calculated for four molecules of tetracycline–urea tetrahydrate, $C_{23}H_{36}N_4O_{13}$, fw 576.56, is 1.459 g cm^{-3}, in good agreement with the value of 1.45 g cm^{-3} determined by flotation. The adduct had previously been reported to be a trihydrate.[6]

The intensity data were measured using previously described techniques.[7] All the reflections in one octant of reciprocal space to a limit of $2\theta \leq 135°$ were measured first and then one half of the hemisphere was measured. The intensities were corrected for a small (maximum 3%) variation in the four standard reflections and then equivalent reflections were averaged. Of the 2682 reflections in the octant, 2395 had an intensity >1.2 times the appropriate background and were considered reliable and used in the analysis. The value of μ for Cu Kα radiation is only 10.4 cm^{-1} and no corrections for absorption were necessary.

Structure Determination and Refinement. The structure was eventually solved by direct methods when we obtained a copy of MULTAN.[8] The correct E map indicated the positions of the tetracycline molecule and many of the other atoms. However, only the tetracycline group was used in calculating a Fourier synthesis which

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Table I. The Final Parameters of Nonhydrogen Atoms for Tetracycline-Urea Complex. All Values are $\times 10^4$. The Estimated Standard Deviations Are Given in Parentheses[a]

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
O(1)	3133 (2)	2003 (2)	1320 (2)	41 (2)	38 (2)	39 (1)	−8 (3)	−19 (2)	14 (2)
O(am)	4916 (2)	3287 (2)	1049 (1)	25 (1)	42 (2)	34 (1)	5 (3)	−5 (2)	0 (2)
O(3)	2525 (2)	5272 (2)	136 (2)	41 (2)	40 (2)	66 (2)	−22 (3)	−20 (3)	49 (3)
O(6)	156 (2)	1187 (2)	2795 (2)	53 (2)	41 (2)	29 (1)	7 (3)	−8 (2)	−7 (2)
O(10)	−962 (2)	−2071 (2)	1156 (2)	53 (2)	32 (1)	38 (1)	−14 (3)	10 (3)	−2 (2)
O(11)	641 (2)	−815 (2)	912 (2)	45 (2)	27 (1)	38 (1)	5 (3)	16 (2)	−7 (2)
O(12)	1907 (2)	496 (2)	317 (2)	37 (2)	28 (1)	41 (1)	−1 (3)	25 (2)	−14 (2)
O(12a)	1894 (2)	2362 (2)	−407 (1)	35 (2)	43 (2)	25 (1)	−19 (3)	0 (2)	−3 (2)
N(am)	4494 (2)	4926 (2)	756 (2)	35 (2)	38 (2)	45 (2)	−22 (4)	−5 (3)	5 (3)
N(4)	447 (2)	4821 (2)	−39 (2)	36 (2)	30 (2)	44 (2)	−10 (3)	−19 (3)	11 (3)
C(1)	2691 (3)	2631 (2)	867 (2)	28 (2)	28 (2)	20 (1)	7 (4)	6 (3)	−7 (3)
C(2)	3072 (3)	3648 (3)	680 (2)	29 (2)	32 (2)	23 (1)	2 (4)	3 (3)	2 (3)
C(3)	2319 (3)	4403 (3)	397 (2)	40 (2)	31 (2)	29 (2)	−20 (4)	−12 (3)	8 (3)
C(4)	1094 (3)	4199 (3)	553 (2)	36 (2)	26 (2)	28 (1)	2 (4)	−9 (3)	1 (3)
C(4a)	707 (2)	3073 (2)	620 (2)	23 (2)	28 (2)	26 (1)	−6 (4)	−10 (3)	1 (3)
C(5)	239 (3)	2846 (3)	1455 (2)	31 (2)	28 (2)	30 (1)	14 (4)	−4 (3)	−5 (3)
C(5a)	−340 (3)	1808 (3)	1459 (2)	26 (2)	30 (2)	26 (1)	6 (4)	−6 (3)	−4 (3)
C(6)	−764 (3)	1458 (3)	2286 (2)	34 (2)	38 (2)	27 (1)	2 (4)	3 (3)	−6 (3)
C(6a)	−1384 (3)	442 (3)	2165 (2)	41 (2)	44 (2)	20 (1)	2 (4)	−8 (3)	10 (3)
C(7)	−2385 (3)	232 (3)	2522 (2)	45 (3)	52 (3)	25 (1)	−5 (5)	13 (3)	6 (3)
C(8)	−2891 (3)	−714 (3)	2394 (2)	38 (3)	68 (3)	28 (2)	−29 (5)	11 (3)	20 (4)
C(9)	−2416 (3)	−1471 (3)	1941 (2)	46 (3)	44 (3)	28 (2)	−34 (5)	−5 (3)	18 (3)
C(10)	−1408 (3)	−1288 (3)	1588 (2)	41 (2)	37 (2)	24 (1)	−7 (4)	−1 (3)	15 (3)
C(10a)	−904 (3)	−319 (3)	1671 (2)	36 (2)	30 (2)	23 (1)	−4 (4)	−5 (3)	13 (3)
C(11)	95 (3)	−82 (3)	1215 (2)	35 (2)	30 (2)	21 (1)	0 (4)	−7 (3)	−5 (3)
C(11a)	388 (3)	988 (2)	1096 (2)	27 (2)	28 (2)	21 (1)	−10 (4)	−9 (3)	0 (3)
C(12)	1283 (3)	1214 (3)	642 (2)	30 (2)	28 (2)	23 (1)	1 (4(	−4 (3)	−7 (3)
C(12a)	1629 (3)	2309 (3)	422 (2)	31 (2)	28 (2)	25 (1)	−8 (4)	4 (3)	3 (3)
C(am)	4222 (3)	3932 (3)	833 (2)	34 (2)	31 (2)	20 (1)	−2 (4)	1 (3)	−6 (3)
C(Me_1)	529 (4)	4461 (3)	−880 (3)	75 (4)	57 (3)	40 (2)	−11 (6)	−37 (5)	28 (4)
C(Me_2)	−724 (3)	4922 (3)	215 (3)	35 (3)	47 (3)	71 (3)	8 (5)	−22 (4)	18 (5)
C(6M)	−1449 (3)	2281 (3)	2688 (2)	53 (3)	53 (3)	33 (2)	14 (5)	15 (4)	−9 (4)
O(1u)	4089 (2)	7840 (3)	1263 (2)	60 (2)	139 (3)	33 (1)	32 (5)	−19 (3)	−11 (4)
N(1u)	5048 (3)	7263 (3)	2319 (2)	64 (3)	61 (3)	35 (1)	16 (5)	−17 (4)	−8 (3)
N(2u)	3954 (3)	8676 (3)	2446 (2)	60 (3)	70 (3)	35 (2)	28 (5)	−13 (3)	−6 (3)
C(1u)	4350 (3)	7923 (3)	1982 (2)	34 (3)	67 (3)	32 (2)	−28 (5)	2 (3)	14 (4)
O(w_1)	2363 (2)	473 (2)	2380 (2)	63 (2)	69 (2)	48 (1)	18 (4)	−15 (3)	28 (3)
O(w_2)	3476 (2)	942 (2)	3769 (2)	53 (2)	79 (2)	47 (1)	30 (4)	−4 (3)	8 (3)
O(w_3)	2406 (2)	2050 (2)	5044 (2)	53 (2)	62 (2)	42 (1)	16 (4)	10 (3)	13 (3)
O(w_4)	4434 (2)	3035 (2)	5019 (2)	59 (2)	50 (2)	52 (1)	−22 (4)	12 (3)	−4 (3)

[a] The temperature factor is of the form $\exp(-\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + \beta_{12}hk + \beta_{13}hl + \beta_{23}kl)$.

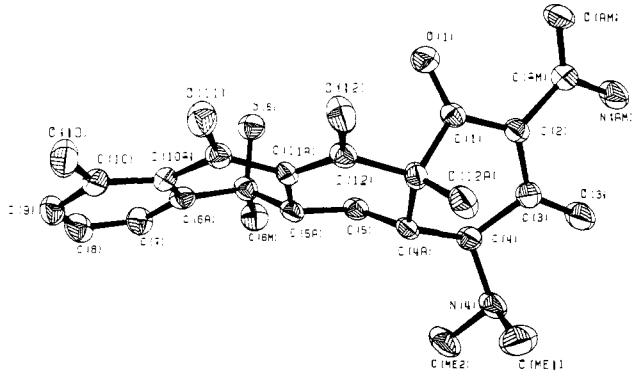

Figure 1. An ORTEP drawing of the tetracycline zwitterion in the tetracycline-urea adduct. The urea molecule which is hydrogen bonded O(am) and O(1) was omitted. The orientation is identical with that given in the preceding paper for the 6-demethyltetracycline cation and the anhydrotetracycline cation.

confirmed the positions of the urea and four water molecules. The initial refinement used the full matrix with individual isotropic thermal parameters. The O and N atoms of the amide group and the urea molecule were initially assigned nitrogen-scattering factors and the O and C on C(6) were given carbon-scattering factors to confirm the postulated assignments by least-squares techniques. After three least-squares cycles in which all the atoms were properly identified, the usual residual R ($=\Sigma\Delta F/\Sigma F(\text{obsd})$) was 0.13 with individual isotropic thermal parameters. Least-squares refinement with anisotropic thermal parameters was carried out using the block approximation (3 × 3 and 6 × 6 blocks) to the full matrix. Three cycles reduced R to 0.084 and a difference Fourier synthesis clearly indicated positions for all the hydrogen atoms. Two least-squares cycles with the hydrogen atoms included as fixed contributions reduced R to 0.053. Four additional cycles in which all parameters were refined, including the hydrogen atoms with isotropic thermal parameters, reduced R to 0.043. At this point the shifts were all less than one-third of an esd and the refinement was considered complete. The final parameters for all atoms are given in Tables I and II.

The quantity $\Sigma w(F(\text{obsd}) - F(\text{calcd}))^2$ was minimized in the least-squares refinement where $w = (F(\text{obsd})/F(\text{min}))^2$ if $F(\text{obsd}) < F(\text{min})$; $w = 1$ if $F(\text{min}) \leq F(\text{obsd}) \leq 2F(\text{min})$; and $w = (2F(\text{min})/F(\text{obsd}))^2$ if $F(\text{obsd}) > 2F(\text{min})$ with $F(\text{min}) = 16.0$. The scattering factors were taken from the usual source.[9,10]

Results and Discussion

The $TC^{\pm}$ molecule (see footnote 11 for the abbreviations used) is shown in Figure 1 with the thermal ellipsoids and the standard numbering used for the tetracycline ring system. The conformation of $TC^{\pm}$ in the urea complex is virtually identical with that found in the free bases $TC^{\pm}\cdot 6H_2O$[13,14] and 5-$HTC^{\pm}\cdot 2H_2O$[14] and the protonated species 6-DM-7-CLTC·

Table II. The Final Parameters of the Hydrogen Atoms in Tetracycline-Urea Tetrahydrate. The Atoms Is Followed by the Positional Parameters ($\times 10^3$), the Isotropic Thermal Parameter and the Distance to the Atom to Which It Is Bonded. The Estimated Standard Deviations Are Given in Parentheses

Atom	x	y	z	B, Å^2	Distance to atom	
H(1)	399 (3)	542 (3)	55 (2)	3.4 (0.9)	0.94 (4)	N(am)
H(2)	520 (3)	515 (3)	91 (2)	2.9 (0.8)	0.94 (4)	N(am)
H(3)	75 (3)	546 (3)	4 (2)	3.2 (0.8)	0.92 (4)	N(4)
H(4)	33 (3)	175 (3)	310 (2)	2.8 (0.8)	0.91 (3)	O(6)
H(5)	−31 (3)	−187 (3)	95 (3)	4.4 (1.0)	0.91 (4)	O(10)
H(6)	156 (3)	−20 (3)	46 (3)	5.0 (1.0)	1.02 (4)	O(12)
H(7)	136 (3)	205 (3)	−66 (2)	2.1 (0.7)	0.87 (3)	O(12a)
H(8)	96 (2)	455 (2)	109 (2)	1.1 (0.6)	1.01 (3)	C(4)
H(9)	15 (2)	292 (2)	23 (2)	1.2 (0.6)	0.97 (3)	C(4a)
H(10)	84 (3)	286 (2)	184 (2)	1.8 (0.7)	0.97 (3)	C(5)
H(11)	−28 (3)	340 (3)	156 (2)	2.0 (0.7)	0.97 (3)	C(5)
H(12)	−95 (3)	187 (2)	110 (2)	1.8 (0.7)	0.96 (3)	C(5a)
H(13)	−276 (3)	71 (3)	292 (2)	2.6 (0.8)	1.01 (4)	C(7)
H(14)	−362 (3)	−88 (3)	264 (2)	2.1 (0.7)	1.00 (3)	C(8)
H(15)	−277 (3)	−216 (3)	181 (2)	3.5 (0.9)	1.01 (4)	C(9)
H(16)	12 (3)	374 (3)	−94 (2)	4.8 (1.0)	1.06 (4)	C(Me_1)
H(17)	15 (3)	500 (3)	−126 (2)	4.7 (1.0)	1.05 (4)	C(Me_1)
H(18)	131 (3)	435 (3)	−103 (2)	4.7 (1.0	0.99 (4)	C(Me_1)
H(19)	−107 (3)	421 (3)	23 (2)	3.4 (0.9)	1.01 (4)	C(Me_2)
H(20)	−107 (3)	541 (3)	−18 (2)	4.6 (1.0)	1.01 (4)	C(Me_2)
H(21)	−71 (4)	523 (3)	74 (3)	6.1 (1.2)	0.97 (5)	C(Me_2)
H(22)	−204 (3)	247 (3)	232 (2)	4.2 (1.0)	0.98 (4)	C(6M)
H(23)	−100 (3)	292 (3)	279 (2)	3.5 (0.8)	1.01 (4)	C(6M)
H(24)	−168 (3)	212 (3)	324 (2)	4.8 (1.1)	0.98 (4)	C(6M)
H(25)	534 (3)	733 (3)	282 (2)	3.7 (0.9)	0.92 (4)	N(1u)
H(26)	542 (3)	676 (3)	198 (2)	4.5 (1.0)	0.97 (4)	N(1u)
H(27)	417 (3)	871 (3)	298 (2)	3.6 (0.9)	0.93 (4)	N(2u)
H(28)	336 (4)	917 (3)	222 (3)	5.4 (1.1)	1.03 (4)	N(2u)
H(29)	260 (4)	93 (4)	206 (3)	6.8 (1.3)	0.85 (5)	O(w_1)
H(30)	166 (4)	55 (4)	247 (3)	7.1 (1.4)	0.88 (5)	O(w_1)
H(31)	305 (4)	126 (3)	414 (3)	5.0 (1.1)	0.90 (4)	O(w_2)
H(32)	299 (4)	69 (4)	336 (3)	5.5 (1.1)	0.96 (4)	O(w_2)
H(33)	196 (4)	241 (3)	475 (2)	5.0 (1.0)	0.87 (4)	O(w_3)
H(34)	198 (4)	198 (4)	547 (3)	7.7 (1.3)	0.88 (5)	O(w_3)
H(35)	483 (3)	281 (3)	461 (2)	4.4 (1.0)	0.89 (4)	O(w_4)
H(36)	380 (4)	274 (4)	503 (3)	8.0 (1.4)	0.86 (5)	O(w_4)

Table III. Torsion Angles in the Tetracycline Molecule

Atoms	Angle, deg	Atoms	Angle, deg
C(1)-C(2)-C(3)-C(4)	18.5	C(11a)-C(12)-C(12a)-C(4a)	−12.5
C(2)-C(3)-C(4)-C(4a)	−28.3	C(12)-C(12a)-C(4a)-C(5)	45.4
C(3)-C(4)-C(4a)C(12a)	−6.3	C(12a)-C(4a)-C(5)-C(5a)	−67.0
C(4)-C(4a)-C(12a)-C(1)	46.5	C(6a)-C(6)-C(5a)-C(5)	176.3
C(4a)-C(12a)-C(1)-C(2)	−58.7	C(10a)-C(11)-C(11a)-C(12)	−176.9
C(12a)-C(1)-C(2)-C(3)	24.5	C(5a)-C(6)-C(6a)-C(10a)	44.7
O(12a)-C(12a)-C(1)-C(2)	60.0	C(6)-C(6a)-C(10a)-C(11)	−7.2
O(1)-C(1)-C(2)-C(am)	18.4	C(6a)-C(10a)-C(11)-C(11a)	−17.7
C(1)-C(2)-C(am)-O(am)	7.3	C(10a)-C(11)-C(11a)-C(5a)	1.0
C(3)-C(4)-N(4)-C(Me1)	69.5	C(11)-C(11a)-C(5a)-C(6)	37.8
C(3)-C(4)-N(4)-C(Me2)	−163.1	C(11a)-C(5a)-C(6)-C(6a)	−58.5
C(11a)-C(12)-C(12a)-C(1)	109.7	C(6a)-C(7)-C(8)-C(9)	2.0
C(12)-C(12a)-C(1)-C(2)	179.2	C(7)-C(8)-C(9)-C(10)	−1.0
C(5a)-C(5)-C(4a)-C(4)	168.7	C(8)-C(9)-C(10)-C(10a)	−2.5
C(5)-C(4a)-C(4)-C(3)	117.3	C(9)-C(10)-C(10a)-C(6a)	4.9
C(4a)-C(5)-C(5a)-C(11a)	49.8	C(10-C(10a)-C(6a)-C(7)	−3.8
C(5)-C(5a)-C(11a)-C(12)	−16.5	C(10a)-C(6a)-C(7)-C(8)	0.4
C(5a)-C(11a)-C(12)-C(12a)	−2.4	N(4)-C(4)-C(4a)-C(5)	−114.9

HCl,[12] 7-CLTC·HCl,[15] 5-HTC·HCl,[16] and 6-DH-5-HTC·HCl.[17] A comparison of the dihedral angles given in Table III for TC$^{\pm}$-urea with the values[12] for 6-DM-7-CLTC·HCl and TC$^{\pm}$·6H_2O shows that the three compounds have virtually identical conformations. The average differences in the dihedral angles of the A, B, and C rings of TC$^{\pm}$-urea compared with 6-DM-7-CLTC·HCl are 6.9, 4.8, and 2.8°, respectively, and, when compared with TC$^{\pm}$·6H_2O, the differences average 6.1, 4.2, and 5.6°, respectively. The largest difference in the three compounds is in the C(12a)-C(1)-C(2)-C(3) torsion angle. The larger angle in TC$^{\pm}$-urea (24.5° vs. 5.4° in TC$^{\pm}$ and 8.3° in 6-DM-7-CLTC·HCl may be required to accommodate the urea molecule which is hydrogen bonded to O(1) and O(am). The interaction between the urea molecule and the A ring of TC$^{\pm}$ is shown in the packing diagram (Figure 2) and in more detail in Figure 3.

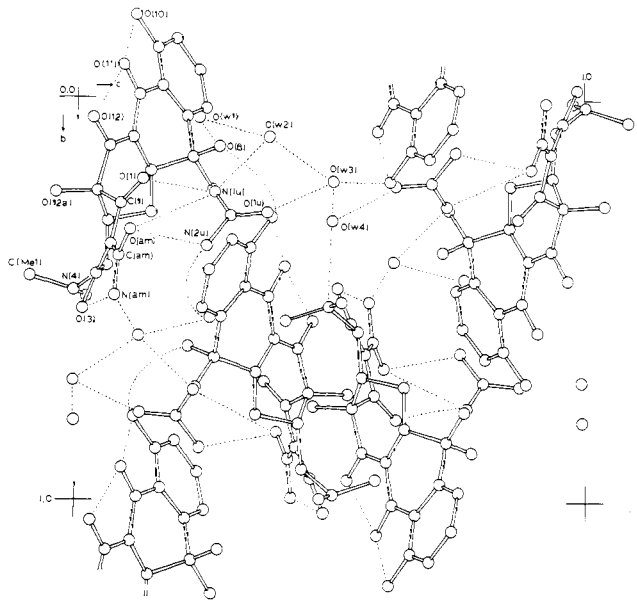

Figure 2. A crystal packing diagram of tetracycline-urea tetrahydrate. The various hydrogen bonds are shown by broken lines which are curved in three places to avoid ambiguities.

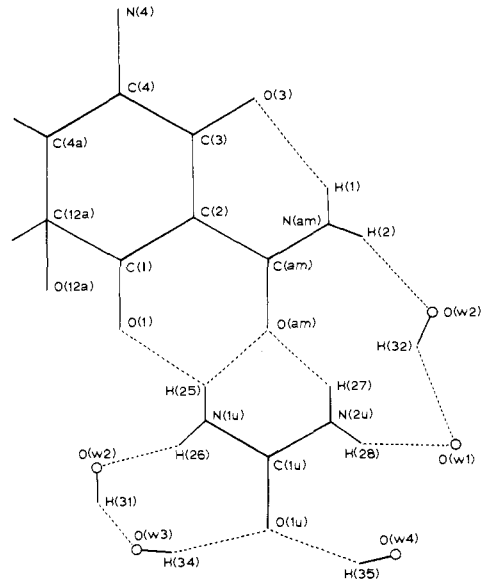

Figure 3. A view of the A ring in the tetracycline molecule, the urea molecule, and parts of the four water molecules showing the hydrogen-bonding pattern between the various groups.

Table IV. Bond Distances

Atoms	Distance Å	Atoms	Distance Å
	A. In the Tetracycline Molecule		
C(1)-O(1)	1.231 (4)	C(6)-C(6a)	1.525 (5)
C(1)-C(12a)	1.552 (5)	C(6)-O(6)	1.452 (4)
C(1)-C(2)	1.426 (5)	C(6)-C(6M)	1.509 (5)
C(2)-C(3)	1.421 (5)	C(6a)-C(7)	1.388 (5)
C(2)-C(am)	1.475 (5)	C(7)-C(8)	1.384 (6)
C(am)-O(am)	1.241 (4)	C(8)-C(9)	1.363 (6)
C(am)-N(am)	1.330 (5)	C(9)-C(10)	1.386 (5)
C(3)-O(3)	1.227 (4)	C(10-O(10)	1.354 (4)
C(3)-C(4)	1.542 (5)	C(10)-C(10a)	1.399 (5)
C(4)-C(4a)	1.530 (5)	C(10a)-C(6a)	1.408 (5)
C(4)-N(4)	1.498 (5)	C(10a)-C(11)	1.470 (5)
N(4)-C(Me_1)	1.480 (6)	C(11)-O(11)	1.263 (4)
N(4)-C(Me_2)	1.498 (5)	C(11)-C(11a)	1.439 (5)
C(4a)-C(12a)	1.532 (5)	C(11a)-C(12)	1.361 (5)
C(4a)-C(5)	1.533 (5)	C(12)-O(12)	1.316 (4)
C(5)-C(5a)	1.514 (5)	C(12)-C(12a)	1.518 (5)
C(5a)-C(11a)	1.508 (5)	C(12a)-O(12a)	1.421 (4)
C(5a)-C(6)	1.539 (5)		
	B. In the Urea Molecule		
C(1u)-N(1u)	1.329 (5)	C(1u)-O(1u)	1.245 (5)
C(1u)-N(2u)	1.331 (6)		

The tetracycline molecule in $TC^{\pm}$-urea exists as a zwitterion with a formal positive charge on N(4) and a negative charge delocalized over the C(1)-C(2)-C(3) grouping. The zwitterionic form is usually found in crystals of the free bases grown at room temperature.[18] The bond distances and angles in the $TC^{\pm}$-urea complex given in Tables IV and V are very similar to those found in $TC^{\pm}\cdot 6H_2O$[14] and reflect the zwitterionic character. A comparison of 35 chemically equivalent bonds in $TC^{\pm}\cdot 6H_2O$ reveals an average bond length difference of 0.009 Å. Using standard tests,[19] the majority of the differences are possibly significant or not significant. However, highly significant differences are found for the C(am)-O(am) and C(12)-O(12) bond lengths, while the difference in C(7)-C(8) appears to be significant. In $TC^{\pm}\cdot 6H_2O$, O(am) forms two strong intermolecular hydrogen bonds to O(12a) and O(6). In $TC^{\pm}$-urea the hydrogen bond between O(12a) and O(am) is weaker (see Table VI) and no interaction occurs with O(6). Instead, O(am) forms two weak bifurcated hydrogen bonds involving the NH groups on the urea molecule. The shorter C(am)-O(am) bond in $TC^{\pm}$-urea is consistent with weaker hydrogen bonding involving O(am) compared with $TC^{\pm}\cdot 6H_2O$. Similarly, the slight lengthening of C(12)-O(12) in $TC^{\pm}\cdot 6H_2O$ relative to that in $TC^{\pm}$-urea can also be related to differences in the hydrogen-bonding patterns. The significant difference in the C(7)-C(8) bond lengths in $TC^{\pm}$-urea vs. $TC^{\pm}\cdot 6H_2O$[14] is more difficult to explain but is probably due to thermal motion. The $TC^{\pm}$-urea data were measured at ambient temperature while the $TC^{\pm}\cdot 6H_2O$ data were collected at −150 °C.[14] There is no significant difference in the C(7)-C(8) bond length in $TC^{\pm}$-urea compared with the determination which utilized room temperature data.[12] Finally, the fact that the bond is apparently shorter in both room temperature studies is consistent with a thermal motion effect. In summary we see that, in both $TC^{\pm}\cdot 6H_2O$ and $TC^{\pm}$-urea$\cdot 4H_2O$, the $TC^{\pm}$ zwitterion has virtually the same shape and dimensions. Consequently, the conformation observed in $TC^{\pm}\cdot 6H_2O$, $TC^{\pm}$-urea$\cdot 4H_2O$, 5-HTC$^{\pm}\cdot 2H_2O$, and all the related protonated species is very stable and is most likely to also be found at the site of biological activity. This conclusion is essential in the development of a model for the molecular interaction of tetracyclines with ribosomes. Our study provides the first structural data for a tetracycline adduct and supports our earlier hypothesis[12] regarding the conformation required for useful therapeutic activity.

The dimensions of the urea molecule are similar to those in urea[20] or in various urea adducts, i.e., the 5,5′-diethylbarbituric acid-urea complex.[21] The urea molecule is hydrogen bonded to $TC^{\pm}$ and water molecules but not to other urea molecules (Figures 2 and 3). In many urea complexes there is extensive hydrogen bonding between the urea molecules but this does not occur in the $TC^{\pm}$-urea complex. The urea forms hydrogen bonds to all four water molecules which are hydrogen bonded to each other as well as to the $TC^{\pm}$. The observed hydrogen bonding patterns may account in part for the insoluble character of the adduct. In $TC^{\pm}$ the amide group is rotated around the C(2)-C(am) bond relative to the orientation found in 7-CLTC·HCl[15] or 5-HTC·HCl,[16] which may explain, in part, the formation of an insoluble urea adduct with only $TC^{\pm}$.

Table V. Bond Angles. The Estimated Standard Deviation is 0.3°

Atoms	Angle, deg	Atoms	Angle, deg
	A. In the Tetracycline Molecule		
O(1)-C(1)-C(2)	126.5	C(7)-C(8)-C(9)	121.7
O(1)-C(1)-C(12a)	119.0	C(8)-C(9)-C(10)	119.5
C(2)-C(1)-C(12a)	114.5	C(9)-C(10)-O(10)	117.2
C(1)-C(2)-C(3)	119.4	C(9)-C(10)-C(10a)	120.1
C(1)-C(2)-C(am)	120.1	O(10)-C(10)-C(10a)	122.7
C(3)-C(2)-C(am)	120.4	C(10)-C(10a)-C(6a)	119.7
C(2)-C(3)-C(4)	117.2	C(10)-C(10a)-C(11)	120.0
C(2)-C(3)-O(3)	127.5	C(6a)-C(10a)-C(11)	120.2
C(4)-C(3)-O(3)	114.6	C(10a)-C(11)-O(11)	119.4
C(3)-C(4)-C(4a)	118.4	C(10a)-C(11)-C(11a)	118.5
C(3)-C(4)-N(4)	108.1	O(11)-C(11)-C(11a)	122.0
C(4a)-C(4)-N(4)	113.0	C(11)-C(11a)-C(5a)	117.9
C(4)-C(4a)-C(5)	111.2	C(11)-C(11a)-C(12)	118.8
C(4)-C(4a)-C(12a)	111.4	C(5a)-C(11a)-C(12)	123.2
C(5)-C(4a)-C(12a)	110.4	C(11a)-C(12)-O(12)	123.0
C(4a)-C(5)-C(5a)	110.3	C(11a)-C(12)-C(12a)	123.8
C(5)-C(5a)-C(6)	114.9	O(12)-C(12)-C(12a)	113.1
C(5)-C(5a)-C(11a)	109.9	C(12)-C(12a)-C(4a)	109.9
C(6)-C(5a)-C(11a)	110.6	C(12)-C(12a)-C(1)	111.5
C(5a)-C(6)-C(6a)	107.5	C(12)-C(12a)-O(12a)	110.1
C(5a)-C(6)-O(6)	109.4	C(4a)-C(12a)-C(1)	110.0
C(5a)-C(6)-C(6M)	112.2	C(4a)-C(12a)-O(12a)	110.3
C(6a)-C(6)-O(6)	104.9	C(1)-C(12a)-O(12a)	105.1
C(6a)-C(6)-C(6M)	112.7	C(2)-C(am)-O(am)	122.4
O(6)-C(6)-C(6M)	109.8	C(2)-C(am)-N(am)	117.4
C(6)-C(6a)-C(7)	123.3	O(am)-C(am)-N(am)	120.1
C(6)-C(6a)-C(10a)	117.9	C(4)-N(4)-C(Me_1)	114.8
C(7)-C(6a)-C(10a)	118.9	C(4)-N(4)-C(Me_2)	111.5
C(6a)-C(7)-C(8)	120.0	C(Me_1)-N(4)-C(Me_2)	111.1
	B. In the Urea Molecule		
N(1u)-C(1u)-O(1u)	121.0	N(1u)-C(1u)-N(2u)	117.1
N(2u)-C(1u)-O(1u)	121.9		

Table VI. Hydrogen Bonds in Tetracycline-Urea Tetrahydrate

D-H···A[a]	Position of A	D-H, Å	H···A, Å	D···A, Å	D-H···A, deg
N(am)-H(1)···O(3)	x, y, z	0.94 (4)	1.93 (4)	2.658 (4)	132 (3)
N(am)-H(2)···O(w_2)	$1-x, \frac{1}{2}+y, \frac{1}{2}-z$	0.94 (4)	1.99 (3)	2.915 (4)	167 (3)
N(4)-H(3)···O(w_4)	$\frac{1}{2}-x, 1-y, z-\frac{1}{2}$	0.92 (4)	1.95 (3)	2.767 (4)	148 (3)
O(6)-H(4)···O(10)	$-x, \frac{1}{2}+y, \frac{1}{2}-z$	0.91 (3)	2.11 (3)	3.010 (4)	171 (3)
O(10)-H(5)···O(11)	x, y, z	0.91 (4)	1.79 (4)	2.574 (4)	144 (4)
O(12)-H(6)···O(11)	x, y, z	1.02 (4)	1.57 (4)	2.497 (4)	149 (4)
O(12a)-H(7)···O(am)	$x-\frac{1}{2}, \frac{1}{2}-y, -z$	0.87 (3)	1.93 (3)	2.774 (3)	161 (3)
N(1u)-H(25)···O(1)	$1-x, \frac{1}{2}+y, \frac{1}{2}-z$	0.92 (4)	2.39 (4)	3.195 (4)	146 (3)
N(1u)-H(25)···O(am)	$1-x, \frac{1}{2}+y, \frac{1}{2}-z$	0.92 (4)	2.27 (4)	3.023 (4)	139 (3)
N(1u)-H(26)···O(w_2)	$1-x, \frac{1}{2}+y, \frac{1}{2}-z$	0.97 (4)	2.12 (4)	3.072 (5)	167 (3)
N(2u)-H(27)···O(am)	$1-x, \frac{1}{2}+y, \frac{1}{2}-z$	0.93 (4)	2.04 (4)	2.907 (4)	154 (3)
N(2u)-H(28)···O(w_1)	$x, 1+y, z$	1.03 (4)	2.10 (4)	3.026 (5)	149 (4)
O(w_1)-H(29)···O(1)	x, y, z	0.85 (5)	1.96 (5)	2.809 (4)	179 (5)
O(w_1)-H(30)···O(6)	x, y, z	0.88 (5)	2.08 (5)	2.934 (4)	163 (5)
O(w_2)-H(31)···O(w_3)	x, y, z	0.90 (4)	1.99 (4)	2.875 (4)	168 (4)
O(w_2)-H(32)···O(w_1)	x, y, z	0.96 (4)	1.82 (4)	2.751 (4)	161 (4)
O(w_3)-H(33)···O(10)	$-x, \frac{1}{2}+y, \frac{1}{2}-z$	0.87 (4)	2.05 (4)	2.898 (4)	165 (4)
O(w_3)-H(34)···O(1u)	$\frac{1}{2}-x, 1-y, \frac{1}{2}+z$	0.88 (5)	1.87 (5)	2.736 (4)	165 (5)
O(w_4)-H(35)···O(1u)	$1-x, y-\frac{1}{2}, \frac{1}{2}-z$	0.89 (4)	1.96 (4)	2.809 (4)	179 (5)
O(w_4)-H(36)···O(w_3)	x, y, z	0.86 (5)	1.93 (5)	2.786 (4)	178 (5)

[a] Donor-hydrogen···acceptor. D-H at x, y, z.

However, attempts to isolate a urea adduct of 6-DM-7-CLTC·HCl, where the amide group has the same orientation as in TC±, have not been successful.[22] The formation of the insoluble TC±-urea·4H_2O must represent a complex, subtle balance between crystal packing and solvation energies which is not readily explicable.

There is extensive hydrogen bonding in the solid state between the TC±, urea, and water molecules. Although the hydrogen-bond dimensions in Table V are fairly normal, there is a rare bifurcated hydrogen bond involving O(1), O(am), and the urea molecules (see Figure 3). We also see in Figure 3 that replacing H(2) on N(am) with a large alkyl group would interfere with the hydrogen-bonding linking O(am), the urea, and water molecules. Indeed, substitution of the hydrogen by large groups such as *tert*-butyl[23] or cycloheptyl[24] narrows the biological spectrum which accents the importance of our re-

sults. Since the urea hydrogen bonds to $TC^{\pm}$ in a manner which could be duplicated by guanine or its derivatives, hydrogen bonding to guanine residues may be important in the biological activity of tetracyclines. We are attempting at present to isolate other tetracycline adducts, particularly with guanine-type compounds, to test these hypotheses.

The optimum antimicrobial activity of tetracyclines is in the pH range[25] where the zwitterion is the predominant species.[26] The zwitterion is also required for adduct formation since protonation of O(am) would prevent adduct formation similar to that observed with urea. Consequently, zwitterion adduct formation could be a significant step in the therapeutic action of tetracyclines. However, whether adduct formation enhances lipid solubility and hence transport or is involved in the inhibition of protein synthesis can not yet be answered.

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Supplementary Material Available: Listing of observed and calculated structure amplitudes of tetracycline-urea tetrahydrate (14 pages). Ordering information is given on any current masthead page.

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Preparation, Crystalline Structure, and Spectral Properties of the Fluorescent Probe 4,4′-Bis-1-phenylamino-8-naphthalenesulfonate[1a]

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Abstract: A method for the preparation and purification of the covalent dimers of 1-phenylamino- and 1-toluidylaminonaphthalenesulfonic acids is described. X-ray analysis of the crystals of the potassium salt of the former (bis-ANS) showed this to be 4,4′-bis-1-phenylaminonaphthalene-8-sulfonate. By similarity of the NMR spectra and optical properties the second dimer is recognized to be 4,4′-bis-1-toluidylaminonaphthalene-8-sulfonic acid. NMR spectra, molar absorption coefficients, fluorescent lifetimes, and the detailed structural parameters derived from the x-ray data are presented. Some of the uses of the compounds as fluorescent probes in solutions and crystals of proteins are briefly discussed.

Introduction[1d]

Rosen and Weber[2] isolated a covalent dimer of 1-phenylaminonaphthalenesulfonic acid (bis-ANS) by treatment of 1-aminonaphthalene-8-sulfonic acid (ANS) with nitrous acid. The covalent character of the dimer was confirmed by elemental analysis and measurements of molecular weight by osmometry and mass spectrometry, and by the rotational diffusion coefficient deduced from measurements of polarized fluorescence and fluorescence lifetime. However, attempts to determine the coupling positions in the monomers by NMR did not yield an unequivocal answer. As bis-ANS is finding application as a fluorescence probe of protein structure, we have now prepared bis-ANS, and also bistoluidylaminonaphthalenesulfonate (bis-TNS), by an improved method and

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