Chemical-Structural Properties of Tetracycline Antibiotics. 4. Ring A Tautomerism Involving the Protonated Amide Substituent as Observed in the Crystal Structure of α-6-Deoxyoxytetracycline Hydrohalides[1]

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Abstract: Crystal structure analyses have been carried out for α-6-deoxyoxytetracycline, DOXY, hydrobromide and hydrochloride. The isomorphous hydrohalide salts display space group symmetry $P2_1$ with a = 18.351 (2), b = 16.195 (2), c = 8.154 (1) Å, and β = 94.74 (1)° for DOXY·HBr at ca. −50 °C and a = 18.203 (2), b = 16.045 (1), c = 8.004 (1) Å, and β = 94.11 (1)° for DOXY·HCl at ca. −150 °C. In each case the asymmetric unit contains two formula units of the hydrohalide, a water molecule, and one ethanol molecule. The absolute configuration, determined for the hydrobromide salt, was found to be consistent with that determined chemically.[10] The symmetry independent molecular cations display an oxygen-protonated amide structure for which the proton is intramolecularly hydrogen bonded to a β-hydroxylate moiety, the position of which is determined by the tautomeric form of the A ring chromophore. The intra- and intermolecular hydrogen bonding displayed by several tetracycline derivatives crystallized under different chemical conditions is examined in some detail.

The broad spectrum antibiotic oxytetracycline·(OXY) has been crystallized under various solvent and chemical conditions. Crystal structure determinations have been reported for a hydrochloride salt,[2] a hydrobromide salt,[3] for two structurally different modifications of the free base,[4] for a diacetyl derivative[5] of the free base, for a mercuric chloride complex, and for a dipotassium salt.[6] In each of the above analyses,[6] the crystal contained one molecule per asymmetric unit, though the crystalline hydrobromide[3] appears to contain a mixture of the conformationally similar fully protonated cation and the zwitterionic free base. The hydrobromide has also been reported to crystallize with space group symmetry $P2_1$. Examples displaying this space group symmetry contain two formula units of the salt and varying water content per asymmetric unit.[7-9]

A derivative of oxytetracycline, α-6-deoxyoxytetracycline (DOXY), has been crystallized as both the hydrobromide and the hydrochloride salts from ethanol-water (initially 90% ethanol) solutions by allowing the solvent to slowly evaporate at 8 °C. The resultant high quality single crystals display space group symmetry $P2_1$ with lattice parameters very similar to those reported for OXY·HBr[7-9] and Z = 4.

The crystal structure of the hydrobromide salt was initially undertaken to utilize x-ray diffraction techniques to determine the validity of the absolute configuration of the tetracyclines as determined by chemical techniques.[10] Examination of the molecular geometry displayed by the carbon, nitrogen, and oxygen atoms of the two symmetry independent cations of the nearly refined model for the hydrobromide salt indicated the

Table I. Crystal Data for the Hydrohalide Salts of 6-Deoxy-5-oxytetracycline (DOXY)

	DOXY·HBr	DOXY·HCl
Crystal size, mm	0.20 × 0.22 × 0.12	0.35 × 0.26 × 0.27
Crystal temp, °C	~−50	~−150
Space group	$P2_1$	$P2_1$
Lattice parameters		
a, Å	18.351 (2)	18.203 (2)
b, Å	16.195 (2)	16.045 (1)
c, Å	8.154 (1)	8.004 (1)
β, deg	94.74 (1)	94.11 (1)
No. of contributing 2θ's[a]	44	62
$2\theta_{min}$-$2\theta_{max}$, deg[a]	25.8-37.2	30.2-43.5
Formula/asymmetric unit	$C_{46}H_{58}Br_2N_4O_{20}$	$C_{46}H_{58}Cl_2N_4O_{20}$
D_{xray}, g cm^{-3}	1.533 (~−50 °C)	1.461 (~−150 °C)
μ, cm^{-1}	18.8	2.3
Intensity data		
No. of unique data	7302	14758
No. obsd ($I > n\sigma(I)$)	4046 (n = 2)	8829 (n = 3)
(sin θ)/λ (max) (λ = 0.71069 Å)	0.704	0.904

[a] Applicable to the lattice parameter refinement only.

Table II. A Summary of the Least-Squares Refinement of the DOXY·HBr and DOXY·HCl Crystal Structure Models

A. Interchanged Assignment of the Amide Nitrogen and Oxygen Atoms in Molecule DOXY(1) of the Hydrobromide Model

	10^2U_{11}	10^2U_{22}	10^2U_{33}	10^2U_{12}	10^2U_{13}	10^2U_{23}
Initial Assigment						
O (2am)	6.4	4.8	6.9	−0.3	0.8	−0.5
N (2am)	2.2	2.1	1.9	−0.2	1.7	−0.8
R_1 = 0.059; R_w = 0.053						
Final Assignment						
O(2am)	4.3	3.8	3.8	0.0	1.6	−0.1
N(2am)	4.0	2.8	4.2	−0.3	0.7	−0.5
R_1 = 0.058; R_w = 0.051						

B. Resultant Precision of Fit for the Refined Structural Models

	DOXY·HBr	DOXY·HCl
No. of contributing reflections	5580	11 987
No. of variables	631	862
R	0.058	0.047
R_w	0.051	0.057
σ	1.098	1.030

Table III. The Structure Factor Magnitudes for the Determination of the Absolute Configuration.

h	k	l	$\|F_o\|$	$\|F_c\|$(E1)[a]	$\|F_c\|$(E2)	h	k	l	$\|F_o\|$	$\|F_c\|$(E1)	$\|F_c\|$(E2)
4	5	2	145.29	152.01	148.66	−2	−2	1	93.54	88.23	103.69
−4	−5	−2	143.02	149.07	151.57	−2	2	1	109.02	103.53	88.39
−4	5	−2	146.65	152.01	148.66	2	−2	−1	94.15	88.23	103.69
4	−5	2	142.94	149.07	151.57	2	1	0	107.72	109.51	117.64
3	2	2	129.93	130.46	129.66	−2	−1	0	114.97	117.66	109.49
−3	−2	−2	128.97	129.56	130.56	−2	1	0	108.03	109.51	117.64
−3	2	−2	130.61	130.46	129.66	2	−1	0	116.35	117.66	109.49
3	−2	2	129.45	129.56	130.56	2	2	2	81.33	78.82	71.70
6	2	−1	88.52	91.58	94.96	−2	−2	−2	72.96	71.60	78.90
−6	−2	1	90.89	94.75	91.76	−2	2	−2	81.60	78.82	71.70
−6	2	1	87.31	91.58	94.96	2	−2	2	73.66	71.60	78.90
6	−2	−1	90.12	94.75	91.76	2	3	3	118.27	117.88	122.15
3	4	0	117.45	116.97	123.85	−2	−3	−3	121.42	122.73	117.29
−3	−4	0	123.42	123.96	116.85	−2	3	−3	117.09	117.88	122.15
−3	4	0	117.87	116.97	123.85	2	−3	3	121.95	122.73	117.29
3	−4	0	126.03	123.96	116.85	2	7	0	106.42	104.89	106.18
0	4	0	115.79	114.30	109.00	−2	−7	0	107.98	106.54	104.47
0	−4	0	110.43	108.97	114.31	−2	7	0	106.92	104.89	106.18
4	5	−1	103.66	103.88	104.46	2	−7	0	108.25	106.54	104.47
−4	−5	1	104.00	104.81	103.52	2	4	−2	127.86	130.05	130.59
−4	5	1	104.61	103.88	104.46	−2	−4	2	128.96	130.66	130.00
4	−5	−1	105.77	104.81	103.53	−2	4	2	127.22	130.05	130.59
1	3	0	109.06	108.04	104.49	2	−4	−2	128.83	130.66	130.00
−1	−3	0	106.59	104.51	108.03	3	2	−4	113.07	120.82	115.21
−1	3	0	110.82	108.04	104.49	−3	−2	4	110.04	115.50	120.52
1	−3	0	106.66	104.51	108.03	−3	2	4	114.39	120.82	115.21
5	2	0	108.92	107.52	111.34	3	−2	−4	108.79	115.50	120.52
−5	−2	0	112.69	111.66	107.22	3	6	−1	91.13	90.09	78.43
−5	2	0	109.01	107.52	111.34	−3	−6	1	80.43	78.36	90.14
5	−2	0	112.47	111.66	107.22	−3	6	1	91.16	90.09	78.43
1	5	−1	138.16	136.05	139.16	3	−6	−1	80.00	78.36	90.14
−1	−5	1	141.55	139.11	136.09	3	2	−1	105.74	105.47	97.03
−1	5	1	138.37	136.05	139.16	−3	−2	1	95.17	97.00	105.46
1	−5	−1	141.23	139.11	136.09	−3	2	1	104.57	105.47	97.03
1	1	2	107.75	104.62	95.63	3	−2	−1	95.44	97.00	105.46
−1	−1	−2	96.77	95.63	104.61	4	1	−1	157.17	153.66	152.23
−1	1	−2	106.42	104.62	95.63	−4	−1	1	155.22	152.52	153.37
1	−1	2	97.97	95.63	104.61	−4	1	1	155.73	153.66	152.23
2	2	−1	108.81	103.53	88.39	4	−1	−1	154.16	152.52	153.37

[a] Enantiomers E1 and E2 are those associated with the $R = 0.021$ and $R = 0.050$ space group enantiomorphs, respectively.

presence of two tautomers in the crystal. Furthermore, the two tautomeric forms involved the A ring amide substituent for which the protonated amide structure had been proposed.[11] While the C-O and C-N distances reported for several hydrohalide salts of tetracycline derivatives[2,3,11] are consistent with those reported by Duntiz and Winkler[12] for protonated cyclic amides, in which the hydrogen atoms were located and their validity tested by crystallographic techniques, the protonated amide structure has not been demonstrated for the tetracyclines in a high-precision crystal structure analysis. This question is particularly interesting for these derivatives because of the array of functional groups associated with the A ring, the possibility that the chromophore may display tautomerism, and because of the unusually strong hydrogen bonding displayed by the enolic form of the free base. The hydrochloride salt presented a very favorable opportunity for the determination of a high-precision crystal structure. The initial model achieved from the analysis of the isomorphous hydrobromide eliminated the difficulty of solving the phase problem, while the smaller number of electrons associated with the chloride ion significantly reduced the bias introduced into the measured intensities by the heavy atom. Finally the crystals proved to be of high quality, enabling a high-resolution data set to be measured from a crystal cooled to ~−150 °C. With these considerations in mind the crystal structure analysis of DOXY·HCl was undertaken.

Experimental Section

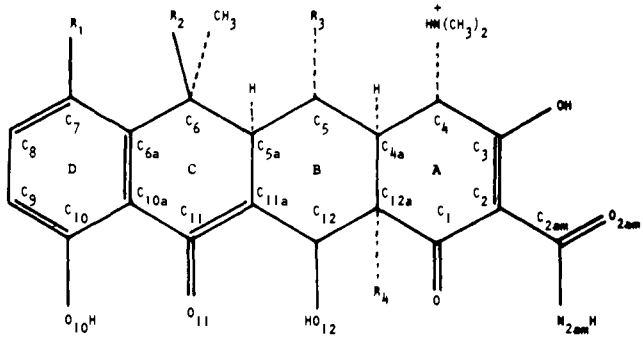
A summary of the crystal data for the hydrohalide salts is presented in Table I. All crystallographic data were measured with monochromatized Mo Kα radiation (λ = 0.71069 Å) on a Syntex P$\bar{1}$ autodiffractometer equipped with a low-temperature device (Syntex LT-1). The tabulated lattice parameters resulted from a least-squares refinement[13] with automatically centered 2θ angles in the angular range tabulated. Integrated diffraction intensities were measured for a 1.0° ω scan; the scan rate was allowed to vary from 1.0 to 24.0° min^{-1} as a function of maximum peak intensity. Background radiation intensity was measured on each side of the reflections for one-half the scan time. Three reference reflections, monitored after each 129 data were measured, displayed neither systematic nor significant deviations from their initial intensities.

A 78 reflection data set was assembled for the determination of the absolute configuration. The data consisted of parent reflections (hkl), selected because the bromine atoms, the principal anomalous scatterers in the crystal, accounted for the major portion of the calculated structure factor, their C_2 symmetry equivalent reflections ($\bar{h}k\bar{l}$), and their respective Friedel pairs ($\bar{h}\bar{k}\bar{l}$ and $h\bar{k}l$). Each reflection in the selected data set was measured six times and the average intensity used.

Table IV. Refined Fractional Atomic Coordinates for the Chlorine, Carbon, Nitrogen, and Oxygen Atoms of the DOXY·HCl Crystal Structure

Atom	10^4x	10^4y	10^4z	Atom	10^4x	10^4y	10^4z
Cl (1)	8155 (0)	5000 (0)	128 (1)	O(1,2)	9410 ()	2903 (1)	7280 (3)
Cl (2)	1324 (0)	2845 (0)	3494 (1)	C(2,2)	9611 (1)	4337 (2)	7816 (3)
C(1,1)[a]	3903 (1)	3316 (1)	4807 (3)	C(2am,2)	9922 (2)	4232 (2)	9529 (3)
O(1,1)	4279 (1)	3452 (1)	3565 (2)	O(2am,2)	10170 (1)	4879 (2)	10379 (3)
C(2,1)	3562 (1)	2543 (1)	5117 (3)	N(2am,2)	9983 (2)	3501 (2)	10260 (3)
C(2am,1)	3682 (1)	1862 (1)	3996 (3)	C(3,2)	9536 (1)	5159 (2)	7216 (3)
N(2am,1)	3409 (1)	1111 (1)	4130 (3)	O(3,2)	9789 (1)	5794 (1)	8004 (3)
O(2am,1)	4071 (1)	2000 (1)	2703 (3)	C(4,2)	9071 (1)	5342 (1)	5595 (3)
C(3,1)	3087 (1)	2465 (1)	6474 (3)	N(4,2)	9428 (1)	6069 (1)	4760 (3)
O(3,1)	2908 (1)	1804 (1)	7111 (3)	C(4m1,2)	10176 (1)	5879 (2)	4186 (4)
C(4,1)	2689 (1)	3260 (1)	6974 (3)	C(4m2,2)	8953 (2)	6441 (2)	3349 (4)
N(4,1)	2496 (1)	3187 (1)	8773 (3)	C(4a,2)	8862 (1)	4590 (1)	4452 (3)
C(4m1,1)	3126 (2)	3279 (3)	10052 (4)	C(5,2)	8027 (1)	4428 (1)	4554 (3)
C(4m2,1)	1876 (2)	3751 (2)	9145 (4)	O(5,2)	7646 (1)	5166 (1)	4010 (3)
C(4a,1)	3025 (1)	4099 (1)	6492 (3)	C(5a,2)	7726 (1)	3673 (1)	3529 (3)
C(5,1)	2573 (1)	4478 (1)	4978 (3)	C(6,2)	7003 (1)	3326 (1)	4155 (3)
O(5,1)	1830 (1)	4556 (1)	5407 (3)	C(6a,2)	6722 (1)	2621 (1)	3015 (3)
C(5a,1)	2861 (1)	5346 (1)	4520 (3)	C(6m,2)	6417 (1)	3989 (2)	4433 (4)
C(6,1)	2580 (1)	5641 (1)	2752 (3)	C(7,2)	5978 (1)	2497 (2)	2566 (3)
C(6a,1)	2848 (1)	6534 (1)	2512 (3)	C(8,2)	5747 (1)	1792 (2)	1631 (4)
C(6m,1)	1752 (1)	5552 (2)	2365 (4)	C(9,2)	6240 (1)	1199 (2)	1159 (3)
C(7,1)	2410 (2)	7151 (2)	1741 (3)	C(10,2)	6987 (1)	1313 (1)	1598 (3)
C(8,1)	2715 (2)	7923 (2)	1362 (3)	O(10,2)	7455 (1)	707 (1)	1172 (2)
C(9,1)	3455 (2)	8093 (2)	1716 (3)	C(10a,2)	7234 (1)	2037 (1)	2470 (3)
C(10,1)	3896 (1)	7486 (2)	2527 (3)	C(11,2)	8025 (1)	2177 (1)	2798 (3)
O(10,1)	4620 (1)	7665 (1)	2896 (3)	O(11,2)	8486 (1)	1607 (1)	2494 (3)
C(10a,1)	3595 (1)	6716 (1)	2984 (3)	C(11a,2)	8280 (1)	2975 (1)	3464 (3)
C(11,1)	4047 (1)	6106 (1)	3946 (3)	C(12,2)	9011 (1)	3059 (1)	3951 (3)
O(11,1)	4742 (1)	6211 (1)	4152 (3)	O(12,2)	9514 (1)	2477 (1)	3704 (2)
C(11a,1)	3696 (1)	5397 (1)	4667 (3)	C(12a,2)	9333 (1)	3817 (1)	4863 (3)
C(12,1)	4134 (1)	4807 (1)	5474 (3)	O(12a,2)	10050 (1)	3992 (1)	4347 (3)
O(12,1)	4863 (1)	4864 (1)	5716 (3)	C(1Et)	4564 (2)	−17 (3)	−324 (5)
C(12a,1)	3832 (1)	4003 (1)	6129 (3)	C(2Et)	3858 (2)	458 (2)	−200 (4)
O(12a,1)	4220 (1)	3760 (1)	7657 (2)	O(Et)	3478 (1)	139 (1)	1178 (3)
C(1,2)	9427 (1)	3629 (2)	6776 (3)	O(w)	1775 (2)	1933 (2)	168 (4)

[a] The numbers following the comma in the parentheses of the atom label designates the symmetry independent molecular anion to which the atom belongs. The numbers in the parentheses following the fractional atomic coordinates are the estimated standard deviations in the last significant digits.



R_1	R_2	R_3	R_4	Deriv
H	H	OH	OH	DOXY
H	OH	OH	OH	OXY
Cl	OH	H	OH	ClTC
H	OH	H	OH	TC
H	OH	$OCOCH_3$	$OCOCH_3$	DAOXY

Figure 1. The chemical structure for tetracycline derivatives: α-6-deoxy-5-oxytetracycline (DOXY); 5-oxytetracycline (OXY); 7-chlorotetracycline (ClTC); tetracycline (TC); and 5,12a-diacetyloxytetracycline (DAOXY). The letters in parentheses are those utilized to identify the various derivatives in the text. The absolute configuration depicted in the drawing is that determined chemically by Dobrynin et al.[10] and subsequently confirmed by anomalous dispersion techniques for DOXY·HBr.

Lorentz and polarization corrections were applied to the intensity data for both derivatives; absorption corrections were applied to all intensity data from the hydrobromide crystal.

Structure Determination and Refinement. The heavy atom technique was utilized to determine the fractional coordinates of the bromine, carbon, nitrogen, and oxygen atoms for the initial structural model. Fractional atomic coordinates for a partial set of hydrogen atoms were obtained from difference electron density maps or calculated employing the constraints of theoretical geometry. The structural model was refined by block diagonal least-squares techniques in which the parameters of one atom were refined in each block; the single scale factor was refined in the block with the variables of one bromine atom. The appropriate fractional atomic coordinates and anisotropic temperature factor coefficients were refined for all bromine, carbon, nitrogen, and oxygen atoms; hydrogen atom contributions to the structure factors were calculated from fixed fractional atomic coordinates and isotropic temperature factors. Those data classified as unobserved for which the calculated intensity exceeded the cutoff criterion, Table I, were included in the refinement. The data were empirically weighted with the equation: $\sigma^2(F) = \sigma^2(F_o) + 0.0125F_o + 0.0001F_o^2$.

A comparative analysis of the bonding geometry of the symmetry independent DOXY cations of the nearly fully refined model for the hydrobromide structure provided an indication that two tautomeric forms of the A ring chlorophore, probably analogous to those reported to exist for model compounds in solution,[14] were present in the crystal. The values of the anisotropic temperature factors for the amide oxygen and nitrogen atoms of one molecule displayed significantly different

Table V. Bond Distances Involving Carbon, Nitrogen, and Oxygen Atoms of the 6-Deoxy-5-oxytetracycline Moiety

Atoms	DOXY·HBr(1)	DOXY·HBr(2)	DOXY·HCl(1)	DOXY·HCl(2)
C(1)-O(1)	1.261 (10)	1.248 (10)	1.266 (3)	1.233 (4)
C(1)-C(2)	1.402 (10)	1.430 (11)	1.417 (3)	1.435 (4)
C(1)-C(12a)	1.544 (10)	1.538 (11)	1.540 (3)	1.558 (3)
C(2)-C(3)	1.462 (11)	1.396 (12)	1.442 (4)	1.406 (4)
C(2)-C(2am)	1.456 (10)	1.447 (11)	1.440 (3)	1.455 (4)
C(2am)-O(2am)	1.306 (10)	1.317 (12)	1.314 (3)	1.305 (4)
C(2am)-N(2am)	1.307 (10)	1.319 (13)	1.311 (3)	1.312 (5)
C(3)-O(3)	1.209 (9)	1.276 (10)	1.230 (3)	1.268 (3)
C(3)-C(4)	1.540 (10)	1.542 (10)	1.534 (3)	1.526 (3)
C(4)-C(4a)	1.526 (10)	1.554 (10)	1.539 (3)	1.546 (3)
C(4)-N(4)	1.498 (10)	1.498 (10)	1.511 (3)	1.515 (3)
N(4)-C(4m1)	1.487 (11)	1.505 (11)	1.489 (4)	1.499 (4)
N(4)-C(4m2)	1.496 (12)	1.508 (11)	1.494 (4)	1.496 (4)
C(4a)-C(5)	1.534 (10)	1.543 (10)	1.540 (3)	1.549 (3)
C(4a)-C(12a)	1.538 (10)	1.520 (10)	1.527 (3)	1.531 (3)
C(5)-C(5a)	1.518 (10)	1.555 (10)	1.542 (3)	1.542 (3)
C(5)-O(5)	1.447 (9)	1.419 (9)	1.425 (3)	1.424 (3)
C(5a)-C(6)	1.558 (10)	1.558 (10)	1.545 (3)	1.546 (3)
C(5a)-C(11a)	1.527 (10)	1.516 (10)	1.518 (3)	1.509 (3)
C(6)-C(6a)	1.530 (10)	1.515 (10)	1.530 (3)	1.519 (3)
C(6)-C(6m)	1.518 (10)	1.511 (11)	1.523 (3)	1.533 (4)
C(6a)-C(7)	1.392 (11)	1.380 (10)	1.388 (4)	1.391 (3)
C(6a)-C(10a)	1.430 (10)	1.413 (10)	1.415 (3)	1.412 (3)
C(7)-C(8)	1.427 (12)	1.429 (13)	1.400 (4)	1.403 (4)
C(8)-C(9)	1.363 (12)	1.360 (12)	1.383 (4)	1.380 (4)
C(9)-C(10)	1.374 (11)	1.393 (11)	1.393 (4)	1.392 (4)
C(10)-C(10a)	1.395 (11)	1.422 (10)	1.411 (4)	1.412 (3)
C(10)-O(10)	1.347 (10)	1.349 (9)	1.359 (4)	1.352 (3)
C(10a)-C(11)	1.449 (10)	1.459 (10)	1.461 (3)	1.462 (3)
C(11)-C(11a)	1.435 (10)	1.436 (10)	1.445 (3)	1.451 (3)
C(11)-O(11)	1.288 (9)	1.287 (9)	1.275 (3)	1.276 (3)
C(11a)-C(12)	1.358 (10)	1.358 (10)	1.370 (3)	1.367 (3)
C(12)-C(12a)	1.501 (10)	1.522 (10)	1.510 (3)	1.514 (3)
C(12)-O(12)	1.353 (9)	1.350 (9)	1.331 (3)	1.332 (3)
C(12a)-O(12a)	1.420 (8)	1.437 (9)	1.422 (3)	1.425 (3)

Table VI. Selected Dihedral Angles within Each Molecular Cation

Angle	DOXY·HBr(1)	DOXY·HBr(2)	DOXY·HCl(1)	DOXY·HCl(2)
C(12)C(12a)C(1)C(2)	−171.5	−171.1	−172.2	−172.1
C(12a)C(1)C(2)C(3)	8.8	15.0	8.9	14.8
C(1)C(2)C(3)C(4)	26.2	16.0	27.9	16.2
C(2)C(3)C(4)C(4a)	−9.5	−7.2	−11.2	−7.8
C(3)C(4)C(4a)C(5)	101.1	108.5	103.9	108.7
C(4)C(4a)C(12a)C(1)	53.9	48.2	53.6	45.9
C(11)C(11a)C(12)-C(12a)	−173.9	−171.9	−174.1	−172.0
C(11a)C(12)C(12a)-C(1)	94.8	97.8	95.1	98.4
C(12)C(12a)C(4a)C(5)	50.5	46.9	50.7	46.3
C(4)C(4a)C(5)C(5a)	176.3	−179.5	176.4	−177.9
C(4a)C(5)C(5a)C(6)	163.9	158.1	163.7	158.1
C(5)C(5a)C(11a)C(12)	−12.8	−13.3	−13.9	−12.7
C(10)C(10a)C(11)-C(11a)	167.8	170.0	168.4	170.8
C(10a)C(11)C(11a)-C(12)	175.8	172.8	176.0	172.2
C(11)C(11a)C(5a)C(6)	40.8	43.4	40.4	42.7
C(5)C(5a)C(6)C(6a)	176.2	176.4	175.1	176.6
C(5a)C(6)C(6a)C(7)	−140.0	−142.3	−138.6	−142.0
C(6)C(6a)C(10a)C(11)	−10.0	−9.4	−10.9	−8.6
C(8)C(9)C(10)C(10a)	−0.4	−1.7	−0.2	−1.8
C(9)C(10)C(10a)C(11)	−175.8	−174.3	−175.3	−175.0
C(10)C(10a)C(6a)C(7)	−4.9	−3.7	−5.9	−4.7
C(6)C(6a)C(7)C(8)	−172.8	−174.9	−171.6	−174.3
C(6a)C(7)C(8)C(9)	2.0	1.9	0.9	1.2
C(7)C(8)C(9)C(10)	−2.8	−1.3	−2.4	−1.2
C(1)C(2)C(2am)-O(2am)	−2.5	172.9	−3.0	171.5
C(2)C(3)C(4)N(4)	−154.4	−147.3	−155.7	−146.8
C(3)C(4)N(4)C(4m1)	76.5	66.4	74.1	65.7
C(3)C(4)N(4)C(4m2)	−155.2	−168.8	−157.7	−167.6

magnitudes, indicating that they may have been misassigned in the initial model and thus their assignment was interchanged. The changes in the anisotropic temperature factor coefficients resulting from refinement of the model with the interchanged assignment of the above atoms and a summary of the conventional residuals from the final refinement of the model are presented in Table 11. Examinations of the observed and calculated structure factors[15] indicated that extinction corrections were not necessary.

The structure factor calculations[15] for the determination of the absolute configuration were made for the enantiomorphic forms of the refined model. Only the scale factor was allowed to change to accommodate the selected data set, which had been collected with the original crystal but at a later date. The standard residuals for the two enantiomorphic models, $R = 0.021$ (E1) and $R = 0.050$ (E2), provide a clear indication of the correct choice of the space group enantiomorph and thus the absolute configuration of the molecular cation. The systematic trend in the observed and calculated structure factors within the data set are also consistent with the selection; Table 111 displays the structure factors for the determination of the absolute configuration.

The atomic coordinates and thermal parameters for the nonhydrogen atoms of the hydrobromide model provided the initial parameters for the hydrochloride (DOXY·HCl) structure analysis. All hydrogen atoms in the latter structure were subsequently located in difference electron density maps. Refinement of the structural model was effected by variable block-block diagonal least-squares techniques. Individual blocks were so constructed that one block contained the scale factor, the appropriate fractional atomic coordinates, and the anisotropic temperature factor coefficients of the two chlorine atoms. The remaining blocks consisted of the fractional atomic coordinates of one carbon, nitrogen, or oxygen atom, its anisotropic temperature factor coefficients, and the coordinates and isotropic thermal parameters of any hydrogen atoms bonded to it. The data were empirically weighted with the same equation applied to the hydrobromide data, and those data subjectively classified as unobserved, Table 1, were screened with the same criterion for utilization in the refinement. Extinction corrections were not found to be necessary.[16] The conventional residuals are presented in Table 11.

Results and Discussion

The refined fractional atomic coordinates for the chlorine, carbon, nitrogen, and oxygen atoms of DOXY·HCl are presented in Table IV. Analogous fractional atomic coordinates for the DOXY·HBr structure, all anisotropic temperature factor coefficients, fractional atomic coordinates, and isotropic temperature factors for hydrogen atoms have been deposited[16] as Tables DI, DII, and DIII, respectively. All fractional atomic coordinates conform to the space group enantiomorph displaying the correct absolute configuration. Bond distances between carbon, nitrogen, and oxygen atoms within the molecular cations are displayed in Table V, bond angles are contained in Table DIV,[16] and the set of dihedral angles previously used to quantitatively characterize the conformation of tetracycline molecular moieties[4-6] is presented for each molecular cation in the DOXY crystal structures in Table VI. The stereochemically correct structure is presented in Figure 1 for several tetracycline derivatives; the abbreviations enclosed in parentheses will be used in subsequent reference to any of the depicted derivatives. As anticipated, the high quality of the hydrochloride crystals and the less dominating character of the contribution of the chloride ion to the diffraction intensities has resulted in a considerable improvement in the precision of the refined parameters of the atoms of the DOXY moieties of this crystal structure analysis in comparison with those of the hydrobromide salt. The improvement is clearly reflected in the

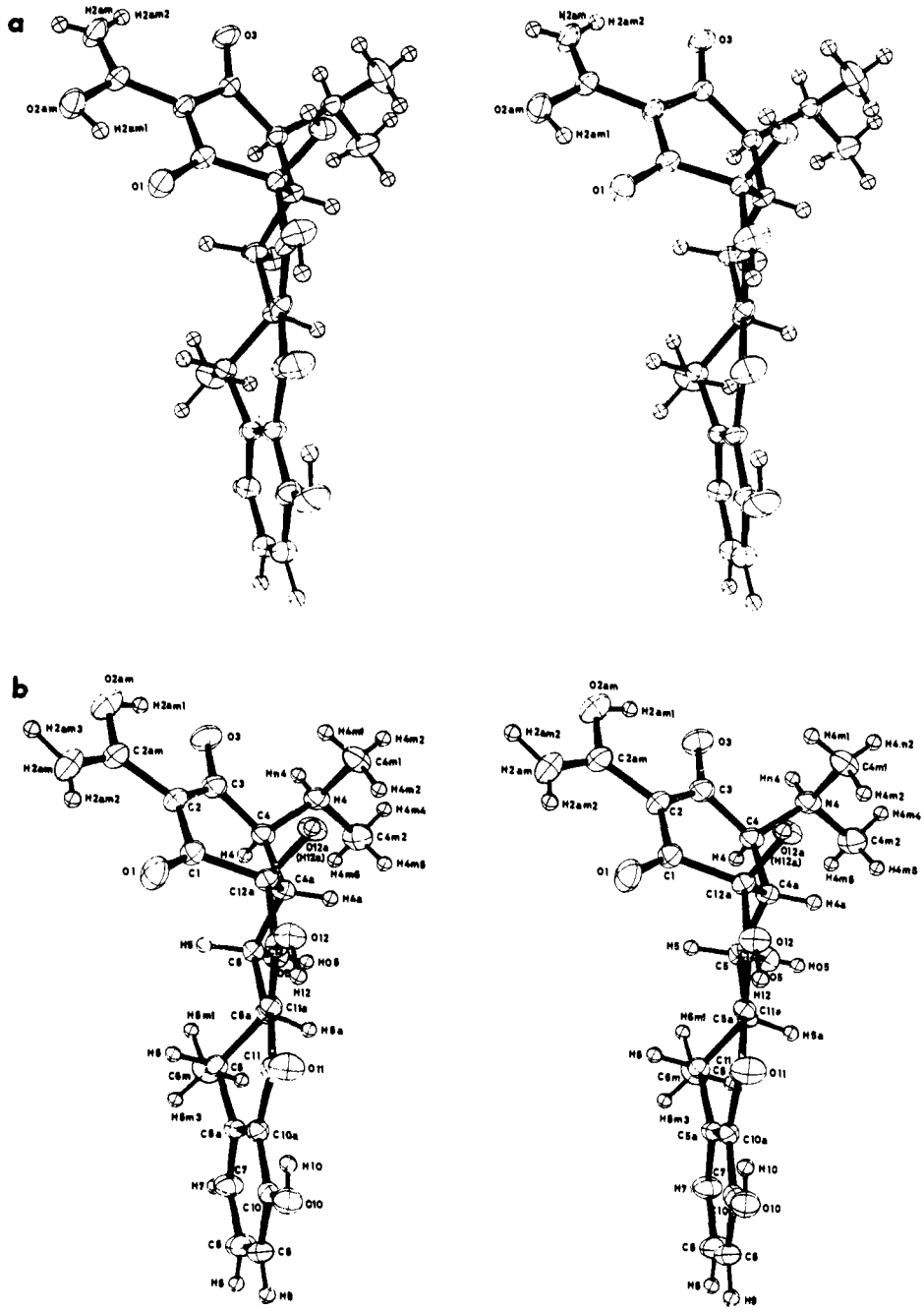
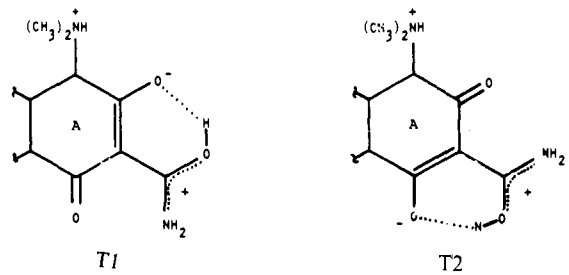


Figure 2. Stereoscopic projections[18] of the symmetry independent DOXY molecular cations. Carbon, nitrogen, and oxygen atoms are depicted with ellipsoids consistent with the 65% probability level for the refined anisotropic thermal parameters. Hydrogen atoms are depicted with uniform isotropic thermal parameters (B = 0.5 Å^2). Molecules 1 and 2 are presented in Figures 2a and 2b, respectively.

precision of the bond distances, Table V. Furthermore, the validity of all hydrogen atoms in the DOXY·HCl model was tested by least-squares refinement. Therefore discussion of the DOXY cation will refer to the structure analysis of the hydrochloride unless otherwise indicated.



T1 T2

Figure 3. The chemical structure of the A ring tautomers observed in the crystalline DOXY hydrochloride and hydrobromide salts.

The two symmetry independent molecular cations display conformations generally similar to those reported previously for the cations in the crystal structure analysis of the hydrohalide salts of other tetracycline derivatives[2,3,11,17] and for the crystalline zwitterionic form of TC and OXY free base.[4a] The symmetry independent cationic molecular moieties are depicted in stereoscopic projection[18] in Figure 2 in an orientation selected to provide a clear view of the A ring chromophore; the applicable labeling scheme is presented with molecule 2 of this figure. The two orientations of the amide substituent are apparent in this figure; the associated tautomerism of the A ring chromophore, Figure 3, is easily confirmed by examination of the appropriate A ring bond distances, Table V. The molecular structure of the cations in crystalline DOXY·HCl and DOXY·HBr[19] may be appropriately characterized as zwit-

Table VII. Bonding Parameters for Oxygen-Protonated Amides

Parameter	DOXY(1)	DOXY(2)	CL(1)[20]	CL(2)[20]	EL[20]	Av
d(C-O), Å	1.305	1.314	1.309	1.313	1.314	1.311
d(C-N), Å	1.312	1.311	1.299	1.304	1.298	1.304
∠(OCN), deg	116.6	117.2	115.2	116.0	116.6	116.4
∠(CCO), deg	118.6	119.6	120.6	119.6	120.4	119.7
∠(CCN), deg	124.8	122.7	124.1	124.0	122.8	123.5
∠($C_{(N)}$NC),[a] deg			127.3	126.8	125.3	126.3

[a] This parameter is the bond angle between the amide carbonyl carbon atom and a secondary amide carbon atom bonded to the amide nitrogen.

terionic cations in which the positive charge of the cation may be formally assigned to the protonated dimethylamine group, the proton of which is intramolecularly hydrogen bonded, see Table VIII, to the oxy substituent at C(3). The positive and negative charge centers of the zwitterionic A ring chromophore are carried, respectively, by the oxygen-protonated amide group and by the ionized oxy moiety at either C(1) or C(3), depending on the tautomeric form of the chromophore, Figure 3. The bonding parameters of the protonated amide group observed here are in accord with the libration corrected values reported[20] for protonated enantholactam, EL, and caprylolactam, CL, Table VII. The average values presented in this table should be suitable as bonding parameters of a standard model for the oxygen-protonated amide group, a group of considerable interest as an intermediate in the mechanism of the acid hydrolysis of amides.[21] The mechanisms which appear to be most probable for the reaction require the formation of either an oxygen- or nitrogen-protonated species prior to the rate-determining step.[21,22] The isolation of this and other crystalline examples of oxygen-protonated amides[2,3,11,12,19,20] contributes to the evidence favoring formation of this species as the suitable precursor.

The chemical structure of the various tetracycline derivatives presents an unusual array of functional groups suitable for intra- and/or intermolecular hydrogen bonding. The determination of several high-precision crystal structure analyses for representatives of these derivatives crystallized under different chemical conditions and in which the validity of the hydrogen atoms of the structural model has been tested by least-squares refinement[4] provides an unusual opportunity to examine the hydrogen bonding displayed by the tetracycline derivatives. An understanding of the interrelationships between the chemical structure, as reflected by the degree of protonation and/or ionization of the molecule, is crucial for the interpretation of much of the physical chemical data[23] available for these derivatives. Table VIII displays the intra- and intermolecular hydrogen bonding parameters displayed by the tetracycline derivatives in crystalline examples of an acid salt, DOXY·HCl, and of the nonionized and zwitterionic free base forms; the zwitterionic form of the free base is designated with the symbol (±) following the abbreviation for the appropriate derivative.

As expected, it is the chemical structure of the A ring substituents that has been affected by the conditions under which the crystals responsible for the tabulated data were obtained. The interrelationships between the intramolecular hydrogen bonding and the protonation state of the dimethylamine group and/or the ionization state of the A ring chromophore may be readily seen from an examination of Table VIII. Both tautomeric forms of the A ring chromophore of the acid salt display a hydrogen bond between the protonated amide carbonyl and a β-hydroxylate group. The bonding parameters (⟨d(O···H)⟩ = 1.63 Å and ⟨∠(OHO)⟩ = 156°) indicate that this hydrogen bond is relatively strong, an observation that is consistent with the unusually high basicity of the protonated amide group (e.g.,

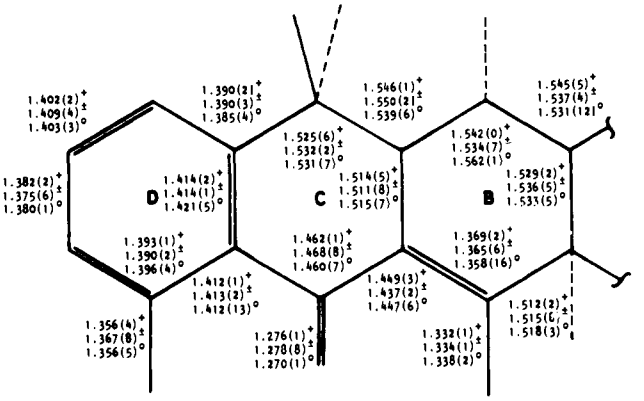


Figure 4. The average bond distances in the B, C, and D rings of tetracycline derivatives displaying different ionization states of the A ring. The number in parentheses is the maximum deviation from the average. The A ring charge is indicated by the symbol following the right parenthesis: +, fully protonated cation; ±, zwitterionic free base,[4a] and o, nonionized free base.[4,5]

protonated *N*-acetylpyrrolidine pK_a = 0.08 (21), whereas OXY·H$^+$ pK_{a_1} = 3.3 (24)).

The examples in which the dimethylamine group is protonated, the fully protonated acid and the zwitterionic free base, display an intramolecular hydrogen bond between the dimethylammonium hydrogen atom, H(n4), and the oxy moiety at C(3). The chemical structure of the molecule is such that this hydrogen bond is representative of the rather unfavorable case of intramolecular hydrogen bonding displaying the geometry of a distorted five membered ring. As may be noted from the Table VIII, the O···H distances and the NHO angles are indicative of rather weak hydrogen bonding, though the interaction does appear to be strong enough to stabilize the orientation of the dimethylammonium group.

Each of the tabulated tetracycline derivatives displays an intramolecular hydrogen bond involving a hydrogen atom of the amide NH_2 moiety. With the exception of the OXY zwitterion, the bonding parameters are very similar and give rise to average hydrogen bonding parameters ⟨d(O···H)⟩ = 2.14 Å and ⟨∠(NHO)⟩ = 132°, parameters which seem to be indicative of only moderately strong hydrogen bonding. The tabulated parameters indicate that this hydrogen bond is unusually strong for OXY(±). This observation is supported by the more accurately determined O···N distance which, at 2.636 Å, is 0.07 Å shorter for OXY(±) than for any of the other structures investigated. It is interesting to note that this example of a zwitterionic free base displays the orientation of the amide group observed in the three crystal structure analyses of nonionized free base derivatives. It seems reasonable to speculate that this structure may be the precursor to an intramolecular proton transfer from the dimethylamine group to the oxy moiety at C(3) and formation of the enolic structure of the A ring chromophore. Such a mechanism clearly favors

Table VIII. Hydrogen Bonding Parameters for Various Tetracycline Derivatives[a]

	DOXY(I)	DOXY(II)	OXY(I)[4a]	OXY(II)[4b]	OXY(±)[4a]	TC(±)[4a]
			A. Intramolecular			
O(D)-H(D)···O(A)[a]	(2am) (2am1) (1)	(2am) (2am1) (3)	(3) (3) (2am)	(3) (3) (2am)		
d(O-H)	0.91 (7)	0.85 (6)	1.08 (4)	1.04 (5)	Dissociated	Dissociated
d(H···O)	1.59 (7)	1.67 (5)	1.39 (5)	1.42 (5)		
∠(OHO)	157 (5)	154 (5)	157 (5)	158 (5)		
N(2am)-H(2am2)···O(A)	(3)	(1)	(1)	(1)	(1)	(3)
d(N-H)	0.84 (4)	0.80 (5)	0.84 (4)	0.87 (4)	0.98 (5)	0.73 (4)
d(H···O)	2.24 (5)	2.10 (4)	2.11 (4)	2.06 (4)	1.80 (5)	2.20 (4)
∠(NHO)	129 (4)	134 (4)	133 (4)	134 (4)	141 (4)	130 (4)
N(4)-H(n4)···O(3)						
d(N-H)	0.86 (5)	0.83 (4)	Not	Not	0.85 (4)	0.97 (4)
d(H···O)	2.38 (4)	2.29 (4)	protonated	protonated	2.22 (4)	2.29 (4)
∠(NOH)	107 (3)	108 (4)			109 (3)	100 (2)
O(10)-H(10)···O(11)						
d(O-H)	0.90 (5)	0.76 (4)	0.96 (4)	0.99 (4)	0.94 (8)	0.84 (4)
d(H···O)	1.71 (5)	1.85 (4)	1.67 (4)	1.59 (4)	1.68 (8)	1.77 (4)
∠(OHO)	151 (4)	151 (4)	152 (4)	157 (4)	150 (7)	152 (4)
O(12)-H(12)···O(11)						
d(O-H)	0.84 (5)	0.83 (6)	0.94 (5)	0.81 (4)	0.80 (8)	0.80 (4)
d(H···O)	1.74 (5)	1.76 (5)	1.62 (5)	1.78 (4)	1.84 (8)	1.76 (4)
∠(OHO)	149 (4)	144 (4)	152 (5)	147 (4)	145 (4)	152 (7)
O(12a)-H(12a)···O(A)		(12)	(1)		(12)	
d(O-H)	Inter-	0.71 (4)	0.84 (5)	Inter-	0.96 (5)	Inter-
d(H···O)		2.30 (4)	2.14 (5)		2.28 (5)	
∠(OHO)		113 (4)	115 (4)		100 (4)	
O(5)-H(5)···O(12a)						
d(O-H)	Inter-	Inter-	0.98 (5)	0.74 (6)	Inter-	
d(H···O)			2.25 (5)	2.22 (6)		
∠(OHO)			118 (4)	132 (5)		
			B. Intermolecular			
O(5)-H(5)···A	Cl(2)	Cl(1)			O(2am)	
d(O-H)	0.73 (5)	0.68 (5)	Intra-	Intra-	0.85 (6)	
d(H···A)	2.60 (5)	2.69 (5)			1.77 (6)	
∠(OHA)	149 (5)	153 (5)			179 (6)	
O(6)-H(6)···O(A)			(2am)	(10)	(w2)	(2am)
d(O-H)			0.71 (4)	0.81 (4)	0.80 (9)	0.74 (4)
d(H···O)			2.14 (5)	2.06 (4)	2.00 (8)	2.08 (4)
∠(OHO)			158 (6)	168 (4)	177 (8)	161 (4)
O(12a)-H(12a)···A	O(10)	Cl(2)		O(5)	O(5)	O(2am)
d(O-H)	0.79 (4)	0.71 (4)	Intra-	0.77 (5)	0.96 (5)	0.81 (5)
d(H···A)	2.07 (4)	2.44 (4)		1.97 (5)	1.74 (6)	1.92 (5)
∠OHA)	155 (4)	149 (4)		166 (5)	162 (5)	170 (4)
N(2am)-H(2am3)···O(5)						
d(N-H)	0.84 (5)		0.91 (4)			
d(H···O)	2.20 (5)		2.05 (4)			
∠(NHO)	147 (4)		174 (4)			
N(2am)-H(2am2)···O(Et)						
d(N-H)	0.74 (6)					
d(H···O)	2.11 (6)					
∠(NHO)	168 (4)					
N(4)-H(4)···O(w)						
d(N-H)	0.86 (5)				0.86 (4)	0.97 (4)
d(H···O)	1.91 (4)				2.17 (4)	1.77 (4)
∠(NHO)	150 (4)				140 (3)	163 (3)
O(1)···H-O(w5)						
d(H-Ow)						0.86 (4)
d(O···H)						2.00 (5)
∠(OHO)						168 (4)
O(6)···H-O(w1)						
d(H-Ow)						0.82 (4)
d(O···H)					*b*	1.96 (6)
∠(OHO)						170 (4)
O(10)···H-O(w2)						
d(H-Ow)						0.74 (5)
d(O···H)					*b*	2.36 (5)
∠(OHO)						144 (4)
O(12)···H-O(w4)						
d(H-Ow)						0.78 (4)
d(O···H)						2.30 (4)
∠(OHO)						136 (4)

[a] Where different atoms of the tetracycline derivatives are involved in hydrogen bonding with a given group, the appropriate atom labels or subscripts are presented above the bonding parameters for that hydrogen bond. [b] The hydrogen atoms of the water molecules of hydration were not located in this structure. The oxygen-oxygen distances in the refined structure indicate hydrogen bonding in which the appropriate atom of the OXY zwitterion serves as the accepter in a manner similar to that found for the TC zwitterion.

formation of the 3-hydroxyl tautomer, the analogue of T1 in Figure 3. The exceptionally strong hydrogen bond between the hydroxyl group and the amide carbonyl clearly stabilizes the enolic structure[4] and may provide a mechanism for some interconversion to the analogue of tautomer T2 via a protonated amide intermediate. Since the stable form of the nonionized free base is the enol and not the protonated amide structure displayed by the DOXY anions, the interconversion of tautomers would be expected to be considerably slower than that of the fully protonated derivatives. It is noteworthy that Dudek and Volpp[14] have reported the observation of the appropriate tautomeric forms of model compounds of the tetracyline A ring that lacked the dimethylamine group, whereas Gulbis and Everett[25] make no mention of similar observations for tetracycline free base derivatives in Me_2SO, a solvent in which the A ring chromophore has been demonstrated to be enolic.[4b]

The chemical conditions under which the tabulated derivatives were crystallized were those which profoundly affected the A ring substituents, but have left the BCD chromophore largely undisturbed, Figure 4. The structural integrity of this chromophore extends to the intramolecular hydrogen bonding as well, Table VIII. The average parameters, $\langle d(O(11)\cdots H)\rangle$ = 1.73 Å, $\langle \angle(O(10)H(10)O(11))\rangle$ = 152°, and $\langle \angle(O(12)$-$H(12)O(11))\rangle$ = 147°, indicate that the strength of these hydrogen bonds is equivalent to that between the protonated amide group and the β-oxy moiety, all of which display similar 6-atom ring geometry. The interrelationships between intramolecular hydrogen bonding and the chemical conditions affecting the structure of the BCD chromophore remain to be determined. The tautomeric form of the BCD chromophore of an OXY divalent anion has been shown to differ from that presented here.[6] Unfortunately the crystals obtained for that investigation were not of adequate quality to permit location of most of the hydrogen atoms. There is also considerable evidence that metal ion complexation involves the BCD chromophore,[6] though solvent effects, including basicity, appear to be important here as well.

There are two more hydroxyl groups that have been shown to form intramolecular hydrogen bonds. The 5-hydroxyl group of OXY is hydrogen bonded, through its hydrogen atom to the 12a-hydroxyl group, in the conformation displayed by the nonionized free base. The hydrogen bonding parameters, Table VIII, indicate that the interaction is relatively weak, particularly since the chemical structure involved is that of a six-membered ring. It has been adequately demonstrated that this hydrogen bond is not the determining factor for the adoption of the conformation of the nonionized free base either in the solid state[4a,5] or in solution.[4b,25] The hydroxyl group at C(12a) has been found to form five-membered ring intramolecular hydrogen bonds to either O(12) or O(1) and has also been found to form intermolecular hydrogen bonds; this is one of differences displayed by the two crystal modifications of nonionized OXY free base, OXY(I) and OXY(II).[4b]

As a result of the constraints imposed by the solid state, the intermolecular hydrogen bonding displayed by the crystalline derivatives is likely to be considerably less representative of that found in solution than the intramolecular variety. Thus it need not be described in detail; however, some points should be mentioned. Except for some of the cases where they are intramolecularly hydrogen bonded, each of the nonchromophoric hydroxyl groups, those at C(5), C(6), and C(12a), are involved in intermolecular hydrogen bonding, often as both donor and acceptor groups. With the exception of the DOXY(II) molecular cation, the hydrogen atom of the protonated dimethylamine group is involved in intermolecular hydrogen bonding with a water molecule as well as in intramolecular hydrogen bonding with the oxy moiety at C(3). The hydrogen bonding parameters, interatomic distances and angles, indicate that the former is considerably stronger than the latter, Table VIII. Of the crystalline examples investigated here, only the zwitterionic free base forms, OXY(±) and TC(±), act as acceptors for hydrogen bonds from water molecules, Table VIII, though it is to be expected that many of the intermolecular hydrogen bonds between the tetracycline moieties in the various crystals are indicative of solvent–TC derivative interactions in aqueous solution.

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Supplementary Material Available: The fractional atomic coordinates for DOXY·HBr; all anisotropic temperature factor coefficients; all parameters associated with the hydrogen atoms; bond angles between carbon, nitrogen, and oxygen atoms; and observed and calculated structure factor amplitudes for the two refined structures (137 pages). Ordering information is given on any current masthead page.

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