Acta Crystallographica Section C
Crystal Structure Communications
ISSN 0108-2701

Tetracycline hydrochloride: a synchrotron microcrystal study

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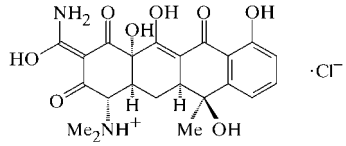
Received 21 June 2000
Accepted 25 July 2000

The title compound, [(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-2-aminohydroxymethylene-1,2,3,4,4a,5,5a,6,11,12a-decahydro-6,10,12,12a-tetrahydroxy-6-methyl-1,3,11-trioxonaphthacen-4-yl]dimethylammonium chloride, $C_{22}H_{25}N_2O_8^+\cdot Cl^-$, a well known antibiotic, has been structurally characterized from an individual coarse powder grain by use of high-intensity synchrotron radiation, in conjunction with an exercise in *ab initio* powder diffraction structure solution. Free refinement of all H atoms establishes the major tautomeric form of the protonated tetracycline molecule without prejudice. The molecule has extensive intramolecular hydrogen bonding involving most of the potential donors and acceptors, and all intermolecular hydrogen bonding uses the chloride anion as acceptor.

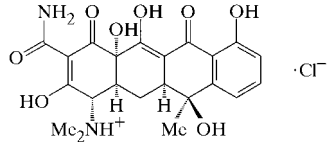
Comment

This determination of the crystal structure of tetracycline hydrochloride, (I), was undertaken as part of a round-robin exercise in *ab initio* structure solution from X-ray powder diffraction data, conducted by Cranswick & Le Bail (1998) and reported at the Eighteenth European Crystallographic Meeting in Prague. Participants in the exercise were provided with sets of powder diffraction data measured from two different materials with conventional laboratory and synchrotron radiation and with no information other than the chemical formulae of the compounds, the unit-cell parameters and possible space groups. In order to provide a benchmark result for one of the samples, a definitive structure determination by single-crystal diffraction was desirable. It was, therefore, essential to measure the data from a crystal selected from the same sample used for the powder diffraction measurements, with no recrystallization to obtain larger single crystals. The individual microcrystals in the sample had maximum dimensions of tens of microns, and so synchrotron radiation was required for adequate intensities. Although crystal quality was good, conventional laboratory X-ray sources would give diffraction data too weak to be useful. Two possible tautomers of (I), *A* and *B*, are illustrated in the Scheme below.

Using the dedicated single-crystal diffraction facility of Station 9.8 at CLRC Daresbury Laboratory Synchrotron Radiation Source (Cernik *et al.*, 1997, 2000), it was possible to



(I), tautomer *A*



(I), tautomer *B*

obtain high quality diffraction data with individual frame exposures of a few seconds, essentially equivalent to what would be obtained from larger crystals and conventional X-ray sources. Procedures for data collection, data reduction and structure solution were standard for this facility (Clegg *et al.*, 1998). H atoms were located in difference syntheses and could be freely refined with no constraints or restraints, as shown in Fig. 1. This enables the tautomeric form of the protonated tetracycline molecule and the hydrogen bonding in the crystal structure to be established without prejudice. A previous report of the structure, under the name of achromycin hydrochloride, is incomplete in that it provides no atomic coordinates and it is also unsatisfactory in that the structure is of relatively low precision (Kamiya *et al.*, 1971). These shortcomings are remedied in this new study.

In addition to the protonation of the dimethylamine N atom to form the hydrochloride salt, this work establishes that the amide function attached to C12 adopts the enol tautomer and the group at C11 adopts the keto form, in accordance with *A* in the chemical scheme. This is the primary classical contribution to the bonding in this part of the molecule, though the geometry of the O19—H19···O11 hydrogen bond (see below) and the fact that H19 has the highest atomic displacement parameter in the whole molecule indicate a significant contribution of an alternative arrangement, *B*, the result being a high degree of conjugation of these substituents. This assignment is supported not only by the successful location and free refinement of all the H atoms but also by the relevant bond lengths (Table 1). Thus, C11═O11 is characteristic of a double bond, while C19—O19 is markedly longer. Lying between them, C12—C19 and C11—C12 have lengths intermediate between single and double bonds, as does C12—C13.

Other than the involvement of the chloride anion, all hydrogen bonding in the structure is intramolecular (Table 2). The H atom attached to O19 is hydrogen bonded to the carbonyl O11 atom, to which it would be directly bonded as a hydroxyl function in the alternative tautomeric form *B*, giving a six-membered ring. The geometry of this hydrogen bond, with relatively long O—H and short H···O distances, together with the high refined displacement parameter of the H atom, is consistent with a strong interaction and a broad shallow

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energy minimum for the H atom between the two O atoms. Other intramolecular hydrogen bonding involves the NH_2 group at N19 and some hydroxyl groups as donors, with carbonyl and hydroxyl O atoms as acceptors, as shown in Fig. 1. All of these produce five- and six-membered rings.

The protonated molecules are linked by hydrogen bonds *via* the chloride anions. Each Cl^- acts as an acceptor for four hydrogen bonds (Table 2), one from NH_2, one from the $NHMe_2$ group and two from hydroxyl groups. The OH group at O6 forms no intramolecular hydrogen bonds, while that at O14 is involved in bifurcated hydrogen bonding, both intramolecular (to O15) and to Cl^-. Thus, the crystal structure is held together *via* a three-dimensional network of N—H···Cl and O—H···Cl interactions.

A search of the Cambridge Structural Database (October 1999 release; Allen & Kennard, 1993) yields structures of about 30 closely related tetracycline derivatives, including some studied more than once. A number of different tautomeric forms have been assigned, including some equivalent to the one found here. Neutral tetracycline itself, as its hexahydrate (Stezowski, 1976; Caira *et al.*, 1977) and in the tetrahydrate of a 1:1 cocrystal with urea (Palenik & Mathew, 1978), is zwitterionic, with a protonated NMe_2 group and a deprotonated adjacent hydroxyl group. Most of the previous crystallographic work on tetracyclines was carried out in the late 1970s and there was some debate about the tautomeric forms of the molecule, especially when protonated as in the present work; structures were variously determined from data collected at room temperature and at reduced temperatures, and free refinement of H atoms was not possible in all cases (Jogun & Stezowski, 1976; Stezowski, 1977; Boggs, 1978; Palenik *et al.*, 1978; Bordner, 1979; Prewo & Stezowski, 1980; Koziol *et al.*, 1992; Carrondo *et al.*, 1994). Even with refined H atoms, the same distribution of H atoms and very similar molecular geometry have been interpreted in terms of different tautomers (Stezowski, 1977; Bordner, 1979). Our results clearly support the conclusions of Bordner (1979) and of Glatz *et al.* (1979) regarding the major contribution of the tautomeric form shown as *A* in the Scheme above, and the nature of the hydrogen bonding between the amide group in its enol form and the adjacent keto group.

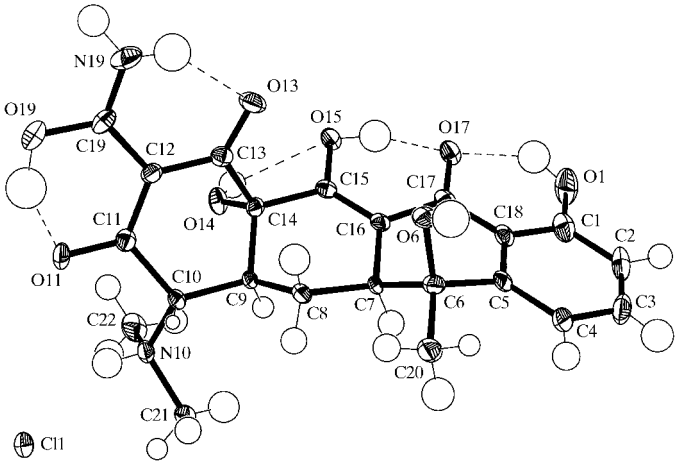

Figure 1
The molecular structure of (I) with atom labels, 50% probability ellipsoids for non-H atoms and 50% probability spheres for H atoms. Intramolecular hydrogen bonds are shown as dashed lines.

Experimental

The crystal was selected from a commercial microcrystalline sample (Aldrich) without recrystallization.

Crystal data

$C_{22}H_{25}N_2O_8^+\cdot Cl^-$
M_r = 480.89
Orthorhombic, $P2_12_12_1$
a = 10.9300 (9) Å
b = 12.7162 (11) Å
c = 15.7085 (13) Å
V = 2183.3 (3) Å^3
Z = 4
D_x = 1.463 Mg m^{-3}
Synchrotron radiation
λ = 0.6883 Å
Cell parameters from 6452 reflections
θ = 2.51–29.20°
μ = 0.228 mm^{-1}
T = 150 (2) K
Prism, yellow
0.04 × 0.03 × 0.02 mm

Data collection

Bruker SMART CCD diffractometer with Oxford Cryosystems open-flow cryostat (Cosier & Glazer, 1986)
ω rotation scans with narrow frames
8955 measured reflections
2957 independent reflections (plus 1958 Friedel-related reflections)
3937 reflections with $I > 2\sigma(I)$
R_{int} = 0.047
θ_{max} = 29.26°
$h = -14 \rightarrow 8$
$k = -17 \rightarrow 17$
$l = -21 \rightarrow 4$
Intensity decay: 15%

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.050
$wR(F^2)$ = 0.116
S = 0.969
4915 reflections
399 parameters
All H-atom parameters refined
$w = 1/[\sigma^2(F_o^2) + (0.0579P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.001
$\Delta\rho_{max}$ = 0.57 e Å^{-3}
$\Delta\rho_{min}$ = −0.24 e Å^{-3}
Extinction correction: *SHELXTL* (Sheldrick, 1997)
Extinction coefficient: 0.0038 (12)
Absolute structure: Flack (1983)
Flack parameter = 0.02 (8)

Table 1
Selected geometric parameters (Å, °).

O1—C1	1.352 (4)	O17—C17	1.277 (3)
O6—C6	1.432 (4)	O19—C19	1.314 (4)
O11—C11	1.260 (4)	N19—C19	1.301 (5)
O13—C13	1.234 (4)	C11—C12	1.403 (5)
O14—C14	1.422 (3)	C12—C13	1.427 (4)
O15—C15	1.330 (3)	C12—C19	1.435 (4)
O11—C11—C12	124.2 (3)	N19—C19—O19	118.4 (3)
C11—C12—C13	121.1 (3)	N19—C19—C12	122.0 (3)
C11—C12—C19	117.5 (3)	O19—C19—C12	119.6 (3)
C13—C12—C19	121.4 (3)		

The observed intensity decline of 15% was due to synchrotron beam decay and was corrected for. All H atoms were located in a difference map and were refined freely with individual isotropic displacement parameters. The absolute configuration, well established for this class of compounds, is confirmed by refinement of the Flack (1983) parameter, the chloride anion providing sufficient anomalous dispersion effect at this wavelength.

Table 2
Hydrogen-bonding geometry (Å, °).

D—H···A	D—H	H···A	D···A	D—H···A
O1—H1···O17	0.92 (5)	1.79 (5)	2.564 (4)	140 (4)
O6—H6···Cl1^{i}	0.94 (4)	2.25 (4)	3.179 (2)	174 (3)
O14—H14···O15	0.70 (4)	2.36 (4)	2.683 (3)	110 (4)
O14—H14···Cl1^{ii}	0.70 (4)	2.56 (4)	3.195 (2)	153 (4)
O15—H15···O17	0.94 (4)	1.63 (5)	2.491 (3)	151 (4)
O19—H19···O11	1.13 (5)	1.49 (5)	2.484 (3)	143 (4)
N10—H10···Cl1	0.87 (4)	2.26 (4)	3.053 (3)	152 (3)
N19—H19*A*···Cl1^{iii}	0.91 (4)	2.27 (4)	3.157 (3)	163 (4)
N19—H19*B*···O13	0.86 (5)	1.97 (5)	2.694 (4)	141 (4)

Symmetry codes: (i) $x-1, y, z$; (ii) $\frac{3}{2}-x, 1-y, \frac{1}{2}+z$; (iii) $x-\frac{1}{2}, \frac{3}{2}-y, -z$.

Data collection: *SMART* (Bruker, 1998); cell refinement: local programs; data reduction: *SAINT* (Bruker, 1998); program(s) used to solve structure: *SHELXTL* (Sheldrick, 1997); program(s) used to refine structure: *SHELXTL*; molecular graphics: *SHELXTL*; software used to prepare material for publication: *SHELXTL* and local programs.

We thank the EPSRC and CCLRC for financial support, and Dr Lachlan Cranswick for providing the microcrystalline sample and suggesting the experiment.

Supplementary data for this paper are available from the IUCr electronic archives (Reference: BM1422). Services for accessing these data are described at the back of the journal.

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