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ORIGINAL PAPER

X-Ray Crystallography of Tetracycline, Doxycycline and Sancycline

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Abstract The molecular and crystal structures of α-6-deoxy-β-5-oxytetracycline (doxycycline) hydrochloride, of tetracycline hydrochloride and tetracycline hexahydrate were re-determined. The crystal structures of 6-deoxy-6-dimethyl-tetracycline (sancycline) hydrochloride were, for the first time, determined by single crystal X-ray diffraction technique. All crystals studied exhibit the orthorhombic space group $P2_12_12_1$ with 4 molecules per unit cell. The starting material tetracycline (TC) hydrochloride crystallizes from aqueous solution, independently of the pH of the mother liquor, as hexahydrate complex of the zwitterion with the same molecular structure ($a = 9.585(3)$ Å, $b = 12.112(3)$ Å, $c = 21.671(6)$ Å). From methanolic solution, tetracycline hydrochloride crystallizes as hydrochloride ($a = 11.001(3)$ Å, $b = 12.852(4)$ Å, $c = 15.795(3)$ Å). Doxycycline hydrochloride crystallizes from acidic aqueous solution as dihydrate complex ($a = 11.115(4)$ Å, $b = 12.768(4)$ Å, $c = 16.921(5)$ Å). In both hydrochloride crystals, the amide group is protonated and oriented such that an intramolecular hydrogen bond is formed between the amide oxygen and O3. In the tetracycline–hexahydrate crystal, the amide group of the zwitterion is rotated by about 180° (vs the cation) with a hydrogen bond being made by one hydrogen of its amino group and $O3^-$. Sancycline hydrochloride crystallized from an acidified solvent mixture (water and methanol) adopts a geometry close to that of the tetracycline cation, e.g., with a hydrogen bond between the protonated amide oxygen and O3 ($a = 6.8944(4)$ Å, $b = 16.815(2)$ Å, $c = 18.190(2)$ Å). But the dimethyl ammonium group of sancycline hydrochloride is disordered with respect to its orientation. In the majority fraction (65.9 %), the proton at N4 is directed towards O3, which has a water molecule next to it. In the minority fraction (34.1 %), the proton at N4 points away from O3 and has a methanol molecule in its neighborhood. The bond length variations in ring A of all compounds studied do not reflect the location of single and double bonds in any of the classical mesomeric structures. By comparison with calculated Wiberg-bond orders we show that this apparent discrepancy is not due to the existence of different tautomers in the crystals as discussed previously but merely a consequence of the π-conjugation in one tautomer.

Keywords Molecular and crystal structures · Tetracycline · Doxycycline · Sancycline · Wiberg-bond order

Introduction

Since the discovery of the antibiotic action of tetracyclines [1] (for chemical structure and generally adopted numbering of atoms see Fig. 1), much work has been devoted to developing an understanding of the protonation–deprotonation equilibria (Scheme 1), the tautomerism of the neutral and mono-anionic form and the geometry adopted in dependence on the state of protonation and kind of environment (solvent). Despite the large effort and the great

In memoriam: Prof. Dr. Dieter Sellmann.

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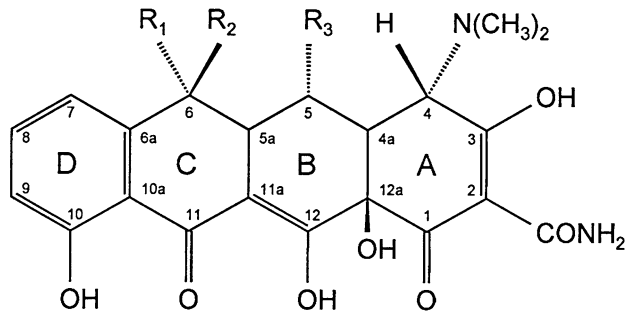


	R_1	R_2	R_3
Tetracycline	CH_3	OH	H
Doxycycline	CH_3	H	OH
Sancycline	H	H	H

Fig. 1 Molecular structure of tetracyclines investigated and conventional numbering of atoms and rings (only one of the possible isomeric/tautomeric forms is shown)

variety of spectroscopic techniques applied many of the addressed questions are still not satisfactorily answered. The elucidation of these properties is, however, considered essential to estimate the pharmacokinetic properties, as, e.g., the bio-availability or the permeability through membranes. With the advent of new applications in various medical fields (non-antibiotic actions) a complete knowledge of the structural properties is more desirable than ever before. (For a review of all topics related to tetracyclines reference [2] is recommended.)

Most crystallographic work on tetracycline and derivatives was done in the late 1970s and early 1980s and free refinement of H atoms was not possible in all cases [3–9]. Consequently, very similar geometries of the heavier atoms have been interpreted in terms of different tautomers [8, 9] with severe consequences on the assignment of experimentally determined pK_a-values. Only recently it could be unambiguously shown that tetracycline hydrochloride exists in the crystalline state as that tautomer in which the proton is located at the amide oxygen rather than at O3 [10, 11].

Tetracycline and oxytetracycline (β-5-oxytetracycline) crystallize in the absence of water in the neutral form with a conformation termed B by Prewo and Stezowski [7], in which both the dimethylamino group and the tricarbonyl system are above the plane spanned by rings B, C and D. The X-ray structure published by Koziol et al. verifies this conclusion [12]. (because there exists a confusing terminology in the literature describing various conformations of tetracyclines, a survey is given in the "Appendix" section).

More recent studies provide strong evidence that in aqueous solution tetracycline hydrochloride adopts the same conformation as in the crystal if the solid material is dissolved directly in aqueos buffer at pH 2 or pH 11, respectively [7, 11, 13–15]. During the course of a titration experiment, the conformation changes with the effect that the mixture of conformers (tautomers) depends on titration history (Remember, drugs taken orally experience during their passage through the intestines an environment of largely different pH-values!).

In order to contribute towards an answer of unresolved questions we present here the molecular structures of tetracycline (zwitterion), tetracycline hydrochloride (cation), doxycycline hydrochloride (cation) and sancycline hydrochloride (cation) obtained by conventional X-ray crystallography from samples crystallized under different experimental conditions. ZDO Atomic Charges and Wiberg-bond orders are calculated for various tautomers of sancycline to rationalize the observed variations of bond lengths.

Experimental Section

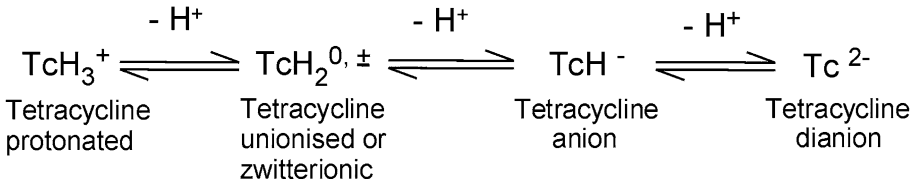
Preparation of (Single) Crystals

Tetracycline hydrochloride was bought from Aldrich (Germany), doxycycline hydrochloride from Fluka (Germany) and sancycline from Clariant (Origgio, Italy).

For the preparation of aqueous solutions of tetracycline, the poly-crystalline material was dissolved in bi-distilled water. For adjustment of the desired pH, either HCl or ammonia water was added [11]. Three different types of tetracycline crystals and one each of doxycycline and sancycline were grown by the following procedures:

(Crystal I) Tetracycline hexahydrate (acidic):
Aqueous solutions of Tc·HCl (69 mM) were adjusted to pH 1.83 and stored in the refrigerator for several days until crystals formed.
(Crystal II) Tetracycline hexahydrate (alkaline):
Aqueous solutions of Tc·HCl were adjusted to pH 9.0 and crystallized in the refrigerator.
(Crystal III) Tetracycline hydrochloride (·0.25H_2O):
Crystallization at room temperature from methanolic solution by slow evaporation of the solvent.
(Crystal IV) Doxycycline hydrochloride dihydrate:
The polycrystalline material was dissolved in water (69 mM) and the pH adjusted to about 1 by addition of concentrated HCl. Afterwards, the pH was raised to 1.84 by addition of ammonia water and the solution stored for 24 h. Several of the separated small crystals were added back to the decanted mother liquor. After several days of storage in the refrigerator, single crystals large enough for X-ray diffractometry could be isolated.

Scheme 1 .



(Crystal V) Sancycline hydrochloride (·hydrate/methanolate): 3.6 g sancycline was given into a beaker containing 100 ml water and 10 ml of 10 M HCl (equivalent to 69 mM). Then, methanol was added slowly until a saturated solution of sancycline hydrochloride was formed. Aliquots of 20 ml of such solutions were made subject to different procedures, which are usually applied for crystallization [16]. Only that sample which rested for several weeks at room temperature in an open beaker produced small crystals suitable for crystallography.

X-Ray Crystal Structure Determinations

Suitable single crystals were sealed in a glass capillary under N_2. Intensity data were either collected on a Nicolet R3m/V (crystals I–IV) or on a Bruker–Nonius KappaCCD (crystal V) diffractometer (using Mo-K_α radiation ($\lambda = 0.71073$ Å, graphite monochromator). Data were corrected for Lorentz and polarization effects while an absorption correction has not been applied except for crystals II and V. Atomic scattering factors used and corrections for anomalous dispersion were taken from Reference [17]. Where appropriate (sufficient anomalous dispersion present, crystals III–V), the absolute structure parameters were determined according to Flack [18]. All structures were solved by direct methods and refined using full-matrix least-squares procedures on F^2 values (SHELXTL NT 6.12) [19]. All non-hydrogen atoms were refined with anisotropic displacement parameters. The positions of the hydrogen atoms of crystals I, III, and IV and those of the solvent water molecules of II were taken from a difference Fourier synthesis and their positional parameters were either kept fixed (I and solvent water hydrogens of II) or refined (III (except solvent water) and IV). The remaining hydrogen atoms of II and all of V were placed in positions of optimized geometry. The isotropic displacement parameters of all hydrogen atoms were tied to those of their corresponding carrier atoms by a factor of either 1.2 or 1.5.

Selected crystallographic data and further details of the data collection and refinement of crystals I–V are summarized in Table 1. Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (CCDC). The corresponding CCDC reference numbers are given in Table 1: CCDC-243335-38 and CCD-C864575.

Results and Discussion

All samples investigated crystallized in the orthorhombic space group $P2_12_12_1$ (no. 19) with four molecules per unit cell. Details of the structure refinement and selected crystallographic data are summarized in Table 1 to allow an easy comparison. Stereo views of the Ortep plots of tetracycline zwitterion and doxycycline cation provide a good impression of the extensively discussed conformation of these compounds.

Tetracycline (Cation and Zwitterion)

Crystallographic *R*-values as well as estimated standard deviations for the sample Tc·$6H_2O$ (alkaline) are worse than those for the sample Tc·$6H_2O$ (acidic). This reflects the fact that the crystal produced from the alkaline solution was smaller and obviously less good than the one produced from the acidic solution. Nevertheless, one can conclude that the crystal structure of Tc·$6H_2O$ is unique and independent of the pH of the mother liquor. In two earlier studies, tetracycline was crystallized from a water–acetone mixture [4] and water [5], respectively. The crystallographic data are in accord with those presented in Table 1.

Neighboring tetracycline molecules are arranged mostly face-to-edge. Intermolecular hydrogen bonds are found between O2AM and the hydroxyl groups attached to C6 and C12A, respectively, of different neighbors. They should have no significant effect on the molecular structure. Figure 2 illustrates the existence of the above described A conformation. Like in all tetracycline derivatives strong intramolecular hydrogen bonds between O11 and O10 and O11 and O12 stabilize the geometry of rings B, C and D.

For the sake of completeness, it should be mentioned that tetracycline was also crystallized from an acidified D_2O solution (CCDC 243339). The crystal structure was found to be identical to that of crystal I produced from a H_2O solution.

The dimensions of the unit cell of crystal III (Tc·HCl·$0.25H_2O$) and the molecular parameters are very close to those reported previously by Clegg and Teat [10]

Table 1 Selected crystallographic data and structure refinement details

	Tc·6H_2O (acidic)	Tc·6H_2O (alcaline)	Tc·HCl·0.25H_2O	Doxy·HCl·2H_2O	Sancycline·H_2O/MεOH[d]
	Crystal I	Crystal II	Crystal III	Crystal IV	Crystal V
CCDC reference number	243335	243336	243337	243338	864575
Formula	$C_{22}H_{36}N_2O_{14}$	$C_{22}H_{36}N_2O_{14}$	$C_{22}H_{25.5}ClN_2O_{8.25}$	$C_{22}H_{29}ClN_2O_{10}$	$C_{21.34}H_{25.68}ClN_2O_8^d$
M_r (g/mol)	552.53	552.53	485.39	516.92	473.65
Crystal size (mm)	0.60 × 0.50 × 0.40	0.36 × 0.30 × 0.22	0.60 × 0.50 × 0.40	0.50 × 0.42 × 0.36	0.24 × 0.07 × 0.05
Temperature (K)	293	298	295	295	100
$F(000)$	1176	1176	1016	1088	995
Crystal system	Orthorhombic	Orthorhombic	Orthorhombic	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a (Å)	9.585(3)	9.603(4)	11.001(3)	11.115(4)	6.8944(4)
b (Å)	12.112(3)	12.108(7)	12.852(4)	12.768(4)	16.815(2)
c (Å)	21.671(6)	21.658(10)	15.795(3)	16.921(5)	18.190(2)
V (Å^3)	2516(2)	2518(2)	2233(1)	2401(2)	2108.8(4)
Z	4	4	4	4	4
$d_{calcd.}$ (g/cm^3)	1.459	1.457	1.444	1.43	1.492
Absorption coeff. μ (mm^{-1})	0.122	0.122	0.225	0.219	0.235
Scan technique	ω scans	ω scans	ω scans	ω scans	ω scans
2θ range (°)	3.7–54.0	3.7–52.0	4.0–56.0	4.0–56.0	3.57–27.1
Meas. reflections	4879	4231	4771	5073	27723
Indep. reflections	4323	3765	4156	4432	4636
R_{int}	0.0188	0.0933	0.0255	0.0256	0.0734
Obs. Reflections[a]	3526	1319	3010	2411	3478
Parameters refined	343	350	382	403	332
R_1^a; wR_2^b	0.0388, 0.0950	0.0665, 0.1962	0.0339, 0.0723	0.0427, 0.0840	0.0476, 0.1052
GooF[b]	0.963	0.783	0.866	0.85	1.038
Absolute structure parameter[18]	n.d.[c]	n.d.[c]	0.00(6)	0.08(8)	0.01(8)
ρ_{fin} max/min) (e/nm^3)	0.187/−0.196	0.318/−0.318	0.195/−0.146	0.170/−0.162	0.294/−0.283

[a] For $I > 2.0\sigma(I)$; [b] for all data, [c] not determined

[d] The data given refer to our crystal (H_2O/MeOH = 0.7/0.3); other crystals can have a different ratio of solvent molecules

employing synchrotron radiation to a commercial microcrystalline sample. The tetracycline cations again adopt kind of an edge-to-face arrangement, but with no intermolecular hydrogen bonds between them. Instead, each cation forms four hydrogen bonds to different chloride anions [10].

The conformation of the cation is very similar to that of the zwitterion as can be concluded from similar values of the dihedral angles characterizing the tilt of ring A versus the plane spanned by rings B, C and D (see Table 2). The most apparent difference between the two structures discussed is the orientation of the amide group. In the tetracycline–hexahydrate crystal the amide group of the zwitterion is oriented such that a hydrogen bond is made by one hydrogen of its amino group, HN2AM, and O3. In the tetracycline cation the amide group is rotated by about 160° versus the zwitterion (compare angles C1–C2–C2AM–O2AM) and a hydrogen bond made between HO2AM and O3.

In the cation, the fragment of ring A that exhibits a π-electronic system can formally be described by a mesomeric structure with three double bonds: C1–O1, C3–O3 and C2–C2AM. The fairly short bonds (1.238 and 1.259 Å, respectively) found for the two C–O bonds are in accordance with such a mesomeric structure. But the 1.434 Å determined for C2–C2AM together with the value 1.398 Å for the length of bond C2–C3 were taken as evidence that the π-electronic system of ring A can not be described properly by such a single mesomeric structure.

The data obtained for the zwitterion show significant differences for several of the bonds discussed above. The bonds C2–C3, C2–C2AM and C2AM–N2AM are relatively longer than in the cation, the bonds C3–O3 and especially C2AM–O2AM significantly shorter (Table 3). The described changes taken together suggest a dominant mesomeric (zwitterionic) structure $N4^+H\cdots O1^-$, again in

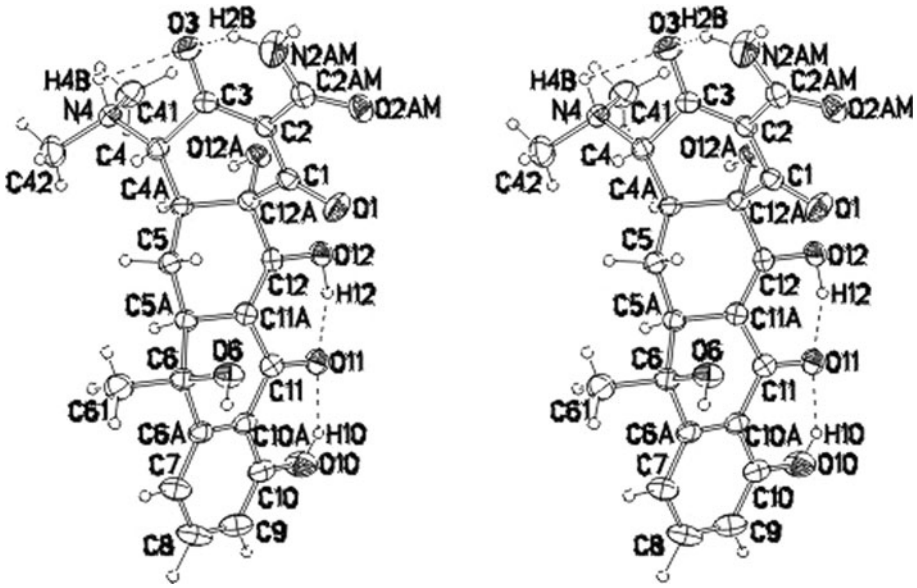


Fig. 2 Stereo drawing of the molecular structure of the Tetracycline zwitterion (crystal I), crystallized from acidic aqueous solution (thermal ellipsoids are drawn at the 50 % probability level, dashed lines indicate intramolecular hydrogen bonds, solvate molecules omitted for clarity)

Table 2 Dihedral angles (°) characterizing the two fragments with tricarbonyl systems (e.s.d.'s in parentheses)

	Tetracycline hexahydrate (zwitterion) (crystal I)	Tetracycline hydrochloride (cation) (crystal III)	Doxycycline hydrochloride (cation) (crystal IV)	Sancycline hydrochloride (cation) (crystal V)
O10–C10–C10A–C11	−1.4(4)	1.8(4)	1.9(6)	0.4(4)
C10–C10A–C11–O11	−12.7(3)	−14.7(3)	−16.1(5)	−11.1(4)
O11–C11–C11A–C12	−1.8(3)	−3.2(3)	−5.2(5)	−3.3(4)
C11–C11A–C12–O12	−0.1(3)	1.6(3)	0.9(5)	5.8(4)
O12–C12–C12A–C1	−78.1(2)	−77.8(2)	−80.3(3)	−81.4(3)
O1–C1–C2–C2AM	5.0(4)	9.8(4)	12.4(6)	0.9(4)
C1–C2–C2AM–N2AM	−169.4(2)	−9.6(4)	−7.7(6)	6.3(4)
C1–C2–C2AM–O2AM	9.4(3)	170.5(2)	173.7(4)	−174.6(3)
C1–C2–C3–O3	−156.6(2)	−168.9(2)	−167.5(3)	174.5(3)
C1–C2–C3–C4	32.9(3)	21.0(3)	20.0(5)	−2.0(4)
C12–C12A–C4A–C4	170.4 (2)	172.1(2)	174.1(3)	173.2(2)
C5–C4A–C12A–C1	−72.5(2)	−73.8(2)	−71.0(3)	−68.5(3)

contrast to the tautomeric form generally assumed in the past ($N4^{+}H\cdots O3^{-}$). A further discussion of this topic will be given below.

Doxycycline

In contrast to tetracycline·HCl, which crystallizes from aqueous solution as zwitterion, doxycycline·HCl crystallizes under essentially the same conditions as dihydrate of the hydrochloride: doxycycline·HCl·$2H_2O$. In our crystal (space group $P2_12_12_1$) the similar intermolecular hydrogen bonding pattern of the four doxycycline molecules per unit cell leads to a similar edge-to-face arrangement within the unit cell and correspondingly to similar cell parameters a, b and c as for the tetracycline cation (Table 1).

Stezowski crystallized doxycycline·HCl from an ethanol–water mixture [6]. In his sample, the unit cell (space group $P2_1$) contained two doxycycline molecules associated with either one water or one ethanol molecule, respectively. Stezowski interpreted his X-ray diffraction data to the effect that in his crystal there were two symmetry-independent molecular cations of doxycycline hydrochloride present which differ in the orientation of the amide group, one having a dihedral angle C1–C2–C2AM–O2AM of 171.3°, the other one of 3.0°. As a consequence the dimensions of the unit cell differ significantly from our values ($a = 18.203$ Å, $b = 16.045$ Å, c = 8.004 Å, β = 94.11°).

The crystal data reported by Bordner [8] for a commercial sample of the α-epimer of doxycycline hydrochloride (Vibramycin) match our cell parameters very well

Table 3 Selected bond distances (Å), bond angles (°) and torsion angles (°) characterizing ring A as determined by X-ray crystallography (e.s.d.'s in parentheses)

	Tetracycline hexahydrate (zwitterion) (Crystal I)	Tetracycline hydrochloride (cation) (Crystal III)	Tetracycline hydrochloride (cation) (from reference 10)	Doxycycline hydrochloride (cation) (Crystal IV)	Sancycline hydrochloride (cation) (Crystal V)
C1–C2	1.430(3)	1.439(3)	1.427(4)	1.449(4)	1.435(4)
C2–C3	1.436(3)	1.398(3)	1.403(5)	1.402(5)	1.417(4)
C1–O1	1.233(3)	1.238(3)	1.234(4)	1.229(4)	1.238(3)
C3–O3	1.241(3)	1.259(3)	1.260(4)	1.271(4)	1.261(3)
C2AM–O2AM	1.255(3)	1.305(3)	1.314(4)	1.329(4)	1.302(3)
C2–C2AM	1.472(3)	1.434(3)	1.435(4)	1.434(4)	1.451(4)
C2AM–N2AM	1.342(3)	1.311(4)	1.301(5)	1.297(5)	1.307(4)
N2AM–C2AM–O2AM	119.2(2)	118.1(2)	118.4(3)	116.0(3)	118.3(3)
N2AM–C2AM–C2	117.4(2)	122.0(3)	122.0(3)	125.0(3)	121.8(3)
O2AM–C2AM–C2–C3	−170.4(2)	−11.7(3)	−12.1(4)	0.7(5)	1.8(4)
O2AM–C2AM–C2–C1	9.4(3)	170.5(2)	170.4(3)	173.7(4)	−174.6(3)

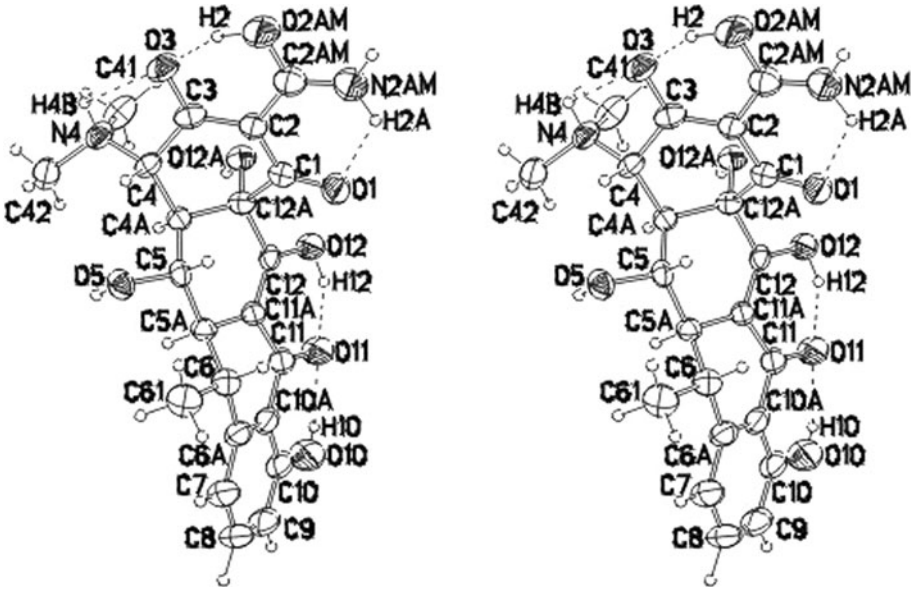


Fig. 3 Stereo drawing of the molecular structure of the doxycycline·cation (crystal IV) crystallized from acidic aqueous solution (thermal ellipsoids are drawn at the 50 % probability level, dashed lines indicate intramolecular hydrogen bonds, chloride ion and solvate molecules omitted for clarity)

($a = 11.638$ Å, $b = 12.177$ Å, $c = 15.840$ Å) although the bond length differ somewhat (Fig. 3).

Sancycline

From an acidified solvent mixture (water/methanol) sancycline hydrochloride crystallizes with either one water or one methanol molecule next to the A-ring of each sancycline molecule protonated at N4. It proved necessary to treat the dimethyl ammonium group (and only it) as a disordered group throughout the refinement process. The respective site-occupancy factors were 65.9 % (water) and 34.1 % (methanol), respectively. The arrangement of the sancycline molecules within the unit cell is essentially independent of the associated solvent molecules and so is the geometry of the rings A, B, C and D (Fig. 4).

A pronounced difference to the three cases discussed above is found in the arrangement of the sancycline molecules within the unit cell with the effect that one dimension of the unit cell is strongly changed versus those determined for tetracycline hydrochloride (Table 1). The reduction of the parameter "*a*" to about 6.8944 Å reflects the side-by-side arrangement of the molecules in staples along this axis (Fig. 5). In the plane orthogonal to the staple axis, the dimensions of the unit cell are nearly the

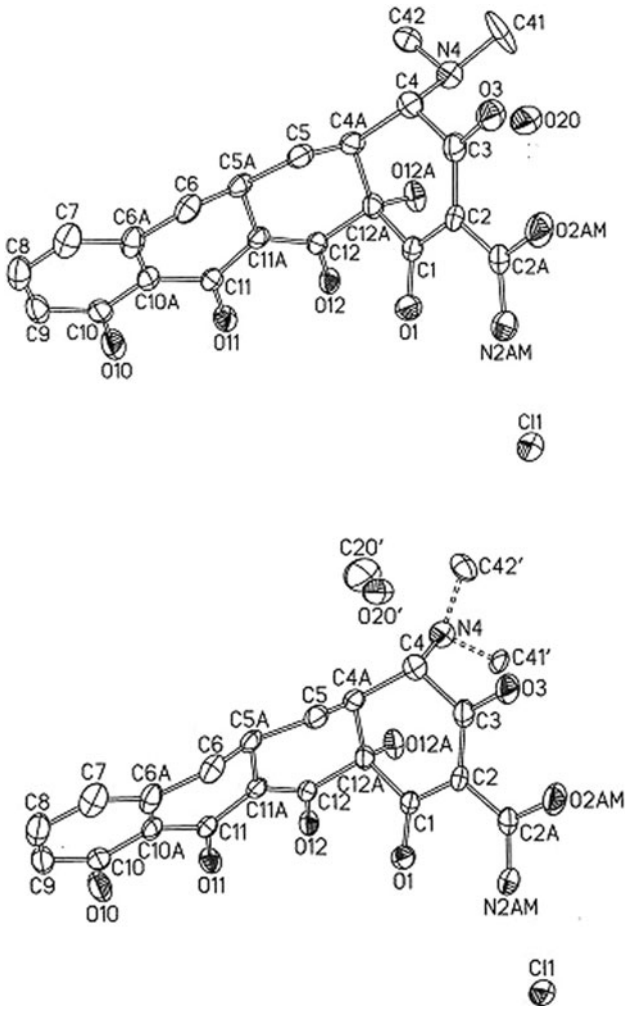


Fig. 4 ORTEP plots of the molecular structure of: top) sancycline·HCl·H_2O and bottom) sancycline HCl·CH_3OH (crystal V) crystallized from an acidified mixed solvent (H_20/CH_3OH). Thermal ellipsoids are drawn at the 50 % probability level

same as for the other derivatives ($b = 16.815$ Å and $c = 18.190$ Å).

Each chloride ion establishes four hydrogen bonds. Two involve the amino group hydrogen atoms HN2AM of two neighboring sancyclines, and the other two are associated with the respective hydrogen atoms HO12A of two sancycline molecules in different staples. In the majority case, $HN4^+$ makes an intermolecular hydrogen bond to the water oxygen (2.11 Å), in the minority case to the methanol oxygen (1.89 Å). In view of the different location of the water and methanol molecule, respectively, relative to ring A the formation of the hydrogen bonds mentioned is possible only because of a rotation of the C4–N4 bond (dihedral angles δ(C3C4N4HN4$^+$) are 59.1° (water) and 124.4° (methanol), respectively).

Due to the different rotation of the C4–N4 bond an intramolecular hydrogen bond between $HN4^+$ and O3 and O12A, respectively, is possible only in the majority case. In both the majority and minority fraction, the amide oxygen is protonated and their tautomeric structure corresponds to the one of tetracycline hydrochloride. The hydrogen bonds made up by the amide group (HOAM–O3 and HNAM–O1) exhibit the same parameters in the majority and minority case. Besides the two strong intramolecular hydrogen bonds located in the tricarbonyl moiety (HO10–O11 and HO12–O11) a hydrogen bond HO12A–O12 helps to stabilise the molecular geometry.

The dihedral angles and bond distances presented in Tables 2 and 3, respectively, confirm that the conformation of sancycline hydrochloride resembles closely the one of tetracycline and doxycycline, respectively. Sancycline lacks the hydroxy group present at C6 in tetracycline and C5 in doxycycline. Because these hydroxy groups are not involved in any intramolecular hydrogen bonding one must not be surprised that the geometries of rings B, C and D of the three hydrochlorides are very similar. The differences in chemical structure are however manifested in the geometry of the tricarbonyl system as seen by the variation of the dihedral angles of this fragment (Table 2) and the concomitant variations of the precise location of the hydrogen atoms O10H and O12H that form the stabilizing intramolecular hydrogen bonding network. In our opinion, these differences can explain the variation in the pK_a-values and binding constants of ions like Mg^{2+} or Ca^{2+} [13–15].

Comparison of Experimental Bond Lengths and Calculated Wiberg-Bond Orders

In the early days the location of hydrogen atoms was less accurate or even impossible. Therefore attempts were made to decide which of the possible tautomers was present in the crystal by comparing the bond lengths between heavier atoms of ring A with the distribution of single and double bonds in the corresponding classical mesomeric structures [4, 6, 8, 10]. As mentioned above the variations in bond length can not be reconciled with any one of the classical mesomeric structures. To disprove the suggested explanation that the observed discrepancies could be due to the presence of several tautomers in the crystal under investigation and to rationalize the variations in bond length we have calculated ZDO Atomic Charges and Wiberg-bond orders [20] for three tautomers of sancycline: San–O2AM–H refers to the structure determined from our crystal with the protonated amide oxygen. San–O3–H (with proton at O3) is the tautomer that was usually assumed in the discussion of titration experiments [21]. Finally, we considered a structure without a proton on either O2AM or O3 (zwitterion).

The calculated ZDO Atomic Charges (Table 4) show for all three structures a strong alternance of the net

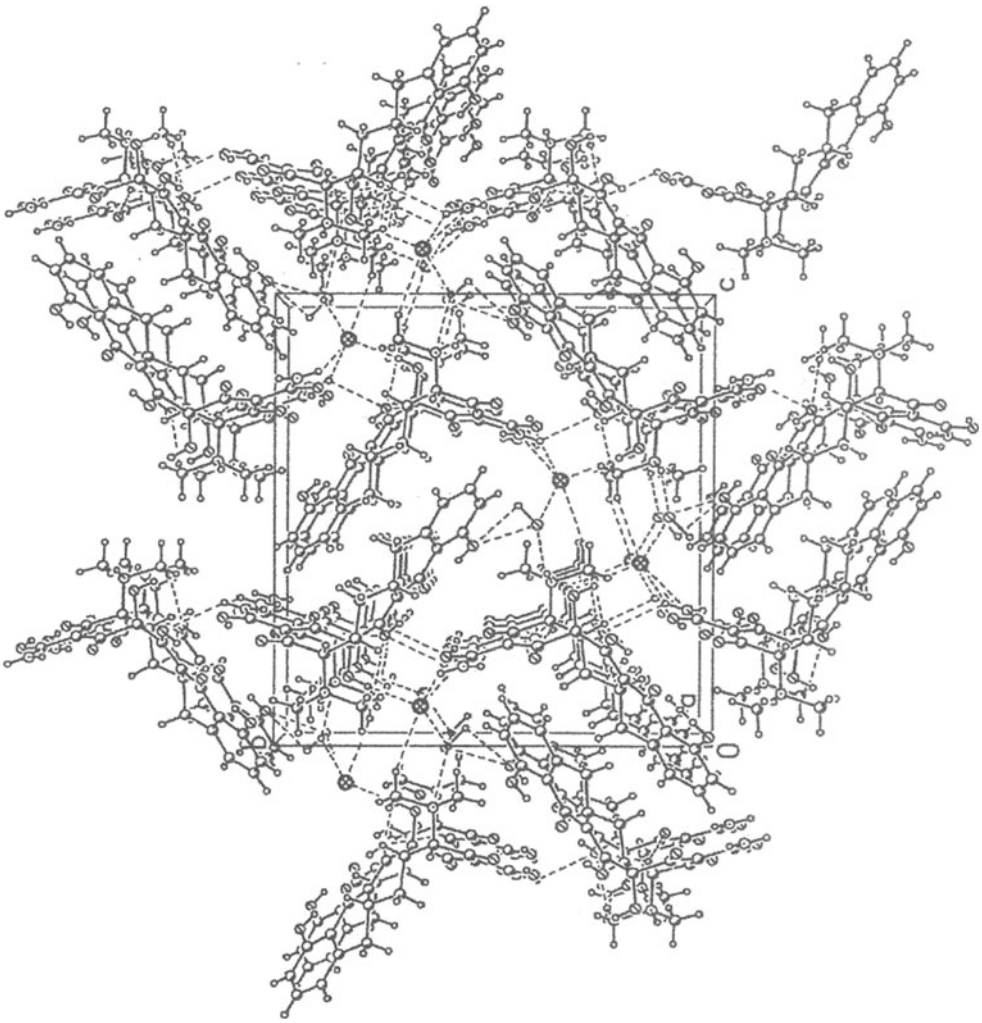


Fig. 5 Packing diagram of sancycline·HCl·H_2O/CH_3OH (crystal V) crystallised from an acidified mixed solvent (H_20/CH_3OH)

charges.The shift of the proton from 2OAM to O3 results in the expected higher negative charge at 2OAM and a lower value at O3. For the other atoms the changes are less pronounced. In the zwitterion the net charges of the three oxygen atoms (O1, 2OAM, and O3) are about the same. This can be taken as a hint that its actual electronic structure must be described by a superposition of three mesomeric structures with the negative charge located on either one of the three oxygen atoms.

The variation of the bond lengths determined for the three compounds under study are of the same magnitude as the variations observed for the same compound by different authors. The conclusions drawn here for sancycline therefore should be valid also for the other derivatives.

Table 5 shows that within each group of bonds the changes of the experimentally determined bond lengths are very well imaged by the corresponding changes of the Wiberg-bond orders tabulated for San–2OAM–H. For the

Table 4 ZDO atomic charges of selected atoms in ring A

	San–O2AM–H	San–O3–H	San–zwitterion
O(1)	−0.29	−0.24	−0.35
O(2AM)	−0.21	−0.46	−0.45
O(3)	−0.38	−0.26	−0.34
C(1)	0.32	0.30	0.31
C(2)	−0.51	−0.39	−0.48
C(2AM)	0.38	0.36	0.36
C(3)	0.28	0.22	0.26
C(4)	−0.06	−0.02	−0.09
C(4A)	−0.13	−0.13	−0.12
C(12A)	0.08	0.07	0.18
N(4)	−0.02	−0.03	−0.01
N(2AM)	−0.23	−0.28	−0.36
O(12A)	−0.30	−0.30	−0.30

Calculated with AM1 Hamiltonian [20]

only C–C bond with unique double bond character, C(11A)–C12), the small bond length (1.384 Å) corresponds to a Wiberg-bond order of 1.61; in contrast, single bonds (∼1.53 Å) exhibit a Wiberg-bond order of about 0.95. The most discussed bonds, C(1)–C(2), C(2)–C(3) and C(2)–C(2A), must therefore be categorized as conjugated bonds both with respect to bond length and Wiberg-bond order. Noteworthy is also the decrease of the bond order parallel to the increase of the bond length found for the four C–O bonds included in Table 5. In summary: For the chosen tautomer, San–O2AM–H, the variations of experimentally determined bond lengths are in perfect agreement with the variations of the calculated bond orders.

In accordance with the differences in the classical mesomeric structures a transfer of the proton from 2OAM to O3 induces a pronounced increase of the Wiberg-bond order of bond C(2)–C(3)and C(2A)–O(2AM), but not to the values characteristic for full double bonds; furthermore, a strong reduction of the bond strength of C(3)–O(3) is predicted, but again not to values characteristic for single bonds. In summary: The tautomer San–O3–H should exhibit a pattern of bond lengths pronouncedly different from that of San–2OAM–H and without typical single and double bonds.

According to the calculations significant changes in bond length versus the cation are predicted for two bonds in the zwitterion: a decrease for C(2A)–O(2AM) and an increase for C(2A)–N(2AM). This prediction is verified by our experimental data for tetracycline: −0.05 Å for C2A)–O(2AM) and +0.03 Å for C(2A)–N(2AM) (for other bonds the trend is also predicted correctly). Taking our data for the doxycycline cation and those presented by Legendre et al. [22] for the zwitterion one gets as bond length differences: −0.08 Å for C(2A)–O(2AM) and +0.11 Å for C(2A)–N(2AM).

Summary and Conclusion

The molecular geometries of the hydrochlorides of tetracycline, doxycycline and sancycline are essentially the same if they are crystallized from a methanolic (tetracycline), from an acidified aqueous solution (doxycycline) and from a water–methanol mixture (sancycline), respectively. In all cases the amide group is oriented such that the protonated amide oxygen can form an intramolecular hydrogen bond with O3. From aqueous solution, tetracycline crystallizes independently of the pH of the mother liquor as a hexahydrate complex with the zwitterion. Because the amide group is rotated, an intramolecular hydrogen bond is formed between a hydrogen atom of the amino group and O3.

The calculated Wiberg-bond orders provide convincing evidence that the pattern of bond lengths can be rationalized by the exclusive presence of the proposed tautomers.

CCDC-243335 (for crystal I), CCDC-243336 (for crystal II), CCDC-243337 (for crystal III), CCDC-243338 (for crystal IV), and CCDC-864575 (for crystal V) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html, by e-mailing data request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax +44(0)1223-336033.

Table 5 Comparison of experimentally determined bond lengths and Wiberg-bond orders of selected bonds in ring A

Bond:	Exp. bond length (Å) San–O2AM–H	Wiberg-bond order San–O2AM–H	Wiberg-bond order San–O3–H	Wiberg-bond order San–zwitterion
C(1)–O(1)	1.237	1.86	1.90	1.78
C(3)–O(3)	1.260	1.71	1.33	1.76
C(2A)–O(2AM)	1.303	1.22	1.49	1.61
C(12A)–O(12A)	1.413	1.00	1.00	0.99
C(2AM)–N(2AM)	1.308	1.41	1.21	1.19
C(4)–N(4)	1.517	0.87	0.89	0.87
C(1)–C(2)	1.435	1.03	1.00	1.12
C(2)–C(3)	1.417	1.17	1.48	1.19
C(2)–C(2AM)	1.451	1.13	0.98	1.00
C(3)–C(4)	1.530	0.90	0.94	0.84
C(4)–C(4A)	1.536	0.96	0.96	0.97
C(4A)–C(12A)	1.533	0.94	0.94	0.94
C(12A)–C(1)	1.556	0.87	0.86	0.83
C(11A)–C(12)	1.384	1.61	1.62	1.57

Calculated with AM1 Hamiltonian [20]

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Appendix

In the literature one finds essentially three different terminologies to describe the conformations of tetracyclines.The conformations "A" and "B" introduced by Prewo and Stezowski [7] correspond to conformations "B" and "E", respectively, in the publications of Mitscher et al. [23], and approximately to the "extended" and "twisted" conformation introduced by Berthon and coworkers [24, 25].

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