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Crystal and molecular structure and absolute configuration of lincomycin hydrochloride monohydrate

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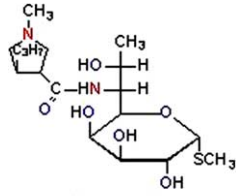
Abstract—Lincomycin is a broad-spectrum antibiotic synthesized by *Streptomyces lincolnensis* that is particularly active against Gram-positive bacteria. It is widely used in human and veterinary applications. The crystal structure of lincomycin has been undertaken with a view to obtain the conformational and structural features of the drug in order to afford a comparison of its structural features with other aminoglycoside antibiotics. We report here the details of its structural and conformational features as determined by single-crystal X-ray crystallography. Crystals of lincomycin hydrochloride are orthorhombic, space group $P2_12_12$, with the cell dimensions $a = 18.5294(3)$ Å, $b = 20.5980(4)$ Å, $c = 6.17380(10)$ Å, $V = 2356.35(7)$ Å^3. The structure was solved using X-ray diffraction data and refined to a final R-value of 0.0391 for 2321 reflections ($I \geqslant 2\sigma$). The absolute configuration was established using the anomalous dispersion of the sulfur and chlorine atoms in the structure. The molecule consists of an amino acid linked by an amide group to a monosaccharide of galactose stereochemistry. A network of hydrogen-bonds stabilizes the crystal structure.

Keywords: Lincomycin; Crystal structure; Stereochemistry; Absolute configuration

1. Introduction

Lincomycin [methyl 6,8-dideoxy-6{[(1-methyl-4-propyl-2-pyrrolidyl)carbonyl]amino}-1-thio-D-*erythro*-α-D-*galacto*-octapyranoside] is a broad-spectrum antibiotic synthesized by *Streptomycin lincolnensis*,[1] which shows in vitro and in vivo activity comparable to that of erythromycin against Staphylococci, streptococci, and diplococci.[2–4] Its mode of action was investigated by Jesten and Allen,[5] who showed that the immediate effect of lincomycin on *S. aureus* is to completely inhibit protein synthesis. Its behavior is comparable to chloramphenicol[6] and puromycin[7] and indicated the activation transfer or polymerization of amino acids as possible sites of action. Its chemical structure was shown by Hoeksema and co-workers[8] as follows:



Lincomycin

In spite of its biological relevance, few reports on the conformational and structural features of the drug and its metal complexes in solution have been published. The structural similarity to aminoglycoside antibiotics and the occurrence of a peptide linkage makes lincomycin a potentially strong ligand for copper and other biologically relevant metals, similar to many other naturally occurring aminoglycosides, such as leucomycin and amikacin, a semisynthetic antibiotic. An assignment of the structure of the carbohydrate moiety of lincomycin

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was established by various physical and chemical properties to be an unbranched 6-aminooctose.[9] A study of the structure and dynamics of the lincomycin–copper complex in water in the solution state[10,11] has provided a molecular model that is helpful in explaining the redox properties of copper–amino sugar complexes toward nucleobases and nucleic acids.

We have undertaken the structural study of lincomycin for the following three reasons: (i) There is a series of antibiotics closely related to lincomycin that possess varying antibacterial activities, depending on the substituents. Consequently, a detailed investigation of the stereochemistry of lincomycin will be a forerunner to the study of the other antibiotics, which might facilitate the understanding of their biological activity. (ii) A detailed study of the structure of lincomycin and its copper complexes in the solid state would provide the necessary database to investigate the specificity of the interaction of the drug with metal atoms. (iii) The structure of lincomycin would provide accurate information of the galactose moiety that could be used in model-building studies of polysaccharides containing this as the monomeric unit. In this paper, we report the structure, stereochemistry, and absolute configuration of lincomycin as deduced from single-crystal X-ray diffraction studies.

2. Experimental

Excellent prismatic crystals of the compound were made available to us through the courtesy of Drs. M. Savage and R. Herr of the Upjohn Company, Kalamazoo, MI. Crystals of lincomycin hydrochloride ($C_{18}H_{34}N_2O_6S$) are orthorhombic, space group $P2_12_12$, with cell dimensions $a=18.5294(3)$ Å, $b=20.5980(4)$ Å, $c=6.17380(10)$ Å, $V=2356.35(7)$ Å^3. A crystal of approximate dimensions $0.2\times0.2\times0.2$ mm^3 was chosen for data collection. Diffraction data were collected at 298 K using a Nonius Kappa-CCD diffractometer with graphite monochromated Mo Kα radiation ($\lambda=0.71073$ Å). A total of 454 frames were collected using phi plus omega scans to fill the asymmetric unit with a scan range of 2° and a counting time of 300 s per degree. The first 10 frames were used for indexing reflections using the DENZO[12] package and refined to obtain final cell parameters. Table 1 gives the crystal data and the parameters used in the refinement of the structure of lincomycin hydrochloride. Data reductions were performed using DENZO-SMN.[12] A total of 39,087 reflections had their intensities integrated and scaled, of which, 2697 were independent, and 2321 were above $2\sigma(I)$. The structure was solved by direct methods and refined by full-matrix least squares on F^2 with anisotropic displacement parameters for the nonhydrogen atoms using Bruker, SHELXTL[13] version 6.10. Hydrogen atoms were included in idealized positions. The structure refined to a goodness-of-fit (GOF)[†] of 1.015 and final residuals of R_1[‡] = 0.0391% ($I>4\sigma(I)$), $wR_2=0.0919\%$ ($I>2\sigma(I)$). A total of 2697 reflections were employed for 270 parameters determination, resulting in data-to-parameter ratio of ~10. The final fractional coordinates, equivalent isotropic displacement parameters [U(eq)] of the atoms in the structure, bond lengths, and angles and torsion angles are deposited with the manuscript.

Table 1. Crystal data and structure refinement for lincomycin

Empirical formula	$C_{18}H_{36}ClN_2O_7S$
Formula weight	460.00
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12$
Unit cell dimensions	$a=18.5294(3)$ Å $\alpha=90°$ $b=20.5980(4)$ Å $\beta=90°$ $c=6.17380(10)$ Å $\gamma=90°$
Volume	2356.35(7) Å^3
Z	4
Density (calculated)	1.297 Mg/m^3
Absorption coefficient	0.290 mm^{-1}
$F(000)$	988
Crystal size	$0.2\times0.2\times0.2$ mm^3
Theta range for data collection	3.44°–21.54°
Index ranges	$-19\leqslant h\leqslant19$, $-21\leqslant k\leqslant21$, $-6\leqslant l\leqslant6$
Reflections collected	39,087
Independent reflections	2697 [R(int) = 0.09]
Completeness to $\theta=21.54°$	98.3%
Absorption correction	Multi-scan
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	2697/0/270
Goodness-of-fit on F^2	1.015
Final R indices [$I>2\sigma(I)$]	$R_1=0.0391$, $wR_2=0.0919$
R indices (all data)	$R_1=0.0507$, $wR_2=0.0974$
Absolute structure parameter	0.10(9)
Largest diff. peak and hole	0.347 and −0.219 e Å^{-3}

3. Results and discussion

The absolute configuration of lincomycin was established by X-ray crystallographic methods as $R(+)$. Both the sulfur and the chlorine atoms scatter anomalously for Mo Kα radiation and, consequently, the intensities of the direct and inverse reflections are no longer equal. Calculated and observed ratio of I_{hkl}/I_{-h-k-l} for few representative reflections are compared in Table 2. The intensities of hkl, $-h-k-l$, $h-kl$, and $-hkl$ were measured. By symmetry, $hkl=-h-kl$, $h-kl=-hkl$. This fixes the configuration of the sugar as that of D-galactopyr-

[†] GOF = $[\sum[w(F_o^2-F_c^2)^2]/(n-p)]^{1/2}$, where n and p denote the number of data and parameters.

[‡] $R_1=(\sum||F_o|-|F_c||)/\sum|F_o|$; $wR_2=[\sum[w(F_o^2-F_c^2)^2]/\sum[w(F_o^2)^2]]^{1/2}$ where $w=1/[\sigma^2(F_o^2)+(a\cdot P)^2+b\cdot P]$ and $P=[(\text{Max};0,F_o^2)+2\cdot F_c^2]/3$.

Table 2. Determination of the Bijvoet ratios for lincomycin hydrochloride monohydrate

h	k	l	I_H/I_{-H} (calculated)	I_H/I_{-H} (observed)
7	7	1	0.58	0.78
3	1	1	1.64	1.59
1	3	2	1.37	1.26
1	1	1	0.68	0.79
4	5	2	1.44	1.43
3	3	2	0.75	0.83
1	1	2	0.82	0.89
1	2	2	1.45	1.27
5	8	1	0.84	0.89
5	1	1	1.17	1.21

anose derivative and the amino acid as L-hygric acid. The R values for $R(+)$ and $R(-)$ are 0.0391 and 0.0404, and the ratio is 1.176. The absolute configuration, as found from X-ray investigation, is in good agreement with that deduced from chemical studies.[8]

An ORTEP diagram of the molecular structure of lincomycin and atomic numbering scheme is shown in Figure 1. Displacement ellipsoids are drawn at 50% probability level, and the water molecule, chloride ion, and hydrogen atoms are omitted for clarity. Figure 2 gives a view of the molecule along with the chloride ion and water molecule. The pyranoside is of the galactopyranose stereochemistry. The molecule has the expected $^4C_1$ chair form with the configuration 1a2e3e4a5e, where the numbers 1, 2, 3, 4, and 5 represent atoms C-2, C-3, C-4, C-5, and C-6. Similar conformation for lincomycin has been observed in the solution state.[14] The average C–C distance is 1.520(5) Å, and the average carbon–hydroxyl distance is 1.427(4) Å. These are similar to the values found in other saccharides, such as methyl-α-D-galactopyranose, [15] β-D-glucopyranose,[16] and those cited in the compilation by Jeffrey and Rosenstein.[17] The linear and angular dimensions of the amide link are closely similar to those usually found in peptides. Figure 3 shows the environment of the chloride ion, with the surrounding atoms O-4, O-5, water oxygen, O-7 and the peptide nitrogen atom, N-1, with coordinating distances ranging from 3.0 Å to 3.5 Å. However, there is no interaction between N-2 and the chloride ion, the distance between them being 5.891 Å.

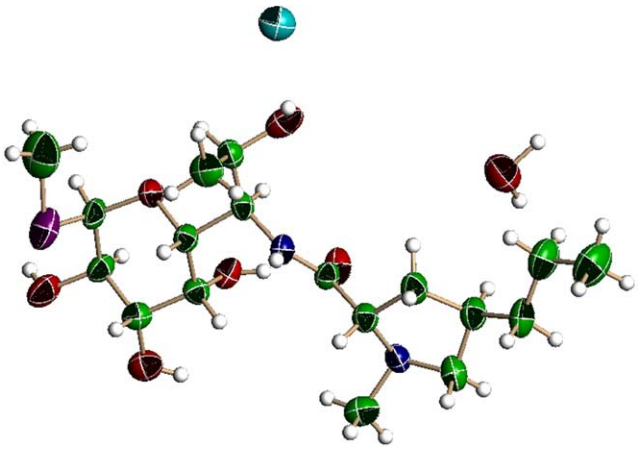

Figure 2. The molecular structure of lincomycin drawn with chloride ion and water molecule.

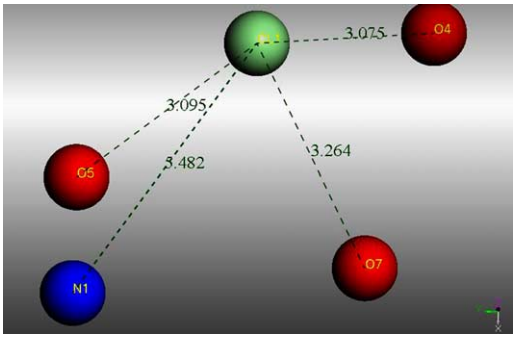

Figure 3. A view of the environment of the chlorine atom viewed perpendicular to the z-axis.

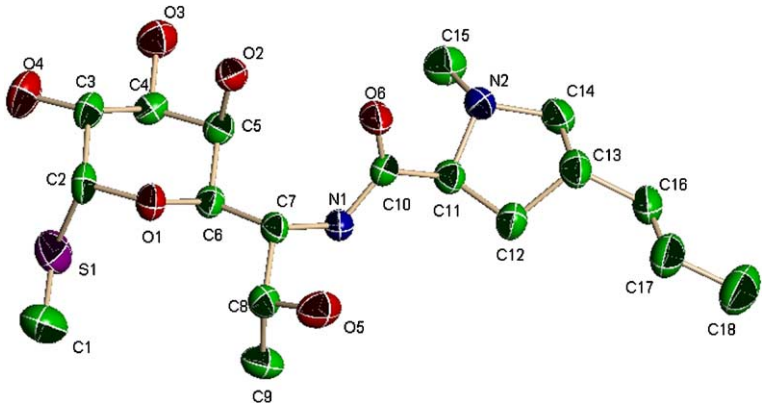

Figure 1. An ORTEP diagram showing the molecular structure of lincomycin and atomic numbering scheme. Displacement ellipsoids are drawn at 50% probability level. The water molecule, chloride ion, and hydrogen atoms are omitted for clarity.

Table 3. Hydrogen-bond distances and angles in lincomycin

Donor D	Hydrogen H	Acceptor A	*d*(D–H) Å	*d*(H···A) (Å)	*d*(D···A) Å	<DHA (deg)	Reference
O-4	H-4	Cl	0.820	2.298	3.075	158.49	$x-1/2, -y+1/2, -z+1$
O-5	H-5	Cl	0.820	2.309	3.095	160.81	$-x+1, -y+1, z-1$
O-2	H-2	O-6	0.820	2.078	2.843	155.16	
O-2	H-2	O-6	0.820	2.287	2.803	121.41	$-x, -y, z$
O-7	H-17	O-4	1.061	1.850	2.879	162.25	$x, y+1, z+1$
O-7	H-27	Cl	1.088	2.196	3.264	166.44	$x-1/2, -y+3/2, -z+1$
N-1	H-1–N	Cl	0.860	2.447	3.281	163.54	$-x+1, -y+1, z-1$

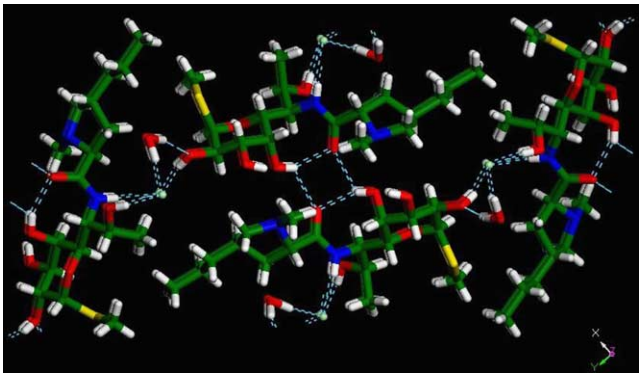

Figure 4. Hydrogen-bonding interactions shown by dashed lines in the crystal structure of lincomycin.

In the five-membered ring containing N-2, the atoms C-11, C-12, C-13, and C-14 are planar, with the nitrogen atom deviating from the mean plane by −0.516 Å, thereby giving it the familiar envelope conformation.

Table 3 lists the hydrogen-bond distances and angles in the structure. Both water hydrogen atoms are involved in hydrogen bonding to the chlorine and the exocyclic oxygen, O-4, of the sugar moiety. Figure 4 shows the network of hydrogen bonding in the structure.

From a review of the magnitude and the directional cosines of the principal axes of the thermal ellipsoids of the atoms, we can divide the molecule into three regions: the constituents on C-13, the atoms C-11, C-10, O-6, and N-1 involved in the amide link and the sugar moiety. The thermal motions of C-13, C-16, C-17, and C-18 are quite similar and have progressively increasing thermal motion. Their maximum direction of vibration seems to be along the *b*-axis. The atoms C-11, C-10, N-1, and O-6 all have very similar thermal motion, with their maximum amplitude of motion along the *c*-axis.

It is interesting to note that both lincomycin and clindamycin, the two important lincosamide antibiotics, have been extensively studied in the solution state in the free, as well as in the complexed state with the bacterial ribosome.[18] The central amide linkage of lincomycin plays an active part in the conformation in the solid state. The studies of the Cu complex of lincomycin provide evidence of the role of the peptide nitrogen N-1 in stabilizing the copper complex by affording a coordination along with the N-2 of the pyrrolidine ring and the deprotonated exocyclic oxygen of the sugar moiety.[10,11] Lincomycin exhibits the characteristics of the sugar-like and the peptide-like moieties. It is very clear that in lincomycin, the existence of the hydrogen bonding, which occurs between the exocyclic oxygen and the carbonyl of the peptide moiety, favors the major conformation in solution in the unbound state. In the bound state, however, the hydroxyl is potentially accessible to the binding site, and this allows the methyl group, the pyrrolidine ring, and the alkyl side chains to occupy a less sterically congested state, allowing the coordination of the copper around the N-1 of the peptide, the nitrogen N-2 of the pyrrolidine ring, and the exocyclic deprotonated oxygen of the pyranose ring. The ability of the copper complexes of lincomycin to promote DNA strand cleavage was tested using pBR322[11] plasmid and gel electrophoresis. This experiment indicated that lincomycin complexes are not able to promote double-strand cleavage of DNA under standard conditions. Both lincomycin and clindamycin inhibit the growth of *Escherichia coli*.[18] It is interesting to point out that lincosamides inhibit the ribosome by binding to the peptidyl site, thus on the large subunit[19] and not at all at the A site like the aminoglycosides. Complexes of lincomycin with the bacterial ribosomes were studied by spectroscopic methods. These studies indicated that the specific conformations are preferred in the bound state. Clindamycin, the more active antibiotic, displayed a stronger NMR response than lincomycin, showing that in lincomycin–ribosome interactions, a low-affinity binding level is associated to the tight-binding level and is related to biological activity.

4. Supplementary material

Crystallographic data for this structure has been deposited with the Cambridge Crystallographic Data Centre as supplementary data No 235119. Copies of the data may be obtained free of charge upon request from The

Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK. (Fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk; WEB: http://www.csdc.cam.ac.uk).

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