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Notes

Models of the Active Site of Dihydroorotase: Structure of Diaquabis(L-dihydroorotato)zinc(II) Reveals an Unexpected Coordination Mode for the L-Dihydroorotato Ligands

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Introduction

Dihydroorotase (DHOase) catalyzes the conversion of *N*-carbamyl-L-aspartate to L-dihydroorotate (Scheme 1), the third reaction in the *de novo* biosynthesis of pyrimidine nucleotides.[1] The active site of DHOase contains a zinc ion coordinated by three histidine groups, and the mechanism of action involves coordination of *N*-carbamyl-L-aspartate to this zinc atom, ring closure, and decoordination of L-dihydroorotate.[1−3] Inhibitors of DHOase are potential chemotherapeutic agents, and the activity of some inhibitors against malaria has been established.[4] As part of a study of the natural substrates and inhibitors of DHOase, we have been investigating their structures.[5,6] Recently, we have commenced an investigation of the structures of their zinc complexes in order to establish the preferred sites of interaction between zinc and the natural and synthetic substrates. L-Dihydroorotate is converted to the pyrimidine orotate (Scheme 1) in the fourth reaction in the biosynthesis of pyrimidines.[1] There have been numerous crystallographic studies of metal complexes of orotate,[7−12] including some of Zn^{II} complexes,[10,11] but none of complexes of dihydroorotate. We describe herein the synthesis of a bis(L-dihydroorotato)-zinc(II) complex and its crystal structure.

Experimental Section

L-Dihydroorotic acid (0.051 g, 3.2 × 10^{-4} mol) was dissolved in 4 mL of water and added dropwise with stirring to a suspension of freshly prepared zinc(II) hydroxide hydrate (1.6 × 10^{-4} mol) made up in 4 mL of water. Crystals suitable for X-ray analysis were grown by slow evaporation of this solution in the dark. Anal. Calcd for $C_{10}H_{14}N_4O_{10}Zn$: C, 28.9; H, 3.4; N, 13.5. Found: C, 30.4; H, 3.6; N, 13.8. Infrared spectra (recorded on a BIO-RAD FTS spectrometer as a KBr pellet), υ_{max} cm^{-1}: 3461sh; 3259s; 3091m; 2950m; 2863m; 1705vs; 1646m; 1578s; 1478m; 1416s; 1356m; 1335s; 1289s; 1262m; 1067w; 992m; 945m; 891m; 818m; 750s; 683w; 596s; 521s; 448m.

Scheme 1

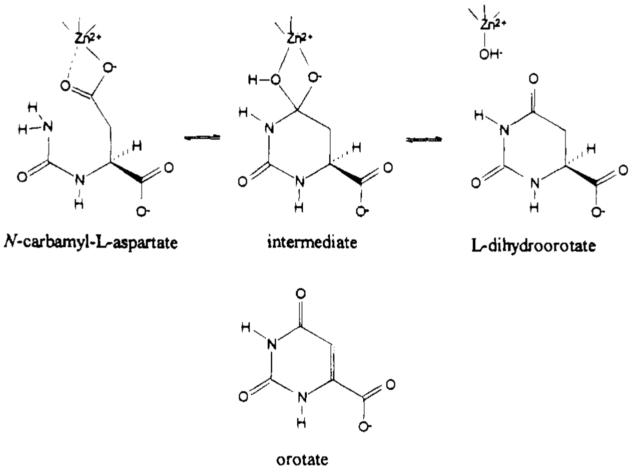

Table 1. Crystallographic Data for $[Zn(L\text{-dihydroorotato})_2(OH_2)_2]$

chem formula	$C_{10}H_{14}N_4O_{10}Zn$	fw	415.63
a, Å	4.815(2)	space group	$P1$ (No. 1)
b, Å	7.793(2)	T, °C	21
c, Å	10.339(2)	λ, Å	0.710 69
α, deg	106.61(2)	D_{calcd}, g cm^{-3}	1.99
β, deg	97.68(3)	abs coeff, cm^{-1}	18.84
γ, deg	106.11(3)	R^a	0.040
V, Å^3	347.9(4)	R_w	0.041
Z	1		

[a] $R = \Sigma(||F_o| - |F_c||)/\Sigma|F_o|$, $R_w = (\Sigma w(|F_o| - |F_c|)^2/\Sigma wF_o^2)^{1/2}$.

Similar reactions between zinc(II) hydroxide and *N*-carbamyl-L-aspartate were carried out but did not yield crystalline products.

The crystallographic data are summarized in Table 1. Cell constants were determined by a least-squares fit to the setting parameters of 25 independent reflections, measured and refined on an Enraf-Nonius CAD4-F diffractometer fitted with a graphite monochromator. Lorentz, polarization, and numerical absorption corrections were applied using the SDP system.[13] The structure was solved by direct methods using SHELXS-86.[14] Refinement, with the zinc atom fixed to define the origin, was performed by full-matrix least-squares methods using SHELX-76.[15] The non-hydrogen atoms were refined anisotropically, and hydrogen atoms were either included at calculated sites (C−H and N−H 0.97 Å) or located and refined with bond distance constraints (O−H 0.87 Å). Graphics were produced using ORTEP.[16] Atomic coordinates are listed in Table 2, and bond lengths and angles are given in Tables 3 and 4.

Results and Discussion

The structure (Figure 1) consists of the Zn^{2+} cation, two L-dihydroorotato ligands, and two aqua ligands. Both of the water molecules are coordinated to the zinc atom, and a fifth O

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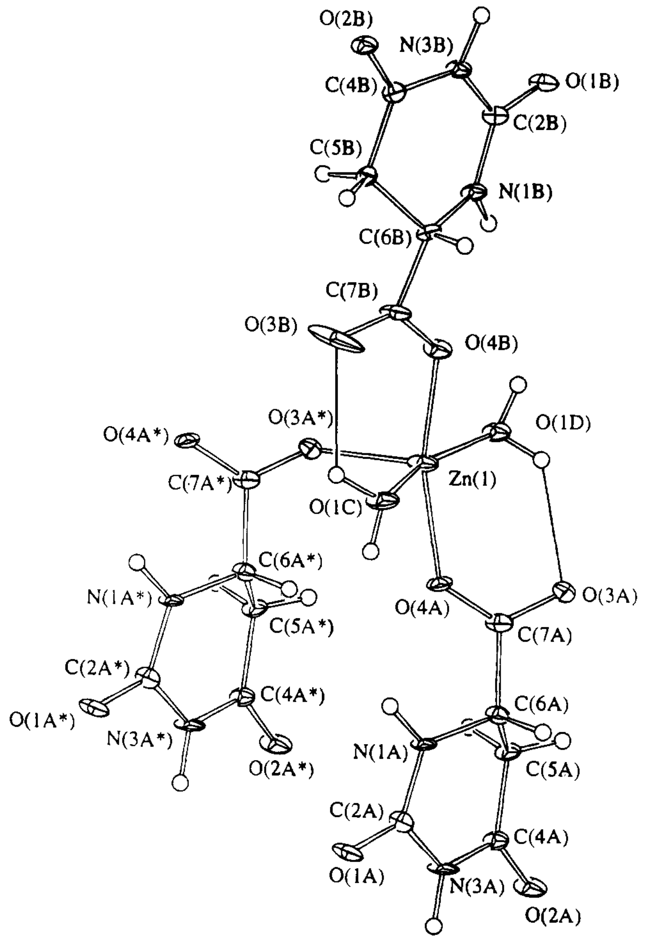

Figure 1. ORTEP[16] plot of $[\mathrm{Zn}(\text{L-dihydroorotato})_2(\mathrm{OH}_2)_2]$ showing atomic labels (30% thermal ellipsoids). The starred atoms are produced by the symmetry operation $x - 1, y, z$.

(carboxylate) atom from a molecule in an adjacent unit cell ($x - 1, y, z$) completes the coordination sphere. There are a number of intermolecular hydrogen bonds between ureido or water donors and carboxylate acceptors. The geometry about zinc is a distorted square-based pyramid with the O atom from the adjacent unit cell occupying the apical position. There are no deviations greater than 0.02 Å from the least-squares plane through the four basal atoms, and the Zn atom lies 0.30 Å from this plane. The bond angles about the Zn atom are also consistent with this description of the coordination geometry. The Zn−O (apical) bond length is longer (2.086(6) Å) than all those in the basal plane (1.986(7)−2.041(7) Å). The geometry of the dihydroorotato ligands can be compared to that of the methyl ester of L-dihydroorotic acid.[5] There are no significant differences between bond lengths and angles, but a number of torsion angles differ significantly as a consequence of the carboxylate group being equatorially disposed in the present structure and axially disposed in the methyl ester. The rings adopt envelope conformations with C6 lying about 0.5 Å out of the planes defined by the other five atoms (all deviations less than 0.08 Å).

Table 2. Fractional Atomic Coordinates ($\times 10^4$) $[\mathrm{Zn}(\text{L-dihydroorotato})_2(\mathrm{OH}_2)_2]$

	x	y	z
Zn(1)	5408	3782	52
N(1A)	9508(15)	4840(11)	−3565(7)
C(2A)	10 021(18)	5319(12)	−4674(8)
O(1A)	8236(14)	4701(10)	−5787(6)
N(3A)	12 793(16)	6602(11)	−4516(7)
C(4A)	14 862(18)	7628(12)	−3289(9)
O(2A)	17 249(14)	8696(10)	−3275(7)
C(5A)	13 996(21)	7323(13)	−2035(10)
C(6A)	11 880(18)	5307(12)	−2371(8)
C(7A)	10 682(19)	5083(13)	−1115(8)
O(3A)	12 588(13)	5368(10)	−48(6)
O(4A)	7978(15)	4655(11)	−1209(8)
N(1B)	2331(14)	1518(10)	3480(7)
C(2B)	1827(18)	1083(11)	4594(8)
O(1B)	3531(13)	1814(9)	5735(6)
N(3B)	−859(15)	−334(10)	4410(7)
C(4B)	−3192(18)	−1053(13)	3277(8)
O(2B)	−5488(13)	−2198(10)	3261(6)
C(5B)	−2628(19)	−334(14)	2124(10)
C(6B)	547(18)	343(12)	2083(8)
C(7B)	1107(21)	1376(15)	1063(8)
O(3B)	−810(21)	978(22)	48(10)
O(4B)	3697(10)	2538(7)	1329(7)
O(1C)	3034(16)	1536(11)	−1686(7)
O(1D)	8607(16)	5514(10)	1764(8)

Table 3. Selected Bond Lengths (Å) for $[\mathrm{Zn}(\text{L-dihydroorotato})_2(\mathrm{OH}_2)_2]$

O(3A)[a]−Zn(1)	2.086(6)	O(4A)−Zn(1)	2.041(7)
O(4B)−Zn(1)	1.986(7)	O(1C)−Zn(1)	2.036(7)
O(1D)−Zn(1)	2.020(7)		

[a] $x - 1, y, z$.

Table 4. Selected Bond Angles (Degrees) for $[\mathrm{Zn}(\text{L-dihydroorotato})_2(\mathrm{OH}_2)_2]$

O(4A)−Zn(1)−O(3A)[a]	98.2(3)	O(4B)−Zn(1)−O(3A)[a]	97.9(3)
O(4B)−Zn(1)−O(4A)	163.9(3)	O(1C)−Zn(1)−O(3A)[a]	96.1(3)
O(1C)−Zn(1)−O(4A)	85.1(3)	O(1C)−Zn(1)−O(4B)	93.9(3)
O(1D)−Zn(1)−O(3A)[a]	101.9(3)	O(1D)−Zn(1)−O(4A)	91.4(3)
O(1D)−Zn(1)−O(4B)	84.5(3)	O(1D)−Zn(1)−O(1C)	162.0(3)

[a] $x - 1, y, z$.

Each of the L-dihydroorotato ligands is coordinated to the zinc atom in a unidentate fashion via an O (carboxylate) atom. This is an unexpected coordination mode because in all structures of complexes with an L-orotato ligand, bidentate coordination via O (carboxylate) and N (ureido) atoms is observed.[7−12] The O (carboxylate) atoms not immediately coordinated to the Zn atom are each involved in one medium to short intramolecular hydrogen bond with one of the water molecules [O(1C)···O(3B) 2.779 Å and H(1C2)···O(3B) 2.25 Å; O(1D)···O(3A) 2.856 Å and H(1D2)···O(3A) 2.22 Å]. This is an interesting motif that may promote deprotonation of the water molecules leading to local control of pH, an important aspect of the catalytic processes of some zinc-containing enzymes. Hydrogen bonding of this type would not be possible if the L-dihydroorotato ligands were coordinated in a O,N-bidentate fashion. There are three other factors that might promote unidentate coordination of L-dihydroorotate rather than the bidentate-O,N coordination of orotate: the pK_a of the ureido nitrogen, N1; the reaction conditions; the geometrical preferences of dihydroorotate. The pK_a's associated with the deprotonation of N2 of orotate and dihydroorotate are in the range 11−12,[17] but those of N1 are unknown, probably because of the instability of the anions at high pH. The difference between dihydroorotate and orotate is that the latter has a double bond between C2 and C3. This is likely to have a substantial effect on the pK_a of N1 because of the additional possibilities the double bond provides for delocalization through the ring of the negative charge arising from deprotonation. It is not known whether the difference is sufficient to account for deprotonation and coordination of N1 in the case of orotate and not dihydroorotate. However, it is possible. The reaction conditions we employed were neutral to slightly basic; more basic conditions might have promoted deprotonation of N1 and bidentate

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coordination. However, [Zn(orotato)$(H_2O)_4$]H_2O was prepared under more acidic conditions than we employed yet yielded orotato bound in a bidentate fashion. Thus, the reaction conditions are unlikely to have been the determining factor. Orotate is planar, and the carboxylate group is rigidly constrained to lie in the same plane. L-Dihydroorotate is puckered, and the carboxylate group can be equatorial to the ring, as observed in the present structure, or lie axially as we observed in the structure of its methyl ester. ^{1}H NMR spectroscopy indicates that in aqueous solution the two coexist and that the equatorial conformation predominates.[3] The axial conformation is unsuitable for forming a N,O-bidentate interaction, but interconversion between axial and equatorial conformations would be rapid and the potential to adopt the axial conformation would not be expected to preclude coordination. However, it is clear that orotate, being rigid, is "preorganized" for bidentate coordination, but dihydroorotate, being more flexible, can adopt two conformations, only one of which is suitable for bidentate coordination. Thus, differences in the structures and conformational flexibilities of orotate and dihydroorotate may be a contributing factor in their adopting different coordination modes, but differences in the pK_a's of the ureido groups are more likely to be the major factor.

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Supporting Information Available: Tables giving full crystallographic details, thermal parameters, bond lengths and angles, H-atom positional and thermal parameters, H-bond contacts, and details of least-squares calculations (7 pages). Ordering information is given on any current masthead page.

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