Complexation of Molybdenum by Siderophores: Synthesis and Structure of the Double-Helical *cis*-Dioxomolybdenum(VI) Complex of a Bis(catecholamide) Siderophore Analogue

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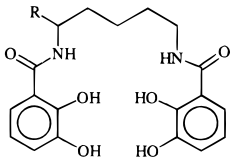
Introduction

Siderophores are high-affinity iron-binding compounds produced by microorganisms to solubilize otherwise hydrolytically insoluble iron and to facilitate its uptake.[1] Iron, as well as molybdenum, is a component of the conventional nitrogenase, and the N_2-fixing cells of *Azotobacter vinelandii*, highly efficient in molybdenum uptake and accumulation,[2] secrete *N*,*N*′-bis(2,3-dihydroxybenzoyl)-L-lysine (LYSCAM),[3] an iron siderophore, which also binds molybdenum.[4,5] Most siderophores have three bidentate binding sites to form six-coordinate octahedral complexes with iron. In contrast bis(bidentate) ligands like LYSCAM appear to be optimal for complexation of the MoO_2^{2+} unit, which has four vacant coordination sites. It has been suggested that, in nitrogen-fixing bacteria, siderophores are involved not only in the uptake of iron but also in the transport of molybdenum.[4a,6,7]

So far, only one molybdenum complex of a siderophore analogue has been structurally characterized by Raymond and co-workers.[8] In this case, the catechol units of a rigid bicyclic ligand are substituted symmetrically and are highly preoriented for metal binding, thereby favoring the formation of a monomer. In contrast, the coordination chemistry of the linear catecholamide ligand LYSCAM and its analogue 5-LICAM is more varied, mono-, di-, and polymeric complexes being possible. Furthermore, not only Δ and Λ optical isomers but also geometric isomers are conceivable because of the asymmetrically substituted catechol units.



R = COOH; LYSCAM
R = H; 5-LICAM

Weitl and Raymond synthesized the linear bis(catecholamide) series, 2-, 4-, 6-LICAM, with two, four, and six methylene group spacers, respectively,[9] but 5-LICAM, the analogue of the naturally occurring siderophore LYSCAM, had not been prepared when we started our investigation. Only recently have the nickel complex and an independent synthesis of 5-LICAM been reported. This synthesis used the acid chloride as an active intermediate for amide bond formation;[10] in contrast, we applied the azolide method.[11]

Experimental Section

General Methods. Starting materials were commercially available (Aldrich, Fluka) and used as supplied. Tetrahydrofuran and dichloromethane were distilled from sodium/benzophenone and phosphorus pentoxide, respectively. NMR data were recorded on a Bruker AM-300, -400, or -500 spectrometer, and acetone was used as a standard to calibrate the variable-temperature spectra. Melting points were obtained on a Laboratory Devices Mel-Temp capillary melting point apparatus and are uncorrected. IR spectra were recorded using a BioRad FTS-7 spectrometer.

Synthesis of *N*,*N*′-Bis(2,3-dimethoxybenzoyl)-1,5-diaminopentane, 1. A 1.622 g sample (10.00 mmol) of solid *N*,*N*′-carbonyldiimidazole was added to a solution of 1.822 g (10.00 mmol) of 2,3-dimethoxybenzoic acid in 10 mL of anhydrous THF. When the CO_2 evolution stopped, a solution of 0.876 g (5.00 mmol) of 1,5-diaminopentane dihydrochloride in 5 mL of H_2O was added and the reaction mixture stirred at room temperature overnight. After evaporation of the solvent, the residue was dissolved in $CHCl_3$ and washed with 2 *N* HCl, saturated $NaHCO_3$ solution, and three portions of water. After drying of the solution over Na_2SO_4, removal of the solvent afforded **1** as an oil (1.851 g, 4.30 mmol, 86%), which was sufficiently pure to be used without further purification: ^{1}H-NMR (300 MHz, $CDCl_3$) $\delta = 1.38-1.48$ (m, 2H, $CH_2CH_2CH_2$), 1.52−1.65 (m, 4H, $HNCH_2CH_2$), 3.35−3.42 (m, 4H, $HNCH_2CH_2$), 3.78 (s, 3H, CH_3), 3.79 (s, 3H, CH_3), 6.93 (dd, 2H, arom H), 7.03 (t, 2H, arom H), 7.56 (dd, 2H, arom H), 7.92 (broad, 2H, NH).

Synthesis of *N*,*N*′-Bis(2,3-dihydroxybenzoyl)-1,5-diaminopentane (5-LICAM), 2. A 1.851 g sample (4.30 mmol) of **1** was dissolved in 25 mL of degassed dry CH_2Cl_2, and the solution was added dropwise to 43 mL of a 1 M solution of BBr_3 in CH_2Cl_2 at room temperature and under argon. The resulting white suspension was stirred overnight. Then 40 mL of H_2O was added slowly (*caution*: HBr evolution!) and the mixture was stirred for 2 h to complete hydrolysis. The precipitate was collected by filtration, washed with H_2O, and dissolved in methanol. The solvent was evaporated three times to remove boron compounds, and the residue was finally recrystallized from methanol/H_2O at pH-meter reading of 7.0 to give thin needles of **2** (1.240 g, 3.31 mmol, 77%): mp 178−179 °C; ^{1}H-NMR (400 MHz, [D$_6$]DMSO) $\delta = 1.35-1.41$ (m, 2H, $CH_2CH_2CH_2$), 1.57−1.64 (m, 4H, $HNCH_2CH_2$), 3.29−3.34 (m, 4H, $HNCH_2CH_2$), 6.68 (t, 2H, arom H), 6.92 (dd, 2H, arom H), 7.30 (dd, 2H, arom H), 8.77 (t, 2H, NH).

Synthesis of $[\{MoO_2(5\text{-LICAM})\}_2]^{4-}$, 3. A 0.187 g sample (0.50 mmol) of solid **2** was added to a solution of 0.121 g (0.50 mmol) of $Na_2MoO_4 \cdot 2H_2O$ in 20 mL of H_2O at ambient temperature, and the mixture was stirred until the formation of the water-soluble complex **3** was complete (approximately 3 h). The resulting clear orange solution was treated with aqueous [PPh_4]Br and the resultant orange precipitate collected and washed well with H_2O. Recrystallization from methanol/H_2O afforded 0.482 g (0.21 mmol, 82%) of the $[PPh_4]^+$ salt **3a.** Crystals of **3** suitable for single-crystal X-ray structure analysis were obtained as the sodium salt **3b** from vapor diffusion of diethyl ether into a

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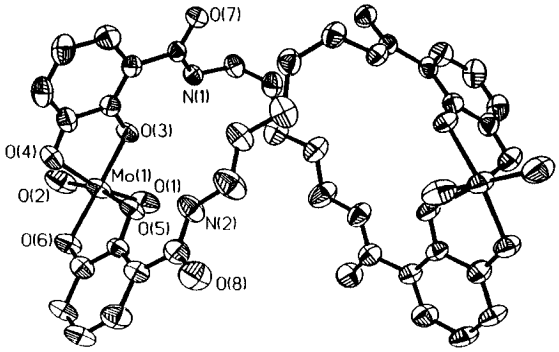

Figure 1. Structure of the anion of **3b**, Λ,Λ-[{MoO_2(5-LICAM)}$_2$]$^{4-}$, showing the atom-labeling scheme. H atoms have been omitted for clarity. Selected interatomic distances (Å) and angles (deg): Mo(1)−O(1) 1.719(4), Mo(1)−O(2) 1.717(4), Mo(1)−O(3) 1.996(3), Mo(1)−O(4) 2.114(4), Mo(1)−O(5) 2.137(4), Mo(1)−O(6) 2.005(3), O(1)−Mo(1)−O(2) 101.7(2), O(3)−Mo(1)−O(4) 75.76(12), O(5)−Mo(1)−O(6) 75.33(12).

solution of Na_2MoO_4 and **2** in a mixture of water, methanol, and tetrahydrofuran (1:4:4) after storing of the sealed flask for 1 month: IR (**3a,b**; KBr) ν = 862, 900 cm^{-1} (*cis*-MoO_2); ^{1}H-NMR (**3b**; 300 MHz, D_2O) δ 0.76 (m, 2H, $CH_2CH_2CH_2$), 0.94 (m, 2H, $HNCH_2CH_2$), 1.04 (m, 2H, $HNCH_2CH_2$), 2.77 (m, 2H, $HNCH_2CH_2$), 3.19 (m, 2H, $HNCH_2CH_2$), 6.55 (t, 2H, arom H), 6.65 (dd, 2H, arom H), 7.06 (dd, 2H, arom H).

Satisfactory elemental analyses were obtained for **1, 2**, **3a**, and **3b**.

X-ray Structure Determination. Crystal data for **3b**·19H_2O: $C_{38}H_{36}N_4O_{35}Mo_2Na_4$, M = 1392.6, red pyramid (0.19 × 0.15 × 0.11 mm), tetragonal, space group $P4_32_12$ (No. 96), a = 15.289(2) Å, c = 27.212(3) Å, V = 6361(1) Å^3, Z = 4, ρ_{calc} = 1.454 g cm^{-3}, F(000) = 2800, μ = 5.1 cm^{-1}, T = 23 °C. The data were collected using synchrotron radiation (λ = 0.68 Å) at EMBL beam line X31 at the DORIS storage ring, DESY, Hamburg, Germany. A MAR imaging plate two-dimensional detector was used, and the single-axis-rotation method for data collection was applied.[12] The crystal was roughly orientated about the a axis, and two passes of 90° total rotation were used (the first with 1.5° rotation and average exposure 6 min per image; the second with 2.5° and 1 min). The images were processed and the intensities were integrated with DENZO.[13] A total of 25 764 reflections were measured (3.06° < 2θ < 48.78°): 3043 unique, R_{int} = 0.064. No absorption correction was applied. The structure was solved by direct methods by means of the SHELXS86 program.[14] The refinement on F^2 was carried out by full-matrix least-squares procedures (SHELXL93)[15] with 393 free parameters and 5035 reflections to final R1 = 0.046, wR2 = 0.1266 [for reflections with $I > 2\sigma(I)$] and R1 = 0.049, wR2 = 0.1395 (for all reflections). The enantiomorph was determined from the Flack parameter x = 0.0429 (±0.0446) to be $P4_32_12$. Hydrogen atoms of the organic ligand were included in the refinement in calculated positions. Non-hydrogen atoms except those of partially occupied water oxygens were refined anisotropically.

Results and Discussion

Crystal Structure of Na$_4$[{MoO$_2$(5-LICAM)}$_2$] (3b). The structural analysis revealed that the crystals are composed of dimer tetraanions that are linked by a network of partly disordered sodium counterions with coordinated as well as free water molecules. The structure of the anion of **3b** is shown in Figure 1. The tetraanion lies on a crystallographic 2-fold axis of rotation, relating the two metal centers to form the dimer based on two bridging ligands. As the space group of the selected crystal is $P4_32_12$, **3b** spontaneously resolves into chiral crystals, each of which contains a single enantiomer, in this case the Λ,Λ form. The geometry around the octahedral MoO_6 centers is distorted in a way typical for MoO_2^{2+} core structures.[8,16] Due to their strong structural trans influence, the two double-bonded oxygen atoms are oriented *cis* to each other, and the Mo−O single bonds *trans* to the oxo ligands experiencing this influence are significantly longer [2.114(4) and 2.137(4) Å] than the *cis*-oriented ones [1.996(3) and 2.005(3) Å]. Interestingly, the two catecholamide subunits coordinating each MoO_2^{2+} ion are orientated differently. In one of the binding subunits, the weaker donor atom [O(5)], the catecholato oxygen *ortho* to the amide substituent, is bound *trans* to the oxo ligand [O(2)], which is to be expected with regard to the strong trans influence exerted by the oxo group. In the other bidentate subunit, the stronger donor atom [O(4)], positioned *meta* to the amide moiety, is orientated *trans* to the oxo ligand [O(1)]. Within each catecholamide subunit, the plane of the amide group is held nearly coplanar with its associated aromatic ring by intramolecular hydrogen bonding between the amide proton and the corresponding catechol oxygens: d(N- -O) = 2.68 and 2.69 Å. The amide group is rotated out of the plane of its associated aromatic ring by *ca.* 8° for the N(1) amide group and *ca.* 5° for the moiety containing N(2).

The most interesting structural feature of [{MoO_2(5-LICAM)}$_2$]$^{4-}$ is certainly the unusual double-helical nature of the binuclear complex. The self-assembly of double and triple helices is controlled by the stereochemical preference of the coordinated metal ions.[17] In general, double-helical structures are obtained by reacting oligobidentate ligands with cations favoring tetrahedral coordination,[18] whereas metal ions showing preference for octahedral coordination are observed to form triple-helical complexes.[19] In contrast, **3b** is a double helicate, although the metal centers are situated in an octahedral coordination environment. A similar arrangement has been observed for bis(bidentate) ligands that are not sufficiently flexible to twist into a triple helicate; thus additional ligands have to complete the coordination sphere of the octahedral metal centers.[20] In **3b**, the third helix strand is missing because two coordination sites of each molybdenum atom are blocked by the oxo ligands. In this case, the "defect" helical structure is predetermined by the metal centers. The finding may be important with regard to biological metal ion recognition, as it demonstrates that the MoO_2^{2+} cations form a helical dimeric complex having a structure slightly different from that expected for the corresponding Fe_2L_3 complex. Thus a triply-bridged binuclear structure is proposed for the iron complex of the bis(bidentate) siderophore rhodotorulic acid, which is supported by the X-ray crystal structure of the iron complex of an analogue ligand. This complex is a triple helicate with a left-handed screw conformation.[21] Furthermore, the self-assembly of triple

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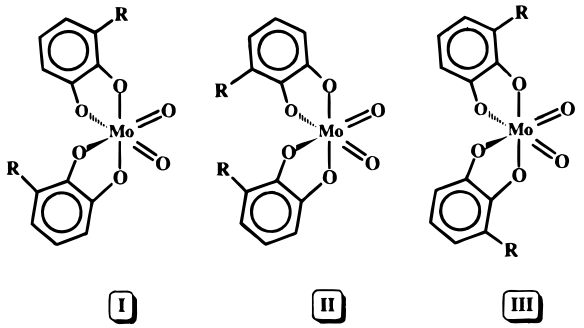

Figure 2. Possible geometric isomers of *cis*-dioxomolybdenum(VI) complexes with two asymmetrically substituted catecholato ligands, shown in Λ configuration.

helical Ga_2 complexes was recently observed for bis(catecholamide) ligands with various three-atom spacers.[19b]

Solution Behavior of 3. A molecular weight distribution analysis of **3a** has been carried out by gel filtration (LH-20, methanol)[22] in order to establish which species dominate in solution. The elution of a single fraction corresponding to a dimer species indicates that the dimeric structure is maintained, at least in methanolic solution, and is not an artifact of the crystallization process.

The proton NMR spectrum of **3b** (Experimental Section) in D_2O at 298 K shows only one set of sharp signals for the aromatic system. Consequently, the protons on each of the aromatic rings are magnetically equivalent at this temperature. This could be due to a dynamic process or the presence of only one isomer in which the two catecholamide subunits are bound symmetrically, such as in **II** and **III** (Figure 2). However, simple molecular model studies show that an isomer analogue **III** is less favorable for $[\{MoO_2(5\text{-LICAM})\}_2]^{4-}$ than for an isomer like **II**. Therefore, it can be proposed that the geometric isomer to be considered is the one in which both *ortho* O atoms are *trans* to the oxo ligands. This assignment is consistent with the proton NMR data reported for the anion *cis*-$[MoO_2(2,3\text{-DHB})_2]^{2-}$ (2,3-H_2DHB = 2,3-dihydroxybenzoic acid).[23]

Variable-temperature experiments over the range 283−343 K in D_2O show that the multiplets assigned to the diastereotopic protons $HNCH_2CH_2$ and $HNCH_2CH_2$ progressively broaden with increasing temperature and coalesce at 323 and 303 K, respectively ($\Delta G^{\ddagger} = 15.3$ kcal mol^{-1}). At 343 K, two broad signals are observed at $\delta = 2.96$ and 1.01. The resonances of the aromatic protons remain sharply resolved over this temperature range. The symmetrization of the diastereotopic protons is due to inversion of configuration at the chiral metal centers. Recently, a similar racemization process was observed for a dimeric complex formed by the self-assembly of two Ti(IV) ions and three bis(catecholato) ligands.[24]

In methanol-d_4 the proton NMR signals of **3b** are broad at room temperature and a minor amount of a second isomer is present. At 313 K, the resonances of the protons of the major isomer sharpen slightly whereas, upon cooling, the signals due to the major isomer decrease while the signals belonging to the minor isomer increase. This is shown in Figure 3 for the protons of the aromatic rings. The two sets of signals observed in a 1:1 ratio at 233 K (Table 1) can be assigned to an isomer with the aromatic rings bound to the metal as in isomer **I**, i.e. the one found in the crystal structure of **3b**. In this geometrical isomer, the protons of one binding subunit are chemically and magnetically distinct from those of the other, as recently established for the corresponding geometric isomer of *cis*-$[MoO_2(2,3\text{-DHB})_2]^{2-}$.[23]

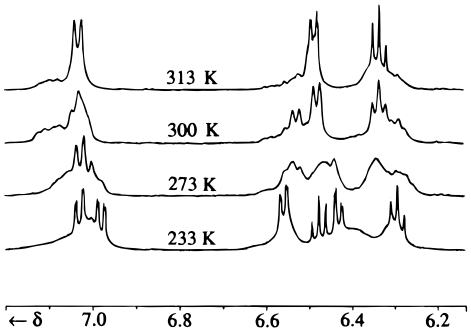

Figure 3. Variable-temperature ^{1}H-NMR spectra of $Na_4[\{MoO_2(5\text{-LICAM})\}_2]$ in methanol-d_4 (aromatic region, 500 MHz).

Table 1. ^{1}H-NMR Data (δ) for $Na_4[\{MoO_2(5\text{-LICAM})\}_2]$ in Methanol-d_4

T (K)	$CH_2CH_2CH_2$	$HNCH_2CH_2$	$HNCH_2CH_2$	aryl CH
313	1.05 (br, 2H)	1.21 (br, 4H)	2.81, 3.30 (2br, 4H)	6.34, 6.49, 7.04 (t, 2d, 6H)
233	0.96 (br, 1H)	1.38 (br, 2H)	2.80, 3.46 (2m, 2H)	6.31, 6.43, 6.98 (t, 2dd, 3H)
	1.09 (br, 1H)	1.49 (br, 2H)	2.98, 3.88 (2m, 2H)	6.49, 6.57, 7.02 (t, 2dd, 3H)

We suggest that the changes occurring on cooling originate from the conversion of an isomer analogue **II** into an isomer like **I**. Warming to room temperature restores the original specrum, demonstrating the reversibility of this geometrical isomerization. Evidently, for **3b**, isomer **I** is enthalpically favored, but as the temperature increases, the entropy factor becomes more important and induces the conversion.

Further experiments are necessary and are in progress in order to gain more insight into the thermodynamic and kinetic features of the system. In addition, the coordination behavior of the naturally occurring optically active siderophore LYSCAM will be investigated, especially with regard to a possible intramolecular asymmetric induction of a given helicity. Competition studies between iron hydroxide and molybdate on LYSCAM at neutral pH have yielded interesting results. Although LYSCAM is able to solubilize Fe(III) at this pH, the complex formation is significantly delayed if a stoichiometric amount of molybdate is present.[25]

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Supporting Information Available: Tables of microanalytical data, atomic coordinates, bond distances, bond angles, anisotropic thermal parameters, and H atom coordinates and isotropic thermal parameters for **3b** (10 pages). Ordering information is given on any current masthead page.

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(22) Unfortunately, aqueous solutions of **3b** containing sodium counterions were not suitable for molecular weight determinations, as elution was retarded due to interactions among these cations, the gel, and the complex anions. The complexes ferrichrome A, $[Fe(MECAM)]^{3-}$ (H_6-MECAM = 1,3,5-tris[(2,3-dihydroxybenzoyl)amino]methylbenzene), $[MoO_2(cat)_2]^{2-}$ ($catH_2$ = catechol), and $[Fe(MALT)_3]$ (H-MALT = maltol) were used as markers to calibrate the column.

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