Orotate Complexes, II[1)]

Preparation and Crystal Structures of Calcium and Zinc Orotate(2−) Hydrates

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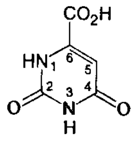
From aqueous solutions containing orotate (dianion of **1**) and calcium or zinc ions in the molar ratio of 2:1, metal orotate (2−) hydrates are readily obtained in crystalline form at pH 9.0 and 5.4, respectively. According to an X-ray crystal structure analysis, calcium orotate tetrahydrate forms a one-dimensional coordination polymer with each orotate ligand coordinated to no less than three neighboring calcium atoms. In this cation triple, the orotate(2−) anion acts as a carboxylate-O/amide-*N*-chelating group for the central calcium ion, but also as a carboxylate-*O*/*O*-chelating group for the first neighboring calcium ion, and as a terminal amide-O donor for the second. Each calcium ion is thus octacoordinated and linked to three orotate(2−) anions and three water molecules. The fourth water molecule is only engaged in hydrogen bonding. The new zinc orotate(2−) phase features $Zn(Or)(H_2O)_4$ units. For the hexacoordinate zinc atom bearing four water molecules the orotate(2−) ligand also acts as an *O*/*N*-chelating group. Unlike a discrete $Zn(Or)(H_2O)_4 \cdot H_2O$ phase reported earlier, the lattice of the new phase contains further disordered water of crystallization, with the overall composition approaching $Zn(Or) \cdot 5.5\ H_2O$. The apparent stability of the M(Or) units probably reflects the well-documented carrier function of the orotate ligand for certain divalent metal ions in biological systems.

Orotic acid [6-uracilcarboxylic acid, OrH_2 (**1**), vitamin B_{13}] and its salts and other derivatives play an important role in the metabolism of pyrimidine nucleotides[2,3)] and are found in cells and body fluids of many living organisms. Metal orotates are also widely applied in medicine. Sodium and potassium orotate(1−) show only limited solubility in water, a feature originally of some importance in analytical chemistry[4,5)]. These and other metal orotates have been used e. g. as uricosurica (for enhanced excretion of uric acid) and for electrolyte substitution (in heart and liver protection)[6)]. Platinum, palladium, and nickel orotates with a wide variety of substituents have been screened as therapeutic agents for cancer[7−9)]. More recent interest has focused on the proposed biological carrier function of orotic acid and the corresponding anionic species for metal ions, which is held responsible for the obviously successful application of metal orotates in curing syndroms associated with a deficiency of a variety of metals such as calcium, magnesium, zinc, or iron.

1, OrH_2



In the light of the importance of orotic acid and its derivatives it is surprising that very little is known about the specific ligand properties of the polydentate orotate species[10)]. In aqueous solution, OrH_2 acts as a dibasic acid, with the carboxylic group (pK = 2.09) and the 1-imino position (pK = 9.28) acting as the primary acid functions[10,11)]. The pK value of the carboxylic acid function indicates that in the physiological pH range (pH 5−9) the obvious site for metal ion coordination is the carboxylate group, whereas polydentate coordination of the metal ion by the Or^{2-} ligand should only become relevant in strong alkaline solutions. The results of potentiometric and preparative studies suggest, however, that with powerful coordination (acceptor) centers, orotate(2−) complexes may play an important role even in the physiological pH range[8−12)]. Magnesium orotate(2−) pentahydrate has been isolated from basic aqueous solutions (pH > 7) and its structure determined by X-ray crystallography. In this study the complex was identified as $[Mg(Or)(H_2O)_4] \cdot H_2O$[13)]. At lower pH, magnesium bis[orotate(2−)] octahydrate has been obtained and shown to feature a hexaaquo complex of magnesium, with the $Mg(H_2O)_6{}^{2+}$ cation associated with hydrated $OrH^- \cdot H_2O$ anions only through hydrogen bonds[1)].

Calcium orotates have gained enormous importance in the therapy of calcium deficiency, but only the crystal structure of calcium 5-ethylidenehydroorotate(2−) sesquihydrate has been determined[14)]. To the best of our knowledge no structure of a calcium compound of the unsubstituted orotate ligand has been reported.

As part of a program oriented towards elucidation of the metal binding properties of orotic acid, we now describe the preparation, crystallization, and X-ray structure determination of a calcium orotate(2−) and a zinc orotate(2−). Much to our surprise, both compounds with a metal-to-ligand ratio of 1:1 have been found to crystallize from aqueous solutions containing the metal ions and the orotate ligand in molar ratios as high as 1:2 (ML_2; M = Ca, Zn; L = orotate) and in an intermediate pH range: The calcium salt has been isolated at pH 9, and the zinc salt is formed at pH 5.4. Crystallization of the zinc compound has been accomplished by layering the aqueous solution with acetone, whereas in the case of the calcium compound crystallization occurred upon slow cooling of the hot solution to room

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temperature. No metal(II) bis[orotates(1 −)] $M(OrH)_2$ could be detected, although the acid/base equilibria and the stoichiometry should have favored their formation. This result indicates the important role of the metal in determining orotate complexation.

In the X-ray structure determinations the crystalline calcium orotate has been identified as calcium orotate(2 −) tetrahydrate, $Ca(Or) \cdot 4\ H_2O$, and the zinc salt to be also a metal(2 +) orotate(2 −) species. In the latter case the overall stoichiometry indicated by a partial occupation model (Exp. Section) approaches the formula $Zn(Or) \cdot 5.5\ H_2O$. The new crystalline zinc orotate(2 −) phase features $Zn(Or)(H_2O)_4$ units, which are also the main building units in a different crystalline form of zinc orotate(2 −), $Zn(Or)(H_2O)_4 \cdot H_2O$, whose structure has been reported in the literature[15]. The crystal parameters of $Zn(Or)(H_2O)_4 \cdot H_2O$ and of the phase isolated by us are, however, completely different [$Zn(Or)(H_2O)_4 \cdot H_2O$: space group $P\bar{1}$ (No. 2), a = 7.243(4), b = 8.390(3), c = 10.105(4) Å, α = 80.10(3), β = 109.23(3), γ = 113.69(3)°, Z = 2]. This reference compound $Zn(Or)(H_2O)_4 \cdot H_2O$ was prepared by the reaction of $ZnCO_3$ with orotic acid in equimolar quantities and crystallized by slow evaporation of the solution[15]. It is an interesting result of our investigation, that apparently at least two different crystalline phases of zinc orotate(2 −) exist, both featuring the same $Zn(Or)(H_2O)_4$ units.

Description of the Crystal Structures

As perhaps expected, the crystal structures of zinc orotate(2 −) and calcium orotate(2 −) as determined by X-ray diffraction at ambient temperature are completely different. While the zinc compound features isolated $Zn(Or)(H_2O)_4$ units, with the hexacoordinate zinc atom at the center of a slightly distorted octahedral environment (Figure 1), the calcium compound adopts a chain structure (Figure 2) with the calcium atom coordinated by eight oxygen or nitrogen donor atoms (Figure 3), some of which are in metal-bridging positions.

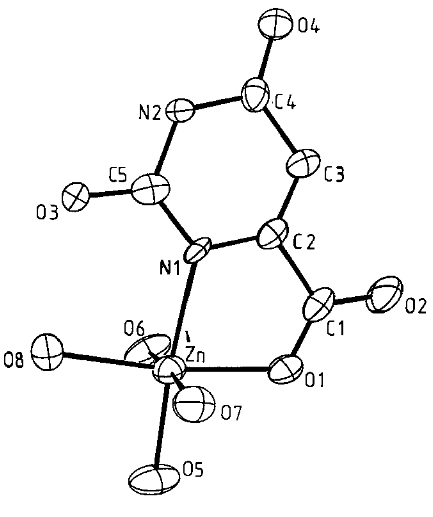

Figure 1. $Zn(Or)(H_2O)_4$ unit in the crystal structure of zinc orotate(2 −)$\cdot$5.5 H_2O with atomic numbering (ORTEP, displacement parameters at the 50% probability level). Selected interatomic distances [Å] and angles [°]: C1−O1 1.256(8), C1−O2 1.248(9), C5−O3 1.264(8), C4−O4 1.265(8), Zn−O1 2.079(5), Zn−O5 2.065(5), Zn−O6 2.104(6), Zn−O7 2.168(5), Zn−O8 2.146(5), Zn−N1 2.088(5); O1−Zn−N1 78.7(2)

Table 1. Prominent hydrogen bonds in the structure of calcium orotate(2 −) tetrahydrate

X - H ··· Y		X - H	H ··· Y	X ··· Y	X - H ··· Y
N1-H1 ···	O7(a)	0.859	2.043	2.900	177.1
O5-H3 ···	O7(b)	0.860	1.880	2.726	167.9
O5-H4 ···	O6(c)	0.776	2.159	2.923	168.6
O6-H5 ···	O8(d)	0.878	1.927	2.790	167.2
O6-H6 ···	O4(e)	0.841	1.862	2.699	173.8
O7-H7 ···	O3(f)	0.836	1.979	2.793	164.5
O7-H8 ···	O1(g)	0.849	1.862	2.704	171.8
O8-H9 ···	O3(c)	0.786	2.235	2.973	156.9
O8-H10 ···	O4(a)	0.902	1.826	2.713	167.7

Symmetry positions of atom Y: (a) $-x + 1, -y + 1, -z + 1$; (b) x, y, z; (c) $0.5 - x, 0.5 + y, 0.5 - z$; (d) $x - 1, y, z$; (e) $x - 0.5, -y + 1.5, z - 0.5$; (f) $x + 1, y + 1, z$; (g) $-x + 1, -y + 2, -z + 1$.

For the hexacoordinate zinc atom, the orotate(2 −) ligand acts as a chelating ligand coordinated by the O1 carboxyl oxygen and the N1 1-imino functions (Figure 1, Table 1). The octahedral coordination sphere of the zinc atom is completed by four water molecules. The geometrical parameters of the $Zn(Or)(H_2O)_4$ unit in the $Zn(Or)(H_2O)_4 \cdot 1.5\ H_2O$ phase show no significant differences as compared with the parameters determined for the same unit in $Zn(Or)(H_2O)_4 \cdot H_2O$[15]. The pyrimidine ring is planar, and the geometry of the orotate ligand is generally consistent with that found in other orotate(2 −) complexes. The carboxylate plane forms an angle of only 1.9° with the plane of the heterocycle. The exocyclic oxygen atoms of the pyrimidine ring are not involved in metal ion binding.

In the chain structure of the calcium compound (Figures 2 and 3, Table 2), each orotate(2 −) ligand is coordinated to no less than three neighboring calcium ions. For this cation triple, it acts as an O2/N chelate for the central Ca atom, but also as an O1/O2 carboxylate chelate for the first neighboring and as a terminal O3 donor for the second Ca atom. This puts the O2 carboxyl oxygen atom into a bridging position between two calcium centers (Figure 2). Each calcium atom is thus octacoordinated and linked to three

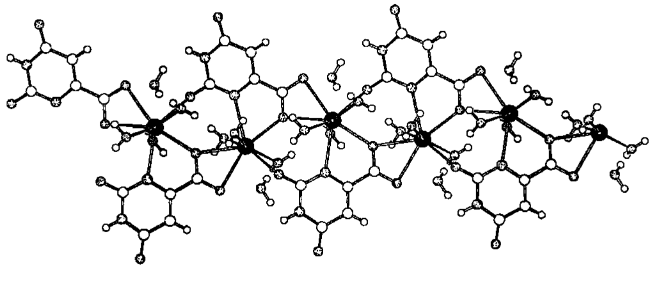

Figure 2. Chain structure of calcium orotate(2 −) tetrahydrate. Ca: black; O: heavy shading; N: light shading; C: large white; H: small white

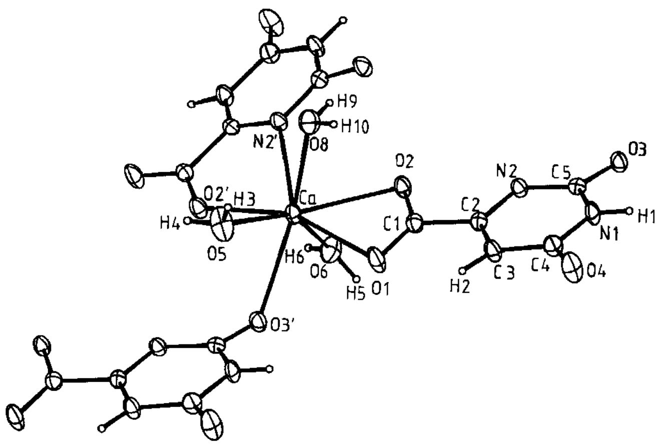

Figure 3. Inner coordination sphere of the calcium ion in the crystal structure of calcium orotate(2−) tetrahydrate with atomic numbering (ORTEP, displacement parameters at the 50% probability level; H atoms with arbitrary radii). Important interatomic distances [Å] and angles [°]: C1−O1 1.249(2), C1−O2 1.255(2), C5−O3 1.270(2), C4−O4 1.249(2), Ca−O1 2.525(1), Ca−O2 2.482(1), Ca−O2′ 2.343(1), Ca−O3′ 2.499(1), Ca−O5 2.366(1), Ca−O6 2.479(1), Ca−O8 2.539(1), Ca−N2′ 2.605(1); N2′−Ca−O2′ 64.9(1)

orotate(2−) anions through the carboxylate oxygen atom and the 1-imino donor nitrogen of the first one of these, two carboxylate oxygen atoms of the second, and one carbonyl oxygen atom of a third, and finally to three water molecules (Figures 2, 3). The fourth water molecule of the formula unit has become integrated in an extensive hydrogen bonding network, which interconnects the coordination polymer chains formed by the calcium atoms and the orotate ligands (Figure 2, Table 3).

The geometrical parameters of the orotate(2−) ligand in Ca(Or)·4 H_2O show no significant deviations from the parameters determined for other metal orotate(2−) salts. The carboxylate plane forms an angle of 12.8° with the pyrimidine plane.

Discussion

The present structure investigation provides further evidence for the relevance of orotate(2−), Or^{2-}, multicoordination even at physiological pH values (5−9), for which only orotate(1−), OrH^-, species are predicted to predominate based on the acid/base equilibria. Both the zinc and the calcium orotates(2−) were readily formed at pH 5.4 and 9, respectively, and with a molar excess of OrH^- present in solution in both cases, which is particularly noteworthy. The formation of orotate(2−) salts appears to be favored over the formation of orotate(1−) compounds, owing to the stabilizing chelate effect operative with orotate(2−) donors. The existence of two phases that both contain $Zn(Or)(H_2O)_4$ units is indicative of a remarkable stability of this unit. It is tempting to assume that the apparent stability of the $M^{2+}(Or)^{2-}$ unit may be related to the well-documented carrier function of orotate for divalent metal ions in biological systems, with the exception of magnesium, however, for which only the hexaaquo complexes are found in the crystalline phase obtained from aqueous solutions near physiological pH values[1].

Regarding the metal ion coordination, our results give additional evidence for the contention that the calcium ion is able to adopt a wide variety of coordination numbers and geometries, whereas other metal ions, and particularly magnesium, are much more restricted to a well-defined environment, e. g. octahedral coordination[16,17]. Zinc can adopt both tetrahedral or octahedral coordination, but occasionally even pentacoordination is not excluded[17]. In agreement with this general rule, $Mg(Or)(H_2O)_4 \cdot H_2O$ contains magnesium atoms octahedrally surrounded by six donor atoms[1]. The great geometrical flexibility of calcium(II) in contrast to the more severe geometrical constraints for other metal ions may play an essential role in the discrimination of metal ions by biological matrices.

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Experimental

All reactions were carried out in pure, desalinated water. Reagents were of p. a. quality. A Knick apparatus (AgCl/KCl) was used for pH readings.

Calcium Orotate(2−) Tetrahydrate: To a suspension of 0.50 g (3.20 mmol) of orotic acid (**1**) in 15 ml of water, ammonia (20% aqueous solution) was added until a pH of 9 was attained. The resulting clear solution was heated to 80°C and 0.24 g (1.60 mmol) of $CaCl_2 \cdot 2H_2O$ added to the hot solution. A white precipitate formed immediately, which was removed by filtration. The clear filtrate was left to cool to room temp. Within 1 d crystals (0.10 g, 0.38 mmol, 23% with respect to $CaCl_2 \cdot 2H_2O$) formed. One of the crystals was selected for X-ray structure investigation.

Zinc Orotate(2−)·5.5 H_2O: A suspension of 0.50 g (3.20 mmol) of orotic acid in 30 ml of water was treated with 0.13 g (1.60 mmol) of ZnO at reflux temp. The resulting suspension (pH 5.4) was filtered, left to cool to room temp., and carefully layered with acetone. Within 1 d a few crystals formed at the acetone/water interphase, and one of these crystals was selected for X-ray structure investigation.

Crystal Structure Determinations: Enraf-Nonius CAD4 diffractometer; Mo-K_α radiation, λ = 0.71069 Å; graphite monochromator; T = 23°C. During data collection three standard reflections were periodically measured as a general check of crystal and instrument stability. No significant change was observed both with the calcium and the zinc compound. Reduced cell calculations did not indicate any higher symmetry (DELOS, LEPAGE). Lp correction and empirical absorption correction were applied [Ca(Or)·$4H_2O$: T_{min} = 0.95, T_{max} = 1.0; Zn(Or)·5.5 H_2O: T_{min} = 0.96, T_{max} = 1.0]. The structures were solved by direct methods (SHELXS-86) and completed by difference Fourier syntheses.

Ca(Or)·4 H_2O: $CaC_5H_{10}N_2O_8$, M = 266.22, monoclinic, a = 6.965(1), b = 8.681(1), c = 16.537(1) Å, β = 93.06(1)°, V = 998.45 $Å^3$, space group $P2_1/n$ (No. 14), Z = 4, D_{calc} = 1.771 g cm^{-3}, $F(000)$ = 552 e, μ(Mo-K_α) = 6.4 cm^{-1}, ω scan. 2480 intensity data were measured up to $(\sin\Theta/\lambda)_{max}$ = 0.637 $Å^{-1}$ (*hkl* range: ±8, 11, 21). After merging of equivalent data (R_{int} = 0.012), 1937 of the remaining 2160 independent structure factors were considered "ob-

Table 2. Fractional atomic coordinates and equivalent thermal displacement parameters of Ca(Or)·4 H_2O

ATOM	X/A	Y/B	Z/C	U(eq.)
O1	0.2413(2)	0.8293(1)	0.41248(6)	0.055
O2	0.2186(2)	0.7046(1)	0.29712(6)	0.040
O3	0.0909(2)	0.1765(1)	0.36230(6)	0.044
O4	0.3019(2)	0.3667(1)	0.60666(6)	0.052
O5	0.5075(2)	1.1486(2)	0.35216(9)	0.105
O6	−0.0530(2)	0.9477(1)	0.22574(8)	0.071
O7	0.8052(2)	1.0269(1)	0.44485(7)	0.055
O8	0.5953(2)	0.8438(1)	0.27779(8)	0.063
N1	0.1986(2)	0.2776(2)	0.48314(7)	0.042
N2	0.1675(2)	0.4322(1)	0.36670(7)	0.036
C1	0.2254(2)	0.7068(2)	0.37311(9)	0.044
C2	0.2170(2)	0.5528(2)	0.41619(8)	0.039
C3	0.2601(2)	0.5429(2)	0.49714(9)	0.043
C4	0.2573(2)	0.3954(2)	0.53405(9)	0.044
C5	0.1513(2)	0.2941(2)	0.40152(9)	0.040
CA	0.27788(5)	0.98538(3)	0.28534(2)	0.037

Table 3. Fractional atomic coordinates and equivalent thermal displacement parameters of Zn(Or)·5.5 H_2O

ATOM	X/A	Y/B	Z/C	U(eq.)
O1	0.7172(2)	0.3140(3)	0.3348(8)	0.032
O2	0.7691(3)	0.4215(3)	0.4296(8)	0.031
O3	0.5281(2)	0.4045(3)	0.0413(7)	0.022
O4	0.6214(2)	0.6379(3)	0.1894(8)	0.025
O5	0.6661(3)	0.1570(3)	0.1905(8)	0.060
O6	0.6905(3)	0.2862(3)	−0.0559(8)	0.062
O7	0.5878(2)	0.2524(3)	0.4335(7)	0.033
O8	0.5528(2)	0.2516(3)	0.0401(8)	0.026
N1	0.6225(2)	0.3965(3)	0.1936(9)	0.023
N2	0.5746(3)	0.5193(3)	0.1292(7)	0.021
C1	0.7237(4)	0.3875(5)	0.353(1)	0.029
C2	0.6706(4)	0.4395(4)	0.2714(9)	0.025
C3	0.6739(4)	0.5183(4)	0.279(1)	0.024
C4	0.6236(3)	0.5632(4)	0.199(1)	0.024
C5	0.5744(4)	0.4375(4)	0.121(1)	0.028
ZN	0.63935(4)	0.27470(5)	0.1860(1)	0.026
O9	0.33028	0.06459	0.01400	0.080
O10	0.08853	0.34833	0.23671	0.080
O11	0.35504	0.06028	0.08373	0.080
O12	0.30350	0.09030	−0.01220	0.080
O13	0.54710	0.15940	−0.25450	0.080

served" [$F_o \geq 1\sigma(F_o)$] and used for refinement. All 10 hydrogen atoms in the asymmetric unit could be located in difference Fourier syntheses. The non-hydrogen atoms were refined with anisotropic, the H atoms with isotropic displacement parameters (number of refined parameters: 185). The function minimized was $\Sigma w(|F_o| - |F_c|)^2$, $w = 1/\sigma^2(F_o)$. Final R and R_w values were 0.027 and 0.024, respectively. Residual electron density. +0.32/−0.26 e/Å^3. Final atomic coordinates of the non-hydrogen atoms are given in Table 2[18].

Zn(Or)·5.5 H_2O: $ZnC_5H_2N_2O_4 \cdot 5.5\ H_2O$, M = 318.54, orthorhombic, a = 20.902(2), b = 16.893(2), c = 7.450(1) Å, V = 2630.58 Å^3, space group *Pbcn* (No. 60), Z = 8, D_{calc} = 1.608 g cm^{-3}, $F(000)$ = 1311 e, $\mu(\text{Mo-}K_\alpha)$ = 19.5 cm^{-1}, Θ-2Θ scan. 2664 intensity data were measured up to $(\sin\Theta/\lambda)_{max}$ = 0.593 Å^{-1} (*hkl* range: 24, 20, 8). After merging of equivalent data (R_{int} = 0.006) 1402 of the remaining 2305 independent structure factors were considered "observed" [$F_o \geq 4\sigma(F_o)$] and used for refinement. 4 hydrogen atoms could be located in difference Fourier syntheses and were included in the refinement with fixed isotropic displacement parameters U_{iso} = 0.05 Å^2. The other hydrogen atoms were neglected. The non-hydrogen H atoms were refined with anisotropic displacement parameters with the exception of the oxygen atoms of the disordered water molecules. These were included in the refinement with fixed isotropic displacement parameters U_{iso} = 0.08 Å^2 and fixed geometrical positions. Only the site occupation factors were refined. The sum of the refined occupation factors equals approximately 1.5. Attempts have also been made to assign the electron density to strongly disordered acetone molecules, but despite numerous efforts this has not been successful within the limits of reasonable geometrical parameters for acetone. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$, $w = 1/\sigma^2(F_o)$. Final R and R_w values were 0.055 and 0.047, respectively (number of refined parameters: 150). Residual electron density: +0.71/−0.55 e/Å^3. Final atomic coordinates of the non-hydrogen atoms are given in Table 3. Supplementary data have been deposited[18].

CAS Registry Numbers

Ca(Or) · 4 H_2O (as coordination compound): 135658-33-2 / Ca(Or) · 4 H_2O (as salt): 135658-35-4 / Zn(Or) · 5.5 H_2O: 135658-34-3

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[18] Further details of the crystal structure determination are available on request from the Fachinformationszentrum Karlsruhe, Gesellschaft für wissenschaftlich-technische Information mbH, D-7514 Eggenstein-Leopoldshafen 2, on quoting the depository number CSD-55621, the names of the authors, and the journal citation.

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