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The Structure of Cobalt Methylmalonate Complexes, $CoClN_4O_7C_{10}H_{28}$ and $CoCl_2N_4O_{5.5}C_{11}H_{28}$, Models for Metal Complexes of γ-Carboxyglutamic Acid

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Abstract

Methylmalonato-2,2′,2″-triaminotriethylamine-cobalt(III) chloride was synthesized by two methods, the first involving the addition of 2,2′,2″-triaminotriethylamine (tren) to a basic solution of cobalt(II) methylmalonate prepared from methylmalonic acid and cobalt(II) chloride and the second involving the addition of disodium methylmalonate to a solution of $[Co(tren)Cl_2]Cl \cdot H_2O$. Crystals of the complex are monoclinic, space group $P2_1/c$, $a = 8.438(2)$, $b = 22.770(7)$, $c = 9.913(3)$ Å, $\beta = 114.6(1)°$, $Z = 4$. The asymmetric unit consists of one complex, a chloride counter ion and three water molecules. A protonated complex was generated in an acidic solution of the first compound and was also characterized crystallographically. The crystals are again monoclinic, space group $P2_1/n$, $a = 9.112(3)$, $b = 16.587(4)$, $c = 12.610(4)$ Å, $\beta = 99.32(2)°$, $Z = 4$. In this case, the asymmetric unit contains the complex, two chloride ions, a water molecule and half of an ethanol molecule. A strong hydrogen bond involving one of the carboxylate oxygens not bound to the metal allows the methylmalonate ion to serve as a uninegative, bidentate ligand. This mode of binding suggests a model for how proteins with two adjacent γ-carboxyglutamic acid residues might bind one Ca^{2+} without causing severe charge balance problems.

Introduction

The dicarboxylic amino acid, γ-carboxyglutamic acid (Gla), has been found in a number of proteins where its calcium binding properties [1] are important for the biochemical function of the proteins. Examples of such Gla-containing proteins include several blood clotting proteins [2–5], bone proteins [6] and a neurotoxin [7]. Crystallographic analyses of Gla [8, 9] and calcium complexes of dicarboxylic acids such as malonate [10] and methylmalonate [11] have been carried out to study the interactions of calcium with the amino acid. In the course of studies aimed at developing reagents useful in analyzing for Gla, we have solved the structures of two methylmalonate complexes of cobalt. The complexes also contain the tetradentate ligand, 2,2′,2″-triaminotriethylamine (hereafter tren), chosen because it limits the remaining coordination sites to mutually *cis* positions and because it is not usually involved in substitution reactions of Co(III) complexes. We report here the synthesis and structural characterization of both a complex containing a partially protonated methylmalonate, $[Co(tren)(Hmmal)]^{2+}$, and a complex containing the dianionic methylmalonate, $[Co(tren)(mmal)]^{+}$. Consideration of the structures of these complexes may provide some insight into the details of how Gla binds calcium in biological systems.

Experimental

All solvents and chemicals used were obtained commercially, were of reagent grade, and were employed without further purification. 2,2′,2″-Triaminotriethylamine (tren) was obtained from Ames Laboratories, Milford, CT.

Preparation of Methylmalonato-2,2′,2″-triaminotriethylaminecobalt(III) Chloride, [Co(tren)(mmal)]Cl

Method A

Sodium hydroxide (4.00 g, 0.100 mol) and methylmalonic acid (5.90 g, 0.050 mol) were added

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to a solution of cobalt(II) chloride hexahydrate (11.95 g, 0.050 mol) in water (100 ml). The solution was filtered after 15 min stirring, and water (250 ml) was added. To the resulting brick-red solution, tren (7.32 g, 0.050 mol) in hydrochloric acid (50 ml, 1.00 N) was added dropwise (with constant stirring and aerating). After 4 h, stirring and aerating were discontinued and the red–brown solution was allowed to stand for two days. The solution was evaporated to dryness and the resulting red–brown glass was dissolved in water (60 ml). Ethanol (180 ml) was added and the walls of the flask were scratched to initiate crystallization (Yield: 5.5 g pink powder, 28%). Red needles were obtained from the crude product by dissolving 5.5 g in water (25 ml), adding ethanol (75 ml) and scratching the walls of the vessel with a glass stirring rod. The product was collected, washed with ethanol, and dried at room temperature at about 1 mm pressure over P_4O_{10} for 12 h. *Anal.* Calc. for [Co(tren)(mmal)]Cl$\cdot$1.5H_2O: C, 31.30; H, 6.57; N, 14.60; Cl, 9.24. Found: C, 31.30; H, 6.64; N, 14.60; Cl, 9.21%. [Note: Crystals used in the single crystal X-ray diffraction study (see below) were not dried under vacuum.]

Method B

[Co(tren)Cl_2]Cl$\cdot H_2O$ (2.00 g, 6.10 mmol) was dissolved in water (20 ml). Methylmalonic acid (0.75 g, 6.10 mmol) dissolved in water (10 ml) was added along with an aqueous solution of sodium hydroxide (12.8 ml, 1.00 N). After 2 h stirring, ethanol (80 ml) was slowly added. Scratching with a glass stirring rod initiated crystallization. Red needles formed over a two day period. (Yield: 1.87 g, 80%).

^{13}C and ^1H NMR spectral data for [Co(tren)(mmal)]Cl prepared by either Method A or B are consistent with the expected structure (for spectral assignments, see ref. 12).

Preparation of Protonated Methylmalonato-2,2′,2″-triaminotriethylaminecobalt(III) Chloride, [Co(tren)(Hmmal)]Cl_2

Red crystals of [Co(tren)(mmal)]Cl$\cdot$3H_2O (1.00 g) were dissolved in concentrated HCl (5.00 ml) forming an orange solution. Ethanol (30 ml) was slowly added. Upon refrigeration at 5 °C, orange rectangular plates formed which were filtered from the mother liquor and washed with ethanol. The crystals were dried at room temperature at about 1 mm pressure over P_4O_{10} for 12 h. *Anal.* Calc. for [Co(tren)(Hmmal)]$Cl_2\cdot$1.5H_2O: C, 28.59; H, 6.24; N, 13.33; Cl, 16.88. Found: C, 28.42; H, 6.22; N, 13.23; Cl, 16.95%. [Note: Crystals used in the single crystal X-ray diffraction study (see below) were not dried under vacuum.]

X-ray Crystallographic Data for [Co(tren)(mmal)]Cl$\cdot$3H_2O

Crystallographic information and data collection details are summarized in Table 1. The position of the cobalt atom was assigned from the Patterson map and used in the initial phasing model. Least-squares refinement of the partial models, followed by calculation of difference maps, yielded the remaining atoms including the chloride ion and the three water molecules. Many of the hydrogen atoms were also located but were not refined.

At an R ($= \Sigma||F_o| - |F_c||/\Sigma|F_o|$) of 0.051, the difference electron density map indicated that the malonate portion of the complex was disordered. Several different models were refined. The model which behaved best in refinement had relative occupancies of 0.75 and 0.25 fixed for the two models involving atoms C(8A), C(8B), C(9A) and C(9B). Isotropic thermal parameters were used for these atoms. One hydrogen atom was found bonded to C(8A) and three were found bound to C(9A). With the hydrogen atoms fixed, the non-hydrogen atoms refined to a final R of 0.038 for 3960 reflections. Least-squares refinement was on F, using $1/\sigma(F)$ weights derived using the expressions $\sigma^2(I) = $ (gross counts + ($k \times$ net counts)^2) and $\sigma(F) = (F^2 + \sigma(F^2))^{1/2} - (F^2)^{1/2}$. The instability constant, k, was 0.02 for [Co(tren)(mmal)]$\cdot$Cl$\cdot$3H_2O. Scattering factors used for C, N, O and Co are those of Cromer and Mann [14] while those of Stewart *et al.* [15] were used for hydrogen. No extinction or anomalous dispersion corrections were made. The XRAY system of crystallographic programs [16] was used throughout the structure analysis. Table 2(a) contains the atomic positional parameters, Table 3 lists the bond lengths and bond angles, and Fig. 1 is an ORTEP [17] view of the complex.

X-ray Crystallographic Data for [Co(tren)(Hmmal)]$Cl_2\cdot H_2O\cdot$0.5CH_3CH_2OH

The crystal for this study was mounted on a glass fiber and coated with epoxy because crystals exposed to air deteriorated rapidly unless protected. Other crystallographic details are given in Table 1. Intensity data were collected on the diffractometer using the higher flux Cu Kα source ($\lambda = 1.5418$ Å) and a limited range, step-scan method (5 steps, 4 s/step) [18] because of crystal deterioration. Background counts were measured between reciprocal lattice rows.

The position of the cobalt atom was obtained from interpretation of the Patterson map and used in the initial phasing model. The remaining non-hydrogen atoms were found in difference Fourier maps. An ethanol of solvation modeled with atoms C(11) (full occupancy) and O(6) (occupancy of 0.5) is located with the crystallographic center of symmetry midway between its methyl and methylene

TABLE 1. Crystallographic data

	$[Co(tren)(Hmmal)]Cl \cdot 3H_2O$	$[Co(tren)(Hmmal)]Cl_2 \cdot H_2O \cdot 0.5CH_3CH_2OH$
Formula	$CoClN_4O_7C_{10}H_{28}$	$CoCl_2N_4O_{5.5}C_{11}H_{28}$
Formula weight	410.8	434.2
Space group	$P2_1/c$	$P2_1/n$
Systematic absences	$(h0l)$: $l = 2n + 1$ $(0k0)$: $k = 2n + 1$	$(h0l)$: $h + l = 2n + 1$ $(0k0)$: $k = 2n + 1$
a (Å)	8.438(2)	9.112(3)
b (Å)	22.770(7)	16.587(4)
c (Å)	9.913(3)	12.610(4)
β (°)	114.6(1)	99.32(2)
V (Å^3)	1731(3)	1881(2)
Z	4	4
D_{calc} (g/cm^3)	1.58	1.55
Crystal size (mm)	0.2 × 0.2 × 0.7	0.7 × 0.2 × 0.1
Crystal color	red	orange
Data collection instrument	Picker FACS-1	Picker FACS-1
Radiation	Mo Kα (0.71069 Å)	Cu Kα (1.5418 Å)
Temperature (°C)	20	20
Scan method	$\omega-2\theta$	$\omega-2\theta$ step scans (5 steps)
Data collection (°)	55	90
μ (cm^{-1})	12.3	102.6
Maximum empirical absorption correction [13]	1.04	1.50
Maximum decay correction	1.04	1.05
No. unique data	3960	1533
Total with F^2 less than $2\sigma(F^2)$	435	230
No. parameters refined	206	217
R	0.038	0.078
R_w	0.047	0.109
Goodness of fit	3.66	1.92

carbon atoms. An anomalous dispersion correction of $f' = -2.2$ electrons and $f'' = 3.9$ electrons [19] was included for the cobalt atom. Hydrogen atoms were not found but the eighteen hydrogen atoms on the tren molecule were added to the model at calculated positions. Least-squares refinement, again on F with $1/\sigma(F)$ weights as defined above ($k = 0.10$), led to a final R of 0.078. The atomic positional parameters are given in Table 2(b). Bond lengths and bond angles for this compound are listed in Table 3, while Fig. 2 shows the thermal ellipsoids for the molecule.

Results and Discussion

Syntheses

[Co(tren)(mmal)]Cl was prepared in aqueous solution by the direct oxidation of $[Co(H_2O)_6]^{2+}$ in the presence of the tren and malonate moieties in an overall reaction represented by eqn. (1).

$$[Co(H_2O)_6]Cl_2 + NaOH + HO_2CCH(CH_3)CO_2H + (tren, HCl, H_2O) \xrightarrow{O_2} [Co(tren)(mmal)]Cl \quad \text{Method A} \quad (1)$$

Products could be isolated only when the tren–HCl solutions were added slowly with vigorous stirring and when the solutions were allowed to stand overnight following the oxidation step (see 'Experimental'). This preparation is one of the few successful direct syntheses of Co(III)–tren complexes via Co(II) oxidation in the presence of tren [20, 21]. (It should be noted that the preparation of Co(II)–tren complexes has resulted from similar synthetic methods [22]. Indeed, the presence of Co(II)–tren complexes may be what prevents the isolation of the desired product from our preparation when the tren is added too rapidly and might also account for the rather low yields of the desired Co(III) compounds.)

A second method used to make [Co(tren)(mmal)]Cl involves a substitution reaction of $[Co(tren)Cl_2]Cl$.

$$[Co(tren)Cl_2]Cl + NaOH + HO_2CCH(CH_3)CO_2H \longrightarrow [Co(tren)(mmal)]Cl \quad \text{Method B} \quad (2)$$

The partially protonated malonate in $[Co(tren)(Hmmal)]Cl_2$, is an intermediate in the acid hydrolysis of that moiety from the cobalt ion. It is synthe-

TABLE 2. Atomic parameters for (a) [Co(tren)(mmal)]Cl$\cdot$3H_2O (coordinates $\times 10^4$, $U_{eq} \times 10^4$) and (b) [Co(tren)(Hmmal)]$Cl_2 \cdot H_2O \cdot 0.5CH_3CH_2OH$ (coordinates $\times 10^5$, $U_{eq} \times 10^3$)

	x	y	z	U_{eq}	PP [a]
(a)					
Co	6621(1)	959(0.2)	95(1)	207	
Cl	5346(1)	1393(1)	3576(1)	506	
N(1)	5937(4)	1597(1)	−1333(3)	324	
N(2)	4342(3)	1030(1)	113(3)	283	
N(3)	5769(3)	427(1)	−1621(3)	302	
N(4)	7601(4)	1580(1)	1603(3)	321	
C(1)	4094(5)	1787(2)	−1684(5)	455	
C(2)	3540(5)	1602(2)	−467(4)	416	
C(3)	6052(5)	1360(2)	−2693(4)	417	
C(4)	5154(5)	767(2)	−3034(4)	411	
C(5)	7208(5)	2082(2)	−647(4)	367	
C(6)	7362(5)	2173(2)	904(4)	389	
C(7)	10164(4)	595(1)	922(4)	301	
C(8A)	10141(6)	412(2)	2463(5)	99	0.75
C(8B)	10213(16)	197(6)	2071(15)	98	0.25
C(9A)	11755(6)	82(2)	3456(6)	125	0.75
C(9B)	11363(23)	473(8)	3580(20)	161	0.25
C(10)	8480(4)	53(2)	2167(4)	330	
O(1)	11469(3)	509(1)	697(3)	438	
O(2)	8850(3)	890(1)	38(3)	287	
O(3)	7020(3)	307(1)	1392(2)	263	
O(4)	8517(3)	−425(1)	2758(3)	433	
O(5)	9431(5)	3661(2)	570(5)	934	
O(6)	1502(4)	1912(1)	2478(4)	578	
O(7)	1031(4)	2304(1)	5057(4)	624	
(b)					
Co	3627(1)	2479(0.9)	3450(1)	42	
Cl(1)	5166(3)	2741(2)	452(3)	64	
Cl(2)	1781(4)	256(2)	2361(4)	103	
N(1)	5638(9)	2320(5)	4175(7)	44	
N(2)	4031(9)	1741(5)	2362(7)	45	
N(3)	3065(9)	1621(5)	4342(7)	49	
N(4)	4405(9)	3389(5)	2710(7)	46	
C(1)	6460(12)	1797(7)	3453(10)	57	
C(2)	5380(13)	1246(7)	2780(10)	55	
C(3)	5555(13)	1963(8)	5216(10)	62	
C(4)	4372(14)	1314(7)	5103(10)	64	
C(5)	6371(12)	3129(7)	4287(10)	54	
C(6)	5999(11)	3556(7)	3222(11)	60	
C(7)	2160(13)	3641(7)	4575(10)	47	
C(8)	1020(12)	3770(6)	3587(9)	53	
C(9)	−283(11)	4281(7)	3761(10)	63	
C(10)	572(15)	2994(7)	2960(9)	50	
C(11)	9272(21)	16(12)	4589(18)	161	
O(1)	2082(7)	4071(4)	5399(6)	50	
O(2)	3284(8)	3186(4)	4569(5)	45	
O(3)	1627(8)	2535(4)	2722(6)	44	
O(4)	−729(9)	2857(5)	2586(6)	55	
O(5)	8913(8)	1273(4)	2001(6)	60	
O(6)	8075(34)	224(15)	5173(27)	180	0.5

[a] Population parameter given if other than 1.00.

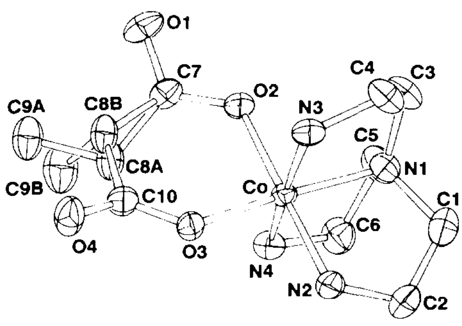

Fig. 1. View of [Co(tren)(mmal)]$^+$.

sized by *protonating* the [Co(tren)(mmal)]$^+$ species in strong acid at room temperature.

$$\text{[Co(tren)(mmal)]Cl} + \text{HCl}_{(\text{conc})} \longrightarrow \text{[Co(tren)(Hmmal)]Cl}_2 \qquad (3)$$

Temperature is an important factor here because placing [Co(tren)(mmal)]Cl in boiling concentrated hydrochloric acid completely removes the malonate moiety from the cobalt ion.

$$\text{[Co(tren)(mmal)]Cl} + \text{HCl}_{(\text{conc})} \xrightarrow{\text{heat}} \text{[Co(tren)Cl}_2\text{]Cl} + \text{H}_2\text{mmal} \qquad (4)$$

In fact, reaction (4) can be used to prepare analytically pure [Co(tren)Cl_2]Cl.

Solid State Structure

In the structures of [Co(tren)(mmal)]Cl and [Co(tren)(Hmmal)]Cl_2, the tren molecule is bound to the cobalt ion through its four nitrogen atoms with the two remaining *cis* sites in the somewhat distorted cobalt coordination octahedron being occupied by two oxygen atoms of the methylmalonate carboxylate groups, see Figs. 1 and 2. As expected, this bidentate mode of binding is seen in other malonate complexes such as that of calcium α-ethylmalonate [10]. In that complex, the carboxylate groups are oriented about the C–C bonds of the malonate ion to allow a Ca–O distance of about 2.45 Å. In the cobalt complexes described here, the carboxylate groups are rotated to position the oxygen atoms closer together to allow the formation of shorter metal–O bonds.

The average bond distances in the tren ligand for [Co(tren)(Hmmal)]Cl_2 are C–C, 1.51(1) Å and C–N, 1.50(1) Å. These values are comparable to those of [Co(tren)(mmal)]Cl which are C–C, 1.51(1) Å and C–N, 1.49(1) Å. The average of the Co–N bond distances in the former structure is 1.94(2) Å and in the latter structure is 1.95(2) Å. A notable characteristic of the binding of the tren molecule in the unprotonated complex is the *trans* influence of the

TABLE 3. Bond lengths (a) and bond angles (b) for [Co(tren)(mmal)]Cl$\cdot$3H_2O and [Co(tren)(Hmmal)]$Cl_2$$\cdot$$H_2O$$\cdot$0.5$CH_3CH_2OH$

[Co(tren)(mmal)]Cl$\cdot$3H_2O		[Co(tren)(Hmmal)]$Cl_2$$\cdot$$H_2O$$\cdot$0.5$CH_3CH_2OH$	
(a) Bond lengths			
Co–N(1)	1.942(3)	Co–N(1)	1.929(8)
Co–N(2)	1.937(3)	Co–N(2)	1.923(9)
Co–N(3)	1.965(3)	Co–N(3)	1.940(9)
Co–N(4)	1.973(3)	Co–N(4)	1.973(9)
Co–O(2)	1.911(3)	Co–O(2)	1.904(7)
Co–O(3)	1.902(2)	Co–O(3)	1.906(7)
C(7)–O(1)	1.227(5)	C(7)–O(1)	1.274(15)
C(7)–O(2)	1.281(4)	C(7)–O(2)	1.276(14)
C(7)–C(8A)	1.592(7)	C(7)–C(8)	1.503(15)
C(7)–C(8B)	1.443(15)		
C(8A)–C(9A)	1.508(6)	C(8)–C(9)	1.506(16)
C(8B)–C(9B)	1.539(20)		
C(8A)–C(10)	1.541(6)	C(8)–C(10)	1.538(15)
C(8B)–C(10)	1.540(16)		
C(10)–O(3)	1.286(4)	C(10)–O(3)	1.302(15)
C(10)–O(4)	1.231(5)	C(10)–O(4)	1.224(15)
		C(11)–O(6)	1.45(4)
		C(11)–C(11)^{i}	1.55(5)
(b) Bond angles			
N(1)–Co–N(2)	87.1(1)	N(1)–Co–N(2)	88.5(4)
N(1)–Co–N(3)	86.6(1)	N(1)–Co–N(3)	86.8(4)
N(1)–Co–N(4)	85.4(1)	N(1)–Co–N(4)	86.8(3)
N(1)–Co–O(2)	92.2(1)	N(1)–Co–O(2)	89.5(3)
N(1)–Co–O(3)	173.5(1)	N(1)–Co–O(3)	174.9(3)
N(2)–Co–N(3)	93.1(1)	N(2)–Co–N(3)	92.3(4)
N(2)–Co–N(4)	92.1(1)	N(2)–Co–N(4)	91.7(4)
N(2)–Co–O(2)	179.0(1)	N(2)–Co–O(2)	177.6(3)
N(2)–Co–O(3)	87.5(1)	N(2)–Co–O(3)	88.1(3)
N(3)–Co–N(4)	170.2(1)	N(3)–Co–N(4)	172.3(3)
N(3)–Co–O(2)	86.2(1)	N(3)–Co–O(2)	86.4(3)
N(3)–Co–O(3)	90.1(1)	N(3)–Co–O(3)	89.6(3)
N(4)–Co–O(2)	88.5(1)	N(4)–Co–O(2)	89.3(3)
N(4)–Co–O(3)	98.4(1)	N(4)–Co–O(3)	97.1(3)
O(2)–Co–O(3)	93.3(1)	O(2)–Co–O(3)	93.9(3)
Co–O(2)–C(7)	127.2(3)	Co–O(2)–C(7)	127.0(7)
O(2)–C(7)–O(1)	123.1(4)	O(2)–C(7)–O(1)	119.4(1.0)
O(2)–C(7)–C(8A)	116.2(4)	O(2)–C(7)–C(8)	121.9(1.1)
O(2)–C(7)–C(8B)	127.0(6)		
O(1)–C(7)–C(8A)	120.2(3)	O(1)–C(7)–C(8)	118.2(1.0)
O(1)–C(7)–C(8B)	108.1(7)		
C(7)–C(8A)–C(9A)	112.9(5)	C(7)–C(8)–C(9)	114.6(1.0)
C(7)–C(8A)–C(10)	109.2(3)	C(7)–C(8)–C(10)	113.3(9)
C(9A)–C(8A)–C(10)	111.3(4)	C(9)–C(8)–C(10)	113.7(9)
C(7)–C(8B)–C(9B)	108.1(1.1)		
C(7)–C(8B)–C(10)	117.8(8)		
C(9B)–C(8B)–C(10)	104.9(1.3)		
C(8A)–C(10)–O(3)	116.4(3)	C(8)–C(10)–O(3)	118.0(1.0)
C(8A)–C(10)–O(4)	122.5(3)	C(8)–C(10)–O(4)	121.0(1.1)
C(8B)–C(10)–O(3)	124.4(6)		
C(8B)–C(10)–O(4)	112.4(6)		
O(4)–C(10)–O(3)	120.7(3)	O(4)–C(10)–O(3)	120.4(1.0)
		C(11)^{i}–C(11)–O(6)	108(2)

'i' denotes atoms related by center of symmetry.

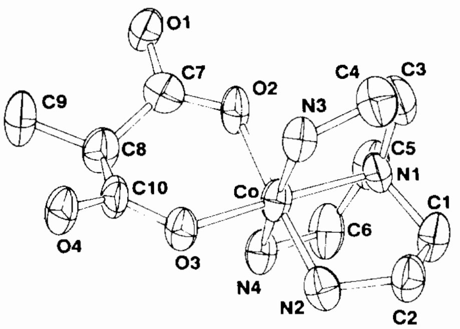

Fig. 2. View of [Co(tren)(Hmmal)]$^{++}$.

TABLE 4. Possible hydrogen bond interactions for [Co(tren)(mmal)]Cl·3H_2O (a) and [Co(tren)(Hmmal)]Cl_2·H_2O·0.5CH_3CH_2OH (b)

Donor and acceptor atoms		Distance	Symmetry operation used to generate primed atom
(a)			
O(5)	O(7)′	2.735(6)	$1 - x, 1/2 - y, z - 1/2$
O(4)	O(5)′	2.775(4)	$2 - x, y - 1/2, 1/2 - z$
O(6)	O(7)′	2.884(5)	$x, 1/2 - y, z - 1/2$
O(6)	O(7)	2.889(5)	
O(1)	N(2)′	2.966(5)	$1 + x, y, z$
O(3)	N(3)′	2.972(4)	$1 - x, -y, -z$
O(1)	N(3)′	3.006(4)	$2 - x, -y, -z$
O(6)	N(4)′	3.128(4)	$x - 1, y, z$
O(4)	N(2)′	3.180(3)	$1 - x, -y, -z$
O(5)	Cl′	3.183(4)	$x, 1/2 - y, z - 1/2$
O(6)	Cl	3.189(3)	
O(1)	O(1)′	3.252(3)	$2 - x, -y, -z$
O(1)	O(2)′	3.255(3)	$2 - x, -y, -z$
N(4)	Cl	3.276(4)	
N(2)	Cl	3.282(3)	
(b)			
O(1)	O(5)′	2.470(10)	$x - 1/2, 1/2 - y, 1/2 + z$
O(4)	O(5)′	2.749(11)	$x - 1, y, z$
O(5)	Cl(2)′	3.087(8)	$1 + x, y, z$
O(2)	O(5)′	3.159(10)	$x - 1/2, 1/2 - y, 1/2 + z$
O(6)	Cl(2)′	3.193(3)	$1 - x, -y, 1 - z$
N(2)	Cl(2)	3.216(9)	
N(4)	Cl(1)	3.224(10)	
N(2)	Cl(1)	3.234(10)	
O(3)	O(5)′	3.263(10)	$x - 1, y, z$
N(4)	Cl(2)′	3.294(9)	$1/2 - x, 1/2 + y, 1/2 - z$

malonate oxygen ligating atoms, the Co–N distances *trans* to Co–O (Co–N(1) and Co–N(2)) being 0.03 Å shorter than the other Co–N distances. A similar trend may exist for the partially protonated complex, but the larger uncertainties in the bond lengths make their comparison less reliable.

Comparison of the binding of the malonate moieties in the two structures is somewhat complicated because the malonate portion of [Co(tren)(mmal)]$^+$ is disordered, requiring a model involving two configurations. The bond lengths and bond angles for the malonate moiety are normal, although as expected, those associated with the disordered atoms are less accurately determined. Nonetheless, the bond lengths and angles are consistent with C(8A) being sp^3 hybridized, and the observation of the hydrogen atom bonded to it substantiates this interpretation. The malonate carbon atom, C(8), in the [Co(tren)(Hmmal)]Cl_2 structure is presumed to be sp^3 hybridized as well as is indicated by the C–C–C bond angles, see Table 3(b). However, the average of these angles around C(8) in this protonated complex, 113.9(9)°, is larger than the averages for the two configurations in the unprotonated complex, 111(1)° and 110(6)°.

The crystal structure of [Co(tren)(mmal)]Cl·3H_2O contains discrete [Co(tren)(mmal)]$^+$ cations, chloride anions and water molecules held together by ionic and van der Waals forces as well as a system of hydrogen bonds. The hydrogen bonding network, see Table 4, consists of the N–H groups of the tren ligand, the carboxylate oxygen atoms, the Cl^- ion and the water molecules in the asymmetric unit. All the tren N–H groups, except one, and all the water hydrogen atoms are involved in hydrogen bonds. The Cl^- ion is the acceptor atom in three such interactions. The structure of [Co(tren)(Hmmal)]Cl_2·H_2O·0.5CH_3CH_2OH also consists of discrete [Co(tren)(Hmmal)]$^{2+}$ cations held together by ionic and van der Waals forces. Two chloride ions which serve as counter ions of the complex as well as a water molecule are involved in hydrogen bonding with the complex cations, see Table 4(b). A disordered molecule of ethanol is present and is held in place by van der Waals forces.

Given that [Co(tren)(Hmmal)]Cl_2 contains two Cl^- ions whereas [Co(tren)(mmal)]Cl contains one Cl^- ion, an extra positive charge (H^+) must be associated with the former. The most likely choices for the location of the H^+ are H_3O^+, HCl_2^- or a protonated carboxylate. The HCl_2 possibility can be ruled out because the two Cl^-s are not close enough to each other in the crystal. Given that the strongest hydrogen bond in the crystal is between O(1) and a water molecule (O···O) = 2.470(10) Å), it is reasonable to place the proton between them. This is also consistent with the pattern of bond lengths in the carboxylate groups. In the protonated complex, the C(7)–O(1) bond distance, 1.27(2) Å, is longer than C(10)–O(4), 1.22(2) Å, and longer than the corresponding bond distances in the unprotonated complex C(7)–O(1), 1.227(5) Å; C(10)–O(4), 1.231(5) Å). The strong hydrogen bond could be accounted for via O(1)–H···OH_2 or O(1)···H_3O^+ or the symmetrical case $\mathrm{O(1)\overset{\cdots}{-}H\overset{\cdots}{-}OH_2}$. Given the observed lengthening of the C(7)–O(1) bond, it seems likely that the first and third cases are the most reasonable. Thus we propose that the malonate moiety in [Co(tren)(Hmmal)](Cl)$_2$ is acting effectively as a uninegative bidentate ligand, and merits the nomenclature Hmmal, indicating its partially protonated state.

The consequences of this type of malonate binding may be important to blood coagulation. In a blood-clotting protein like prothrombin, –NH and –OH groups in the vicinity of a proposed Ca(Gla)$_2$ complex [23] could hydrogen bond with the malonate moieties in the Gla side-chains. This could influence the binding constant of metals by favoring certain orientations of the carboxylate groups. It could also cause the malonate moieties to act as bidentate ligands with a net charge of -1 if the groups involved in this hydrogen bond had a net charge of zero, e.g., –COOH$\cdots$H$_2$N–. It would be much easier for this to happen in the Gla system than with the model complex, [Co(tren)(mmal)]Cl. The charge on a Ca(Gla)$_2$ complex in the absence of outside protonation would be -2. This charge would facilitate the addition of protons whereas the $+1$ charge on the model complex must make protonation more difficult. Presumably, this is the reason the Co(III) complex reported here is protonated only under extremely acidic conditions.

The generation of hydrogen bonds to the dicarboxylate groups in Gla (in proteins) or methylmalonate (in these studies) to enable them to serve as uninegative, bidentate ligands is one means by which the binding of metals such as Ca^{2+} to these types of ligands might be modulated. Outside protonation of the [Ca(Gla)$_2$]$^{2-}$ complex in blood coagulation proteins would serve to lower the charge and facilitate the binding of an anionic phospholipid vesicle for initiation of the blood clotting cascade.

Supplementary Material

Lists of anisotropic temperature factors, hydrogen atom coordinates and structure factors are available on request from R.E.S.

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