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Pentaamminecobalt(III) as a Carboxyl Protective Group for Threonine

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The pentaammine(L-threonine-*O*)cobalt(III) ion has been synthesized and its chemical and spectral properties have been studied.

The crystal structure of $[Co(NH_3)_5(C_4H_9NO_3)]Br_3$ has been determined by X-ray diffraction technique. The crystals are monoclinic, space group *I*2, with a=16.732(4) Å, b=7.555(9) Å, c=13.891(4) Å and β=104.8(2)°. The structure has been refined to R=0.093. The threonine ligand is bonded to the metal only through a carboxylate oxygen atom; the Co–O distance is 1.91(1) Å. The absolute configuration has been established.

As a demonstration of its applicability in peptide synthesis the threonine complex has been coupled to *N*-protected L-alanine by use of a standard procedure for peptide bond formation.

In a recent paper Isied *et al.*[1] have developed the idea of using the pentaamminecobalt(III) group for carboxyl protection in peptide synthesis.

This protective group is distinguished by a visual red colour, a tripositive charge and ease of removal.[1] As shown below it also exhibits a distinctive circular dichroism effect in the visible region when attached to optically active amino acids. As these special features of the pentaamminecobalt(III) group might be profitable in certain practical applications[2] involving carboxyl protection of threonine, the present study was undertaken.

NOTATION

The following abbreviations have been used in this paper. A=NH_3. H–Thr–OH=L-threonine. thr=H–Thr–O^- (threoninate ion) as ligand. thrH=$^+H_2$–Thr–O^- (zwitterion) as ligand. DCC=*N*,*N*′-dicyclohexylcarbodiimide. HONSu=*N*-hydroxysuccinimide. In chemical formulae Z=benzyloxycarbonyl. NMM=*N*-methylmorpholine. CD=circular dichroism. ε-values are given in M^{-1} cm^{-1}.

EXPERIMENTAL

Chemicals. L-threonine, Z–L-alanine, DCC, HONSu and NMM were of analytical grade.

$[(H_2O)CoA_5](ClO_4)_3$ was prepared from the bromide[3] by precipitation with excess $NaClO_4$.

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Preparation of [(thrH)CoA$_5$]Br$_3$. 6.0 g [(H$_2$O)CoA$_5$](ClO$_4$)$_3$ (13.0 mmol) and 4.5 g L-threonine (37.8 mmol) were dissolved in 60 ml H$_2$O, and the solution was kept at 60 °C for 10 h. Then the solution was evaporated to dryness in a rotatory evaporator, and the precipitate was extracted with 60 ml methanol. The red-coloured extract containing dissolved [(thrH)CoA$_5$](ClO$_4$)$_3$ was filtered. The bromide was crystallized from the extract by gradual addition of an equal volume of methanol containing 20 g NaBr per 100 ml, and subsequent cooling to 0 °C. The red, crystalline [(thrH)CoA$_5$]Br$_3$ was finally isolated by filtration and washed with a small volume of ethanol. Yield 2.89 g or 44 %. Calc. for [(C$_4$H$_9$O$_3$N)Co(NH$_3$)$_5$]Br$_3$: C 9.55; H 4.81; N 16.71; Br 47.67. Found: C 9.77; H 4.81; N 16.14; Br 46.83. ^{13}C NMR [22.63 MHz, D$_2$O]: δ 177.6 (C-1), 60.4 (C-2), 66.3 (C-3), 20.0 (C-4). Absorption [H$_2$O (ε)]: 501 (65), 344 (64) nm. CD [H$_2$O ($\Delta\varepsilon$)]: 505 (−0.088) nm. Crystals to be used for X-ray crystallography were grown by allowing ethanol vapour to diffuse slowly into a saturated solution of [(thrH)CoA$_5$]Br$_3$ in a water/ethanol mixture.

DCC/HONSu coupling. Using the general guidelines[2,4] for DCC-induced peptide bond formation we have arrived at the following detailed conditions.

0.23 g [(thrH)CoA$_5$]Br$_3$ (0.45 mmol), 0.10 g Z−L−Ala (0.45 mmol) and 0.11 g HONSu (0.93 mmol) were dissolved in a mixture of 7.5 ml DMF and 1.5 ml H$_2$O. After cooling to 0 °C, 0.097 g DCC (0.47 mmol) and 0.05 ml NMM (0.45 mmol) dissolved in 1.4 ml DMF was added under stirring. The reaction mixture was kept cooled and stirred for *ca.* 10 h; then it was allowed to stand at room temperature for *ca.* 1 h. After filtration the reaction mixture was added to ethylacetate, and the red-coloured [Z−Ala−Thr−OCoA$_5$]$^{2+}$ together with unreacted [(thrH)CoA$_5$]$^{3+}$ was extracted into 5 % aqueous acetic acid using a separatory funnel. The aqueous layer was separated and extracted again with ethylacetate to remove organic impurities.

Chromatography. [Z−Ala−Thr−OCoA$_5$]$^{2+}$ was purified on a cation exchanger column of SP-Sephadex C-25 (5 cm diameter, *ca.* 8 cm bed height). The extracted reaction mixture

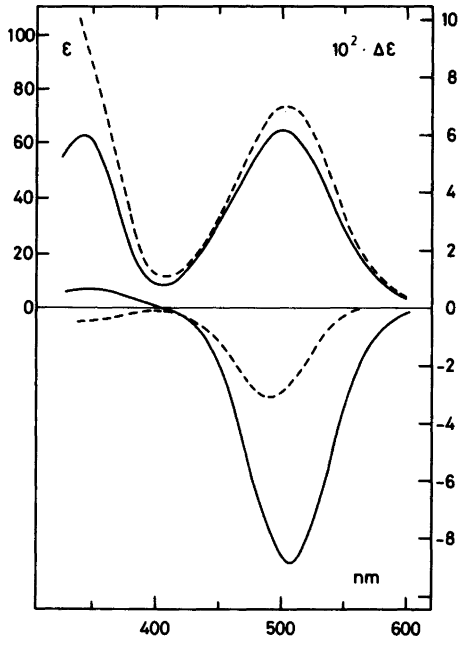

Fig. 1. Absorption and circular dichroism spectra in aqueous solution.
——: [(thrH)CoA$_5$]$^{3+}$ (pH 5.39).
---: [(thr)CoA$_5$]$^{2+}$ (pH 9.74).

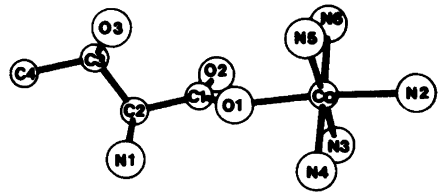

Fig. 2. The structure of [(thrH)CoA$_5$]$^{3+}$.[21]

Table 1. Crystal data.

M	502.9
μ(Cu$K\alpha$) (cm^{-1})	168.2
Crystal system	monoclinic
V(Å^3)	1697.4
a (Å)	16.732(4)
b (Å)	7.555(9)
c (Å)	13.891(4)
β (°)	104.8(2)
Space group	$I2$
D_c (g cm^{-3})	1.97
Z	4
Total number of reflections	1805
Number of independent observations [$I \geq 2\sigma(I)$]	1513
$R=\sum\|\|F_o\|-\|F_c\|\|/\sum\|F_o\|$	0.093
$R_w=\left[\frac{\sum w(\|F_o\|-\|F_c\|)^2}{\sum w\|F_o\|^2}\right]^{1/2}$	0.115

from above was applied to the column and eluted with *ca.* 1 l 0.07 M aqueous NH_4Br. At this point two red zones had separated, the slow zone being $[(thrH)CoA_5]^{3+}$, the chromatographic behaviour of which was known from separate experiments. The desired $[Z-Ala-Thr-OCoA_5]^{2+}$ in the fast zone was isolated by extrusion of the Sephadex bed from the column, mechanical segregation of the pertinent zone, transfer to a smaller column, and quantitative elution with 0.5 M aqueous NH_4Br. The total yield of the protected dipeptide after synthesis and chromatography was 46 %.

Amino acid analysis: The ratio Thr/Ala was 0.98/1.00.

Spectroscopy. A Cary 11 spectrophotometer and a Roussel-Jouan Dichrographe II were used to measure absorption and CD spectra, respectively.

Proton-decoupled 22.63 MHz ^{13}C NMR spectra were obtained with a Bruker WH90 spectrometer. Dioxane (δ 67.40) served as an internal reference.

Analyses. Elemental and amino acid analyses were performed by Novo Microanalytical Laboratory.

X-Ray technique. The possible space groups were established from Weissenberg photographs using Cu$K\alpha$-radiation. Unit cell dimensions and their standard deviations were determined by least-squares refinement based on reflections measured on a four-circle diffractometer. For data collection a crystal of dimensions 0.09×0.11×0.75 mm was used. However, the Weissenberg photographs showed some splitting of the diffraction spots, indicating a crystal of poor quality. This feature was found for all the examined crystals. Three-dimensional data with $\theta \leq 70°$ were measured on a four-circle diffractometer (CAD-4F) using monochromated Cu$K\alpha$-radiation with ω-scan technique. The intensities were corrected for Lorentz and polarization effects, but not for absorption. The structure was solved by Patterson technique.[5] The calculations included full-matrix least-squares refinements of positional and anisotropic thermal parameters for non-hydrogen atoms. The hydrogen atoms were not located. The atomic scattering factors for Br^-, Co^{3+}, O, N and C are those given by Cromer and Mann.[6] The anomalous dispersion corrections for bromine and cobalt were those given by Cromer and Liberman.[7] The absolute configuration was determined by the R-factor method.[8] The ratio of the R-factors for the coordinates of the structure shown in Fig. 2 and of its mirror image was 0.930, establishing that the structure in Fig. 2 is in the correct absolute configuration. The weights were of the form $w^{-1}=a+b\sigma^2(F_o)+c|F_o|+d|F_o|^2$ with the coefficients adjusted to give as uniform a distribution of $w|\Delta F|^2$ as possible.[9] Crystal data and R-values are listed in Table 1. The final positional parameters with estimated standard deviation are listed in Table 2 and the labelling of the ligand atoms are shown in Fig. 2. Lists of thermal parameters and observed and calculated structure factors may be obtained from the authors on request.

Table 2. Atomic coordinates $\times 10^4$. The estimated standard deviations $\times 10^4$ are given in parentheses. The isotropic temperature factors are estimated from anisotropic values.[20]

Atom	*x*	*y*	*z*	B_{eq}
Br1	8047(1)	4874(6)	6789(2)	4.2
Br2	9235(1)	4947(6)	1614(3)	6.1
Br3	6380(1)	5398(6)	685(1)	4.0
Co	5571(1)	4950(0)	7517(2)	2.8
O1	4415(8)	4617(20)	6963(9)	3.4
C1	3941(11)	4641(23)	6056(12)	2.9
O2	4180(7)	5049(32)	5317(8)	4.9
C2	3065(11)	4255(31)	5967(13)	3.3
N1	2987(12)	3167(26)	6867(14)	3.7
C3	2546(13)	5868(35)	5878(14)	4.2
C4	1619(11)	5485(31)	5818(16)	4.1
O3	2835(9)	6939(18)	6771(12)	3.6
N2	6726(10)	5108(29)	8270(12)	4.2
N3	5818(11)	3103(25)	6628(15)	3.9
N4	5424(14)	3148(27)	8478(15)	4.2
N5	5279(11)	6791(26)	8342(13)	3.7
N6	5695(11)	6753(25)	6574(13)	3.5

RESULTS

Chemical and spectral properties of $[(thrH)CoA_5]^{3+}$. It has been claimed[10] that most pentaamminecobalt(III) complexes in which an amino acid is coordinated as a unidentate ligand *via* its carboxylate group are very difficult to prepare as pure samples. We have prepared $[(thrH)CoA_5]^{3+}$ from $[(H_2O)CoA_5]^{3+}$ and excess L-threonine in aqueous solution, as described previously.[11] But we have improved the purification procedure by taking advantage of the extreme solubility of $[(thrH)CoA_5]^{3+}$ and the insolubility of unreacted $[(H_2O)CoA_5]^{3+}$ and threonine in methanol. Finally, $[(thrH)CoA_5]Br_3$ has been isolated as pure crystals.

$[(thrH)CoA_5]Br_3$ has been characterized by its absorption and CD spectra (Fig. 1), and by ^{13}C NMR. The composition and atomic arrangement of the complex have been confirmed by the X-ray structure analysis (Figs. 2 and 3).

It is known that the aquation rate for pentaammine(aminoacid-*O*)-cobalt(III) complexes generally is slow,[1,12] even slower than for the corresponding acetato and trifluoroacetato complexes.[12] By measuring CD as a function of time, we have made sure that

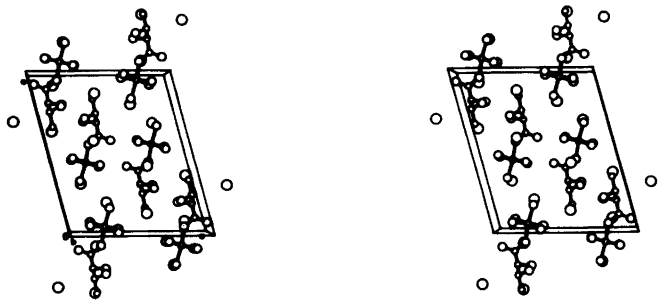

Fig. 3. Stereo view along the *b*-axis in the structure of $[(thrH)CoA_5]Br_3$.

Table 3. Bond distances (Å) and bond angles (°) with estimated standard deviations.

Atoms	Distance	Atoms	Angle
Co–O1	1.91(1)	O1–Co–N3	90.4(7)
Co–N2	1.95(2)	O1–Co–N4	83.8(7)
Co–N3	1.98(2)	O1–Co–N5	87.1(7)
Co–N4	1.97(2)	O1–Co–N6	95.4(7)
Co–N5	1.94(2)	N2–Co–N3	92.4(8)
Co–N6	1.94(2)	N2–Co–N4	87.6(8)
O1–C1	1.31(2)	N2–Co–N5	90.2(8)
C1–O2	1.23(2)	N2–Co–N6	93.3(8)
C1–C2	1.47(3)	N3–Co–N4	91.0(9)
C2–N1	1.53(3)	N3–Co–N6	89.9(8)
C2–C3	1.48(3)	N4–Co–N5	90.0(9)
C3–O3	1.46(3)	N5–Co–N6	89.0(8)
C3–C4	1.56(3)	O1–C1–O2	124.0(1.6)
		O1–C1–C2	115.1(1.6)
		O2–C1–C2	120.7(1.4)
		C1–C2–N1	109.3(1.4)
		C1–C2–C3	113.2(1.9)
		N1–C2–C3	109.9(1.7)
		C2–C3–O3	109.0(1.4)
		C2–C3–C4	113.9(2.0)
		O3–C3–C4	104.9(1.7)

$[(thrH)CoA_5]^{3+}$ is sufficiently inert for use in peptide synthesis; an aqueous solution of the bromide retained 96 % of its CD signal after standing 10 days at room temperature.

It has been suggested[1] that the presence of Cl^- and other halide ions would promote aquation. We have found no evidence for this by comparing CD *vs.* time curves for solutions of $[(thrH)CoA_5](ClO_4)_3$ in pure water and in 0.15 NaCl, respectively.

The acid dissociation constant of the ammonium group in $[(thrH)CoA_5]^{3+}$ was determined in aqueous solution by titration with 0.01 M NaOH and it was found to be 7.6, the corresponding value in free L-threonine being 9.1.

Deprotonation of the ammonium group causes a marked change in the CD spectrum; see Fig. 1. The change is reversible if reacidification is carried out within an hour, whereas prolonged standing in basic solution results in irreversible reactions of $[(thr)CoA_5]^{2+}$.

The crystal structure of $[(thrH)CoA_5]Br_3$. Bond lengths and bond angles with estimated standard deviations are listed in Table 3. The structure consists of $[(thrH)CoA_5]^{3+}$ ions and Br^- ions. The Co atom shows octahedral coordination with one Co–O (carboxyl) bond [1.91(1) Å] and five Co–N(ammonia) bonds [average distance 1.96(2) Å]. The threonine ligand binds the metal atom only through its O(carboxyl) atom. To our knowledge, the only previously published crystal structure showing this type of unidentate coordination of an amino acid is $[Fe(H_2O)_6][Fe(glyH)_2(H_2O)_4](SO_4)_2$.[13] Bond lengths and bond angles of the threonine ligand are in agreement with values found in other L-threonine compounds.[14–18]

The configuration of the amino acid is given by the angles ψ^1[N1–C2–C1–O1][−24°] and ψ^2[N1–C2–C1–O2][60°] describing the torsion of the two C–O bonds about C1–C2. The corresponding values for L-threonine,[14] L-*allo*-threonine[15] and glycyl-L-threonine dihydrate[17] are [−26,156°], [−15,167°] and [−9,170°], respectively. The sidechain conformation is given by the torsion angles $\chi^{2,1}$[N1–C2–C3–C4][−56°] and

$\chi^{2,2}$[N1−C2−C3−O3][61°] about C2−C3 giving the position of C4 and O3 with respect to N1. The corresponding angles for L-threonine,[14] glycyl-L-threonine dihydrate[17] and *N*-(purin-6-ylcarbamoyl)-L-threonine[18] are [186,−55°], [−62,61°] and [67.1,54.5°], respectively.

The packing of the structure is shown in Fig. 3. The Co atoms and the Br atoms are all located at $y\sim0$ and $y\sim\frac{1}{2}$. The complex ions are linked *via* hydrogen bonds. The N···O distance is N1−O3($\frac{1}{2}-x,-y-\frac{1}{2},\frac{3}{2}-z$) [2.77(3) Å]. The rather short distances N6−O2($1-x,y,1-z$) [2.98(2) Å] and N3−O2($1-x,y,1-z$)[3.08(3) Å] suggest hydrogen bonds N−H···O. There are also hydrogen bonds between the complex ion and the bromide ions. The N−H···Br^- distance is N1−Br2($x-\frac{1}{2},y-\frac{1}{2},z+\frac{1}{2}$) [3.28(2) Å] and the O−H···Br^- distance is O3−Br2 ($x-\frac{1}{2}$, $y+\frac{1}{2},z+\frac{1}{2}$) [3.31(2) Å]. The fairly short intermolecular distances N2−Br1 [3.39(2) Å], N6−Br2($x-\frac{1}{2},y+\frac{1}{2},z+\frac{1}{2}$) [3.44(2) Å], N1−Br3($x-\frac{1}{2},y-\frac{1}{2},z+\frac{1}{2}$) [3.47(2) Å] and N5−Br3($x,y,1+z$) [3.47(2) Å] suggest possible hydrogen bonds.

Peptide synthesis. In order to examine the synthetic applicability of $[(thrH)CoA_5]^{3+}$ the formation of a simple model peptide have been studied.

Carboxyl protected L-threonine, $[(thrH)CoA_5]^{3+}$, and Z−L-alanine were coupled to give $[Z-Ala-Thr-OCoA_5]^{2+}$. A standard one-step DCC/HONSu coupling procedure[4] in aqueous DMF was used. After extraction[1] of the protected dipeptide it was purified on a SP-Sephadex column.

The visual colour of the cobalt(III) group proved to be very advantageous in carrying out the extraction and the chromatography.

The ^{13}C NMR spectrum of $[Z-Ala-Thr-OCoA_5]^{2+}$ was found fully consistent with the formulation by comparison with the spectra of $[(thrH)CoA_5]^{3+}$, alanine and the Z-group.[19]

An amino acid analysis showed the presence of Ala and Thr in the ratio 1:1.

The visible absorption and CD spectra of $[Z-Ala-Thr-OCoA_5]^{2+}$ in aqueous solution were very similar to those given for the protonated form of the threonine complex in Fig. 1.

The -CoA_5 protective group is easily removed by $NaBH_4$, NaHS or $(NH_4)_2S$.[1] In addition, we have found that a mild deprotection may be performed by adding an Fe(II)/edta solution. The progress of the reduction reaction could conveniently be monitored measuring the decrease of the CD signal in the visible region.

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