

Cyclic Peptide–Metal Salt Adducts.

I. Crystal Structure of the Hexaquoocopper(II) Perchlorate Cyclosarcosylsarcosine 1:2 Adducts*

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Abstract

The crystal structure analysis of the 2:1 adduct of cyclosarcosylsarcosine with copper(II) perchlorate shows that the independent unit is composed of six water molecules octahedrally coordinated to the Cu(II) ion, two tetrahedral perchlorate ions and four independent halves of cyclosarcosylsarcosine molecules lying on crystallographic centers of symmetry. All available hydrogens of water molecules are involved in hydrogen bonding as donors and all carbonyl oxygen atoms of the cyclic peptide molecules function as acceptors. Two other oxygen atoms for each perchlorate anion participate in the hydrogen bonding scheme, which leads to the absence of the orientational disorder usually observed in these ions. Layers of inorganic material and layers of organic material, roughly parallel to the *ab* plane, pack alternatively with each other. Electrostatic and ion-dipole interactions together with hydrogen bonds are responsible for the building up of the crystals.

Introduction

The interaction between metal cations and cyclic peptides is closely related to the activities of cyclic peptide antibiotics and of great relevance to the understanding of the transport mechanism of metal cations through a biological membrane by complexation. Studies on the interactions occurring between metal cations and cyclic peptides can provide structural information on the stereochemical requirements for binding and can help to determine the factors responsible for coordination and the way it occurs.

Cyclosarcosylsarcosine forms crystalline complexes with several metal salts [1], such as perchlorates of copper, lithium, barium and silver. By infrared spectroscopy, the suggestion has been made that the carbonyl oxygen atoms of the cyclic

peptide coordinate to the metal cations in these complexes. The crystal structure of the lithium complex [2] has confirmed the IR data, showing that the cyclosarcosylsarcosine molecules are linked together by coordination of the carbonyl groups to the Li⁺ ion.

As part of our continuing research on peptide transport through membranes, we report the crystal structure analysis of the 2:1 complex of cyclosarcosylsarcosine with copper(II) perchlorate. Our analysis shows that the complex should be considered instead as a hexaquoocopper(II) perchlorate–cyclosarcosylsarcosine 1:2 adduct.

Experimental

The cyclic peptide–copper(II) perchlorate complex was obtained by mixing ethyl acetate solutions of the dipeptide and the copper(II) salt in a 2:1 ratio. Needle-like crystals of the copper(II) complex were prepared by slow evaporation of the ethyl acetate solution. Preliminary Weissenberg photographs indicate the monoclinic system with *P2₁/c* space group, because of the systematic absences of the *h0l* and *0k0* reflections with *l* = $2n + 1$ and *k* = $2n + 1$, respectively. Density measurements obtained by flotation techniques indicate the presence in the unit cell of six water molecules and two cyclic peptide molecules for each copper perchlorate unit.

A CAD 4 Enraf-Nonius diffractometer equipped with PDP-8 and PDP-11 Digital computers was used for the data collection, structure determination, and refinement. The SDP package of crystallographic programs (structure determination programs) was used throughout. Unit cell parameters, orientation matrix, and intensity data collection were carried out using a graphite monochromator and Cu K α radiation. Crystallographic data are reported in Table I.

Data collection was carried out using the following set of conditions. An ω - 2θ scan mode with a range of $(0.8 + 0.15 \tan\theta)^\circ$ was chosen for the peak measurements; background counts were taken at

*A preliminary account of the results has been reported at the VIII American Peptide Symposium [3].

TABLE I. Crystal Data for $\text{Cu}(\text{H}_2\text{O})_6(\text{ClO}_4)_2 \cdot 2(c\text{-Sar}_2)$

Formula unit	$\text{C}_{12}\text{H}_{32}\text{N}_4\text{O}_{18}\text{Cl}_2\text{Cu}$
Formula weight	654.85 a.m.u.
Crystal system	monoclinic
space group	$P2_1/c$
Z	4 formula unit/unit cell
a	13.879(7) Å
b	14.504(7) Å
c	13.083(8) Å
β	90.98(10)°
V	2633.2(9) Å ³
D_m (floatation)	1.65 g/cm ³
D_c	1.652 g/cm ³
Reflections, measured	4055
Reflections, with $I \geq 3.0\sigma(I)$	3802
R factor	0.083
Temperature	23 °C, ambient

each end of each scan. A distance crystal counter of 368 mm was used with a counter entrance aperture of 4 mm. The tube placed between the goniometer head and the detector was evacuated using a vacuum pump.

Prescan runs were made with a speed of 5 deg/min. Reflections with a net intensity $I \leq 0.5\sigma(I)$ were flagged as 'weak'; those having $I > 0.5\sigma(I)$ were measured at lower speed (in the range 1–5 deg/min) depending on the value of $\sigma(I)/I$. The maximum time allowed for the scan was set to 60 s. Two intensity-control reflections were recorded after every 60 min of X-ray exposure time; no significant change in their intensity was observed during data collection. Orientation checks were made with respect to the scattering vectors of four strong reflections every 200 reflections; reorientation was made using 25 high-angle reflections if displacement exceeded the calculated value by 0.15°.

A total of 4055 reflections in the range 1–130° of 2θ , corresponding to two octants, were collected; of these, 3802 reflections had a net intensity greater than $3.0\sigma(I)$ and were considered as 'observed'. All reflections were corrected for Lorentz and polarization effects.

The structure has been solved by interpretation of the Patterson vectors. The copper and chlorine atoms were located, and, by successive Fourier syntheses, the positions of first the water molecules and then the cyclic dipeptide molecules and the oxygen atoms of the perchlorate ions were determined.

Refinement by full-matrix least-squares procedure with weights $w = 1/\sigma(F_o^2)$, anisotropic temperature factors for Cu, Cl, O, N, and C atoms and isotropic temperature factors for H atoms, led to a final R factor of 0.083 for all reflections with $F_o > 3.0\sigma(F_o)$. The positions of the hydrogen atoms in the water molecules were determined by difference Fourier

synthesis, while those of the other hydrogen atoms (methylene and methyl groups in the cyclosarcosyl-sarcosine molecules) were calculated according to stereochemical considerations. A temperature factor equal to that of the atom to which it is linked was assigned to each hydrogen atom. The atomic parameters of the H atoms were kept fixed.

Refinement was ended when the maximum shift in the atomic coordinates and anisotropic thermal

TABLE II. Final Atomic Parameters with Estimated Standard Deviations (in unit of the last significant figure). Fractional Coordinates are Multiplied by 10⁴

Atom	x/a	y/b	z/c	B_{eq}
Cu	7491(1)	2683(1)	2682(1)	2.19(4)
O(1)	6139(4)	2605(4)	3174(4)	2.98(21)
O(2)	7031(4)	2736(4)	1250(4)	3.19(23)
O(3)	7889(4)	2646(4)	4124(4)	3.17(22)
O(4)	8845(3)	2828(4)	2220(4)	3.12(23)
O(5)	7644(4)	1128(4)	2436(5)	4.01(26)
O(6)	7356(4)	4280(4)	2700(5)	3.74(24)
Cl(1)	5991(1)	9048(2)	2596(2)	3.98(8)
O(7)	5080(5)	8739(6)	2953(7)	8.45(41)
O(8)	6621(7)	9124(10)	3404(7)	10.05(61)
O(9)	6263(7)	8576(8)	1747(7)	10.79(54)
O(10)	5887(9)	9962(7)	2239(11)	11.32(72)
Cl(2)	9144(2)	6134(2)	2478(2)	4.24(9)
O(11)	8726(11)	6158(10)	3382(9)	13.84(83)
O(12)	9135(8)	5265(7)	2072(9)	11.31(60)
O(13)	10051(10)	6232(13)	2894(16)	17.93(95)
O(14)	9069(18)	6768(10)	1820(10)	17.39(91)
Molecule A				
C ^α	5334(6)	898(6)	4731(6)	3.61(35)
C'	5959(6)	248(5)	5357(6)	3.25(32)
O	6761(4)	527(4)	5650(5)	4.06(26)
N	5606(5)	-590(4)	5572(5)	3.29(28)
C ^N	6190(9)	-1229(8)	6165(10)	6.90(57)
Molecule B				
C ^α	5947(6)	155(6)	-374(8)	4.24(39)
C'	5364(6)	912(5)	66(6)	3.17(30)
O	5733(4)	1708(4)	71(5)	4.42(26)
N	5490(5)	-749(5)	-419(6)	4.19(31)
C ^N	6069(9)	-1483(8)	-829(10)	6.59(57)
Molecule C				
C ^α	10199(6)	-883(5)	470(7)	3.33(33)
C'	9064(5)	396(6)	172(6)	3.10(31)
O	8271(4)	794(4)	288(5)	4.06(26)
N	9282(4)	-420(4)	563(5)	3.20(27)
C ^N	8546(7)	-917(7)	1152(10)	5.79(48)
Molecule D				
C ^α	9038(6)	134(6)	4661(7)	3.68(34)
C'	9603(5)	898(5)	5133(6)	3.00(29)
O	9192(4)	1665(4)	5236(4)	4.27(26)
N	9502(5)	-755(5)	4586(5)	3.43(28)
C ^N	8897(7)	-1503(7)	4182(9)	5.01(46)

parameters were less than 1/5 and 1/3 of the corresponding standard deviation, respectively. Atomic scattering factors for all atomic species were calculated from Cromer and Waber [4]. All calculations were carried out on a PDP-11 Digital computer using the SDP system. The final atomic parameters for the copper complex are reported in Table II.

Results and Discussion

The independent crystallographic unit in the unit cell is composed of six water molecules nearly octahedrally coordinated to the Cu(II) ion, giving rise to the hexaquocopper(II) cation, two grossly tetrahedral perchlorate anions, and four independent halves of cyclosarcosylsarcosine molecules lying on centers of symmetry. The crystal is held together by coulombic interactions between ions and by a three-dimensional network of hydrogen bonds in which all donor O–H groups from the six water molecules are extensively linked to the carbonyl oxygens of the cyclosarcosylsarcosine molecules and partially linked to the perchlorate ions. Thus, the present structure is completely different from that

observed in the 2:1 complex of cyclosarcosylsarcosine with lithium perchlorate [2], where the cyclic dipeptide molecules are linked by direct coordination of their carbonyl groups to the Li^+ ion. In fact, the structure reported here is characterized by extensive H-bonding between the hexaquocopper(II) ion and the acceptor groups in both the cyclic organic molecules and the inorganic perchlorate anions.

The Hexaquocopper(II) Ion

Six water molecules are nearly octahedrally coordinated to the copper(II) ion (Fig. 1). Distortion from ideal octahedral coordination occurs. In Fig. 1 the observed geometry of the resulting octahedron is given. Two Cu–O bond distances show larger values (2.289 and 2.325 Å) with respect to the other four Cu–O bond distances (mean value 1.980 Å). This stretching of the octahedron along one axis is typical of the Jahn–Teller effect of ions with d^9 configuration [5]. The O–Cu–O bond angles are all, within few degrees, close to 90° or 180° . The observed deviations from the ideal values are in most cases less than 4° , and are due to the need to form better hydrogen bonds between water molecules of the ligand and both the cyclosarcosylsarcosine molecules and the

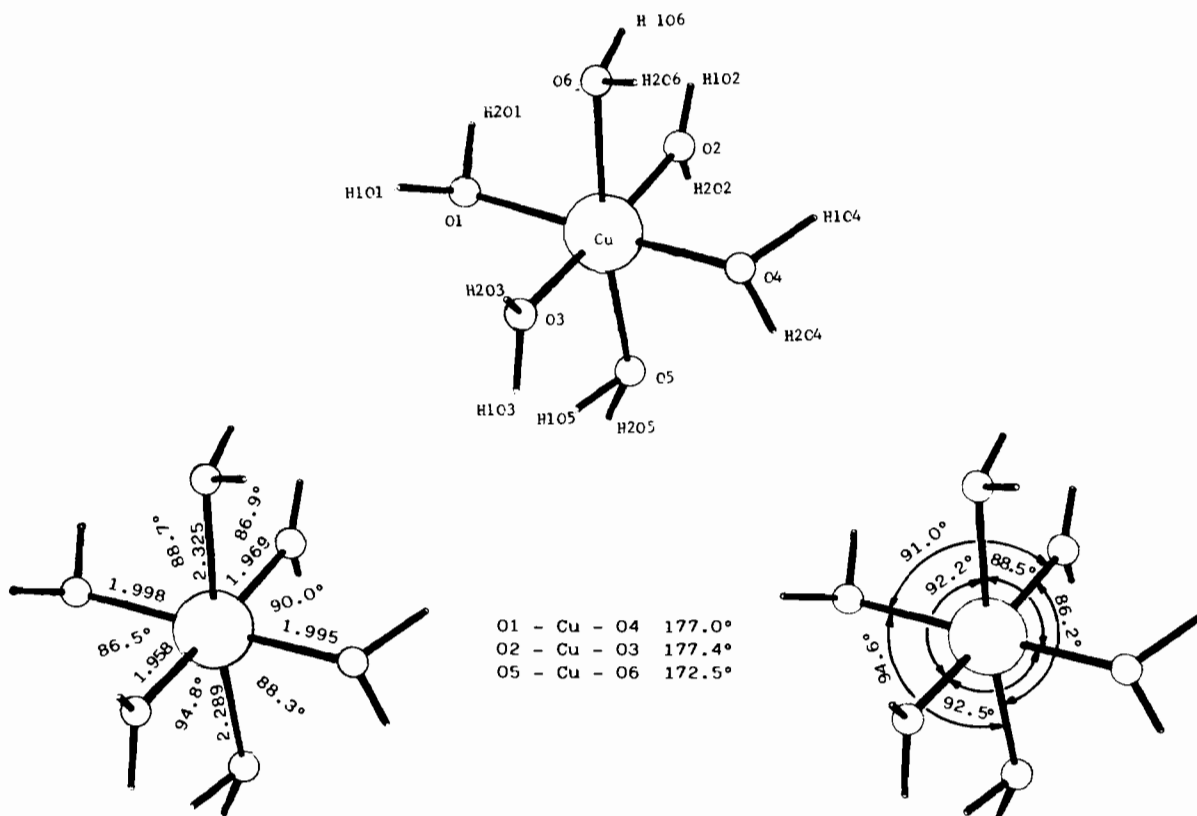


Fig. 1. Geometry of the $\text{Cu}(\text{H}_2\text{O})_6^{2+}$ ion. Bond lengths (Å). Estimated standard deviations of bond lengths and bond angles are 0.006 Å and 0.5° , respectively.

perchlorate ions. These deviations, on the other hand, require a very small amount of energy which is largely regained by the establishment of more appropriate contacts in the crystal, with a consequent better packing. In the subtle balance among electrostatic interactions, ion-dipole interactions, hydrogen bonds and van der Waals forces, the distortion of bond angles is fully justified, representing the easiest pathway for relaxing unfavourable interactions.

The Perchlorate Ions

In contrast with most of the literature data, the two independent perchlorate anions do not show orientational disorder; they are, instead, rather rigidly fixed in their position, because of the involvement in the H-bonding scheme of half of the oxygen atoms in each anion. The oxygen atoms of these ions, however, still show much larger temperature factors, indicative of large thermal vibrations in the crystal. (With the exception of the hydrogen atoms, the temperature factors of the perchlorate oxygens are more than twice the average temperature factor of any other atoms in the structure, as shown in Table II.) No correction has been applied for thermal motion and, consequently, the observed geometry shows large fluctuations of Cl-O bond

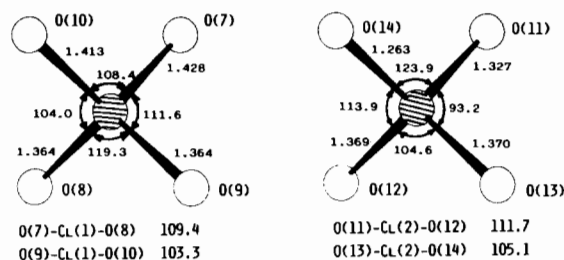


Fig. 2. Geometry of the perchlorate anions (bond lengths (Å) and bond angles ($^{\circ}$)). Estimated standard deviations are 0.04 Å and 4° for bond lengths and bond angles, respectively.

distances and O-Cl-O bond angles (Fig. 2), with values ranging between 1.263–1.428 Å and 93.2 – 123.9° , respectively. The average Cl-O bond distance (1.36 Å) is much lower than the value reported for this distance in the literature [6] of 1.44 Å. Furthermore, in each perchlorate ion, two of the Cl-O distances are shorter than the other two (Fig. 2). The two longer Cl-O distances in both anions involve oxygen atoms which participate in the H-bonding scheme with water molecules of the hexa-aquocopper(II) cation.

TABLE III. Geometric and Conformational Parameters of $c(\text{Sar}_2)$

	Molecule				Reference 7	Reference 8 ^b
	A	B	C	D		
Bond lengths ^a						
C ^α -C'	1.51(1)	1.48(1)	1.51(1)	1.48(1)	1.506(4)	1.504(6)
C'-N	1.34(1)	1.30(1)	1.32(1)	1.31(1)	1.348(3)	1.338(11)
C'-O	1.24(1)	1.26(1)	1.25(1)	1.26(1)	1.234(3)	1.230(3)
C ^α -N	1.43(1)	1.46(1)	1.45(1)	1.45(1)	1.455(3)	1.462(13)
N-C ^N	1.45(1)	1.44(1)	1.48(1)	1.47(1)	1.475(3)	1.457(5)
Bond angles ^c						
O-C'-N	124(1)	122(1)	124(1)	123(1)	123.6(2)	122.7(5)
O-C'-C ^α	118(1)	117(1)	117(1)	118(1)	118.3(2)	118.9(12)
C ^α -C'-N	118(1)	121(1)	119(1)	119(1)	118.1(2)	118.2(9)
C'-N-C ^α	124(1)	123(1)	125(1)	123(1)	124.6(2)	123.3(12)
C'-N-C ^N	119(1)	121(1)	119(1)	121(1)	119.7(2)	119.1(10)
C ^α -N-C ^N	117(1)	116(1)	116(1)	116(1)	115.5(2)	117.4(14)
N-C ^α -C'	118(1)	116(1)	116(1)	117(1)	117.0(2)	113.2(16)
Conformational Angle ^{c,d}						
(C'-C ^α -N-C')	φ	1.9	2.6	2.9	3.3	
(N-C ^α -C'-N)	ψ	-1.8	-2.5	-2.7	-3.1	
(C ^α -C'-N-C ^α)	ω	1.9	2.7	3.0	3.3	

^aBond lengths are in Å. ^bData reported in this column are the average of the values given in the original paper (ref. 8). ^cBond angles and conformational angles are in degrees. ^dBecause of the retention of a crystallographic center of symmetry by each molecule, within the cyclic structure the three conformational angles, φ, ψ, and ω are followed by three angles φ', ψ', and ω' with same absolute value and sign changed.

The Cyclosarcosylsarcosine Molecules

In Table III bond lengths and bond angles in the cyclic dipeptide molecules are given and compared with previous literature data [7, 8]. Bond lengths are within 0.03 Å with respect to the values given by Groth [7] for *c*-Sar₂ and Benedetti *et al.* [8] for other *N*-methylated cyclic dipeptide molecules. Bond angles show an average deviation from literature data not greater than 4°.

Each cyclic molecule, sitting on a crystallographic center of symmetry, must present either a fully planar or a very flat chair conformation. In fact, all molecules in the crystal show the latter conformation because the ϕ , ψ and ω conformational angles [9] of the cyclic molecules have values close to 0° with alternating plus or minus signs as in a chair conformation of a six-membered ring structure. This conformation has been observed in cyclosarcosylsarcosine itself [7] as well as in other *N*-methylated cyclic peptide molecules [8].

All C=O groups of the four cyclosarcosylsarcosine molecules are involved as acceptors in the H-bonding scheme. Each oxygen atom is H-bonded to two water molecules of the hexaaquocopper(II) ions in the crystal. The C=O...O distance is, on average, 2.78 Å, with a range of 2.66–2.99 Å. Each C=O group shows an H-bonded C=O...O distance slightly longer than the other (Table IV).

TABLE IV. Hydrogen Bonds in the Cu(H₂O)₆(ClO₄)₂·2*c*-(Sar₂)

Acceptor	Donor ^a	Length (Å)	Symmetry operation (A)
O(7)–perchlorate 1	O(1)	2.767	1 - x, y - 1/2, 1/2 - z
O(10)–perchlorate 1	O(5)	2.976	x, y - 1, z
O(13)–perchlorate 2	O(4)	2.782	2 - x, y - 1/2, 1/2 - z
O(12)–perchlorate 2	O(6)	2.980	x, y, z
O(molecule A)	O(2)	2.662	x, 1/2 - y, z - 1/2
O(molecule A)	O(6)	2.806	x, 1/2 - y, z - 1/2
O(molecule B)	O(1)	2.742	x, 1/2 - y, 1/2 + z
O(molecule B)	O(2)	2.785	x, y, z
O(molecule C)	O(3)	2.773	x, 1/2 - y, 1/2 + z
O(molecule C)	O(5)	2.994	x, y, z
O(molecule D)	O(3)	2.706	x, y, z
O(molecule D)	O(4)	2.748	x, 1/2 - y, z - 1/2

^aAll donors are oxygen atoms of water molecules.

Packing in the Crystal

The mode of packing of the hexaaquocopper(II) perchlorate–cyclosarcosylsarcosine 1:2 adduct along the *b* direction is given in Fig. 3. Layers of inorganic material and layers of organic material, roughly parallel to the *ab* plane, pack alternately with each other. Electrostatic interactions, ion–dipole interactions and hydrogen bonds are mainly responsible for the building up of the crystals. The

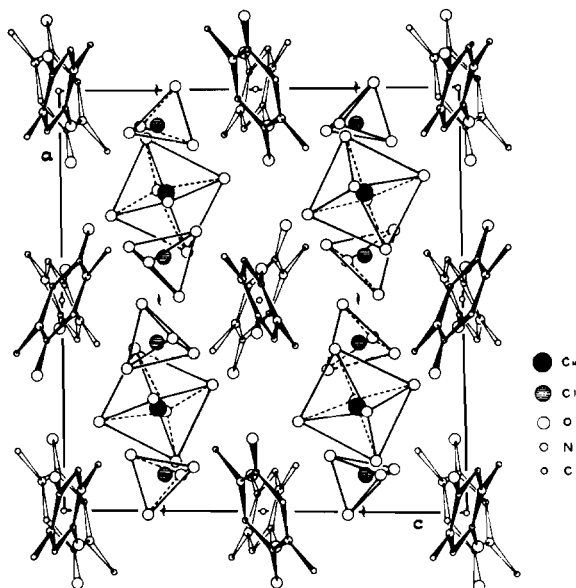


Fig. 3. Mode of packing of the adduct as viewed along the *b* axis.

involvement of each donor and each acceptor group in the hydrogen bonding scheme, together with the O–H...O distances is given in Table IV.

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