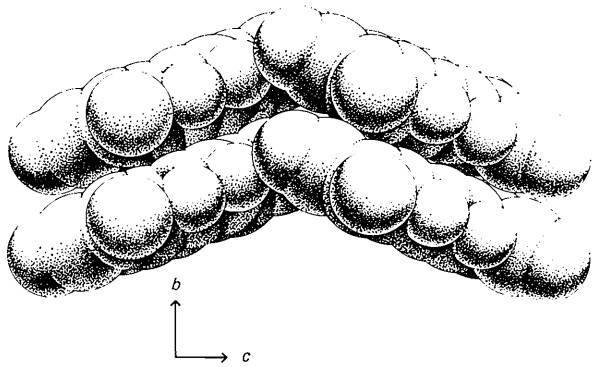

Fig. 3. A view of the structure along **a***.

molecules is evident in Fig. 3 which is a view along **a***; the angle between the planes of molecules in adjacent stacks, related by the twofold axis, is 48°. The perpendicular distance between the planes of adjacent molecules within a given stack is 3·45 Å, only about 0·1 Å more than that in graphite.

We are most grateful to the U.C.L.A. Computing Facility for their cooperation in this work, to Dr R. E. Merrifield and Dr Paul Arthur, Jr., of the Central Research Department, E.I. DuPont de Nemours and Company, who supplied us with the crystals and with preliminary data on them, to Maryellin Reinecke for the illustrations, and to the Air Force Office of Scientific Research for their generous support.

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Crystallographic Studies of Metal–Peptide Complexes. III. Disodium Glycylglycylglycylglycino Cuprate(II) Decahydrate

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(*Received* 3 *July* 1964)

The structure of the pink complex disodium glycylglycylglycylglycino cuprate(II) decahydrate has been determined by X-ray diffraction methods from three-dimensional intensity data recorded on Weissenberg films and estimated visually. The structure (without hydrogen atoms) has been refined by full-matrix least-squares with anisotropic temperature factors to an *R* index of 0·092. The environment of the copper(II) atom is approximately square-planar, the copper being coordinated by the four nitrogen atoms of one peptide molecule. The discrete glycylglycylglycylglycino cuprate(II) ions are extensively hydrogen bonded to water molecules. Both sodium ions exhibit distorted octahedral coordination, the octahedra sharing a face of three water molecules.

Introduction

This is the third in a series of papers describing a crystallographic study of complexes between metals and small peptides (Freeman, Robinson & Schoone, 1964; Freeman, Schoone & Sime, 1965). From the study of a number of these complexes it is hoped to establish possible stereochemical relationships for metal–protein interaction. Disodium glycylglycylglycylglycino cuprate(II) decahydrate is the first complex to be studied in this series where there exists the possibility of fourfold coordination of a metal ion by nitrogen atoms of the same peptide molecule. Koltun, Roth & Gurd (1963)

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have recently presented evidence, from *p*H titration and spectrophotometric measurements, that the metal ion is coordinated to the terminal amino and three peptide nitrogen atoms, accompanied by the ionization of the three peptide hydrogen atoms. It was expected that the two negative charges on the $CuGGGG^{2-}$* ion in this complex would produce some interesting changes in the normally constant peptide-group dimensions.

A preliminary account of this structure analysis has been published (Freeman & Taylor, 1964).

Experimental

The preparation of crystals of a pink complex between copper(II) and GGGG* from alkaline solution was reported by Rising, Parker & Gaston (1934). By chemical analysis they established the empirical formula as $Na_2CuC_8H_{10}N_4O_5 \cdot 2H_2O$.

Pink solutions of the complex were readily obtained by a similar procedure. Moist, freshly prepared cupric hydroxide (10^{-3} mole) and solid GGGG (10^{-3} mole) were mixed and heated for thirty minutes on a steam bath. Some of the GGGG dissolved, to give a deep blue solution, presumably of a neutral complex. When a solution containing 2×10^{-3} mole of sodium hydroxide was added to this mixture, all the GGGG immediately dissolved to give a pink solution. The compound crystallized as long, pink needles with rectangular cross-section from a solution containing acetone, ethanol, and water. Not enough crystals were obtained for chemical analysis, and this crystallization could not be successfully repeated†.

Crystal data for the Delaunay reduced cell are:

$C_8H_{30}N_4O_{15}CuNa_2$ F.W. = 531·88

$Na_2[CuNH_2\{CH_2CON\}_3CH_2COO]10H_2O$

Triclinic, $a = 7{\cdot}665 \pm 0{\cdot}006$ Å, $b = 10{\cdot}204 \pm 0{\cdot}009$ Å,
$c = 14{\cdot}872 \pm 0{\cdot}010$ Å, $\alpha = 93°48' \pm 2'$,
$\beta = 107°39' \pm 3'$, $\gamma = 94°17' \pm 3'$,
$K = 0{\cdot}08 \pm 0{\cdot}04$, $S = -0{\cdot}006 \pm 0{\cdot}01$,
$U = 1100{\cdot}5 \pm 1{\cdot}6$ Å^3, $D_m = 1{\cdot}67 \pm 0{\cdot}1$ g.cm^{-3},
$Z = 2$, $D_x = 1{\cdot}600$ g.cm^{-3}.

Space group $P\bar{1}$ (C_i^1).

The unit-cell parameters were obtained from 79 high sin θ values measured on three zero-level photographs. The $0kl$ data were recorded on a double radius camera using the Straumanis method of film mounting, and the $h0l$ and $hk0$ data on normal radius, platinum-calibrated Weissenberg photographs. The six unit-cell parameters and two parameters K and S were fitted to the data by least-squares. K and S are coefficients of terms which allow for systematic errors and are defined by $\Delta\theta_{abs} = K\cos^2\theta$, and $\Delta\theta_{ecc} = S\sin 2\theta$ (Buerger, 1942).

The density was measured by flotation in methylene bromide–ethanol mixtures. This density was measured after the intensity data had been recorded. At this stage the only crystals available were of very poor quality, and so coated with extraneous material that the value of the density is not considered to be accurate.

The intensity data were recorded on equi-inclination Weissenberg photographs with unfiltered CuK X-radiation. One crystal of dimensions $0{\cdot}160 \times 0{\cdot}050 \times 0{\cdot}106$ mm^3 was used to collect data from layers $h = 0$–6, and another, $0{\cdot}210 \times 0{\cdot}030 \times 0{\cdot}060$ mm^3 to collect layers $k = 0$ and 1. Data were obtained for 4319 of the 5000 unique reflexions within the Cu $K\alpha$ limit. The intensities were estimated visually. 629 were too weak to measure and were given a value of one-third of the minimum observable intensity (Hamilton, 1955). Absorption corrections were calculated by the method outlined by Busing & Levy (1957). A least-squares method equivalent to that described by Hamilton, Rollett & Sparks (1965) was used to calculate the scale factors required to correlate the intersecting layers of data.

Statistical tests to distinguish between $P1$ and $P\bar{1}$ gave ambiguous results.

The solution of the structure

The routine application of the heavy-atom method was complicated by the lack of knowledge of the empirical formula, the space group, and the accurate density. The formula weight calculated from the observed density indicated that the compound was not the same as that prepared by Rising, Parker & Gaston (1934). It did indicate, however, that provided there was at least one tetrapeptide molecule associated with one copper atom there could only be two copper atoms in the unit cell. In view of these uncertainties the structure was solved in space group $P1$.

Table 1. *Observed and calculated structure factors*

Each group of reflexions is headed by

$h\ k$

and for each reflexion the data are presented in the order

$l \quad 10F_c \quad 10|F_o|$

The symbol following $|F_o|$ indicates the weight that was given to the reflexion during the least-squares refinement.

Symbol	Weight of $\|F_o\|$
none	$1{\cdot}0/\sigma^2$
V	$2{\cdot}0/\sigma^2$
W	$2{\cdot}5/\sigma^2$
X	$1{\cdot}5/\sigma^2$
Y	$16/\|F^2_{\min}\|$
Z	Zero

Y also denotes an unobservably weak reflexion.

Standard deviations in $|F_o|$ used in least-squares refinement

$\|F_o\|$	0	2·5	10·0	20·0	35·0	50·0	95·0
$\sigma\|F_o\|$	0·40	0·35	0·57	1·05	1·90	3·00	35·00

* $CuGGGG^{2-}$ is written for the glycylglycylglycylglycino cuprate(II) ion and GGGG for the glycylglycylglycylglycine molecule.

† Since the completion of this work Mr R. L. Sinclair has prepared the substance reproducibly by adding excess cupric hydroxide to GGGG (0·1 g) and sodium hydroxide (0·1 g) in water (0·75 ml). Undissolved cupric hydroxide is filtered off and absolute alcohol is added until a faint cloudiness just appears. Pink crystals appear if the solution is allowed to stand in a stoppered tube for 48 hours.

Table 1. *Observed and calculated structure factors*

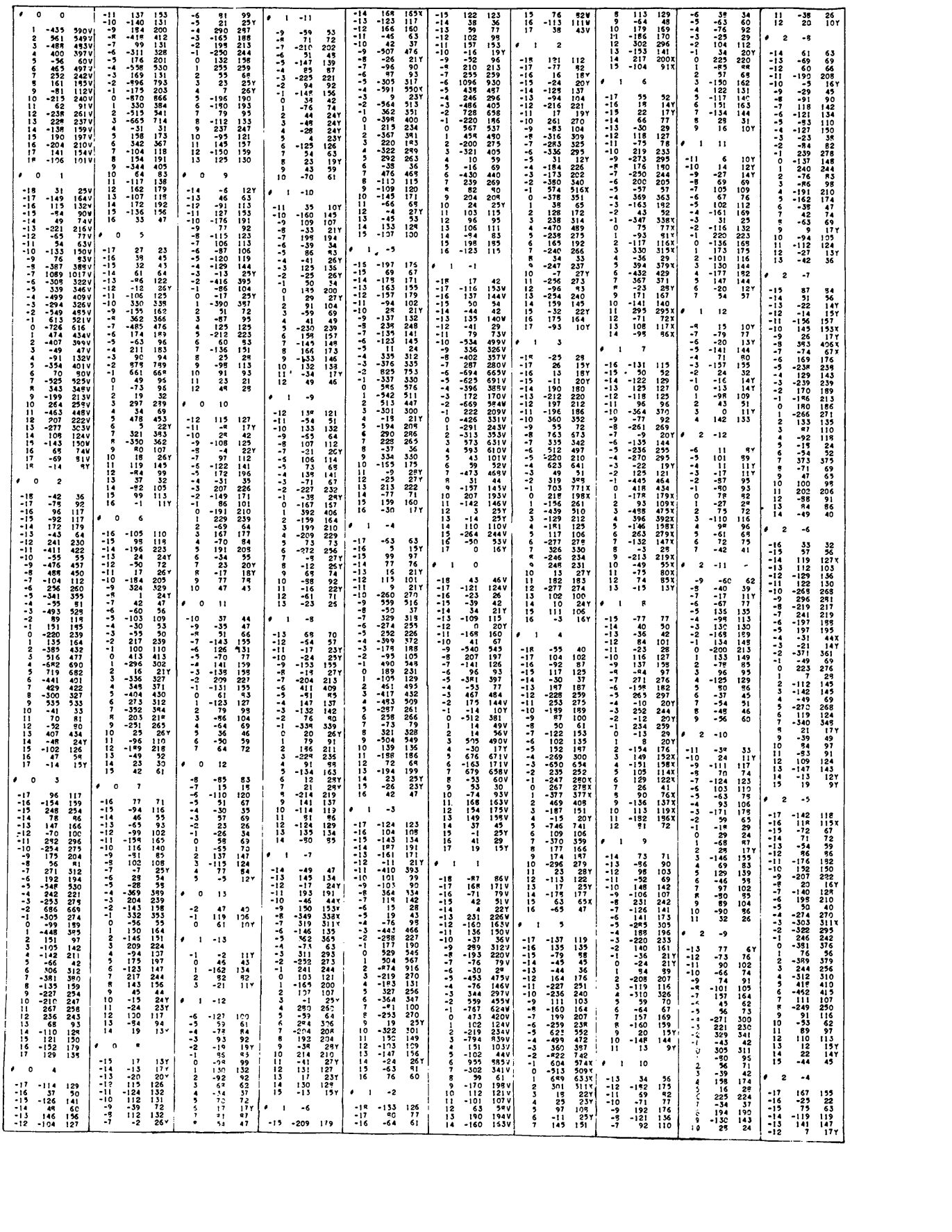

Table 1. (*cont.*) *Observed and calculated structure factors*

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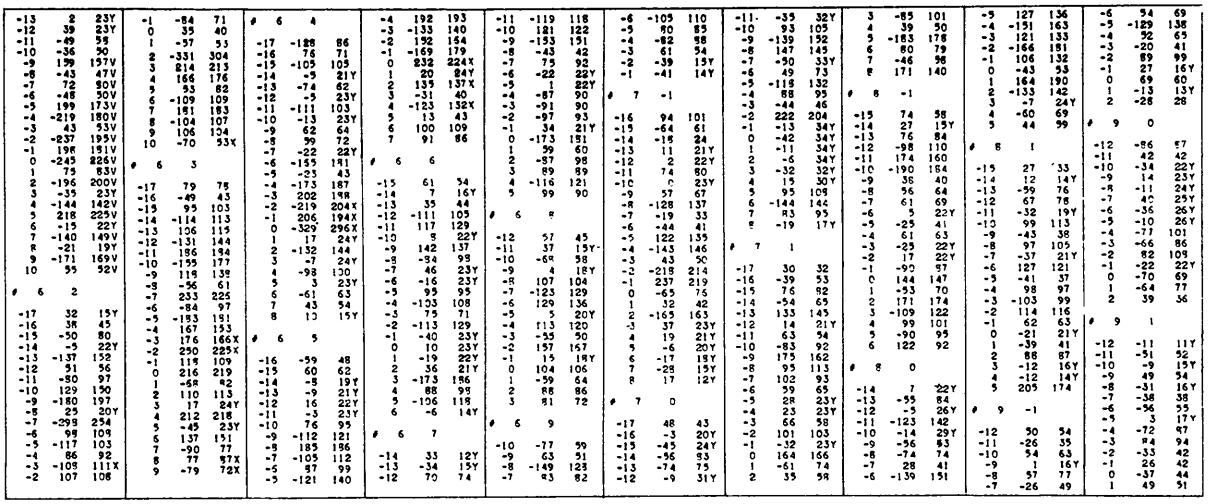

The positions of two copper atoms were obtained from a three-dimensional Patterson synthesis. Four small peaks surrounding the copper atom–copper atom vector indicated the orientation of the coordination square of at least one of these atoms. A three-dimensional Fourier synthesis was computed with phases based on the contributions to the structure factors from two copper atoms and four nitrogen atoms surrounding one of them. All the observed data were included in the computation, the terms being weighted to minimize the errors in electron density due to errors in phase angle (Rossmann & Blow, 1961).

All the atoms in the structure were identified in this and one more Fourier synthesis. The presence of a centre of symmetry was also established. The R index at this stage was 0·27.

The scattering factors used were those of Berghuis, Haanappel, Potters, Loopstra, MacGillavry & Veenendaal (1955) for carbon, nitrogen, oxygen and Cu^+ and that of Freeman (1959) for Na^+. The curve for Cu^+ was used instead of that for Cu^{2+}, consistent with the principle (Pauling, 1948) that the partial ionic character of the metal–ligand bonds reduces the electrical charge on the central metal ion in a complex to +1 or less. To allow for the real part of the anomalous scattering by copper, 2·1 electrons were subtracted over the whole $\sin\theta$ range before the temperature factor was applied (Dauben & Templeton, 1955).

Values of $\sigma(|F_o|)$ for weighting the refinement were found from the independent observations used to calculate $|F_o|$. A similar method has been described by Ibers (1956). The data were divided into three groups: those where $|F_o|$ was derived from more than one observation, those where there was only one observation, and those that were too weak to be measured.

For each of the reflexions in the first group, the population variance (*i.e.* the variance of a single observation) was estimated from the sample variance. This estimate is likely to be more accurate than the range estimate recommended by Ibers (1956) (Tippett, 1925; Davies & Pearson, 1934). The standard errors of these individual variances are high so that an average of them was used. The mean variance for a range of $|F_o|$ was plotted against $|F_o|$, a smooth curve drawn through the points, and the appropriate variances read off (see legend to Table 1).

The variance of the mean $|F_o|$ rather than the population variance is required for least-squares weighting. This was obtained from the relation $\sigma^2(|\bar{F}_o|) = \sigma^2(|F_o|)/n$. The variances needed for the second group of reflexions were given by $\sigma^2(|F_o|)$ ($n=1$ in the above equation). The variance of reflexions in the third group was obtained by the method outlined by Hamilton (1955). In some cases where two or more observations were not considered to be independent, non-integral values for n were used. A complete list of the weights used is given in the legend to Table 1.

Four cycles of full-matrix least-squares refinement using isotropic temperature factors lowered R to 0·14 and $\Sigma w(\Delta F)^2$ to 3·5. Large systematic trends in ΔF with the h index indicated that the atoms of the approximately planar $CuGGGG^{2-}$ ion had their major component of anisotropic thermal motion normal to the plane. Accordingly the refinement was continued with anisotropic thermal parameters.

With the full-matrix least-squares program and computer available (see last paragraph) only 205 of the 271 variables could be refined in the same cycle. Different but overlapping blocks of parameters were therefore refined in successive cycles. (i) The scale factor and the parameters of the copper atom were refined in every cycle. (ii) All or none of the positional parameters of a particular atom were included in the same refinement cycle. (iii) All or none of the thermal parameters of a particular atom were included in the same refinement cycle. (iv) All thermal parameters were var-

ied in the first cycle since the changes in these were expected to be far larger than the changes in the positional parameters. (v) All positional parameters were varied in every cycle after the first, and the thermal parameters were chosen so that each of them was varied at least every second cycle.

The R index fell to 0·097 after the first cycle, then more slowly through three more cycles to 0·092. With few exceptions, the shifts in the parameters had decreased to less than one-half of their standard deviations. Consequently, the refinement was terminated. The final parameters and standard deviations are presented in Table 2. The final scale factor, applied to the F_c, was 1·0372 with a standard deviation of 0·0033.

Description of the structure

Arabic numerals have been used to distinguish between atoms of the same type and Roman numerals to distinguish between atoms in different asymmetric units. The Arabic numerals have been assigned sequentially, beginning at the N-terminus of the peptide molecule. The oxygen atoms of water molecules are numbered from six to fifteen.

The asymmetric units are denoted as follows:

Coordinates			Superscript
x	y	z	none
$1-x$	$1-y$	$1-z$	i
x	$-1+y$	z	ii
$1-x$	$-y$	$1-z$	iii
$-1+x$	y	z	iv
$-x$	$1-y$	$1-z$	v
$-1+x$	$-1+y$	z	vi
x	y	$-z$	vii
x	$1+y$	z	viii
$1-x$	$1-y$	$2-z$	ix
$1+x$	y	z	x
$2-x$	$1-y$	$2-z$	xi
$1+x$	y	$1+z$	xii

The structure consists of layers of discrete $CuGGGG^{2-}$ anions alternating with layers of sodium ion–water molecule octahedra, the layers being parallel to (001). The approximately planar $CuGGGG^{2-}$ anions within the layers do not lie parallel to (001). They are inclined 25° to this plane and are linked edge to edge by hydrogen bonding through water molecule O(15) to form infinite chains parallel to the b axis. The ions in neighbouring chains have no bonded contacts with each other (Fig. 1).

One GGGG molecule is coordinated to one copper atom as a tetradentate chelate with its four nitrogen atoms as ligand atoms, thus forming three five-membered chelate rings. The four Cu–N bonds range from 1·912 to 2·028 Å in length. The $CuGGGG^{2-}$ ion [the

Table 2. *Final atomic parameters*

All values are $\times 10^4$. Standard deviations from the least-squares refinement are in parentheses.
Temperature factor = exp $\{-(b_{11}h^2+b_{22}k^2+b_{33}l^2+2b_{12}hk+2b_{13}hl+2b_{23}kl)\}$.

Atom	x/a	y/b	z/c	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
Cu	3203(07)	6508(06)	5299(06)	123(2)	44(1)	21(1)	−15(1)	7(1)	−4(0)
*Na(1)	5475(26)	1458(21)	8782(22)	222	53	39	−9	−20	−3
*Na(2)	1805(21)	6059(19)	9914(19)	133	58	33	−14	10	O(3)
*C(1)	1590(63)	8335(50)	3967(53)	207	48	38	−20	−13	−2
*C(2)	812(49)	6933(45)	3539(43)	108	54	28	−5	15	−O(4)
*C(3)	894(50)	4608(44)	3832(43)	130	46	26	−19	3	−6
C(4)	1849(48)	3876(44)	4676(41)	104(9)	49(3)	25(2)	−7(4)	13(3)	−6(2)
C(5)	4031(49)	4225(42)	6289(42)	128(9)	42(3)	26(2)	−11(5)	3(3)	−3(2)
C(6)	5134(47)	5435(45)	6921(41)	91(8)	62(4)	24(2)	−17(5)	11(3)	−8(2)
C(7)	5955(51)	7736(45)	7169(43)	139(9)	55(4)	26(2)	−37(5)	17(3)	−14(2)
C(8)	5245(48)	8285(43)	7957(41)	110(9)	50(4)	26(2)	−1(5)	2(3)	0(2)
*N(1)	3182(53)	8337(43)	4843(44)	219	51	35	−13	−5	−4
*N(2)	1461(42)	6017(38)	4065(37)	127	49	26	−6	1	−5
*N(3)	2960(43)	4649(38)	5398(36)	141	50	22	−19	-0_2	−2
*N(4)	4865(41)	6574(36)	6586(34)	117	52	19	−21	5	−6
O(1)	−403(38)	6792(35)	2725(33)	153(8)	64(3)	29(1)	−6(4)	−4(2)	2(2)
O(2)	1547(42)	2632(32)	4631(34)	224(9)	39(3)	32(2)	−23(4)	−8(3)	−5(2)
O(3)	6246(39)	5223(36)	7715(32)	160(8)	69(3)	25(1)	−5(4)	−13(2)	−1(2)
O(4)	3829(37)	7744(38)	8076(36)	129(7)	74(3)	41(2)	−27(4)	26(3)	−10(2)
O(5)	6206(37)	9290(35)	8462(34)	145(7)	59(3)	36(2)	−18(4)	12(3)	−20(2)
O(6)	2760(40)	1229(39)	7503(36)	156(8)	80(4)	36(2)	3(4)	9(3)	3(2)
O(7)	4957(47)	3644(52)	9264(55)	161(9)	113(5)	81(3)	43(6)	−2(4)	−20(3)
O(8)	2564(40)	8316(38)	9620(35)	161(8)	78(4)	34(2)	13(4)	17(3)	3(2)
O(9)	2606(53)	1631(43)	9721(42)	313(12)	71(4)	41(2)	−34(6)	−1(4)	2(2)
O(10)	7805(49)	2946(48)	8410(41)	256(11)	109(5)	43(2)	3(6)	46(4)	2(3)
O(11)	8547(40)	6081(40)	9496(36)	158(8)	76(4)	36(2)	−13(4)	−11(3)	4(2)
O(12)	2280(44)	5348(40)	8471(36)	230(10)	73(4)	32(2)	−18(5)	12(3)	−6(2)
O(13)	667(42)	8740(41)	6978(40)	183(9)	76(4)	43(2)	−6(5)	−4(3)	−13(2)
*O(14)	9797(40)	9825(47)	8533(40)	133	114	44	−16	5	−8
*O(15)	3510(46)	959(39)	5768(41)	246	64	48	20	36	−4

* The thermal parameters of these atoms were not refined in the final refinement cycle.

Table 3(*a*). *Interatomic distances* (Å) *and angles* (°) *of the glycylglycylglycylglycino cuprate(II) ion*

Standard deviations in parentheses are for the last place listed

			Present work				Average in peptides
Cu —N(1)	2·028(5)	Cu —N(2)	1·923(4)	Cu —N(3)	1·912(4)	Cu —N(4)	1·944(4)
N(1)–C(1)	1·490(8)	N(2)–C(3)	1·458(6)	N(3)–C(5)	1·444(6)	N(4)–C(7)	1·460(7)
C(1)–C(2)	1·527(7)	C(3)–C(4)	1·523(7)	C(5)–C(6)	1·527(7)	C(7)–C(8)	1·527(7)
C(2)–O(1)	1·276(6)	C(4)–O(2)	1·267(6)	C(6)–O(3)	1·272(6)		
C(2)–N(2)	1·290(7)	C(4)–N(3)	1·316(6)	C(6)–N(4)	1·301(6)		
						C(8)–O(4)	1·246(6)
						C(8)–O(5)	1·268(6)

			Present work				Average in peptides
Cu —N(1)–C(1)	108·8(3)						
N(1)–C(1)–C(2)	111·5(3)	N(2)–C(3)–C(4)	108·9(3)	N(3)–C(5)–C(6)	108·6(3)	N(4)–C(7)–C(8)	115·6(3)
C(1)–C(2)–N(2)	114·9(3)	C(3)–C(4)–N(3)	113·9(3)	C(5)–C(6)–N(4)	116·7(3)		
C(1)–C(2)–O(1)	117·7(3)	C(3)–C(4)–O(2)	119·7(3)	C(5)–C(6)–O(3)	116·6(3)	C(7)–C(8)–O(4)	120·4(3)
O(1)–C(2)–N(2)	127·4(3)	O(2)–C(4)–N(3)	126·4(3)	O(3)–C(6)–N(4)	126·7(3)		
C(2)–N(2)–C(3)	125·4(3)	C(4)–N(3)–C(5)	125·8(3)	C(6)–N(4)–C(7)	117·3(3)		
C(2)–N(2)–Cu	119·0(3)	C(4)–N(3)–Cu	118·9(3)	C(6)–N(4)–Cu	115·3(2)	C(7)–C(8)–O(5)	115·1(3)
Cu —N(2)–C(3)	115·5(3)	Cu —N(3)–C(5)	115·3(2)	Cu —N(4)–C(7)	127·1(2)	O(4)–C(8)–O(5)	124·5(3)
N(1)–Cu —N(2)	83·5(2)	N(2)–Cu —N(3)	82·8(2)	N(3)–Cu —N(4)	84·0(2)	N(4)–Cu —N(1)	109·8(2)

			Average in peptides
N— C(α)	1·47	N— C(α)–C	110
C(α)–C	1·53	C(α)–C — N	114
C — O	1·24	C(α)–C — O	121
C — N	1·32	O — C — N	125
		C — N — C(α)	123

Table 3 (*b*). *Intervector angles for the hydrogen bonded contacts of the glycylglycylglycylglycino cuprate(II) ion*

Atoms	Angle*
Cu ———N(1)–H · · · O(15^{i})	111·5°
Cu ———N(1)–H · · · O(15^{viii})	133·6
C(1) ———N(1)–H · · · O(15^{i})	104·5
C(1) ———N(1)–H · · · O(15^{viii})	103·7
O(15^{i}) · H–N(1)–H · · · O(15^{viii})	90·7
C(1) ———N(1)———Cu	108·8
C(2) ———O(1) · · · H–O(6^{v})	111·9
C(2) ———O(1) · · · H–O(10^{i})	96·8
C(2) ———O(1) · · · H–O(12^{v})	133·9
O(6^{v})–H · · O(1) · · · H–O(10^{i})	113·0
O(6^{v})–H · · O(1) · · · H–O(12^{v})	107·0
O(10^{i})–H · O(1) · · · H–O(12^{v})	89·8
C(4) ———O(2) · · · H–O(13^{v})	120·5
C(4) ———O(2) · · · H–O(15)	128·0
O(13^{v})–H · O(2) · · · H–O(15)	109·4
C(6) ———O(3) · · · H–O(10)	132·5
C(6) ———O(3) · · · H–O(11)	151·5
O(10)–H · · O(3) · · · H–O(11)	75·9
C(8) ———O(4) · · · H–O(8)	125·6
C(8) ———O(4) · · · H–O(12)	143·0
C(8) ———O(4) · · · H–O(13)	111·8
O(8)–H · · · O(4) · · · H–O(12)	73·0
O(8)–H · · · O(4) · · · H–O(13)	86·4
O(12)–H · · O(4) · · · H–O(13)	100·1
C(8) ———O(5) · · · H–O(9^{ix})	102·4
C(8) ———O(5) · · · H–O(14)	120·1
C(8) ———O(5) xxxxxx Na(1^{viii})	131·9
O(9^{ix})–H · O(5) · · · H–O(14)	89·6
O(9^{ix})–H · O(5) xxxxxx Na(1^{viii})	103·2
O(14)–H · · O(5) xxxxxx Na(1^{viii})	100·0

xxxx is not a hydrogen bond.

* Subtended by atoms other than hydrogen.

carboxyl group C(8), O(4) and O(5) excepted] is close to planar. There are several significant deviations from the least-squares plane of these atoms, but the most remarkable one is that of N(1) which is nearly 0·25 Å away (plane 1, Table 4). The copper atom exhibits fourfold, approximately square-planar, coordination. The fifth and sixth 'octahedral' positions are vacant, the atoms nearest to these positions all being further than 3·65 Å from the copper atom. The four copper atom–nitrogen atom bonds are not coplanar but are

Table 4(*a*). *Coefficients for best* (*least-squares*) *planes*

Each plane is represented by $lX+mY+nZ+p=0$ where X, Y and Z are coordinates in Å, referred to orthogonal axes*

Plane	*l*	*m*	*n*	*p*
1	0·917930	−0·050559	−0·393508	3·67810 Å
2	0·901160	−0·082735	−0·425518	4·01351
3	0·924557	−0·068801	−0·374781	3·61062
4	0·927410	−0·066111	−0·368156	3·55018
5	0·925832	−0·106302	−0·362678	3·71053
6	0·903541	−0·024073	−0·427825	3·66153
7	0·926578	−0·070519	−0·369433	3·58339
8	0·904818	−0·060396	−0·421493	4·02246
9	−0·427456	0·654180	−0·623963	2·10007

* The coordinates X, Y, Z are obtained from the fractional non-orthogonal coordinates x, y, z by the transformation:

$$X = 7{\cdot}6650x - 0{\cdot}7623y - 4{\cdot}0598z$$
$$Y = 10{\cdot}1755y - 1{\cdot}3286z$$
$$Z = 14{\cdot}1093z$$

Table 4(*b*). *Distances* (Å) *from the planes listed in Table* 4(*a*)

	Plane							
Atom	1	2	3	4	5	6	7	8	9
Cu	0·041	−0·046	0·003	0·007	(−0·029)†	(−0·069)	(0·004)	(0·124)	
N(1)	(0·243)	0·114			0·046				
N(2)	0·064	0·056	−0·006		−0·048	0·015			
N(3)	0·000		−0·001	−0·002	0·040		0·004		
N(4)	−0·035			−0·016	−0·039			−0·012	(−0·004)
C(1)	−0·034	−0·113				−0·015			
C(2)	0·001	−0·010				0·009			
C(3)	0·060		0·008			−0·012	0·000		
C(4)	0·008		−0·003				−0·003		
C(5)	−0·052			−0·009			−0·002	0·011	
C(6)	−0·032			0·020				−0·011	
C(7)	0·030							0·013	−0·001
C(8)									0·004
O(1)	−0·053					0·002			
O(2)	−0·010						0·002		
O(3)	0·013							−0·001	
O(4)									−0·001
O(5)									−0·001

† A distance in parentheses indicates that the atom was not included in the least-squares computation.

arranged so that the copper atom is near the centre of an extremely flattened tetrahedron (plane 5, Table 4). The chelate ring Cu,N(1),C(1),C(2),N(2) is not planar but the other two are planar within the limits of experimental error (planes 2, 3 and 4, Table 4).

The three peptide groups Cα–CO–N–C′α and the carboxyl group are each planar (planes 6, 7, 8 and 9, Table 4). Despite the coplanarity of the atoms of the chelate rings, the copper atom is significantly out of the planes of the peptide groups containing N(2) and N(4).

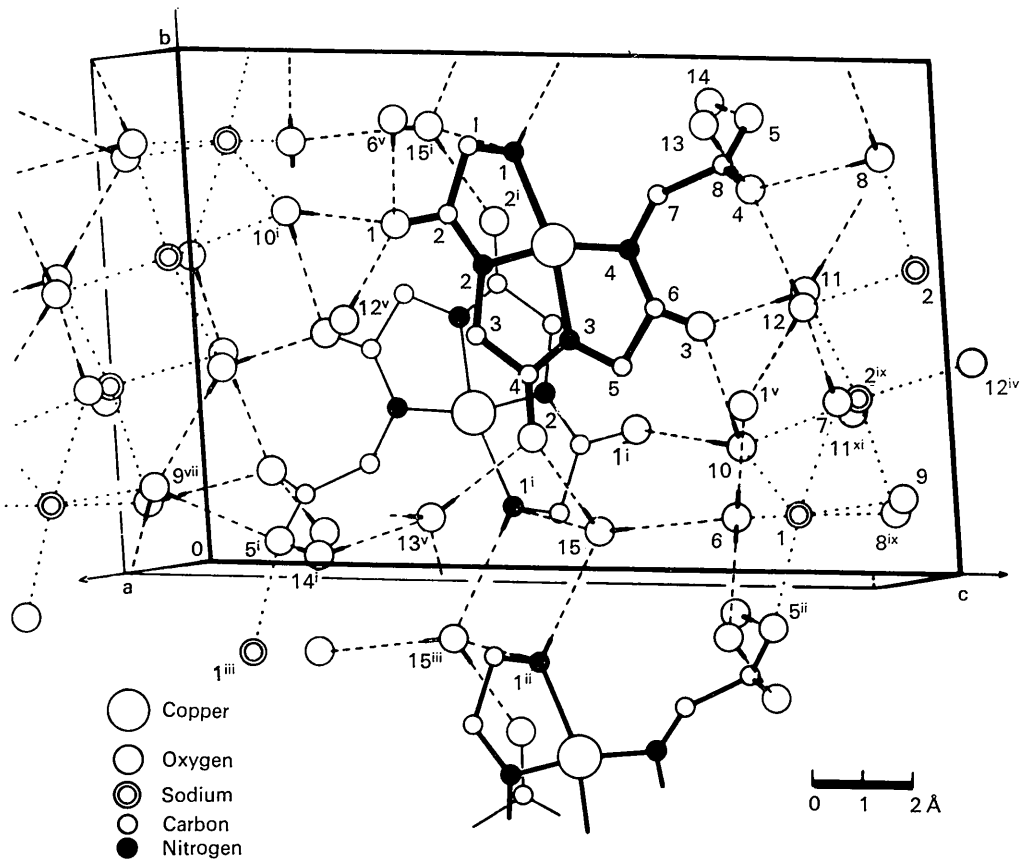

Fig. 1. The structure of disodium glycylglycylglycylglycino cuprate(II) decahydrate viewed along the normal to the (100) plane. Hydrogen bonds are dashed and sodium ion–water molecule contacts dotted. Proton donation in a hydrogen bond is represented by a short full line at the appropriate end of the bond.

The carboxyl group is twisted so that it makes an angle of 79·6° with the mean plane (plane 1, Table 4) of the remaining atoms of the $CuGGGG^{2-}$ ion.

The bond lengths and angles in the ligand are listed in Table 3(*a*) and illustrated in Fig. 4.

Na(1) is surrounded by a distorted octahedron of five water molecules and the carboxyl oxygen atom O(5), while Na(2) is surrounded by a more regular octahedron of six water molecules (Figs. 2 and 3). The relevant interatomic distances and angles are listed in

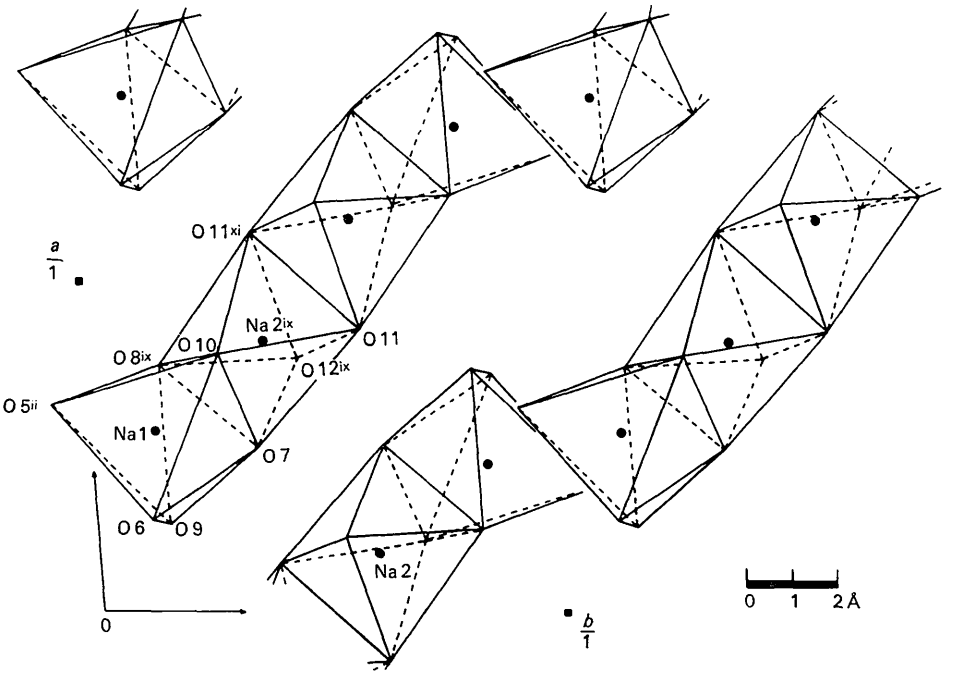

Fig. 2. The sodium ion–water molecule polyhedra viewed along the normal to the (001) plane. Both full and broken lines represent polyhedron edges.

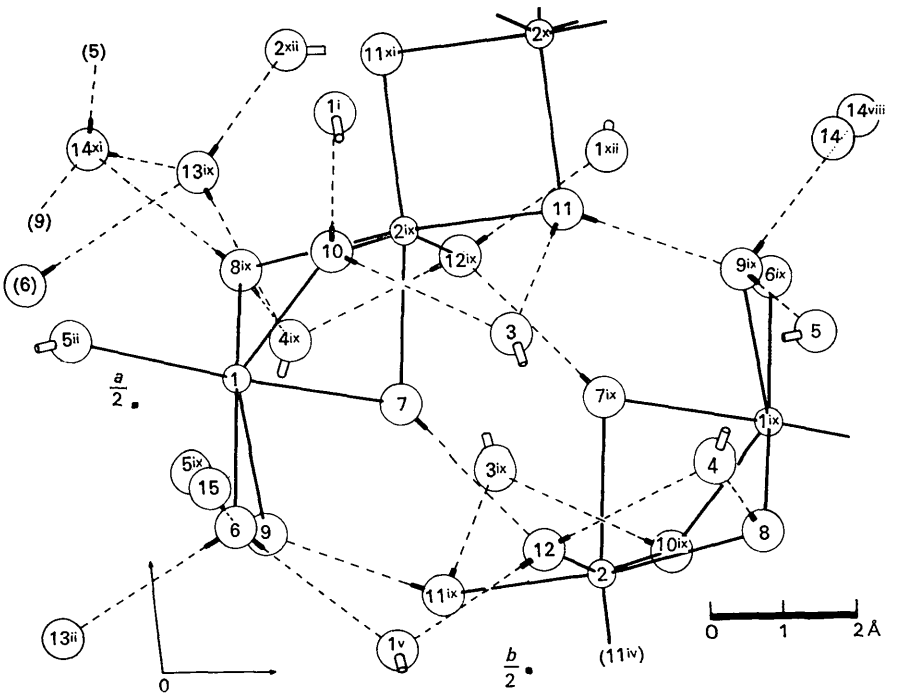

Fig. 3. Bonding associated with the sodium ion–water molecule polyhedra. The region near the origin in Fig. 2 is shown in more detail. Full lines represent electrostatic interactions and broken lines hydrogen bonds. Proton donation in a hydrogen bond is represented by a short full line at the appropriate end of the bond. Small circles represent sodium ions and large circles oxygen atoms.

Table 5. *Environment of water molecules*

−H indicates proton donation to the hydrogen bond from the water molecule and H− donation from the neighbour atom.

Water molecule	Proton assignment	Neighbour atom	Distance (σ)	Angle neighbour-water-neighbour	
O(6)	H−	O(15)	2·809(6) Å	O(15), Na(1)	111·2°
		Na(1)	2·339(5)	O(15), O(1^{v})	103·8
	−H	O(1^{v})	2·779(6)	O(15), O(13^{ii})	87·8
	−H	O(13^{ii})	2·829(6)	Na(1), O(1^{v})	119·6
				Na(1), O(13^{ii})	119·4
				O(1^{v}), O(13^{ii})	109·4
O(7)		Na(1)	2·395(6)	Na(1), Na(2^{ix})	85·8
		Na(2^{ix})	2·397(6)	Na(1), O(9)	67·2
	*	O(9)	2·883(7)	Na(1), O(12)	133·3
	−H	O(12)	2·822(6)	Na(2^{ix}), O(9)	120·7
				Na(2^{ix}), O(12)	134·7
				O(9), O(12)	98·6
O(8)		Na(1^{ix})	2·381(4)	Na(1^{ix}), Na(2)	85·3
		Na(2)	2·433(5)	Na(1^{ix}), O(4)	122·9
	−H	O(4)	2·794(6)	Na(1^{ix}), O(14^{iv})	134·3
	−H	O(14^{iv})	2·868(6)	Na(2), O(4)	98·1
				Na(2), O(14^{iv})	121·2
				O(4), O(14^{iv})	92·0
O(9)		Na(1)	2·950(6)	Na(1), O(11^{ix})	128·9
	H−	O(11^{ix})	2·857(7)	Na(1), O(5^{ix})	110·5
	−H	O(5^{ix})	2·825(6)	Na(1), O(14^{vi})	101·2
	−H	O(14^{vi})	2·780(7)	O(11^{ix}), O(5^{ix})	90·1
				O(11^{ix}), O(14^{vi})	116·0
				O(5^{ix}), O(14^{vi})	108·9
O(10)		Na(1)	2·471(6)	Na(1), Na(2^{ix})	81·0
		Na(2^{ix})	2·550(5)	Na(1), O(1^{i})	145·3
	−H	O(1^{i})	2·984(7)	Na(1), O(3)	109·7
	−H	O(3)	2·807(6)	Na(2^{ix}), O(1^{i})	128·9
				Na(2^{ix}), O(3)	88·9
				O(1^{i}), O(3)	90·3
O(11)		Na(2^{ix})	2·434(5)	Na(2^{ix}), Na(2^{x})	91·9
		Na(2^{x})	2·385(5)	Na(2^{ix}), O(3)	122·3
	−H	O(3)	2·743(6)	Na(2^{ix}), O(9^{ix})	114·1
	−H	O(9^{ix})	2·857(7)	Na(2^{x}), O(3)	92·8
				Na(2^{x}), O(9^{ix})	120·9
				O(3), O(9^{ix})	111·9
O(12)		Na(2)	2·360(5)	Na(2), O(7)	95·6
	H−	O(7)	2·822(6)	Na(2), O(1^{v})	125·3
	−H	O(1^{v})	2·732(6)	Na(2), O(4)	99·3
	−H	O(4)	2·814(6)	O(7), O(1^{v})	86·4
				O(7), O(4)	112·6
				O(1^{v}), O(4)	130·2
O(13)	H−	O(6^{viii})	2·829(6)	O(6^{viii}), O(14^{iv})	74·3
	H−	O(14^{iv})	2·780(6)	O(6^{viii}), O(2^{v})	137·3
	−H	O(2^{v})	2·702(6)	O(6^{viii}), O(4)	85·2
	−H	O(4)	2·785(6)	O(14^{iv}), O(2^{v})	129·6
				O(14^{iv}), O(4)	94·1
				O(2^{v}), O(4)	121·1
O(14^{iv})	H−	O(8)	2·868(6)	O(8), O(9^{viii})	73·1
	H−	O(9^{viii})	2·797(7)	O(8), O(5^{iv})	120·1
	−H	O(5^{iv})	2·734(6)	O(8), O(13)	85·1
	−H	O(13)	2·780(6)	O(9^{viii}), O(5^{iv})	131·2
				O(9^{viii}), O(13)	113·0
				O(5^{iv}), O(13)	114·8
O(15)	H−	N(1^{i})	2·997(7)	N(1^{i}), N(1^{ii})	89·3
	H−	N(1^{ii})	2·884(7)	N(1^{i}), O(2)	91·6
	−H	O(2)	2·695(6)	N(1^{i}), O(6)	134·2
	−H	O(6)	2·809(6)	N(1^{ii}), O(2)	113·0
				N(1^{ii}), O(6)	118·1
				O(2), O(6)	107·7

* Not a hydrogen-bond.

Tables 5 and 6. The octahedra surrounding Na(1) and Na(2^{ix}) have three water molecules in common [O(7), O(8^{ix}), and O(10)] and therefore may be said to share a face. This unit is related to another across a centre of symmetry, so that the octahedra surrounding adjacent atoms of type Na(2) share an edge of two water molecules [O(11) and O(11^{xi})]. These short chains of four octahedra extend obliquely across the sodium ion–water molecule layer, being linked at each side of the layer to a $CuGGGG^{2-}$ ion *via* its carboxyl oxygen atom O(5) (Fig. 2).

Table 6. *Interatomic distances and intervector angles of the sodium octahedra*

Distances from Na(1)

Atom	D(Å)	σ(Å)
O(5^{ii})	2·376	0·004
O(6)	2·339	0·005
O(7)	2·395	0·006
O(8^{ix})	2·381	0·004
O(9)	2·950	0·006
O(10)	2·471	0·006

Distances from Na(2^{ix})

Atom	D(Å)	σ(Å)
O(11)	2·434	0·005
O(11^{xi})	2·385	0·005
O(7)	2·397	0·006
O(8^{ix})	2·433	0·005
O(12^{ix})	2·360	0·005
O(10)	2·550	0·005

Other distances

Atoms	D(Å)	σ(Å)
O(7)–O(8^{ix})	3·076	0·007
O(7)–O(10)	2·946	0·007
O(8^{ix})–O(10)	3·358	0·007

Angles subtended at Na(1) by

Atoms	Angle
O(5^{ii}), O(6)	93·5°
O(5^{ii}), O(7)	174·1
O(5^{ii}), O(8^{ix})	94·0
O(5^{ii}), O(9)	115·4
O(5^{ii}), O(10)	105·3
O(6), O(7)	92·1
O(6), O(8^{ix})	159·3
O(6), O(9)	77·3
O(6), O(10)	109·1
O(7), O(8^{ix})	80·2
O(7), O(9)	64·3
O(7), O(10)	74·5
O(8^{ix}), O(9)	82·1
O(8^{ix}), O(10)	87·4
O(9), O(10)	138·6

Angles subtended at Na(2^{ix}) by

Atoms	Angle
O(11), O(11^{xi})	88·1°
O(11), O(7)	92·4
O(11), O(8^{ix})	169·2
O(11), O(12^{ix})	99·0
O(11), O(10)	86·4
O(11^{xi}), O(7)	163·9
O(11^{xi}), O(8^{ix})	98·2
O(11^{xi}), O(12^{ix})	103·5
O(11^{xi}), O(10)	91·0
O(7), O(8^{ix})	79·1
O(7), O(12^{ix})	92·4
O(7), O(10)	73·0
O(8^{ix}), O(12^{ix})	88·2
O(8^{ix}), O(10)	84·7
O(12^{ix}), O(10)	164·7

No attempt was made to find the positions of hydrogen atoms but their presence in hydrogen bonds has been inferred by considering interatomic distances and angles. Eight of the thirty hydrogen atoms present are bonded to carbon atoms and do not form hydrogen bonds. Nineteen hydrogen atoms of water molecules and two bonded to N(1) have been assigned to hydrogen bonds. One hydrogen atom of water molecule O(7) does not seem to be involved in a hydrogen bond. The only two atoms apart from O(12) which could be hydrogen bonded to O(7) are O(9) (2·833 Å) and O(3) (3·242 Å). A hydrogen bond to O(9) would be directed along the O(7)–O(9) edge of the Na(1) coordination polyhedron and is therefore unlikely (Templeton, 1960). Such a bond would also disrupt the very reasonable tetrahedral environment proposed for O(9). A bond to O(3) would be too long and O(3) is not in a favourable orientation to the O(7)–O(12) vector.

The $CuGGGG^{2-}$ ion makes twelve acceptor and two donor hydrogen bonds with neighbouring water molecules, ranging from 2·695 Å to 2·997 Å in length (Table 5).O(1) and O(4) each make three hydrogen bonds and O(2), O(3) and O(5) each make two. The environments of O(1), O(4) and O(5) are approximately tetrahedral [O(5) has an interaction with Na(1)] and those of O(2) and O(3) are approximately trigonal planar. N(1^{i}) is hydrogen bonded to O(15) and O(15^{iii}). Intervector angles for these hydrogen bonds are listed in Table 3(*b*).

There are no direct links between neighbouring peptide chains but there are many links *via* one or two water molecules. Water molecule O(15) links three peptide chains. Every water molecule [except O(7)] is attached to atoms of the $CuGGGG^{2-}$ ion and is bound to four neighbouring atoms arranged at the corners of a more or less distorted tetrahedron.

Discussion

The copper atom–amide nitrogen atom bond lengths are similar to those found in other metal–peptide structures, but they are not equal. The difference between the lengths of Cu–N(3) and Cu–N(4) of 0·032 Å (s.d. 0·006 Å) is probably significant. It should be noted that the short Cu–N(3) bond is complemented by a C(4)–N(3) bond which is longer than the two other examples of this type of bond (Table 3).

The non-planarity of the four nitrogen ligand atoms is not caused by direct steric interference from other elements of the structure since all non-bonded contacts of these atoms are at normal van der Waals lengths or longer, and the environment of each atom is similar on both sides of the planar anion. The non-planarity is possibly caused by the strain which results from closing the five-membered chelate rings. The first ring [Cu, N(1), C(1), C(2), N(2)] has a tetrahedrally hybridized nitrogen atom in place of the trigonal one in the other two rings. The strain is therefore greatest in the first ring which is buckled so that N(1) is 0·24 Å from the plane of the other four atoms. In this connected system, this deviation causes N(1) to be out of the plane of N(2), N(3) and N(4).

Rings corresponding to the first ring of this compound also occur in glycylglycino copper(II) trihydrate (Strandberg, Lindqvist & Rosenstein, 1961) and sodium glycylglycylglycino cuprate(II) hydrate (Freeman, Schoone & Sime, 1965). In both these compounds this ring is non-planar. Calculations have shown that the tetrahedrally hybridized nitrogen atoms are 0·196 Å

and 0·206 Å out of the plane of the other four atoms of the rings in the two crystallographically distinct examples in the first compound, and 0·210 Å out of the plane in the second compound.

There are some indications of a correlation between the lengths of the peptide C–N and C=O bonds. The C–N bond-lengths (C(4)–N(3), 1·316 Å; C(6)–N(4), 1·301 Å; C(2)–N(2), 1·290 Å) run in the reverse order to the corresponding C=O bond-lengths (C(4)–O(2), 1·267 Å; C(6)–O(3), 1·272 Å; C(2)–O(1), 1·276 Å). While the difference between the two extreme C=O bond-lengths is only on the threshold of significance, the trend in these bonds parallels the number of hydrogen bonds (two, two, three) in which the carbonyl oxygens are acceptors. The hydrogen bonds possibly stabilize the contribution of

$$-C=N<^{(+)}_{O(-)}$$

to the resonance hybrid and so contribute to the shortening of the C–N and the lengthening of the C=O bonds. A similar situation was found in cytosine monohydrate by Jeffrey & Kinoshita (1963).

The environment of the copper atom in this complex is similar to that of the copper in potassium bisbiureto cuprate(II) tetrahydrate (Freeman, Smith & Taylor, 1961). In both complexes the copper(II) atoms are square-planar coordinated by four nitrogen ligand atoms, of which three are deprotonated in the present complex, and four in the other. The similarity of the

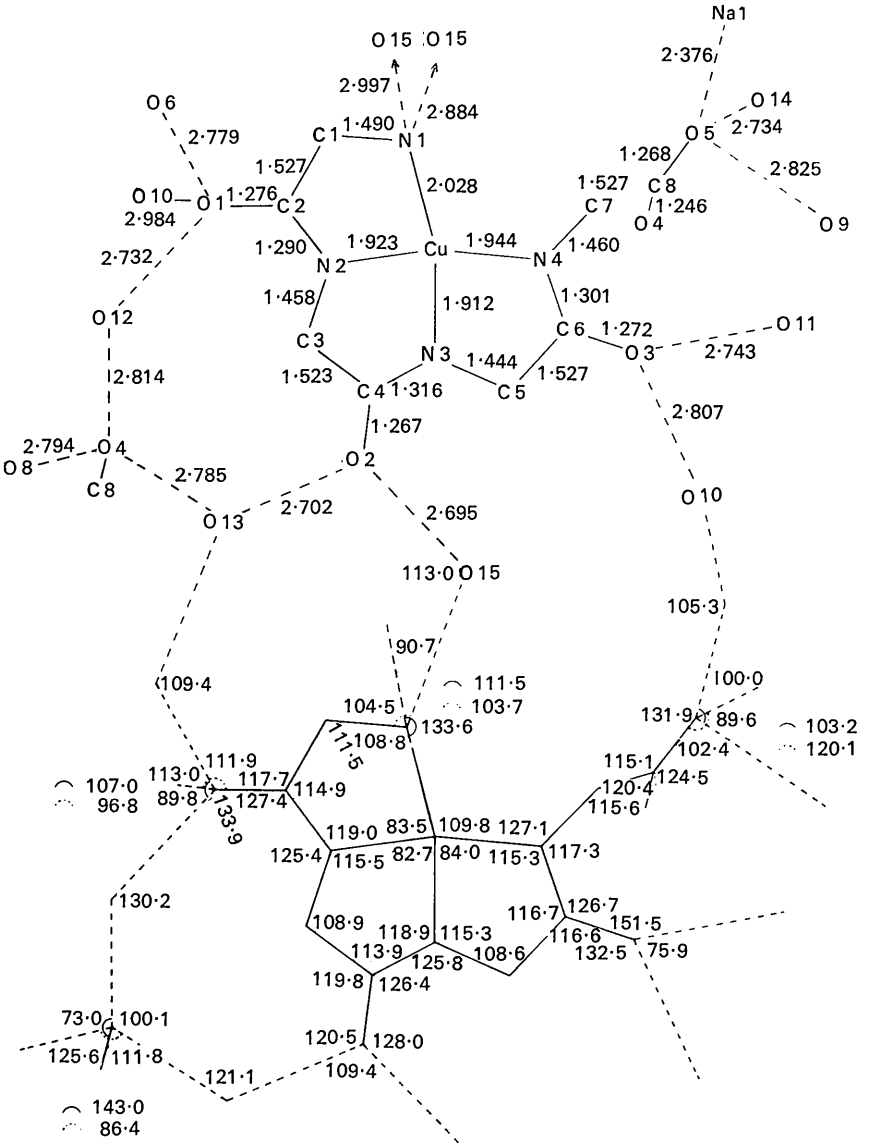

Fig. 4. Interatomic distances (above) and intervector angles (below) of the glycylglycylclycylglycino cuprate(II) anion and its contacts. This diagram is not to scale though the general relationship between atomic positions has been retained. Roman superscripts have been omitted.

ligand fields results in a similarity of the colours (pink) of the crystals. The structure of the complexes appears to persist in solution, for the solution spectra also resemble each other,

$[\lambda_{max}(CuGGGG^{2-}) = 520$ mμ (Koltun *et al.*, 1963);
$\lambda_{max}(Cu(biu)_2^{2-}) = 505$ mμ (Ferguson, private communication in Freeman, Smith & Taylor, 1961],

and the solution spectrum of $Cu(biu)_2^{2-}$ is almost identical with the crystal spectrum (Ferguson, private communication).

In the solid state this structure, therefore, supports the deductions made by Koltun, Roth & Gurd (1963) from the results of *p*H titration and spectrophotometric studies, that the hydrogen atoms of the three peptide nitrogen atoms are ionized in alkaline solution. It also supports the structure proposed for the oxytocin complex of copper(II) by Breslow (1961), since her conclusions included a consideration of the similarity between the spectra of the copper-GGGG and the copper–oxytocin complexes.

In the crystalline metal–peptide complexes examined to this date, every terminal amino nitrogen atom is bonded to the copper(II) ion, indicating that this may be an essential requirement for chelation of a peptide chain to copper(II). This view is supported by Breslow's (1961) observation that complex formation with deamino–oxytocin (in which the free amino group is substituted by hydrogen) occurs only at *p*H's higher than 12 and then only slightly.

The calculations for this analysis were made on the SILLIAC with programs devised by Dr F. M. Lovell, Dr J. G. Sime and M.R.T., and on the IBM 7090 computer at the Weapons Research Establishment, Salisbury, with programs ORXLS, ORXFE, and ORFLS (Busing & Levy, 1959*a*, *b*; Martin, Busing & Levy, 1962). The research was undertaken during the tenure of an Australian Institute of Nuclear Science and Engineering Post-Graduate Research Studentship by M.R.T., and was supported by Grant GM10867 from the Division of General Medical Sciences, U.S. Public Health Service.

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