The Crystal and Molecular Structure of L-Citrulline Hydrochloride*

By P. S. Naganathan and K. Venkatesan

Centre of Advanced Study in Physics, University of Madras, Madras-25, India

The crystal structure of L-citrulline hydrochloride, $C_6H_{13}N_3O_3 . HCl$, has been determined using three-dimensional photographic data. The crystals are monoclinic, space group $C2$, with cell dimensions $a = 17 \cdot 86$, $b = 5 \cdot 09$, $c = 11 \cdot 77$ Å and $\beta = 106 \cdot 8°$. Refinement was carried out with full-matrix least-squares methods, including anisotropic thermal parameters for all non-hydrogen atoms, to a final R index of 0·071 for 920 observed reflexions. The crystal structure is stabilized by a three-dimensional network of hydrogen bonds. The backbone and side-group conformations of the citrulline molecule have been studied. The side-group conformation is similar to that of arginine in the structure of L-arginine dihydrate.

Introduction

The X-ray crystallographic investigation of amino acids, peptides and related compounds is being carried out in this laboratory to obtain information regarding the backbone and side-chain conformations. It has been shown that comparative studies of the crystal structures of amino acids and peptides are useful in determining the effect of molecular environment on the conformation of amino acid side-chains (Lakshminarayanan, Sasisekharan & Ramachandran, 1967). L-Citrulline is of interest because this molecule, with ornithine and arginine, is an intermediate in the physiologically important urea cycle. The crystal and molecular structure of ornithine (Chiba, Ukei, Ashida, Sasada & Kakudo, 1967; Kalyanaraman & Srinivasan, 1970) and arginine (Karle & Karle, 1964; Dow, Jensen, Mazumdar, Srinivasan & Ramachandran, 1970; Mazumdar, Venkatesan, Mez & Donohue, 1969) in their different modifications have already been studied. The present paper gives an account of the investigation of L-citrulline hydrochloride (I).
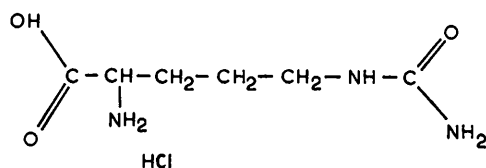
(I)

Experimental

L-Citrulline was dissolved in concentrated hydrochloric acid and crystals in the form of thin plates were obtained by slow evaporation. Rotation, Weissenberg and precession photographs were taken with Cu $K\alpha$ ($\lambda = 1 \cdot 5418$ Å) radiation. The crystal data are

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Crystal system	Monoclinic
Cell dimensions	$a = 17 \cdot 86 \pm 0 \cdot 02$ Å
	$b = 5 \cdot 09 \pm 0 \cdot 01$
	$c = 11 \cdot 77 \pm 0 \cdot 02$
	$\beta = 106° 48' \pm 12'$
Space group	$C2(hkl$, absent for
	$h + k = 2n + 1)$
Molecular formula	$C_6N_3O_3H_{14}Cl$
F.W.	211·6
Number of molecules in the cell	$Z = 4$
F(000)	448
Measured density by flotation	1·352 g.cm^{-3}
Calculated density	1·371 g.cm^{-3}

The intensity data were collected by rotation about the b axis by the multiple-film Weissenberg equi-inclination technique using Cu $K\alpha$ radiation ($\mu = 33 \cdot 3$ cm^{-1}) from $k = 0$ to 4 layers. A crystal of cross section $0 \cdot 10 \times 0 \cdot 15$ mm was employed and absorption corrections were neglected. The intensities were measured visually using a calibrated set of intensities and corrected for Lorentz and polarization factors. They were placed on an approximate scale by layerwise Wilson (1942) plots. Of 1126 reflexions within the region surveyed 920 were observed.

Structure determination and refinement

Because of the shortness of the b axis (5·09 Å) attempts were made to solve the structure from the $h0l$ projection. The position of the chlorine was deduced from the Patterson projection on the b plane. A chlorine-phased Fourier synthesis and a weighted β synthesis (Ramachandran & Raman, 1959) were computed for this projection. By comparing these two maps and after a few trials a satisfactory orientation of the molecule was obtained. The approximate x and z coordinates for all the light atoms were derived and the R index after the inclusion of the light atoms for the

h0l data was 0·35. A chlorine-phased three-dimensional Fourier synthesis was used to determine the *y* coordinates of all the light atoms. The *R* index, with chlorine alone, for the three-dimensional data was 0·48. After introducing contributions from the light atoms the *R* index dropped to 0·40.

Four cycles of full-matrix least-squares refinement were carried out on a CDC 3600 computer using the program of Gantzel, Sparks & Trueblood (1961). The quantity minimized was $\Sigma w(|F_o| - k|F_c|)^2$. All reflexions were given unit weight during the initial stages of refinement. This refinement of the positional coordinates, individual isotropic vibration parameters and the layer scale factors reduced the *R* index to 0·14. At this stage a final editing was done in which a few reflexions were corrected. Further refinement of two cycles with a weighting function of the form

$$w = \frac{1}{14 \cdot 0 + |F_o| + 0 \cdot 02|F_o|^2}$$

(Cruickshank, 1961) brought the agreement factor to 0·106. A difference electron density map showed peaks at positions where the hydrogen atoms were expected to occur, with heights varying from 0·2 to 0·4 e.Å^{-3}. Further, the map showed that the chlorine atom was vibrating anisotropically.

At this stage all the observed data were placed on the same scale and two more cycles of least-squares refinement, using anisotropic thermal parameters for chlorine alone and refining the overall scale factor, resulted in an *R* index 0·087. The hydrogen atoms were included in the structure factor calculations only and were given the isotropic temperature factors of the heavy atom to which they are bonded. A further three cycles of refinement with anisotropic thermal parameters for the chlorine atoms as well as for the twelve C, N and O atoms and an overall scale factor reduced the *R* index for the 920 observed reflexions to 0·071. In the last cycle the maximum parameter shift for the non-hydrogen atoms was less than one fifth of the standard deviation. The final positional and thermal parameters for the non-hydrogen atoms and their standard deviations are listed in Table 1(*a*) and (*b*), and those for hydrogen atoms in Table 2. It must be emphasized that the limitations of the data and the scaling procedure preclude any extensive discussion of anisotropic thermal motion in this structure. The final electron density distribution is shown in Fig. 1. The observed and calculated structure factors are recorded in Table 3. Of the 206 unobserved reflexions, 56 calculated about 1·5 times F_{min}. The reflexions too weak to be observed were assigned an intensity of $I_{min}/2$ (Hamilton, 1955). However in the case of unobserved (*h0l*) reflexions an intensity of $I_{min}/3$ was assigned.

Table 1. *Positional and thermal parameters of the non-hydrogen atoms and their standard deviations* ($\times 10^4$)

(*a*) Positional parameters

	X	Y	Z
Cl	0·1568 (1)	0·2500	0·1480 (1)
C(1)	0·1490 (4)	0·6076 (18)	0·4834 (5)
C(2)	0·1354 (4)	0·8172 (17)	0·3910 (6)
C(3)	0·0505 (4)	0·8501 (17)	0·3238 (6)
C(4)	0·0094 (4)	0·6064 (18)	0·2688 (6)
C(5)	−0·0719 (4)	0·6777 (17)	0·1897 (6)
C(6)	−0·1624 (4)	0·3141 (17)	0·1711 (5)
N(1)	0·1825 (3)	0·7653 (17)	0·3106 (4)
N(2)	−0·1146 (3)	0·4606 (16)	0·1287 (5)
N(3)	−0·2083 (3)	0·1399 (17)	0·0965 (5)
O(1)	0·1921 (3)	0·4240 (13)	0·4835 (4)
O(2)	0·1146 (4)	0·6495 (16)	0·5603 (5)
O(3)	−0·1648 (3)	0·3293 (13)	0·2741 (4)

Table 2. *Positional parameters of the hydrogen atoms*

	Bonded to	x	y	z	B
H(1)	N(1)	0·167	0·567	0·267	2,98 Å^2
H(2)	N(1)	0·167	0·933	0·267	2·98
H(3)	N(1)	0·233	0·733	0·367	2·98
H(21)	C(2)	0·150	0·983	0·433	3·16
H(31)	C(3)	0·033	1·000	0·250	3·19
H(32)	C(3)	0·017	0·900	0·383	3·19
H(41)	C(4)	0·000	0·467	0·333	2·71
H(42)	C(4)	0·033	0·533	0·200	2·71
H(51)	C(5)	−0·100	0·767	0·233	3·19
H(52)	C(5)	−0·067	0·833	0·133	3·19
H(4)	N(2)	−0·100	0·400	0·050	3·13
H(5)	N(3)	−0·200	0·116	0·017	3·36
H(6)	N(3)	−0·250	0·033	0·130	3·36
H(7)	O(2)	0·125	0·486	0·617	4·41

Table 1 (*cont.*)

(*b*) Thermal parameters

	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Cl	0·0037 (0)	0·0310 (10)	0·0058 (1)	0·0005 (5)	0·0040 (1)	0·0034 (6)
C(1)	0·0027 (2)	0·0271 (37)	0·0039 (4)	−0·0013 (16)	0·0017 (5)	0·0028 (20)
C(2)	0·0025 (2)	0·0279 (45)	0·0061 (5)	0·0031 (15)	0·0013 (6)	0·0023 (21)
C(3)	0·0027 (2)	0·0262 (41)	0·0054 (5)	0·0031 (15)	0·0008 (6)	0·0022 (20)
C(4)	0·0025 (2)	0·0260 (36)	0·0052 (5)	0·0011 (16)	0·0016 (5)	−0·0015 (22)
C(5)	0·0025 (2)	0·0291 (47)	0·0061 (5)	−0·0004 (14)	0·0012 (6)	−0·0004 (21)
C(6)	0·0022 (2)	0·0270 (42)	0·0051 (4)	−0·0016 (13)	0·0019 (5)	−0·0032 (19)
N(1)	0·0026 (2)	0·0283 (31)	0·0058 (4)	−0·0009 (16)	0·0024 (4)	0·0032 (24)
N(2)	0·0026 (2)	0·0400 (39)	0·0051 (4)	0·0051 (14)	0·0021 (5)	−0·0011 (19)
N(3)	0·0026 (2)	0·0465 (39)	0·0055 (4)	−0·0050 (15)	0·0021 (5)	−0·0055 (21)
O(1)	0·0032 (2)	0·0275 (29)	0·0059 (4)	0·0019 (13)	0·0017 (4)	0·0020 (16)
O(2)	0·0050 (2)	0·0544 (43)	0·0074 (5)	0·0132 (17)	0·0053 (6)	0·0115 (22)
O(3)	0·0032 (2)	0·0404 (35)	0·0049 (3)	−0·0040 (12)	0·0029 (4)	−0·0035 (16)

The temperature factor is in the form $T = \exp[-(B_{11}h^2 + B_{22}k^2 + B_{33}l^2 + B_{12}hk + B_{13}hl + B_{23}kl)]$

Atomic scattering factors used for Cl⁻, O, N, C and H are those listed in *International Tables for X-ray Crystallography* (1962).

Description of the structure

(a) Crystal packing and hydrogen bonding

The packing of the citrulline molecules is characterized by a three-dimensional network of hydrogen bonds. Fig. 2. illustrates the packing of the molecules in the crystal lattice. There are seven protons in the molecule which can take part in hydrogen bonding and all of them are involved in intermolecular hydrogen bonding. The hydrogen bond distances and angles are recorded in Table 4. Each nitrogen is a donor in

the structure; N(1) for three, N(2) for one and N(3) for two hydrogen bonds. As in many other amino acid and peptide structures (*e.g.* Chiba, Ukei, Ashida, Sasada & Kakudo, 1967; Mallikarjunan, Thyagaraja Rao, Venkatesan & Sarma, 1969), the amino nitrogen has a fourth neighbour at a distance N(1)···O(3) 2·898 Å. From consideration of the various angles and distances involving N(1), H(3), O(1) and O(3) (Table 4 and Fig. 3) it appears that atoms O(1) and O(3) form a bifurcated hydrogen bond with N(1) as found to occur, for example, in α-glycine (Albrecht & Corey, 1939; Marsh, 1958; Donohue, 1967). In the light of large positional errors associated with hydrogen atoms it is possible that the N(1)···O(3^{iv}) interaction is an ionic contact. In order to find out the nature of the

Table 3. *Observed and calculated structure factors*

contact N(1)$\cdots$O(3^{iv}) the positions of the hydrogen atoms of the NH$_3^+$ group were fixed from geometrical considerations using (i) the bond length N–H = 1·0 Å; (ii) a tetrahedral disposition of the hydrogen atoms at the nitrogen atom and (iii) a staggered disposition of the N–H bonds. The pertinent bond lengths and

angles involving atoms N(1), H(3), O(1) and O(3) are given within brackets in Table 4. The values seem to indicate that atoms O(1) and O(3) form a bifurcated hydrogen bond with N(1)

The hydrogen bond between O(2) and O(3) is strong and is of length 2·500 Å. This observation coupled with the bond lengths of C(1)–O(2) (1·250 Å) shows that the proton is attached to O(2). Further, the smaller value for the angle O(2)–C(1)–C(2) (113°) than the angle O(1)–C(1)–C(2) (121·5°) is consistent with the observation.

The chlorine ion accepts five hydrogen bonds.

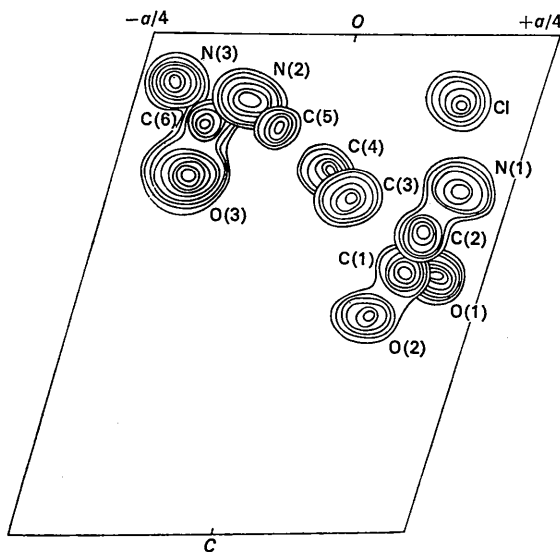Fig. 1. Composite diagram of the final three-dimensional electron density map. The contours are drawn at intervals of 1e.Å^{-3} for light atoms and for chlorine at intervals of 4e.Å^{-3}.
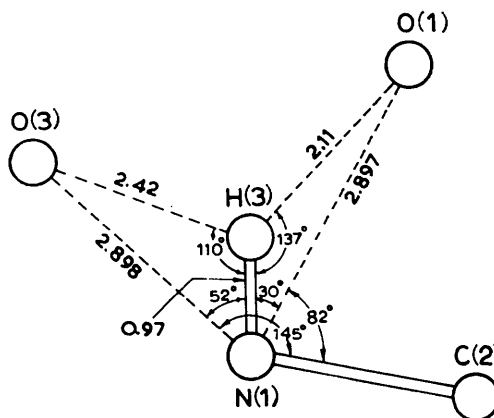

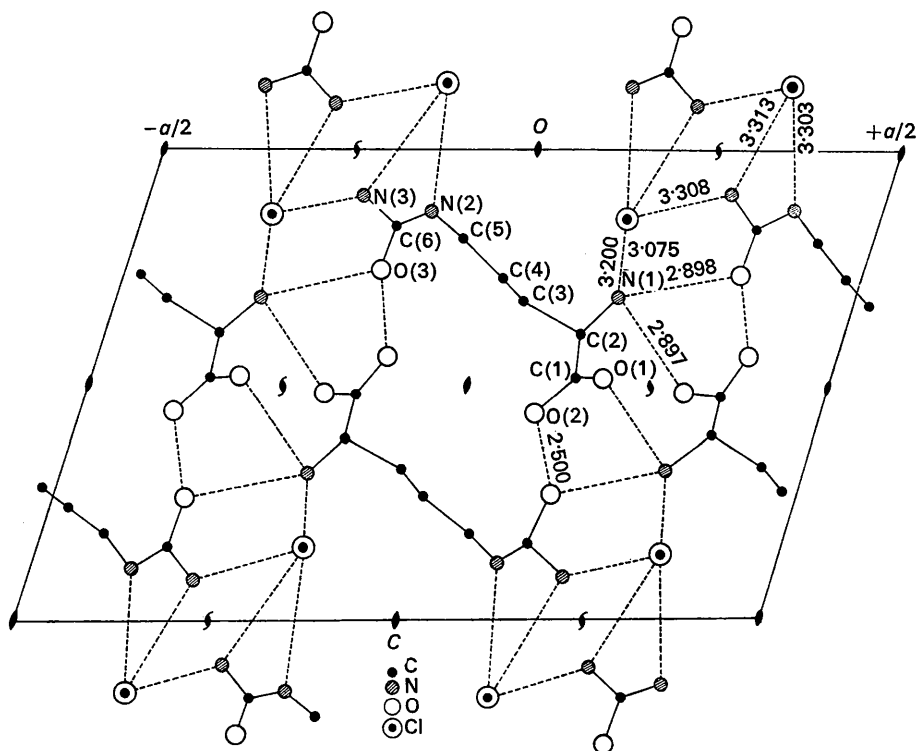Fig. 3. Surroundings of amino nitrogen N(1) in the structure.

Fig. 2. Packing of the structure viewed down the b axis.

Table 4. *Hydrogen bond lengths and angles*

Donor	Acceptor	$D \cdots A$	$H \cdots A$	$C - D \cdots A$	$D - H \cdots A$	$H - D \cdots A$
C(2)–N(1)–H(1)···Cl^i		3·200 Å	2·11 Å	122°	161°	12°
C(2)–N(1)–H(2)···Cl^{ii}		3·074	2·11	104	163	11
C(2)–N(1)–H(3)···O(1^v)		2·897	2·11	82	137	30
			(2·10)		(136)	(30)
C(2)–N(1)–H(3)···O(3^{iv})		2·898	2·42	145	110	52
			(2·23)		(123)	(40)
C(6)–N(2)–H(4)···Cl^{iii}		3·303	2·38	101	142	26
C(6)–N(3)–H(5)···Cl^{iii}		3·312	2·38	101	154	18
C(6)–N(3)–H(6)···Cl^{iv}		3·308	2·25	128	163	13
O(1)–O(2)–H(7)···O(3^{iii})		2·500	1·50	108	156	14

Symmetry code

i	$x,$	$y,$	z
ii	$x,$	$1+y,$	z
iii	$-x,$	$y,$	$-z$
iv	$\frac{1}{2}+x,$	$\frac{1}{2}+y,$	z
v	$\frac{1}{2}-x,$	$\frac{1}{2}+y,$	$1-z$

(b) *Bond distances and angles*

The bond lengths and angles in the citrulline molecule are given in Fig. 4. The estimated standard deviation of the bond lengths and angles among C, N and O are about 0·011 Å and 0·8° respectively. The average value of the four C–C bond lengths is 1·503 Å and the average of the four C–C–C angles is 112·2°. The bond
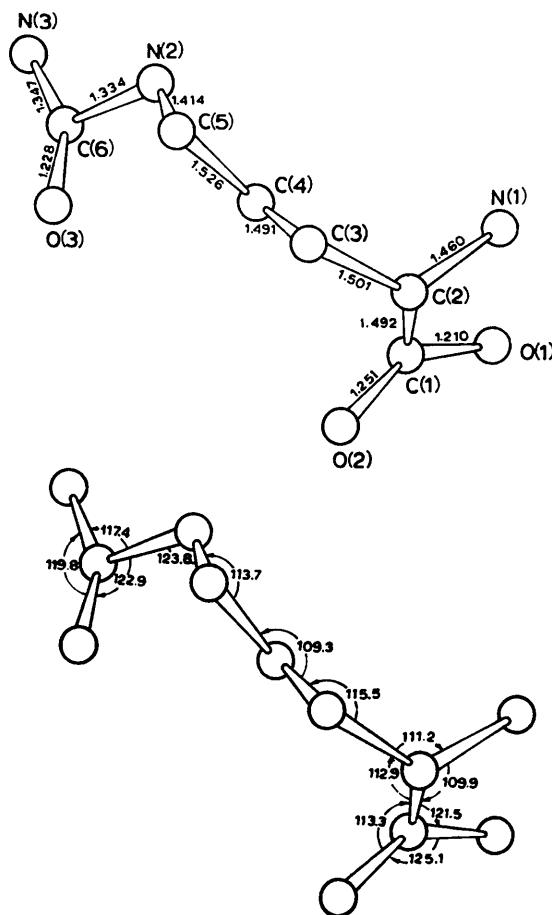

Fig. 4. Bond lengths and angles in the molecule.

lengths involving hydrogen atoms range from 0·93 to 1·13 Å. It is interesting to note that the value of 115·5° for the angle at C^β [C(2)–C(3)–C(4)] is significantly larger than the tetrahedral value. A similar situation has been observed in other amino acids such as L-lysine hydrochloride dihydrate (Wright & Marsh, 1962), L-arginine dihydrate (Karle & Karle, 1964), L-arginine hydrochloride (Mazumdar et al., 1969) and L-arginine hydrochloride monohydrate (Dow et al., 1970); the average value of the angle at C^β in the above mentioned structures is 114°. The values of the bond angle at C^δ [C(4)–C(5)–N(2)] in the present structure and in L-arginine HCl . H$_2$O (Dow et al., 1970) are 113·7 and 113·4° respectively. The widening of the angles in these structures appears to be due to overcrowding of atoms in the regions close to these atoms.

The C–NH$_3^+$ length of 1·461 Å is smaller than the average value of 1·487 Å for this distance in amino acids and peptides (Marsh & Donohue, 1967). Further the bond length C(5)–N(2) is rather short (1·415 Å) and this value differs significantly from the value of 1·472 Å suggested by Sutton (1965).

The side chain terminal group OCNH$_2$NH, which corresponds to the urea fraction, has dimensions close to the bond lengths and angles obtained in the structure of urea (Worsham, Levy & Peterson 1957; Vaughan & Donohue, 1952) and also the urea fraction in the structure of potassium salt of allantoic acid (Ringertz, 1969). The values of bond lengths and angles pertaining to urea are recorded in Table 5 along with the values observed in citrulline.

Table 5. *Bond lengths and angles observed in urea*

	X-ray analysis*	Neutron diffraction analysis†	This work
C–O	1·262 Å	1·243 Å	1·228 Å
C–N	1·335	1·351	1·347
C–N	1·335	1·351	1·334
N–C–O	121·0°	121·5°	122·9°
N–C–N	118·0	117·0	117·4
N–C–O	121·0	121·5	119·8

* Vaughan & Donohue (1952)
† Worsham, Levy & Peterson (1957)

As may be seen from Table 6, the citrulline molecule is characterized by three planar groups, the carboxylate group, the urea fraction and the aliphatic chain. The equations for the best planes of the carboxylate group, the urea fraction and the group of atoms C(2), C(3), C(4), C(5) and N(2) are given in Table 6 together with their deviations from the respective planes. It may also be seen from Table 6 that the deviation of amino nitrogen, N(1), from the plane of the carboxylate group is very small (0·091 Å). The dihedral angle between the planes I and II is 91° and that between II and III is 87°.

(c) Molecular configuration

The conformation of the amino acid is best understood from a study of the sequence of partial conformations along the chain C(1)–C(2)–C(3)–C(4)–C(5)–N(2). Fig. 5(a) shows the configuration looking down the single bonds C(1)–C(2), C(2)–C(3), C(3)–C(4), C(4)–C(5), C(5)–N(2). As in many other amino acids (Marsh & Donohue, 1967) it may be seen that the torsion angle N(1)–C(2)–C(1)–O(1) is very small (−2°) which results from the electrostatic attraction between positively charged amino nitrogen N(1) and negatively charged oxygen O(1). As for the side group conformation, it is concerned with rotations about the various single bonds in the side chain and is described by a set of dihedral angles χ which are close to 60, 180 and 300° (Lakshminarayanan et al., 1967). The C^γ atom, C(4), occupies position I with $\chi = 70°$ as found to occur in L-arginine dihydrate (Karle & Karle, 1964; Ramachandran, Mazumder, Venkatesan & Lakshminarayanan, 1966). The orientation of the side group as a whole in relation to the backbone is also shown in Fig. 5(b). The manner in which the side group juts out is similar to that observed in L-arginine dihydrate (Ramachandran et al., 1966).
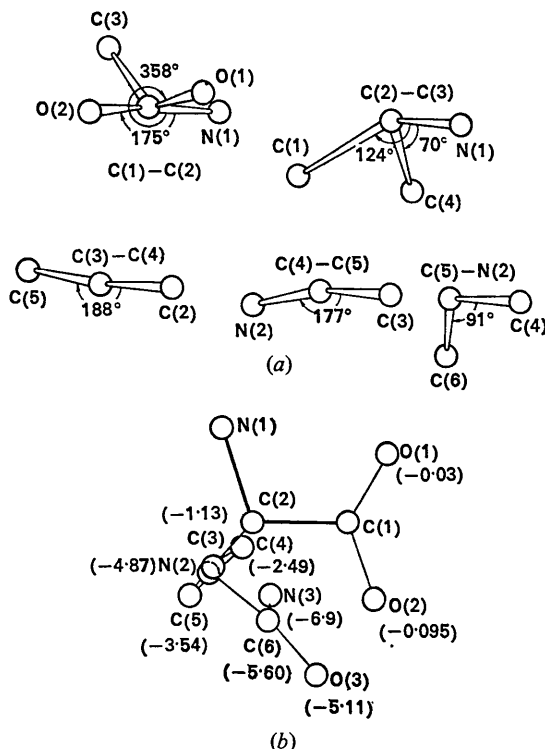
Fig. 5. (a) Conformation about the various bonds. (b) The projection of the side group on the $NC^\alpha C'$ plane.

Table 6. *Equations of least-squares planes and deviations (Å) of atoms from these planes*

X, Y and Z are expressed in Angström units relative to the orthogonal system defined by the a, b and c^* axes

	Atom	Deviation	Equation to the plane
Plane I			
Carboxyl group	C(1)	−0·0148	$0·6522X + 0·5159Y + 0·5554Z = 5·2985$
C(1)–C(2)–O(1)–O(2)	C(2)	0·0040	
	O(1)	0·0055	
	O(2)	0·0051	
	†N(1)	0·091	
Plane II			
Aliphatic chain	C(2)	−0·071	$0·5824X − 0·1612Y + 0·7968Z = 2·2766$
C(2)–C(3)–C(4)–C(5)–N(2)	C(3)	0·049	
	C(4)	0·074	
	C(5)	−0·006	
	N(2)	−0·052	
Plane III			
Urea fraction	C(6)	0·006	$0·6619X + 0·6960Y − 0·2783Z = 2·8749$
C(6)–N(2)–N(3)–O(3)	N(2)	−0·002	
	N(3)	−0·002	
	O(3)	−0·002	

† Not included in the calculation of the plane.

References

ALBRECHT, G. & COREY, R. B. (1939). *J. Amer. Chem. Soc.* **61**, 1967.

CHIBA, A., UKEI, T., ASHIDA, T., SASADA, Y. & KAKUDO, M. (1967). *Acta Cryst.* **22**, 863.

CRUICKSHANK, D. W. J. (1961). In *Computing Methods and the Phase Problem in X-ray Crystal Structure Analysis.* London: Pergamon Press.

DONOHUE, J. (1967). In *Structural Chemistry and Molecular Biology*, p. 441. Edited by A. RICH & N. DAVIDSON. London: Freeman.

DOW, J., JENSEN, L. H., MAZUMDAR, S. K., SRINIVASAN, R. & RAMACHANDRAN, G. N. (1970). *Acta Cryst.* **B26**, 1662.

GANTZEL, P. K., SPARKS, R. A. & TRUEBLOOD, K. N. (1961). *UCLALS* 1, Univ. of California Program.

HAMILTON, W. C. (1955). *Acta Cryst.* **8**, 185.

International Tables for X-ray Crystallography (1962). Vol. III. Birmingham: Kynoch Press.

KALYANARAMAN, A. R. & SRINIVASAN, R. (1970). Private communication.

KARLE, I. L. & KARLE, J. (1964). *Acta Cryst.* **17**, 835.

LAKSHMINARAYANAN, A. V., SASISEKHARAN, V. & RAMACHANDRAN, G. N. (1967). In *Conformation of Biopolymers*, Vol. 1, p. 61. Edited by G. N. RAMACHANDRAN. London: Academic Press.

MALLIKARJUNAN, M., THYAGARAJA RAO, S., VENKATESAN, K. & SARMA, V. R. (1969). *Acta Cryst.* **B25**, 220.

MARSH, R. E. (1958). *Acta Cryst.* **11**, 654.

MARSH, R. E. & DONOHUE, J. (1967). *Advanc. Protein Chem.* **22**, 235.

MAZUMDAR, S. K., VENKATESAN, K., MEZ, H. & DONOHUE, J. (1969). *Z. Kristallogr.* **130**, 328.

RAMACHANDRAN, G. N., MAZUMDAR, S. K., VENKATESAN, K. & LAKSHMINARAYANAN, A. V. (1966). *J. Mol. Biol.* **15**, 232.

RAMACHANDRAN, G. N. & RAMAN, S. (1959). *Acta Cryst.* **12**, 957.

RINGERTZ, H. (1969). *Acta Cryst.* **B24**, 1686.

SUTTON, L. E. (1965). *Interatomic Distances Supplement*, Special Publ. No. 18, S19s. London: The Chemical Society.

VAUGHAN, P. & DONOHUE, J. (1952). *Acta Cryst.* **5**, 530.

WILSON, A. J. C. (1942). *Nature, Lond.* **150**, 152.

WORSHAM, J. E., LEVY, H. A. & PETERSON, S. W. (1957). *Acta Cryst.* **10**, 319.

WRIGHT, D. A. & MARSH, R. E. (1962). *Acta Cryst.* **15**, 54.

Composition and Crystal Structure of Hexagonal Cu-rich Rare Earth–Copper Compounds

BY K. H. J. BUSCHOW AND A. S. VAN DER GOOT

Philips Research Laboratories, N.V. Philips' Gloeilampenfabrieken, Eindhoven, The Netherlands

Compounds RCu_5 with the hexagonal $CaCu_5$ structure have been observed for R = La, Ce, Pr, Nd, Sm, Gd, Tb and Y. Their lattice constants are given. For R = Gd, Tb, Dy and Y a compound of the approximate composition RCu_7 has been observed. The structure and the lattice constants of these compounds are presented. The compounds RCu_7, in which R = Gd, Dy and Tb, decompose on annealing at low temperatures into RCu_5 and elementary Cu. The pycnometric density of $TbCu_7$ has been determined.

Introduction

In a previous investigation it has been shown that for the heavy rare earth elements the compounds of the form RCu_5 have the cubic $AuBe_5$ structure (Buschow, van der Goot & Birkhan, 1969). For the light rare earth elements and incidentally also for some of the heavy rare earth elements a hexagonal compound of the $CaCu_5$ type has been reported in literature (Dwight, 1961; Wernick & Geller, 1959; Haszko, 1960). The stoichiometric composition of these hexagonal phases does not seem well established, as some rare earth elements have been observed to have the $CaCu_5$ structure type in compounds of composition RCu_4 as well as in compounds of composition RCu_5 (Gschneidner, 1961). During a recent investigation of the structure of rare earth–copper compounds of the form RCu_6 indications were obtained that some rare earth–copper

systems in the concentration range 80–90 at.% Cu probably have more than one hexagonal phase. To clarify this situation we have investigated this concentration range in most of the rare earth–copper systems by X-ray diffraction and metallography.

Experimental

The samples were prepared by arc melting. The purity of the starting materials was 99·99% for Cu and 99·9% for the rare earth elements. Vacuum annealing was performed in sintered Al_2O_3 crucibles sealed into quartz tubes. This procedure was found to lead to significant losses in weight of the Sm–Cu samples. Some of these samples were therefore also prepared by sealing the proper amounts of Sm and Cu under purified argon into a molybdenum container. This container was heated subsequently to a temperature suf-