Crystal and Molecular Structure of ω-Amino Acids, ω-Amino-Sulfonic Acids and Their Derivatives. VII. The Crystal and Molecular Structure of DL-Carnitine Hydrochloride

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The crystal structure of DL-carnitine hydrochloride, $C_7H_{15}NO_3 \cdot HCl$, was determined by the heavy atom method using X-ray diffraction data on a Weissenberg photograph, and refined by the least-squares method to an *R*-factor of 0.13 for 1969 independent reflections. The crystals are monoclinic space group $P2_1/c$, $Z=4$, with $a=6.77(1)$, $b=11.41(2)$, $c=15.10(3)$ Å and $\beta=120.1(1)°$. By protonation at carboxyl oxygen atom, carnitine molecule has the cationic form, $N^+(CH_3)_3CH_2CH(OH)CH_2COOH$, with *trans-zigzag* skeletal configuration as similar as γ-amino-β-hydroxybutyric acid. The Cl^- ion mediates between adjacent carnitine cations by two O–H···Cl^- hydrogen bonds to form infinite chain elongated along the *c*-axis. The correlation between molecular structure and biological activity was also discussed.

Carnitine is a constituent of striated muscle and liver, being isolated from meat extract.[1] Its chemical structure was identified by M. Tomita et al. as L-γ-trimethylamino-β-hydroxybutyrobetaine by synthesis from L-γ-amino-β-hydroxybutyric acid.[2] Carnitine is well known as a vitamin B_T, a growth factor of certain insects, and medically used as a stimulant of gastric juice secretion. Furthermore, carnitine and its acetyl-derivatives have the muscarinic action in nervous system.[3] As its chemical structure is very similar to that of acetylchlorine and γ-amino-β-hydroxybutyric acid (GABOB), both chemical transmitters, we have carried out a single crystal X-ray analysis of DL-carnitine hydrochloride as a part of a series on the structural studies of ω-amino acids, and we also discuss very interesting correlation between structural specificity and biological activity.

Experimental

Using the commercial DL-carnitine hydrochloride, a colorless plate crystal was obtained from aqueous methanol solution by slow evaporation at room tempreature. The space group and cell constants were determined from precession photograph: the systematic absences of $0k0$ reflections with k odd and $h0l$ with l odd indicate that the space group is $P2_1/c$. The density was measured by flotation method using carbon tetrachloride-benzene mixture. The obtained crystallographic data are shown in Table 1. The intensities of 1969 independent reflections were collected from equi-inclination Weissenberg photographs around the *a*- and *b*-axes ($h=0$—5, $k=0$—3) with nickel-filtered Cu$K\alpha$ radiation and estimated by visual comparison with a calibrated scale. The usual corrections for Lorentz and polarization factors were made but none for absorption or extinction.

Table 1. Crystal data

DL-Carnitine hydrochloride, $C_7H_{15}NO_3 \cdot HCl$	
Colorless transparent plates, Monoclinic system	
$a=6.77\pm0.01$ Å	$b=11.41\pm0.02$ Å
$c=15.10\pm0.03$ Å	$\beta=120.1\pm0.1°$
Volume of unit cell	1008.9 Å^3
Density (by flotation)	1.290 g/cm^3
Density (calculated)	1.301 g/cm^3
$Z=4$	$F(000)=428$
Space group: $P2_1/c$	

Structure Determination and Refinement

The structure was solved by heavy atom method. The position of the chlorine atom was easily determined from the inspection of sharpened Patterson map and all the non-hydrogen atoms were located by successive Fourier syntheses with phases based on the chlorine atom. By block-diagonal least-squares refinement with unit weight for the non-zero reflections and isotropic temperature factor, the initial *R*-factor, 0.26, was reduced to 0.19. Several cycles of refinement with anisotropic temperature factor for all non-hydrogen atoms were carried out to reduce *R*-factor to 0.17. The used anisotropic temperature factors are of the form: $\exp\{-(B_{11}h^2+B_{22}k^2+B_{33}l^2+B_{12}hk+B_{13}hl+B_{23}kl)\}$.

A three-dimensional difference Fourier synthesis was then calculated and the positions of seven hydrogen atoms except those of methyl groups, were properly found. Each one hydrogen atom peak of three methyl groups having reasonable bond length and angle in the difference Fourier map was fixed and the remaining two hydrogen atom positions in each methyl group were calculated. The final cycles of refinement including the hydrogen atoms with isotropic temperature factors reduced *R*-factor to 0.13 (0.14 including $|F_0|=0$).

Observed and calculated structure factors for each reflection are listed in Table 2. The atomic scattering factors used in all calculations were taken from International Tables for X-ray Crystallography (1692).[4] The final positional and thermal parameters together with their estimated standard deviations are gived in Tables 3(a) and 3(b).

All the computations were carried out on an NEAC 2200—700 Computer at this University using the programs of UNICS system.

Results and Discussion

Molecular Dimensions. The bond lengths and angles are shown in Table 4 with their estimated stand-

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TABLE 2. OBSERVED AND CALCULATED STRUCTURE FACTORS

Table 3(a) Final positional and thermal parameters with their standard deviations in parenthesis ($\times 10^4$)

Atom	x/a	y/b	z/c	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Cl	870(3)	2888(2)	4220(1)	44(4)	35(1)	16(1)	49(5)	24(3)	21(2)
C(1)	5589(17)	6422(9)	2880(7)	182(30)	34(7)	32(6)	−140(24)	61(21)	−6(10)
C(2)	5876(15)	4453(8)	3526(6)	101(24)	22(6)	20(5)	74(21)	−51(16)	4(9)
C(3)	3330(15)	5856(8)	3639(6)	128(24)	35(6)	16(4)	−35(21)	57(17)	−31(9)
C(4)	2418(12)	5091(7)	1933(5)	29(18)	16(5)	1(4)	−10(16)	−3(11)	3(7)
C(5)	455(12)	4365(6)	1855(5)	55(19)	3(4)	1(4)	6(15)	15(11)	9(6)
C(6)	−1282(11)	4224(7)	717(5)	13(18)	27(5)	2(3)	−21(17)	−14(13)	6(7)
C(7)	−3536(11)	3776(7)	553(5)	20(18)	7(4)	7(4)	−8(15)	−1(13)	−2(7)
N	4271(10)	5439(5)	2999(4)	58(17)	3(4)	4(3)	−4(13)	11(11)	3(5)
O(1)	1204(10)	3256(5)	2291(4)	163(18)	6(3)	15(3)	28(14)	56(12)	29(5)
O(2)	−4190(10)	3869(5)	1159(5)	118(18)	56(5)	29(4)	−68(17)	112(14)	−40(7)
O(3)	−4729(10)	3249(5)	−343(4)	94(16)	55(5)	10(3)	−96(16)	19(11)	−25(7)

Table 3(b). Positional and thermal parameters of hydrogen atoms

Atom	Bound to	x/a(×10³)	y/b(×10³)	z/c(×10³)
H(1)	O(3)	−634	271	−45
H(2)	C(6)	−73	346	41
H(3)	C(6)	−97	504	51
H(4)	C(5)	−54	485	214
H(5)	O(1)	35	296	264
H(6)	C(4)	187	591	158
H(7)	C(4)	318	459	154
H(8)	C(2)	632	408	301
H(9)	C(2)	736	468	412
H(10)	C(2)	480	379	366
H(11)	C(3)	459	618	416
H(12)	C(3)	308	508	391
H(13)	C(3)	155	624	325
H(14)	C(1)	709	660	364
H(15)	C(1)	605	619	227
H(16)	C(1)	447	719	269

Over-all isotropic temperature factor is 1.244 Å²

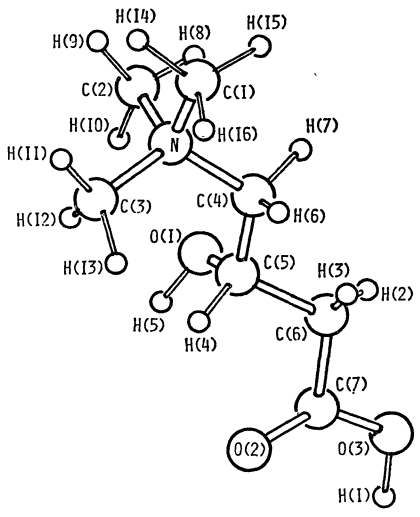

Fig. 1. The perspective drawing of the molecule of carnitine.

ard deviations in parentheses, in which the atomic numbering corresponds to that in Fig. 1, the perspective drawing of the molecule of carnitine as observed in crystals of the hydrochloride. They are in good agreement with those reported in the literatures. It is evident, from the difference in bond length between C_7–O_2 and C_7–O_3, that the protonation occurs at O_3 atom, being confirmed by difference Fourier synthesis.

The carnitine molecule has two planes, one is defined by the skeletal carbon, C_4, C_5, and C_6, and nitrogen N (plane 1), and another one by C_6, C_7, O_2 and O_3 (plane 2). The equations of least squares planes and deviations of the individual atoms from each plane are listed in Table 5. The experimental value of the dihedral angle between these two planes is 29.2°, indicating that all the non-hydrogen atoms in main carnitine molecule compose roughly a *trans-zigzag* configuration except two methyl carbon and hydroxyl oxygen atoms. The torsion angles around relevant bonds are shown in Fig. 2, with which it will be discussed later.

The hydrogen atoms of the trimethylammonium group have been observed to be in a staggered conformation and the C_4–N–C_1–H_{14}, C_4–N–C_2–H_9 and C_4–N–C_3–H_{11} torsion angles are all nearly 180°. This staggered conformation is also observed in the crystal

TABLE 4. BOND LENGTHS(Å) AND ANGLES(°) OF CARNITINE
Estimated standard deviations are listed in parentheses except the bonds including hydrogen atoms.

	Bond length		Bond angle		Bond angle
N-C(1)	1.499(13)	C(1)-N-C(2)	108.1(7)	H(6)-C(4)-N	101
N-C(2)	1.487(12)	C(1)-N-C(3)	108.4(7)	H(7)-C(4)-C(5)	108
N-C(3)	1.475(12)	C(1)-N-C(4)	107.1(7)	H(7)-C(4)-N	110
N-C(4)	1.518(10)	C(2)-N-C(3)	109.5(7)	H(8)-C(2)-H(9)	104
C(4)-C(5)	1.519(11)	C(2)-N-C(4)	111.4(7)	H(8)-C(2)-H(10)	108
C(5)-C(6)	1.529(11)	C(3)-N-C(4)	112.3(7)	H(8)-C(2)-N	108
C(5)-O(1)	1.419(10)	N-C(4)-C(5)	116.8(7)	H(9)-C(2)-H(10)	118
C(6)-C(7)	1.507(11)	C(4)-C(5)-O(1)	111.3(7)	H(9)-C(2)-N	115
C(7)-O(2)	1.203(10)	C(4)-C(5)-C(6)	107.2(6)	H(10)-C(2)-N	104
C(7)-O(3)	1.324(10)	C(5)-C(6)-C(7)	111.5(6)	H(11)-C(3)-H(12)	106
		C(6)-C(7)-O(2)	124.9(7)	H(11)-C(3)-H(13)	126
O(3)-H(1)	1.19	C(6)-C(7)-O(3)	111.8(7)	H(11)-C(3)-N	100
C(6)-H(2)	1.14	C(6)-C(5)-O(1)	108.7(7)	H(12)-C(3)-H(13)	101
C(6)-H(3)	1.03	O(2)-C(7)-O(3)	123.3(8)	H(12)-C(3)-N	101
C(5)-H(4)	1.11			H(13)-C(3)-N	119
O(1)-H(5)	1.01	H(1)-O(3)-C(7)	115	H(14)-C(1)-H(15)	114
C(4)-H(6)	1.05	H(2)-C(6)-H(3)	115	H(14)-C(1)-H(16)	106
C(4)-H(7)	1.12	H(2)-C(6)-C(7)	101	H(14)-C(1)-N	107
C(2)-H(8)	1.05	H(2)-C(6)-C(5)	108	H(15)-C(1)-H(16)	113
C(2)-H(9)	0.99	H(3)-C(6)-C(5)	95	H(15)-C(1)-N	110
C(2)-H(10)	1.14	H(3)-C(6)-C(7)	127	H(16)-C(1)-N	105
C(3)-H(11)	0.91	H(4)-C(5)-C(4)	112		
C(3)-H(12)	1.02	H(4)-C(5)-C(6)	102		
C(3)-H(13)	1.13	H(4)-C(5)-O(1)	115		
C(1)-H(14)	1.11	H(5)-O(1)-C(5)	111		
C(1)-H(15)	1.15	H(6)-C(4)-H(7)	109		
C(1)-H(16)	1.10	H(6)-C(4)-C(5)	112		

TABLE 5. EQUATIONS OF LEAST-SQUARES PLANES AND DEVIATIONS OF INDIVIDUAL ATOMS FROM THESE PLANES

Equation[a]	Deviations of atoms in Å		
$0.55309X-0.83308Y+0.00833Z+4.7564=0$ (plane 1)	C_4: −0.0291	C_5: 0.0247	C_6: 0.0378
	N: −0.0327		
$0.25745X-0.87242Y+0.41545Z+4.17759=0$ (plane 2)	C_6: 0.0009	C_7: −0.0012	O_2: 0.0012
	O_3: 0.0010		

a) X, Y, and Z are orthogonal coordinates in Å parallel to a, b, and c*.

structure of acetylcholine and its derivatives[5].

Molecular Enrivonment. Figure 3 shows the arrangement of the molecules in crystals as viewed along the *a*-axis. The chlorine ion mediates between adjacent carnitine cations by two O–H···Cl^- hydrogen bonds, $O_3H_1\cdots Cl^-$ (3.00 Å) and $O_1–H_5\cdots Cl^-$ (3.06 Å).

The contacts of the trimethylammonium group with the same group of neighboring molecules related by either a center of symmetry or two-fold screw axis have the normal van der Waals distances. There are no unusual short contacts in this crystal.

The Correlation between Molecular Structure and Biological Activity. As already mentioned in the introductory section, carnitine and its acetyl-derivatives have the muscarinic action in nervous system, and R. W. Baker *et al.* showed a strong correlation between structure and activity of muscarinic stimulants.[5] They pointed out that the torsion angles around relevant bonds and intramolecular distances between the nitrogen atom of trimethylammonium group and acetyl oxygen atom and so on in acetylcholine or its derivatives are of very important significance in performance of their activities. The torsion angle (τ) of the bonded group X–A–B–Y is defined as the angle between the plane X–A–B and the plane A–B–Y, and it is positive if clockwise and negative if anticlockwise from the near atom X to the further atom Y. As shown in Fig. 2, τ_1 of carnitine is +197.0°, τ_2 +174.8°, τ_3 +193.8° and τ_4 +203.3°, which correspond to all the anti-planar region of torsion angles defined by Klyne *et al.*[6] For comparison certain, torsion angles and interatomic distances observed in crystals of muscarinic stimulants are shown in Table 6(a) and (b), with that of carnitine hydrochloride and γ-amino-β-hydroxybutyric acid (GABOB). In this table, *ap*, *sc* or *ac* are the abbreviations of antiperiplanar, syn-clinal or anti-clinal, respectively. R. W. Baker *et al.* concluded that for potent muscarinic activity, τ_1 should be 180°, τ_2 in the range +73° to +137°,

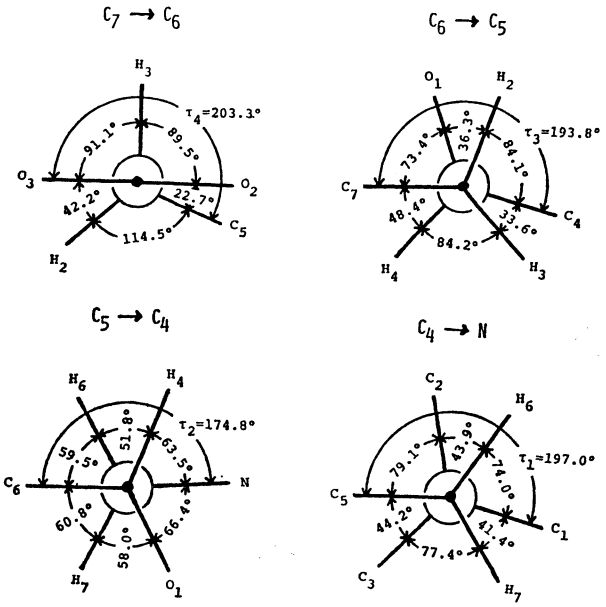

Fig. 2. The torsion angles around relevant bonds.

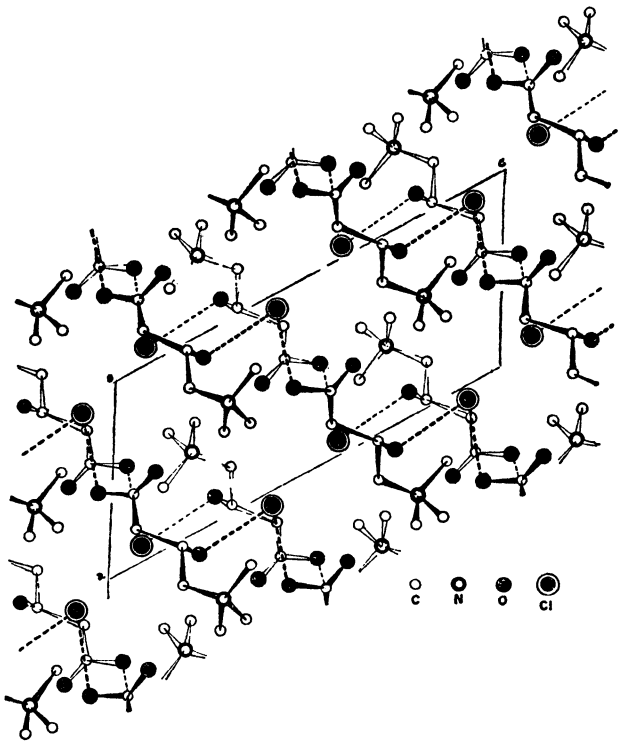

Fig. 3. The arrangement of the molecule in crystals as viewed along the *a*-axis.

τ_3 180±35° and τ_4 either 180° or −137°. The interatomic distances are: N^+–O (ester oxygen)=3.20 Å, N^+–C(carbonyl carbon)=4.50 Å and N^+–C(methyl carbon)=5.40 Å. Furthermore, the conformation of carbamoylcholine in crystals of its bromide is the only exceptional example of a potent muscarinic agonist with anti-periplanar conformation at τ_2(178°), and therefore, the relevant interatomic distances are significantly longer than those of the first three compounds in Table 6(b). On the other hand, the torsion angles and interatomic distances in crystals of carnitine hydrochloride and CABOB are very similar to those of carbamoylcholine because of having the *trans-zigzag* main chain configuration. It may be considered that there are two structurally different stimulants for muscarinic activity even if their conformations on interaction with the receptor molecule were modified from those observed in crystals. In other words, it may strongly suggest that the existence of two different receptor sites for muscarinic stimulants.

TABLE 6(a). CERTAIN TORSION ANGLES

Compound	τ_1	τ_2	τ_3	τ_4
Acetylcholine chloride[7]	*ap*	+*sc*	*ap*	*ap*
L(+)-Muscarine iodide[8]	*ap*	+*sc*	+*ac*	−*ac*
L(+)-*S*-Acetyl-*β*-methylcholine iodide[9]	*ap*	+*sc*	−*ac*	*ap*
Carbamoylcholine bromide[10]	*ap*	*ap*	*ap*	*ap*
Carnitine chloride (this work)	*ap*	*ap*	*ap*	*ap*
γ-Amino-*β*-hydroxybutyric acid[11]	*ap*	*ap*	*ap*	*ap*

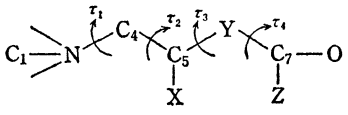

TABLE 6(b). CERTAIN INTERATOMIC DISTANCES

Compound	N-Y	N-C_7	N-Z
Acetylcholine	3.26 Å	4.40 Å	5.38 Å
L(+)-Muscarine iodide	3.07	4.49	5.40
L(+)-*S*-Acetyl-*β*-methylcholine iodide	3.21	4.25	5.16
Carbamoylcholine bromide	3.69	4.82	5.96
Carnitine chloride (this work)	3.87	5.08	6.18
γ-Amino-*β*-hydroxybutyric acid	3.79	4.94	6.05

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References

1) W. Gulewitsch and R. Krimberg, *Z. Physiol. Chem.*, **45**, 326 (1905); G. Fraenkel, *Biol. Bull.*, **104**, 359 (1953).
2) M. Tomita and Y. Sendju, *Z. Physiol. Chem.*, **169**, 263 (1927).
3) E. A. Hosein and P. Proulx, *Arch. Biochem. Biophys.*, **106**, 267 (1964); P. Weger, *Biochem. Z.*, **287**, 424 (1936).
4) "International Tables for X-Ray Crystallography," Vol. 3, Kynoch Press, England (1962).
5) R. W. Baker, C. H. Chothia, P. Pauling, and T. J. Petcher, *Nature*, **230**, 439 (1971).
6) W. Klyne and V. Perlog, *Experientia*, **16**, 521 (1960).
7) J. K. Herdklotz and R. L. Sass, *Biochem. Biophys. Res. Commun.*, **40**, 583 (1970).
8) F. Jellinek, *Acta Crystallogr.*, **10**, 277 (1957).
9) C. H. Chothia and P. J. Pauling, *Chem. Commun.*, **1969**, 626.
10) Y. Barrans and J. Clastre, *C. R. Acad. Sci., Paris, Ser. C*, **270**, 306 (1970).
11) K. Tomita, M. Harada, and T. Fujiwara, This Bulletin, **46**, 2854 (1973).