THE MOLECULAR STRUCTURE OF PALMITYLCARNITINE CHLORIDE

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The structure of palmitylcarnitine chloride has been studied by single crystal X-ray diffraction techniques. The final structure of this molecule shows that the carnitine moiety has a conformation similar to that of acetylcarnitine and acetylcholine. The molecular packing of this compound is interesting and rarely found in solid structures of amphipathic molecules.

INTRODUCTION

Carnitine and its derivatives are found in a large number of plant and animal tissues.(1) Carnitine has been shown to be an intermediate of fatty acid transport into mitochondria through the formation of a carnitine fatty acid ester.(2) It is now established that carnitine is available to mammals both from dietary sources and from the biosynthesis of essential amino acids of lysine and methionine.(3) It has also been shown that the carboxyl ester derivatives of carnitine possess acetylcholine-like activity.(4) Carnitine in the fatty acid oxidase-deficient insect flight muscle affects carbohydrate utilization via a role in pyruvate metabolism.(5) The phospholipid membrane permeability of acetylcarnitine and palmitylcarnitine is different; artificial phospholipid membranes are permeable to palmitylcarnitine but not to acetylcarnitine.(6) The mechanism of acyl carnitine transport into membranes has been studied by means of the electrochemical gradient of H^+ ions and it may be concluded that transport is due to a movement of protonated acyl carnitine down the H^+ ion gradient generated by the redox of ATPase.(6)

The structural analysis of acetylcarnitine chloride has recently been done by single crystal X-ray diffraction techniques.(7) The relation-

ship between the structural and functional similarity of acetylcarnitine and acetylcholine was discussed. This paper reports the crystallographic study of palmitylcarnitine chloride and compares the conformation of the carnitine moiety in this molecule with that of acetylcarnitine. Also, the crystal structure study of this molecule is important to an understanding of the relation between membrane structure and the stereostructure of molecules active in membrane transport systems.

EXPERIMENTAL

The compound palmitylcarnitine chloride was prepared by "method C" of Ziegler, Bruckner and Binon.[8] Crystals suitable for X-ray diffraction study were obtained by ether diffusion into a 95% ethanol solution of this compound at room temperature. The crystals are monoclinic (space group $P2_1/n$) with cell dimensions, a = 8.38Å, b = 51.390Å, c = 6.28Å and β = 97.904 degrees. The density of crystalline palmitylcarnitine chloride was measured to be 1.07g/ml by "flotation method". This compares with the calculated density of 1.08g/ml assuming four molecules per unit cell. Three dimensional data were collected on a Philips PAILRED diffractometer employing equi-inclination geometry and using silicon(111) monochromatized molybdenum K_α radiation. All reflections for which the statistical counting error exceeded 40% were rejected.[9] A total of 6450 reflections were measured. Of these, 2414 independently observed reflections were used in the final structure analysis.

The chlorine atom position was determined from a three-dimensional sharpened, origin removed Patterson synthesis. The chlorine atom was then used as a phasing model from which the fifteen carbon atoms on the long fatty acid chain were located. Scale factors, positional parameters and isotropic temperature factors were refined to an R value of 0.239. Refinement of anisotropic temperature factors reduced R to 0.156. Hydrogen atom positions were then located from a three-dimensional difference Fourier synthesis. Refinement was continued on all positional parameters,

TABLE 1

Final Positional and Thermal Parameters for Non-Hydrogen Atoms and Their Estimated Standard Deviations

The Positional Coordinates in this Table Have Been Multiplied by 10^4

Temperature Factors are Multiplied by 10^2

Atom	X	Y	Z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
Cl	366(3)	2261(0)	961(4)	698(15)	573(17)	270(17)	- 53(12)	278(12)	- 85(12)
C(1)	3083(18)	6347(2)	811(24)	1481(111)	684(67)	918(121)	171(68)	558(96)	- 49(71)
C(2)	3147(16)	6201(2)	8699(20)	1201(91)	753(66)	613(96)	175(64)	366(76)	-150(63)
C(3)	4427(13)	6003(2)	8708(16)	918(76)	570(54)	387(78)	25(53)	160(63)	29(52)
C(4)	4384(10)	5844(2)	6671(14)	846(69)	614(54)	276(72)	137(50)	- 23(58)	5(50)
C(5)	5665(10)	5642(1)	6650(14)	826(69)	540(50)	281(71)	56(48)	47(56)	41(48)
C(6)	5597(11)	5480(1)	4625(15)	892(70)	637(56)	245(69)	- 38(51)	175(57)	- 58(49)
C(7)	6888(10)	5275(1)	4614(14)	869(69)	478(46)	259(66)	72(46)	120(55)	- 25(44)
C(8)	6803(10)	5116(1)	2578(14)	826(65)	478(47)	317(71)	53(44)	104(56)	32(45)
C(9)	8108(10)	4916(1)	2543(14)	887(70)	481(48)	304(70)	41(45)	151(57)	- 8(45)
C(10)	8032(10)	4756(1)	4976(14)	951(72)	403(44)	288(68)	34(45)	87(57)	3(45)
C(11)	9359(10)	4558(1)	469(15)	861(66)	526(48)	281(69)	109(45)	131(55)	24(46)
C(12)	9262(10)	4401(1)	8434(13)	831(66)	425(45)	208(63)	104(42)	89(52)	- 65(42)
C(13)	597(10)	4201(1)	8462(14)	941(71)	489(46)	304(70)	- 47(46)	153(59)	- 90(45)
C(14)	476(10)	4034(1)	6433(13)	903(66)	391(41)	207(62)	57(44)	86(53)	- 3(41)
C(15)	1701(10)	3822(1)	6541(14)	950(71)	413(45)	268(67)	81(44)	106(56)	- 71(43)
C(16)	1566(10)	3659(1)	4543(15)	770(65)	357(43)	481(82)	41(41)	221(61)	51(39)
O(1)	1264(9)	3733(1)	2726(10)	1956(85)	320(31)	213(50)	220(40)	175(52)	108(32)
O(2)	1870(6)	3403(1)	5044(8)	816(40)	328(26)	53(36)	39(26)	100(31)	17(24)
C(17)	1899(9)	3216(1)	3226(12)	727(58)	374(39)	32(52)	15(37)	124(45)	- 57(36)
C(18)	3416(9)	3215(1)	2469(14)	737(59)	331(37)	322(64)	-141(36)	138(49)	-107(39)
C(19)	3439(9)	3064(1)	338(12)	514(55)	330(38)	208(61)	- 32(38)	7(49)	137(39)
O(3)	2258(7)	2971(1)	9407(9)	722(45)	856(44)	102(42)	-126(37)	90(36)	- 34(34)
O(4)	4841(6)	3054(1)	252(11)	515(37)	777(39)	354(48)	- 24(30)	118(34)	11(34)
C(20)	1394(8)	2954(1)	4195(12)	494(47)	414(39)	94(51)	102(35)	106(40)	- 50(36)
N	9672(7)	2908(1)	4433(9)	652(45)	413(33)	12(44)	- 3(32)	27(37)	- 60(30)
C(21)	8582(10)	2885(1)	2433(15)	764(67)	578(51)	417(80)	170(46)	137(60)	23(50)
C(22)	9029(9)	3113(1)	5838(14)	832(61)	348(39)	366(65)	- 24(38)	380(52)	- 85(40)
C(23)	9603(10)	2657(1)	5699(13)	867(61)	417(42)	117(54)	- 17(41)	60(46)	5(37)

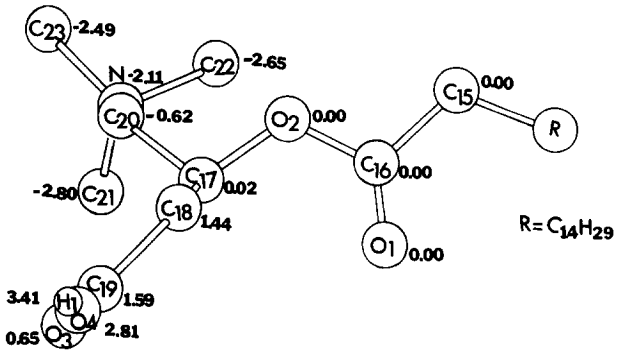

Figure 1 The structure of the palmitylcarnitine molecule in crystals of palmitylcarnitine chloride.

scale factors, anisotropic temperature factors on the non-hydrogen atoms and isotropic temperature on the hydrogen atoms. The final conventional R value was 0.097. Final positional parameters for all the non-hydrogen atoms are listed in Table 1.

RESULT AND DISCUSSION

The conformation of the carnitine moiety in the molecule of palmitylcarnitine is shown in Figure 1. The distance in anstroms of each atom from the plane defined by C(15), C(16), O(2) and O(1) is given. Figure 2 shows the calculated bond lengths and bond angles for the non-hydrogen atoms. The estimated standard deviations of bond lengths involving non-hydrogen atoms are approximately 0.011Å. The estimated standard deviations of bond angles are approximately 0.6°. The average C-C bond distance on the fatty acid chain is 1.509Å, and the average C-C-C angle is 115.2°. Relatively short bond lengths and large bond angles have previously been reported for long hydrocarbon chains, e.g., 1.51Å and 114.6° for β-tricaprin[10], 1.511Å and 114.2° for DL-2-methyl-7-oxododecanoic acid[11], 1.512Å and 113.9° for 13-oxoisosteric acid[12], 1.516Å and 113.9° for triacetylsphingosine.[13] These values can most probably be explained as the result of thermal motion of the chain. The O(4)-C1 distance of 2.96Å is very close to the reported value of 2.97Å

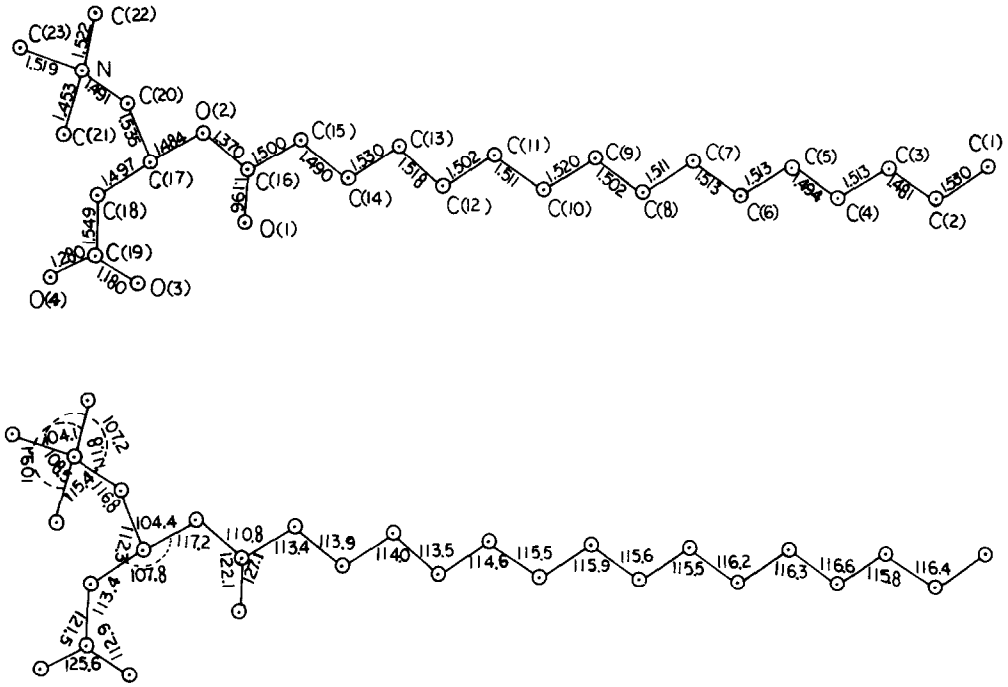

Figure 2 Intramolecular bond length and bond angles of Palmitylcarnitine molecule.

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for acetylcarnitine chloride but is smaller than the reported value of 3.04Å for choline chloride.[14] The chlorine atom to the nearest nitrogen atom distance is 3.98Å and to the nearest nitrogen methylcarbon atom is 3.60Å.

The configuration of the carnitine moiety in the molecule of palmitylcarnitine and that of acetylcarnitine are very similar. This configuration is observed in many cholinergic molecules. Referring to Figure 1, the N-O(2) distance of 3.13Å for palmitylcarnitine compares with values of 3.22Å and 3.26Å observed in acetylcarnitine and acetylcholine chloride[15], respectively. The distance from O(2) to the nearest nitrogen bonded methyl group is 3.17Å for acetylcholine chloride, 3.02Å for acetylcarnitine and 2.91Å for palmitylcarnitine. Table 2 lists selected torsional angles found in palmitylcarnitine. The torsion angle N-C(20)-

TABLE 2.

Selected Torsional Angles for Palmitylcarnitine Chloride Molecules

C(23)-N-C(20)-C(17)	172.7°
N-C(20)-C(17)-O(2)	82.0°
C(20)-C(17)-O(2)-C(16)	154.4°
C(17)-O(2)-C(16)-O(1)	- 0.4°

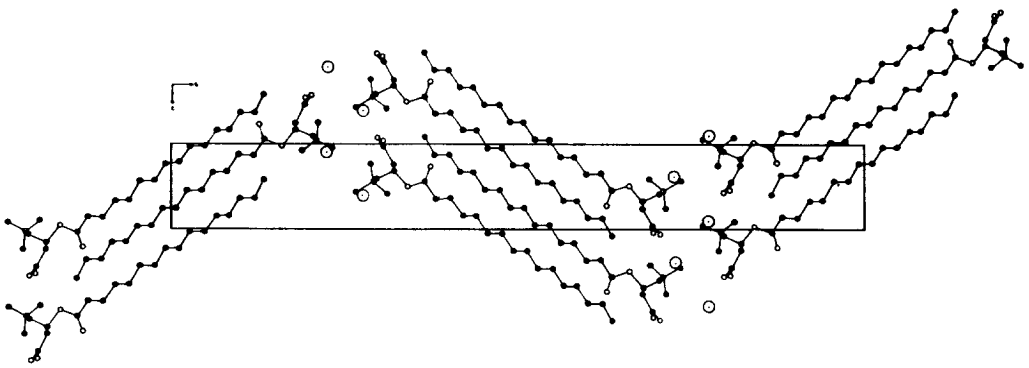

Figure 3 The crystal packing diagram of [100] projection of the unit cell.

C(17)-O(2) of 82° compares with the value of 87.9° for acetylcarnitine. This is consistent with the shorter N-O(2) distance in palmitylcarnitine. The torsion angle C(20)-C(17)-O(2)-C(16) of 154.4° is in a near trans configuration and agrees with the trans C-C-O-C conformation normally found in primary esters.[16] The gauche configuration for N-C(20)-C(17)-O(2) and the trans configuration for C(20)-C(17)-O(2)-C(16) are found in a number of cholinergic molecules.[7]

Figure 3 shows the crystal packing diagram of the a-axis

projection of the unit cell. The interdigitating crystal packing pattern of this molecule is interesting in that it is rarely observed for molecules with a polar head and a long nonpolar tail. The molecules are arranged in a zig-zag layer pattern along the b-axis direction. In the molecule the chain axis forms an angle of 78.3° with the plane defined by C(16), O(1) and O(2). Regions of chain packing alternate with regions of carnitine and chloride polar groups.

From the mechanism study of acyl carnitine transport into mitochondria three molecular structures of palmitylcarnitine have been proposed.[6] Inner salt and zwitter ion structure are electroneutral while the cationic form is reported as the active one in the membrane translocation. The crystal structure of palmitylcarnitine chloride may thus be indicative of the structure of the membrane active species.

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