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# The Crystal and Molecular Structure of Cholesteryl Isobutyrate

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The structure of cholesteryl isobutyrate, $(CH_3)_2CHCOOC_{27}H_{45}$, was determined by single crystal X-ray diffraction methods. Cholesteryl isobutyrate crystallized monoclinic space group $P2_1$, with $a$ = 15.115 (8)Å, $b$ = 9.636 (5)Å, $c$ = 20.224 (9)Å, $\beta$ = 93.15 (5)°, $z$ = 4, $Dc$ = 1.03 g/cm$^3$ and $Dm$ = 1.04 g/cm$^3$. The intensity data were measured for the 3417 reflections, within $\sin\theta/\lambda = 0.59$Å$^{-1}$, using an automatic four-circle diffractometer and graphite monochromated Mo-$K_\alpha$ radiation. The structure was solved by fragment search Patterson methods and direct methods and refined by full-matrix least-squares methods. The final R factor was 0.129 for 2984 observed reflections. The two symmetry-independent molecules (A) and (B) are almost fully extended. The molecules are in antiparallel array forming monolayers with thickness $d_{100}$ = 15.2Å, and molecular long axes are nearly parallel to the [$\bar{1}$01] directions. The two distinct molecules form separate stacks with almost the same orientations, but with differing degrees of steroid overlap. Thers is a close packing of cholesteryl groups within the monolayers. The packing type is similar to those of cholesteryl hexanoate and cholesteryl oleate.

## Introduction

Cholesterol[1-3] which is the most abundant steroid in the animal kingdom, and is found mainly as a component in cell membranes and lipoproteins. In addition to being a primary metabolic precursor for many of the steroid hormones, it and some of its esters play an important role in the structural stabilization of membranes[4]. Thus an important first step towards deriving detailed structural models of membranes is a study of the stereochemistry and packing of cholesterols and its derivatives. Although cholesterol first attracted the interest of X-ray crystallographers[2] more than four decades ago, it is only recently that the detailed crystal structures of cholesterol and some of cholesterol derivatives have been determined. The reason for this delay is undoubtedly the complexity of the crystal structures, arising from a consistent tendency of cholesterol to crystallize with more than one independent molecules in the crystallographic asymmetric unit. Another remarkable feature of these cholesterol structures is the presence of local pseudo-symmetry, that is, noncrystallographic symmetry which is satisfied locally, to remarkably high degree. In addition to the complexity and the pseudo-symmetry, an object of interest is the characteristic bilayer nature of the structure of cholesterol crystals, with a molecular arrangement generally similar to that of cholesterol in biological membranes[5].

Barnard and Lydon have conducted a crystallographic study on fourteen straight chain cholesteryl esters[6]. Examination of their unit cell parameters in addition to other crystallographic data, suggests that majority of esters may have one of the three common crystal packing arrangements. These arrangements differ with respect to the portion of the ester molecules that are involved in intermolecular interactions and therefore determine the crystal structure. These structure types differ according to the relative importance of three kinds of molecular interactions, namely cholesteryl-cholesteryl (Type II Monolayer)[7-12], cholesteryl-fatty acid (Type I Monolayer)[13] and fatty acid-fatty acid (Bilayers)[14].

The crystal structure analysis of the cholesteryl isobutyrate is one of a series of cholesteryl ester structure determinations which we have undertaken. From consideration of the crystal data of the cholesteryl isobutyrate, it seemes interesting to study its crystal structure, because the different mode of crystal packing type tends to be present in this compound.

## Experimental

Cholesteryl isobutyrate from Tokyo Kasei Kogyo Company Ltd. was recrystallized by slow evaporation of an ace-

**Table 1. Crystal Data**

| | | |
|---|---|---|
| Chemical Formula | : | $(CH_3)_2CHCOOC_{27}H_{45}$ |
| Molecular Weight | : | 456.8 |
| Crystal System | : | monoclinic |
| Space Group | : | $P2_1$ |
| | | from systematic absences |
| | | $0\,k\,0;\ k = 2n + 1$ |
| Unit Cell Parameter | : | $a$ = 15.115(8)Å |
| | | $b$ = 9.636(5)Å |
| | | $c$ = 20.224(9)Å |
| | | $\beta$ = 93.15(5) ° |
| | | $z$ = 4 |
| $\mu$(Mo-K$\alpha$) | : | 0.58 $cm^{-1}$ |
| Density | : | Dm = 1.04 $g/cm^3$ |
| | | (by the flotation method in a methanol-KI aqueous solution) |
| | | $Dc$ = 1.03 $g/cm^3$ |
| F(000) | : | 1015.9 |

tonitrile solution at room temperature. The resulting monoclinic, colorless, lath-shaped crystals melted at 132.0 °C. The melting point of cholesteryl isobutyrate was measured by differential thermal analysis method.

Preliminary crystal data of cholesteryl isobutyrate obtained from X-ray oscillation and Weissenberg photographies, which showed systematic absent reflections for $0k0$ when $k$ is odd, indicated that the crystals were monoclinic with the space group $P2_1$. The unit cell parameters were determined by least-squares fit to observed $2\theta$ angles for 25 centered reflections within $35° < 2\theta < 50°$ measured with Mo-K$\alpha$ radiation on an automated Nicolet R3m diffractometer. The intensity data were collected with graphite monochromated Mo-K$\alpha$ radiation using $\theta$-$2\theta$ scan technique over a scan rate which was a function of count rate (4.9 °/min ~ 29.3 °/min). Three standard reflections were monitored every 100 reflections throughout the data collection. Of the 3417 independent reflections measured, range of $hkl$: $-15 \le h \le 15$, $0 \le k \le 9$, $0 \le l \le 20$, within $(\sin\theta/\lambda)_{max} = 0.59$ Å$^{-1}$, 1190 reflections were considered unobserved as defined by $|Fo| < 2\sigma|Fo|$. Data were corrected for Lorentz and polarization effect, but the absorptions were ignored. All of the crystal data are listed in Table 1.

## Structure Determination and Refinement

The phase problem was solved by fragment search Patterson methods and direct methods.

The tetracyclic system of cholesterol provides a rigidly bonded framework exhibiting a characteristic pattern of interatomic vectors. When the effects of this pattern are recognized in a systematic search of the observed Patterson function, the cholesterol fragments in the crystal structure can be assigned to their positions and orientations in the unit cell. The remaining atoms can then be located by Fourier methods.

First, the sharpened Patterson function were calculated with 200 reflections for $2\theta \le 42°$ of cholesteryl isobutyrate. With the artificial 20-atoms cholesterol fragment, rotation-translation Patterson methods were performed using the program PATSEE[15]. The atomic coordinates of the cholesterol ring system that was used as the search model were derived

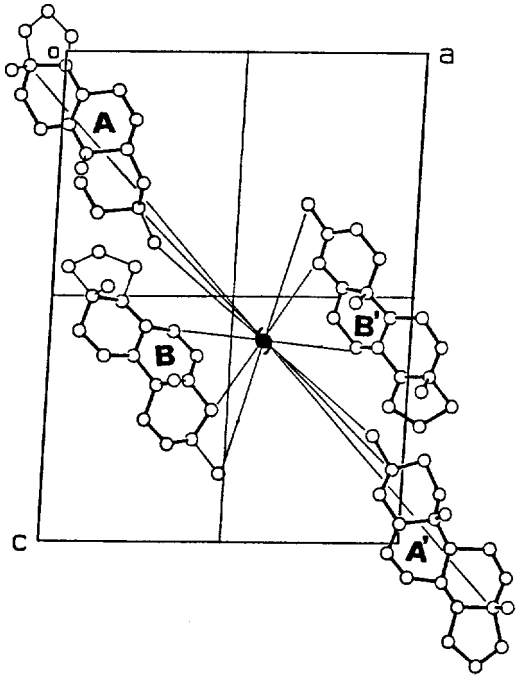


**Figure 1.** The four tetracyclic fragments located in the artifical triclinic crystal systems for cholesteryl isobutyrate. Atoms with thinney bonds were missed on E maps.

from the crystal structure of 3-chloro-5-en-androsten-17-ol[16]. This fragment was positioned with the approximate center at the origin of a unit cell with parameters; $a$ = 13.000, $b$ = 18.000, $c$ = 12.000Å and $\alpha = \beta = \gamma = 90.0°$, and space group P1.

The one tetracyclic ring with the best figure of merit was selected among the three rotation-translation search solutions.

Using this fragment as a partial structure, two tetracyclic ring systems for each of the independent cholesteryl isobutyrate molecules were found by the direct method of the program SHELXS-86[17]. However the remaining 26 non-hydrogen atoms of the ester groups and cholesterol C(17) side chains were not found in subsequent electron density maps. No trial structure could be found that gave $R$ better than 0.33.

In this stage, the monoclinic data set of cholesteryl isobutyrate were expanded into the triclinic data set using the following relationships; $|F(hkl)| = |F(h\bar{k}l)|$ and $|F(\bar{h}kl)| = |F(\bar{h}\bar{k}\bar{l})|$.

With previously determined 40-atoms of the two tetracyclic ring systems as the partial structure expansion, the direct methods were applied to 500 reflections with $E \ge 1.5$ using SHELXS-86.

As can be seen in Figure 1, the four tetracyclic fragments were found in the artificial triclinic crystal system. These four cholesteryl fragments in the artificial triolinic system were shifted to two cholesteryl fragments properly positioned in the original monoclinic system. In this way, both cholesterol fragments (A) and (B) were correctly positioned.

The structure gave an $R$ value of 0.35 for 40-atoms with 352 reflections whose $E$ values are greater than 1.6. The remaining atoms were found by direct method and Fourier method using the program SHELXS-86 and SHELX-76[18].

**Table 3. Anisotropic Thermal Parameters($Å^2 \times 10^3$) for Nonhydrogen Atoms of Cholesteryl Isobutyrate. The Temperature Expression used for Anisotropic Thermal Parameters is $\exp[-2\pi^2(U_{11}h^2a^{*2}+U_{22}k^2b^{*2}+U_{33}l^2c^{*2}+\ldots\ldots+2U_{12}hka^*b^*0]$. The e.s.d.'s are in Parentheses**

| Atom | $U_{11}$ | $U_{22}$ | $U_{33}$ | $U_{23}$ | $U_{13}$ | $U_{12}$ |
|---|---|---|---|---|---|---|
| C(A1) | 111( 8) | 89( 8) | 77( 6) | 6( 6) | −6( 6) | −2( 7) |
| C(A2) | 187(12) | 89( 9) | 89( 7) | 20( 7) | −29( 8) | 3( 9) |
| C(A3) | 130( 9) | 108( 9) | 79( 7) | −25( 7) | −47( 7) | 33( 8) |
| C(A4) | 102( 7) | 110( 9) | 87( 7) | −9( 7) | −7( 6) | 11( 7) |
| C(A5) | 93( 7) | 72( 7) | 72( 6) | −9( 6) | −14( 6) | −3( 6) |
| C(A6) | 87( 7) | 117( 9) | 89( 7) | −19( 7) | 4( 6) | −6( 7) |
| C(A7) | 99( 7) | 94( 8) | 71( 6) | −7( 6) | 15(5) | −11( 7) |
| C(A8) | 78( 6) | 67( 6) | 65( 5) | 0( 5) | 1( 5) | 12( 5) |
| C(A9) | 111( 7) | 63( 6) | 63( 5) | −12( 5) | 3( 5) | 14( 6) |
| C(A10) | 71( 6) | 59( 6) | 74( 6) | −3( 5) | 10( 4) | 2( 5) |
| C(A11) | 72( 6) | 175(12) | 75( 6) | −14( 7) | 6( 5) | −20( 8) |
| C(A12) | 119( 8) | 149(11) | 69( 6) | 9( 7) | 9( 6) | −20( 8) |
| C(A13) | 89( 7) | 113( 9) | 65( 6) | −7( 6) | 7( 5) | −5( 7) |
| C(A14) | 64( 6) | 93( 7) | 71( 6) | 2( 5) | 5( 4) | −1( 5) |
| C(A15) | 107( 7) | 9( 9) | 70( 6) | 24( 6) | 5( 5) | 0( 7) |
| C(A16) | 80( 6) | 138(10) | 85( 6) | 8( 7) | −7( 5) | −4( 7) |
| C(A17) | 81( 6) | 119( 9) | 63( 5) | −11( 6) | 1( 4) | 25( 6) |
| C(A18) | 108( 8) | 91( 9) | 114( 8) | −30( 7) | 1( 6) | 30( 7) |
| C(A19) | 142( 9) | 81( 8) | 87( 7) | −20( 6) | 5( 6) | 18( 7) |
| C(A20) | 110( 7) | 200(14) | 89( 7) | 4( 9) | −12( 6) | −29(10) |
| C(A21) | 102( 8) | 446(32) | 100( 7) | 30(15) | −12( 7) | −77(16) |
| C(A22) | 120( 8) | 198(14) | 73( 6) | −22( 8) | −13( 6) | 8( 9) |
| C(A23) | 103( 8) | 301(21) | 113( 9) | −9(12) | −39( 7) | 14(12) |
| C(A24) | 111( 8) | 267(18) | 101( 8) | −38(11) | −41( 7) | 12(11) |
| C(A25) | 183(17) | 581(53) | 105(11) | −79(21) | −21(11) | 130(27) |
| C(A26) | 133(12) | 940(79) | 131(12) | −9(31) | −17(10) | 78(29) |
| C(A27) | 299(28) | 345(34) | 373(34) | −196(29) | −211(25) | 178(27) |
| C(A28) | 105(10) | 174(15) | 138(12) | −11(11) | 15( 9) | 23(11) |
| C(A29) | 91( 9) | 553(42) | 78( 7) | −8(17) | −41( 7) | 32(19) |
| C(A30) | 102(10) | 1345(101) | 223(18) | −362(39) | −27(12) | 56(32) |
| C(A31) | 219(18) | 191(17) | 170(13) | −21(14) | −58(13) | −18(15) |
| O(A3) | 171( 7) | 149( 8) | 122( 6) | −17( 6) | −39( 5) | 20( 7) |
| O(A28) | 187(10) | 275(15) | 245(13) | −89(12) | −119( 9) | 117(11) |
| C(B1) | 79( 7) | 105( 8) | 97( 7) | −28( 7) | −13( 5) | 8( 6) |
| C(B2) | 90( 7) | 115( 9) | 99( 7) | −13( 7) | −22( 6) | 18( 7) |
| C(B3) | 86( 6) | 131(10) | 93( 7) | −8( 8) | −45( 6) | −14( 8) |
| C(B4) | 81( 7) | 129( 9) | 108( 8) | 13( 8) | 1( 6) | 15( 7) |
| C(B5) | 58( 5) | 133( 9) | 78( 6) | 13( 7) | 4( 5) | −9( 7) |
| C(B6) | 85( 7) | 153(11) | 76( 7) | −31( 7) | 13( 6) | 20( 7) |
| C(B7) | 111( 7) | 143(10) | 64( 6) | −18( 6) | 8( 6) | 5( 8) |
| C(B8) | 65( 5) | 72( 6) | 83( 6) | 7( 5) | 3( 5) | 11( 5) |
| C(B9) | 72( 5) | 59( 6) | 63( 5) | 0( 4) | −2( 4) | −3( 5) |
| C(B10) | 85( 7) | 81( 7) | 94( 7) | 3( 6) | 15( 5) | 3( 6) |
| C(B11) | 79( 6) | 108( 8) | 100( 7) | −20( 7) | −18( 5) | 20( 6) |
| C(B12) | 96( 6) | 119( 9) | 62( 6) | −26( 6) | −3( 5) | 18( 7) |
| C(B13) | 80( 6) | 60( 6) | 84( 6) | 1( 5) | 3( 5) | −12( 6) |
| C(B14) | 66( 5) | 94( 7) | 64( 5) | −1( 5) | 2( 4) | 24( 6) |
| C(B15) | 103( 8) | 204(14) | 66( 6) | −22( 8) | −16( 6) | 46( 9) |
| C(B16) | 107( 8) | 193(13) | 88( 7) | −33( 8) | 13( 6) | 30( 9) |
| C(B17) | 92( 6) | 79( 7) | 69( 5) | −12( 5) | 1( 5) | 10( 6) |
| C(B18) | 138(10) | 90( 8) | 139( 9) | 53( 8) | −43( 8) | −46( 8) |
| C(B19) | 121( 9) | 81( 8) | 149(10) | 13( 8) | −40( 8) | −18( 7) |
| C(B20) | 119( 8) | 82( 7) | 101( 7) | 14( 6) | −40( 6) | −8( 7) |
| C(B21) | 102( 7) | 120( 9) | 128( 8) | −31( 8) | −28( 6) | 54( 7) |
| C(B22) | 115( 7) | 139(10) | 83( 6) | −4( 7) | −34( 6) | 29( 8) |
| C(B23) | 131( 9) | 172(13) | 140( 9) | 18(10) | −51( 8) | 18(10) |
| C(B24) | 197(12) | 217(16) | 96( 7) | −44(10) | −97( 9) | 82(13) |
| C(B25) | 219(18) | 183(18) | 213(18) | −30(16) | −60(15) | 1(16) |
| C(B26) | 326(25) | 368(35) | 230(17) | −154(22) | −178(19) | 168(26) |
| C(B27) | 152(14) | 324(31) | 242(21) | 109(23) | −49(14) | −32(18) |
| C(B28) | 358(27) | 137(15) | 211(18) | 6(15) | −165(18) | 18(18) |
| C(B29) | 221(16) | 380(30) | 103( 9) | −58(15) | −105(11) | 52(21) |
| C(B30) | 260(20) | 337(28) | 170(14) | −43(18) | −61(14) | −19(20) |
| C(B31) | 157(13) | 372(31) | 314(25) | −86(24) | −116(15) | 82(18) |
| O(B3) | 142( 6) | 152( 8) | 147( 7) | 20( 7) | −47( 5) | −14( 6) |
| O(B28) | 181(10) | 280(17) | 223(12) | 49(13) | −69( 9) | 47(12) |

*The final observed and calculated structure factors are available from the author (YJP).

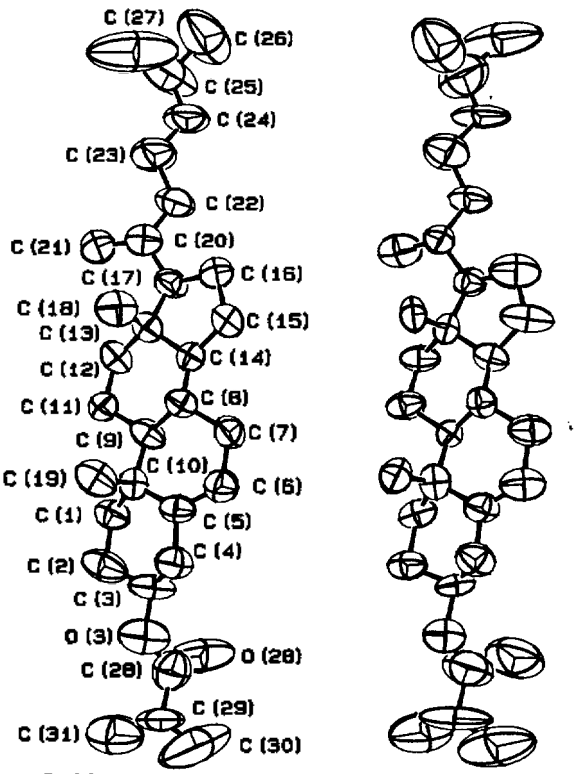


**Figure 2.** Molecular structures of cholesteryl isobutyrate. Molecules (A) and (B), which are not related by crystal symmetry are shown in the observed conformation within their tetracyclic systems in the same orientation. Atoms are represented as 50% probability ellipsoids.
Left: molecule(A), Right: molecule(B).

0.6-2.5° for bond angles. In the cholesterol ring system, C-C bond distances range from 1.48(1)Å for C(5)-C(10) to 1.60 (1)Å for C(11)-C(12) in molecule (A), and from 1.46(1)Å for C(8)-C(14) to 1.61(1)Å for C(13)-C(17) in molecule (B). The C(5) = C(6) double bond distances are 1.35(1)Å for molecule (A) and 1.30(2)Å for molecule (B). The single bond angles of cholesteryl isobutyrate range from 100.5(8)° for

**Table 4. Bond distances (Å) for nonhydrogen atoms of Cholesteryl Isobutyrate. The e.s.d.'s are in parentheses**

| Bond | Molecule(A) | Molecule(B) |
|---|---|---|
| C(1)-C(2) | 1.521(15) | 1.583(14) |
| C(1)-C(10) | 1.508(13) | 1.552(14) |
| C(2)-C(3) | 1.494(19) | 1.521(15) |
| C(3)-C(4) | 1.519(16) | 1.477(14) |
| C(3)-O(3) | 1.437(14) | 1.510(13) |
| C(4)-C(5) | 1.524(14) | 1.489(13) |
| C(5)-C(6) | 1.350(14) | 1.300(16) |
| C(5)-C(10) | 1.477(12) | 1.555(14) |
| C(6)-C(7) | 1.486(13) | 1.512(13) |
| C(7)-C(8) | 1.543(12) | 1.535(15) |
| C(8)-C(9) | 1.527(12) | 1.527(11) |
| C(8)-C(14) | 1.502(12) | 1.463(11) |
| C(9)-C(10) | 1.560(12) | 1.550(12) |
| C(9)-C(11) | 1.570(13) | 1.578(13) |
| C(10)-C(19) | 1.530(15) | 1.513(16) |
| C(11)-C(12) | 1.600(13) | 1.573(13) |
| C(12)-C(13) | 1.575(14) | 1.533(12) |
| C(13)-C(14) | 1.496(12) | 1.514(13) |
| C(13)-C(17) | 1.575(12) | 1.609(12) |
| C(13)-C(18) | 1.556(16) | 1.544(15) |
| C(14)-C(15) | 1.535(12) | 1.605(12) |
| C(15)-C(16) | 1.564(14) | 1.565(15) |
| C(16)-C(17) | 1.573(14) | 1.562(14) |
| C(17)-C(20) | 1.480(14) | 1.506(14) |
| C(20)-C(21) | 1.532(17) | 1.577(15) |
| C(20)-C(22) | 1.532(16) | 1.600(15) |
| C(22)-C(23) | 1.495(16) | 1.525(17) |
| C(23)-C(24) | 1.451(17) | 1.604(20) |
| C(24)-C(25) | 1.444(21) | 1.354(24) |
| C(25)-C(26) | 1.508(22) | 1.584(31) |
| C(25)-C(27) | 1.296(42) | 1.439(36) |
| C(28)-C(29) | 1.554(24) | 1.570(31) |
| C(28)-O(3) | 1.347(18) | 1.231(24) |
| C(28)-O(28) | 1.224(23) | 1.360(28) |
| C(29)-C(30) | 1.212(29) | 1.567(25) |
| C(29)-C(31) | 1.361(21) | 1.376(32) |

**Table 5. Bond angles (°) for the Cholesteryl Isobutyrate. The e.s.d.'s are in Parentheses**

| | Molecule(A) | Molecule(B) |
|---|---|---|
| C(3)-C(2)-C(1) | 107.9( 9) | 105.1( 9) |
| C(4)-C(3)-C(2) | 110.4(10) | 111.7( 9) |
| C(5)-C(4)-C(3) | 110.1( 9) | 113.4( 8) |
| C(5)-C(10)-C(1) | 108.4( 8) | 106.0( 8) |
| C(6)-C(5)-C(4) | 118.3( 8) | 123.0( 9) |
| C(7)-C(6)-C(5) | 124.1( 8) | 125.1(10) |
| C(8)-C(7)-C(6) | 113.5( 7) | 114.1( 9) |
| C(9)-C(8)-C(7) | 108.2( 7) | 107.1( 7) |
| C(9)-C(10)-C(1) | 109.2( 7) | 106.9( 7) |
| C(9)-C(10)-C(5) | 109.4( 7) | 108.6( 8) |
| C(10)-C(1) -C(2) | 116.1( 9) | 113.0( 7) |
| C(10)-C(5) -C(4) | 117.6( 8) | 114.6( 9) |
| C(10)-C(5)-C(6) | 124.1( 9) | 122.4( 8) |
| C(10)-C(9)-C(8) | 113.4( 7) | 113.7( 7) |
| C(11)-C(9)-C(8) | 113.4( 7) | 111.2( 7) |
| C(11)-C(9)-C(10) | 111.6( 7) | 112.6( 7) |
| C(12)-C(11)-C(9) | 113.4( 7) | 113.2( 8) |
| C(13)-C(12)-C(11) | 109.4( 9) | 107.7( 7) |
| C(13)-C(14)-C(18) | 116.7( 7) | 115.2( 8) |
| C(14)-C(8)-C(7) | 111.9( 7) | 111.3( 8) |
| C(14)-C(8)-C(9) | 109.8( 7) | 111.4( 6) |
| C(14)-C(13)-C(12) | 108.0( 8) | 106.8( 7) |
| C(15)-C(14)-C(8) | 117.3( 8) | 117.9( 7) |
| C(15)-C(14)-C(13) | 104.5( 7) | 104.4( 7) |
| C(16)-C(15)-C(14) | 103.1( 8) | 103.4( 8) |
| C(16)-C(17)-C(13) | 100.5( 8) | 103.9( 7) |
| C(17)-C(13)-C(12) | 112.6( 9) | 114.3( 7) |
| C(17)-C(13)-C(14) | 102.8( 7) | 101.6( 7) |
| C(17)-C(16)-C(15) | 107.8( 8) | 107.6( 8) |
| C(18)-C(13)-C(12) | 110.8( 8) | 110.4( 8) |
| C(18)-C(13)-C(14) | 114.0( 9) | 114.6( 8) |
| C(18)-C(13)-C(17) | 108.4( 8) | 108.9( 7) |
| C(19)-C(10)-C(1) | 110.2( 7) | 111.7( 9) |
| C(19)-C(10)-C(5) | 106.4( 8) | 111.1( 9) |
| C(19)-C(10)-C(9) | 112.4( 7) | 112.2( 8) |
| C(20)-C(17)-C(13) | 122.4( 9) | 118.3( 8) |
| C(20)-C(17)-C(16) | 113.2( 8) | 112.9( 8) |
| C(21)-C(20)-C(17) | 117.7( 9) | 111.5( 8) |
| C(22)-C(20)-C(17) | 112.2( 9) | 108.4( 8) |
| C(22)-C(20)-C(21) | 113.4(11) | 106.1( 8) |
| C(23)-C(22)-C(20) | 115.9(11) | 113.0(11) |
| C(24)-C(23)-C(22) | 114.1(11) | 107.2(12) |
| C(25)-C(24)-C(23) | 118.8(12) | 118.2(16) |
| C(26)-C(25)-C(24) | 115.9(15) | 113.8(18) |
| C(27)-C(25)-C(24) | 124.3(25) | 115.1(17) |
| C(27)-C(25)-C(26) | 107.4(23) | 97.9(17) |
| C(28)-O(3)-C(3) | 120.6(11) | 120.8(14) |
| C(30)-C(29)-C(28) | 108.6(23) | 95.7(15) |
| C(31)-C(29)-C(28) | 112.0(15) | 113.7(21) |
| C(31)-C(29)-C(30) | 120.2(17) | 116.6(17) |
| O(3)-C(3)-C(2) | 107.1( 9) | 104.1( 9) |
| O(3)-C(3)-C(4) | 108.5(10) | 108.8( 8) |
| O(3)-C(28)-C(29) | 108.1(16) | 112.1(18) |
| O(28)-C(28)-C(29) | 134.9(16) | 115.8(19) |
| O(28)-C(28)-O(3) | 116.7(13) | 112.6(17) |

C(13)-C(17)-C(16) to 124.3(3)° for C(24)-C(25)-C(27) in molecule (A), and from 95.7(2)° for C(28)-C(29)-C(30) to 105.1(1)° for C(5)-C(6)-C(7) in molecule (B).

The bond distances and angles of cholesteryl isobutyrate are listed in Table 4 and 5. These are in agreement, within experimental error, with those found in other cholesterol esters[21]. The bond distances in the tail and the isobutyrate groups showed the apparent shortening which is characteristic of cholesteryl esters. In these case, it is especially pronounced in the C(25)-C(27) bond (1.30(4)Å) and C(29)-C(30) bond (1.21(3)Å) for molecule (A).

The overall distance of the tetracyclic system, taken as the C(3)···C(16) distance were 8.95Å in molecule (A), and

**Table 6. Selected torsion angles (°) and asymmetry parameters in Cholesteryl Isobutyrate. The e.s.d.'s are in parentheses**

| | Molecule (A) | Molecule (B) |
|---|---|---|
| (1) Steroid skeleton | | |
| Ring A | | |
| C(10)-C(1)-C(2)-C(3) | -58.5(10) | -62.4( 9) |
| C(1)-C(2)-C(3)-C(4) | 59.7(10) | 60.1( 9) |
| C(2)-C(3)-C(4)-C(5) | -56.3(10) | -58.0(10) |
| C(3)-C(4)-C(5)-C(10) | 50.6( 9) | 52.9(10) |
| C(4)-C(5)-C(10)-C(1) | -45.7( 9) | -50.6( 9) |
| C(2)-C(1)-C(10)-C(5) | 49.9( 9) | 56.8( 9) |
| *$\Delta C_s$(1) | 10.0 | 7.0 |
| $\Delta C_s$(2) | 3.8 | 2.0 |
| $\Delta C_s$(3) | 5.5 | 6.7 |
| $\Delta C_2$(1-2) | 10.2 | 5.7 |
| $\Delta C_2$(2-3) | 1.6 | 4.2 |
| $\Delta C_2$(3-4) | 11.1 | 9.8 |
| Ring B | | |
| C(10)-C(5)-C(6)-C(7) | 2.0( 9) | 3.8( 9) |
| C(5)-C(6)-C(7)-C(8) | 11.7( 9) | 10.5(10) |
| C(6)-C(7)-C(8)-C(9) | -41.2( 8) | -41.5( 8) |
| C(7)-C(8)-C(9)-C(10) | 60.9( 8) | 62.8( 8) |
| C(8)-C(9)-C(10)-C(5) | -47.4( 8) | -49.0( 8) |
| C(6)-C(5)-C(10)-C(9) | 15.3( 9) | 14.7(10) |
| $\Delta C_s$(5) | 25.6 | 27.6 |
| $\Delta C_s$(6) | 18.6 | 19.3 |
| $\Delta C_s$(7) | 44.2 | 46.3 |
| $\Delta C_2$(5-6) | 5.1 | 6.1 |
| $\Delta C_2$(6-7) | 44.4 | 46.7 |
| $\Delta C_2$(7-8) | 49.3 | 52.6 |
| Ring C | | |
| C(14)-C(8)-C(9)-C(11) | -48.2( 8) | -47.0( 8) |
| C(8)-C(9)-C(11)-C(12) | 47.3( 9) | 48.2( 8) |
| C(9)-C(11)-C(12)-C(13) | -50.0( 9) | -55.6( 8) |
| C(11)-C(12)-C(13)-C(14) | 54.8( 9) | 60.4( 8) |
| C(12)-C(13)-C(14)-C(8) | -62.4( 9) | -65.2( 8) |
| C(9)-C(8)-C(14)-C(13) | 58.5( 8) | 58.0( 8) |
| $\Delta C_s$( 8) | 10.9 | 12.0 |
| $\Delta C_s$( 9) | 6.6 | 3.2 |
| $\Delta C_s$(11) | 4.7 | 9.8 |
| $\Delta C_2$( 8-9 ) | 11.8 | 9.7 |
| $\Delta C_2$( 9-11) | 2.9 | 6.3 |
| $\Delta C_2$(11-12) | 11.4 | 15.5 |
| Ring D | | |
| C(17)-C(13)-C(14)-C(15) | 47.0( 8) | 43.7( 7) |
| C(13)-C(14)-C(15)-C(16) | -33.3( 8) | -33.3( 8) |
| C(14)-C(15)-C(16)-C(17) | 7.0( 8) | 8.6( 8) |
| C(15)-C(16)-C(17)-C(13) | 19.9( 8) | 17.3( 8) |
| C(14)-C(13)-C(17)-C(16) | -40.4( 8) | -37.9( 8) |
| $\Delta C_s$(13) | 10.6 | 12.0 |
| $\Delta C_s$(14) | 25.5 | 22.0 |
| $\Delta C_s$(15) | 50.8 | 46.5 |
| $\Delta C_s$(16) | 10.4 | 7.0 |
| $\Delta C_s$(17) | 31.8 | 39.8 |
| $\Delta C_2$(13-14) | 10.4 | 7.0 |
| $\Delta C_2$(14-15) | 51.2 | 46.3 |
| $\Delta C_2$(15-16) | 72.4 | 67.9 |
| $\Delta C_2$(16-17) | 65.9 | 63.6 |
| $\Delta C_2$(17-13) | 34.3 | 35.0 |
| (2) Chain | | |
| C(1)-C(2)-C(3)-O(3) | 177.2(12) | 177.3(10) |
| C(2)-C(3)-O(3)-C(28) | 131.4(14) | 128.5(17) |
| C(28)-C(28)-O(3)-C(3) | -5.8(13) | 38.5(15) |
| C(29)-C(28)-O(3)-C(3) | 169.8(17) | 171.1(23) |
| C(4)-C(3)-O(3)-C(28) | -109.6(14) | -112.3(17) |
| O(3)-C(3)-C(4)-C(5) | -173.4(12) | -172.3(12) |
| O(3)-C(28)-C(29)-C(30) | -155.7(25) | -138.1(26) |
| O(3)-C(28)-C(29)-C(31) | 69.3(17) | 99.6(20) |
| O(28)-C(28)-C(29)-C(30) | 18.8(24) | -7.1(19) |
| O(28)-C(28)-C(29)-C(31) | -116.3(28) | -129.4(22) |
| (3) Tail | | |
| C(13)-C(17)-C(20)-C(21) | -51.9(12) | -64.9( 9) |
| C(13)-C(17)-C(20)-C(22) | 173.7(15) | 178.6(11) |
| C(16)-C(17)-C(20)-C(21) | -172.4(15) | 173.6(12) |
| C(16)-C(17)-C(20)-C(22) | 53.3(10) | 57.1( 9) |
| C(17)-C(20)-C(22)-C(23) | -166.8(16) | -166.8(12) |
| C(21)-C(20)-C(22)-C(23) | 56.9(13) | 73.4(10) |
| C(20)-C(22)-C(23)-C(24) | 171.2(18) | -178.8(13) |
| C(22)-C(23)-C(24)-C(25) | 168.7(21) | 164.3(18) |
| C(23)-C(24)-C(25)-C(26) | 174.4(28) | 165.2(23) |
| C(23)-C(24)-C(25)-C(27) | 37.3(25) | 53.3(18) |

* $\Delta C_s$: mirror plane asymmetry parameter

$$\Delta C_s = [\sum_{i=1}^{m} (\phi_i + \phi_i')^2/m]^{\frac{1}{2}}$$

$\Delta C_2$: twofold asymmetry parameter

$$\Delta C_2 = [\sum_{i=1}^{m} (\phi_i - \phi_i')^2/m]^{\frac{1}{2}}$$

where $\Delta C_s(n)$ is a measure of the deviations from mirror symmetry about a plane passing through atom $n$ and the diametrically opposed atom $o$, and $\Delta C_2(n\text{-}o)$ is a measure of the deviations from twofold symmetry about an axis bisecting bond $(n\text{-}o)$. The symmetry related torsion angles are $\phi i$ and $\phi i'$, and $m$ is the number of such pairs.

8.99Å in molecule (B) for cholesteryl isobutyrate. These are in good agreement with other cholesteryl esters which ranged from 8.85Å to 9.02Å[21]. A measure of the twist of the ring system about its long axis, is given by the C(19)-C(10)····C(13)-C(8) pseudo-torsion angle which has a values of 5.8° in molecule (A) and 7.3° in molecule (B). These pseudo-torsion angles for the other cholesteryl esters range from 7.9° to 18.0°[21].

The appropriate ring torsion angles, along with the torsion angles within the tail and chain are given Table 6. Also given are the appropriate mirror plane and the twofold asymmetry parameters of the ring as defined by Duax and Norton[22]. *A*-ring and *C*-ring assumed to be chair conformations, with *C*-ring somewhat distorted (for A-ring, $<\Delta C_s>$ = 6.44 and $<\Delta C_2>$ = 7.65; whereas C-Ring, $<\Delta C_s>$ = 7.40 and

**Table 7. Least-squares Planes and Deviation(Å) of Individual Atoms from These Planes in Cholesteryl Isobutyrate. The Equation of Plane is Expressed in the form Ax + By + Cz = D, where *x,y* and *z* are in Å and with Respect to Orthogonal Axes**

| Atoms included in plane | Atoms not included in plane | Distance in Å from the best plane Molecule(A) | Molecule(B) | Given constant Molecule(A) | Molecule(B) |
|---|---|---|---|---|---|
| (1) tetra-cyclic ring system, C(1) through C(17) | | | | | |
| | | | | A = 0.227 | A = -0.409 |
| | | | | B = 0.966 | B = 0.853 |
| | | | | C = -0.126 | C = 0.325 |
| | | | | D = 1.206 | D = 7.932 |
| (2) ethylenic group, C(4) through C(7) and C(10) | | | | | |
| C(5) | | 0.009 | 0.007 | A = 0.073 | A = -0.519 |
| C(6) | | 0.021 | 0.032 | B = 0.996 | B = 0.681 |
| C(10) | | 0.001 | 0.004 | C = 0.060 | C = 0.517 |
| C(4) | | -0.017 | -0.018 | D = -0.222 | D = 6.012 |
| C(7) | | -0.015 | -0.022 | | |
| (3) alkanoate chain, C(30), O(3), C(3) and O(28) | | | | | |
| C(3) | | 0.054 | 0.016 | A = 0.645 | A = -0.722 |
| O(3) | | -0.044 | -0.038 | B = -0.486 | B = 0.110 |
| C(28) | | -0.014 | 0.313 | C = -0.590 | C = 0.683 |
| O(28) | | -0.013 | -0.060 | D = -0.216 | D = 3.876 |
| C(29) | | 0.124 | -0.034 | | |
| | C(2) | -1.048 | 0.783 | | |
| | C(4) | 1.406 | -1.364 | | |
| | C(30) | 0.624 | 1.404 | | |
| | C(31) | -1.003 | -0.533 | | |
| (4) C(17) side chains, C(17), C(20), C(22) through C(26) | | | | | |
| C(17) | | -0.061 | -0.043 | A = -0.270 | A = -0.722 |
| C(20) | | 0.001 | 0.037 | B = 0.933 | B = 0.110 |
| C(22) | | 0.194 | 0.103 | C = 0.239 | C = 0.683 |
| C(23) | | -0.038 | -0.136 | D = 1.792 | D = 3.876 |
| C(24) | | -0.044 | -0.010 | | |
| C(25) | | -0.011 | 0.141 | | |
| C(26) | | -0.153 | -0.114 | | |
| | C(21) | 0.882 | 1.362 | | |
| | C(27) | -0.628 | -0.851 | | |

Dihedral angles (°) between the planes.

| (A) | (B) | | (A) | (B) |
|---|---|---|---|---|
| (1)/(3) 104.4 | 52.3 | (1)/(4) | 36.0 | 45.8 |

$<\Delta C_2>$ = 8.70 in molecule (A), and for *A*-ring, $<\Delta C_s>$ = 5.22 and $<\Delta C_2>$ = 6.55; whereas C-ring, $<\Delta C_s>$ = 8.32 and $<\Delta C_2>$ = 10.50 in molecule (B)). But the *B*-rings are 8 to 9 half-chair conformations, and the D-rings are near a 13, 14-twist conformation.

In the fatty acid cholesteryl esters, the twist at the ester linkage C(3)-O(3) is important for determining the overall shape of the molecules. The bond O(3)-C(28) is almost trans either to bond C(3)-C(2) and to bond C(3)-C(4). The torsion angles of C(2)-C(3)-O(3)-C(28) are 131° in molecule (A) and 129° in molecule (B), the C(4)-C(3)-O(3)-C(28) torsion angles are -110° and -112° for molecules (A) and (B), respectively.

The C(17) side chains are fully extended in both molecules, The C(17)···C(25) distances, taken as a measure of the extension of the tail, are 6.27(2)Å and 6.28(2)Å for molecules (A) and (B), respectively. This is characteristic of the antiperiplanar conformation, which is generally expected for the alkane chain, about C(22)-C(23) and C(23)-C(24). (see Table 6)

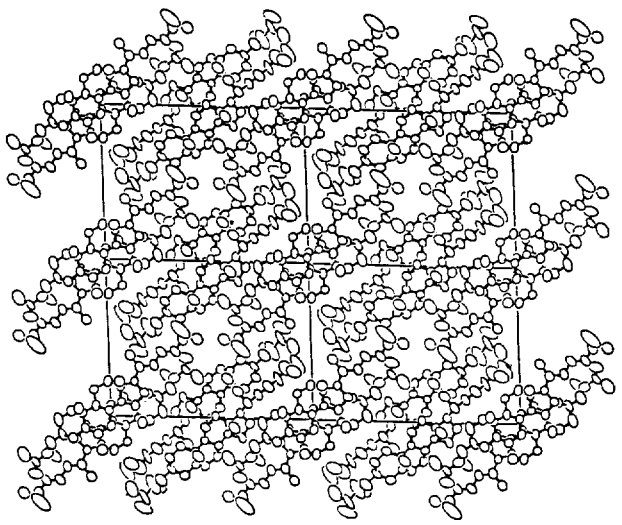

**Figure 3a.** The crystal structure cholesteryl isobutyrate in projection down the *b*-axis. Atoms are shown as 50% probability ellipsoids. Four unit cells are shown.

The least-squares planes and the deviations of neighboring atoms from each plane are listed in Table 7. The atoms within ethylenic group are nearly coplanar. The atoms of C(17), C(20), C(22) through C(26) are in a zigzag chain and C(21) and C(27) are out of the plane. Cholestrol tetracyclic ring system nucleus least-squares plane makes an angle of 104.4° and 52.3°, with the isobutyrate group and of 35.9° and 45.8° with the plane of C(17) side zigzag chain atoms for molecules (A) and (B), respectively.

The packing diagrams are shown in Figure 3a and 3b. The plane of the cholesterol fragments are parallel to the *ac* plane with the entire molecular long axes being nearly parallel to the [$\bar{1}$01] directions. The structure of cholesteryl isobutyrate consists of antiparallel molecules arranged to form monolayers that are parallel to the crystal plane (100) and thickness of $d_{100}$ = 15.12Å. Each layer is made up of row of a pair of molecules (A) and (B) packed tail to tail. The most interesting feature is that the two distinct molecules form separated stacks which have similar orientations but with differing degrees of steroid overlap; more efficient cholesteryl packing of the molecule in (A) than molecule in (B) with each other in stacks along the crystallographic *b*-axis.

The monolayers are regions of closely packed molecules which are separated by interface regions where atoms are more loosely packed. The efficiency of cholesteryl packing arrangements is in contrast to the packing of the isobutyrate chain. It is loosely packed to form the monolayer interface region. The overall packing type of cholesteryl isobutyrate is similar to those of cholesteryl hexanonate[7], formate[8], hexyl cabonate[9], octanonate[10], oleate[11] and chloroformate[12] which are called Type II Monolayer, while cholesteryl isobutyrate has two independent molecules in the asymmetric unit.

A notable feature of Type II monolayer structure is that cholesteryl-cholesteryl interactions may be the principal crystal packing forces since these forces may be greater than

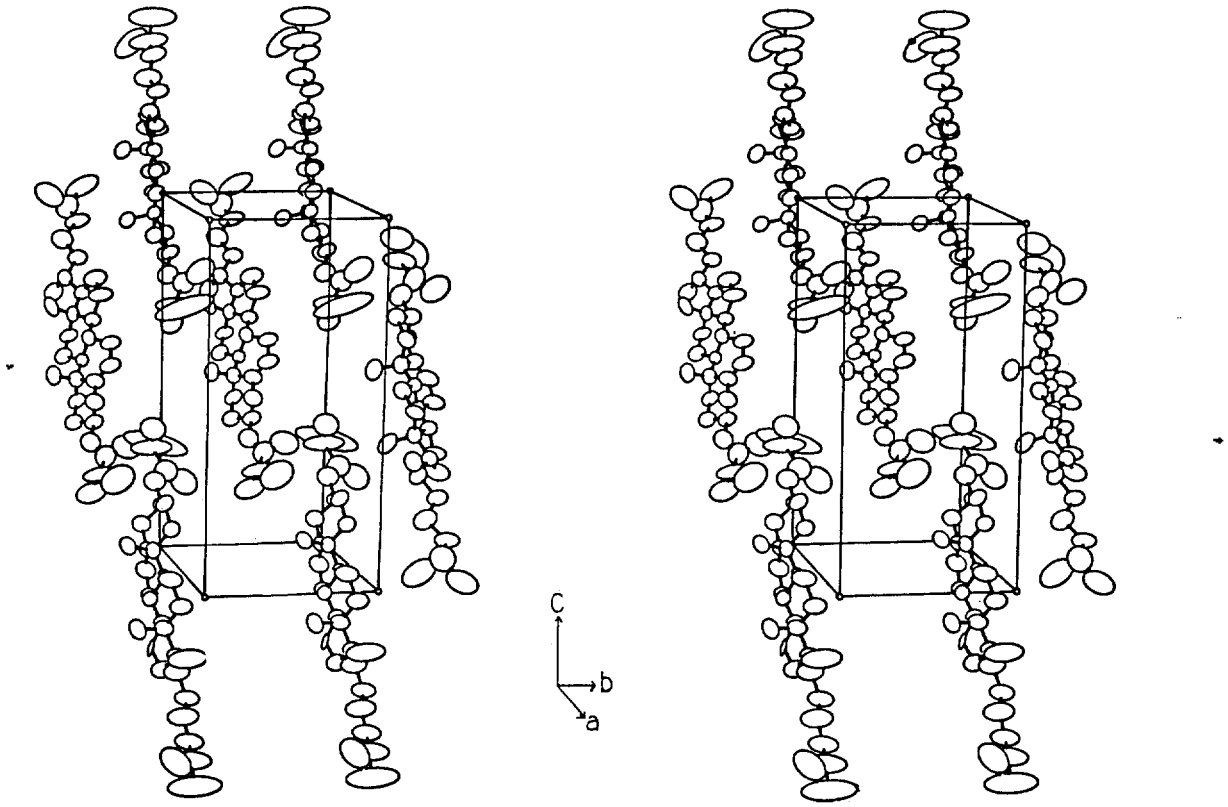


**Figure 3b.** Stereoview of the crystal structure of cholesteryl isobutyrate in view down the *a*-axis.

**Table 8. Intermolecular distances(Å) less than 4.0Å in Cholesteryl Isobutyrate**

| | | |
|---|---|---|
| C(A18) ... O(B26) | 3.84 | 1 / 0 1 0 |
| C(A23) ... O(B28) | 3.95 | 1 / 1 1 1 |
| C(A27) ... O(B28) | 3.60 | 1 / 1 1 1 |
| C(A30) ... C(B4) | 3.90 | 2*/ 1 1 1 |
| C(A31) ... C(B7) | 3.82 | 1 / 0 1 0 |
| O(A28) ... C(B31) | 3.89 | 2 / 1 1 1 |

*Distance is between C(A30) at symmetry position 1($x,y,z$) and C(B4) at symmetry position 2($-x$, $1/2+y$, $-z$) and translated 1 unit cell along $a$, 1 unit cell along $b$, 1 unit cell along $c$.

any other interactions involving the shorter ester chains.

In the directions which are more or less parallel to the *b*-axis, there are multiple intermolecular distances less than van der Waals distance between methylene groups (4.0Å) of which the shortest is C(A27)-O(B28) of 3.60Å (Table 8).

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# Computer Graphics / Molecular Mechanics Studies of β-Lactam Antibiotics. Geometry Comparison with X-Ray Crystal Structures

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Geometries for a number of representative β-lactam antibiotics (penams, cephems and monobactams) have been calculated by computer graphics/molecular mechanics energy minimization procedures using both MM2 and AMBER force fields. The calculated geometries have been found in reasonable agreement with the geometries reported in the X-ray crystal structures, especially in terms of the pyramidal character of the amide nitrogen in the β-lactam ring and the Cohen distance. Based on these calculations, it is suggested that the nitrogen atom in the monobactams may also have pyramidal geometries in the biologically active conformations.

## Introduction

Computer-assisted molecular design (CAMD) is recently becoming on important new tool in the molecular research areas such as organic synthesis, enzyme catalysis, drug-receptor interactions and protein engineering.[1-10] The CAMD technique commonly involves the interactive manipulation of three-dimensional molecular structure information by means of a sophisticated computer graphics system; the requisite 3-D structural information is typically obtained from X-ray crystallographic data and from theoretical computations. In the case of "small molecules" (excluding proteins and other macromolecules) X-ray crystallographic methods provide highly precise unambiguous information of molecular structure and conformation in the solid state. Subject to certan limitations, *e.g.* disorder in the crystal littice, occluded solvent, polymorphism, unusual intermolecular interactions or crystal packing forces, the X-ray experiment provides a time-averaged model of the low energy conformations of the given molecule within a given crystal lattice environment. More recently, 2D-NMR techniques such as NOE measurments are becoming an increasingly powerful tool for exploring molecular conformations in solution.

Theoretical calculation methods including *ab initio*, semiempirical quantum mechanics and molecular mechanics (energy minimization, grid searching of conformational space, Monte Carlo searches and molecular dynamics simulations) have been employed to generate and study 3-D molecular conformations. *Ab initio* and semiempirical quantum mechanics calculations have profitably been applied to a wide range of chemical problems but they have not yet been shown practical for studying molecular interactions involved with, for example, enzyme catalysis and ligand-receptor interactions.[4] Molecular mechanics calculations have been very useful in studying organic molecules in non-polar solvents and in the gas phase. They use simple analytical functions to represent bond stretching, bending, and torsional and non-bonded (dispersion, attraction exchange repulsion and electrostatic interaction) energies of molecules.[11] Since evaluation of these analytical functions is computationally rapid and efficient the molecular mechanics methods can be applied to the study of complex molecular systems and interactions.

In connection with our research program of applying the CAMD techniques to the design of physiologically important molecules,[12] we desired to evaluate the reliability and utility of the computer graphics/molecular mechanics method in the molecular design of several types of antibiotics. Thus, we have generated a number of energy minimized molecular conformations of the β-lactam antibiotics by using molecular mechanics calculations, compared them with the corresponding solid state X-ray crystallographic conformation and herein report the results.

## Results and Discussions

Among the representative β-lactam antibiotics whose X-ray crystal structures are available either through the published literature or accessible Cambridge Structures Database(CSD), we have selected three penams(**1-3**), two cephems(**4,5**), three oxa/carbapenams(**6-8**), and two monobactams(**9,10**) as test examples. The structures were interac-

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