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Structure and pseudosymmetry of cholesterol at 310 K

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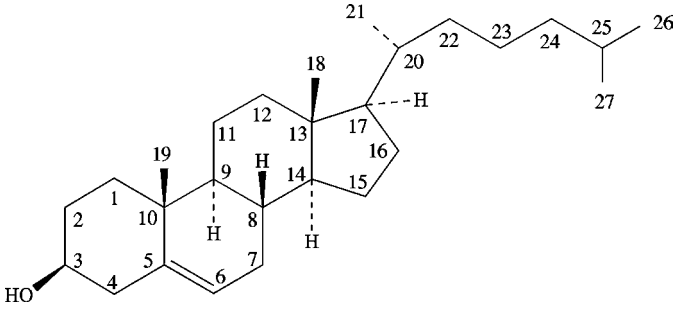
The structure of cholesterol above the (304.8 K) phase transition, previously published in preliminary form [Hsu & Nordman (1983). *Science*, **220**, 604–606], has been fully refined using augmented X-ray data. The crystals are triclinic, space group $P1$, with (reassigned) cell parameters $a = 27.565\ (10)$, $b = 38.624\ (16)$, $c = 10.748\ (4)$ Å, $\alpha = 93.49\ (3)$, $\beta = 90.90\ (3)$, $\gamma = 117.15\ (3)^\circ$, and $V = 10151\ (7)$ Å^3. The unit cell contains $Z = 16$ molecules, of which eight are related to the other eight by unusual twofold rotational pseudosymmetry. The structure is related to the room-temperature phase, with $Z = 8$, by a rearrangement of some of the molecules, and by a doubling of the a axis.

1. Introduction

Background information on the 304.8 K phase transition in crystalline cholesterol has been given by Hsu & Nordman (1983), hereafter HN, and will not be repeated here. In Fig. 1 of HN the 310 K (37°C) structure was shown in a nonstandard cell in order to display its relationship to the published room-temperature structure as a doubling of the a axis. Here we describe the 310 K structure in terms of a standard cell; the simple relationship of a doubling of the a axis of the room-temperature cell is preserved if the unit cell axes of the latter (Shieh *et al.*, 1981) are transformed as $\mathbf{a}_{new} = \mathbf{a}_{old}$, $\mathbf{b}_{new} = \mathbf{b}_{old} - \mathbf{a}_{old}$, $\mathbf{c}_{new} = \mathbf{c}_{old}$. With this transformation the cell parameters of the room-temperature phase are $a = 14.172\ (7)$, $b = 38.443\ (18)$, $c = 10.481\ (5)$ Å, $\alpha = 93.88\ (4)$, $\beta = 90.67\ (4)$, $\gamma = 117.81\ (4)^\circ$, and $V = 5033\ (4)$ Å^3. As before, the eight independent molecules in the room-temperature cell are labeled $A, B, \ldots, H$. In the doubled 310 K cell the 16 molecules are correspondingly labeled $A1, B1, \ldots, H1, A2, B2, \ldots, H2$ (Fig. 1).



2. Data collection and refinement

The original (HN) X-ray data were collected over one hemisphere of reciprocal space, on a Syntex $P1$bar diffractometer,

with Cu $K\alpha$ radiation, using eight consecutive crystals. The data from each crystal were corrected for radiation decay, about 10%, and merged to yield 18 047 reflections with $I > 1.5\sigma(I)$. Initial isotropic refinement was carried out with a restrained rigid-group refinement program based on the Gauss–Seidel algorithm (Hoard & Nordman, 1979). This program, intended for macromolecules, did not provide for anisotropic displacement parameters. Further anisotropic block diagonal refinement, without parameter restraints, was attempted next. However, the behavior of the hydrocarbon tails of the molecules, which exhibited extreme thermal motion, or disorder, made it clear that a program allowing both restraints and anisotropic displacement parameters was needed for satisfactory refinement. The best result at this point was $R = 0.080$ for the 18 047 independent reflections, as reported in HN.

The problem was revisited in 1999. As a Bruker CCD detector was now available, it was felt that a new and, to be hoped, better data set could be collected. A large number of crystals were grown from dioxane solution at 310 K, as before, and maintained at that temperature during transfer and mounting. During the data collection the crystals were kept in a stream of nitrogen gas at 310 K. For unknown reasons the crystals were somewhat smaller than in the previous batch. Complete data sets were collected from two specimens. Disappointingly, the CCD data sets were clearly inferior to the diffractometer data. Nonetheless, a judicious merging of the three sets resulted in an improvement over the original diffractometer data.

The data in the two CCD sets were processed with *SAINT* (Bruker, 1999) and *SADABS* (Sheldrick, 1996). Within each set, average $F^2(hkl)$ and $\sigma[F^2(hkl)]$ values were obtained by weighted averaging over the multiple observations, assuming $F^2(hkl) = F^2(-h,-k,-l)$. Acceptance criteria for the CCD data sets were established by comparing them, without refining, with the original HN set of F_c^2 values. Accepting CCD F^2 values exceeding three times their sigmas and having d values between 16.5 and 1.4 Å, the two sets were scaled and merged, yielding 4555 reflections with R_2merg = 0.0582 for 4223 reflections common to the two CCD sets. This set of 4555 combined CCD reflections was similarly scaled to and merged with the old diffractometer data set, giving R_2merg = 0.0940 and a new total of 18 051 reflections.

The structure was refined with *SHELX*97 (Sheldrick, 1997). Restraints were imposed on all bond lengths and second nearest-neighbor distances in the hydrocarbon tails, starting at C20. Similarly, restraints were imposed on the components of the displacement parameters in the direction of the interatomic vectors of bonded and second nearest neighbors in the hydrocarbon tails. These restraints were taken as particularly soft, with standard deviations of 0.04 and 0.08 Å, respectively, in order not to bias the choice between extreme thermal motion and disorder. H atoms bonded to carbon were in calculated positions with 'riding' refinement; the U_{eq} values of CH_2 and OH H atoms were taken as 1.2 times the carbon or oxygen values, those of CH_3 H atoms as 1.5 times. All H atoms attached to O atoms are involved in hydrogen bonds. These were taken to form tetrahedral C—O—H angles, and the O—H distances were softly restrained, with $\sigma = 0.05$ Å. The H···O distances were unrestrained. The hydroxyl hydrogen positions in the room-temperature structure (Shieh *et al.*, 1981), in which all OH H atoms were found in difference Fouriers, were taken as starting positions in the *SHELX*97 refinement. The refinement was stable and led to reasonable geometry for all 16 hydrogen bonds.

The parameters $a = 0.015$ and $b = 0.0$ in the weight expression (WGHT) were chosen by a trial and error procedure which minimized the mean standard deviations of the coordinates of the O and ordered C atoms. The standard deviations achieved in this way were ∼13% lower than those resulting from refinement with weight parameters recommended by *SHELX*97 (Sheldrick, 1997), namely $a = 0.104$ and $b = 1.035$. Details of the data collection and structure refinement are given in Table 1.[1]

All crystal structures of cholesterol and its solvates show extreme thermal motion in the hydrocarbon tails. The present structure, determined above room temperature, presents particular difficulties in deciding whether to model the behavior as thermal motion or disorder. Guided by prominent difference electron density peaks, we have, conservatively,

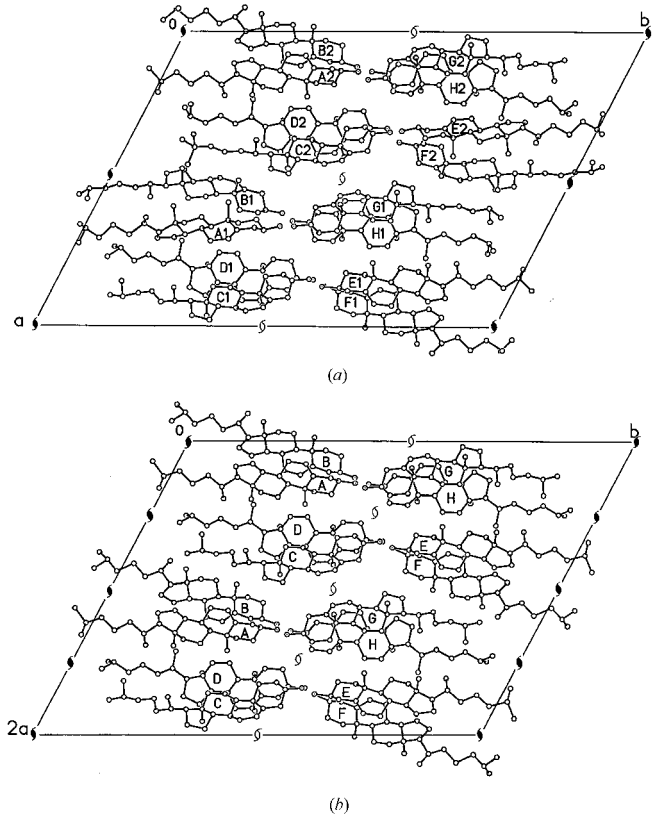


Figure 1
(*a*) One cell of the 310 K structure and (*b*) two cells of the room-temperature structure. The 'screw axis' symbols are explained in the text.

[1] Supplementary data for this paper are available from the IUCr electronic archives (Reference: BK0099). Services for accessing these data are described at the back of the journal.

Table 1
Experimental details.

Crystal data	
Chemical formula	$C_{27}H_{46}O$
Chemical formula weight	386.64
Cell setting, space group	Triclinic, *P*1
a, *b*, *c* (Å)	27.565 (10), 38.624 (16), 10.748 (4)
α, β, γ (°)	93.49 (3), 90.90 (3), 117.15 (3)
V (Å^3)	10151 (7)
Z	16
D_x (Mg m^{-3})	1.012
Radiation type	Mo $K\alpha$ (CCD), Cu $K\alpha$ (*P*bar)
No. of reflections for cell parameters	4586
θ range (°)	2.15–18.98
μ (mm^{-1})	0.435
Temperature (K)	310 (2)
Crystal form, color	Plate, colorless
Crystal size (mm)	0.60 × 0.30 × 0.04
Data collection	
Diffractometer	Syntex *P*1bar, Bruker CCD
Data collection method	ω and φ scans
No. of measured, independent and observed parameters	18 051, 18 051, 16 847
Criterion for observed reflections	$I > 2\sigma(I)$
R_{int}	0.0000
θ_{max} (°)	52.54
Range of *h*, *k*, *l*	$0 \rightarrow h \rightarrow 28$ $-39 \rightarrow k \rightarrow 35$ $-11 \rightarrow l \rightarrow 11$
No. and frequency of standard reflections	3 every 50 reflections
Intensity decay (%)	10
Refinement	
Refinement on	F^2
$R[F^2>2\sigma(F^2)]$, $wR(F^2)$, *S*	0.0637, 0.0955, 1.993
No. of reflections and parameters used in refinement	18 051, 4091
H-atom treatment	Mixed
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.0150P)^2 + 0.0000P]$, where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$	0.004
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.173, −0.159

Computer programs used: *SMART*, *SAINT* (Bruker, 1999), *SHELXL*97 (Sheldrick, 1997), *SHELXTL* (Bruker, 1997).

treated one of the 16 hydrocarbon chains as partly disordered. In molecule C2 the terminal isopropyl group, atoms C26 and C27, occupies two rotational positions. An apparent disorder also exists in the mid-section of the chain of molecule C1, where atoms C22 and C23 each occupy two (at least) slightly different positions. Refining these as split atoms did not significantly improve the agreement and we choose not to model this as a disorder.

It is noteworthy that all carbon–carbon distances at the ends of the chains are short, although restrained to nominal values. This implies a wagging motion of the chain ends, with presumably kidney-shaped atoms modeled as ellipsoids with shortened bonds.

3. Description of the structure

As in all structures of cholesterol and its solvates, the molecules are in a bilayer arrangement, with alternating hydrophilic and hydrophobic layers. In the hydrophilic layer there are four hydrogen-bonded chains parallel to the *c* axis, as shown in Fig. 2. As in the room-temperature form, the H atoms of all OH groups point in the positive *c* direction. Both forms of anhydrous cholesterol differ from the hydrate (Craven, 1976, 1979) and the hemiethanolates (Shieh *et al.*, 1982) in that the hydrogen-bonded chains are not coplanar, but staggered in the *b* direction by *ca* ±1.6 Å about their mean plane, forming a corrugated sheet parallel to the *ac* plane (see Fig. 1). Correspondingly, the hydrocarbon tails are also staggered, resulting in considerable interdigitation in the hydrophobic layer. Such interdigitation is not found in the solvated cholesterol structures.

An ellipsoid plot of the 310 K structure is given in Fig. 4.

Liang *et al.* (1995) have reported force-field studies of pairwise cholesterol–cholesterol molecular interactions, without, however, considering hydrogen bonding. They found several low-energy molecular pairings, some of which can be recognized in the crystal structure. Denoting the 'flat' face of the molecule α, and the opposite face, with two protruding methyl groups, β, we find a clear $\alpha\cdots\alpha$ packing in the pair $A2\cdots B2$ (Fig. 3). These molecules have the shortest center-to-center distance, 4.91 Å. The $\beta\cdots\beta$ packing is much less favorable; it could be said to exist between *B*1 and *C*2, whose center$\cdots$center distance is quite long, over 10 Å. Low energy, so called β (major groove), contacts exist between *B*2(+**c**) and *A*2 (8.09 Å), *B*1 and *A*1 (6.36 Å), *D*2 and B1 (>10 Å), and also between *D*1 and *B*2 (+**a**) (9.93 Å). These authors (Liang *et al.*, 1995) also did a molecular mechanics study of the rigidity of the tetracyclic ring system, *in vacuo*, at 500 K. The variability of several torsion angles in the molecule may be compared to the variability under 16 different packing environments in the crystal structure, although the latter is obviously much smaller. We find that the variability in the torsion angles of ring *B* is greater than the variability in either ring *A* or ring *C*. This parallels the result obtained by Liang *et al.* in their 500 K molecular mechanics study. In ring *B* we find that the torsion angles C05—C06—C07—C08 cover a range of 9.4° (81° at 500 K), whereas C09—C08—C14—C13 and C08—C14—

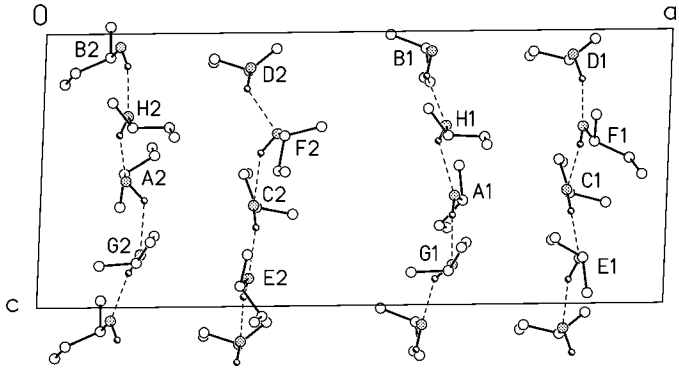


Figure 2
The four hydrogen-bonded chains. The OH groups and their adjacent carbon atoms C01, C02, C03 and C04 of all 16 molecules are shown. The view is along the *b* axis, toward the origin. Compare with Fig. 1(*a*).

C13—C12 in ring *C* have the ranges 5.2 and 6.3°, respectively (36 and 38° at 500 K). Remarkably, the torsion angle C10—C05—C06—C07, across a double bond, shows a range of 10.6° (65° at 500 K). The torsion angles in ring *A* are also more constant than those of ring *B*, varying over the ranges 8.1, 8.2, 7.5, 5.6 and 5.3°. The conformation of the hydrocarbon tails can be described in terms of the torsion angles C20—C22—C23—C24 and the less accurately determined C22—C23—C24—C25. With two exceptions, the former angle is within 7.2° of ±180°, *i.e.* pure *trans*. The exceptions are molecules *C*2 (−169.7°) and *G*2 (−165.9°); these two molecules are related by pseudosymmetry. The latter torsion angle shows much greater variability; again, with two exceptions, the values are within 35.2° of pure *trans*. The exceptions are molecule *C*1 (−91.9°) and *G*2 (−59.4°), these two molecules are related by pseudosymmetry. Within the remaining seven pseudosymmetrically related pairs of molecules the agreement is much closer; the greatest difference is 9.1°, the average difference only 4.4°.

4. Pseudosymmetry and phase transition

The remarkable pseudosymmetry present in the structures is indicated in Fig. 1. The 'open' screw-axis symbols indicate pseudoscrew axes, essentially along the *c* axis, with alternating *z* translations of nearly 1/4 and 3/4. The pseudoasymmetric unit *A*1,*B*1,*C*1,*D*1,*A*2,*B*2,*C*2,*D*2, when rotated about *x*,*y* = 0.5,0.5 and translated by $\Delta z \simeq 0.25$, matches *E*2,*F*2,*G*2,*H*2,*E*1,*F*1,G1,*H*1 with an r.m.s. atom–atom misfit of only 0.31 Å for 8 × 28 = 224 atom pairs. Similarly, the $E2,\ldots,H1$ unit is rotated onto the $A1,\ldots,D2(+\mathbf{c})$ unit with $\Delta z \simeq 0.75$. The 'filled' screw-axis symbols denote 'regular' pseudoscrew axes which superimpose $A1,\ldots,D2$ on $E2,\ldots,H1(-\mathbf{b})$ with $\Delta z \simeq 0.5$ and likewise $E2,\ldots,H1(-\mathbf{b})$ on $A1,\ldots,D2(+\mathbf{c})$. Coaxial with the 'filled' pseudoscrew axes are twofold pseudorotation axes which transform for example $E2,\ldots,H1$ into $A1,\ldots,D2(-\mathbf{b})$ with $\Delta z \simeq 0$; these units are more distant by *b* in the *y* direction than the above-mentioned pairs. The fact that $b\cos\alpha \simeq 0.25c$ underlies the relationship between pseudoscrew and rotation axes. The pseudosymmetry in the 310 K form is quite analogous to the pseudosymmetry in the room-temperature structure, except that the pseudoasymmetric unit in the 310 K structure is twice as big. A diagrammatic view of the axis arrangement in the room-temperature structure has been given by Shieh *et al.* (1977).

The 310 K structure is compared with the room-temperature structure in Fig. 1. As reported in HN, the main difference between the two is in molecules *A*1 and *E*2, which rotate by 157° about their long axes, in going from the room-temperature to the 310 K structure. Similarly, molecules *B*1 and *F*2, which neighbor *A*1 and *E*2, respectively, rotate by 87°. The pair *A*2*B*2 shows a slight movement, but no rotation, as does the pseudosymmetrically related pair *E*1*F*1. The rest of the molecules, *i.e.* the pairs *C*, *D*, *G* and *H*, are essentially unchanged from the room-temperature structure, except for a change in the tail conformations of *C*2 and *G*1. Since in the room-temperature structure *A*2*B*2 and *E*2*F*2 are related to *A*1*B*1 and *E*1*F*1 by an **a** translation, the *a* axis is doubled in the 310 K structure. A possible rationale for this unusual phase transition may be found in the short intermolecular distances between the interdigitated tails of molecules *B* and *F* in the room-temperature structure, corresponding to *B*1 and *F*2 in the 310 K form. The hydrophobic contacts between C16 of *B* and C27 of *F* (−**b**–**c**) (3.47 Å) and between C27 of *B* and C16 of *F* (−**b**) (3.48 Å) are the shortest intermolecular C···C distances found in either structure. One might speculate that these hydrophobic contacts influence the simultaneous rotation of the pairs *A*1,*B*1 and *E*2,*F*2. The closest contacts between the interdigitated tails of B1 and F2 in the 310 K structure is 3.83 Å, from C21 of *B*1 to C26 of *F*2 (−**b**). Identically close contacts exist between the room-temperature counterparts of *B*2 and *F*1. These are also relieved in the phase transition as a result of the movement of the pairs *A*2,*B*2 and *E*1,*F*1.

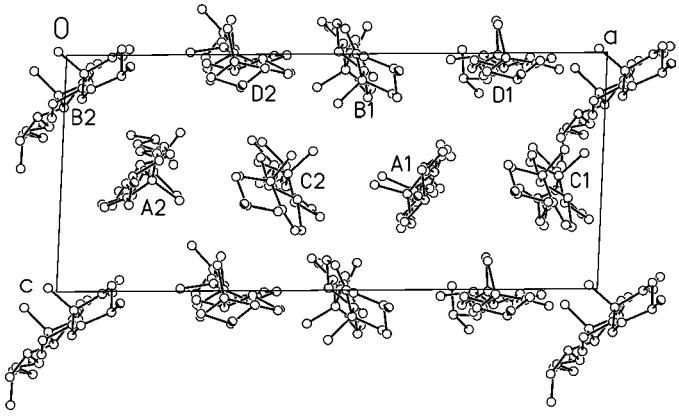


Figure 3
One half of the unit cell viewed along *b*, towards the origin. The figure represents the left half of the cell shown in Fig. 1(*a*), and shows the lateral packing of molecules, and the reorientation of molecules *A* and *B*.

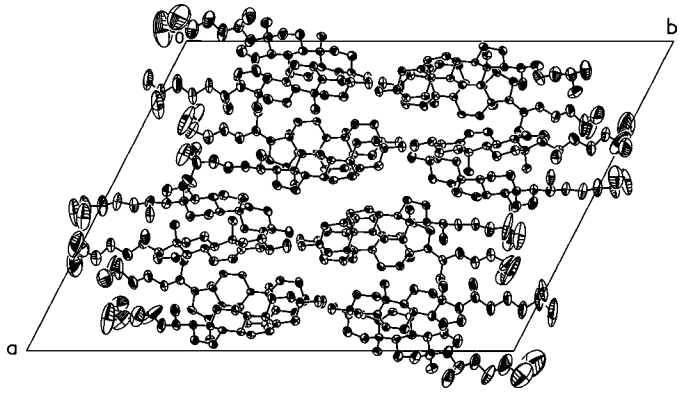


Figure 4
Ellipsoid plot, at 25% probability, of one cell of the 310 K structure of cholesterol, viewed along the *c* axis. The apparent disorder in the vicinity of C23 of molecule *C*1, discussed in the text, can be seen in the lower left of the figure.

As expected, the packing of molecules is less tight in the high-temperature phase. This can be seen in the number of short C···C intermolecular distances in the two structures. In the (doubled) room-temperature cell there are 34 such contacts less than 3.7 Å, 12 less than 3.6 Å. The corresponding numbers for the 310 K cell are 12 less than 3.7 and 2 less than 3.6 Å.

Finally, it should be pointed out that a phase transition where the unit cell is (approximately) *doubled* on heating is highly unusual, if not unique. The normal transition behavior is that the *crystallographic symmetry increases* on heating. This frequently includes translational symmetry; in such cases the unit cell size is *reduced* by a rational factor, often it is halved. The volume of the doubled cell, and the density, change in the expected directions: on going from the room-temperature to the 310 K phase (Fig. 1), 2*a* contracts by 2.7%, *b* and *c* expand by 0.5 and 2.5%, respectively, and 2*V* expands by 0.8%.

References

Bruker (1997). *SHELXTL*, Version 5.10. Bruker Analytical X-ray Instruments, Madison, Wisconsin, USA.

Bruker (1999). *SAINT PLUS*, Version 6.02. Bruker Analytical X-ray Instruments, Madison, Wisconsin, USA.

Craven, B. M. (1976). *Nature*, **260**, 727–729.

Craven, B. M. (1979). *Acta Cryst.* B**35**, 1123–1128.

Hoard, L. G. & Nordman, C. E. (1979). *Acta Cryst.* A**35**, 1010–1015.

Hsu, L.-Y. & Nordman, C. E. (1983). *Science*, **220**, 604–606.

Liang, C. (1995). *J. Comput. Chem.* **16**, 883–897.

Sheldrick, G. M. (1996). *SADABS*. University of Göttingen, Germany.

Sheldrick, G. M. (1997). *SHELXL*97. University of Göttingen, Germany.

Shieh, H.-S., Hoard, L. G. & Nordman, C. E. (1977). *Nature*, **267**, 287–289.

Shieh, H.-S., Hoard, L. G. & Nordman, C. E. (1981). *Acta Cryst.* B**37**, 1538–1543.

Shieh, H.-S., Hoard, L. G. & Nordman, C. E. (1982). *Acta Cryst.* B**38**, 2411–2419.