

## Crystal Structure and Thermal Vibrations of Cholesteryl Acetate from Neutron Diffraction at 123 and 20 K

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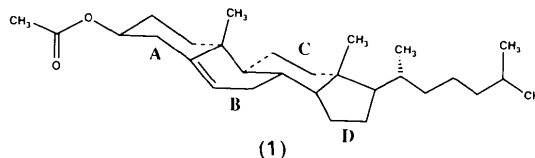
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### Abstract

Cholesteryl acetate ( $C_{29}H_{48}O_2$ ) at 20 K is monoclinic, space group  $P2_1$  with  $a = 16.521$  (4),  $b = 9.220$  (2),  $c = 17.620$  (5) Å,  $\beta = 107.18$  (2)° and  $Z = 4$  (two molecules in the asymmetric unit). The crystal structure, earlier determined by X-ray diffraction at 123 K, has been redetermined at 123 and 20 K by neutron diffraction (7447 reflections at 123 K, 7281 at 20 K;  $\sin\theta/\lambda < 0.69$  Å<sup>-1</sup>) and refined by full-matrix least squares with 1423 variables to give  $R(F^2) = 0.10$  at 123 K, 0.06 at 20 K. Results at 20 K are more accurate not only because nuclear thermal vibrations have reduced amplitudes but also because a larger crystal was used for data collection. At 20 K, the average of 44 methylene C—H bond lengths is 1.102 (8) Å ( $\sigma$  from the observed distribution) and 1.103 (6) Å for 16 methine groups. The 22 methylene H—C—H angles [ $106.2$  (9)°] all fall within a narrow range. Analysis of nuclear anisotropic thermal parameters shows that intramolecular vibrations of the H nuclei are highly significant with similar mean-square amplitudes at 123 and 20 K. Torsional vibrations around the C—CH<sub>3</sub> bonds of the C18 and C19 methyl groups have r.m.s. amplitudes of about 7°. For methylene groups, mean-square amplitudes are a minimum for C—H bond stretching (0.006 Å<sup>2</sup>) and a maximum for CH<sub>2</sub> in-plane vibrations (0.024 Å<sup>2</sup>). Values of  $B_{eq}$  for the H-atom intramolecular vibrations are 0.8, 1.2 and 1.6 Å<sup>2</sup> for methine, methylene and methyl H atoms respectively. These results can be used to estimate  $B_{eq}$  for H atoms in crystal structure determinations carried out by X-ray diffraction. The anisotropic intramolecular vibrations of the H nuclei contribute to the effective shape of the H atoms which, from the observed anisotropy in the distribution of intermolecular H···H distances, appears to be flattened along the direction of the C—H bond.

### Introduction

Inspection of a space-filling molecular model for cholesteryl acetate (1) indicates that almost the entire outer surface of the lipid molecule consists of H atoms from methylene, methine or methyl groups. In



order to study the details of the H-atom configuration and the intermolecular H···H distances, the crystal structure of cholesteryl acetate has been determined by neutron diffraction. The structure has been determined at two low temperatures (20 and 123 K) for a better understanding of the thermal vibrations, particularly the intramolecular vibrations of the H nuclei. The present study complements an earlier X-ray structure determination at 123 K (Sawzik & Craven, 1979; henceforth SC), in which the molecular framework was accurately defined. One other steroid crystal structure has been determined by neutron diffraction, namely [20-C<sup>2</sup>H<sub>3</sub>]-20-methylpregnenediol methanolate at 123 K. However, this work has been published only in preliminary form (Fronckowiak & McMullan, 1980; Fronckowiak, 1981; hereafter FM).

### Experimental

Cholesteryl acetate was obtained from Supelco Chemical Company and crystallized by slow evaporation from *n*-pentanol at room temperature, as described by SC. The first of two crystals used for neutron data collection was a prism of volume 3.1 mm<sup>3</sup> and mass 3.3 mg, with maximum dimension 2.6 mm along *b*, exhibiting the faces {100}, {001}, (010), (0 $\bar{1}\bar{1}$ ) and (0 $\bar{1}$ 1). Neutron data were measured using the four-circle automatic diffractometer at the

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H6-S station of the High Flux Beam Reactor at Brookhaven National Laboratory. The crystal, glued to a hollow aluminium pin, was enclosed in a helium-filled aluminium can and the assembly was mounted so that the crystal was oriented with the  $-b$  axis at  $5^\circ$  from the diffractometer  $\varphi$  axis. The crystal was cooled by a closed-cycle refrigerator\* with temperature control  $< 0.5$  K. The neutron beam was obtained by (022) reflection from a germanium monochromator crystal. The neutron wavelength,  $\lambda = 1.1593$  (1) Å, was determined previously from a least-squares fit of 32  $\sin^2\theta$  values measured with a standard KBr crystal ( $a_0 = 6.6000$  Å at 298 K).

The first set of neutron data for cholesteryl acetate was collected at 123 K, this being the estimated temperature for the previous X-ray study. Unit-cell dimensions (Table 1), which were derived from a least-squares fit of  $\sin^2\theta$  values for 32 neutron reflections in the range  $0.32 < \sin\theta/\lambda < 0.40$  Å $^{-1}$ , agree well with those of SC. The integrated intensity data were obtained from  $\omega/2\theta$  scans for reflections in the quadrant of reciprocal space having indices ranging from  $-24$  to  $24$  in  $h$ ,  $-13$  to  $0$  in  $k$  and  $0$  to  $25$  in  $l$ . Two check reflections ( $\bar{1}\bar{3}, \bar{3}, 9$  and  $1, 0, \bar{1}\bar{3}$ ) were remeasured at intervals of 75 reflections and showed no significant variation. For data collection, reflections were grouped into four spherical shells with limiting  $\sin\theta/\lambda$  values of  $0.40$ ,  $0.50$ ,  $0.61$  and  $0.69$  Å $^{-1}$ . Scan widths were constant in the first shell ( $\Delta 2\theta = 2.6^\circ$ ) but variable in the others ( $\Delta 2\theta = 0.22^\circ + 4.46\tan\theta^\circ$ ). For  $(0\bar{2}0)$ , a strong reflection occurring in the first shell,  $\omega/2\theta$  scans were recorded at  $10^\circ$  intervals in  $\psi$  (rotation about the scattering vector). The shells were collected in order of increasing  $2\theta$ , except that the fourth was completed before the third. Limited time was available and it was considered more important to have the complete fourth shell, which consisted of the data with the highest resolution of the diffractometer. As a consequence, the data in the third shell were collected only for index  $k$  from  $-3$  to  $0$ . A total of 6254 reflections were measured. All  $\omega/2\theta$  scan profiles were inspected and, if necessary, contributions from overlapping aluminium powder diffraction were subtracted before integration. Integrated intensities,  $I$ , were derived assuming the backgrounds to be given by the first and last tenths of each scan. Neutron absorption corrections with  $\mu = 3.2$  cm $^{-1}$  were applied using an analytical procedure (de Meulenaer & Tompa, 1965; Templeton & Templeton, 1973). The corrections to the intensities ranged from 1.203 to 1.676. After absorption correction, the  $\psi$  scan for  $(0\bar{2}0)$  showed a variation as much as 19% from the maximum intensity, indicating significant extinction effects in the data. The  $F^2 (= I\sin 2\theta)$  values were then

Table 1. Unit-cell data for cholesteryl acetate

N: measured by neutron diffraction,  $\lambda = 1.1593$  Å. X: measured by X-ray diffraction,  $\lambda = 0.7093$  Å (Sawzik & Craven, 1979).

	20 K (N)	123 K (N)	123 K (X)
$a$ (Å)	16.521 (4)	16.547 (4)	16.537 (6)
$b$ (Å)	9.220 (2)	9.297 (2)	9.289 (3)
$c$ (Å)	17.620 (5)	17.645 (4)	17.640 (5)
$\beta$ (°)	107.18 (2)	106.96 (2)	106.95 (3)

averaged for symmetry-related and repeated observations. This gave 5904 independent reflections with  $R_{\text{int}} = 0.07$  and  $wR_{\text{int}} = 0.01$ , where  $w = 1/\sigma^2(F^2)$  and  $\sigma(F^2)$  are from counting statistics. It was found that  $F^2 < 3\sigma(F^2)$  for 42% of the reflections, and  $F^2 < 0$  for 10%, but no value was more negative than  $2\sigma$ . All reflections were included in the subsequent structure refinement (see next section).

Neutron data collection was later resumed in order to complete the intensity measurements at 123 K, and to obtain an additional data set at 20 K. The earlier experience had shown the desirability of enhancing the peak intensity to background ratio, especially at the larger Bragg angles, for both greater accuracy and speed of measurement. Accordingly, larger crystals of cholesteryl acetate were obtained, this time by crystallization from methyl ethyl ketone solution. An elongated prismatic crystal was cut approximately parallel to  $(010)$  to give a fragment of volume 8.0 mm $^3$ , mass 8.6 mg and longest dimension 3.7 mm. In morphology, this crystal was like the first one. Neutron data collection made use of the same experimental facility and the data were processed as before. At 123 K, 3362 reflections were measured in the second, third and fourth shells. These data and those from the smaller crystal were scaled by means of common reflections and were merged to give a total of 7447 non-symmetry-related reflections. The value of  $R_{\text{int}} = 0.11$  was somewhat high because many of the common reflections were weak.

On further cooling of the crystal, there was no indication of a phase transformation although the  $b$ -axis length decreased by 0.8% (Table 1). A complete set of neutron data ( $\sin\theta/\lambda < 0.69$  Å $^{-1}$ ) was collected at this temperature 19.6 (5) K (henceforth designated 20 K for brevity). A total of 7702 reflections were measured which, after averaging, gave 7281 non-symmetry-related observations with  $R_{\text{int}} = 0.02$  for averaged reflections. It was found that  $F^2 < 3\sigma(F^2)$  for 29% of all reflections, and  $F^2 < 0$  for 3%. As a result of using a larger crystal and a lower temperature, the desired enhancement of the weaker reflections appeared to have been achieved. For a group of 25 randomly chosen reflections with  $\sin\theta/\lambda > 0.62$  Å $^{-1}$ , it was found that the average ratio of  $F^2$  at 20 to 123 K was 2.6 (7). However, there was also an increase in neutron extinction because of the larger crystal. This was indicated in the  $\psi$ -scan data for  $(0\bar{2}0)$  at 20 K. After absorption

\* Displex, model CS-202; Air Products and Chemicals, Inc.

corrections, there were greater variations from the maximum intensity (27%) than were found for the smaller crystal (19%).

### Structure refinements

The crystal structure was first refined using the incomplete 123 K data set. Initial nuclear parameters, taken from the X-ray study by SC\* were subjected to three cycles of differential-synthesis refinement† using the computer program *DIFSYN* (McMullan, 1976). An approximate scale factor and H-nuclear positional and anisotropic thermal parameters were obtained. At this stage the conventional  $R(F)$  value was 0.12. Refinement was continued by a block-diagonal least-squares procedure using the program *NOOT* (Weber & Craven, 1983). Computing facilities available at the time were inadequate for full-matrix least-squares refinement. The variables consisted of the scale factor and isotropic extinction parameter (type I crystal, Lorentzian mosaicity; Becker & Coppens, 1974) in the first block, and positional and anisotropic thermal parameters for all nuclei in another 23 blocks for each of the independent molecules, *A* and *B*. Each block was designed to contain all parameters for a chemically bonded group, such as a methylene. Coherent neutron-scattering lengths (6.648, 5.803 and  $-3.741$  fm for C, O and H) were from Koester (1977). The residual which was minimized was  $\sum w(F_o^2 - F_c^2)^2$  with  $w = 1/\sigma^2(F^2)$  and  $\sigma^2(F^2) = \sigma^2 + (0.02F^2)^2$ , where  $\sigma^2$  was the variance from counting statistics. Refinement was terminated at  $R(F^2) = 0.13$ ,  $wR(F^2) = 0.10$ ,  $S = 1.31$  with the largest parameter change being  $0.15\sigma$ . Nuclear anisotropic thermal parameters obtained at this stage were used in the analysis of molecular rigid-body and internal-H vibrations (see below).

Subsequently, refinement using the complete 123 K data set (including those reflections with  $F^2 < 0$ ) was carried out by full-matrix least squares, using a VAX11/750 computer. After three cycles, refinement was terminated with the largest parameter shift in the final cycle being  $0.11\sigma$ . The agreement indices for all 7448 reflections were  $R(F^2) = 0.10$ ,  $wR(F^2) = 0.10$ ,  $S = 0.98$ . For the 2748 reflections having  $\sin\theta/\lambda < 0.50 \text{ \AA}^{-1}$ , the value of  $R(F^2)$  was considerably lower (0.06) than for the remaining reflections (0.25). The latter were generally weaker, with only about one

third (1679) having  $F^2 > 3\sigma$ . A similar trend was not present in the refinement using 20 K data. Final nuclear parameters for 123 K are included in the supplementary material.\* Parameter values obtained were at most only marginally different ( $< 3\sigma$ ) from those previously derived from the partial data set.

The refinement of the structure at 20 K was entirely by full-matrix least-squares methods, assuming the 123 K nuclear parameters as the starting point. After nine cycles, there was no further reduction in the residual and the largest parameter change was  $0.3\sigma$ . The final values were  $R(F^2) = 0.06$ ,  $wR(F^2) = 0.06$  and  $S = 1.06$ , including all 7281 reflections. For the larger crystal used to collect data at 20 K, the extinction parameter obtained was  $g = 0.76 (2) \times 10^{-4} \text{ rad}^{-1}$ , much larger than for the smaller crystal used at 123 K [ $0.066 (8) \times 10^{-4} \text{ rad}^{-1}$ ]. For the larger crystal, the reflections most affected by extinction were 211 with  $0.61F_c^2$  and 020 with  $0.36F_c^2$ . For the smaller crystal, these values became  $0.92F_c^2$  and  $0.85F_c^2$  respectively. Final nuclear parameters are given in Table 2.

## Results and discussion

### Molecular geometry

Atomic nomenclature and the conformation of molecule *A* are shown in Figs. 1 and 2. This molecule and also molecule *B* have conformations which are not significantly changed by cooling from 123 to 20 K. Thus, when viewed in superposition, the *A* molecules at the two temperatures are indistinguishable, as are also the *B* molecules. However, as a result of differences in the crystal environment of molecules *A* and *B*, considerable differences in molecular conformation can be seen when they are superposed (Fig. 3). While the steroid *C* and *D* rings overlap closely, there is a relative flexing of rings *A* and *B* involving bond torsion-angle differences as great as  $11.6 (3)^\circ$  (see Table 3). Bond torsion-angle differences of a similar magnitude occur in the ester linkage and in the C17 tail, all these being associated with an overall bowing of molecule *A* with respect to molecule *B*. For details of the crystal packing and molecular-framework conformations, see SC. The molecular flexibility indicated in the crystal structure of cholesteryl acetate may well be a feature of cholesterol itself in its interactions with other lipids in biological membranes and in its binding with enzymes.

\* In that paper, atomic labels should be interchanged for C26 and C27 in both molecules *A* and *B*.

† Refinement by differential synthesis (Cruickshank, 1959) has fallen into disuse, perhaps because it involves unweighted structure factors and thus lacks the flexibility of least-squares procedures. However, the method is computationally rapid and, by providing knowledge of the gradient and curvature in neutron-scattering density (electron density in the case of an X-ray study) at the assumed nuclear positions, it can be valuable in the early stages of a structure determination.

\* Tables of experimental and calculated  $F^2$  data obtained for both temperatures, together with nuclear parameters at 123 K, have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 53548 (84 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 2. Nuclear positional ( $\times 10^4$ ) and anisotropic thermal parameters ( $\text{\AA}^2 \times 10^3$ ) for cholesteryl acetate at 19.6 KTemperature factors are of the form:  $T = \exp[-2\pi^2 \sum_i \sum_j h_i h_j a_i^* a_j^* U_{ij}]$ .

	x	y	z	U11	U22	U33	U12	U13	U23
C1A	-2254 (1)	4374	451 (1)	5 (1)	8 (1)	5 (1)	0 (1)	1 (1)	0 (1)
C2A	-2544 (1)	4517 (4)	1198 (1)	4 (1)	10 (1)	6 (1)	-1 (1)	3 (1)	0 (1)
C3A	-1780 (1)	4315 (4)	1923 (1)	5 (1)	7 (1)	6 (1)	0 (1)	2 (1)	1 (1)
C4A	-1131 (1)	5501 (4)	1954 (1)	6 (1)	8 (1)	3 (1)	-1 (1)	1 (1)	0 (1)
C5A	-851 (1)	5468 (4)	1210 (1)	6 (1)	7 (1)	5 (1)	0 (1)	1 (1)	0 (1)
C6A	-23 (1)	5456 (4)	1275 (1)	5 (1)	9 (1)	4 (1)	0 (1)	0 (1)	0 (1)
C7A	344 (1)	5430 (4)	594 (1)	4 (1)	8 (1)	4 (1)	0 (1)	0 (1)	2 (1)
C8A	-315 (1)	5675 (4)	-214 (1)	4 (1)	8 (1)	3 (1)	0 (1)	0 (1)	0 (1)
C9A	-1157 (1)	4905 (4)	-251 (1)	3 (1)	4 (1)	3 (1)	0 (1)	1 (1)	0 (1)
C10A	-1543 (1)	5441 (4)	409 (1)	4 (1)	6 (1)	3 (1)	0 (1)	0 (1)	0 (1)
C11A	-1779 (1)	4981 (4)	-1092 (1)	3 (1)	10 (1)	6 (1)	0 (1)	2 (1)	0 (1)
C12A	-1403 (1)	4419 (4)	-1738 (1)	5 (1)	8 (1)	5 (1)	0 (1)	3 (1)	-2 (1)
C13A	-589 (1)	5235 (4)	-1728 (1)	5 (1)	6 (1)	3 (1)	0 (1)	0 (1)	0 (1)
C14A	17 (1)	5091 (4)	-873 (1)	4 (1)	7 (1)	6 (1)	0 (1)	1 (1)	2 (1)
C15A	860 (1)	5680 (4)	-944 (1)	5 (1)	9 (1)	4 (1)	0 (1)	2 (1)	0 (1)
C16A	877 (1)	5119 (4)	-1764 (1)	6 (1)	7 (1)	5 (1)	0 (1)	3 (1)	-1 (1)
C17A	-27 (1)	4555 (4)	-2205 (1)	5 (1)	6 (1)	3 (1)	0 (1)	1 (1)	1 (1)
C18A	-783 (1)	6822 (4)	-1975 (1)	7 (1)	7 (1)	7 (1)	1 (1)	1 (1)	0 (1)
C19A	-1926 (1)	6976 (4)	233 (1)	9 (1)	4 (1)	8 (1)	2 (1)	4 (1)	2 (1)
C20A	-263 (1)	4810 (4)	-3107 (1)	6 (1)	5 (1)	4 (1)	0 (1)	1 (1)	-1 (1)
C21A	-1160 (1)	4279 (4)	-3544 (1)	7 (1)	12 (1)	7 (1)	1 (1)	2 (1)	-1 (1)
C22A	398 (1)	4074 (4)	-3440 (1)	6 (1)	8 (1)	6 (1)	1 (1)	2 (1)	0 (1)
C23A	307 (1)	4402 (4)	-4313 (1)	7 (1)	12 (1)	6 (1)	2 (1)	3 (1)	0 (1)
C24A	1057 (1)	3815 (4)	-4560 (1)	9 (1)	11 (1)	5 (1)	2 (1)	2 (1)	0 (1)
C25A	972 (1)	3940 (4)	-5447 (1)	7 (1)	10 (1)	6 (1)	0 (1)	1 (1)	0 (1)
C26A	1711 (2)	3175 (4)	-5633 (1)	10 (1)	13 (1)	13 (1)	1 (1)	7 (1)	-2 (1)
C27A	920 (2)	5514 (4)	-5723 (1)	26 (1)	13 (1)	11 (1)	7 (1)	10 (1)	4 (1)
C28A	-1609 (1)	3637 (4)	3270 (1)	6 (1)	9 (1)	5 (1)	0 (1)	2 (1)	1 (1)
C29A	-1941 (2)	3878 (4)	3969 (1)	12 (1)	14 (1)	5 (1)	0 (1)	2 (1)	0 (1)
O3A	-2064 (2)	4392 (4)	2629 (1)	6 (1)	10 (1)	5 (1)	2 (1)	2 (1)	3 (1)
O28A	-1012 (2)	2879 (4)	3276 (2)	9 (1)	15 (1)	8 (1)	6 (1)	2 (1)	3 (1)
H11A	-2027 (3)	3248 (6)	423 (3)	24 (2)	17 (2)	28 (3)	3 (2)	12 (2)	0 (2)
H12A	-2806 (3)	4512 (7)	-78 (3)	17 (2)	29 (3)	13 (2)	-2 (2)	0 (2)	1 (2)
H21A	-2824 (3)	5606 (7)	1222 (3)	26 (3)	17 (3)	24 (3)	6 (2)	9 (2)	0 (2)
H22A	-3032 (3)	3704 (7)	1193 (3)	16 (2)	31 (3)	21 (2)	-8 (2)	5 (2)	5 (2)
H31A	-1500 (3)	3240 (6)	1910 (3)	20 (2)	16 (2)	20 (2)	4 (2)	6 (2)	1 (2)
H41A	-581 (3)	5378 (7)	2477 (3)	17 (2)	30 (3)	12 (2)	0 (2)	1 (2)	1 (2)
H42A	-1433 (3)	6557 (6)	2013 (3)	27 (3)	17 (3)	24 (3)	0 (2)	13 (2)	-4 (2)
H61A	425 (3)	5455 (7)	1869 (3)	13 (2)	31 (3)	14 (2)	0 (2)	0 (2)	0 (2)
H71A	658 (3)	4365 (7)	592 (3)	25 (3)	19 (3)	18 (2)	11 (2)	6 (2)	3 (2)
H72A	853 (3)	6253 (7)	687 (3)	18 (2)	36 (3)	20 (2)	-14 (2)	8 (2)	0 (2)
H81A	-428 (3)	6852 (6)	-295 (3)	19 (2)	14 (2)	17 (2)	4 (2)	5 (2)	6 (2)
H91A	-993 (3)	3748 (6)	-119 (3)	20 (2)	11 (2)	22 (2)	3 (2)	9 (2)	6 (2)
H111A	-1991 (3)	6117 (6)	-1233 (3)	25 (3)	12 (2)	18 (2)	6 (2)	5 (2)	3 (2)
H112A	-2355 (3)	4350 (7)	-1118 (3)	11 (2)	35 (3)	21 (2)	-5 (2)	6 (2)	-3 (2)
H121A	-1891 (3)	4509 (7)	-2325 (3)	15 (2)	36 (3)	7 (2)	0 (2)	0 (2)	-2 (2)
H122A	-1252 (3)	3248 (6)	-1636 (3)	23 (2)	15 (2)	20 (2)	1 (2)	7 (2)	2 (2)
H141A	100 (3)	3899 (6)	-768 (3)	18 (2)	13 (2)	16 (2)	1 (2)	4 (2)	0 (2)
H151A	1407 (3)	5330 (7)	-456 (3)	11 (2)	34 (3)	17 (2)	0 (2)	0 (2)	4 (2)
H152A	855 (3)	6869 (6)	-925 (3)	26 (3)	16 (2)	25 (3)	-4 (2)	11 (2)	-2 (2)
H161A	1344 (3)	4265 (7)	-1706 (3)	14 (2)	26 (3)	23 (2)	7 (2)	3 (2)	-4 (2)
H162A	1055 (3)	5993 (7)	-2116 (3)	26 (3)	24 (3)	19 (2)	-9 (2)	8 (2)	2 (2)
H171A	-38 (3)	3373 (6)	-2108 (3)	18 (2)	14 (2)	21 (2)	0 (2)	7 (2)	-2 (2)
H181A	-1283 (3)	6891 (7)	-2544 (3)	29 (3)	26 (3)	15 (2)	2 (2)	-2 (2)	4 (2)
H182A	-1005 (4)	7440 (7)	-1546 (3)	42 (3)	18 (3)	23 (3)	9 (2)	18 (2)	-3 (2)
H183A	-236 (3)	7409 (7)	-2048 (4)	20 (3)	17 (3)	43 (3)	-6 (2)	13 (2)	7 (2)
H191A	-2492 (4)	6966 (7)	-281 (4)	23 (3)	30 (3)	26 (2)	6 (2)	-3 (2)	-1 (3)
H192A	-2113 (4)	7413 (7)	736 (3)	41 (3)	26 (3)	21 (3)	12 (3)	16 (2)	1 (2)
H193A	-1467 (3)	-7743 (6)	118 (4)	24 (3)	14 (3)	46 (3)	1 (2)	16 (2)	9 (2)
H201A	-225 (3)	5985 (6)	-3206 (3)	26 (2)	12 (2)	16 (2)	1 (2)	4 (2)	5 (2)
H211A	-1235 (4)	3152 (7)	-3387 (4)	27 (3)	17 (3)	41 (3)	-6 (2)	3 (2)	2 (2)
H212A	-1645 (3)	4941 (7)	-3393 (3)	18 (2)	38 (3)	28 (3)	9 (2)	9 (2)	-6 (2)
H213A	-1292 (3)	4363 (8)	-4190 (3)	26 (3)	57 (4)	6 (2)	0 (3)	2 (2)	-2 (2)
H221A	368 (4)	2890 (7)	-3353 (3)	38 (3)	17 (3)	26 (3)	5 (2)	19 (2)	6 (2)
H222A	1036 (3)	4412 (7)	-3085 (3)	15 (2)	39 (3)	14 (2)	0 (3)	2 (2)	0 (2)
H231A	263 (4)	5600 (7)	-4393 (3)	38 (3)	18 (3)	27 (3)	13 (2)	15 (2)	10 (2)
H232A	-277 (3)	3953 (8)	-4699 (3)	14 (2)	45 (4)	16 (2)	-3 (2)	0 (2)	-2 (2)
H241A	1144 (4)	2658 (7)	-4387 (3)	36 (3)	25 (3)	24 (3)	14 (2)	15 (2)	12 (2)
H242A	1641 (3)	4367 (8)	-4222 (3)	14 (2)	44 (4)	21 (2)	-5 (2)	4 (2)	-7 (2)
H251A	380 (3)	3391 (7)	-5780 (3)	18 (2)	28 (3)	20 (2)	-2 (2)	9 (2)	-7 (2)
H261A	2316 (3)	3671 (8)	-5306 (4)	15 (2)	52 (4)	39 (3)	-6 (3)	9 (2)	-14 (3)
H262A	1740 (4)	2023 (7)	-5467 (4)	43 (3)	20 (3)	48 (4)	8 (3)	22 (3)	5 (3)
H263A	1656 (4)	3229 (8)	-6261 (3)	42 (3)	45 (4)	18 (3)	10 (3)	18 (2)	1 (3)
H271A	392 (5)	6077 (8)	-5620 (4)	59 (4)	33 (3)	54 (4)	29 (3)	42 (4)	23 (3)
H272A	849 (4)	5580 (8)	-6364 (4)	59 (4)	39 (4)	22 (3)	9 (3)	21 (3)	12 (3)
H273A	1499 (5)	6108 (8)	-5409 (5)	54 (4)	24 (3)	49 (4)	-15 (3)	18 (3)	-4 (3)
H291A	-2618 (4)	3775 (9)	3796 (4)	21 (3)	75 (5)	25 (3)	1 (3)	11 (2)	0 (3)
H292A	-1655 (4)	3104 (8)	4431 (3)	44 (4)	45 (4)	18 (3)	15 (3)	11 (3)	12 (3)
H293A	-1786 (6)	4993 (8)	4194 (4)	90 (6)	27 (4)	34 (3)	-14 (4)	34 (4)	-12 (3)
C1B	3738 (1)	-53 (4)	3980 (1)	5 (1)	8 (1)	5 (1)	-1 (1)	3 (1)	-1 (1)
C2B	3380 (1)	337 (4)	4663 (1)	6 (1)	11 (1)	5 (1)	0 (1)	2 (1)	0 (1)

Table 2 (cont.)

	x	y	z	U <sub>11</sub>	U <sub>22</sub>	U <sub>33</sub>	U <sub>12</sub>	U <sub>13</sub>	U <sub>23</sub>
C3B	4087 (1)	425 (5)	5443 (1)	4 (1)	8 (1)	5 (1)	0 (1)	1 (1)	1 (1)
C4B	4798 (1)	1433 (4)	5389 (1)	5 (1)	10 (1)	6 (1)	-1 (1)	3 (1)	-2 (1)
C5B	5100 (1)	1110 (4)	4677 (1)	6 (1)	7 (1)	4 (1)	0 (1)	2 (1)	0 (1)
C6B	5924 (1)	871 (4)	4765 (1)	6 (1)	10 (1)	5 (1)	0 (1)	1 (1)	1 (1)
C7B	6281 (1)	605 (4)	4084 (1)	5 (1)	13 (1)	4 (1)	1 (1)	1 (1)	1 (1)
C8B	5668 (1)	1076 (4)	3290 (1)	4 (1)	8 (1)	5 (1)	0 (1)	1 (1)	0 (1)
C9B	4778 (1)	467 (4)	3211 (1)	4 (1)	6 (1)	5 (1)	0 (1)	2 (1)	0 (1)
C10B	4414 (1)	1037 (4)	3879 (1)	5 (1)	7 (1)	3 (1)	-1 (1)	1 (1)	0 (1)
C11B	4167 (1)	722 (4)	2371 (1)	5 (1)	11 (1)	4 (1)	0 (1)	1 (1)	0 (1)
C12B	4520 (1)	222 (4)	1692 (1)	5 (1)	8 (1)	6 (1)	-1 (1)	2 (1)	0 (1)
C13B	5386 (1)	914 (4)	1766 (1)	4 (1)	5 (1)	5 (1)	0 (1)	1 (1)	0 (1)
C14B	5964 (1)	510 (4)	2600 (1)	4 (1)	8 (1)	4 (1)	2 (1)	1 (1)	1 (1)
C15B	6854 (1)	917 (4)	2574 (1)	5 (1)	16 (1)	4 (1)	0 (1)	2 (1)	0 (1)
C16B	6842 (1)	495 (4)	1720 (1)	4 (1)	12 (1)	6 (1)	1 (1)	2 (1)	1 (1)
C17B	5894 (1)	257 (4)	1229 (1)	6 (1)	6 (1)	4 (1)	0 (1)	1 (1)	0 (1)
C18B	5296 (1)	2564 (4)	1645 (1)	10 (1)	8 (1)	6 (1)	2 (1)	4 (1)	0 (1)
C19B	4007 (1)	2552 (4)	3671 (1)	9 (1)	7 (1)	6 (1)	2 (1)	2 (1)	2 (1)
C20B	5705 (1)	813 (4)	369 (1)	6 (1)	8 (1)	3 (1)	0 (1)	2 (1)	0 (1)
C21B	4781 (1)	585 (4)	-118 (1)	6 (1)	13 (1)	5 (1)	0 (1)	0 (1)	0 (1)
C22B	6308 (1)	118 (4)	-46 (1)	7 (1)	11 (1)	5 (1)	1 (1)	3 (1)	1 (1)
C23B	6238 (1)	708 (4)	-875 (1)	12 (1)	10 (1)	7 (1)	4 (1)	6 (1)	3 (1)
C24B	6967 (1)	212 (4)	-1180 (1)	9 (1)	13 (1)	7 (1)	4 (1)	3 (1)	3 (1)
C25B	6825 (1)	390 (4)	-2070 (1)	11 (1)	9 (1)	9 (1)	2 (1)	6 (1)	1 (1)
C26B	7609 (2)	-107 (4)	-2297 (1)	11 (1)	17 (1)	10 (1)	5 (1)	6 (1)	0 (1)
C27B	6607 (2)	1949 (4)	-2352 (1)	10 (1)	11 (1)	9 (1)	0 (1)	3 (1)	0 (1)
C28B	4121 (1)	785 (4)	6794 (1)	8 (1)	9 (1)	4 (1)	-1 (1)	3 (1)	0 (1)
C29B	3666 (2)	1421 (4)	7341 (1)	11 (1)	13 (1)	9 (1)	0 (1)	5 (1)	-1 (1)
O3B	3695 (2)	995 (4)	6025 (2)	5 (1)	10 (1)	5 (1)	1 (1)	2 (1)	1 (1)
O28B	4782 (2)	129 (4)	7019 (2)	8 (1)	15 (1)	6 (1)	1 (1)	1 (1)	1 (1)
H11B	4025 (3)	-1131 (6)	4090 (3)	25 (3)	14 (2)	25 (3)	6 (2)	10 (2)	3 (2)
H12B	3218 (3)	-126 (7)	3423 (3)	19 (2)	31 (3)	12 (2)	-2 (2)	2 (2)	-3 (2)
H21B	3053 (3)	1386 (6)	4551 (3)	20 (2)	22 (3)	20 (2)	7 (2)	3 (2)	1 (2)
H22B	2913 (3)	-488 (7)	4716 (3)	19 (2)	33 (3)	23 (3)	-11 (2)	10 (2)	-1 (2)
H31B	4341 (3)	-662 (6)	5644 (3)	20 (2)	15 (2)	18 (2)	3 (2)	5 (2)	4 (2)
H41B	5332 (3)	1351 (7)	5942 (3)	13 (2)	35 (3)	12 (2)	-5 (2)	-1 (2)	0 (2)
H42B	4557 (3)	2557 (6)	5359 (3)	25 (2)	16 (3)	26 (3)	0 (2)	11 (2)	0 (2)
H61B	6366 (3)	884 (7)	5368 (3)	16 (2)	41 (4)	12 (2)	0 (2)	-2 (2)	3 (2)
H71B	6432 (3)	-560 (3)	4059 (3)	26 (2)	24 (3)	18 (2)	8 (2)	4 (2)	0 (2)
H72B	6894 (3)	1175 (7)	4200 (3)	13 (2)	38 (3)	19 (3)	-8 (3)	1 (3)	1 (3)
H81B	5649 (3)	2272 (6)	3278 (3)	21 (2)	13 (2)	19 (2)	0 (2)	7 (2)	1 (2)
H91B	4857 (3)	-718 (6)	3292 (3)	17 (2)	12 (2)	23 (2)	2 (2)	7 (2)	3 (2)
H111B	4013 (3)	1877 (7)	2281 (3)	29 (3)	15 (3)	19 (2)	9 (2)	9 (2)	2 (2)
H112B	3556 (3)	172 (7)	2309 (3)	11 (2)	34 (3)	23 (3)	-11 (2)	4 (2)	-3 (2)
H121B	4048 (3)	474 (7)	1126 (3)	14 (2)	32 (3)	13 (2)	-2 (2)	1 (2)	0 (2)
H122B	4596 (3)	-969 (6)	1715 (3)	24 (2)	14 (3)	29 (3)	-2 (2)	13 (2)	0 (2)
H141B	5940 (3)	-682 (6)	2632 (3)	22 (2)	14 (2)	18 (2)	1 (2)	6 (2)	1 (2)
H151B	7355 (3)	373 (8)	3030 (3)	18 (2)	49 (4)	14 (2)	9 (3)	1 (2)	10 (2)
H152B	6949 (3)	2090 (7)	2667 (3)	26 (3)	26 (3)	30 (3)	-11 (2)	13 (2)	-8 (2)
H161B	7212 (3)	-490 (7)	1726 (3)	22 (2)	32 (3)	19 (2)	8 (2)	2 (2)	-3 (2)
H162B	7137 (3)	1336 (7)	1451 (3)	26 (3)	32 (3)	15 (2)	-8 (2)	7 (2)	3 (2)
H171B	5764 (3)	-927 (6)	1189 (3)	21 (2)	12 (2)	20 (2)	0 (2)	10 (2)	0 (2)
H181B	4869 (4)	2823 (7)	1067 (3)	34 (3)	24 (3)	16 (2)	7 (2)	-4 (2)	4 (2)
H182B	5058 (4)	3106 (7)	2087 (3)	39 (3)	19 (3)	24 (3)	6 (2)	20 (2)	-1 (2)
H183B	5902 (3)	3088 (7)	1673 (4)	19 (2)	21 (3)	48 (4)	-5 (2)	14 (2)	2 (3)
H191B	3404 (3)	2483 (7)	3195 (3)	18 (2)	27 (3)	24 (3)	2 (2)	-2 (2)	2 (2)
H192B	3873 (4)	3075 (7)	4179 (3)	45 (3)	26 (3)	18 (2)	10 (3)	18 (2)	-1 (2)
H193B	4426 (3)	3298 (7)	3474 (4)	26 (3)	23 (3)	33 (3)	-2 (2)	14 (2)	9 (2)
H201B	5827 (3)	1991 (6)	391 (3)	18 (2)	12 (2)	20 (2)	0 (2)	6 (2)	2 (2)
H211B	4608 (4)	-561 (7)	-106 (4)	25 (3)	20 (3)	44 (3)	-9 (2)	4 (2)	-4 (3)
H212B	4343 (3)	1231 (7)	105 (3)	18 (2)	31 (3)	26 (3)	9 (2)	8 (2)	-4 (2)
H213B	4675 (3)	885 (8)	-744 (3)	23 (2)	46 (4)	14 (2)	-1 (3)	2 (2)	4 (2)
H221B	6210 (3)	-1067 (6)	-76 (3)	30 (3)	16 (3)	28 (3)	4 (2)	14 (2)	2 (2)
H222B	6970 (3)	262 (8)	322 (3)	13 (2)	39 (3)	20 (2)	1 (2)	6 (2)	0 (2)
H231B	6197 (4)	1891 (7)	-867 (4)	44 (3)	14 (3)	32 (3)	7 (2)	20 (3)	6 (2)
H232B	5637 (3)	343 (8)	-1314 (3)	17 (2)	43 (3)	18 (2)	-1 (2)	4 (2)	1 (2)
H241B	7124 (4)	-908 (7)	-1014 (4)	47 (3)	24 (3)	31 (3)	16 (3)	22 (3)	12 (2)
H242B	7547 (3)	814 (8)	-853 (3)	22 (3)	51 (4)	20 (2)	-4 (3)	4 (2)	-1 (3)
H251B	6287 (3)	-296 (7)	-2378 (3)	24 (3)	27 (3)	26 (3)	-1 (2)	7 (2)	-2 (2)
H261B	8159 (3)	578 (9)	-2020 (4)	14 (2)	56 (4)	46 (3)	-13 (3)	6 (2)	-19 (3)
H262B	7781 (4)	-1232 (8)	-2100 (4)	48 (4)	27 (3)	40 (3)	18 (3)	21 (3)	12 (3)
H263B	7492 (4)	-100 (8)	-2945 (3)	38 (3)	45 (4)	20 (3)	4 (3)	12 (2)	0 (3)
H271B	6017 (4)	2313 (8)	-2276 (4)	32 (3)	33 (4)	44 (4)	14 (3)	24 (3)	10 (3)
H272B	6539 (5)	2050 (8)	-2986 (3)	60 (4)	30 (3)	18 (3)	7 (3)	15 (3)	10 (2)
H273B	7090 (4)	2699 (8)	-2034 (4)	38 (3)	29 (3)	46 (4)	-12 (3)	1 (3)	-3 (3)
H291B	3347 (5)	558 (8)	7554 (4)	64 (4)	32 (4)	48 (4)	-16 (3)	44 (3)	-8 (3)
H292B	4123 (4)	1920 (9)	7844 (4)	31 (3)	51 (4)	20 (3)	-4 (3)	3 (2)	-13 (3)
H293B	3195 (4)	2222 (8)	7042 (4)	37 (3)	47 (4)	25 (3)	26 (3)	9 (3)	6 (3)

The e.s.d.'s in bond lengths and angles are generally greater for the neutron diffraction results at 123 K (0.006 Å for C—C bond lengths and 0.4° for C—C—C angles) than at 20 K (0.003 Å, 0.2°). The

e.s.d.'s for the molecular framework obtained at 20 K are very similar to those obtained from X-ray diffraction at 123 K by SC. The e.s.d.'s given here are average values which exclude the atoms of the

terminal isopropyl and acetyl groups. At 123 K, atoms of these groups, owing to their greater thermal vibrational motion, have e.s.d.'s almost twice those of the rest of the molecule. The bond-length differences between molecules *A* and *B* are greatest for the bond C25—C26. Values are 1.493, 1.518 (7) Å from X-ray diffraction at 123 K,\* 1.52 and 1.53 (1) Å from neutron data at 123 K, and 1.525, 1.529 (6) Å from neutron data at 20 K. We believe that none of the differences is significant and that all these bond-length estimates are subject to thermal-vibration corrections difficult to determine but likely to be appreciable even at 20 K. Thus, if it is assumed that the C26 methyl group vibrates while riding on C25 (Busing & Levy, 1964), the bond lengths become 1.531 and 1.538 Å at 20 K. Corresponding distances and angles obtained at 123 and 20 K by neutron diffraction are in satisfactory agreement at the  $3\sigma$  level. Because they are more accurate, we show in Fig. 1 only the values from the 20 K study.

Comparison of Fig. 1 with Fig. 3 of SC shows very good overall agreement in framework bond lengths and angles uncorrected for thermal-vibration effects. The most notable difference in a bond length

\* The two bond-length values cited here and henceforth refer to values obtained for the two independent molecules *A* and *B* respectively.

involves the ethylenic C5=C6 bond (1.325 and 1.319 Å at 123 K; 1.340 and 1.342 Å at 20 K).\* We suggest that the C5=C6 bond length obtained from X-ray diffraction is apparently slightly shorter because the assumed spherical-atom model brings the atoms closer together than the true nuclear separation in order to compensate for the effect of the charge density in the ethylenic bond. Such an effect would be absent in neutron diffraction.

The 20 K neutron study confirms some unusually long C—C bond lengths reported by SC, such as C16—C17 (1.561 and 1.567 Å at 123 K; 1.556 and 1.565 Å at 20 K). As found by SC in the previous X-ray study, corresponding bond lengths in molecules *A* and *B* all agree within experimental error. As was also reported by SC, there are significant bond-angle differences between the two molecules. They occur in the *A* and *B* rings. At 20 K, the largest difference is 2.9 (3)° in C1—C10—C5. These effects are considered to be associated with the conformational differences between molecules *A* and *B* (Fig. 3), which are also greatest in the steroid *A* and *B* rings.

Bond lengths and angles involving H atoms are listed in Table 4. These values are uncorrected for

\* The uncorrected C5=C6 bond lengths from neutron diffraction at 123 K are 1.350 and 1.336 (6) Å.

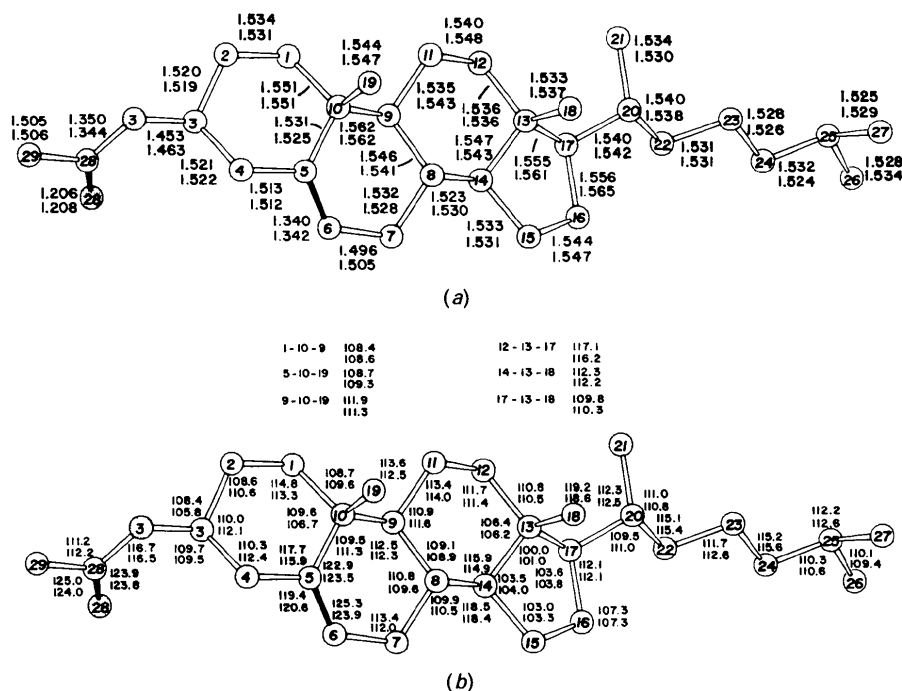


Fig. 1. Molecular framework with atom numbering for cholesteryl acetate viewed approximately normal to plane of steroid nucleus (Johnson, 1976). Double bonds are indicated by solid lines; O atoms are stippled. (a) Bond lengths (Å) determined at 20 K are given with values from molecule *A* above those from *B*. (b) Bond angles (°) at 20 K, with values from molecule *A* above those from *B*.

thermal-vibration effects. The C—H bond lengths from neutron diffraction seem to require two corrections which are almost equal and opposite, with magnitude about 0.02 Å. They are a lengthening correction due to rigid-body and intramolecular harmonic librations and a shortening due to anharmonic bond-stretching vibrations. Craven & Swaminathan (1984) reported that after applying both corrections, the average of 12 C—H bond lengths obtained from three different crystal structures was 1.098 (2) Å, while the average uncorrected value was 1.095 (2) Å. These values are both in good agreement with the  $r_e$  value of 1.096 (2) Å for the methylene C—H bond lengths in propane as determined spectroscopically (Lide, 1960). In cholesteryl acetate at 20 K, the average of 44 uncorrected C—H methylene bond lengths is 1.102 (8) Å where the e.s.d. is from the statistical distribution. The average for 16 uncorrected methine C—H bond lengths is very similar, [1.103 (6) Å]. The C—H bond-length e.s.d.'s obtained from the distribution are very similar to those obtained from the least-squares structure refinement (0.005 to 0.008 Å).

The most remarkable feature of the methylene H—C—H bond angles (Table 4a) is that all 22 values fall in a narrow range with an average value 106.2° and an e.s.d. of 0.9° obtained from the distri-

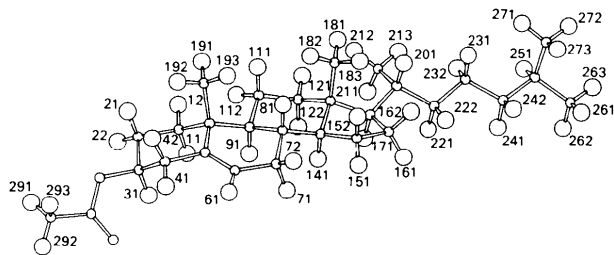


Fig. 2. Molecular conformation for molecule *A*. Framework atoms are represented as small spheres (Johnson, 1976). The H atoms are the larger spheres, numbered as in Table 2.

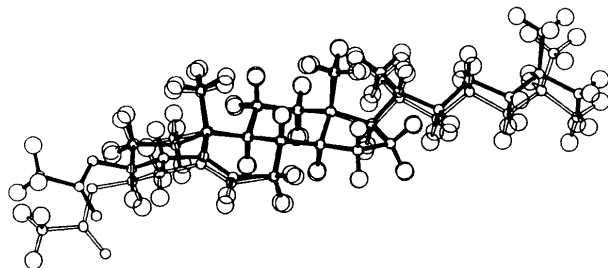


Fig. 3. Superposition of molecules *A* and *B*. Molecule *B* is shown with solid bonds. Molecule *A* is taken from Fig. 2. These molecules are in the same orientation defined with respect to an axial reference system involving atoms of the steroid *C* and *D* rings.

Table 3. Selected bond torsion angles (°) at 20 K

E.s.d.'s are 0.3° in C—C—C—C and 0.4° in C—C—C—H.

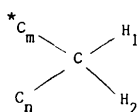
	Molecule <i>A</i>	Molecule <i>B</i>
C2—C3—O3—C28	151.3	161.5
C10—C1—C2—C3	-57.0	-58.0
C1—C2—C3—C4	62.3	53.1
C2—C3—C4—C5	-58.8	-49.9
C3—C4—C5—C10	50.3	51.9
C4—C5—C10—C1	-42.7	-53.0
C5—C10—C1—C2	45.8	55.8
C2—C1—C10—C9	165.3	175.8
C10—C5—C6—C7	0.6	4.4
C10—C5—C6—H61	-178.8	-176.4
C4—C5—C6—H61	0.9	1.8
C4—C5—C6—C7	-179.7	-177.3
C3—C4—C5—C6	-129.4	-126.5
C5—C6—C7—C8	9.7	17.0
C1—C10—C5—C6	136.9	125.3
C13—C17—C20—C22	178.2	176.1
C17—C20—C22—C23	-172.5	-174.0
C20—C22—C23—C24	171.7	168.0
C22—C23—C24—C25	173.3	163.7
C23—C24—C25—C26	-173.9	178.5
C1—C10—C19—H193	-172.3	-168.1
C12—C13—C18—H183	-172.7	-177.9
C17—C20—C21—H213	-174.0	-174.2
C24—C25—C26—H263	178.7	173.7
C24—C25—C27—H273	-179.8	175.3
O28—C28—C29—H293	108.5	163.2

bution. Similar results for the H—C—H bond angles were noted by FM in their study of 20-methylpregnenediol, where the average for eight methylene groups was 106.5 (5)°. They reported a significant linear dependence whereby the H—C—H angle decreases slightly with increasing C—C—C angle at the same central C atom. In the cholesteryl acetate structure, there is also such a trend, but the linearity of the relationship is not well established. The correlation coefficient between the angles H—C—H and C—C—C in the linear regression is smaller (-0.53) than the value reported by FM (-0.90).

In the methyl groups of cholesteryl acetate, the H—C—H bond angles (Table 4b) have an average value 107.6° for 36 observations, with an e.s.d. of 1.1°. For the methyl C—C—H bond angles, the corresponding values are 111.3 and 1.1°. Within each C—CH<sub>3</sub> group, the observed departures from three-fold rotational symmetry have at most a marginal significance, the largest angle differences being 3.1 (8)° for H—C—H in the C21A methyl group and 3.2 (7)° for C—C—H in C29B. However, there are significant departures from an ideally staggered configuration about the C—CH<sub>3</sub> bonds, as can be seen from the selected bond torsion angles in Table 3. For example, the C19 methyl group has  $\tau = -168.1$  (4)° in molecule *B*, this being a twist of 12° from the *trans* configuration. In molecule *A*, the corresponding twist is 6° smaller. As can be seen in Fig. 3, such differences in methyl torsions have no simple explanation because they occur in concert with a number of other conformational differences between molecules *A* and *B*. The largest difference in methyl configuration in *A* and *B* involves the acetyl C29 group, which has a relative twist of 55° about the

Table 4. Bond lengths (Å) and angles (°) involving H at 20 K

These values are for molecule *A* (top) and *B* (below). E.s.d.'s are 0.005 to 0.008 Å for C—H bond lengths, 0.5° for H—C—H, 0.4° for C—C—H angles.



## (a) Methylene groups

C	C <sub>m</sub>	C <sub>n</sub> *	C—H1	C—H2	H—C—H	C <sub>m</sub> —C—H1	C <sub>m</sub> —C—H2	C <sub>n</sub> —C—H1	C <sub>n</sub> —C—H2
C1	C10	C2	1.110 1.094	1.102 1.099	106.1 106.6	108.7 108.7	109.6 109.7	108.3 108.8	109.0 109.6
C2	C1	C3	1.112 1.098	1.099 1.109	107.7 107.5	110.5 110.5	110.4 110.2	109.3 108.8	110.4 109.2
C4	C3	C5	1.092 1.106	1.113 1.106	107.2 106.8	110.9 109.5	107.5 107.6	109.8 110.1	111.0 110.2
C7	C6	C8	1.111 1.107	1.108 1.105	105.7 105.3	108.5 109.6	109.9 109.2	109.4 109.6	109.7 111.0
C11	C9	C12	1.109 1.095	1.105 1.104	106.1 105.4	109.7 110.2	109.8 110.1	108.8 107.6	108.8 109.2
C12	C11	C13	1.110 1.094	1.111 1.105	106.9 106.7	108.8 108.2	109.0 109.4	111.6 112.6	108.7 108.5
C15	C14	C16	1.096 1.091	1.097 1.098	106.4 107.4	113.0 113.1	109.7 109.4	113.4 112.9	111.5 110.8
C16	C15	C17	1.086 1.093	1.107 1.096	106.4 105.8	111.2 110.8	111.2 111.3	110.9 110.7	109.9 111.0
C22	C20	C23	1.105 1.103	1.100 1.102	106.0 104.7	108.5 109.2	109.1 109.8	109.7 109.7	108.0 107.5
C23	C22	C24	1.085 1.093	1.113 1.115	106.7 105.3	107.9 109.3	111.1 110.8	110.1 111.2	109.4 107.4
C24	C23	C25	1.106 1.085	1.098 1.110	106.0 103.6	108.2 109.9	109.8 108.6	108.6 109.4	108.6 109.1

## (b) Methyl groups

C	C <sub>m</sub>	C—H1	C—H2	C—H3	C <sub>m</sub> —C—H1	C <sub>m</sub> —C—H2	C <sub>m</sub> —C—H3	H1—C—H2	H2—C—H3	H3—C—H1
C18	C13	1.097 1.080	1.094 1.092	1.093 1.100	110.7 111.0	112.7 113.2	113.3 112.3	106.6 107.4	106.4 106.4	106.8 106.1
C19	C10	1.093 1.098	1.098 1.093	1.099 1.103	111.0 111.1	111.8 112.6	111.3 111.6	107.2 107.0	106.7 106.3	108.7 107.9
C21	C20	1.092 1.096	1.101 1.096	1.097 1.100	110.0 110.2	111.5 112.3	111.3 111.4	108.8 108.3	106.0 107.3	109.1 107.3
C26	C25	1.096 1.096	1.099 1.104	1.083 1.100	110.9 111.4	111.3 111.0	111.6 111.5	107.5 107.3	107.5 106.3	107.9 109.1
C27	C25	1.076 1.076	1.100 1.093	1.100 1.079	112.1 112.8	111.1 110.8	110.7 111.5	107.5 106.1	107.7 108.2	107.6 107.1
C29	C28	1.073 1.082	1.081 1.082	1.104 1.088	110.8 108.8	109.6 109.3	109.2 112.0	110.0 108.8	109.9 109.6	107.2 108.4

## (c) Methine groups

C	C <sub>i</sub>	C <sub>m</sub>	O C <sub>n</sub>	C—H	C <sub>i</sub> —C—H	C <sub>m</sub> —C—H	C <sub>n</sub> —C—H
C3	C2	C4	O3	1.096 1.104	110.0 111.1	110.7 110.3	107.9 107.8
C6	C5	C7	-	1.089 1.097	118.0 118.2	116.7 117.9	- -
C8	C7	C9	C14	1.103 1.103	108.2 108.0	109.3 110.0	109.5 109.9
C9	C8	C10	C11	1.108 1.105	105.8 106.0	106.1 106.9	107.4 107.1
C14	C8	C13	C15	1.116 1.102	106.0 106.0	105.1 105.6	106.9 107.2
C17	C13	C16	C20	1.104 1.111	106.0 106.9	108.0 108.4	107.5 106.6
C20	C17	C21	C22	1.103 1.103	107.7 108.1	109.1 107.2	107.1 106.9
C25	C24	C26	C27	1.104 1.094	107.9 107.7	108.2 108.7	108.0 107.7

C28—C29 bond. In this case, the effect is attributed to the considerable differences in crystal environment.

The shortest H...H nonbonded intramolecular distances at 20 K are H121...H212 (2.08, 2.12 Å in molecules *A* and *B*) and H42...H192 (2.10 Å) in molecule *B*. In molecule *A*, the latter increases to

2.34 Å because of conformational differences in the steroid *A* ring.

*Molecular thermal vibrations*

In Fig. 4, it can be seen that the 90% probability ellipsoids enclosing H atoms are consistently larger



than those for the C and O atoms. The differences are notable at 123 K and become even greater at 20 K. It appears that the C and O ellipsoids change more with temperature than the H ellipsoids. This indicates that for the H atoms, intramolecular nonrigid C—H thermal vibrations are predominant over molecular rigid-body vibrations at both temperatures. The analysis of the nuclear anisotropic thermal vibrations (Table 2) using a set of computer programs by Craven, He & Weber (1986) has been directed towards determining details of the C—H intramolecular harmonic vibrations. Our procedure was similar to that of Johnson (1970) in his study of phenyl-group vibrations in norbornyl derivatives. The assumptions made were firstly that molecular rigid-body or lattice vibrations are not correlated with intramolecular nonrigid modes, so that the corresponding mean-square amplitudes are simply additive (Higgs, 1953). Secondly, the contributions from the nonrigid motion of the heavier-atom framework were assumed to be sufficiently small to allow a meaningful rigid-body fit to the total mean-square amplitudes of vibration of the C nuclei. Independent justification for this approach comes from a normal coordinate calculation (presumably for 295 K) car-

ried out by Johnson (1970) for the much simpler molecule, cyclobutane. Resulting principal values for the nonrigid vibrations of the C atoms were in the range 0.0008 to 0.0022 Å<sup>2</sup>. This would correspond to about 25% of the observed mean-square amplitudes for the C atoms in cholesteryl acetate at 20 K (Table 2).

Initially,  $U^{ij}$  values for the nuclei of the complete heavier-atom molecular framework of cholesteryl acetate were fitted to a rigid-body model by least squares (Schomaker & Trueblood, 1968). For molecules *A* and *B* at 123 K, the fit was only moderately good with  $wR = 0.31, 0.22$  and  $S = 2.45, 1.93$ .<sup>\*</sup> Tests of various models involving nonrigid-framework vibrations were carried out by introducing librations about selected C—C bonds, according to the procedure of Dunitz & White (1973). For molecule *A*, there was some improvement ( $wR = 0.24, S = 1.98$ ), with a mean-square torsional libration around the C24—C25 bond of about 100 (17) deg<sup>2</sup> being indicated in most models. For molecule *B*, there was no significant improvement over the rigid-framework model.

It was decided to continue the analysis considering only the molecular fragment consisting of the steroid-ring system C1 through C19 and the H atoms bonded to it. As shown in Table 5, the heavier-atom framework then gives a satisfactory fit to a rigid body for both fragments *A* and *B* at 20 K and fragment *B* at 123 K. Fragment *A* at 123 K gives the poorest fit ( $S = 1.67$ ). In this fragment, discrepancies from the model seem to be widely distributed over the atoms and did not seem to involve any particular vibrational direction. For both fragments, mean-square amplitudes of rigid-body librational motion have principal values almost zero at both temperatures, except for  $L_3$ .<sup>†</sup> This is a libration about an axis within a few degrees of the long axis of the molecule.

The thermal parameters in Table 5 were then used to calculate the rigid-body contribution to the thermal vibrations for each H nucleus. After subtracting this from the observed  $U^{ij}$  for each H nucleus, the residual components were assumed to represent the intramolecular anisotropic thermal vibration. These components were transformed to a local Cartesian axial system with the  $x$  axis along the C—H bond,  $y$  along the normal to the plane of the CH<sub>2</sub> nuclei in the methylene group (CCH in a methyl or methine group) and  $z$  normal to the C—H bond, completing the right-handed system. For most H nuclei, the principal values for the nonrigid vibrations were found to be in directions within 20° of

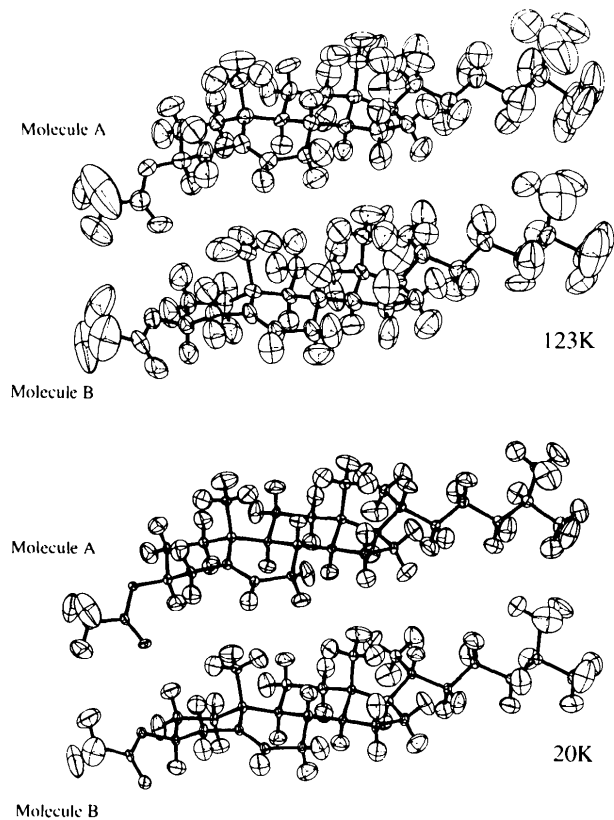


Fig. 4. Thermal-ellipsoid plots for cholesteryl acetate at 90% probability for enclosing the atomic nuclei (Johnson, 1976).

<sup>\*</sup> In the thermal analysis,  $wR = [(\sum w\Delta^2)/(\sum wU_{\text{obs}}^2)]^{1/2}$ , where  $\Delta = U_{\text{obs}} - U_{\text{calc}}$ ,  $w = \sigma^{-2}(U)$  and the sum includes the six  $U^{ij}$  components for all nuclei;  $S = [\sum w\Delta^2/(m - n)]^{1/2}$  with  $(m - n) = 94$ .

<sup>†</sup> Corrections to the bond lengths as a result of librational motion are small ( $< 0.002$  Å at 123 K) and have not been applied.

Table 5. Rigid-body thermal vibrations

Tensor components for translation  $T$  ( $\text{\AA}^2 \times 10^4$ ), libration  $L$  ( $\text{deg}^2$ ) and screw motion  $S$  ( $\text{deg} \times 10^3$ ) are referred to crystal Cartesian axes ( $a, b, c^*$ ) with the molecular center of mass as origin. Values were determined only for the steroid framework (atoms C1 through C19).

	20 K		123 K	
	<i>A</i>	<i>B</i>	<i>A</i>	<i>B</i>
$T_{11}$	41 (3)	44 (3)	138 (10)	154 (7)
$T_{22}$	59 (5)	61 (4)	117 (15)	154 (11)
$T_{33}$	41 (3)	45 (3)	171 (9)	137 (6)
$T_{12}$	-2 (3)	-3 (2)	2 (10)	-30 (7)
$T_{13}$	-4 (3)	-1 (2)	-24 (8)	-10 (6)
$T_{23}$	-5 (3)	-2 (3)	8 (9)	12 (7)
$L_{11}$	1.0 (3)	1.3 (3)	4.9 (9)	5.4 (7)
$L_{22}$	0.3 (1)	0.1 (1)	0.6 (4)	0.1 (3)
$L_{33}$	1.8 (4)	3.3 (4)	7.0 (11)	11.7 (8)
$L_{12}$	0.0 (2)	0.2 (2)	0.1 (5)	-0.1 (4)
$L_{13}$	-1.2 (3)	-1.9 (3)	-4.5 (8)	-6.6 (6)
$L_{23}$	0.0 (2)	-0.2 (2)	0.0 (5)	-0.3 (4)
$S_{11}$	-4 (13)	-8 (12)	14 (33)	37 (24)
$S_{12}$	5 (6)	-10 (6)	-11 (13)	-50 (9)
$S_{13}$	-22 (8)	-7 (7)	34 (21)	-7 (16)
$S_{21}$	-5 (4)	-1 (3)	7 (9)	0 (7)
$S_{22}$	-7 (9)	2 (8)	5 (26)	-22 (19)
$S_{23}$	-3 (3)	1 (3)	28 (10)	-5 (7)
$S_{31}$	3 (9)	13 (8)	-56 (22)	-72 (17)
$S_{32}$	-7 (5)	18 (5)	-18 (14)	49 (11)
$S_{33}$	11	6	-19	-15
Principal values				
$T_1$	36	42	128	189
$T_2$	61	62	114	124
$T_3$	44	46	184	133
$L_1$	0.2	0.3	1.3	1.4
$L_2$	0.3	0.0	0.5	0.1
$L_3$	2.6	4.4	10.6	15.9
Agreement criteria				
$wR$	0.21	0.17	0.23	0.15
$S$	1.09	1.00	1.67	1.25

these local axes. For the methyl groups, the mean-square amplitudes for C—C—H out-of-plane vibration correspond to a torsional libration around the C—C bond with r.m.s. amplitude of about  $7^\circ$ . For methylene groups, the mean-square amplitude along  $x$  is an estimate of C—H bond stretching, along  $y$  is termed out-of-plane motion and along  $z$  is in-plane. In Table 6 are shown the averages of these values for the H nuclei in the 16 methylene groups associated with fragments *A* and *B* at each temperature, together with e.s.d.'s from the distribution. Although the e.s.d.'s at 123 K are three times greater than at 20 K, the average values at the two temperatures are in good agreement. We have carried out an analysis in the same way (see Table 6), using the  $U^{ij}$  values obtained by FM for the same molecular fragment in their refinement of the crystal structure of 20-methylpregnenediol methanolate. This crystal was also determined by neutron diffraction at 123 K. It differs from cholesteryl acetate in having only one independent molecule in the cell and in having quite a different packing arrangement, since there is no C17 tail and the molecules are hydrogen bonded to form a three-dimensional network. Also included in Table 6 are the results obtained by Craven & Swaminathan (1984) for the *n*-propyl segment of

Table 6. Mean-square amplitudes ( $\text{\AA}^2 \times 10^4$ ) for non-rigid vibrations of H nuclei

	No. of C—H bonds	C—H stretch	C—C—H out-of-plane	C—C—H in-plane
<b>Methylene groups</b>				
Cholesteryl acetate at 20 K <sup>a</sup>	32	57 (22)	148 (22)	235 (37)
Cholesteryl acetate at 123 K <sup>a</sup>	32	65 (76)	172 (63)	253 (113)
20-Methylpregnenediol methanolate at 123 K <sup>b</sup>	16	57 (34)	154 (25)	247 (63)
$\gamma$ -Aminobutyric acid at 123 K <sup>c</sup>	6	63 (11)	142 (31)	225 (59)
<b>Methyl groups</b>				
Cholesteryl acetate at 20 K <sup>a</sup>	12	52 (24)	370 (43)	172 (17)
Cholesteryl acetate at 123 K <sup>a</sup>	12	57 (70)	360 (118)	216 (37)
20-Methylpregnenediol methanolate at 123 K <sup>b</sup>	6	40 (22)	394 (44)	163 (42)
<b>Methine groups</b>				
Cholesteryl acetate at 20 K <sup>a</sup>	12	50 (16)		Normal to C—H 151 (36)
Cholesteryl acetate at 123 K <sup>a</sup>	12	53 (53)		145 (64)
20-Methylpregnenediol methanolate at 123 K <sup>b</sup>	6	30 (23)		141 (29)

(a) Present report; (b) Fronckowiak & McMullan (1980); (c) Craven & Swaminathan (1984) and Weber, Craven & McMullan (1983).

$\gamma$ -aminobutyric acid. These values were based on  $U^{ij}$  parameters determined by neutron diffraction at 123 K (Weber, Craven & McMullan, 1983).

There is remarkable consistency in the results obtained from the three different crystal structures and for cholesteryl acetate over the temperature range 20 to 123 K. The internal vibrations of the methylene H nuclei are anisotropic, having a minimum mean-square amplitude for C—H bond stretching ( $0.006 \text{\AA}^2$ ) and a maximum for in-plane motion ( $0.024 \text{\AA}^2$ )\*. The principal values in Table 6 agree well with the normal-coordinate analysis values for the H atoms in cyclobutane ( $0.0069$ ,  $0.0139$  and  $0.0292 \text{\AA}^2$ ; Johnson, 1970) and with the value for C—H stretching in methane from electron diffraction ( $0.0061 \text{\AA}^2$ ; Bartell, Kuchitsu & DeNeui, 1961). The standard deviations in mean-square amplitudes (Table 6) estimated from the observed distribution about the mean value are larger than the  $\sigma(U^{ij})$  for individual H nuclei as obtained from the crystal structure least-squares refinements. The latter range from  $0.0009 \text{\AA}^2$  in  $\gamma$ -aminobutyric acid to  $0.0006 \text{\AA}^2$  in cholesteryl acetate at 123 K. The larger error estimates could be the result of small real differences in nonrigid vibrations of the H nuclei owing to their different molecular or crystal environments.

In crystal structure determinations by X-ray diffraction, isotropic thermal parameters for H atoms bonded to a C atom are often assigned fixed values based on  $B_{\text{eq}}$  for the C atom. Appropriate H-

\* It must be remembered that the diffraction data give the average over all vibrational modes without regard to the phase of concerted atomic vibrations.

atom thermal parameters are obtainable from  $B(H) = B_{eq}(C) + \Delta B$ , where  $\Delta B$  is given by  $B_{eq}$  for the nonrigid component of the H vibrations. For temperatures up to 123 K, the values for  $\Delta B$  determined from our neutron diffraction study of cholesteryl acetate are 0.8, 1.2 and 1.6 Å<sup>2</sup> for methine, methylene and methyl H atoms respectively. The resulting value for  $B(H)$  should be taken as a lower limit because if the H atom is directed to the outside of the molecule, it will have a rigid-body librational component of its vibration slightly greater than that of the bonded C atom.

#### Intermolecular H...H distances

Nyburg, Faerman & Prasad (1987) carried out a search of neutron diffraction results in the Cambridge Structural Database and found an anisotropic distribution of intermolecular H...H distances. They concluded that the effective shape of the H atom was a polar-flattened spheroid of revolution about the C—H bond. We confirm this conclusion by considering the crystal structure of cholesteryl acetate at 20 K, in which there are 230 distinct H...H intermolecular distances less than 3.0 Å. In Fig. 5 is shown a polar scatter plot with points for 61 of these, selected because the H...H vector makes similar angles  $\theta_1$  and  $\theta_2$  with the two C—H bond vectors. This discrimination leaves only the points most likely to reveal anisotropy in the effective shape of the H atoms. In Fig. 5, the outer boundary is a circle of radius 3 Å and the inner curve represents the surface for closest H...H contacts as derived by Nyburg *et al.* (1987). Whereas the usual H...H sum of van der Waals radii is 2.4 Å, the inner boundary in Fig. 5 ranges from 2.5 to 2.0 Å along the C—H bond direction. Although the distribution from cholesteryl acetate is sparse close to the C—H bond direction, the results in Fig. 5 give further support for a polar-flattened H atom.

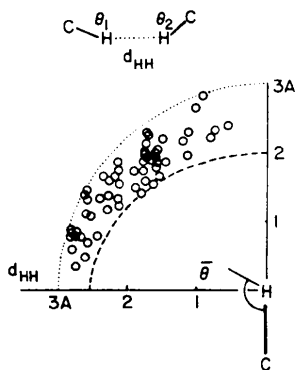


Fig. 5. Polar scatter plot of  $d(H...H)$  versus  $\bar{\theta}$  for 61 intermolecular distances having  $\bar{\theta} = (\theta_1 + \theta_2)/2$  and  $\Delta\theta = |\theta_1 - \theta_2| < 20^\circ$ . The dotted and dashed lines are from analysis of Nyburg *et al.* (1987).

The explanation for the polar flattening would seem to involve two properties of the C—H group. First, the electron density distribution around the H nucleus is aspherical because charge density is built up in the C—H covalent-bonding region at the expense of density on the other side of the H nucleus. From diffraction studies of the charge density in a number of simple organic crystal structures, this effect has been shown to give rise to a local dipole of about 1 debye at the H nucleus (Craven, 1987). Secondly, as described in the previous section, the intramolecular vibrations of H atoms in C—H groups are highly anisotropic. They have r.m.s. amplitudes ranging from 0.08 Å along the C—H bond to 0.15 Å perpendicular to the bond. These vibrations occur even at the lowest temperatures and therefore they constitute an inherent property of the C—H group. The internal vibrations and the asphericity in the charge density both contribute to the polar-flattening effect. We consider both to be important for determining the effective shape of the H atoms in organic molecules and hence the surface configuration of all lipid molecules.

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## Structure of the Pig Insulin Dimer in the Cubic Crystal

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### Abstract

Atomic coordinates for pig insulin in the cubic crystal have been refined by reciprocal-space methods to an *R* factor of 0.173 for data between 10.0 and 1.7 Å resolution with structure-factor amplitudes greater than two standard deviations. Stereochemical parameters for the refined model are close to standard values and the estimated error in the positions of well-ordered atoms is about 0.1 Å. Residues directly involved in the formation of the exact (crystallographic) cubic insulin dimer are oriented similarly to

those in the non-crystallographic 2Zn insulin dimer. Other residues, which make different molecular contacts in the different crystal forms, have locally altered conformations. The cubic insulin molecule is significantly more similar to one of the two independent molecules in the 2Zn insulin dimer than the other. This more similar molecule is expected to be the more stable conformer.

### 1. Introduction

Insulin is a small (51 amino acid) hormone important for regulating the cellular uptake of glucose and which, over longer time periods, is necessary for normal cell growth and proliferation. In solution, pig insulin forms monomers, dimers or hexamers depending on the concentration. Insulin molecules are stored as hexameric arrays in the pancreatic B

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