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The Crystal Structure of Ergosterol Monohydrate

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The monohydrate of ergosterol (provitamin D_2) crystallizes in space group $P2_1$ with $Z=4$ (formula unit: $C_{28}H_{44}O.H_2O$) and $a=9{\cdot}95$, $b=7{\cdot}59$, $c=35{\cdot}5$ Å, $\beta=100{\cdot}4°$. Data were collected by four-circle diffractometry and the structure was solved by direct methods. The final R was 0·242 on 2972 reflexions. The high R is mainly due to the fact that the crystals suffered severe radiation damage during data collection. The two molecules in the asymmetric unit are similar in conformation: rings A and C have the chair conformation; rings B are distorted half-chairs; rings D are slightly distorted envelopes. The molecules are linked with each other and with the water of crystallization in a hydrogen-bonded network so that they form bilayers with thickness approximately twice the length of the ergosterol molecule.

Introduction

The first X-ray diffraction studies of ergosterol (provitamin D_2, systematic name: ergosta-5,7,22-trien-3β-ol) were made by Bernal (1932), as part of a series of investigations into the dimensions of molecules in the vitamin D_2 series; the results were important in the determination of the chemical structure of steroids (Bernal & Crowfoot, 1935). However at that time, it was not possible to determine the structure fully because no methods of solving structures with as many as 60 atoms in the asymmetric unit were available. Subsequent attempts to investigate the crystal structure of ergosterol were hindered by the difficulty of obtaining crystals suitable for X-ray analysis (*e.g.* Braun, Hornstra, Knobler, Rutten & Romers, 1973). The structure of the orthorhombic form of ergocalciferol (vitamin D_2), another of the compounds examined by Bernal (1932), has also been determined in this laboratory (Hull, Leban, Main, White & Woolfson, 1976). Both ergosterol and ergocalciferol have two molecules in the asymmetric unit.

Experimental

Crystals of ergosterol were obtained as flat, colourless needles by recrystallization from acetone, at room temperature, of material supplied by the Sigma London Chemical Co. The crystals were slightly sensitive to light, becoming yellow after prolonged exposure, and decayed fairly rapidly in the X-ray beam. Crystal data are given in Table 1.

Data were measured automatically on a Hilger and Watts computer-controlled Y290 four-circle diffractometer. No standard deviations are given in Table 1 for the cell dimensions because the crystal decay caused a change in these dimensions with time. This is illustrated by measurements on a crystal which was not used for data collection. The unit cell was measured nine times at intervals of a few days, during which the crystal was exposed to X-rays, by centring 12 reflexions automatically and then refining the orientation matrix and unit-cell parameters by least squares. Throughout these measurements a and β consistently increased and c consistently decreased. The behaviour of b was less obvious, although in the last few measurements it seemed to be decreasing. The difference between the maximum and minimum values of the cell parameters was about 1% for a and $\cos\beta$, and about 0·3% for b and c. For the data collection a large crystal (2·0 × 0·5 × 0·2 mm) was selected and a less intense X-ray beam used. These changes lessened, but did not remove, the previously observed effects of radiation damage and during data collection the crystal had to be reset several times. It is hoped that it will be possible to recollect the data at low temperature, and that this will greatly reduce the radiation damage to the crystal. During the data reduction the usual corrections were applied and the data were rescaled to correct for loss of scattering by the crystal during data collection. No absorption correction was applied.

Table 1. *Crystal data*

Chemical formula: $C_{28}H_{44}O.H_2O$, $M=414{\cdot}7$, $F_{000}=920$
Monoclinic space group: $P2_1$ ($0k0$: $k=2n+1$ absent)
$a=9{\cdot}95$, $b=7{\cdot}59$, $c=35{\cdot}5$ Å, $\beta=100{\cdot}4°$
$D_c=1{\cdot}045$, $Z=4$
λ(Cu $K\alpha$) = 1·5418 Å, μ(Cu $K\alpha$) = 4·9 cm^{-1}
Data measured to $\theta=51°$
Total number of unique reflexions measured: 3118
observed: 2571
unobserved: 547

Structure solution

The structure was solved with *MULTAN* (Main, Woolfson, Lessinger, Germain & Declercq, 1974). $|E|$ values were calculated with *NORMAL*; the calculated overall temperature factor was 6·1 Å^2. Spherically averaged molecular scattering factors were calculated

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for a group of 20 atoms forming the main steroid skeleton with the positions given for an analogue of provitamin D by Braun *et al.* (1973).

MULTAN generated 32 phase sets for 410 reflexions. It was necessary to use 4000 $\sum_2$ relationships to solve the structure, because the use of only 2000 $\sum_2$ relationships produced 'chicken-wire' type *E* maps. The effects of increasing the number of phase relationships are discussed by Lessinger (1976). In order to determine the order in which to calculate *E* maps, *MULTAN* calculates a combined figure of merit (Declercq, Germain, Main & Woolfson, 1973) from the absolute figure of merit, the 'psi zero' figure of merit and the Karle residual. For all the sets generated in this case the values of the first of these figures of merit lay between 1·0 and 1·1, which is in the expected range for a correct set and therefore could not be used to discriminate between the sets. The combined figure of merit calculated from the other two figures of merit was therefore used to determine the order in which to calculate *E* maps. The first *E* map showed the steroid framework of one of the molecules in the asymmetric unit unambiguously. However there were two possible interpretations for the steroid framework of the second molecule, related to each other by a rotation through 180° about the longest axis of the molecule and a shift of about 2 Å parallel to the length of the molecule. One of these interpretations proved to be correct. The structure was completed by recycling the phases calculated from the incomplete structure through the tangent formula and by weighted Fourier synthesis; towards the end of this procedure the two water molecules were located.

Refinement

The structure was refined by block-diagonal least-squares with *GENSFLS* (Alcock, Alcock & Bailey, 1971). The six blocks contained 59, 56, 60, 56, 8 and 2 parameters respectively and were so arranged that the parameters for each ergosterol molecule were contained in two of the larger blocks. Scattering factors were taken from *International Tables for X-ray Crystallography* (1962). The final *R* was 0·242 and the final

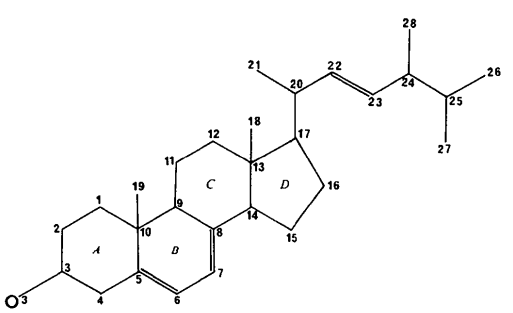


Fig. 1. The atomic numbering scheme for ergosterol. Numbers for molecule *B* are obtained by adding 100 to those of molecule *A*.

Table 2. *Fractional coordinates and isotropic thermal parameters* ($\times 10^4$)

Estimated standard deviations are given in parentheses.

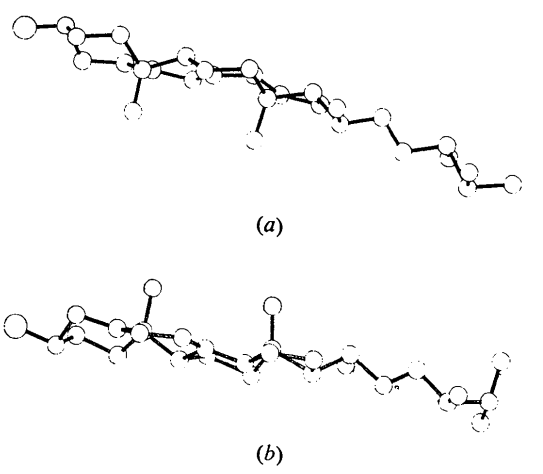
	x	*y*	*z*	*B*
Molecule *A*				
C(1)	5986 (21)	3418 (30)	3835 (6)	58 (5)
C(2)	6109 (20)	3239 (29)	4264 (6)	51 (5)
C(3)	5735 (22)	1082 (34)	4354 (7)	67 (6)
C(4)	6901 (23)	86 (31)	4235 (6)	63 (6)
C(5)	6920 (21)	272 (29)	3829 (6)	54 (5)
C(6)	7114 (23)	−1003 (31)	3572 (7)	60 (6)
C(7)	7400 (19)	−827 (27)	3199 (5)	42 (4)
C(8)	7210 (22)	805 (32)	2996 (6)	56 (5)
C(9)	6625 (20)	2350 (28)	3233 (5)	45 (5)
C(10)	7117 (21)	2307 (29)	3672 (6)	53 (5)
C(11)	7060 (20)	4209 (28)	3071 (6)	42 (5)
C(12)	6938 (21)	4287 (30)	2639 (6)	51 (5)
C(13)	7916 (19)	2847 (30)	2507 (5)	42 (4)
C(14)	7151 (19)	991 (27)	2603 (5)	38 (4)
C(15)	7782 (21)	−387 (31)	2379 (6)	55 (5)
C(16)	8046 (21)	521 (31)	2010 (6)	56 (5)
C(17)	7664 (20)	2709 (31)	2063 (6)	52 (5)
C(18)	9317 (23)	2896 (35)	2688 (6)	66 (6)
C(19)	8541 (23)	2767 (37)	3814 (6)	76 (6)
C(20)	8655 (21)	3672 (28)	1845 (6)	52 (5)
C(21)	8140 (24)	5713 (37)	1854 (7)	79 (7)
C(22)	8384 (24)	3145 (35)	1434 (6)	70 (6)
C(23)	9512 (24)	2675 (37)	1285 (7)	70 (6)
C(24)	9235 (33)	2294 (47)	854 (9)	111 (10)
C(25)	9630 (39)	−93 (52)	853 (11)	139 (12)
C(26)	10605 (33)	3086 (54)	716 (10)	114 (10)
C(27)	10433 (36)	2651 (58)	280 (10)	148 (12)
C(28)	10094 (46)	5096 (72)	679 (13)	185 (17)
O(3)	5861 (15)	1013 (–)	4754 (5)	79 (4)
Molecule *B*				
C(101)	2855 (24)	6152 (37)	3885 (7)	74 (6)
C(102)	2116 (23)	6031 (35)	4237 (6)	67 (6)
C(103)	2406 (26)	7903 (43)	4450 (7)	85 (7)
C(104)	1428 (23)	9273 (34)	4192 (7)	63 (6)
C(105)	1964 (22)	9372 (34)	3834 (6)	63 (6)
C(106)	2156 (22)	10926 (32)	3631 (6)	60 (6)
C(107)	2382 (21)	10980 (31)	3238 (6)	53 (5)
C(108)	2783 (23)	9668 (34)	3046 (7)	60 (6)
C(109)	3108 (21)	7750 (32)	3314 (6)	52 (5)
C(110)	2097 (23)	7509 (33)	3592 (6)	61 (6)
C(111)	3010 (22)	6172 (32)	3042 (6)	55 (5)
C(112)	3743 (23)	6342 (32)	2671 (6)	61 (6)
C(113)	2913 (24)	7965 (33)	2420 (6)	58 (5)
C(114)	3337 (22)	9679 (31)	2705 (6)	53 (5)
C(115)	3040 (22)	11230 (32)	2449 (6)	57 (5)
C(116)	3341 (23)	10647 (34)	2053 (6)	68 (6)
C(117)	3774 (24)	8457 (34)	2094 (7)	75 (7)
C(118)	1474 (23)	7830 (35)	2318 (6)	66 (6)
C(119)	655 (26)	7098 (35)	3407 (7)	82 (7)
C(120)	3232 (26)	7688 (40)	1696 (7)	85 (7)
C(121)	3563 (27)	5400 (38)	1762 (8)	84 (7)
C(122)	4292 (26)	8209 (39)	1451 (7)	89 (8)
C(123)	3841 (32)	8721 (43)	1108 (9)	109 (10)
C(124)	4952 (41)	9240 (57)	890 (12)	134 (12)
C(125)	4747 (36)	11436 (50)	779 (9)	122 (10)
C(126)	5015 (52)	8406 (71)	506 (14)	164 (16)
C(127)	5684 (57)	6767 (89)	632 (16)	241 (25)
C(128)	3695 (53)	8209 (78)	303 (14)	204 (19)
O(103)	1715 (16)	7725 (27)	4796 (5)	88 (5)
Water molecules				
O(200)	6327 (18)	7458 (28)	5021 (5)	102 (5)
O(201)	8921 (22)	6149 (37)	4879 (6)	147 (7)

weighted R 0·183. The refinement was based on 2972 reflexions with weights based on $|F_o|$ for observed and set at 0·1 for unobserved reflexions. The 146 reflexions observed with negative intensities were omitted. The C and O atoms were refined with isotropic temperature factors and the H atoms were excluded. The fractional coordinates and thermal parameters are listed in Table 2.* The atomic numbering is shown in Fig. 1.

Discussion

Because the data are of poor quality it is not possible to draw definite conclusions about the detailed geometry of the structure. Therefore the following discussion concentrates on the general molecular conformation and the crystal packing. Fig. 2 shows views of the two molecules in the asymmetric unit. The overall steroid conformation is similar to that reported for an analogue of provitamin D by Braun *et al.* (1973). The *A* rings of both molecules have chair conformations with the hydroxyl group in an equatorial position. The rings *B* are similar to those in the provitamin D analogue, being distorted half-chairs with the atoms of the butadiene (C=C–C=C) fragment lying in a plane (r.m.s. deviations 0·045 and 0·055 Å for the two molecules) and the other two atoms lying on opposite sides of this plane. The out-of-plane atoms are up to 0·45 Å from the plane. The rings *C* have chair conformations in both molecules, in contrast to lumisterol$_3$ (de Kok & Romers, 1974) in which the corresponding ring has a boat conformation. The rings *D* are slightly distorted envelopes in ergosterol; in the provitamin D analogue they are distorted towards half-chairs. In both ergosterol molecules C(14), C(15), C(16) and C(17) lie in a

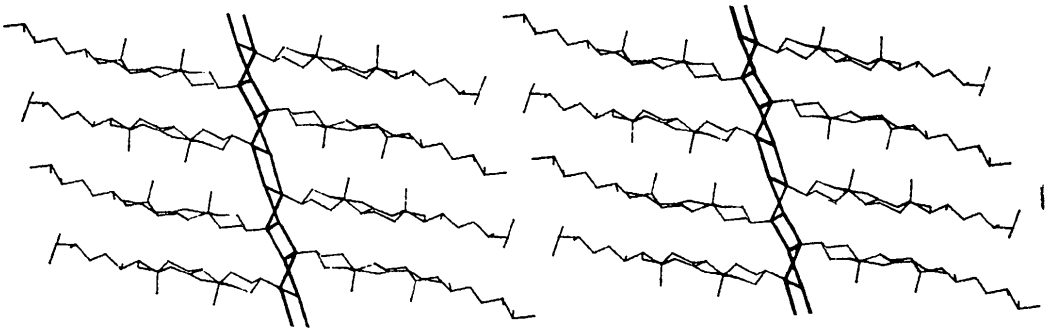
* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 31668 (12 pp., 1 microfiche). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1 NZ, England.



(*a*)

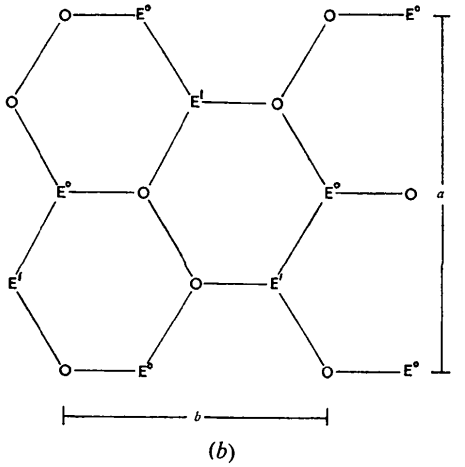
(*b*)

Fig. 2. Projections of the two molecules viewed along the unique axis; (*a*) molecule *A*, (*b*) molecule *B*.



(*a*)

Fig. 3. (*a*) The molecular packing viewed along the unique axis, showing the hydrogen bonding.



(*b*)

Fig. 3 (*cont.*). (*b*) A diagrammatic view of the hydrogen bonding projected onto *ab*. *O* represents water and *E* represents the oxygen atom of ergosterol. The superscripts (0 and 1) indicate molecules extending into and out of the plane of the paper.

plane (r.m.s. deviations 0·033 and 0·018 Å) and C(13) is about 0·8 Å from this plane.

Despite the high temperature factors of the atoms forming the side chains, it can be seen that the conformations of these chains are the same in both molecules, except for the arrangement about C(24)–C(25). In molecule *A* this bond has a staggered conformation, in *B* C(23)–C(24) and C(25)–C(27) are almost eclipsed.

The structure is held together by hydrogen bonding between the water molecules and the hydroxyl groups on the *A* rings of the steroid molecules. The packing is shown diagrammatically in Fig. 3. The molecules pack in sheets as shown in Fig. 3(*a*); each sheet is the width of the unit cell in the **c** direction (35·5 Å), or approximately the length of two molecules. The outsides of the sheets consist of the hydrophobic hydrocarbon tails of the ergosterol molecules; at the centre of each sheet is a puckered two-dimensional array of hydrogen-bonded O atoms holding the ergosterol molecules together. A diagrammatic projection of the hexagonal array of O atoms is shown in Fig. 3(*b*). Each six-membered ring contains three water molecules and three ergosterol molecules, and the average O···O hydrogen-bond length is 2·91 Å for the six unique bonds. Each ergosterol molecule donates one H atom and accepts two H atoms.

The packing is such that steroid molecules which are directly linked by hydrogen bonds extend towards opposite sides of the sheets. The packing in crystals of ergosterol is very different from that in either ergocalciferol or lumisterol and is similar in its bilayer structure to that found in lipids such as the phospholipid studied by Hitchcock, Mason, Thomas & Shipley (1974). This is not surprising since both the lipid and ergosterol contain polar groups at the ends of long hydrophobic chains. The important part which water plays in the crystal structure of ergosterol explains why the preparation of anhydrous ergosterol has been found to be almost impossible (Bills & Honeywell, 1928). Apart from the hydrogen bonds, the closest intermolecular contacts are about 3·5 to 3·7 Å between C atoms.

Remeasurement of the X-ray data at low temperature should greatly improve the accuracy of the positional parameters, allowing a fuller discussion of the molecular geometry, and reduce the temperature factors, especially for atoms in the hydrocarbon tail of the molecule.

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