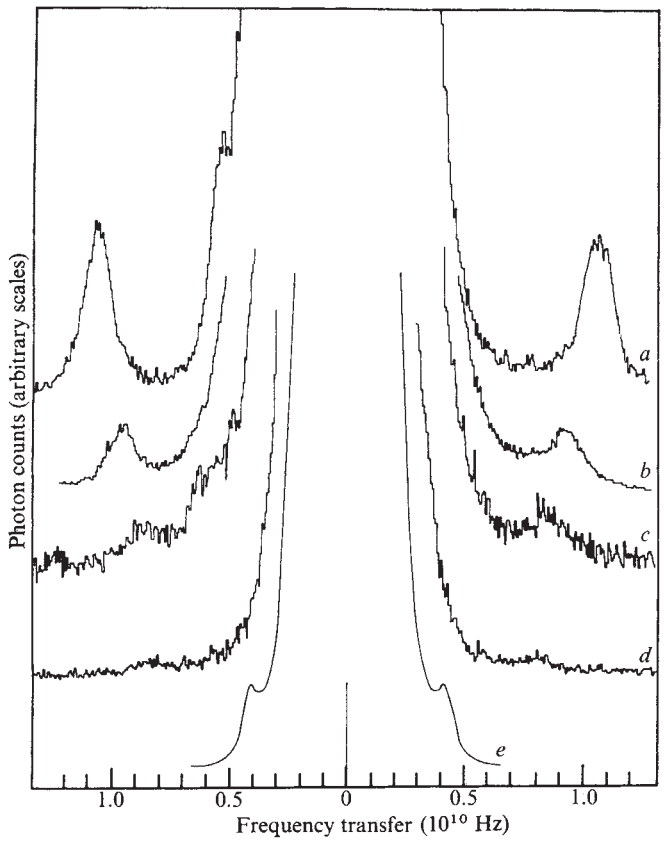

Fig. 4 Dependence of the Brillouin frequencies on degree of hydration for rat tail collagen at 25 °C. Obtained at lowest possible laser power at relative humidities of *a*, 0%; *b*, 30%; *c*, 84%; *d*, 100%. *e*, Spectrum of ordinary distilled water (not dust free).

from collagen and muscle give values for the velocities of acoustic waves and elastic moduli in the medium. For dry collagen this leads to an estimate of the hydrogen bond strength. For material in the native state the measurements are capable of giving information about the dynamics of water molecules in the material.

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Crystal structure of anhydrous cholesterol

CHOLESTEROL, the most abundant steroid in the animal kingdom, is found mainly as a component in cell membranes and lipoproteins. The presence of the hydrophilic C(3) hydroxyl group in this otherwise hydrophobic molecule allows cholesterol to occupy a position at polar–nonpolar interfaces. Crystals of 3-hydroxy steroids and their hydrates show a tendency towards double layer structures with an end-for-end arrangement of approximately parallel molecules[1]. As this molecular organisation is generally similar to that in membranes, it can be expected that some of the principles of intermolecular association in membranes might be revealed in the molecular packing in the crystal structures. Another common feature in these structures is the presence of more than one molecule in the crystallographic asymmetric unit. While this is a complicating factor in structure analysis, it clearly increases the amount of information regarding preferred conformation and intermolecular association to be gained from a structure determination. Craven[2] has recently reported the remarkable crystal structure of one form of cholesterol monohydrate. The arrangement of the eight crystallographically independent molecules in the triclinic cell shows unusual local pseudo-symmetry. One pair of molecules is related to another by a non-crystallographic $b/2$ translation, while a third pair, oriented end-for-end relative to the first, is related to the fourth by a similar $a/2$ translation. Non-crystallographic twofold rotation symmetry relates members of the first and the third pairs. The overall structure is a stacking of bilayers of 33.9 Å total thickness. We report here that anhydrous cholesterol crystals are also triclinic, with eight independent molecules per cell. This structure offers additional insight into the packing of cholesterol molecules, and a comparison with the monohydrate structure.

Colourless lath-shaped crystals were obtained by cooling a saturated acetone solution of cholesterol. The cell dimensions are $a=14.172(7)$, $b=34.209(18)$, $c=10.481(5)$ Å, $\alpha=94.64(4)$, $\beta=90.67(4)$ and $\gamma=96.32(4)°$, in agreement with values reported by Bernal *et al.*[1], and converted to a reduced cell[3]: $a=14.00$, $b=33.71$, $c=10.46$ Å, $\alpha=94.5°$, $\beta=90.0°$ and $\gamma=95.9°$. Since cholesterol is an optically active compound, the space group of the triclinic crystals is P1. Each unit cell contains eight $C_{27}H_{46}O$ molecules; thus there are 224 non-hydrogen atoms per asymmetric unit. The calculated density is 1.021 g cm^{-3}; the experimental value (flotation) is 1.03 g cm^{-3}. A total of 10,399 unique reflections were measured to the limit 2θ (CuKα)=100° using a Syntex P1 diffractometer with a graphite monochromator.

The structure was solved by a combination of vector search methods[4] and direct methods[5]. Vectors representing the known geometry of the rigid 21-atom 3-hydroxy steroid group were used in Patterson-space orientation searches which gave several probable orientations of this group in the crystal. Vectors between steroid groups in these orientations were used in translation searches. In this way it was possible to position three oriented steroid groups in relation to each other in such a way that searches with the three sets of inter-group vectors gave a consistent result. This 63-atom partial structure was used to calculate input

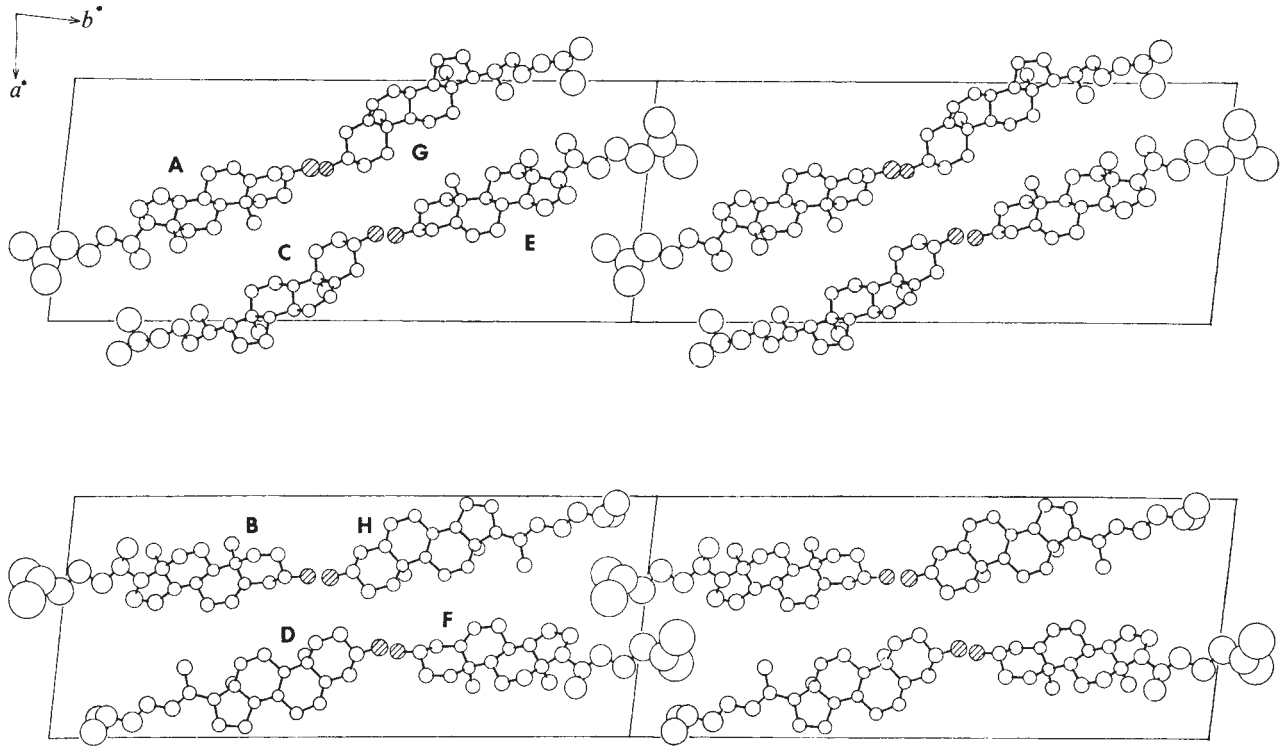

Fig. 1 The crystal structure of anhydrous cholesterol viewed along *c* (out of page). The upper figure is to be superposed on the lower one to give the complete structure. Two unit cells ($x = 0$–1; $y = 0$–2) are shown, displaying non-crystallographic twofold symmetry axes at $x \approx 0.5$; $y \approx 0.5$, 1.0 and 1.5. Atoms are shown as 50% probability thermal motion spheres. Oxygen atoms shaded.

phases to the MULTAN programme[5], modified to hold selected input phases fixed throughout the phase extension and refinement. The structure then emerged in a straightforward manner. About 80% of the hydrogen atoms were found in difference Fouriers, including those eight which are involved in hydrogen bonding. Refinement by block-diagonal least squares, with anisotropic C and O thermal parameters, gave $R=0.056$ for 8032 reflections with $I > 2.33\sigma(I)$.

There are no significant differences between the steroid skeletons of the eight molecules in this structure, the average atom–atom misfit in superposing steroid skeletons being 0.07 Å. The conformation of the tail part of the cholesterol molecule shows considerable variation, however, as shown in Fig. 1, where molecules A and C represent two extreme cases in their C(17)–C(25) distances of 6.60 and 5.51 Å, respectively. This variability in the hydrocarbon chains is less pronounced in the monohydrate[2] where the chains are partially folded to an approximately equal degree.

The hydrogen bonding pattern (Fig. 2) consists of two independent chains of hydrogen bonds running parallel to

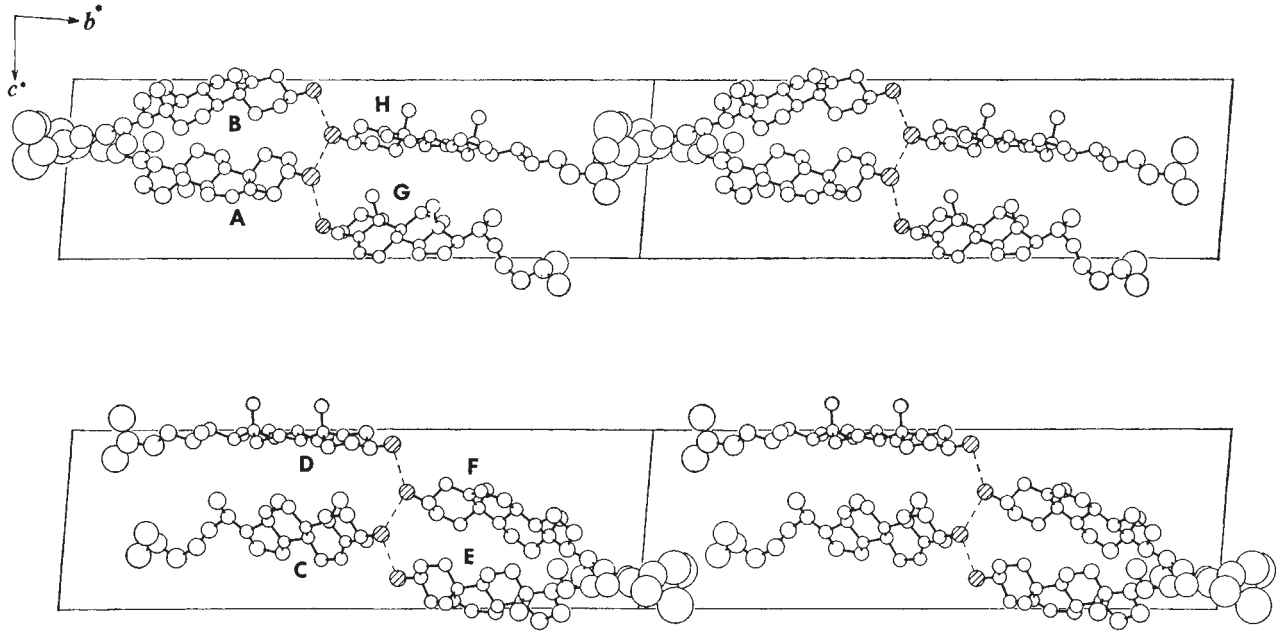

Fig. 2 The structure viewed along *a* (into page). Two unit cells ($y = 0-2$; $z = 0-1$) are shown, as in Fig. 1. The dashed lines indicate hydrogen bonding.

c with an average O . . . O distance of 2.87 Å. The hydrogen bond chains are staggered by ±2.18 Å about a mean plane parallel to *ac*. This corrugated sheet of separated, parallel chains is to be compared to the pleated sheet of hydrogen bonds in the monohydrate.

The steroid planes of the eight molecules show no tendency towards parallel packing. The dihedral angles between least-squares planes of adjacent molecules range from 3° to 88°. The long molecular axes, while generally oriented along *b*, show considerable variation in direction. The angles between the least-squares lines calculated from any two molecules range from 4° to 36°. This is in contrast to the monohydrate, in which all the ring systems are approximately parallel and aligned.

Careful examination of the adjacent unit cells (Fig. 1) reveals an approximate local symmetry axis at $x=0.5$, $y=1.0$ along a direction making angles of 90.0, 94.5 and 0.7° with the crystal axes *a*, *b* and *c*, respectively. A 180°

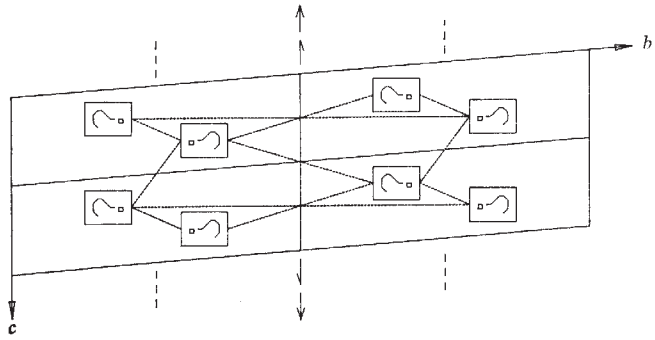

Fig. 3 Schematic representation of the non-crystallographic symmetry in four adjacent cells. The left- and right-leaning question marks represent, respectively, the molecular clusters ABCD and EFGH. Local symmetry elements shown are coaxial twofold rotation and screw axes at $y = 1$ and a 'twofold screw axis' with alternating translational components of 0.25 and 0.75 *c* at $y = 0.5$ and 1.5. This diagram applies at $x = 0$ and 0.5.

rotation about this axis brings the leftmost molecules A, B, C and D into close coincidence with the molecules E, F, G and H of the adjacent (right hand) cell. The mean atom–atom misfit between the 112 atoms of the first four molecules, turned as a rigid group, and the second 112 atoms is only 0.24 Å. This fit is as good in the C(17) chain regions as it is in the steroid skeleton regions. The remaining molecules E, F, G, H of the left hand cell are related to A, B, C, D of the right hand cell by a pseudo-twofold screw axis which is coaxial with the pseudo-twofold rotation axis. Figure 3 illustrates in diagrammatic fashion the relationships among the molecules of four adjacent unit cells. Non-crystallographic symmetry axes can also be seen to exist at $x=0.5$, $y=0.5$ and 1.5 in Fig 3. These are 'screw axes' whose translation components alternate between (very nearly) $c/4$ and $3c/4$, instead of the usual one-half cell translation.

With the presence of this pseudo-symmetry, the non-crystallographic asymmetric unit contains four molecules. These four 'unique' molecules are A, B, C and D. As can be seen in Fig. 2, there is no hydrogen bonding between these molecules. It seems that this unique structural unit is held together primarily by hydrophobic interactions.

The number of 'unique' molecules, that is, molecules not related to each other by either local or strict symmetry, is the same in the monohydrate[2] and the anhydrous form. The nature of the non-crystallographic symmetry in the two structures is entirely different; there is no non-crystallographic half-cell translation in the anhydrous structure. Yet, in each case, the non-crystallographic symmetry is obeyed locally to a remarkably high degree.

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Direct evidence for an interaction of β-adrenergic blockers with the 5-HT receptor

Advances in neurotransmitter receptor identification and quantification by biochemical techniques involving radiolabelled analogues of putative neurotransmitters have led to the characterisation of a number of receptor systems in mammalian brain tissue[1]; these include the catecholamines[2,3], 5-HT[4], glycine[5] and GABA[6] receptors. The development of these techniques has provided a means of screening chemical compounds for their effects on different receptor systems[7]. The central nervous system effects of (±)-propranolol (Inderal, ICI) in animals are well documented[8,9]. This drug has therapeutic benefit in man in anxiety[10], and is also reported to have similar benefit in schizophrenia[11,12], essential tremor[13] and drug dependence[14]. The origin of these effects is not understood but theories about its mode of action include β-adrenergic blockade[15], membrane stabilisation[8] and a 5-HT receptor blocking action[16,17]. We report here that propranolol a stereospecific affinity for the 5-HT receptor from rat brain which is similar in magnitude to the putative 5-HT receptor blocking drug methylsergide.

Specific binding of ^{3}H-5-HT was measured by the method of Bennett and Snyder[4]. The whole brain (minus the cerebellum) of a freshly killed Alderley Park rat (250–350 g) was rapidly removed and homogenised in 30 ml of ice-cold 0.32 M sucrose with five passes of an air-driven glass–Teflon homogeniser. After centrifugation at 1,000*g* for 10 min, the supernatant was subjected to 17,000*g* for 30 min to obtain a crude mitochondrial pellet. The pellet was lysed with 30 ml of ice-cold distilled water and briefly homogenised as before. The lysate was then spun at 10,000*g* for 20 min. The pellet from this spin, a bilayer with a soft, buffy upper layer was washed carefully to collect the upper layer and the resultant supernatant centrifuged at 48,000*g* for 20 min. The pellet was suspended in 6 ml of 75 mM Tris-HCl buffer (*p*H 7.4) containing 25 mM $MgCl_2.6H_2O$ and 50 μg ml^{-1} of nialamide.

Incubations (15 or 16.5 min, 37 °C) were carried out in glass test tubes in a 1 ml final volume containing 75 mM Tris-HCl (*p*H 7.4), 25 mM $MgCl_2.6H_2O$, 5 nM ^{3}H-5-HT (specific activity 14 Ci mmol^{-1}, Radiochemical Centre, Amersham), 640–840 μg of protein, the appropriate concentration of the drug under test and in the presence or absence