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# X-Ray Crystallography and the Chemistry of the Steroids. Part I

J. D. Bernal, Dorothy Crowfoot and I. Fankuchen

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# X-RAY CRYSTALLOGRAPHY AND THE CHEMISTRY OF THE STEROIDS. PART I

*By* J. D. BERNAL, F.R.S., DOROTHY CROWFOOT AND I. FANKUCHEN

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A survey has been made of the X-ray crystallography of some eighty sterol derivatives belonging mainly to the cholesterol and ergosterol series but including also calciferol and other photoderivatives of ergosterol and some higher plant and animal sterols. The measurements are recorded in four tables and include determinations of unit cell size, space group and some data on the crystal morphology and optics. In three cases, cholesteryl chloride, bromide and cholesteryl chloride hydrochloride, Patterson projections have also been derived from the intensities of the X-ray reflexions of the *h*0*l* planes. These confirm earlier deductions on the shape and size of the sterol molecules, proving that these are roughly lath-shaped, $20 \times 7 \times 4$ A, and the details of the patterns can also to some degree be correlated with the actual arrangement of the carbon atoms in the sterol ring system and with the positions of the chlorine and bromine atoms. The arrangement of the molecules in the crystal units is closely that given by the preliminary examination from the optic orientation, and this has therefore been employed to suggest in each of the remaining sterol crystal structures the probable molecular arrangement.

Among the eighty compounds studied a number of different types of crystal structure appear. An attempt is made to group these in a general geometric classification depending upon the relative orientation of the molecular axes, thickness, width, and length to the crystallographic axes *a*, *b*, and *c*. In monoclinic crystals three main groups are distinguished in which the monoclinic *b* axis is the direction of the molecular thickness, width or length, and the orthorhombic and triclinic crystals are classified by comparison with the monoclinic varieties. Inside each group divisions are based first on the multiplicities, the numbers of molecules present, and secondly on the space groups. Altogether 105 different sterol structures are included in the classification, and of these seventy-seven fall into the second of the three main groups described above, which includes cholesteryl chloride and bromide and has been called the normal type of sterol structure.

The crystallographic measurements as a whole are discussed in their bearing on specific chemical problems under the following headings:

(*a*) *Characterization and identification*

The X-ray data have been applied particularly among the higher plant and animal sterols both to identify individual constituents (e.g. stigmasterol in phytosterol mixtures), to distinguish additional crystal forms (e.g. two types of cerevisterol) and to characterize new sterols and relate these to previously known compounds (e.g. a sterol from rubber).

(*b*) *Molecular weight determinations*

Molecular weight measurements have been carried out on cholestenone, ergotetraene, $\gamma$-spinastenol acetate, ergosterol $H_2O$, stigmasterol $H_2O$, $\gamma$-sitosterol $H_2O$ and $\beta$ sitosterol from rubber. In the case of crystals of cholestanol, $\beta$-ergostadienetriol and $\Delta$-4-cholestene-7-ol, the measurements are used to estimate water of crystallization.

(*c*) *The stereochemistry of the carbon skeleton*

The X-ray measurements indicate that the general configuration of the sterol ring system must be flat, but so far it has been impossible to correlate stereochemical changes at particular ring junctions with crystallographic changes, e.g. between rings *C* and *D* ($\alpha$-ergostenol, $\beta$-ergostenol), *B* and *C* (lumisterol), and *A* and *B* (coprostane, cholestane).

(*d*) *The effect of substituents on the crystallography of the sterols*

(i) *The position of the hydroxyl group.* A hydroxyl group at C 3 of the sterol skeleton generally, but not invariably, leads to double-layer formation in the crystal structure and vice versa, a double-layer crystal structure usually indicates the presence of a terminal hydroxyl group. Exceptions are cholestane 6-ol which shows a double layer, and pyrocalciferol which does not. Brassicasterol, cerevisterol, and the spinasterols all probably have hydroxyl groups at C 3. The position of *i*-cholesterol (which shows no double layer) is obscure.

(ii) *The stereochemistry of the hydroxyl group at* C 3. It is not possible to distinguish at this stage between the two possible configurations of the hydroxyl group at C 3. In cholesteryl chloride and bromide, the chlorine and bromine atoms must lie closely in the plane of the ring system. $\alpha$-Chlor-cholestane is correlated crystallographically with cholesteryl chloride.

(iii) *The position of the double bonds.* The introduction of either hydroxyl groups or halogens at the double-bond system usually produces radical changes in the crystallography which makes simple comparison difficult. The Patterson analysis of cholesteryl chloride hydrochloride provides, however, definite evidence that the extra chlorine atom is at C 5. The crystallography of both the hydroxyl and maleic anhydride derivatives of ergosterol is in agreement with their present chemical formulae but provides no certain proof of the correctness of these.

(*e*) *A comparison of the crystallography of different sterols—monohydroxy compounds*

There is a group of sterols, including ergosterol and many of the higher plant and animal sterols, which show particularly close resemblances in crystal structure to one another. While inclusion in this group must indicate close similarity both in sterol skeleton and molecular arrangement, deviations do not necessarily seem to have a chemical significance.

(*f*) *The structure of calciferol*

Calciferol, while showing certain differences from the characteristic sterol group mentioned above, also shows similarities, particularly in *c*-plane intensities. It seems unlikely therefore that the actual distribution of the atoms in the molecule differs considerably from that in ergosterol.

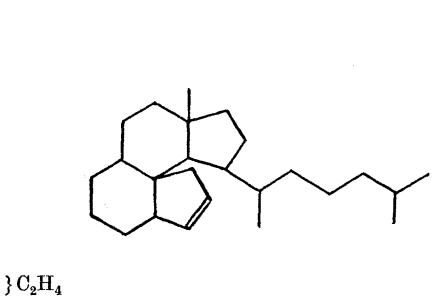
*Conclusion*

Many of the outstanding problems of the chemical structure of the sterols can only be settled by exact analysis. The present survey has indicated a number of compounds that are suitable for such treatment.

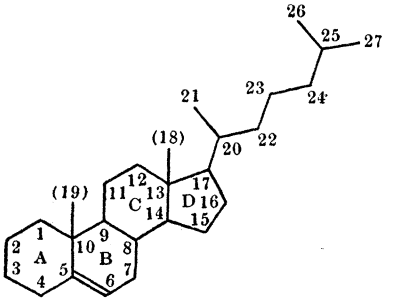
The use of X-ray crystallography for the solution of problems of the structure of chemical molecules is only beginning, but it has already shown itself as a promising method of attack. X-ray analysis of any crystalline material can yield, according to the degree to which it is pushed, a succession of data bearing in greater and greater detail on the structure of the material. Briefly, the successive stages of analysis can yield information on: I, Identification and comparison of substances from different sources; II, Molecular weight to any desired degree of accuracy; III, Symmetry of

molecules; IV, General size and shape of molecules; V, Position of substituent groups; VI, Position of all the atoms in the molecules (Crowfoot and Bernal 1937; Robertson 1937). The first three stages are straightforward and unequivocal, the next two are less direct and usually require a degree of collaboration between crystallographic and chemical methods. The last stage is the most exact and can, though as yet only under certain conditions, give information absolutely independent of chemical considerations. It is, however, extremely laborious and limited as yet to fairly simple compounds.

The investigations recorded in this paper have not been carried to this last stage. Their aim has been to use the methods of crystallography as an auxiliary in determining the structural formula of an important group of naturally occurring chemical substances, the steroids. In this field up to a few years ago chemical methods had not seemed to yield unequivocal results. Here an early application of crystallographic methods by one of us (Bernal 1932) gave the clue to a fundamental revision of the structural formula of the carbon skeleton of cholesterol, I (Windaus 1928; Wieland 1928) and led to the proposal of that associated with the names of Rosenheim and King (1932) and Wieland and Dane (1932), II, and now generally accepted. The crystal structures studied at that time also suggested that in cholesterol and ergosterol the hydroxyl group was in a terminal position, and that in the sex hormone, oestrone, the active groups were at opposite ends of the molecule.




I




II

Both these deductions have been supported by later chemical work, but in view of the simplicity of the crystallographic methods employed in reaching them, more confirmation would be desirable. Naturally no complete test will be possible without either a chemical synthesis on the one hand or an exact crystallographic analysis on the other—both of which are now within the bounds of possibility. In the meantime, it has been possible to add to the weight of evidence by extending the method of preliminary crystallographic examination and comparison to a larger number of sterols and their derivatives. We feel it is useful to present now the results so far obtained, to discuss the conclusions of different degrees of probability that may be drawn from them, and to suggest the lines of further attack on the most outstanding problems in this field.

The present investigation arose directly out of the original examination of cholesterol, ergosterol and calciferol in 1932, and has been carried on parallel with much of the chemical work of the last eight years. The material to be considered is concerned with some eighty compounds belonging to the main sterol series and including cholesterol, ergosterol and some of the higher plant and animal sterols, but the actual derivatives studied are chemically a somewhat haphazard collection. For purposes of reference the crystallographic data are listed in four tables: 1, The cholesterol series; 2, The ergosterol series; 3, The photoderivatives of ergosterol; 4, Higher plant and animal steroids; and each group is further subdivided according to the chemical character of the available compounds, hydrocarbons, hydroxyl and halogen derivatives and so on.

The information given in tables 1–4 covers the first stages only of the crystallographic investigation, determination of cell size, space group and some data on the crystal optics and morphology. The details of most of the actual measurements carried out, together with estimated intensities of some of the X-ray reflexions, are recorded in a thesis by one of us (Crowfoot 1936). It is possible to make direct use of these data for the identification of individual sterol derivatives, and though in most cases the cell constants are not given correct to more than 1 or 2 %, in a few examples (see table 6) they were measured with sufficient precision to be employed for molecular weight determination. Apart from the recognition of molecular symmetry, which is not important here since all sterol molecules are asymmetric, the further use of the data depends upon how much correlation can be effected between crystallographic character and chemical structure. Some of the crystal structures listed, such as those of anhydrous ergosterol, of cholesterol and of coprostanol, are obviously very complicated and can hardly be expected to yield much chemical information. Others are comparatively simple and would probably repay an attempt at intensive analysis. Short of this it is possible, as shown in the first sterol investigation, to derive the most probable arrangement of the molecules and their approximate dimensions from a combination of the optic with the X-ray data; and this is the method that has been followed with all the sterol crystals now examined. In order to facilitate comparison between different compounds the nomenclature of the crystallographic axes in tables 1–4 is arranged as far as possible to coincide with that of the optic axes, $a$, $\alpha$; $b$, $\beta$; $c$, $\gamma$; most of the crystals examined are plates showing one dominating face, the $c$ plane. Deviations in crystal character which make such a nomenclature impossible or undesirable then provide one type of distinction that will be important in further crystallographic classification. But in view of the assumption as to the character of the molecules which underlies such a process it is worth while first to review shortly some direct evidence which has been obtained in the course of the investigations in support of the original deductions of the size and shape of sterol molecules.

## 1. The dimensions and orientation of sterol molecules in crystals

It is worth considering first how far the data now collected add to the certainty with which the dimensions of the sterol molecule are known. The smallest unit-cell dimension actually measured on any of the crystals so far examined is 5·85 A in *cis*-$\Delta$-5-6-cholestene-3-4-diol (*b*), and this proves definitely that in one direction the molecule is at least as small as 5·85 A. This can be combined with the evidence of the crystal cell of $\beta$-ergostadienetriol (leaflet) to prove that in another direction the molecule must not be shorter than 15 A, while the long unit-cell dimensions measured in dibromocholesteryl chloride and in *i*-cholesteryl ethyl ether suggest that the sterol molecule can assume a configuration in which it is at least 21 A long.

The unit-cell dimensions alone do not take the determination very far. But a further direct test is possible through a more detailed examination of cholesteryl chloride and bromide, including an application of Patterson analysis. A preliminary account of the crystallography of these two compounds has already appeared (Bernal and Crowfoot 1933). They both crystallize in a simple monoclinic arrangement with only two molecules in the unit cell related to one another by a twofold screw axis. Intensity data on the (*h*0*l*) planes were derived from Weissenberg photographs taken with the crystals rotating about the *b* axes, and from the $F^2$ values calculated Patterson series were formed. The diagrams obtained (figure 1 B, C) show two irregular series of maxima running along the lines $x=0$ and $x=\frac{1}{2}$ and corresponding to two main sets of interatomic distances. One of these is between atoms in the same molecule, which, if they lie almost directly along the *c* axis would, with varying distances between atoms in the *c* direction, have a common *a* interatomic distance of about zero. The second corresponds to distances between the two molecules, where again with varying distances towards *c* the *a* difference is approximately $\frac{1}{2}$. The break-up of the field into these two ridges at $x=0$ and $x=\frac{1}{2}$ can only be explained by the presence of long molecules relatively thin in the *a* direction, molecules which agree well both in character and dimensions with the requirements of the present sterol formula. This is illustrated by figure 1 A, which shows a projection of two cholesteryl bromide molecules placed in the unit cell as required by the Patterson projection. The drawings are made assuming a puckered arrangement of the carbon atoms in the sterol ring system, with ring junctions *trans* throughout, and some even of the more intimate features of this structure can be correlated with the X-ray data. The main ridge in the Patterson diagram at $x=0$ is composed of a series of peaks at approximately 2·2, 4·4 and 6·6 A from the origin, and these distances correspond closely with those between carbon atoms concentrated around the junctions between the rings of the sterol skeleton. And the peak heights are probably further enhanced by their coincidence with the main carbon-halogen distances (cf. Robertson 1939*a*). It is not possible to fix the stereochemistry of the side chain at this stage, but it is obvious that it must continue the main line of the ring system, and the same is true of the bromine and chlorine atoms.

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TABLE 1. THE CHOLESTEROL SERIES

| Compound | Source | Space group | $n$ | $a$ | $b$ | $c$ | $\beta$ | Morphology | Optics | Structure type |
|---|---|---|---|---|---|---|---|---|---|---|
| (*a*) Hydrocarbons: | | | | | | | | | | |
| Cholestane | H. | $P2_1$ | 4 | 11·2 | 11·0 | 19·8 | 104° | Plates, [100], {001}, {10*l*}, {01*l*}, {11*l*} | +, $b=\alpha$, $\gamma$ 80° from (001), 2 V = 70° | *ab* (22)1 |
| Coprostane | H. | $P22_12_1$ | 4 | 10·85 | 7·62 | 30·3 | 90° | Needles, [010], {102}, {101}, {110}, {001}(1) | +, $a=\alpha$, $b=\beta$, $c=\gamma$ | *a* 212 |
| Cholestene | H. | $P2_1$ | 4 | 13·2 | 19·45 | 11·1 | 94° | Irregular pyramids, frequently twinned | $b=\gamma$ | *c* 221 |
| Coprostene | H. | $P2_1$ | 2 | 11·05 | 7·67 | 20·1 | 133° | Laths, [010], {001}, {10$\bar{1}$}, {11*l*} | +, $b=\beta$, $\gamma$ 45° from (001) | *a* 211 |
| Cholesterylene | H.G. | $P2_12_12_1$ | 4 | 15·85 | 7·66 | 19·25 | 90° | Needles, [010], {101}, {120} (?) (2) | +, $a=\alpha$, $b=\beta$, $c=\gamma$, 2 V = *c*. 80° | *a* 411 |
| (*b*) Monoketones: | | | | | | | | | | |
| Cholestenone | H. | $P2_1$ | 2 | 10·61 | 7·86 | 19·98 | 135° | Needles, [010], {10$\bar{1}$}, {001}, {011}, and very small {100}, {101}, {10$\bar{2}$}(3) | +, $b=\beta$, $\gamma$ 30° from (001), 2 V = 60° (3) | *a* 211 |
| (*c*) Monohydroxy compounds: | | | | | | | | | | |
| Cholestanol $2H_2O$ | H. | $P1$ | 4 | 9·79 | 7·76 | 36·8 | $\alpha$ 83° $\beta$ 106° $\gamma$ 88½° | Laths, [010], {001}, {10*l*}, {01*l*}, {11*l*}, frequently twinned | +, $\beta$ very approx. 10° from [010], $\gamma$ 64° from (001) | *a* 212 |
| Epicholestanol | H. | $P1$ | 4 | 9·93 | 7·5 | 33·2 | $\alpha$ 95° $\beta$ 98·5° $\gamma$ *c*. 90° | Needles, fibrous, [010], {001}, frequently twinned | +, *b* approx. = $\beta$, $\gamma$ 72° from (001), 2*E* small | *a* 212 |
| Coprostanol | H. | $P2_1$ | 16 | 7·7 | 35·0 | 31·4 | 100° | Stout needles, [100], {011}, {100} | −, $b=\alpha$, $\gamma$ 30° to *a*, 2 V *c*. 40° | ? |
| Cholesterol (anhydrous) | H. | $P1$ | 8 | 14·0 | 10·46 | 37·8 | $\alpha$ 94°(4) $\beta$ 117½° $\gamma$ 90° | Laths, [010], {001}, {10$\bar{2}$}, {01*l*}, frequently twinned | +, $b=\alpha$, $\gamma$ 60° to (001), 2*E* small | *b* 222 |
| Cholesterol $H_2O$ | B. | C1 | 32 | 15·8 | 19·1 | 74·0 $d_{001}$ 68·4 | $\alpha$ *c*. 90°(4) $\beta$ 112½° $\gamma$ 92° | Rhomb-shaped plates, {001}, {11*l*}, {01*l*}, frequently twinned | +, *b* approx. = $\beta$, $\gamma$ inclined to (001) | *ab* (8)4 |
| Cholesterol MeoH | M. | $P1$ | 4 | 12·3 | 6·23 | 42·3 $d_{001}$ 34·9 | $\alpha$ *c*. 90°(4) $\beta$ 124° $\gamma$ *c*. 90° | Laths, [010], {001}, {100}, {102}, {10$\bar{4}$} | +, $\alpha'$ 16° from [010] in (001), $\gamma'$ 61° from (001) in (010) | *ab* (21)2 |
| Cholestane-6-ol-EtOH | H. | C2 | 16 | 15·4 | 19·7 | 32·7 | 106° | Rhomb-shaped plates, {001}, {11*l*} | +, $b=\beta$, $\gamma$ inclined to (001) | *ab* (8)2 |
| $\Delta$-4-Cholestene-7-ol $H_2O$ ($\psi$-cholesterol) | H. | $P2_1$ | 2 | 13·96 | 6·15 | 17·75 | 123½° | Needles, [010], {001}, {10$\bar{1}$}, sometimes {100} | +, $b=\alpha$, $\gamma$ 75–80° from (001), 2 V = 49° | *ab* (21)1 |

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|---|---|---|---|---|---|---|---|---|---|
| (*d*) Polyhydroxy and keto compounds: | | | | | | | | | |
| *cis*-Δ-5-6-Cholestene-3-4-diol | H. | C 2 | 8 | 30·8 | 5·85 | 30·6 | 120° | Needles, [010], {001}, {401}, {10$\bar{1}$}, {20$\bar{1}$} | +, $b=\alpha$, $\gamma$ 49° from (001), 2 V *c.* 40° | *ab* (41)2 |
| *trans*-Δ-5-6-Cholestene-3-4-diol | H. | $P2_12_12_1$ | 4 | 10·3 | 7·7 | 30·2 | 90° | Needles, [010], habits (*a*) {101} only, (*b*) {001} with {101}, {11*l*} | −, $a=\alpha$, $c=\beta$, $b=\gamma$, 2 V = 54° | *a* 212 |
| α-Cholestanetriol I (from solution) | H. | $P2_1$ | 4 | 11·0 | 7·5 | 43·8 | 131° | Laths, [010], {001} | +, $b=\beta$, $\gamma$ inclined to (001) | *a* 212 |
| α-Cholestanetriol II (from melt) | H. | $P2_12_12_1$ | 16 | 26·05 | 7·43 | 71·0 | 90° | Laths, [010], {001} | $a=\alpha$, $b=\beta$, $c=\gamma$, 2*E* large | *a* 414 |
| Cholestane-5-ol-3-6-dione | H. | $P2_1$ | 2 | 8·32 | 7·7 | 20·1 | 91° | Laths, [010], {10$\bar{1}$} or leaf-shaped twins on {001} | +, $b=\beta$, $\gamma$ nearly ⊥ to (001), 2 V = 88° | *a* 211 |
| (*e*) Ethers: | | | | | | | | | |
| Dicholesteryl ether | M. | *A* 2 | 4 | 11·3 | 6·23 | 76·3 | 110° | Hair-like needles, [010], {001}, {101} | +, $b=\alpha$, $\gamma$ 50° from (001) | *ab* (21)2 |
| Cholesteryl methyl ether | M. | $P2_1$ | 2 | 11·65 | 7·58 | 22·2 | 138° | Laths, [010], {001}, {10$\bar{1}$}, {10$\bar{2}$} | $b=\beta$, $\gamma$ 44° from (001), 2 V *c.* 90° | *a* 211 |
| *i*-Cholesteryl methyl ether | M. | $P2_1$ | 2 | 10·9 | 7·67 | 22·2 | 136°(5) | Laths, [010], {001}, {10$\bar{1}$} (?), {10$\bar{2}$} | +, $b=\beta$, $\gamma$ 50° from (001), 2 V = 73° | *a* 211 |
| Cholesteryl ethyl ether | M. | $P2_1$ | 2 | 12·9 | 9·33 | 24·7 | 152° | Laths, [010], {001}, {100}, {40$\bar{7}$}, {20$\bar{5}$} | +, $b=\alpha$, $\gamma$ 36° from (001), 2 V = 72° | *b* 211 |
| *i*-Cholesteryl ethyl ether | M. | $P2_12_12_1$ | 4 | 8·23 | 7·58 | 43·3 | 90° | Laths, [010], {001} | +, $a=\alpha$, $b=\beta$, $c=\gamma$, 2 V = 70° | *a* 212 |
| (*f*) Monohalogen derivatives: | | | | | | | | | |
| α-Chlorcholestane | H. | $P2_1$ | 2 | 10·4 | 7·7 | 19·9 | 128° | Laths, [010], {001}, {10*l*}, {11*l*} | $b=\beta$, $\gamma$ inclined to (001), 2 V = 90° | *a* 211 |
| β-Chlorcholestane | H. | $P2_1$ | 2 | 11·4 | 7·8 | 20·2 | 134° | Laths, [010], {10$\bar{1}$}, frequently twinned on {100} | $b=\beta$, $\gamma$ inclined to (001) | *a* 211 |
| Cholesteryl chloride | H.M. | $P2_1$ | 2 | 10·6 | 7·55 | 21·7 | 132°(6) | Laths, [010], {001}, {10$\bar{1}$}, {10$\bar{2}$}, {11*l*} | +, $b=\beta$, $\gamma$ 53° from (001), 2 V = 86° | *a* 211 |
| Cholesteryl bromide | H.M. | $P2_1$ | 2 | 11·0 | 7·55 | 21·6 | 134° | Laths, [010], {001}, {10$\bar{2}$}, {11*l*} | +, $b=\beta$, $\gamma$ 47° from (001), 2 V = 87° | *a* 211 |
| Cholesteryl iodide | M. | $P2_1$ | 2 | 11·0 | 10·42 | 21·8 | 149° | Laths, [010], {001}, {100}, {10$\bar{2}$} | +, $b=\alpha$, $\gamma$ 37° from (001) | *b* 211 |
| Cholestene hydrochloride | H. | $P2_12_12_1$ | 4 | 14·6 | 8·9 | 19·2 | 90° | Prisms, [010], {101}, {011}, {11$\bar{1}$}, {210}, rarely {111}, {110}(7) | +, $a=\alpha$, $b=\beta$, $c=\gamma$, $2E=c.$ 50° | *b* 221 |

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TABLE 1 (*continued*)

| Compound | Source | Space group | $n$ | $a$ | $b$ | $c$ | $\beta$ | Morphology | Optics | Structure type |
|---|---|---|---|---|---|---|---|---|---|---|
| (*g*) Polyhalogen derivatives: | | | | | | | | | | |
| Cholesteryl chloride hydrochloride | H. | $P2_1$ | 2 | 10·3 | 7·57 | 21·01 | 128° | Laths, [010], {001} | +, $b=\beta$, $\gamma$ much inclined to (001), 2 V = 56° | *a* 211 |
| Dichlorcholestane-3-6 ? | G. | $P2_1$ | 2 | 11·24 | 7·60 | 18·75 | 128° | Plates, {001} | +, $b=\beta$, $\gamma$ 45° from (001), 2 V = 27° | *a* 211 |
| $\alpha$-Cholestene dibromide | H. | $P2_12_12_1$ | 4 | 11·27 | 10·6 | 20·8 | 90° | Prisms or plates, [010], {001}, {101}, {102}, {011} (8) | $a=\alpha$, $b=\beta$, $c=\gamma$, $2E=45°$ | *ab* (22)1 |
| $\beta$-Cholestene dibromide | H. | $P1$ | 2 | 10·5 | 7·7 | 20·4 | 127½° | Laths, [010], {001}, {11*l*}, {01*l*}, {10*l*}. Twins | $\alpha$ about 4° from (010), $\gamma$ nearly $\perp$ to (001) | *a* 211 |
| Coprostene dibromide | H. | $P2_12_12_1$ | 4 | 11·6 | 6·5 | 35·5 | 90° | Prisms, [010], {001}, {101}, {102}, {104}, {112}? | +, $a=\alpha$, $b=\beta$, $c=\gamma$, $2E$ medium | *a* 212 |
| Dibromocholesteryl chloride | H. | $P2_12_12_1$ | 4 | 8·07 | 7·7 | 42·8 | 90° | Prisms, [010], {001}, {102}, also {112} | +, $b=\alpha$, $a=\beta$, $c=\gamma$, $2E$ small | *a* 212 |
| Dibromocholesteryl bromide | H.M. | $P2_12_12_1$ | 4 | 12·0 | 12·3 | 18·25 | 90° | Stout prisms, [010], {101} | $a=\alpha$, $b=\beta$, $c=\gamma$ | *ab* (22)1 |
| Dibromocholesterol | H. | $P22_12_1$ | 4 | 10·32 | 7·52 | 33·5 | 90° | Laths, [010], {001}, {010}, {100} | +, $a=\beta$, $b=\alpha$, $c=\gamma$, $2E$ large | *a* 212 |

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TABLE 2. THE ERGOSTEROL SERIES

| Compound | Source | Space group | $n$ | $a$ | $b$ | $c$ | $\beta$ | Morphology | Optics | Structure type |
|---|---|---|---|---|---|---|---|---|---|---|
| (*a*) Hydrocarbons: | | | | | | | | | | |
| α-Ergostene | H. | $P2_12_12_1$ | 4 | 10·97 | 6·44 | 35·2 | 90° | Laths, [010], {001}, {100}, {010} | +, $a=\alpha$, $b=\beta$, $c=\gamma$, $2E$ medium | *a* 212 |
| Ergotetraene | H. | $P2_1$ | 2 | 7·73 | 9·67 | 16·13 | 97° | Laths, [010], {001}, {100} | +, $b=\alpha$, $\gamma$ 74° to (001), $2E$ small | *b* 211 |
| (*b*) Monohydroxy compounds: | | | | | | | | | | |
| α-Ergostenol | H. | $P2_1$ | 4 | 12·1 | 6·1 | 35·6 | 93° | Needles, [010], {001}, {101}, {10$\bar{1}$}, {11*l*} | +, $b=\alpha$, $\gamma$ nearly ⊥ to (001), $2E$ small | *ab* (21)2 |
| β-Ergostenol | H. | $P2_1$ | 4 | 10·3 | 7·35 | 38·0 | 91° | Plates, [001], {01*l*}, {11*l*} | +, $b=\beta$, $\gamma$ nearly ⊥ to (001) | *a* 212 |
| Ergosterol (anhydrous) | H. | C2 | 32 | 52·7 | 12·56 | 32·8 | 101° | Sheets from melt, {001} | +, $b=\beta$, $\gamma$ inclined to (001), $2E$ small | *ab* (82)2 |
| Ergosterol $H_2O$ | H. | $P2_1$ | 4 | 9·92 | 7·57 | 38·5 | 115° | Laths, [010], {001}, {100}, {10$\bar{2}$}, {10$\bar{4}$} | +, $b=\beta$, $\gamma$ 62° from (001) | *a* 212 |
| Dehydroergosterol $H_2O$ | H. | $A2$ | 8 | 9·5 | 7·5 | 75·2 | 96° | Laths, [010], {001} | $b=\beta$, $\gamma$ ? inclined to (001) | *a* 214 |
| Neoergosterol | G.C. | $P2_12_12_1$ | 12 | 15·2 | 7·45 | 67·5 | 90° | Laths, [010], {001}, {010}, {100} | +, $a=\alpha$, $b=\beta$, $c=\gamma$, $2E$ medium | *a* 314 |
| (*c*) Polyhydroxy and keto derivatives, etc.: | | | | | | | | | | |
| Ergosterol peroxide | H. | $P2_1$ | 4 | 14·8 | 6·15 | 36·7 | 127½° | Silky needles, [010], {001}, {201}, {11*l*} | +, nearly uniaxial, $b=\beta$, $\gamma$ 50° from (001) | *ab* (21)2 |
| α-Ergostadienetriol | H. | C2 | 4 | 15·4 | 7·35 | 35·0 | 137° | Plates {001}, {11*l*}, {10*l*}, also laths [010], {001} | +, $b=\beta$, $\gamma$ 44° from (001) | *a* 212 |
| β-Ergostadienetriol I $2H_2O$ (leaflet) | H. | $P2_1$ | 2 | 6·74 | 7·16 | 33·0 | 119° | Plates, [010], {001}, {11*l*}, {10*l*} | +, $b=\beta$, $\gamma$ 70° from (001), 2 V=72° | *a* 112 |
| β-Ergostadienetriol II (needle) | H. | C2 | 8 | 23·7 | 6·3 | 36·3 | 91° | Laths, [010], {001} | +, nearly uniaxial, $b=\beta$, $\gamma$ ⊥ to (001) | *a* 412 |
| (*d*) Maleic anhydride adducts: | | | | | | | | | | |
| Ergosterol acetate maleic anhydride adduct I | G.H. | $A2$ | 4 | 10·5 | 9·5 | 33·2 | 112°(9) | Prisms, [010], or plates {001}, rich in faces, including {100}, {101}, {10$\bar{2}$} | +, $b=\beta$, $\gamma$ 66° from (001) | *a* 212 |
| Ergosterol acetate, maleic anhydride, adduct dibromide | H. | $P2_1$ | 2 | 10·75 | 7·5 | 38·2 | 149° | Laths, [010], {10$\bar{2}$}, {001} | +, $b=\beta$, $\gamma$ 25° from (001) | *a* 112 |

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TABLE 3. PHOTODERIVATIVES OF ERGOSTEROL

| Compound | Source | Space group | $n$ | $a$ | $b$ | $c$ | $\beta$ | Morphology | Optics | Structure type |
|---|---|---|---|---|---|---|---|---|---|---|
| (*a*) Lumisterol group: | | | | | | | | | | |
| Lumisterol | G., H., M. | $P2_1$ | 4 | 20·3 | 7·25 | 38·4 | 152½° | Needles, [010], {001}, {10$\bar{1}$}, {10$\bar{2}$} | +?, $b=\beta$, $\gamma$ 31° from (001), 2$E$ small | $a$ 212 |
| Lumisterol acetate | M. | $P2_1$ | 4 | 21·40 | 7·33 | 17·44 | 99° 18′ | Needles, [010], {001}, {100}, also {10$\bar{1}$}, {$\bar{2}$01} | +, $b=\beta$, $\gamma$ 66° from (001), 2 V = 72° | $a$ 411 |
| Dihydrolumisterol acetate | M. | $P2_12_12_1$ | 4 | 8·93 | 7·51 | 41·2 | 90° | Laths, [010], {001}, {10$l$}, {11$l$} | $a=\alpha$, $b=\beta$, $c=\gamma$, 2 V $c$. 90° | $a$ 212 |
| Tetrahydrolumisterol acetate | M. | $C2$ | 4 | 11·3 | 7·58 | 37·4 | 119° | Rhomb-shaped plates, {001}, {11$l$} | $b=\beta$, $\gamma$ 61° from (001), 2 V $c$. 90° | $a$ 212 |
| Hexahydrolumisterol acetate I | M. | $P2_1$ | 2 | 15·0 | 6·81 | 24·7 | 146° | Needles, [010], {001}, {10$\bar{1}$}, {10$\bar{2}$} | +, $b=\beta$, $\gamma$ 43° to (001), 2 V = 27° | $a$ 211 |
| Hexahydrolumisterol acetate II | M. | $P2_1$ | 2 | 14·2 | 7·58 | 24·1 | 147° | Needles or laths, [010], {001}, {10$\bar{2}$} | +, $b=\beta$, $\gamma$ 40° to (001), 2 V = 50° | $a$ 211 |
| (*b*) Calciferol group: | | | | | | | | | | |
| Calciferol | H. | $P2_1$ | 8 | 20·5 | 7·2 | 35·6 | 102° | Laths, [010], {001}, {01$l$}, {10$l$} | +, $b=\beta$, $\gamma$ nearly ⊥ to (001) | $a$ 412 |
| Pyrocalciferol-calciferol | H. | $C2$ | 8 | 20·2 | 7·35 | 35·4 | 100° | Laths, [010], {001} | +, $b=\beta$, $\gamma$ nearly ⊥ to (001) | $a$ 412 |
| Pyrocalciferol | H. | $P2_1$ | 4 | 18·2 | 7·15 | 20·5 | 92° | Laths, [010], {001}, {01$l$}, {10$l$}, frequently twinned | $b=\alpha$, $a=\beta$, $c=\gamma$ | $a$ 411 |
| Dihydrocalciferol | H. | $P2_1$ | 4 | 11·5 | 7·26 | 36·4 | 117° | Laths, [010], {001} | +, $b=\beta$, $\gamma$ 64–60° from (001) | $a$ 212 |
| Perhydroepidihydro pyrocalciferol | G. | $P1$ | 4 | 11·4 | 7·2 | 37·6 | $\beta$ 93° $\gamma$ 73° | Plates, [010], {001}, {10$l$}, {01$l$} | +, $\beta$ $c$. 8° from [010], $\gamma$ inclined to (001) | $a$ 212 |
| Calciferol $m$-dinitro benzoate | H. | $P2_1$ | 2 | 13·75 | 10·25 | 12·55 | 112° | Yellow plates, [010], {001} | −, $b=\beta$, $\gamma$ nearly ⊥ to (001) | — |
| Pyrocalciferol $m$-dinitro benzoate | H. | $P2_1$ | 2 | 11·0 | 11·25 | 26·2 | 150° | Orange plates, [010], {001} | +, $b=\beta$, $\gamma$ 31° from (001) | — |
| (*c*) Suprasterol group: | | | | | | | | | | |
| Suprasterol I | G. | $C2$ | 8 | 25·0 | 7·5 | 34·8 | 129° | Laths, [010], {001} | ? normal | $a$ 412 |
| Suprasterol II | G. | $P22_12_1$ | 8 | 13·4 | 10·4 | 35·4 | 90° | Prisms, [010], {001} | +, $a=\alpha$, $b=\beta$, $c=\gamma$, 2$E$ small | $b$ 222 |

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TABLE 4. HIGHER PLANT AND ANIMAL STEROIDS

| Compound | Source | Space group | $n$ | $a$ | $b$ | $c$ | $\beta$ | Morphology | Optics | Structure type |
|---|---|---|---|---|---|---|---|---|---|---|
| (*a*) Monohydroxy compounds: | | | | | | | | | | |
| $\gamma$-Sitostanol (and $\alpha\beta$-sitostanol?) $2H_2O$ | A. | $P1$ | 4 | 9·94 | 7·74 | 37·4 | $\alpha$ 93° $\beta$ 97° $\gamma$ *c.* 90° | Plates, [010], {001}, {10*l*}, {11*l*} | +, $b=\beta$, $\gamma$ 81° from (001) | *a* 212 |
| $\gamma$-Sitosterol $H_2O$ | A. | $A2$ | 16 | 20·44 | 7·58 | 82·5 | 120° | Laths, [010], {001}, {100}, {120}(10) | +, $b=\beta$, $\gamma$ 65° from (001), $2E$ large | *a* 414 |
| $\beta$-Sitosterol $H_2O$ (from rubber) | R. | $P2_1$ | 4 | 10·33 | 7·55 | 41·3 | 122° | Rhomb-shaped plates, {001}, {11*l*} | +, $b=\beta$, $\gamma$ inclined to (001) | *a* 212 |
| Stigmasterol $H_2O$ | H. | $P2_1$ | 4 | 9·51 | 7·59 | 37·2 | 100° | Rhomb-shaped plates, {001}, {11*l*}, {201} | +, $b=\beta$, $\gamma$ 78° from (001) | *a* 212 |
| Actiniasterol VI (lugworm sterol) | M. | $A2$ | 8 | 10·12 | 7·58 | 82·0 | 121° | Plates, {001}, {11*l*}, or {10*l*}, {11*l*} | +, $b=\beta$, $\gamma$ inclined to (001) | *a* 214 |
| Cerevisterol I | S. | $A2$ | 16 | 9·8 | 7·6 | 148·0 | 93° | Laths, [010], {001}, {10*l*}, {11*l*} | +, $b=\beta$, $\gamma$ nearly $\perp$ to (001) | *a* 218 |
| Cerevisterol II | S. | $P2_1$ | 4 | 10·6 | 7·45 | 39·0 | 120° | Laths, [010], {001}, {10*l*}, {11*l*} | +, $b=\beta$, $\gamma$ 58° from (001) | *a* 212 |
| Ostreasterol | S. | $A2$ | 8 | 10·1 | 7·65 | 78·6 | 117° | Irregular plates, {001} | $b=\beta$, $\gamma$ inclined to (001) | *a* 214 |
| Brassicasterol | S. | — | — | 9·6 | 7·7 | 37 ($d_{001}$) | — | Very thin irregular plates giving very few X-ray reflexions | $b=\beta$, $\gamma$ inclined to (001) | *a* 212? |
| $\beta$-Dihydrofucosterol | M. | $P2_1$ | 16 | 38·5 | 7·62 | 36·0 | 94° | Laths, [010], {001}, {10*l*}, {41*l*} | $b=\beta$, $\gamma$ *c.* 80° from (001) | *a* 812 |
| Spinastanol $2H_2O$ (?) | L. | $P1$ | 4 | 10·00 | 7·74 | 37·5 | $\alpha$ *c.* 90° $\beta$ 97° $\gamma$ 88° | Laths, [010], {001}, twins, $\rho=1{\cdot}031$ | $\beta'$ 7° from [010], $\gamma$ inclined to (001) | *a* 212 |
| $\alpha$-Spinastenol $2H_2O$ (?) | L. | $P1$ | 4 | 10·4 | 7·35 | 37·4 | $\alpha$ 96° $\beta$ 93° $\gamma$ 83° | Plates, {001}, {2$\bar{1}l$}, {10*l*}, {01*l*}, $\rho=1{\cdot}032$ | +, $b=\beta$, $\gamma$ nearly normal to (001), 2 V = 70° | *a* 212 |
| $\beta$-Spinastenol $H_2O$ | L. | $P2_12_12_1$ | 8 | 10·30 | 7·28 | 74·3 | 90° | Prisms, [010], {001}, {10*l*}, {11*l*}, $\rho=1{\cdot}030$ | −, $a=\alpha$, $b=\beta$, $c=\gamma$, 2 V = 70° | *a* 214 |

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TABLE 4 (*continued*)

| Compound | Source | Space group | $n$ | $a$ | $b$ | $c$ | $\beta$ | Morphology | Optics | Structure type |
|---|---|---|---|---|---|---|---|---|---|---|
| (*b*) Acetates: | | | | | | | | | | |
| $\beta$-Sitosterol acetate | R. | $P2_1$ | 4 | 10·43 | 7·63 | 42·1 | 120° | Laths, [010], {001}, {10*l*}, {11*l*}, $\rho=1{\cdot}049$ | +, $b=\beta$, $\gamma$ inclined to (001) | *a* 212 |
| Actineasterol acetate | M. | $P2_1$ | 4 | 16·55 | 9·56 | 17·8 | 106° | Laths, [010], {001} | $b=\alpha$, $\gamma$ 51° from (001) | *b* 221 |
| Spinastanol acetate | L. | $A2$ | 8 | 10·20 | 7·72 | 80·25 | 110° | Laths, [010], {001}, {10*l*}, {11*l*}, twins badly, $\rho=1{\cdot}37$ | +, $b=\beta$, $\gamma$ 74° from (001), 2 V = 60° | *a* 214 |
| $\alpha$-Spinasterol acetate | L. | $C2$ | 4 | 10·75 | 7·69 | 40·2 | 119° | Plates, {001}, {11*l*}, {01*l*}, $\rho=1{\cdot}057$ | +?, $b=\beta$, $\gamma$ inclined to (001) | *a* 212 |
| $\gamma$-Spinasterol acetate | L. | $P2_12_12_1$ | 4 | 13·28 | 6·95 | 31·0 | 90° | Needles, [010], {201}, $\rho=1{\cdot}064$ | +, $a=\alpha$, $b=\beta$, $c=\gamma$, 2 V = 72° | *a* 212 |
| (*c*) Other esters: | | | | | | | | | | |
| $\beta$-Spinastenol *p*-nitro-benzoate | L. | $P2_12_12_1$ | 8 | 12·2 | 7·38 | 76·8 | 90° | Laths, [010], {001}, {10*l*}, {11*l*}, $\rho=1{\cdot}114$ | +, $b=\alpha$, $a=\beta$, $c=\gamma$ | — |
| $\gamma$-Spinasterol gluco-side tetra-acetate | L. | $P2_12_12_1$ | 4 | 13·1 | 6·12 | 55·9 | 90° | Needles [010] | +, $a=\alpha$, $b=\beta$, $c=\gamma$, 2 V = 48° | — |

## Notes to Tables 1–4

(1) *Source*. The sterol preparations used came to us from a number of different research workers and research laboratories. These are indicated as follows:

H. Dr O. Rosenheim, Dr H. King, Dr R. K. Callow, National Institute for Medical Research, Hampstead, London.
G. Professor A. Windaus, University Laboratory, Göttingen.
B. Dr T. V. Barker, Department of Mineralogy, Oxford (cholesterol from brain).
M. Professor I. Heilbron and Dr F. S. Spring, Manchester University.
C. Professor A. H. Cook, Cancer Hospital, London.
R. Dr K. C. Roberts, Kuala Lumpur, Malay States.
A. Dr R. J. Anderson (given to Dr Rosenheim).
S. Dr R. Schönheimer, Columbia Medical School, New York City (given to Dr J. Needham).
L. Dr C. D. Larsen, School of Medicine and Dentistry, Rochester, New York.

(2) *Morphology*. The crystals are classified first according to habit, e.g. as plates, prisms, needles, laths (platy crystals markedly elongated in one direction), and this description is followed by a zone symbol to show direction of elongation, if any, e.g. [010], and then a form symbol, e.g. {001}, to indicate the dominating face. Any other forms observed are then noted. Very commonly these were only measured as edges bounding the main face and the indexing is then recorded in an incomplete form, e.g. {10*l*}.

(3) *Optics*. The crystals were all biaxial. The sign of the birefringence is first recorded followed by the directions of the principal refractive indices with reference to the crystal axes. In some cases the optic axial angle was measured by immersion in glycerine and this is then recorded as 2 V.

(4) *Structure type*. This column refers to the crystallographic classification of table 5.

## References to Tables 1–4

(1) Cf. Steinmetz, *Ber. dtsch. chem. Ges.* **59**, 2064 (1926); Steinmetz' $c=b$ above.
(2) Cf. A. Pelikan, *S.B. Akad. Wiss. Wien*, **104**, **116**, 824; *Z. Kristallogr.* **29**, 303; Pelikan's $c=b$ above.
(3) F. M. Jaeger, *Z. Kristallogr.* **44**, 568 (1908); Groth III, 536, axes changed, Jaeger's $c=a$. Jaeger's $(001)=(10\bar{1})$.
(4) Cf. F. Klötzer, *Z. Kristallogr.* A, **95**, 338 (1936); axes changed.
(5) Measurements partly carried out by F. Bell.
(6) Cf. J. D. Bernal and D. Crowfoot, *Trans. Faraday Soc.* **29**, 1032 (1933).
(7) Cf. Becke and Karny, *S.B. Akad. Wiss. Wien*, **116**, II*b*, 1019 (1907); *Z. Kristallogr.* **47**, 697 (1910), axes changed, Becke and Karny's $c=b$ above.
(8) Cf. A. Pelikan, *S.B. Akad. Wiss. Wien*, **103**, 27 (1894); *Z. Kristallogr.* **26**, 619 (1896). Pelikan's $b=c$ above; also X-ray measurements, G. E. R. Schulze, *Z. Phys. Chem.* A, **171**, 436 (1935). Schulze's $b=a$, $a=c$, above.
(9) Cf. G. E. R. Schulze, *loc. cit.*, Schulze's $a=c$ above.
(10) Cf. Mugge, *Z. Untersuch. Nahr.- u. Genussm.* **1**, 45 (1898).


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The position of the molecules in the unit cell given by the Patterson synthesis is closely that deduced from the preliminary data, 'smear' lines and, most important, the optic orientation. $\gamma$, the greatest refractive index, is along the ridge which indicates

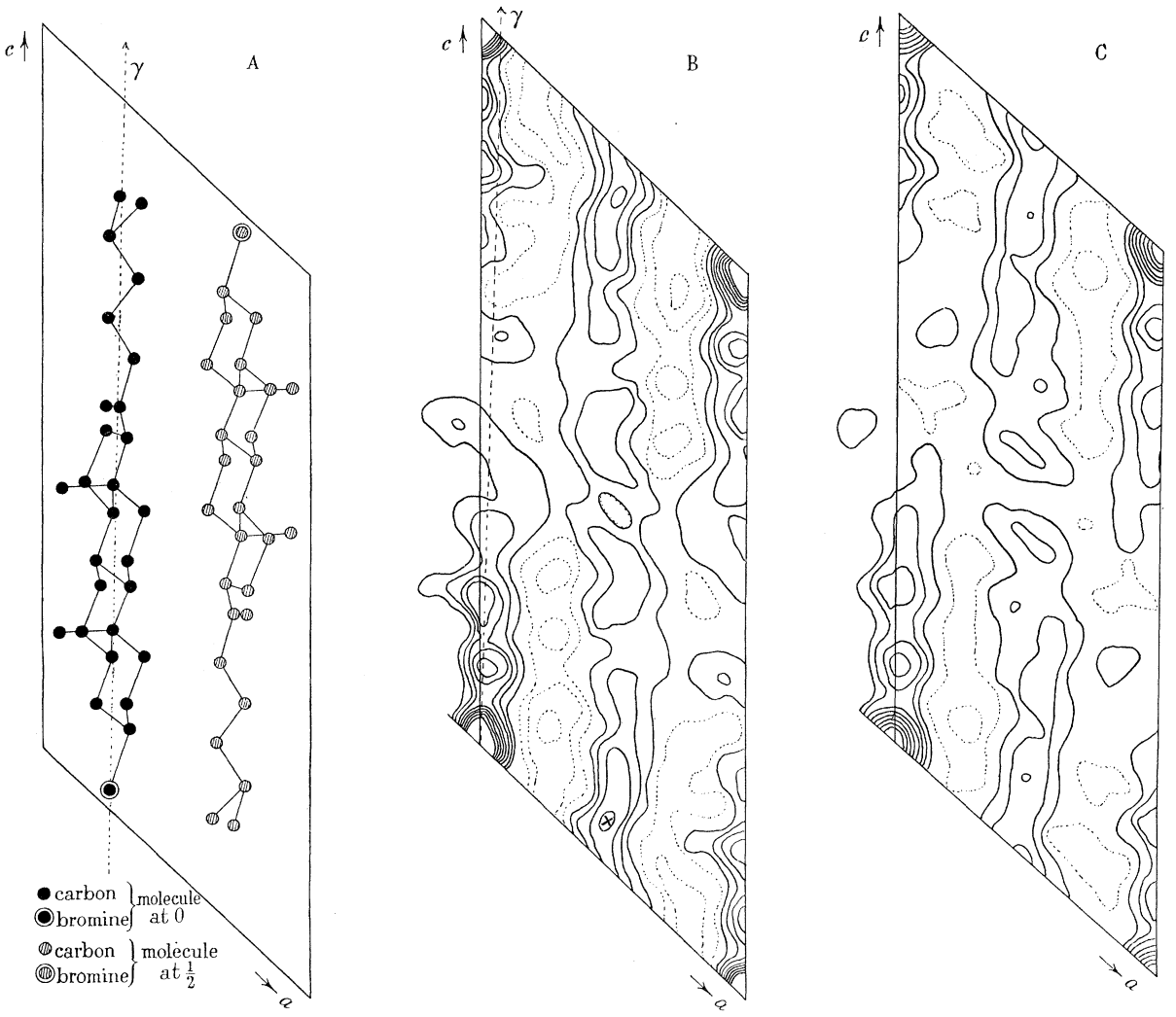



FIGURE 1. Cholesteryl bromide and chloride. (A) Suggested crystal structure of cholesteryl bromide projected on (010). The $z$ parameter of the two molecules is given by a correlation of the bromine-bromine distance with peak $x$ in the Patterson projection (B). (B) Patterson projection, *Pxz*, for cholesteryl bromide. (C) Patterson projection, *Pxz*, for cholesteryl chloride. The contours in these and in figure 8 are plotted at 100 units apart, those in negative areas being dotted. Contours at the origin above 700 are omitted.

the direction of the long axis of the molecule, close to $c$, $\alpha$ approximately at right angles to the plane of the ring system in the $b$ plane, $\beta$ normal to the $b$ plane, evidently nearly in the plane of the ring system. Crudely, the dimensions of the molecules may be given as about 21 A (length), 7·7 A (width) and 4 A (thickness), similar to those originally derived for quite different sterol crystals (Bernal 1933). The principal

remaining inaccuracy in this description is probably due to the indeterminacy of the exact slope of the plane of the ring system to the *b* plane. But preliminary calculations on intensities suggest this slope to be about 20–25°, which does not greatly affect the rough estimate of molecular size and shape.

In any one crystal structure the molecular dimensions deduced from the combined cell dimensions and optic orientation will not necessarily present the rigid picture suggested above owing to the packing of the individual atoms in the actual molecular arrangement adopted. It does seem true, however, that no very grave changes in the molecular configuration as a whole—such as the curling up of the side chain, for example—do occur in the course of crystallization. In all the sterol crystal structures examined so far it has been found possible, using as guide the optic orientation, to fit molecules corresponding in outline with those required by the present chemical formula. And, for the purposes of a discussion of the varieties of crystal structure found, it is a great advantage to picture the sterol molecule as constructed approximately on rectangular co-ordinates, having a definite length, width and thickness. The variation of crystal structures found can then be expressed by the changing orientation of the molecular axes to the crystal axes and the combination of these orientations within the crystallographic space groups.

## 2. An attempt at a classification of sterol crystal structures

To assist in tracing the chemical implications of the different crystal structures actually observed it is essential to bring these into some kind of geometrical classification. One type may be based on the relation between the crystallographic axes and symmetry and the orientations of the sterol molecules treated simply as lath-shaped objects, groups within the classification being described by a nomenclature indicating directly the relation of the molecular axes, thickness, width and length, to the crystal axes *a*, *b*, and *c*. Such a classification may evidently be extended to lath-shaped molecules in general, though any scheme suggested now cannot avoid being somewhat arbitrary; partly because the relations between the different variables are not exactly known and, in any case, need several dimensions in which to express them, and partly because intermediate structure types may exist which are not shown by our limited experimental evidence. Certain relations between the directions of the crystallographic axes and the probable molecular orientation do, however, seem to be particularly common among the actual structures observed, and these have therefore been chosen as a basis for the crystallographic classification.

The most usual type of sterol crystal among all those examined is still that first described by Bernal, which we have come to speak of as the 'normal' type. These crystals are monoclinic plates tending to be elongated along the *b* axis which is the direction of the $\beta$ refractive index, $\gamma$ being inclined at varying angles to the plane of the plate (001). A study of the unit-cell dimensions of this type of crystal shows that

these are roughly multiples of $4\frac{1}{2}$ A, the molecular thickness, $a \sin \beta$; 7 A, the width $b$; and 20 A, the length $c$. Crystals showing these characteristics can therefore be classified as belonging to a class by themselves, which has as unit-cell dimensions $a \sin \beta = n_t \times$ thickness, $b = n_w \times$ width, $c = n_1 \times$ length (figure 2). For purposes of classification the orientation of the molecular axes, thickness, width and length (taken in this order) can then be described by the symbols *abc* (also in order), and these symbols followed by numerals $n_t n_w n_1$ to represent the multiplicities. For example, the symbol *abc* 214 indicates a crystal structure in which, roughly, $a \sin \beta$ is a measure of twice the molecular thickness, $b$ of the width, and $c$ of four times the length. For convenience the symbol may be shortened to *a* 214. It will be realized that the product of the figures gives the number of molecules in the unit cell.

Opposed to this, the *a* class is a much smaller group of monoclinic crystals in which the symmetry axis is more nearly some multiple of 5 A and optically coincides with the direction of the $\alpha$ refractive index. Here the symmetry axis, $b$, evidently corresponds in direction more nearly with the molecular thickness and this may be considered the distinguishing character of a second type, the 'reversed' or *b* type for which the molecular dimensions are roughly given by thickness $= b/n_t$, width $= a \sin \beta / n_w$ and length $= c/n_1$ (figure 3). The symbol for classification is therefore *bac*, $n_t n_w n_1$ or shortly *b*, $n_t n_w n_1$.

As might be expected there does seem to be a gradual transition between these two groups, and a third small division appears of crystal structures which it is very difficult to classify as either one or the other. These have been separated into a group by themselves, *ab* or crossed. The length of the molecules is still along the *c* direction, but the plane of the ring system is probably nearly at 45° to the *a* and *b* axes (figure 4). The group symbol becomes $ab.c\ (n_t \times n_w).n_1$. It is possible for $n_t \times n_w$ to be compounded in different ways, *a* and *b* being either roughly equal to one another or one of them some multiple of the other. This can be indicated by splitting the figures within the bracket—e.g. *ab*(22)1 and *ab*(41)1 are two possible varieties in each of which $a \times b$ is equal to $4 \times$ molecular width $\times$ thickness. But in the first case *a* and *b* are roughly equal, in the second *a* is four times *b*.

The third variety of crystal structure which is geometrically compatible with monoclinic symmetry is that in which the molecular length is mainly along the *b* axis. Only one sterol derivative, cholestene, shows this orientation (with the possible very doubtful exception of coprostanol which is omitted from the scheme), but it is known in several compounds of the oestrin series (figure 5). If the nomenclature of the crystal axes is arranged so that the directions of the molecular thickness, width and length are given by the axes *c*, *a*, *b* respectively, the group symbol becomes *cab*, shortened to *c*, $n_t n_w n_1$.

Any attempt to include the orthorhombic or triclinic crystal structures in the above classification must to a certain degree be arbitrary, since in the orthorhombic system all three crystallographic axes are symmetry axes, in the triclinic none, and it is a

matter of choice as to which unit-cell dimension is named *a*, *b* or *c*. But the relation of certain orthorhombic and triclinic crystal structures to each of the above groups is very marked, and the attempt has therefore been made to introduce these into the classification following particularly the secondary consideration of crystal habit as a

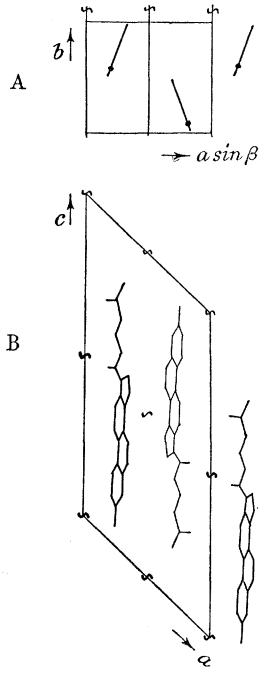



FIGURE 2

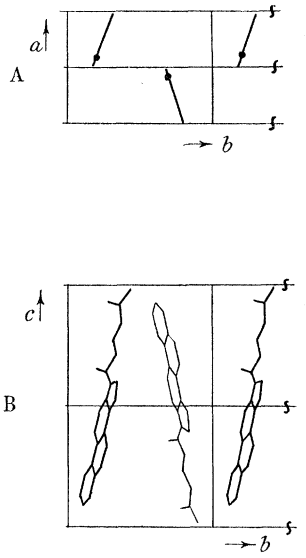



FIGURE 3

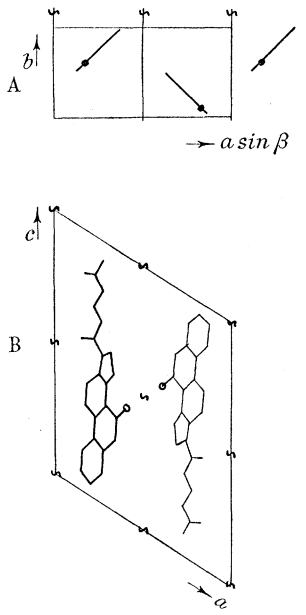
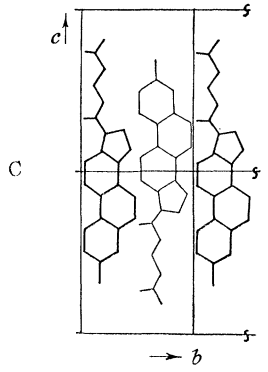
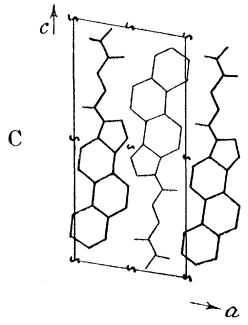



FIGURE 4



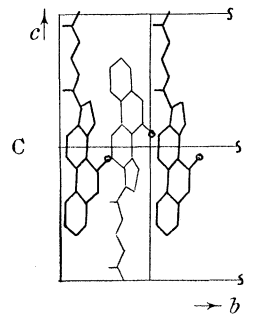




FIGURE 2. The probable arrangement of the molecules in cholesteryl bromide, structure type *a* 211. (A) Projection normal to [001]. (B) Projection on (010). (C) Projection on (100). – molecule at 0, – molecule at $\frac{1}{2}$, in this and in figures 3–6 and 9.

FIGURE 3. The probable arrangement of the molecules in ergotetraene, structure type *b* 211. (A) Projection on (001). (B) Projection on (100). (C) Projection on (010).

FIGURE 4. The probable arrangement of the molecules in $\Delta$-4-cholestene-7-ol, structure type *ab* (21)1. (A) Projection normal to [001]. (B) Projection on (010). (C) Projection on (100).

guide. In the *a* type structures, the crystals are markedly elongated in the *b* direction (width, $\beta$), in the *b* type also *b* (here the direction of the thickness, $\alpha$) is the needle axis. Orthorhombic or triclinic crystals showing elongation in the apparent direction of the width are therefore classified as *a* type, in the direction of the thickness, *b* type. The optic orientation in these crystals is often anomalous owing to the really considerable deviation of the molecular axes from the crystal axes. The *ab* or crossed type is accepted on the near equality of the *a* and *b* dimensions.

Inside each main division, *a*, *b*, *ab* and *c*, further subdivision is based first on the varying multiplicities of *n* and secondly on the space groups shown. Within each space group the structures are arranged approximately in order of increasing molecular slope to the *c* plane, so that horizontal relationships between compounds showing nearly equal angles of slope can easily be traced. Purposely, no formal numbering of the structure types is set out, since the varieties already discovered certainly do not exhaust the crystallographic possibilities. The symbols themselves fall into a reasonable sequence into which new structures can be inserted when found without disturbance of the general order.

The result of this classification is table 5. This includes not only the crystal structures listed in tables 1–4 but also for completeness a few whose measurements have been recorded elsewhere, particularly the compounds studied by Schulze, a group mainly of *i*-cholesterol derivatives measured by Miss F. Bell, and certain of the sex hormones.

There are in all 105 different crystal structures listed in table 5 and of these seventy-eight fall into the *a* group or normal type—which is some justification for the adoption of this title. Of the three remaining groups, nine structures are listed under *b* (reversed), fourteen under *ab* (crossed), and four under *c*. Within the *b*, *ab* and *c* series there are almost as many subdivisions as crystal structures named. But these subdivisions are worth stating since each represents a distinct type of structure which can be related to one or other of the subdivisions of the normal series.

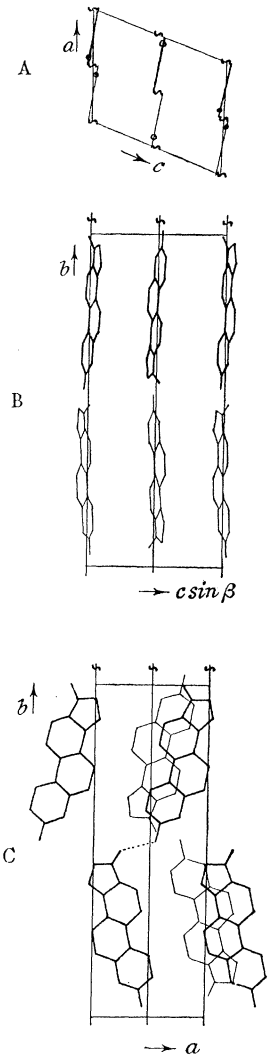



FIGURE 5. The probable arrangement of the molecules in oestrone 3, structure type *c* 212. (A) Projection on (010). (B) Projection normal to [100]. (C) Projection on (001). ···, hydrogen or hydroxyl bond (in this and succeeding figures).

Inside the normal series there are two structure types which occur most frequently, *a* 211 and *a* 212. *a* 211 (eighteen compounds) is one of the most homogeneous groups

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TABLE 5

| Structure type | Space group | $n$ | Compound | Thickness $a \sin\beta$ | Width $b$ | Length $c$ | $\beta$ | $a$ | $c \sin\beta$ | Ref.* |
|---|---|---|---|---|---|---|---|---|---|---|
| *abc* normal: | | | | | | | | | | |
| *a* 111 | $P1$ | 1 | Cholesteryl salicylate | (4·19) | (9·8) | 23·7 | 67·5° | 6·4 | — | H. |
| *a* 112 | $P2_1$ | 2 | $\beta$-Ergostadienetriol 1 | 5·85 | 7·16 | $2 \times 16{\cdot}5$ | 119° | 6·74 | 28·77 | II *c* |
| | | | Ergosterol maleic anhydride acetate dibromide | 5·54 | 7·5 | $2 \times 19{\cdot}1$ | 149° | 10·75 | 19·7 | II *d* |
| *a* 211 | $P1$ | 2 | $\beta$-Cholestene dibromide | $2 \times 4{\cdot}16$ | 7·7 | 20·4 | $127\frac{1}{2}$° | 10·5 | 16·2 | I *g* |
| | $P2_1$ | 2 | Cholestane-5-ol-3-6-dione | $2 \times 4{\cdot}16$ | 7·7 | 20·1 | 91° | 8·32 | 20·1 | I *d* |
| | | | Cholestane-3-6-dione | $2 \times 3{\cdot}95$ | 7·62 | 19·6 | 93° | 7·9 | 19·6 | S. |
| | | | Lactone from digoxigenin, $C_{23}H_{36}O_2$ | $2 \times 5{\cdot}2$ | 7·7 | 11·7 | 101° | 10·6 | 11·5 | G. |
| | | | Androsterone | $2 \times 4{\cdot}4$ | 7·7 | 11·95 | 111° | 9·44 | 11·2 | O. |
| | | | Cholesteryl chloride HCl | $2 \times 4{\cdot}05$ | 7·57 | 21·01 | 128° | 10·3 | 16·6 | I *g* |
| | | | Cholesteryl chloride | $2 \times 3{\cdot}95$ | 7·55 | 21·7 | 132° | 10·6 | 16·1 | I *f* |
| | | | $\alpha$-Chlorcholestane | $2 \times 4{\cdot}1$ | 7·7 | 19·9 | 128° | 10·4 | 15·7 | I *f* |
| | | | Cholesteryl bromide | $2 \times 3{\cdot}96$ | 7·55 | 21·6 | 134° | 11·0 | 15·5 | I *f* |
| | | | Coprostene | $2 \times 4{\cdot}02$ | 7·67 | 20·1 | 133° | 11·05 | 14·7 | I *a* |
| | | | *i*-Cholesteryl methyl ether | $2 \times 3{\cdot}80$ | 7·67 | 22·2 | 136° | 10·9 | 15·4 | I *e* |
| | | | 'Oxycholestenone dibromide' | $2 \times 4{\cdot}52$ | 7·58 | 19·6 | $128\frac{1}{2}$° | 11·54 | 15·35 | S. |
| | | | Cholesteryl methyl ether | $2 \times 3{\cdot}90$ | 7·58 | 22·2 | 138° | 11·65 | 14·9 | I *e* |
| | | | Dichlorcholestane (3-6?) | $2 \times 4{\cdot}43$ | 7·60 | 18·75 | 128° | 11·24 | 14·8 | I *g* |
| | | | $\beta$-Chlorcholestane | $2 \times 4{\cdot}1$ | 7·8 | 20·2 | 134° | 11·4 | 14·6 | I *f* |
| | | | Cholestenone | $2 \times 3{\cdot}8$ | 7·86 | 19·98 | 135° | 10·61 | 14·1 | I *b* |
| | | | Hexahydrolumisterol acetate I | $2 \times 4{\cdot}19$ | 6·81 | 24·7 | 146° | 15·0 | 13·8 | III *a* |
| | | | Hexahydrolumisterol acetate II | $2 \times 3{\cdot}87$ | 7·58 | 24·1 | 147° | 14·2 | 13·1 | III *a* |
| *a* 212 | $P1$ | 4 | $\gamma$-Sitostanol $2H_2O$ | $2 \times 4{\cdot}94$ | 7·74 | $2 \times 18{\cdot}7$ | 97° | 9·94 | 37·1 | IV *a* |
| | | | Spinastanol $2H_2O$ | $2 \times 4{\cdot}96$ | 7·74 | $2 \times 18{\cdot}75$ | 97° | 10·00 | 37·2 | IV *a* |
| | | | Perhydro-epi-dihydro-pyro-calciferol | $2 \times 5{\cdot}7$ | 7·2 | $2 \times 18{\cdot}8$ | 93° | 11·4 | 37·6 | III *b* |
| | | | $\alpha$-Spinastenol $2H_2O$? | $2 \times 5{\cdot}2$ | 7·35 | $2 \times 18{\cdot}7$ | 93° | 10·4 | 37·4 | IV *a* |
| | | | Cholestanol $2H_2O$ | $2 \times 4{\cdot}7$ | 7·76 | $2 \times 18{\cdot}4$ | 106° | 9·79 | 35·42 | I *c* |
| | | | Epicholestanol | $2 \times 4{\cdot}90$ | 7·5 | $2 \times 16{\cdot}6$ | 98·5° | 9·93 | 32·8 | I *c* |

* The references in this column are to the entries in tables 1–4 and to work carried out by the following authors:

H. S. B. Hendricks, *Z. Kristallogr.* A, **89**, 427 (1934); axes changed; the classification of this compound here is however doubtful.

S. G. E. R. Schulze, *Z. Phys. Chem.* A, **171**, 436 (1935).

G. J. D. Bernal and D. Crowfoot, *Chem. Ind. Rev.* **12**, 953 (1934).

O. J. D. Bernal and D. Crowfoot, *Z. Kristallogr.* A, **93**, 464 (1936).

C. J. D. Bernal, *Nature, Lond.*, **129**, 277 (1932).

B. Miss F. O. Bell (unpublished).

G., K. G. Giacomello and O. Kratky, *Z. Kristallogr.* A, **95**, 459 (1936).

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TABLE 5 (*continued*)

| Structure type | Space group | $n$ | Compound | Thickness $a \sin\beta$ | Width $b$ | Length $c$ | $\beta$ | $a$ | $c \sin\beta$ | Ref.* |
|---|---|---|---|---|---|---|---|---|---|---|
| | $P2_1$ | 4 | $\beta$-Ergostenol? | $2\times5{\cdot}15$ | 7·35 | $2\times19{\cdot}0$ | 91° | 10·3 | 38·0 | II *b* |
| | | | Stigmasterol $H_2O$ | $2\times4{\cdot}68$ | 7·59 | $2\times18{\cdot}6$ | 100° | 9·51 | 36·64 | IV *a* |
| | | | Brassicasterol | — | 7·7 | — | — | 9·6 | 37·0 | IV *a* |
| | | | Ergosterol $H_2O$ | $2\times4{\cdot}49$ | 7·57 | $2\times19{\cdot}25$ | 115° | 9·92 | 34·82 | II *b* |
| | | | $\beta$-Sitosterol $H_2O$ (from rubber) | $2\times4{\cdot}33$ | 7·55 | $2\times20{\cdot}6$ | 122° | 10·33 | 35·0 | IV *a* |
| | | | $\beta$-Sitosterol acetate | $2\times4{\cdot}52$ | 7·63 | $2\times21{\cdot}05$ | 120° | 10·43 | 36·4 | IV *b* |
| | | | Ergosterol acetate | — | 7·58 | — | — | 10·48 | 34·8 | S. |
| | | | Cerevisterol II | $2\times4{\cdot}59$ | 7·45 | $2\times19{\cdot}5$ | 120° | 10·6 | 33·8 | IV *a* |
| | | | $\alpha$-Cholestanetriol 1 | $2\times4{\cdot}16$ | 7·5 | $2\times21{\cdot}9$ | 131° | 11·0 | 33·0 | I *d* |
| | | | Dihydrocalciferol | $2\times5{\cdot}12$ | 7·26 | $2\times18{\cdot}2$ | 117° | 11·50 | 32·4 | III *b* |
| | | | Lumisterol | $2\times4{\cdot}67$ | 7·25 | $2\times19{\cdot}2$ | 152½° | 20·3 | 17·7 | III *a* |
| | C2 | 4 | $\alpha$-Spinasterol acetate | $2\times4{\cdot}71$ | 7·69 | $2\times20{\cdot}1$ | 119° | 10·75 | 35·2 | IV *b* |
| | | | Tetrahydrolumisterol acetate | $2\times4{\cdot}94$ | 7·58 | $2\times18{\cdot}7$ | 119° | 11·30 | 32·6 | III *a* |
| | | | $\alpha$-Ergostadienetriol | $2\times5{\cdot}2$ | 7·35 | $2\times17{\cdot}5$ | 137° | 15·4 | 23·8 | II *c* |
| | *A*2 | 4 | Ergosterol acetate maleic anhydride adduct I | $2\times4{\cdot}85$ | 9·5 | $2\times16{\cdot}6$ | 112° | 10·5 | 31·0 | II *d* |
| | $P22_12_1$ | 4 | Dibromocholesterol | $2\times5{\cdot}16$ | 7·52 | $2\times16{\cdot}8$ | 90° | 10·32 | 33·5 | I *g* |
| | | | Coprostane | $2\times5{\cdot}42$ | 7·62 | $2\times15{\cdot}15$ | 90° | 10·85 | 30·3 | I *a* |
| | $P2_12_12_1$ | 4 | Dibromocholesteryl chloride | $2\times4{\cdot}03$ | 7·7 | $2\times21{\cdot}4$ | 90° | 8·07 | 42·8 | I *g* |
| | | | *i*-Cholesteryl EtOH | $2\times4{\cdot}10$ | 7·6 | $2\times21{\cdot}2$ | 90° | 8·20 | 42·4 | B. |
| | | | *i*-Cholesteryl ethyl ether | $2\times4{\cdot}11$ | 7·58 | $2\times21{\cdot}65$ | 90° | 8·23 | 43·3 | I *e* |
| | | | *i*-Cholesteryl acetate | $2\times4{\cdot}15$ | 7·6 | $2\times21{\cdot}5$ | 90° | 8·30 | 43·0 | B. |
| | | | Dihydrolumisterol acetate | $2\times4{\cdot}46$ | 7·51 | $2\times20{\cdot}6$ | 90° | 8·93 | 41·2 | III *a* |
| | | | Oestrone 2 | $2\times4{\cdot}95$ | 7·7 | $2\times\ 9{\cdot}1$ | 90° | 9·90 | 18·2 | O. |
| | | | *trans*-$\Delta$-5-6-Cholestene-3-4-diol | $2\times5{\cdot}15$ | 7·7 | $2\times15{\cdot}1$ | 90° | 10·3 | 30·2 | I *d* |
| | | | Nor-nor-cholanic acid ethyl ether | $2\times5{\cdot}02$ | 7·49 | $2\times14{\cdot}13$ | 90° | 10·04 | 28·27 | G. and K. |
| | | | Nor-cholanic acid ethyl ether | $2\times5{\cdot}08$ | 7·23 | $2\times14{\cdot}84$ | 90° | 10·16 | 29·69 | G. and K. |
| | | | Cholanic acid ethyl ether | $2\times5{\cdot}39$ | 7·49 | $2\times14{\cdot}53$ | 90° | 10·78 | 29·06 | G. and K. |
| | | | $\gamma$-Spinasterol acetate | $2\times6{\cdot}64$ | 6·95 | $2\times15{\cdot}5$ | 90° | 13·28 | 31·0 | IV *b* |
| | | | $\alpha$-Ergostene | $2\times5{\cdot}48$ | 6·44 | $2\times17{\cdot}6$ | 90° | 10·97 | 35·2 | II *a* |
| | | | Coprostene dibromide | $2\times5{\cdot}8$ | 6·5 | $2\times17{\cdot}75$ | 90° | 11·6 | 35·5 | I *g* |
| *a* 214 | *A*2 | 8 | Dehydroergosterol? | $2\times4{\cdot}75$ | 7·5 | $4\times18{\cdot}8$ | 96° | 9·5 | 75·0 | II *b* |
| | | | Spinastanol acetate | $2\times4{\cdot}79$ | 7·72 | $4\times20{\cdot}06$ | 110° | 10·2 | 75·3 | IV *b* |
| | | | Ostreasterol | $2\times4{\cdot}5$ | 7·65 | $4\times19{\cdot}65$ | 117° | 10·10 | 72·0 | IV *a* |
| | | | Actiniasterol VI (lugworm sterol) | $2\times4{\cdot}33$ | 7·58 | $4\times20{\cdot}5$ | 121° | 10·12 | 70·3 | IV *a* |
| | $P2_12_12_1$ | 8 | $\beta$-Spinastenol $H_2O$ | $2\times5{\cdot}15$ | 7·28 | $4\times18{\cdot}6$ | 90° | 10·30 | 74·3 | IV *a* |
| *a* 218 | *A*2 | 16 | Cerevisterol 1 | $2\times4{\cdot}9$ | 7·6 | $8\times18{\cdot}5$ | 93° | 9·8 | 148·0 | IV *a* |
| *a* 314 | $P2_12_12_1$ | 12 | Neoergosterol | $3\times5{\cdot}06$ | 7·45 | $4\times16{\cdot}9$ | 90° | 15·20 | 67·5 | II *b* |
| *a* 411 | $P2_1$ | 4 | Pyrocalciferol? | $4\times4{\cdot}55$ | 7·15 | 20·5 | 92° | 18·20 | 20·4 | III *b* |
| | | | Lumisterol acetate | $4\times5{\cdot}27$ | 7·33 | 17·44 | 99° 18′ | 21·40 | 17·2 | III *a* |
| | $P2_12_12_1$ | 4 | Oestrone 1 | $4\times4{\cdot}07$ | 7·46 | 12·15 | 90° | 16·28 | 12·15 | O. |
| | | | Cholesterylene | $4\times3{\cdot}97$ | 7·66 | 19·25 | 90° | 15·85 | 19·25 | I *a* |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *a* 412 | $P2_1$ | 8 | Ergosterol maleic anhydride acetate II (?) | 4 × 5·8 | 7·91 | 2 × 16·1 | 92° | 23·2 | 32·2 | S. |
| | | | Calciferol | 4 × 5·02 | 7·2 | 2 × 17·8 | 102° | 20·5 | 34·8 | III *b* |
| | C2 | 8 | Calciferol-pyrocalciferol | 4 × 4·97 | 7·35 | 2 × 17·7 | 100° | 20·2 | 34·9 | III *b* |
| | | | Suprasterol I | 4 × 4·85 | 7·5 | 2 × 17·40 | 129° | 25·0 | 27·0 | III *c* |
| | | | *β*-Ergostadienetriol II | 4 × 5·9 | 6·3 | 2 × 18·15 | 91° | 23·7 | 36·3 | II *c* |
| *a* 414 | *A*2 | 16 | *γ*-Sitosterol $H_2O$ | 4 × 4·5 | 7·58 | 4 × 20·6 | 120° | 20·44 | 71·4 | IV *a* |
| | $P2_12_12_1$ | 16 | *α*-Cholestanetriol II | 4 × 6·51 | 7·43 | 4 × 17·75 | 90° | 26·05 | 71·0 | I *d* |
| *a* 612 | C2 | 12 | Dihydroergosterol ½EtOH (?) | 6 × 4·37 | 7·4 | 2 × 20·4 | 121½° | 30·78 | 35·1 | C. |
| *a* 812 | $P2_1$ | 16 | *β*-Dihydrofucosterol | 8 × 4·8 | 7·62 | 2 × 18·0 | 94° | 38·5 | 35·9 | IV *a* |
| *a* 224 | $B2_1$? | 8 | 4-Hydroxycholestane | 2 × 4·64 | 2 × 7·7 | 4 × 18·5 | 102° | 9·5 | 72·3 | B. |
| *bac* reversed: | | | | | | | | | | |
| *b* 211 | $P2_1$ | 2 | Ergotetraene | 2 × 4·83 | 7·66 | 16·13 | 97° | 7·73 | 16·00 | II *a* |
| | | | Cholesteryl iodide | 2 × 5·21 | 5·66 | 21·8 | 149° | 11·0 | 11·3 | I *f* |
| | | | Cholesteryl ethyl ether | 2 × 4·66 | 6·07 | 24·7 | 152° | 12·9 | 11·5 | I *e* |
| *b* 221 | $P2_1$ | 4 | Cholesteryl acetate | 2 × 4·7 | 2 × 8·1 | 17·6 | 103° | 16·6 | 17·1 | B., S. |
| | | | Actiniasteryl acetate | 2 × 4·78 | 2 × 7·95 | 17·8 | 106° | 16·55 | 17·1 | IV *b* |
| | $P2_12_12_1$ | 4 | Bromomethoxy oestrone | 2 × 4·5 | 2 × 6·9 | 12·8 | 90° | 13·8 | 12·8 | O. |
| | | | Cholestene hydrochloride | 2 × 4·45 | 2 × 7·3 | 19·2 | 90° | 14·6 | 19·2 | I *f* |
| *b* 222 | *P*1 | 8 | Anhydrous cholesterol | 2 × 5·23 | 2 × 6·2 | 2 × 18·9 | 117½° | 14·0 | 33·5 | I *c* |
| | $P22_12_1$ | 8 | Suprasterol II | 2 × 5·20 | 2 × 6·7 | 2 × 17·7 | 90° | 13·4 | 35·4 | III *c* |
| (*ab*)*c* crossed: | | | | *a* sin *β* | *b* | *c* | *β* | *a* | *c* sin *β* | |
| *ab* (21)1 | $P2_1$ | 2 | *Δ*-4-Cholestene-7-ol | 2 × 5·83 | 6·15 | 17·75 | 123½° | 13·96 | 14·80 | I *c* |
| *ab* (21)2 | *P*1 | 4 | Cholesterol MeOH | 2 × 5·1 | 6·23 | 2 × 21·15 | 124° | 12·3 | 34·9 | I *c* |
| | $P2_1$ | 4 | *α*-Ergostenol | 2 × 6·05 | 6·1 | 2 × 17·8 | 93° | 12·1 | 35·6 | II *b* |
| | | | Pregnane | 2 × 5·9 | 6·29 | 2 × 11·3 | 100° | 12·0 | 22·4 | O. |
| | | | Ergosterol peroxide | 2 × 5·85 | 6·15 | 2 × 18·3 | 127½° | 14·8 | 29·1 | II *c* |
| | *A*2 | 4 | Dicholesteryl ether | 2 × 5·32 | 6·23 | 2 × 38·15 | 110° | 11·3 | 71·3 | I *e* |
| *ab* (22)1 | $P2_1$ | 4 | Cholestane | 10·85 | 11·0 | 19·8 | 104° | 11·2 | 19·25 | I *a* |
| | | | Testosterone | 12·05 | 11·08 | 11·01 | 125° | 14·72 | 9·05 | O. |
| | $P2_12_12_1$ | 4 | *α*-Cholestene dibromide | 11·27 | 10·6 | 20·8 | 90° | 11·27 | 20·8 | I *g* |
| | | | Dibromo-cholesteryl bromide | 12·0 | 12·30 | 18·25 | 90° | 12·0 | 18·25 | I *g* |
| *ab* (41)2 | C2 | 8 | *cis*-*Δ*-5-6-Cholestene-3-4-diol | 4 × 6·65 | 5·85 | 2 × 15·3 | 120° | 30·8 | 26·5 | I *d* |
| *ab* (8)2 | C2 | 16 | Cholestane-6-ol-EtOH | 14·8 | 19·7 | 2 × 16·35 | 106° | 15·4 | 31·4 | I *c* |
| *ab* (8)4 | C1 | 32 | Cholesterol $H_2O$ | 14·6 | 19·1 | 4 × 18·5 | 112½° | 15·8 | 68·4 | I *c* |
| *ab* (82)2 | C2 | 32 | Anhydrous ergosterol | 4 × 12·9 | 12·56 | 2 × 16·4 | 101° | 52·7 | 32·1 | II *b* |
| *cab*: | | | | *c* | *a* | *b* | *β* | | | |
| *c* 112 | $P2_1$ | 2 | Hydroxyketone from cholestanol (androstane-3-(*β*)-ol-17-one) | 6·3 | 6·62 | 2 × 11·05 | 109° | — | — | O. |
| *c* 212 | $P2_1$ | 4 | Oestrone 3 | 2 × 4·61 | 7·60 | 2 × 11·05 | 112° | — | — | O. |
| | | | Oestriol | 2 × 4·53 | 7·50 | 2 × 11·4 | 112° | — | — | O. |
| *c* 221 | $P2_1$ | 4 | Cholestene | 2 × 5·5 | 2 × 6·6 | 19·45 | 94° | — | — | I *a* |

of all listed. Apart from androsterone, the lactone from digoxigenin and cholestane-5-ol-3-6-dione, it includes only compounds such as ethers, esters and halogen and keto derivatives, in none of which are there any hydroxyl groups. The length of *b* is fairly constant at 7·6–7·9 A; the plane of the ring system is approximately in the *a* plane, $a \sin \beta$ corresponding to twice the molecular thickness. The slope of the long axes of the molecules to the *c* plane is generally pronounced, varying from about 127 to 135°. (There are deviations at either end of the series.) This molecular arrangement (figure 2) can be considered as a fundamental from which a great many of the others described here are built up, in fact, all the remaining normal type structures. Its relation to the type of ergotetraene (*b* 211) (figure 3) in which the *a* and *b* axes are interchanged is obvious from the nomenclature. But $\Delta$-4-cholestene-7-ol, *ab* (21)1 (figure 4), can also be very closely derived from it by a gradual tilting of the plane of the ring system to the *b* plane.

The structure type *a* 212 (thirty-six compounds) is essentially the same as *a* 211 and can be considered to be built up of two units of this placed end to end in the *c* direction. This can be achieved in two ways to give monoclinic and orthorhombic symmetry respectively and the significance of these two divisions is rather different. In the main monoclinic series—seventeen compounds belonging to the space groups $P2_1$, and, for convenience, $P1$, the doubling of *c* doubles the asymmetric unit and suggests that the molecules are united end to end by hydroxyl bonds (figure 6). In the orthorhombic group the asymmetric unit is the molecule itself and, while it is possible for association to occur in the space group $P22_12_1$, this is not so in $P2_12_12_1$. Chemically both orthorhombic groups are rather mixed, while the monoclinic section includes almost exclusively compounds with terminal hydroxyl groups. The space groups C 2 and A 2 fall rather into the $P22_12_1$ category.

The relation between the *a* 211 structures and that of $\Delta$-4-cholestene-7-ol, *ab* (21)1 may be paralleled by one between the *a* 212 structures and those of, say, $\alpha$-ergostenol and ergosterol peroxide, *ab* (21)2. It is one of the drawbacks of the classification as it stands that the groups *a*, *ab* and *b* necessarily merge into one another—so that it is doubtful,

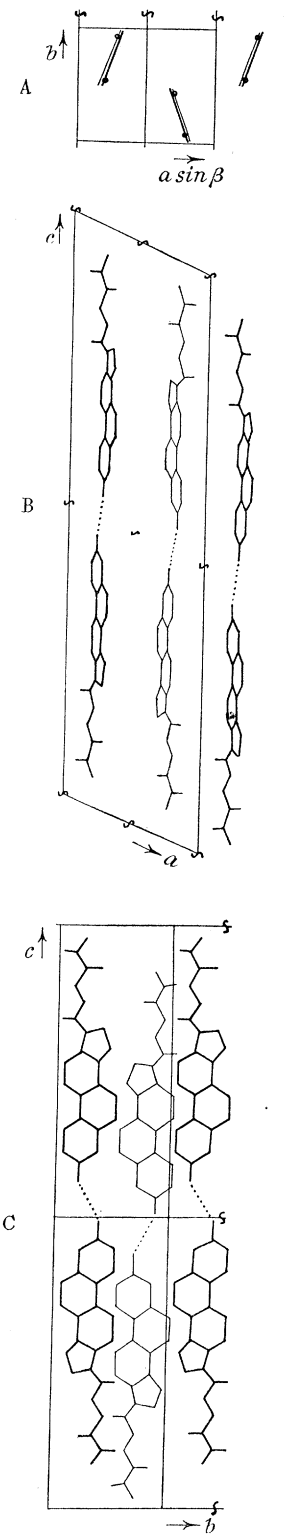


FIGURE 6. The probable arrangement of the molecules in ergosterol $H_2O$, structure type *a* 212. (A) Projection normal to [001]. (B) Projection on (010). (C) Projection on (100).

for example, whether α-ergostene and coprostene dibromide should be classified as *a* 212 or *ab* (21)2, and β-ergostadienetriol II as *a* 412 or *ab* (41)2. Such relations are more easily indicated by a use of the multiplicities alone as the basis of classification. But against this use it is, on the one hand, very convenient for experimental purposes to distinguish the main *a*, *b* and *c* groups, and, on the other hand, differences based only on multiplicities are often equally arbitrary in character. γ-Sitosterol $H_2O$ is, for example, exceedingly similar crystallographically to ergosterol $H_2O$—the doubling of *a* which removes it from the *a* 21 group is evidently a very minor crystallographic variation shown only by a weak superstructure on the principal X-ray reflexions. The likeness to ergosterol is easily recognized in the tables by reference to the actual values of β and the cell dimensions. There are accordingly three principal directions in which geometrical comparisons may be made between the different crystal structures listed in the table. First, the division into *a*, *b* or *c* groups indicates roughly the orientation of the molecular axes to the crystallographic axes. Secondly, within a single multiplicity group the change from *a* to *ab* to *b* indicates a gradual alteration in the slope of the short axes of the molecules to the *a* and *b* axes. Thirdly, the change in β and *c* sin β shows approximately the change in the relative slope of the long axes of the molecules to the *c* plane.

The chief difficulty we have to contend with throughout is that though in the classification we have suggested that the molecules are oriented in certain crystallographic directions it is actually very seldom if ever that the molecular and crystallographic axes exactly coincide (cf. Hendricks 1934). There are only two or three crystal structures of all those examined in which a single X-ray reflexion seems to be of a different order of magnitude from all the others observed. Among these are α-ergostadienetriol and ergosterol peroxide and possibly suprasterol I, and here it is probable that the plane of the ring system coincides fairly accurately with the *a* plane. In all other cases we are dealing with various degrees of approximation to a parallel orientation.

## 3. Chemical implications of the crystallographic investigation

### (*a*) *Characterization and identification*

Physical measurements may be employed in the routine examination of an unknown substance, first to characterize it as belonging to some established chemical series, and finally to identify it as a particular compound known through previous examination or synthesis. These uses depend on rather different characteristics of the data concerned, the first on the existence of certain similarities within particular chemical series, the second on detailed differences.

The general classification of sterol crystals given in table 5 includes a great variety of types of crystal structure. Some of these are very individual in character, and if found in an unknown compound could not be considered necessarily suggestive of

sterol structure. But there is, in general, considerable similarity which extends between the different sterol series. The lines in the tabulation do not divide the cholesterol, ergosterol and phytosterol groups. In some of the structure types, particularly *a* 212 and to a lesser degree *a* 211, crystal structures of such similarity are found that it would be difficult not to conclude that the chemical compounds present were in all cases closely related.

This general similarity of many sterol crystal structures has had its disadvantages in the past in the difficulty with which compounds can be isolated pure and their chemical individuality established. Crystals belonging to the *a* 211 and *a* 212 groups commonly appear as lath-shaped plates bounded only by the edges (100), (110) and (010), and since *a* and *b* have almost the same sizes in all these compounds, morphological crystallographic measurements are of little or no assistance in the identification of the substance present. It can be safely stated that the sterol crystals that are easily recognizable by morphological examination are those which do not belong to the crystallographically defined normal type—such as hydrated cholesterol or cholestene hydrochloride. But the X-ray method introduces a very rapid and simple means of identification which can actually be applied most easily in just the group which is most difficult to characterize in other ways. The *b* axis is an easily recognizable crystallographically unique direction, and X-ray photographs taken over a small oscillation range starting with the X-ray beam parallel to this axis are consequently also unique. These photographs give the (*h*0*l*) reflexions, both the positions and intensities of which are extremely sensitive to small changes in molecular structure and shifts in orientation. The identity or otherwise of two compounds is most easily recognized by direct comparison of photographs. In default of a set in each of the various laboratories studying sterol mixtures, measurements of the cell sizes and records of the intensities serve the purpose equally well if rather more laboriously.

These processes of characterization and identification by the X-ray method may be illustrated by the work on the mixed sterols of rubber recorded in table 4. A crystalline material isolated by Dr K. C. Roberts from rubber and which was considered probably to be a terpene alcohol related to amyrin was submitted to X-ray examination. This showed the presence in the preparation of two compounds, one of which gave X-ray photographs identical with those of stigmasterol. The second and main constituent proved exceedingly similar to ergosterol, and even more so to a constituent measured by accident in one soya-bean mixed sitosterol preparation. The measurements recorded, combined with the form of the X-ray reflections observed, justified its characterisation as a sterol closely related to ergosterol, possibly $\alpha$- or $\beta$-sitosterol. Since this paper was first written, chemical work in Professor Heilbron's laboratories at Imperial College has shown that two of the constituents of the mixed sterols of rubber are $\beta$-sitosterol and 24 : 25-dehydrostigmastanol (private communication from Dr K. C. Roberts).*

* Added in proof.

Another sterol preparation in which X-ray examination has shown the presence of two crystalline forms is that of cerevisterol—the two varieties are listed as cerevisterol I and II in table 4. But since neither of these is identical with any other sterol so far studied, it is not at this stage possible to say whether the difference between them is only crystallographic or chemical as well—though the latter seems most likely.

It is probable that many of the preparations so far examined are not altogether pure, as such great similarity in crystallography frequently results in mixed crystal formation. This is comparatively easy to recognize where the pure compounds have previously been studied, but more difficult where the suspected preparation contains new derivatives. The comparison between $\gamma$-sitostanol and the preparation of $\alpha\beta$-sitostanol, for example (table 4), was undertaken to settle the question of the identity or otherwise of these compounds. The X-ray photographs are exceedingly similar, much more so than photographs so far studied of different individuals, and suggest that the main constituents of the two preparations are identical. But the fact that the two sets of photographs are not exactly the same indicates that there must be one or more different constituents in either or both samples. And one possible constituent is suggested by the fact that spinastanol also gives photographs practically identical with those of the sitostanols in the spacings, though markedly different from them in the intensities of the X-ray reflexions. Other similar examples of mixed crystals appear among the sitosterols themselves. In the soya-bean sitosterol preparation it is common to find some crystals which give X-ray reflexions very similar in position and intensities to $\gamma$-sitosterol but not showing the superstructure which gives $\gamma$-sitosterol a doubled $a$ dimension, while others show a much weaker superstructure. Still it is noticeable that such mixed crystal formation seems only to occur when the crystal structures are very much alike not only in type but in the relative slope of the molecules. The same soya-bean sitosterol crystals give in addition weak reflexions of stigmasterol which forms layer growths over, and not included in, the sitosterol complex, owing presumably to the marked difference of slope of the molecules to the $c$ plane in the two structures.

It would obviously be valuable in applying the X-ray method for identification purposes in this field to examine first in more detail the crystallographic behaviour of sterol mixtures. Four possible varieties of crystallographic interaction between two compounds have already been observed:

(i) The two components separate in two distinct crystals which can be recognized by optical or morphological means, e.g. stigmasterol and $\beta$-sitosterol.

(ii) The two distinct crystal structures form layer structures recognizable on X-ray photographs, e.g. stigmasterol on the soya-bean sitosterol.

(iii) A mixed crystal is formed, e.g. probably $\alpha$-, $\beta$- and $\gamma$-sitosterols. Such crystals give rather blurred X-ray reflexions intermediate in character between those of the two components.

(iv) A definite crystal compound is formed giving X-ray reflexions different in position and intensities from either component, e.g. calciferol-pyrocalciferol.





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These four types of behaviour are not sharply distinguished from one another. (i) and (ii) may occur in the same preparation. The difference between (ii) and (iii) may become one only of degree, and between (iii) and (iv) only one of regularity.

### (*b*) *Molecular weight determination*

The use of X-ray measurements to determine chemical molecular weights follows from the relation between the crystal density and the volume and mass of the crystal unit cell:

$$\rho = \frac{nM}{V},$$

where $\rho$ is the density, $V$ the volume of the unit cell, $n$ the number of molecules in the unit, and $M$, the mass of the individual molecule, is equal to the chemical molecular weight multiplied by the mass of an atom of hydrogen. The routine measurement of the molecular weight depends therefore on the precision with which the quantities $\rho$, $V$ and $n$ can be determined. Of these $n$ must be an integer and is fixed by the arrangement of the molecules in the unit cell. Where the order of magnitude of the required molecular weight is known, $n$ can easily be assigned by an approximate calculation. The exact numerical accuracy of the measurement depends therefore on the determination of $\rho$ and $V$.

By the employment of precision X-ray goniometers it is now possible to measure lattice constants with an accuracy of a few parts in 100,000, and these determine the cell volumes. The limiting factor in the accuracy of the molecular weight measurement is therefore probably the density, though Hendricks (1933) has shown that with careful choice of crystals and the use of a laboratory centrifuge to follow their flotation in suitable solutions, it is possible to measure crystal densities with a probable error of not more than 0·0001. No measurements in the sterol series have yet been made to this degree of precision, though it is probable that such measurements would be valuable in the case of, for example, calciferol, where the number of hydrogen atoms has been in doubt. For the first order examination of the sterols, as of most organic compounds, it has been considered sufficient to use methods that will determine the number of carbon atoms in the molecule, and for this purpose an accuracy of $\pm 1{\cdot}5$ % is adequate. The standard laboratory X-ray goniometers have therefore been used, and for the density determination the flotation method under centrifugal force, preferably with a dust of small crystals (Bernal and Crowfoot 1934*a*). The solution generally employed has been sodium chloride in water with a drop or two of sodium taurocholeate solution to assist the wetting of the crystals. Occasionally zinc sulphate solution was used instead. The measurements on a number of different sterols are recorded in table 6.

In the study of a series of compounds it is evidently unnecessary to measure exactly the molecular weight of every compound. One set of precision measurements on a suitable compound should suffice to define the series. The choice of such a compound depends first on its occurrence in good crystals and secondly, if an absolute determina-

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TABLE 6. MOLECULAR WEIGHT DETERMINATIONS IN THE STEROL SERIES

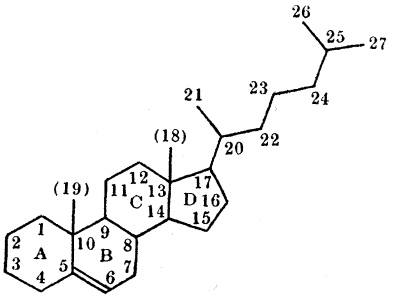
| Compound | $a$ | $b$ | $c \sin\beta$ | $\rho$ | No. in unit cell | No. in asymm. unit | Molecular wt. measured | Molecular wt. calculated | Formula |
|---|---|---|---|---|---|---|---|---|---|
| Cholestenone | $a'$ 14·63 ±0·05 | 7·86 ±0·015 | $d'_{001}$ 10·26 ±0·02 | 1·078 ±0·002 | 2 | 1 | 385 ±3 | 384 | $C_{27}H_{44}O$ |
| Ergotetraene | 7·73 ±0·04 | 9·67 ±0·04 | 16·00 ±0·05 | 1·065 ±0·005 | 2 | 1 | 384 ±7 | 378 | $C_{28}H_{42}O$ |
| $\gamma$-Spinasterol acetate | 13·28 ±0·02 | 6·95 ±0·03 | 31·0 ±0·20 | 1·064 ±0·004 | 4 | 1 | 460 ±8 | 456 | $C_{31}H_{52}O_2$ |
| Ergosterol $H_2O$ | 9·92 ±0·04 | 7·57 ±0·03 | 34·82 ±0·04 | 1·055 ±0·003 | 4 | 2 | 18+401 ±7 | 396 | $C_{28}H_{44}O$ |
| Stigmasterol $H_2O$ | 9·51 ±0·09 | 7·59 ±0·02 | 36·64 ±0·06 | 1·055 ±0·004 | 4 | 2 | 18+406 ±7 | 412 | $C_{29}H_{48}O$ |
| $\gamma$-Sitosterol $H_2O$ | 20·44 ±0·10 | 7·58 ±0·02 | 71·40 ±0·20 | 1·035 ±0·002 | 16 | 4 | 18+415 ±6 | 414 | $C_{29}H_{50}O$ |
| $\beta$-Sitosterol $H_2O$ | 10·33 ±0·10 | 7·55 ±0·01 | 35·00 ±0·10 | 1·049 ±0·004 | 4 | 2 | 18+414 ±7 | 414 | $C_{29}H_{50}O$ |
| $\beta$-Sitosterol acetate | 10·43 ±0·04 | 7·63 ±0·02 | 36·4 ±0·1 | 1·049 ±0·002 | 4 | 2 | 460 ±6 | 456 | $C_{29}H_{50}O.CO.CH_3$ |
| Cholestanol $x$ $H_2O$ | 9·79 ±0·03 | 7·76 ±0·01 | 35·42 ±0·06 | 1·040 ±0·006 | 4 | 4 | 425 ±5 | 424 | $C_{27}H_{48}O.2H_2O$ |
| $\beta$-Ergostadienetriol $x$ $H_2O$ | 6·738 ±0·010 | 7·762 ±0·004 | 28·77 ±0·04 | 1·10 ±0·01 | 2 | 1 | 463 ±6 | 466 | $C_{28}H_{46}O_3.2H_2O$ |
| $\Delta$-4-Cholestene-7-ol $x$ $H_2O$ | 13·96 ±0·04 | 6·15 ±0·02 | 14·80 ±0·05 | 1·051 ±0·003 | 2 | 1 | 405 ±5 | 404 | $C_{27}H_{46}O.H_2O$ |

tion is desired, on the actual crystal structure adopted. In the sterol series, particularly among hydroxy compounds, the asymmetric unit of the crystal structure is frequently composed of two or more molecules, and the crystallographic maximum molecular weight is this multiple of the chemical minimum value. For an unambiguous measurement it is generally possible to find some member of the series in which the space group alone determines $n$, the number of molecules in the unit cell. This is the case with cholestenone, ergotetraene and $\gamma$-spinasterol acetate recorded below and with calciferol-pyrocalciferol measured by Bernal (1932), and the measurements on these compounds may be considered to define the cholesterol, ergosterol, spinasterol and calciferol series. Cholestenone in particular forms very good crystals of which morphological measurements were made by Jaeger in 1908. The X-ray results given in table 6 are recorded using the axes chosen by Jaeger, though these have been changed in tables 1–5 for the sake of the general classification. The density now measured is slightly but not significantly higher than that found by Jaeger (1·071). The crystals of ergotetraene used were much less good and had a fibrous appearance. Though fragile, they show no cleavage. The results on $\gamma$-spinasterol acetate are of particular interest, since they show that the spinasterols, like sitosterol and stigmasterol (below), belong to the C 29 group of plant sterols and not to the C 28, ergosterol, group as previously accepted on chemical grounds (Heyl and Larsen 1934).

For most of the measurements of molecular weight in the higher plant sterol series only hydroxy compounds were available. The crystals of these suffer not only from the formal disadvantage that the asymmetric unit contains two or four chemical molecules but also that there is present water of crystallization, the proportion of which should be determined by separate chemical analysis. But the crystal structures of these compounds are extremely similar to that of hydrated ergosterol and it seems consequently reliable to place the same interpretation upon them all. The weight of ergosterol itself is crystallographically defined by that of ergotetraene, and there is the further evidence of Tanret's analyses of the water content of ergosterol hydrate crystals (Tanret 1889, 1908) to demonstrate that the ergosterol molecule has a weight of $C_{28}H_{44}O$, and that two are present each associated with a molecule of water in the crystal asymmetric unit. The crystallographic data on stigmasterol, $\beta$- and $\gamma$-sitosterol may be interpreted by comparison with this conclusion. The value so deduced for stigmasterol agrees with that found by chemical methods (Sandqvist and Gorton 1930; Windaus, v. Werder and Gschaider 1932), though it is a little low, probably owing to the cracked nature of the crystals used. The weight found for $\gamma$-sitosterol agrees very exactly with that suggested by earlier data on mixed sitosterol preparations, and although it is unlikely that the sample used now is quite pure, it may be taken as evidence of the C 29 formula for this sterol. The value for $\beta$-sitosterol from rubber is supported by the X-ray measurements on the acetate and has been chemically confirmed.*

* Private communication from Dr K. C. Roberts.

Where the weight of the sterol molecule present is known the X-ray measurement may be applied rather differently, to the exact determination on a small quantity of material of the proportion of solvent of crystallization present. This application is illustrated by the measurements recorded on $\Delta$-4-cholestene-7-ol ($\psi$-cholesterol), cholestanol and $\beta$-ergostadienetriol (leaflet form). The X-ray photographs of cholestanol invariably show in addition traces of lines corresponding to a smaller unit cell with a $c$ spacing 34·2 A, probably due to the formation of a lower hydrate.




II

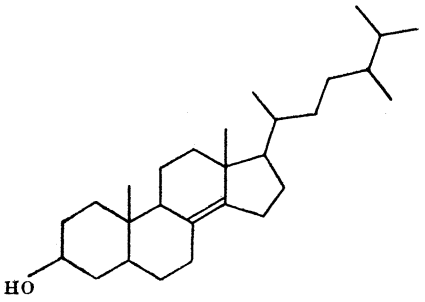
### (c) *The stereochemistry of the carbon skeleton of the sterols*

If the carbon skeleton written above (II) is accepted as in outline a correct representation of sterol structure, there are still a number of stereochemical alternatives among which the exact arrangement of the atoms in the individual molecules must be sought. A large proportion of these can be excluded by the crystallographic evidence, particularly on cholesteryl chloride and bromide, which requires the presence in these derivatives of a reasonably flat molecule. And bearing this in mind it is a simplification to consider separately the nature of the junctions between rings $A$ and $B$, $B$ and $C$, and $C$ and $D$ and where these differ in the different sterols studied, their relation, if any, to the crystallographic data. The descriptions *cis* and *trans* may be applied here as in the *cis* and *trans* decalins.

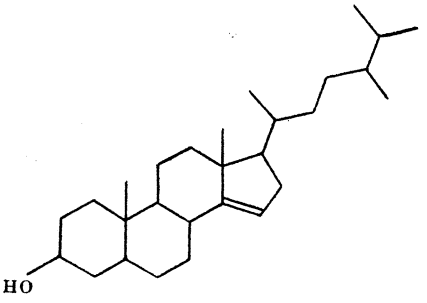
There is as yet no X-ray evidence on the nature of the junction between rings $C$ and $D$, which is usually, on evidence supplied by Wieland and Dane (1933), considered to be *trans*. The configuration here is almost certainly the same in all the compounds so far examined. But a certain modification in the relative positions of the atoms surrounding this junction is to be expected in compounds having double bonds impinging on it as in the structures proposed for $\alpha$- and $\beta$-ergostenol III, IV (Achtermann 1934; F. Laucht, 1935). These two compounds differ markedly from one another, $\alpha$-ergostenol crystallizing in the *ab* (21) group, $\beta$-ergostenol in the normal *a* 212 group, but it is not possible from the data so far collected to associate their differences with their molecular configuration. The same may be said of $\beta$-dihydrofucosterol for which a structure with a double bond in ring $D$ has been suggested (V) (Heilbron *et al.* 1936), and which crystallizes in a complex variant of the *a* type structure, so far peculiar to itself.

The junction between rings $B$ and $C$ is generally considered to be *trans* to fit the

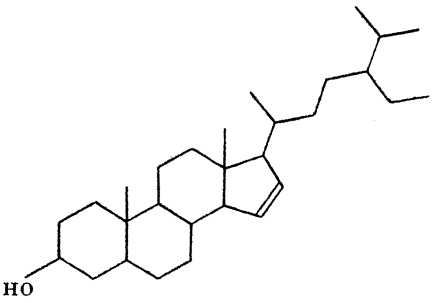
general requirements of the X-ray measurements (cf. Ruzicka and Thomann 1933). It should be stated that in the sterols themselves this determination has not anything like the certainty of that of the parallel decision in the case of the *cis* and *trans* hexahydrochrysenes (Bernal 1933). In the completely reduced ring system there is much greater freedom of movement and models can be constructed with a *cis* central junction nearly as flat as those where the junction is *trans*. But where one ring is aromatic as in oestrone, the more rigid conditions hold, and here it is certain that the central junction must be *trans*. The relations between the sterol and sex-hormone group justify one in extending this condition to all normal members of the series, especially when it is realized that any change of the configuration of the central junction made without corresponding changes at the junction *C* to *D* completely alters the shape of the molecule. The X-ray evidence also applies a restriction on the possibilities of isomerism at C10, since to keep the molecule flat the substituent hydrogen or methyl at C10 must in the general case be *trans* to that at C9.




III




IV

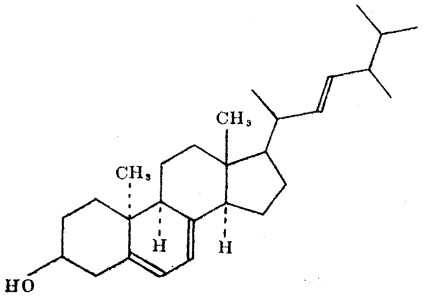



V

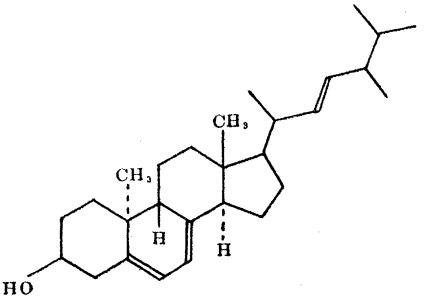
These considerations are particularly relevant to the question of the structures of lumisterol and of pyrocalciferol. Chemical evidence leads to the conclusion that the molecule of lumisterol differs from that of ergosterol only in the stereochemistry of the ring system at C10 (VI), while in pyrocalciferol the arrangement of the atoms at C9 is also affected (VII) (Windaus and Dimroth 1937; Heilbron, Kennedy, Spring and Swain 1938). Both these changes produce considerable alteration in the molecular shape, and it seems significant therefore that neither of these two compounds is crystallographically of a simple type. Lumisterol and its derivatives, the hexahydrolumisterol acetates, show a marked approximation to pseudo-hexagonal symmetry, and while the simplest interpretation of the structure following from the optics leads to the deduction of relatively flat molecules, it is possible to see in their greatly sloped arrangement an adjustment to some change in molecular configuration. Pyrocalciferol shows certain relations crystallographically to lumisterol. The asymmetric unit is doubled, but there is no apparent double layer formation, and this makes it impossible at this stage to draw further conclusions from the X-ray data on the molecular shape.

In the junction between rings *A* and *B* the arrangement may be either *cis* or *trans*, and the chemical evidence shows it to be *trans* in the cholestane allo-cholanic acid

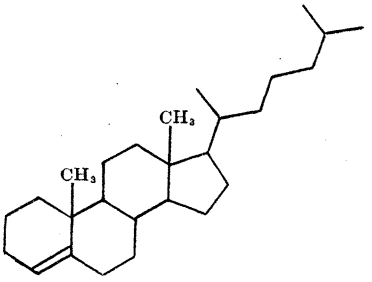
series, passing to *cis* in the coprostane-cholanic acid series. Compounds of both groups have been examined crystallographically, but the differences between them do not at this stage appear to be diagnostic. It is perhaps interesting, however, that coprostene (VIII) and coprostane (IX) are crystallographically closely related—a fact which may have some stereochemical significance. Cholestene (X) and cholestane (XI), on the other hand, both crystallize in rather irregular structures.



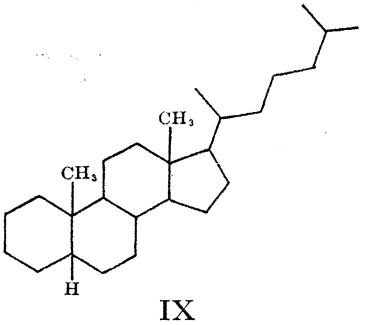

VI



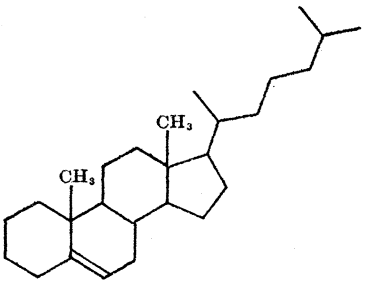

VII



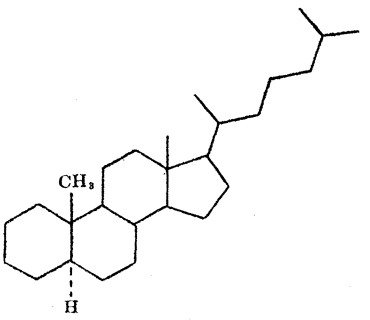

VIII




IX




X




XI

(*d*) *The effect of substituents on the crystallography of the sterols*

(i) *The position of the hydroxyl group.*

One of the sharpest distinctions in the geometrical classification of sterol crystal structures may be drawn between the single- and double-layer structure types. It has been already suggested (p. 156) that the difference between the two is generally due to association between terminal hydroxyl groups, which in the double-layer structures causes doubling of the crystal asymmetric unit in the direction of the molecular length.

Such behaviour is well known among the fatty acids and long-chain alcohols, and its occurrence in the sterol series receives additional support from the direct relation of the characteristic sterol double-layer crystal structures to the crystal structure of dicholesteryl ether. This compound crystallizes in the space group $A2$, and the unit cell which contains four chemical molecules shows very much the same dimensions as those of ostreasterol and actiniasterol for which the space group is also $A2$, but the asymmetric unit double the chemical molecule: cf.

| | $a$ | $b$ | $c$ | | $n$ |
|---|---|---|---|---|---|
| Dicholesteryl ether | 11·30 | 6·23 | 76·3 | 110° | 4 |
| Ostreasterol | 10·10 | 7·65 | 78·6 | 117° | 8 |
| Actiniasterol | 10·12 | 7·58 | 82·0 | 121° | 8 |

In dicholesteryl ether the doubling of the molecular length is produced by the chemical elimination of water between the hydroxyl groups, and these must evidently be terminally placed in the sterol skeleton to produce the effects observed—the crystal structure of this compound might indeed be quoted as the best single piece of evidence from the crystallographic side for the terminal position of the hydroxyl group of cholesterol. Actually the removal of one hydroxyl group in its formation probably forces the two component cholesterol chains of the ether to lie at a certain angle to one another which may account for some of the deviations of its crystal structure from the strictly normal double-layer types. But the general relation between the doubling of the molecule in dicholesteryl ether and the formation of many of the sterol double-layer structures is plain. And since it was the consideration of these that was first employed to demonstrate from the crystallographic side that the hydroxyl group of ergosterol and cholesterol was terminally placed at position C 3, it is worth considering how far the formation of a crystal double layer among sterols is absolutely specific for the presence of a terminal hydroxyl group.

In the general classification (table 5) the single-layer structure types are $a$ 111, $a$ 211, $a$ 411, $b$ 211, $b$ 221, $ab$ (21)1, $ab$ (22)1 and $c$ 221, to which must be added certain groups in which no association is possible between equivalent atoms, $a$ 112; $a$ 212, C 2, $A2$, $P2_12_12_1$; $ab$ (21)2, $A2$; and $c$ 112. All the rest can be classified as double-layer types, and it is very significant that out of the forty-eight compounds in the table belonging to this class thirty-three are known to have a hydroxyl group at the same position as cholesterol, C 3. In the case of seven others, actiniasterol, brassicasterol, the spinasterols, and the cerevisterols, the position of the hydroxyl group has not yet been chemically proved but is almost certainly again C 3. The remaining eight are intrusions into the group whose nature requires more careful consideration. They include two hydrocarbons, pregnane and coprostane, four acetates, ergosteryl acetate, $\beta$-sitosterol acetate, ergosterol maleic anhydride acetate 2 and spinastanol acetate and the hydroxy derivatives cholestane-4-ol and cholestane-6-ol. Of these pregnane, coprostane and spinastanol acetate all show either orthorhombic or a close approxima-

tion to orthorhombic symmetry, and even the preliminary examination might suggest that the doubling of the asymmetric unit is here rather different from that produced by association of hydroxyl groups and due to some more purely geometrical aspect of molecule packing. The same may be said of the remaining exceptions, but it is plain that it would be practically impossible to distinguish at least between the other acetates and normal double-layer structures on the data so far collected. The remaining two compounds, cholestane-4-ol and 6-ol, raise still further problems. The examination of cholestane-4-ol has not yet been taken as far as space group determination, but it is certain that the structure type is not exactly that shown by any of the derivatives examined with the hydroxyl group at C 3. This seems very reasonable. The hydroxyl group is in a nearly terminal position, and a double layer would be expected, as observed, rather different in character from the normal variety. Cholestane-6-ol EtOH is, on the other hand, exceedingly similar morphologically to cholesterol $H_2O$. It is, in fact, the only derivative in the table that might on cursory inspection be mistaken for cholesterol $H_2O$. The crystal structure in both cases is of an irregular and complicated variety from which it is impossible to expect to draw exact conclusions at this stage. And while a very natural deduction from the direct comparison of the two structures would certainly be that in both the hydroxyl group is at C 3, one fact, the further doubling of the *c* dimension in cholesterol $H_2O$ compared with cholestane-6-ol, is against it. In any case there is evidently a relation of an intimate geometrical character between cholestane-6-ol and cholesterol $H_2O$.

In the formation of double-layer structures the terminal hydroxyl groups may either interact directly with one another or through water molecules. Examples of both varieties of interaction are well known already in the crystal structures of oxalic acid and oxalic acid dihydrate (Hendricks 1935; Robertson 1936). Among the sterols the first process seems frequently attended by some difficulty, witness the extremely complicated structures of anhydrous cholesterol and ergosterol and the great readiness with which crystals, particularly of the latter and many closely related sterols, take up water or alcohol of crystallization. The crystal structures belonging to the simplest type, the normal *a* 212 structures (figure 6), probably nearly all contain such water, and it seems likely from the structure of cholesterol MeOH that methyl alcohol can play a similar part to water in joining hydroxyl groups. The case of ethyl alcohol, however, is more doubtful. That this is associated with the sterol hydroxyl group in crystallization is demonstrated by the exceedingly close relation between *i*-cholesterol ethyl ether and *i*-cholesterol EtOH, which show not only approximate identity of unit-cell dimensions but also of X-ray intensities. Both structures are essentially single-layer structures, and it might be argued that here ethyl alcohol of crystallization has closed the hydroxyl bond chain and so prevented further interaction, a type of behaviour that would be relevant also to the problem of the cholestane-6-ol structure.

The single-layer class is characteristically the structure type of hydrocarbons, halogen derivatives, ethers, esters and ketones, in none of which are there specific





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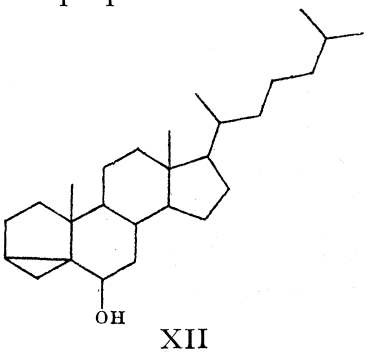
chemical groups likely to cause association between molecules. But the intrusions of hydroxyl compounds into this division are both common and varied. In table 5 the list includes androsterone, $\alpha$- and $\beta$-ergostadienetriol, *trans*-$\Delta$-5-6-cholestene-3-4-diol, pyrocalciferol, oestrone-1 and androstane-3-($\beta$)-ol-17-one, all of which from chemical evidence have at least one hydroxyl group at C 3, and two compounds in which the hydroxyl group is known to be differently placed, $\Delta$-4-cholestene-7-ol ($\psi$-cholesterol) and cholestane-5-ol-3-6-dione together with *i*-cholesterol EtOH, in which there is still some doubt about the exact position of the hydroxyl group. In all of these it seems probable that hydroxyl or hydrogen bond formation does occur, though this does not lead to the appearance of a double layer. The classical example is provided by oestrone (figure 5) in which hydrogen bonds can be formed between the terminal hydroxyl and keto groups of neighbouring molecules, and this interaction takes the place of double-layer formation. The process is better illustrated by the crystal structures of androsterone and the isomeric hydroxyketone from cholesterol where the molecule is itself the asymmetric unit rather than by oestrone and oestriol where for other reasons the asymmetric unit is doubled. Among the polyhydroxy compounds, $\alpha$- and $\beta$-ergostadienetriol, and *trans*-$\Delta$-5-6-cholestene-3-4-diol interaction is possible either (or both) between hydroxyl groups within a single molecule or between non-equivalent hydroxyl groups in neighbouring molecules. In cholestane-3-6-dione-5-ol, a hydrogen bond may be formed between the hydroxyl group and a neighbouring keto group within one molecule. And it is likely that the double layer of *cis*-$\Delta$-5-6-cholestene-3-4-diol is not quite of the normal type suggested superficially by its classification, but that here again the hydroxyl bonds, though leading to double-layer formation, are between non-equivalent groups on the two molecules concerned. The remaining two compounds, pyrocalciferol and $\Delta$-4-cholestene-7-ol, are in a slightly different category, since the essential association may in these cases be between equivalent groups though no double layer is shown. Pyrocalciferol provides the most awkward exception so far encountered—the only compound for which there is chemical evidence that the hydroxyl group is at C 3 and in which there is only the one hydroxyl group and yet no double layer has been formed. The asymmetric unit is actually doubled in another direction, so that it is possible for association between the hydroxyl groups to have occurred in some other way, but the crystal structure certainly calls for further investigation. In $\Delta$-4-cholestene-7-ol, on the other hand, the crystal structure shows precisely the behaviour that might be expected from a compound having a single hydroxyl group remote from any terminal position. The crystals contain water of crystallization presumably associated with the hydroxyl groups, and this may even be employed to link them together if the molecules are so arranged in the unit cell that the hydroxyl groups are brought not far from 3·05 A away from one another, the closest approach which is crystallographically permissible (figure 4).

Taking altogether the evidence now collected on sterol crystal structures, it is clear that this adds considerable weight to the correlation of the double-layer structures

with the presence of terminal hydroxyl groups. But since on the one side there are compounds which show a crystallographic double layer though they contain no terminal hydroxyl group, and on the other, at least one compound, pyrocalciferol, which, although chemical evidence indicates the presence of a terminal hydroxyl group, shows no double layer, it is clear that some caution must be exercised in applying this criterion to place hydroxyl groups on the sterol skeleton. Where there is very great similarity between the crystal structures of the compounds under investigation and a sterol structure of known type it seems safe to apply the argument rigidly. The crystal structures, both in cell dimensions and character of the X-ray intensities, of brassicasterol and the cerevisterols are so like those of ergosterol, ostreasterol and actiniasterol that it is impossible to doubt that the hydroxyl group is in all cases in the same position. But it is much more difficult to place crystallographically the hydroxyl group of *i*-cholesterol. The chemical evidence on this compound has been somewhat conflicting, but the work of Wallis, Fernholz and Gephart (1937) and Heilbron, Hodges and Spring (1938) strongly favours the formula below (XII) with the hydroxyl group at C 6 and not C 3 as first proposed:




XII

Crystallographically Miss Bell finds that the acetate, ethyl ether and *i*-cholesterol EtOH are all very much alike. The only cholesterol derivative, so far examined, which they at all closely resemble is, as table 5 shows, dibromocholesteryl chloride in which there are substituents both at C 3, and at C 5 and C 6. This crystal structure is one in which the molecules have an imbricated arrangement which renders it particularly difficult to place the substituent groups from the preliminary examination. It is also impossible to place the methoxy group of *i*-cholesterol methyl ether from the data at present collected, owing again to the unspecific character of the structure type, *a* 211, in which it crystallizes. From the table it appears as closely related to oxycholestenone dibromide and dichlorcholestane, which probably have substituents at C 5 or C 6, as it is to cholesterol methyl ether with the substituent at C 3. But the crystal structure of this methyl ether is sufficiently simple to hope that it may be submitted to an exact analysis, the solution of which would be the more interesting in view of the somewhat paradoxical relations which exist chemically between the *i*-cholesterol compounds, cholesterol and cholestane 6-ol on the one hand and crystallographically, as mentioned above, between cholesterol and cholestane 6-ol.

(ii) *The stereochemistry of the hydroxyl group at position* 3.

*A priori* one might imagine that the crystal structure would be one of the physical properties of a compound most sensitive to a stereochemical change such as that of the hydroxyl group of the sterols between the epi and normal series. Models show that with a *trans* configuration of the ring system between C 5 and C 10, in one isomer which, following Ruzicka, Furter and Goldberg (1938), we may call *cis*, the hydroxyl group should project vertically from the ring system, in the other, *trans*, it should lie almost in the plane of the ring system (figure 7). We might expect that *cis* isomers would show rather different crystal structures from *trans*, or at least a definite shortening of the molecular length.

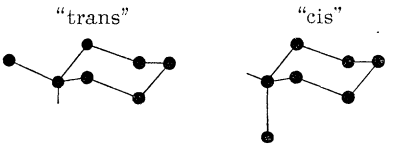



FIGURE 7. The stereochemistry of the sterol hydroxyl group at C 3.

As far as the present examination goes there does not appear to be any definite change in crystal structure which one can associate with the change from *cis* to *trans*. An examination of molecular lengths of twelve compounds all of which crystallize in the normal type of structure and have only a terminal hydroxyl group does, however, show a certain separation into two groups, the first having apparent lengths 37–39 A, the second 34–35 A. (For this comparison the lengths are calculated from the slope of $\gamma$ to the $c$ plane in the crystals.)

| | | | |
|---|---|---|---|
| Cholestanol $2H_2O$ | 39·5 | Epicholestanol | 34·5 |
| Ergosterol $H_2O$ | 39·4 | Lumisterol | 34·4 |
| $\gamma$-Sitosterol $H_2O$ | 39·6 | Calciferol-pyrocalciferol | 34·9 |
| $\gamma$-Sitostanol | 37·8 | Calciferol | 34·8 |
| $\beta$-Ergostenol | 38·0 | Suprasterol I | 34·8 |
| Stigmasterol $H_2O$ | 37·5 | Dihydrocalciferol | 36·1–37·6 |

Further, this separation does correspond in scope to the chemical distinction between the normal and epi series and is paralleled among the sex hormones by the relation between androsterone and the hydroxy ketone from cholestanol. But the reliability of the natural deduction that therefore the normal cholesterol-ergosterol series have the *trans* and the epi series the *cis* configuration is much open to question. The normal series nearly all crystallize with water of crystallization which might independently cause an increase in length (it also might even be argued to be due to this being a more difficultly crystallizable *cis* form). And with the exception of epi-cholestanol itself the entire 'short length' division consists of the photoderivatives of ergosterol about whose structures there is, to say the least, considerable doubt.

Another approach to the problem can be made through a more detailed examination of the halogen derivatives of cholesterol. Calculations on the intensities of X-ray

reflexions from cholesteryl chloride and bromide show quite clearly that in these compounds the halogen atoms are not far from the plane of the ring system and so directed as to increase the molecular length. The most natural interpretation is that they have the *trans* configuration. But here the double bond at C 5 : 6 may very easily affect the actual position in space adopted by a group at C 3, and it is stereochemically possible for atoms attached both at the *cis* and *trans* positions to lie fairly closely in the plane of the ring system. A better test is the study of the reduced derivatives, $\alpha$- and $\beta$-chlorcholestanes, derived by the action of phosphorus pentachloride on epi-cholestanol and cholestanol respectively. The X-ray analysis has here not been carried so far, but the general crystallography and in particular the intensities of the *c* plane reflexions leave no doubt that it is the $\alpha$ compound that has the same configuration as cholesteryl chloride. This $\alpha$ compound can also be prepared by the direct reduction of cholesteryl chloride while reduction of cholesterol followed by the action of phosphorus pentachloride gives cholestanol first and then $\beta$-chlorcholestane. Further correlation of the configuration of cholesteryl chloride and $\alpha$-chlorcholestane with epi-cholestanol cannot therefore be carried through at this stage, since Walden inversion must have occurred at least once, if not several times, in the chemical reactions involved. But the X-ray evidence does show that no such inversion occurs during the hydrogenation of cholesteryl chloride. It would probably be most useful to examine in more detail the crystallographically very similar cholesteryl methyl ether.

It is interesting that the general similarity between the epi and normal cholesterol series in relation to the packing together of the molecules in the crystals is paralleled by their behaviour in surface films (Adam, Askew and Danielli 1935). The limiting surface pressure areas show no significant differences between the two series though there does appear to be a change in surface potential. And while this should be valuable to assist in distinguishing an epi from a normal compound it does not provide a clue as to which has which configuration.

(iii) *The positions of the double bonds.*

The two most important types of reaction by which the double bond in cholesterol is characterized are perhaps its behaviour on oxidation and towards halogens. Products of both courses of treatment are recorded in table 5.

As the general review of these crystal structures has shown, the introduction of hydroxyl groups has a very marked influence on the kind of molecular arrangement adopted. The five polyhydroxy sterols described, $\alpha$-cholestanetriol, $\alpha$- and $\beta$-ergostadienetriol, *cis* and *trans* $\Delta$-5-6-cholestene-3-4-diols show crystal structures classified in seven different divisions of the table—five different space groups including polymorphic forms. A direct comparison of these structures, either among themselves or with others, from the point of view of establishing intensity relations is therefore rather difficult. But in all there are signs that the structure adopted is largely determined by the tendency to form hydroxyl bonds between the hydroxyl groups and, if this is

accepted, it is possible to put forward plausible suggestions as to their whereabouts. As far as it goes the crystallographic evidence is fairly well in agreement with the chemical as to the position of the hydroxyl groups in these compounds. In the case of $\alpha$-ergostadienetriol and ergosterol peroxide it is inclined to favour a C 5-6 configuration of the oxygen atoms. But the crystal structures of these two compounds are of fairly simple types and might be worth more exact study.

The halogen derivatives examined are most promising crystallographically though still inclined to appear a very miscellaneous collection. Eight of them crystallize in the *a* 211 group and are nearly isomorphous. A direct comparison between two of these, cholesteryl chloride and cholesteryl chloride hydrochloride, has therefore been possible. Chemically cholesteryl chloride hydrochloride differs from cholesteryl chloride by the addition of hydrogen chloride at the double bond. Crystallographically this alteration affects mainly the intensities of the X-ray reflexions, and the change can be followed by forming the Patterson projection, *Pxz*, from the intensities of the *h*0*l* planes. Compared with the corresponding projection of cholesteryl chloride (figure 1 C) the resulting diagram (figure 8 A) shows a new series of peaks due both to new chlorine-chlorine interatomic distances and to distances between the additional chlorine atom and the carbon skeleton. Figure 8 B shows a probable arrangement of the molecules which correlates the observed effects. From the chemical point of view the most significant feature of the pattern is the fact that the new peaks lie between the old lines, showing that the additional chlorine atom projects from the main line of the carbon skeleton, and the peak *A* in particular from its strength can be identified as due to the chlorine-chlorine distance within the molecule. This chlorine-chlorine distance, 4·2 A, accurately fixes the additional chlorine atom of cholesteryl chloride hydrochloride—and therefore one end of the cholesterol double-bond system—in the neighbourhood of C 5, which is in agreement with present chemical theory.

Unfortunately all the compounds so far examined in which *two* halogen atoms have been added to the double bond show different types of structure from one another. The orthorhombic forms in which imbrication occurs such as dibromocholesteryl chloride and bromide are most difficult to solve, but coprostene dibromide and dibromocholesterol should prove possible. Work has been started on a survey of the intensities of these compounds by G. Knott.

One of the most characteristic reactions of the double-bond system of ergosterol is that with maleic anhydride. The two ergosterol acetate maleic anhydride adducts that can be obtained have both been examined by G. E. R. Schulze (1935), and a further study of that melting at 216° (I) has been made by us. This shows, in its crystal structure, some deviations from the normal type, while the second adduct appears, like the dibromide, to show more usual cell dimensions. Schulze gives for II:

$$c = 25{\cdot}3, \quad b = 7{\cdot}91, \quad a = 32{\cdot}2\ \text{A}, \quad \beta = 92{\cdot}1°,$$
$$\text{(our } a\text{)} \qquad\qquad \text{(our } c\text{)}$$

while for the adduct I the measurements are

$$a = 10{\cdot}5, \qquad b = 9{\cdot}5, \qquad c = 33{\cdot}2, \qquad \beta = 111^\circ \qquad \text{(table 2)}.$$

Accepting the chemical interpretation of adduct formation as likely to occur normal to the ring system and hence produce an increase in the thickness of the molecule and seeing that it is $b$ that has increased in size in the adduct I compared with the normal structure, Schulze draws the conclusion that $b$ in the normal type sterol

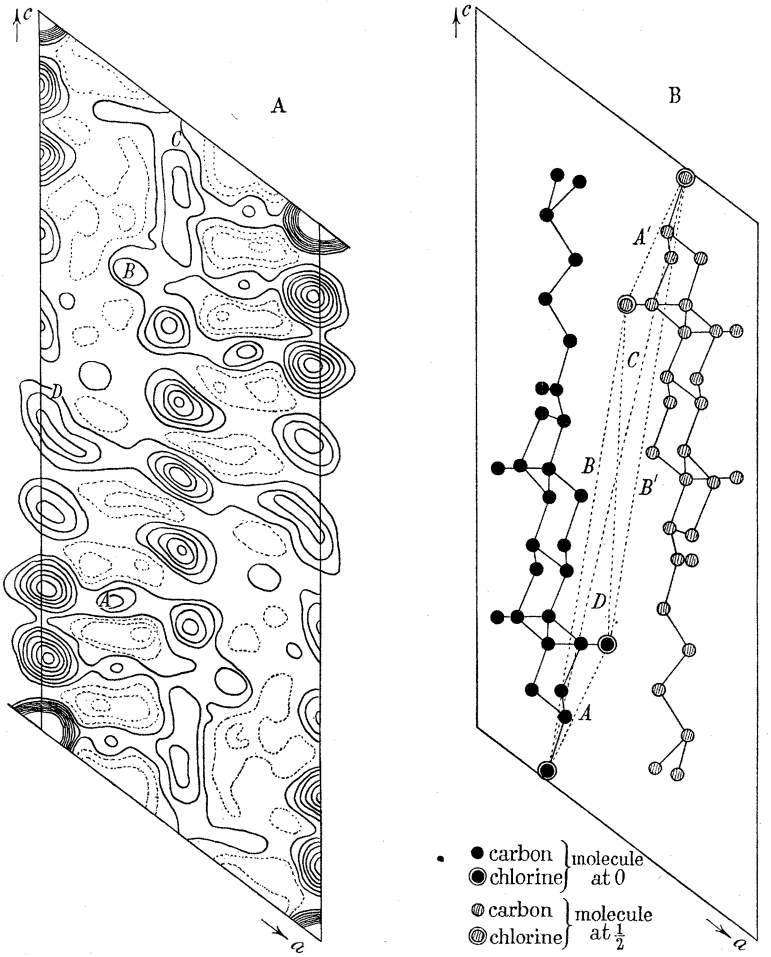
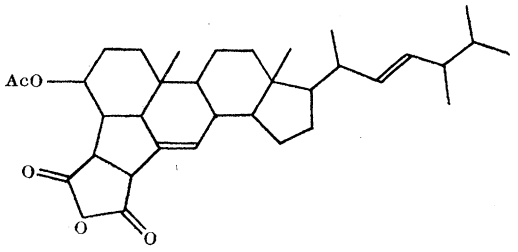



FIGURE 8. Cholesteryl chloride hydrochloride. (A) Patterson projection, *Pxz*. (B) Probable arrangement of the molecules, projected on (010). The dotted lines show chlorine to chlorine distances.

structures represents molecular thickness and $a$ the width. This is a conclusion that cannot be accepted for sterol crystals in general. It is contrary to the evidence of both the optics and the X-ray intensities, both in this compound and in the normal structures. And since Schulze's paper was written the calculations on cholesteryl bromide show that only an orientation with the thickness mainly in (100) can give the experimentally observed effects.

An alternative explanation might therefore be given that the maleic anhydride adds on to the molecule at the side of the ring system, so that, as observed, the dimension corresponding to the width is increased in the adduct. A possible formulae satisfying this requirement is XIII




XIII

While this is a crystallographically plausible explanation there is another which seems more probable. As discussed above, direct deductions of molecular dimensions from lattice dimensions must not be interpreted too rigidly. In the first place it is probable that the plane of the ring system, if 'mainly' in the *a* plane, is still at a considerable angle, as much as 30°, to it. And further, in the adduct the space group, and consequently the relative arrangement of the molecules, has changed compared with the strictly normal type. Since the molecule is here the asymmetric unit, the crystal structure can be fixed with a relative degree of certainty. The orientation with the length of the molecule along *c*, thickness roughly in the *a* plane and width in *b*, is supported both by the optics and by the appearance of 'smear' lines along the layer lines on photographs taken about [001] (figure 9). This arrangement shows that the molecules following each other along the *c* axis are separated by a distance $\frac{1}{2}b$ compared with those in, for example, cholesteryl bromide. In such a structure it is possible to insert the extra atoms of the maleic anhydride with adduct formation (as suggested by chemical investigation) at C 5-8 provided that the adduct is given the stereochemical form which brings the atoms nearest to the plane of the sterol ring system.

These widely varying interpretations that have been put on the crystallographic measurements on ergosterol maleic anhydride adduct acetate are evidence of a weakness in the X-ray method of attacking this particular problem by preliminary measurement alone. The adducts do, however, appear much more promising subjects for further investigation than might have been expected on general considerations.

### (*e*) *A comparison of the crystallography of different sterols* (*monohydroxy compounds*)

The changes in the structure of the carbon skeleton of the sterols with which we have to deal are chemically of four main types: (1) stereochemical only, e.g. in the disposition of carbon atoms around the ring junctions; (2) alterations in the number or distribution of double bonds; (3) introduction or removal of carbon atoms, e.g. as methyl groups; (4) actual breakdown of the sterol ring system as in the proposed

structure for calciferol. Of these types of change we have already to a certain extent considered (1) in relation to problems of sterol stereochemistry as a whole and (2) in relation to the identification of double bonds through chemical modification. (3) might be treated as an additional case of the effect of substituent groups on sterol crystallography. But actually, in studying new sterol derivatives, we must expect to find modifications of all these kinds occurring at once. It seems useful therefore to review the experimental data from a somewhat different angle in order to discover how far the observed crystallographic character can be associated with the sterol skeleton as such, and from this how far it may be sensitive to changes of each of the above types in turn and particularly to those of type (4). The actual similarities and differences that do exist among as wide as possible a group of sterol derivatives must evidently be considered, and for this purpose only compounds with a single hydroxyl group at C 3 have been selected in order to limit as far as possible the deviations in crystallography to those due to changes in the sterol skeleton itself.

There are in table 5 twenty-seven sterol derivatives which have according to the available evidence a single hydroxyl group at position 3 and differ from one another either stereochemically or in the structure of the sterol skeleton. Of these it is noticeable that the four completely reduced compounds examined, $\gamma$-sitostanol, spinastanol, cholestanol, epicholestanol and perhydro epidihydro pyrocalciferol, together with $\alpha$-spinastenol, all occur in one group with the space group $P1$, class $a$ 212. These crystal structures are, however, not so closely alike among themselves as are those of a second group which includes all the higher plant and animal sterols examined and which may be referred to as the ergosterol $H_2O$ group after the type structure, ergosterol $H_2O$. These ten compounds, ergosterol, stigmasterol, $\beta$- and $\gamma$-sitosterol, brassicasterol, ostreasterol, actiniasterol, $\beta$-dihydrofucosterol, $\beta$-ergostenol and also dehydroergosterol, although they fall into a number of different classification groups in table 5, all have crystal structures based on the fundamental $a$ 212 type and show similarities in spacings and the general

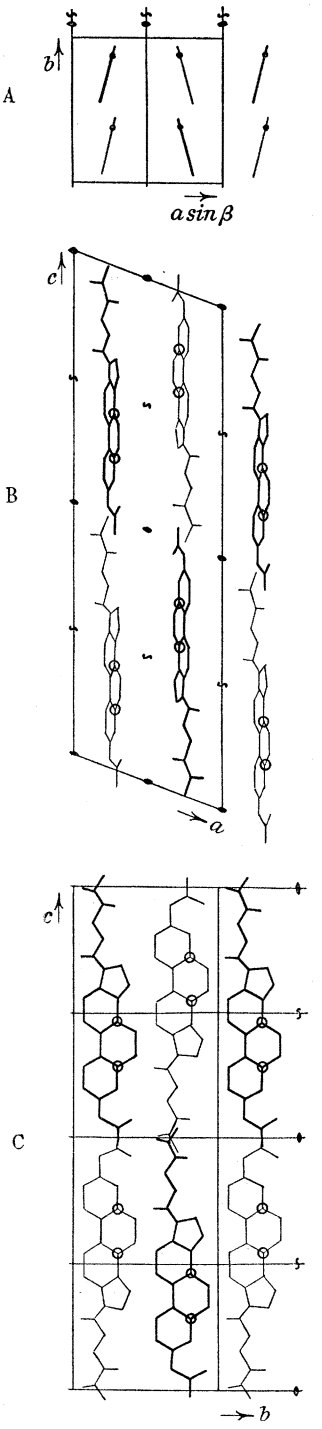



FIGURE 9. Suggested crystal structure of ergosterol acetate maleic anhydride adduct I. (A) Projection normal to [001]. (B) Projection on (010). (C) Projection on (100). $o$, point of attachment of maleic anhydride required by present chemical theory.

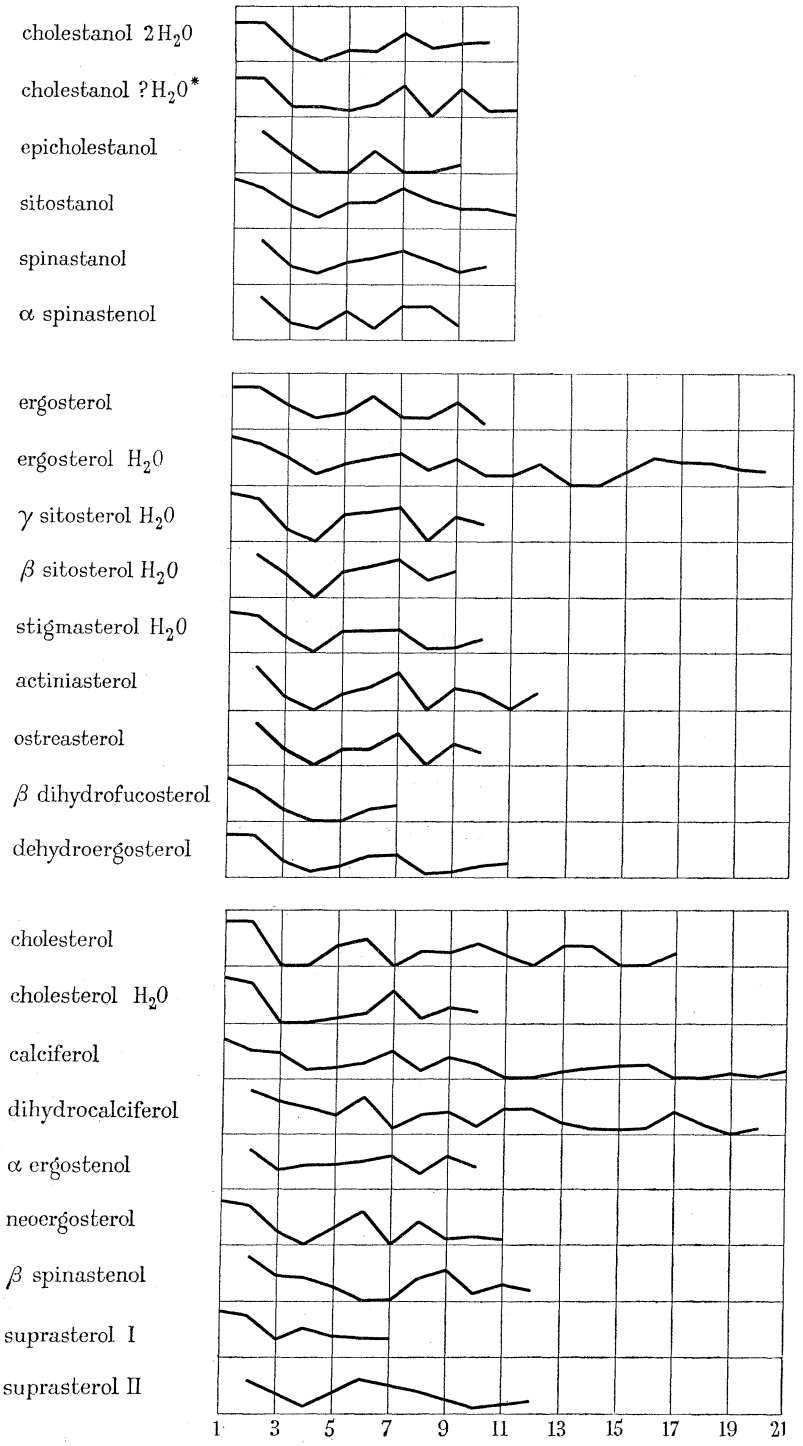



FIGURE 10. Intensities of the X-ray reflexions from the *c* planes of sterols with hydroxyl groups at C3.

run of the intensities of reflexions which are of a different order of magnitude from any other likenesses observed.

The remaining eleven structures are very miscellaneous both chemically and crystallographically. While they do include a number in which the sterol skeleton may have radically altered, such as neoergosterol and all the photoderivatives of ergosterol, they also include the crystal structures of cholesterol, anhydrous and hydrated, and that of anhydrous ergosterol which prevents the main distinction from having much significance. Neoergosterol may be mentioned as crystallographically of particular interest. The crystal structure is of a curious and complicated kind, unlike any other, and the X-ray photographs taken about the *b* axis show marked layer line 'smearing', indicating some degree of irregularity in the arrangement of the molecules. Some of the other crystal structures are of the same structure type as the ergosterol $H_2O$ group, e.g. dihydrocalciferol, others are much more complicated. It is possible, however, to attempt a further comparison of these structures with those of the characteristically normal group by a consideration of the intensities of the X-ray reflections from the *c* plane.

Variations in the intensities of the X-ray reflexions from one crystal structure to another can be analysed as depending on two factors, the variation in the molecular arrangement and in the molecular structure. By considering the *c* plane intensities only of structures, all of which are of a double-layer type, it may be hoped to exclude, as far as possible in a preliminary survey, variations of the first kind and to obtain records which will be sensitive mainly to differences in molecular structure. Figure 10 provides a list of the *c* plane intensities of all the sterol derivatives of the type now considered which show double-layer structures and for which sufficient data were obtained. The records are arranged to correspond roughly in spacing, i.e. the fourth order in the cholesterol $H_2O$ and ostreasterol structures corresponds to the second in ergosterol $H_2O$ and is therefore written in column 2. The only compounds which are excluded from the list as not possessing comparable double-layer structures are lumisterol and pyrocalciferol, for both of which there is chemical evidence that stereochemical changes of type (1) have occurred (cf. p. 164).

The comparison of the *c* plane intensities illustrates again the very close relation between all the members of the ergosterol $H_2O$ group. Through the individual variations the same general curve can be traced and the form of this is illustrated in a rather interesting way by the data on $\beta$-dihydrofucosterol and dehydroergosterol. Both crystals were very poor and certainly impure, and the result on the X-ray intensities appears as a blurring of individual differences while the general 'sterol' form remains. After these very close similarities other resemblances can be traced, particularly between anhydrous ergosterol and cholesterol and again, with a possibly characteristic difference at the seventh order, between these and the normal hydrated structures. Epicholestanol, on the other hand, shows certain differences perhaps significant which are paralleled, though very approximately, by dihydrocalciferol. The member of the





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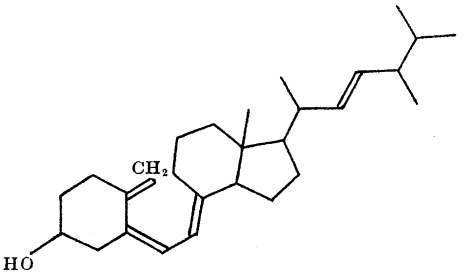
group which deviates most is $\alpha$-ergostenol, and the differences here are probably to be associated rather with change in molecular arrangement than directly with molecular structure. It is clear that differences are in general much less significant in comparisons of this kind than are similarities and it is consequently the more important that the calciferol group, particularly calciferol itself, and to a lesser degree the suprasterols, do all show a formal likeness in $c$ plane intensities to the normal sterol group. At the same time their differences in structure type can all be paralleled among normal sterols.

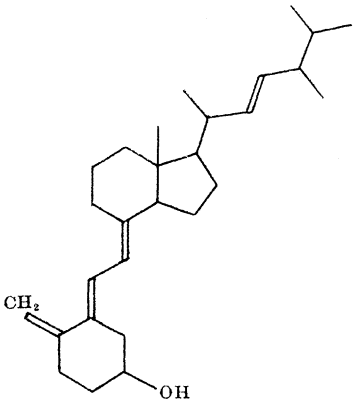
From the general grouping it is clear that the crystallographic character is much more sensitive to changes in the structure of the ring system than to changes in the side chain. All the compounds in which radical alteration of the ring system is suspected do belong, if in somewhat different degrees, to the deviating group. But beyond this it is difficult to make any generalization of diagnostic value.

### (*f*) *The structure of calciferol*

The structure of calciferol must be closely related to the position of the double-bond system in ergosterol and as far as this goes the crystallographic evidence is not contrary to the chemical evidence. The structure of $\beta$-ergostadienetriol is in good agreement with the presence of one double bond in ergosterol at C 5-6 and that of $\Delta$-4-cholestene-7-ol ($\psi$-cholesterol) is some confirmation for a more distant situation of the second double bond. But calciferol itself, according to the chemical evidence accumulated in the last four years, is formed from ergosterol through lumisterol and tachysterol by the rupture of ring $B$ at the tachysterol stage of the transformation (Lettré 1934, Müller 1935; Windaus and Thiele 1935; Heilbron, Samant and Spring 1935). In the present accepted structure for calciferol, ring $A$ is therefore joined by a short chain to rings $C$ and $D$. While this formula is usually written in the configuration XIV, it would seem almost certain physically that such a molecule should assume configuration XV:




XIV




XV

Calciferol would in this case be expected to show marked differences in crystal structure from the normal sterol structures whereas actually on its preliminary examina-

tion it was its similarity that appeared most significant (cf. Bernal and Crowfoot 1935).

It is possible now to carry the crystallographic comparison of calciferol and its derivatives with other sterols a stage further from the material discussed in the preceding section. This has made it clear that actually none of the calciferol group do belong to the most characteristically 'steroid' ergosterol $H_2O$ group, though amongst themselves they deviate from this in very different degrees. Dihydrocalciferol crystallizes in the normal *a* 212 structure type and its divergence from the ergosterol group is comparatively minor, a certain shortening of *b* and lengthening of $a \sin \beta$ which also occurs in calciferol-pyrocalciferol, pyrocalciferol and calciferol itself. Pyrocalciferol is perhaps the most radically different in crystal structure, and this compound is agreed on chemical grounds to have an abnormal sterol skeleton. Calciferol and pyrocalciferol-calciferol are, on the other hand, more closely related to the normal group; the relation of calciferol in particular is shown by the comparison of the *c* plane intensities with those of the ergosterol $H_2O$ group. They show very marked similarities suggesting the presence of closely similar atomic groupings. Further, the crystallographic interpretation of the data on the suprasterols, particularly suprasterol I, is definitely against the presence of a spiro ring system here (Müller 1935), though it cannot exclude other ring systems which would conform to lath shape in type (cf. Rosenheim and King 1935).

It is, however, admittedly impossible to exclude the broken-ring formula for calciferol on the present crystallographic data without some information on the behaviour of compounds known to possess such structures. If it is actually possible for a molecular configuration approximating to XIV to be maintained in the crystal there is no reason to consider the crystallographic data conflicting with the chemical. So far the only compound examined crystallographically in which a ring-chain configuration at all comparable exists is *cis* azobenzene. And here the structure is definitely unstable and the planes of the benzene rings are disposed at a considerable angle to one another (Robertson 1939 *b*). It may, however, be argued that the aromatic system in *cis* azobenzene is not sufficiently close to that suggested for calciferol to make the comparison of much value. But it should be possible to find other analogous compounds, perhaps in other series, to investigate.

There is one rather interesting point in connexion with the vitamin-D problem that seems worth mentioning. Crystallographically $\beta$- and $\gamma$-sitosterols are very much more similar to ergosterol than is stigmasterol. The slope of the molecules to the *c* plane and the *c* plane intensities in the three compounds are practically identical. Chemically antirachitic products can be obtained by irradiation of both ergosterol and 7-dehydrositosterol, whereas that from stigmasterol is almost if not quite inactive. There seems to be a relation between the two sets of properties of the sitosterols and ergosterol for which the present chemical formulae, which make stigmasterol the most similar to ergosterol, provide no clue.



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## Conclusion

The survey of sterol crystal structures which has been undertaken in this paper has illustrated very plainly the limitations of preliminary crystallographic investigation as a source of chemical information. Even in matters of straight identification there may be difficulties where impure preparations consisting largely of mixed crystals or where polymorphic modifications are concerned. Double-layer structures, though most frequently, are not quite exclusively associated with terminal hydroxyl groups, and deviations in crystal-structure type very often make it impossible to apply simple comparisons of X-ray intensities to fixing the positions of substituent groups. It is still desirable to extend the survey method to a number of problems which have been mentioned in the course of the discussion, for example, the behaviour of sterol mixtures, and also to cover more systematically different sterol types, to include, among others, epi-cholesterol and epi-coprostanol. But it is clear that the solutions of the most important problems of sterol structure must be sought from the crystallographic side by exact X-ray analysis and it is probably in this direction that the general survey should prove most useful.

Where, as in the case of the sterols, the molecules, and therefore necessarily the crystal structures, have no centres of symmetry, it is essential to find comparatively simple crystal structures for intensive analysis. And at the same time it is desirable to choose those whose solution will answer the most outstanding questions of chemical structure. Of the structures listed in the tables the halogen and ether derivatives of cholesterol, and particularly $\alpha$- and $\beta$-chlorcholestane should be useful both on account of simplicity of crystal structure and for their relation to the fundamental problem of the position and stereochemistry of the hydroxyl group of cholesterol. These have, however, one crystallographic disadvantage. The easiest approach to exact analysis in monoclinic crystals is through an analysis of the $h0l$ reflexions since an effective centre of symmetry appears for projections on the $b$ plane at the position of the twofold axis. But for $\alpha$- and $\beta$-chlorcholestane this projection should give the less instructive view of the sterol skeleton as a whole, roughly normal to the plane of the ring system. The alternative view over the extended ring system might be obtained from certain of the $b$ or $ab$ type structures. Of these $\Delta$-4-cholestene-7-ol is probably the most hopeful and its relation to the preparation of 7-dehydrocholesterol and the antirachitic vitamin should make it chemically one of the most valuable. The direct analysis of calciferol itself is too difficult to attempt yet and it would possibly be useful in this connexion to extend the survey to the antirachitic products derived from cholesterol and sitosterol and their derivatives in the hopes of finding more suitable subjects. Of the calciferol derivatives already examined, dihydrocalciferol is the most promising, particularly if its analysis can be combined with that of ergosterol itself. Other compounds that may be mentioned here as both crystallographically and chemically eligible for further study are $i$-cholesterol methyl ether, $\alpha$- and $\beta$-ergostadienetriol and ergosterol maleic anhydride adduct I.

The work that has already been carried out would have been impossible without the close co-operation of a number of workers in the field of sterol chemistry. We are particularly indebted both for advice and material to Drs O. Rosenheim, H. King and R. K. Callow (Hampstead), Professor A. Windaus (Gottingen), Professor A. H. Cook (London) and Professor I. Heilbron and Dr F. S. Spring (Manchester) and also for material to Dr B. H. Blount (Oxford), Dr C. D. Larsen (Rochester, N.Y.), Dr J. Needham (Cambridge), Dr K. C. Roberts (Malay States), Dr R. Schonheimer (New York City). We should also like to acknowledge grants towards apparatus from the Royal Society and from Imperial Chemical Industries, Ltd.

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## References

Achtermann, T. 1934 *Hoppe-Seyl. Z.* **225**, 141.
Adam, N. K., Askew, F. K. and Danielli, J. F. 1935 *Biochem. J.* **29**, 1792.
Bernal, J. D. 1932 *Nature, Lond.*, **129**, 277; *Chem. Ind. Rev.* **10**, 466.
— 1933 *Chem. Ind. Rev.* **11**, 288.
Bernal, J. D. and Crowfoot, D. 1933 *Trans. Faraday Soc.* **29**, 1032.
— — 1934*a* *Nature, Lond.*, **134**, 809.
— — 1934*b* *Chem. Ind. Rev.* **12**, 953;.
— — 1935 *Chem. Ind. Rev.* **13**, p. 699.
Coffey, D. H., Heilbron, I. M., Spring, F. S. and Wright, H. R. 1935 *J. Chem. Soc.* p. 1205.
Crowfoot, D. 1936 X-ray crystallography and the chemistry of the sterols. Thesis for Ph.D. degree, University of Cambridge.
Crowfoot, D. and Bernal, J. D. 1937 *Chem. Weekbl.* **34**, 2, 1.
Dimroth, K. 1936 *Ber. dtsch. chem. Ges.* **69** B, 1123.
Heilbron, I. M., Coffey, D. H. and Spring, F. S. 1936 *J. Chem. Soc.* p. 738.
Heilbron, I. M., Hodges, J. and Spring, F. S. 1938 *J. Chem. Soc.* p. 759.
Heilbron, I. M., Kennedy, T., Spring, F. S. and Swain, G. 1938 *J. Chem. Soc.* p. 869.
Heilbron, I. M., Samant, K. M. and Spring, F. S. 1935 *Nature, Lond.*, **135**, 1072.
Heilbron, I. M., Spring, F. S. and Stewart, P. A. 1935 *J. Chem. Soc.* p. 1221.
Hendricks, S. B. 1933 *J. Opt. Soc. Amer.* **23**, 299.
— 1934 *Z. Kristallogr.* A, **89**, 427.
— 1935 *Z. Kristallogr.* A, **91**, 48.
Heyl, F. W. and Larsen, D. 1934 *J. Amer. Chem. Soc.* **56**, 942.
Laucht, F. 1935 *Hoppe-Seyl. Z.* **237**, 236.
Lettré, H. 1934 *Annalen. d. Chem.*, **511**, 280.
Müller, M. 1935 *Hoppe-Seyl. Z.* **233**, 233.
Robertson, J. M. 1936 *J. Chem. Soc.* p. 1817.
— 1937 *Reports on Progress in Physics*, p. 332.
— 1939*a* *Nature, Lond.*, **143**, 75.
— 1939*b* *J. Chem. Soc.* p. 232.

* Now National Research Fellow in Protein Chemistry, Massachusetts Institute of Technology, U.S.A.


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Rosenheim, O. and King, H. 1932 *Chem. Ind. Rev.* **10**, 464; *Nature, Lond.*, **130**, 315; *Chem. Ind. Rev.* **10**, 954.
— — 1935 *Chem. Ind. Rev.* p. 699.
Rosenheim, O. and Starling, W. W. 1937 *J. Chem. Soc.* p. 377.
Ruzicka, L. and Thomann, G. 1933 *Helv. Chim. Acta*, **16**, 221.
Ruzicka, L., Furter, M. and Goldberg, M. W. 1938 *Helv. Chim. Acta*, **21**, 498.
Sandqvist, H. and Gorton, J. 1930 *Ber. dtsch. chem. Ges.* **63**, 1935.
Schulze, G. E. R. 1935 *Z. Phys. Chem.* A, **171**, 436.
Tanret, C. 1889 *C.R. Acad. Sci., Paris*, **108**, 98.
— 1908 *C.R. Acad. Sci., Paris*, **147**, 75.
Wallis, E. S., Fernholz, E. and Gephart, F. T. 1937 *J. Amer. Chem. Soc.* **59**, 137.
Wieland, H. 1928 Les Prix Nobel en 1928 (Stockholm).
Wieland, H. and Dane, E. 1932 *Hoppe-Seyl. Z.* **210**, 268.
— — 1933 *Hoppe-Seyl. Z.* **216**, 91.
Windaus, A. 1928 Les Prix Nobel en 1928 (Stockholm).
Windaus, A. and Dimroth, K. 1937 *Ber. dtsch. chem. Ges.* **70**, 376.
Windaus, A. and Thiele, W. 1935 *Ann. Phys., Lpz.*, **521**, 160.
Windaus, A., v. Werder, F. and Gschaider, B. 1932 *Ber. dtsch. chem. Ges.* **65**, 1006.


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