887. *A Crystallographic Examination of the Structure of Lumisterol.*

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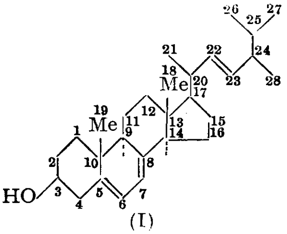
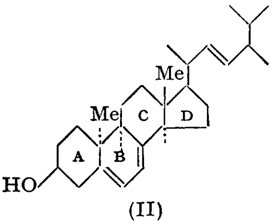
Most of the stereochemical features of the molecule of lumisterol have been elucidated by an *X*-ray diffraction study of the crystal structure of lumisteryl 4-iodo-3-nitrobenzoate. The configuration deduced earlier is confirmed for positions 3, 9, 10, 13, 14, and 17, with rings A and C in the chair form and rings C and D *trans*-fused. As proposed on chemical evidence, the change from the ergosterol structure that has occurred is inversion at $C_{(10)}$. But the retention of the chair form of ring A requires also a change of orientation of the $C_{(3)}$–OH bond from a direction nearly parallel with the nucleus to one approximately at right angles to it (κ–ε in Hassel's notation).

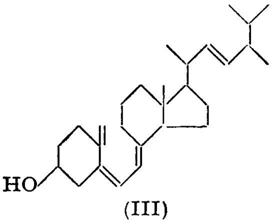
The present study is the third in a series of sterol crystal structure determinations carried out in this laboratory, the previous two compounds examined having been cholesteryl iodide (Carlisle and Crowfoot, *Proc. Roy. Soc.*, 1945, *A*, **184**, 64) and calciferyl 4-iodo-3-nitrobenzoate (Crowfoot and Dunitz, *Nature*, 1948, **162**, 608). Lumisterol (II) was selected for study as one of the group of compounds found during the photochemical transformation of ergosterol (I) into calciferol (III). Chemical evidence (Dimroth, *Ber.*, 1936, **69**, 1123; Kennedy and Spring, *J.*, 1939, 250) had indicated that, in the case of lumisterol, the transformation involved inversion at $C_{(10)}$, and this has been confirmed by the present investigation.



(I)



(II)



(III)

The compound chosen for *X*-ray analysis, lumisteryl 4-iodo-3-nitrobenzoate, was very kindly prepared for us by Dr. J. W. Cornforth. It crystallised in three different forms, of which two appeared to be solvated. The third, which was the one studied in detail, occurs in pale yellow, solvent-free prisms. Preliminary crystallographic data on these crystals may be summarised as follows :

Crystal Data.—$C_{35}H_{46}O_4NI$; $M = 671$. Found : *d*, 1·34. Calc. : *d*, 1·35. Pale yellow, monoclinic prisms, $\{001\}$, $\{\bar{1}01\}$ slightly elongated along the *b* axis. Optically biaxial, birefringence probably negative, $2V$ large, with $\alpha \parallel b$ and γ 48° to (001) in the acute angle. Unit-cell dimensions : $a = 10{\cdot}55$, $b = 7{\cdot}63$, $c = 20{\cdot}57$ Å, $\beta = 93{\cdot}5°$. Space group, $P2_1$. Two molecules per unit cell. Absorption coefficient for Cu-$K\alpha$ *X*-rays, $\mu = 86$ cm.$^{-1}$. Total number of electrons per unit cell = F(000) = 696.

Structure Analysis.—The *X*-ray analysis was a straightforward application of the heavy-atom technique. From the *b* axis projection of the Patterson function (Fig. 1) the *x* and *z* co-ordinates of the iodine atoms were found directly. On account of the crystal symmetry, the *y* co-ordinates of one atom can be arbitrarily chosen and the co-ordinates of the iodine atoms in the crystal could be given as : $x = \pm\ 0{\cdot}267, y = \pm\ 0{\cdot}25, z = \pm\ 0{\cdot}0354$.

By using signs based on the contributions of the iodine atoms alone and the observed structure factors, a first electron-density projection was calculated along the *b* axis. This projection is centrosymmetric, the structure factors are real, and the signs appropriate to the structure factors are very largely those based on the contributions of the iodine atom alone. The first *b*-axis projection accordingly approximates closely to a correct representation of the electron density (Fig. 2).

Although most of the individual atomic positions are unresolved, it proved possible to recognise the molecule in this first projection and to correlate the several concentrations of electron density with definite portions of the lumisterol structure. Study of models showed that of the different possible stereochemical forms of the sterol skeleton only one possessed the general features demanded by this projection. Thus the main line of peaks across the centre of the cell must correspond with the sterol nucleus, viewed edge on, while the nitrobenzoate group projects nearly at right angles to this line with the plane of the benzene ring close to the plane of the projection. This generally L-shaped form of the molecule as a whole, and the presence on opposite sides of the nucleus of two peaks which can hardly be other than the 18- and 19-methyl groups, establish the configurations at the asymmetric centres $C_{(3)}$, $C_{(10)}$, and $C_{(13)}$, and also the chair form of ring A. The straight-line appearance of the nucleus establishes the configuration at $C_{(9)}$, the chair form of ring C, and the configuration at $C_{(14)}$, *i.e.*, the *trans*-fusion of rings C and D. Finally the general location of the side chain establishes the configuration $C_{(17)}$.

FIG. 1. *Projection of Patterson function,* Pxz, *calculated for lumisteryl* 4-*iodo*-3-*nitrobenzoate.*

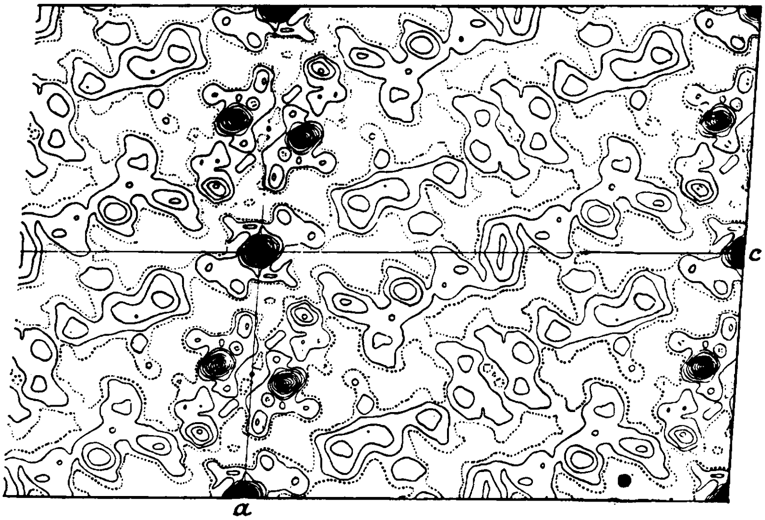
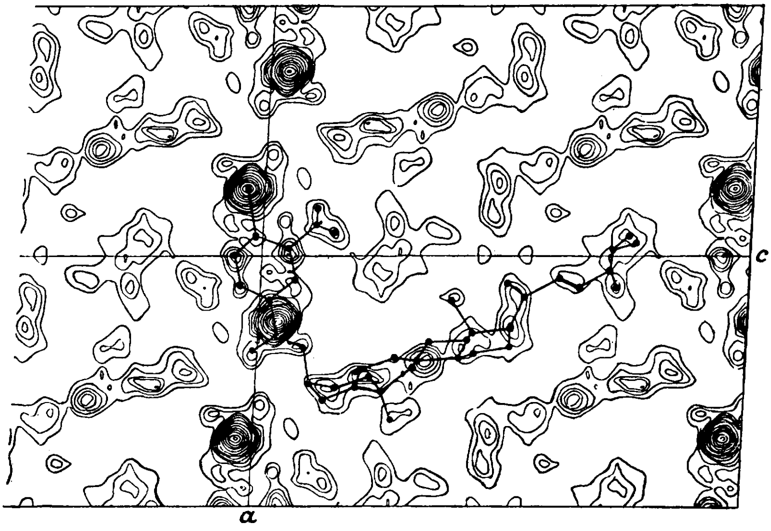
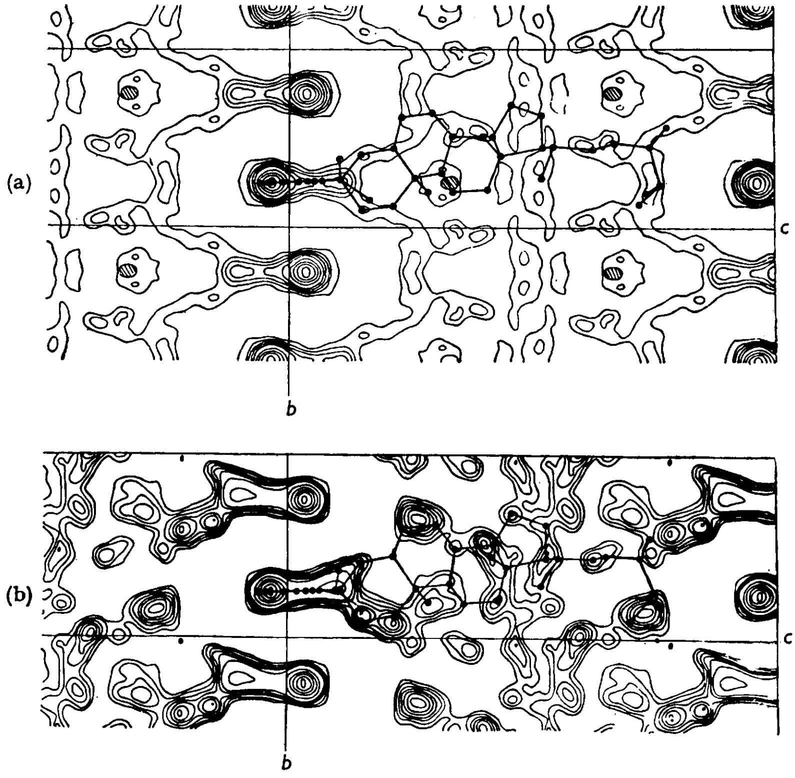


FIG. 2. *Projection of electron density along the* b *axis calculated for lumisteryl* 4-*iodo*-3-*nitrobenzoate, based on signs calculated on iodine positions only.*



The remaining calculations were carried out mainly as a further check on the atomic arrangement deduced at this stage. The *b*-axis projection was refined once, but little change occurred. The non-centrosymmetrical *a*-axis projection was then calculated with signs based, in the first instance, on the iodine positions only. Since the distribution of the iodine atoms is, by itself, centrosymmetrical, these signs correspond to a centrosymmetrical electron-density distribution. The *a* projection, as first calculated, accordingly possesses a spurious centre of symmetry and corresponds approximately to the correct projection of the two molecules in the unit cell, superimposed on those of two mirror-image molecules (Fig. 3*a*). Examination showed that it had the appearance expected of overlapping sterol molecules viewed along a line roughly at right angles to the plane of the ring systems. The model of the molecule which had been obtained from the *b*-axis projection was therefore placed over this second projection in the manner which seemed best to account for the observed electron-density maxima. From the tentative atomic positions thus obtained, phases were calculated and the non-centrosymmetrical distribution, Fig. 3*b*, was derived. This distribution confirms very satisfactorily the proposed structure on which it was based.

FIG. 3. *Projection of electron density along the* a *axis, calculated for lumisteryl* 4-*iodo*-3-*nitrobenzoate,* (a) *based on signs calculated on iodine positions only,* (b) *once refined.*



By the further use of models projected over the latest *a* and *b* electron-density projections together, the set of atomic co-ordinates listed in the Table was derived. We lay no claim to accuracy of these co-ordinates in detail. The validity of the molecular structure they define rests principally on its agreement with the general character of the projections observed, particularly the *b*-axis projection, but it is supported also by structure-factor calculations based on these co-ordinates. The reliability indices found were $R = 26\%$ for $(h0l)$ structure factors and $R = 21\%$ for $(0kl)$ structure factors.

The chemical form of the molecule deduced is illustrated by (II) and Fig. 4, which refer to the sterol portion of the structure only. The relations between the different asymmetric centres in the nucleus are those derived from chemical arguments as illustrated in (II). In addition rings A and C are in the chair form. The evidence obtained for the arrangement of atoms in the side chain is less strong than that for the nucleus. It confirms the configuration at $C_{(20)}$ found for cholesterol and calciferol but appears to contradict the configuration for $C_{(24)}$ tentatively proposed for calciferol (Crowfoot and Dunitz, *loc. cit.*). However, the evidence obtained for this configuration, both here and with calciferol, is not conclusive and this detail remains in doubt.

There are a number of interesting points about the crystal and molecular structure. As expected, the stereochemical difference between lumisterol and ergosterol is in the change of the 19-methyl group from the β- to the α-position. This change is associated with a rearrangement in the packing of the neighbouring atoms in ring A. The chair form is retained in ring A, through alternate puckering of the carbon atoms of the ring system from that likely in ergosterol. As a result the $C_{(3)}$-OH bond, which in ergosterol presumably lies near the general plane of the molecule (κ-bond in the notation of Hassel, *Tidsskr. Kjemi*, 1943, **3**, 32) is rotated to a position approximately normal to the molecular plane (ε-bond). In this position the arrangement of the hydroxyl group simulates that of the *epi*-configuration first proposed for lumisterol to account for its similarity to *epi*cholesterol in the digitonin precipitation reaction.

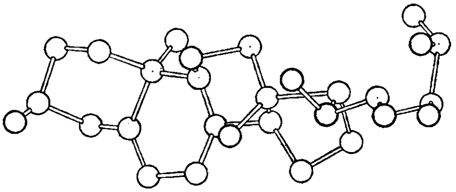
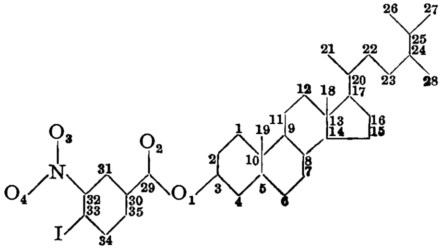


FIG. 4. *Perspective drawing of lumisterol in the stereochemical configuration found. Only carbon and oxygen atoms are shown.*

Final co-ordinates.



C at position	*x*	*y*	*z*
1	0·460	−0·120	0·226
2	0·515	−0·090	0·156
3	0·516	−0·263	0·116
4	0·595	−0·409	0·155
5	0·536	−0·448	0·222
6	0·485	−0·603	0·242
7	0·436	−0·625	0·311
8	0·438	−0·487	0·344
9	0·486	−0·298	0·337
10	0·536	−0·269	0·266
11	0·368	−0·187	0·361
12	0·353	−0·202	0·437
13	0·320	−0·389	0·451
14	0·429	−0·514	0·425
15	0·385	−0·686	0·462
16	0·377	−0·625	0·535
17	0·336	−0·439	0·535
18	0·177	−0·446	0·410
19	0·663	−0·194	0·290
20	0·188	−0·440	0·556
21	0·110	−0·286	0·522
22	0·167	−0·433	0·629
23	0·059	−0·451	0·654
24	0·037	−0·445	0·727
25	0·048	−0·250	0·755
26	0·128	−0·117	0·711
27	−0·100	−0·183	0·760
28	−0·079	−0·551	0·742
29	0·331	−0·257	0·038
30	0·188	−0·256	0·019
31	0·103	−0·261	0·068
32	−0·027	−0·260	0·052
33	−0·069	−0·252	−0·011
34	0·017	−0·247	−0·062
35	0·145	−0·250	−0·046
O			
1	0·374	−0·263	0·100
2	0·393	−0·252	−0·013
3	−0·085	−0·155	0·147
4	−0·212	−0·360	0·098
N	−0·107	−0·263	0·098
I	−0·267	−0·250	−0·0354

The change of orientation of the hydroxyl group in lumisterol is a consequence of the preferred arrangement of the atoms of ring A in the staggered chair form of the *cyclo*hexane ring. The situation is parallel with that found in *cis*-decalin (Bastiansen and Hassel, *Nature*, 1946, **157**, 765) and probably also in the choleic acids * and, as in these cases, results in the production of a very awkwardly shaped molecule. However, the overall shape of the molecule of lumisteryl 4-iodo-3-nitrobenzoate is completely masked in the preliminary crystallographic data through the intricate way in which the large substituent groups are fitted between the ends of the sterol ring systems. The molecular arrangement is determined by the relative packing of the main part of the sterol nuclei and side chains of neighbouring molecules in a way exactly similar to that expected for normal steroids and this is reflected in the unit-cell dimensions, which are very similar to those of many normal steroid crystal structures. The crystal refractive indices, on the other hand, are largely controlled by the orientation of the substituent nitrobenzoate group and do not conform to the normal type of relation to the cell dimensions (Bernal, Crowfoot, and Fankuchen, *Phil. Trans. Roy. Soc.*, 1940, *A*, **139**, 135).

These observations emphasise the difficulty of drawing conclusions about certain types of stereochemical configuration from preliminary *X*-ray data and suggest a resolution of an apparent conflict in the literature between present theory and early crystallographic data on coprostane, which has been discussed by Shoppee (*Ann. Reports*, 1946, **43**, 200) and by Turner (Fieser and Fieser, " Natural Products related to Phenanthrene," Chap. X, Reinhold Publ. Corpn., N.Y.). That coprostane has unit-cell dimensions belonging to one of the normal steroid types can easily be accounted for by the lath-like character of the major part of the molecule; it does not provide any real evidence of a boat-shaped structure for ring A. As in the lumisterol crystal structure, the unit-cell dimensions almost certainly conceal the presence in this molecule of the chair form of ring A.

A final point of a rather different kind may be made. The mechanism of the transformation of ergosterol into lumisterol and its relation to the production of calciferol need further investigation. Stereochemically, it is unlikely that the 19-methyl group migrates directly to the other side of the steroid nucleus; this would involve its passage between carbon atoms initially themselves only 2½ Å apart. Most probably the initial stage is rupture between $C_{(10)}$ and one of the carbon atoms of the ring system; either $C_{(9)}$–$C_{(10)}$, which is certainly broken in calciferol, or $C_{(5)}$–$C_{(10)}$ † (cf. the production by an irradiation reaction of lumiœstrone and lumiandrosterone) are likely points of attack. In either case, lumisterol itself would then be formed by closure of the ring system in the new stereochemical configuration. It would not correspond with a definite stage in the transformation of ergosterol into calciferol but would be the product of a side-reaction, and would be capable itself of undergoing transformation into calciferol. These conclusions are in agreement with the early observations of Setz (*Z. physiol. Chem.*, 1933, 215) that the production of lumisterol and calciferol is favoured by light of different wave-lengths, but in detail the course of the reactions is still obscure.

EXPERIMENTAL

Preparation and Characterisation of the Three Crystalline Forms.—Recrystallisation of Dr. Cornforth's original material was accomplished by cooling or evaporation of acetone or benzene solutions. The properties of the three modifications are tabulated below. Forms A and B, if uncoated, deteriorate rapidly and become opaque after a few days, but C remains unchanged for months. For this reason, and because form C has the smallest unit cell and alone can be

	Source	Form	*a*	*b*	*c*	*β*
A	Original material; also by evaporation of benzene solution at 25°	Prism	10·5	7·6	24·6	102°
B	Evaporation of acetone solution at 25°	Needle	17·8	7·5	27·3	103°
C	Cooling acetone or benzene solution from 55°	Prism	10·55	7·63	20·57	93·5°

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* This has not been established in detail but is strongly suggested by the electron-density projections calculated by Giacomello and Caglioti, *Gazzetta*, 1939, **69**, 245.

† As suggested to us by Dr. Cornforth.

obtained from either acetone or benzene, it is presumed to be the only unsolvated form, and all the further work was done on it.

Analysis of the original materials by Drs. Weiler and Strauss, Oxford, supported the view that this preparation, which largely consisted of form A, m. p. 136·5—137°, contained benzene of crystallisation (Found: C, 65·2; H, 7·0; loss at 100°, 9·9. $C_{35}H_{46}O_4NI,C_6H_6$ requires C, 65·5; H, 6·95; C_6H_6, 10·4. Found, on dried material: C, 62·9; H, 7·5; I, 18·75. $C_{35}H_{46}O_4NI$ requires C, 62·6; H, 6·85; I, 18·9%).

X-*Ray Measurements*.—Copper-$K\alpha$ radiation was used throughout. The intensities of the ($h0l$) and ($0kl$) reflections were estimated visually from multiple-film Weissenberg photographs; 392 out of a possible 564 F($h0l$)'s, and 127 out of a possible 217 F($0kl$)'s were estimated. On the edges of these Weissenberg photographs had been recorded the diffraction pattern of a fine copper wire, and the unit-cell dimensions were determined with the aid of this calibrating pattern. The lattice constant of copper was taken to be 3·6153 Å.

The dimensions of the crystal used were 0·12 × 0·15 × 0·15 mm. It was estimated that the extremes of X-ray transmission for this crystal were 0·32 and 0·39, and it was decided that this variation was not severe enough to make absorption corrections necessary.

The absolute scales were obtained by correlation with the final calculated F($h0l$)'s and F($0kl$)'s.

Calculations.—The b-axis Patterson projection (Fig. 1) is a " sharpened " projection, based on the ($h0l$) intensities divided through by the square of the f curve of iodine with a temperature factor $B = 3{\cdot}6 \times 10^{-16}$. All Fourier sums were performed with Beevers–Lipson strips and a desk adding machine, at intervals of 1/60 along c and 1/30 along a and b. All structure-factor calculations were carried out by a technique which reduces structure-factor calculation to Fourier series summation (Sayre, *Acta Cryst.*, 1951, **4**, 362) and which, it is estimated, required only about 1/3 of the usual time. In all the structure-factor calculations CH_3 was treated as a carbon atom of atomic number 9, O and CH_2 as of 8, N and CH as of 7, and C as one of 6. Viervoll and Ögrim's f curve (*Acta Cryst.*, 1949, **2**, 277) was used for carbon, and that of the " Internationale Tabellen zur Bestimmung von Kristallstructuren " for iodine. In the earlier calculations a temperature factor $B = 3{\cdot}5 \times 10^{-16}$ was applied to all atoms; in the final calculations an empirical overall correction curve was used instead, chosen to bring about the best agreement between $F_{obs.}$ and $F_{calc.}$, and approximately equivalent to a temperature correction with $B = 6{\cdot}5 \times 10^{-16}$.

We acknowledge gratefully grants from the Rockefeller and Nuffield Foundations and also personal assistance from Mrs. U. K. Ledwith in the calculations.

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[*Received, May 14th*, 1952.]