366. *Steroids of Unnatural Configuration. Part IX.* Oxidation of 9α-Lumisterol (Pyrocalciferol) and 9β-Ergosterol (Isopyrocalciferol) with Perbenzoic Acid*

By G. M. L. Cragg and G. D. Meakins

The oxidations of 9α-lumisterol (5) and 9β-ergosterol (20) with perbenzoic acid in benzene at 5° proceed rapidly, the half-lives of the reactions being less than 30 seconds. In both cases oxidation occurs preferentially at the 5,6-double bond, and predominantly on the side opposite to the 10-methyl group, *i.e.* on the β-face in 9α-lumisterol and on the α-face in 9β-ergosterol.

The major products from 9α-lumisterol are the 6-benzoates, (6) and (7), of the 3β,5β,6β- and 3β,5β,6α-triols: a minor product is considered to be the 5α-hydroxy-3β,6β-epoxide (9) rather than the expected 3β-hydroxy-5β,6β-epoxide. With 9β-ergosterol the 6α-benzoyloxy-3β,5α-diol (22) was accompanied by dehydroergosterol (24). Oxidations of the corresponding 3-acetates, (17) and (21), showed surprising differences from those of the parent sterols, the main product from 9α-lumisteryl acetate being the 3β-acetoxy-5α-hydroxy-$\Delta^{6,8}$-diene (19).

In this series of compounds the positions and configurations of the hydroxyl groups established by chemical methods are fully confirmed by spectrographic studies.

In continuing work on the oxidation of steroid ring-B dienes [1,2] we have studied the reactions of 9α-lumisterol and 9β-ergosterol with perbenzoic acid. Determination of the structures of the products, particularly the stereochemical details, was facilitated by examining the O–H stretching absorptions of dilute solutions under high dispersion,[3] and the configurations of the diols and triols obtained can be deduced from the spectrographic data.[4] However, in the present account, the structural arguments are based mainly on chemical evidence, and to simplify presentation the structures finally established for the products are used from the outset.

Whilst treatment of lumisterol (1) with perbenzoic acid in chloroform affords the triol monobenzoate (3) as the main product [5] the oxidation is slower in benzene solution, and after 22 hours at 5° the 5,6-epoxide (2; R = H) is obtained in good yield.[1] It seemed reasonable that 9α-lumisterol (5) would similarly yield a 5,6-epoxide, but in fact a complex mixture of products was formed. Chromatographic separation gave the 3β,5β-dihydroxy-6β-benzoyloxy-compound (6) (66%), the corresponding 6α-benzoate (7) (14%), and a hydroxy-epoxide (13%), m. p. 165°, which (see later) was not a 5,6-epoxide. This prompted an investigation of the rate of the oxidation by following the disappearance of 9α-lumisterol ($\lambda_{max.}$ 287 mμ) spectrographically. The oxidation was surprisingly fast, half the 9α-lumisterol having reacted in 22 seconds: working up after 10 minutes, followed by chromatography, afforded the 6β-benzoate (6) (19%), the 6α-benzoate (7) (9%), the hydroxy-epoxide (m.p. 165°; 12%), and the 3β,5β,6α-triol (8) (36%).

The chemical approach to the structures of the triol monobenzoates (6) and (7) is described only briefly since it resembles previous work in the lumisterol [1] and ergosterol [2] series. Hydrolysis of the 6β-compound gave a triol (10), called the first triol, which readily formed an acetonide (12). Oxidation of the latter afforded a product (14) in which the carbonyl group was not conjugated and did not become so on treatment with alkali: the

* Part VIII, J. Castells, G. D, Meakins, and R. Swindells, *J.*, 1962, 2917.

[1] P. A. Mayor and G. D. Meakins, *J.*, 1960, 2792; G. D. Meakins and M. W. Pemberton, *J.*, 1961, 4676.

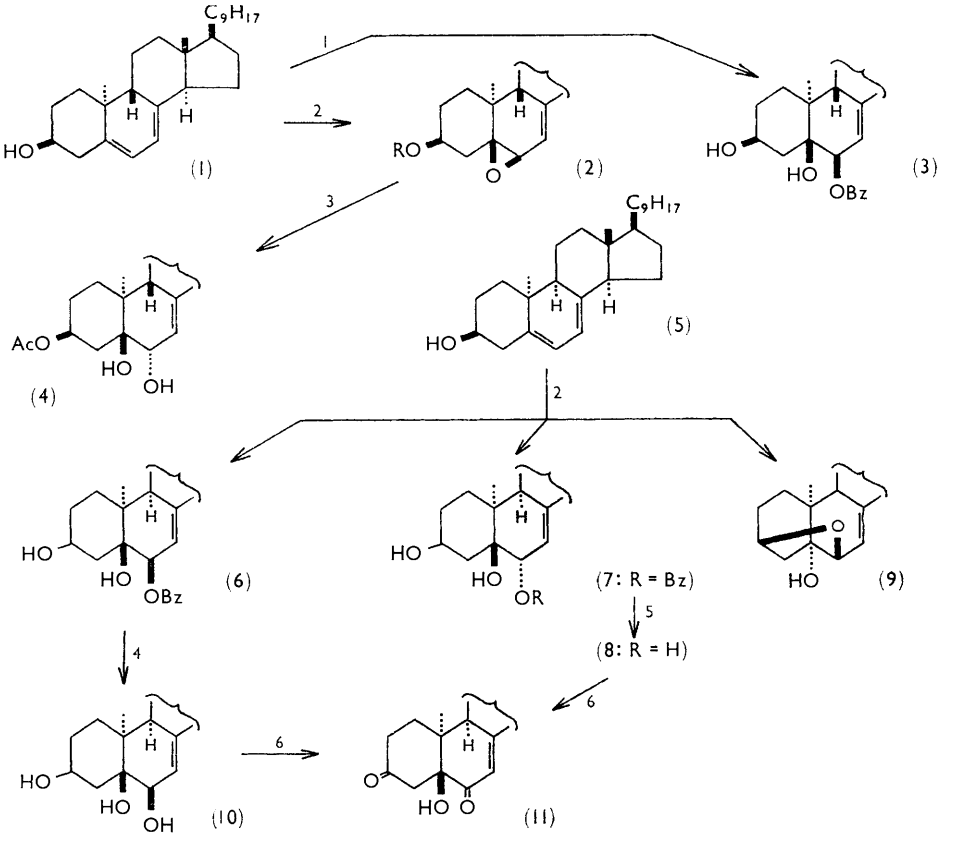
[2] F. Dalton and G. D. Meakins, *J.*, 1961, 1880.

[3] F. Dalton, J. I. McDougall, and G. D. Meakins, *J.*, 1963, 4068.

[4] G. M. L. Cragg and G. D. Meakins, unpublished work.

[5] I. M. Heilbron, F. S. Spring, and P. A. Stewart, *J.*, 1935, 1221.

carbonyl group is therefore at position 3, the triol is a 3,5,6-trihydroxy-compound, and formation of the acetonide involves *cis*-hydroxyl groups at positions 5 and 6. Reduction of the other triol monobenzoate (7) with lithium aluminium hydride afforded a second triol (8), which did not form an acetonide. Both triols were converted by mild oxidation into the hydroxy-diketone (11), showing that they differ only in their configurations at position



Reagents: 1, BzO_2H–$CHCl_3$; 2, BzO_2H–C_6H_6; 3, Al_2O_3(active); 4, KOH; 5, $LiAlH_4$; 6, CrO_3–$COMe_2$

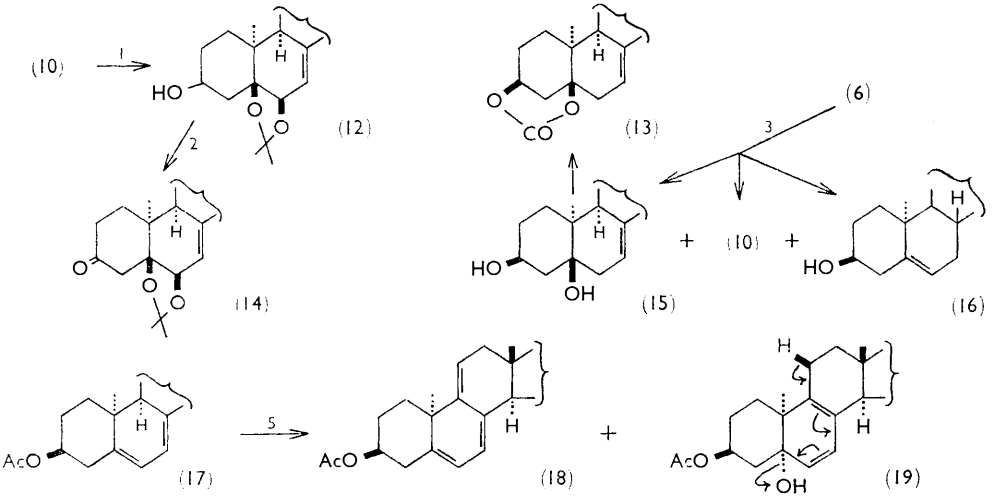
6. [Hydrolysis of the 6α-benzoate (7) with alkali gave a mixture of the second triol (8) and a third triol. The hydroxy-diketone (11) was obtained in low yield (27%) by oxidising the mixture, whose behaviour towards acetylation suggested that the third triol has two tertiary hydroxyl groups. This triol, which was not isolated, may be a 3,5,6-trihydroxy-Δ^6-compound.]

To establish the configuration of the 5-hydroxyl group in these compounds one of the triol monobenzoates [the 6β-compound (6)] was reduced with lithium in ethylamine. The required 3,5-diol (15), (30%), was obtained, together with the first triol (10) and a mixture of dihydro-9α-lumisterols in which the $\Delta^{5,22}$ isomer (16) predominated. Bridging of the diol's hydroxyl groups with phosgene to the cyclic carbonate (13) showed that the 5-hydroxyl group has the same (β) configuration as the 3-hydroxyl group, thus completing the structural investigation of the triols and their derivatives.

The hydroxy-epoxide, m.p. 165°, a minor oxidation product of 9α-lumisterol, was originally thought to be the expected 3-hydroxy-5,6-epoxide. However, its stability towards attempted acetylation, reduction (with lithium aluminium hydride), and ring-opening (with benzoic acid) contrasts sharply with the behaviour of lumisterol 5,6-epoxide.[1]

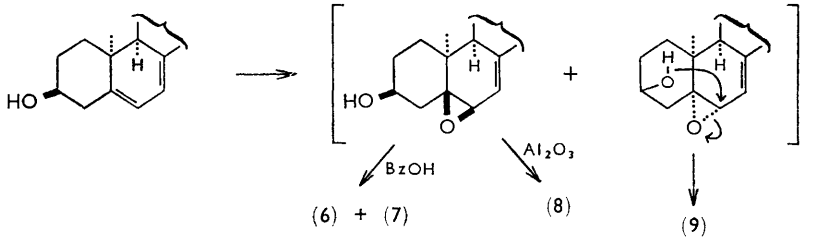
Formulation of the product as the 5-hydroxy-3,6-epoxide (9) is supported by the strong infrared absorption at 975 cm.$^{-1}$,[6] and by the interpretation of the course of the oxidations which follows.

Comparison of the results obtained in the peracid oxidations shows that the main reaction involves the rapid formation of an unstable intermediate which is slowly converted into the two triol monobenzoates (6) and (7) in the long-period oxidation, and which



Reagents: 1, $COMe_2$–$CuSO_4$; 2, CrO_3–$COMe_2$; 3, Li–Et·NH_2; 4, $COCl_2$; 5, BzO_2H–C_6H_6

generates the 3β,5β,6α-triol (8) during the working up of the short-period oxidation product. The most probable explanation is that 9α-lumisterol [7] quickly forms the 5β,6β-epoxide as the major intermediate together with a smaller amount of the α-isomer and that both these oxides are unstable. In the α-compound the stereochemistry is favourable for an intramolecular rearrangement to the more stable 3,6-epoxide (9). With the β-epoxide, which cannot undergo a similar rearrangement, ring-opening by benzoic acid in the long-period oxidation leads to the triol monobenzoates, *cis*-opening of the oxide ring predominating:



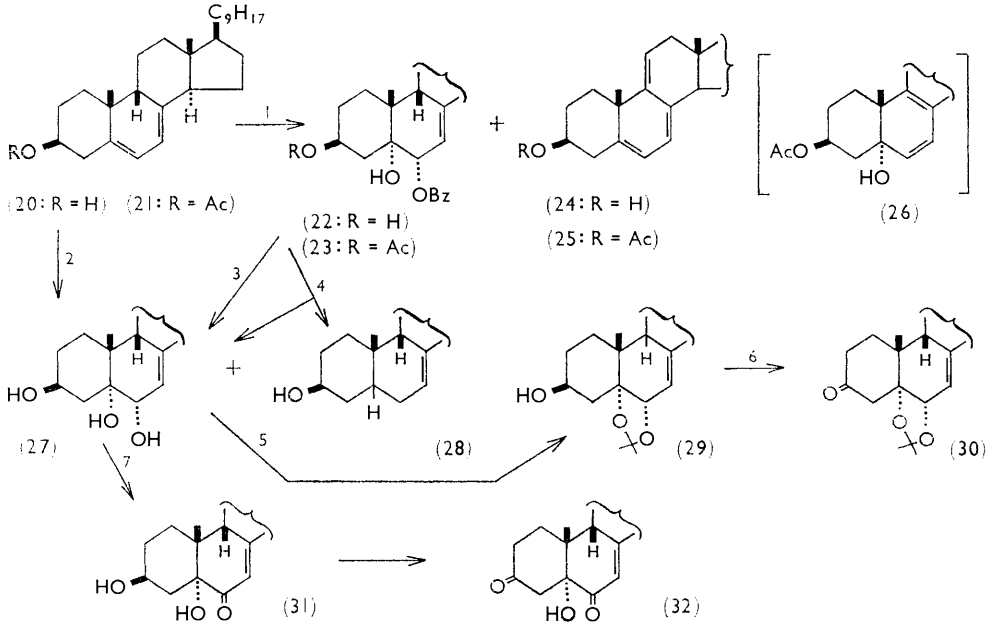
chromatography following the short-period reaction causes *trans*-opening to give the 3β,5β,6α-triol (8). The salient facts supporting these proposals are: (*a*) the triols, their 6-benzoates, and the 3,6-epoxide (9) are stable under the chromatographic conditions used in this work, (*b*) opening of lumisterol 5β,6β-epoxide (2; R = H) with benzoic acid gives mainly the 6-benzoate of the 3β,5β,6β-triol,[1] and (*c*) although the 5β,6β-epoxide of lumisteryl acetate (2; R = Ac) is stable on deactivated alumina [1] contact with more active material produces the 3-acetate (4) of the 3β,5β,6α-triol in high yield. Thus, ring-opening by benzoic acid is of S_N1 character and the stereochemistry is probably governed by hindrance of the 10α-methyl group to approach of the benzoyloxy-anion at the α-face: conversely the

[6] H. R. Arthur and W. H. Hui, *J.*, 1961, 551.
[7] J. Castells, E. R. H. Jones, G. D. Meakins, S. Palmer, and R. Swindells, *J.*, 1962, 2907.

S_N2-type reaction on alumina requires an appreciable " push " from the entering nucleophile which must then adopt the α-configuration.

Two more oxidations were carried out to confirm the structures of the products described above, but neither followed the expected path. In the first, 9α-lumisterol was treated with osmium tetroxide since this reagent with lumisterol gives the 3β,5β,6β-triol.[1] However a complex mixture was obtained from 9α-lumisterol, and after acetylation two hydroxyacetates were isolated in low yield. In these compounds (*M*, 454) the acetoxyl : hydroxyl ratio was much higher than 2 : 1, and their structures remain obscure. The second experiment, oxidation of 9α-lumisteryl acetate (17) with perbenzoic acid, produced a mixture of dehydrolumisteryl acetate (18), (8%), and a compound (86%) for which structure (19) is proposed, mainly from its ultraviolet absorption (characteristic of a $\Delta^{6,8}$-diene system) and the *trans*-relationship (demonstrated by infrared examination [4]) between the 3-acetoxyl and 5-hydroxyl groups. It is likely that the dehydrolumisteryl acetate is formed from the $\Delta^{6,8}$-diene (19) either during the reaction or in the subsequent chromatography.

With 9β-ergosterol (20) [8] attack by oxidising agents was found to occur predominantly on the α-face, as would be expected with a molecule having the natural (β) 10-methyl configuration. In the long-period reaction with perbenzoic acid the products were the triol monobenzoate (22), (74%), and dehydroergosterol (24), (19%). The products were also isolated in the shorter-period oxidation but the yields were appreciably lower (50 and 7%, respectively) and a spectrographic examination showed that the initial attack on the 9β-ergosterol was extremely fast (half-reaction time 15 seconds). It seems most likely that the main reaction involves very rapid formation of the 5α,6α-epoxide followed fairly quickly



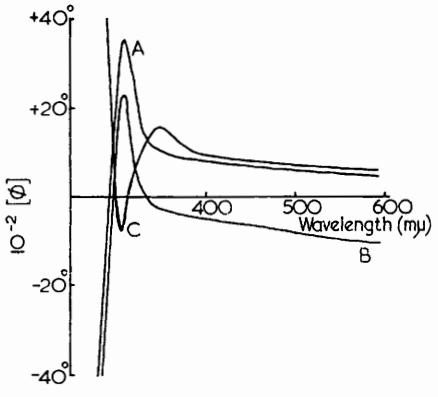
Reagents: BzO_2H–C_6H_6; 2, O_5O_4; 3, $LiAlH_4$ or KOH; 4, Li–Et·NH_2; 5, $COMe_2$–$CuSO_4$; 6, CrO_3–$COMe_2$; 7, CrO_3–C_5H_5N

by *cis*-ring opening: a competing minor reaction leads to dehydroergosterol, possibly by hydride abstraction from position 9. The apparently trivial variation of using the 3-acetate (21) again influenced the course of the reaction, but not so markedly as with

[8] H. B. Henbest and S. Palmer (unpublished work) have made a systematic study of the reduction products of 9β-ergosterol.

9α-lumisterol. In the long-period oxidation of 9β-ergosteryl acetate the triol derivative (23), (50%), and dehydroergosteryl acetate (25), (8%) were accompanied by a substance which, although it could not be fully purified, showed light absorption corresponding to the $\Delta^{6,8}$-diene structure (26).

9β-Ergosterol behaved normally with osmium tetroxide giving a good yield of the triol (27) obtained by hydrolysis or hydride reduction of the triol monobenzoate (22). Formation and examination of the hydroxy-acetonide (29) and the oxo-acetonide (30) established all structural details exept the relative configurations of the 3-hydroxyl group and the *cis*-5,6-dihydroxy-system. Attempts to investigate this point by preparing a 3,5-diol were unsuccessful, reduction of the triol monobenzoate (22) with dissolving metals under various conditions giving only the triol (27) and the 5β-dihydro-compound (28). However, selective oxidation of the triol (27) with chromic oxide in pyridine gave a dihydroxy-ketone which was further oxidised in acetone solution to the hydroxy-diketone (32). In the dihydroxy-ketone, shown to be the 6-oxo-compound (31) by spectrographic examination,



Optical rotatory dispersion curves of (A) 5α,6α-isopropylidenedioxy-9β-ergosta-7,22-dien-3-one, (B) 5α,6α-isopropylidenedioxy-ergosta-7,22-dien-3-one, and (C) 5β,6β-isopropylidenedioxy-9α-lumista-7,22-dien-3-one

the hydroxyl groups at positions 3 and 5 do not exhibit intramolecular hydrogen bonding [4] and therefore have opposite configurations. The α-orientation of the 5-hydroxyl group in the oxidation products is strongly supported by the optical rotatory dispersion curves of the oxo-acetonides, shown in the Figure. (We are grateful to Professor W. Klyne for these examinations.) It is clear that the ergosterol and 9β-ergosterol derivatives are structurally similar whilst the 9α-lumisterol compound is of the enantiomeric type.

Inspection of the molecular rotation data (see Table) shows a consistent pattern for the

Molecular rotations ($[M]_D$) in chloroform

Lumista-7,22-dien-5β-ols					Ergosta-7,22-dien-5α-ols				
Substituents					Substituents				
3	6	9α	9β	$\Delta[M]_D$	3	6	9α	9β	$\Delta[M]_D$
β-OH	α-OH	+630°	+198° *	+432°	β-OH	β-OH	+125° †‡	−202°	+327°
β-OAc	α-OAc	+1180	+792 *	+388	β-OAc	β-OAc	+216 †	−545	+741
β-OH	—	+505	+33 *	+472	β-OH	β-OBz	+262 †	−475	+737
β-OAc	—	+474	+132 *	+342	β-OAc	β-OBz	+253 †	−651	+904
β-OH	β-OH	+485	−21	+506	O	O	−55	+9	−64
β-OAc	β-OAc	+231	−211	+442					
β-OH	β-OB_2	+16	−352	+368					
O	O	+895	−72	+967					

* Values from ref. 1. † Values from Elsevier's "Encyclopaedia of Organic Compounds," Vol. XIV and supplements. ‡ In pyridine.

lumisterol and 9α-lumisterol series which confirms the structural proposals. In all cases the $\Delta[M]_D$ (9α − 9β) value is positive, and with the exception of the dihydroxy-ketones the figures are reasonably close to an average of 425 units. With the 9β-ergosterol–ergosterol

pairs the numerical agreement is somewhat less satisfactory: the hydroxy-diketones are again anomalous and here they lead to a negative $\Delta[M]_D$ value.

The results of the present and previous [1] studies show that lumisterol, 9α-lumisterol, and 9β-ergosterol differ markedly in their behaviour to oxidation with perbenzoic acid, and that in two of the cases there are further differences between the sterols and their acetates. Although the reaction between ergosteryl acetate and perphthalic acid has been investigated in detail,[9] only the nature of the main product is known in the oxidation of ergosterol with perbenzoic acid.[10] A closer examination of the latter reaction is required before the influence of stereochemical changes at positions 9 and 10 on the peracid oxidation can be satisfactorily discussed.

Experimental

Melting points were determined on a Kofler hot-stage apparatus and are corrected. Rotations were determined at 20° using a Bendix–Ericcson automatic polarimeter with chloroform solutions. Ultraviolet spectra were recorded for ethanol solutions with a Cary 14M spectrometer, and routine infrared spectra for carbon tetrachloride or carbon disulphide solutions with Perkin-Elmer model 21 or 237 spectrometers: more precise infrared data for many of the products will be reported later.[4] Neutral alumina was prepared by stirring P. Spence material (grade H) with an excess of ethyl acetate for 2 days, filtration, washing repeatedly with hot water and then hot distilled water, and heating at 250° for 2 days. Deactivated alumina was prepared by shaking neutral material with water (5% by weight) for 1 day. The silica gel used was B.D.H. chromatographic grade, and the purity of products was checked using " unbaked " silica chromatoplates. Light petroleum refers to the fraction with b. p. 40—60°.

Oxidation of 9α-Lumisterol with Perbenzoic Acid.—(*a*) *Long-period reaction.* An 0·18M-solution of perbenzoic acid in dry benzene (35 ml.) was added at 5° to 9α-lumisterol (2·5 g.) in benzene (25 ml.). After 22 hr. at 5° the mixture was poured on to a column of deactivated alumina (200 g.). Elution with benzene gave 3β,5β-*dihydroxy*-9α-*lumista*-7,22-*dien*-6β-*yl benzoate* (6) (1·9 g.), m. p. 154—155° (from aqueous acetone), $[\alpha]_D$ +30° (*c* 0·6) (Found: C, 78·6; H, 9·45. $C_{35}H_{50}O_4$ requires C, 78·6; H, 9·4%), $\nu_{max.}$ 1723 cm.$^{-1}$.

Elution with benzene–ether (19 : 1) gave material (536 mg.) which was rechromatographed (see below) followed by 3β,5β-*dihydroxy*-9α-*lumista*-7,22-*dien*-6α-*yl benzoate* (7) (400 mg.), m. p. 181—182° (from aqueous acetone), $[\alpha]_D$ +230° (*c*, 0·5) (Found: C, 78·65; H, 9·3%), $\nu_{max.}$ 1729 cm.$^{-1}$.

The above material (536 mg.) was dissolved in light petroleum–benzene (1 : 1) and chromatographed on alumina (50 g.). In graded development, elution with benzene–ether (4 : 1) afforded a compound formulated as 3β,6β-*epoxy*-9α-*lumista*-7,22-*dien*-5α-*ol* (9) (320 mg.), m. p. 165—166° (from aqueous acetone), $[\alpha]_D$ +17° (*c* 1·1) (Found: C, 81·45; H, 10·75. $C_{28}H_{44}O_2$ requires C, 81·5; H, 10·75%), $\nu_{max.}$ 3580 and 975 cm.$^{-1}$.

(*b*) *Short-period reactions.* Perbenzoic acid in benzene (0·3 ml. of an 0·2M-solution) and 9α-lumisterol (15·6 mg.) in benzene (25 ml.) were mixed at 5°. The intensity of absorption at 287 mμ decreased to half the original value in 22 sec.

0·19M-Perbenzoic acid in benzene (16 ml.) and 9α-lumisterol (1·2 g.) in benzene (13 ml.) were kept at 5° for 10 min. and then poured on to deactivated alumina (100 g.). Elution with benzene gave the 6β-benzoate (6) (200 mg.) m. p. 154—155°, identical with authentic material. Elution with benzene–ether (19 : 1) gave impure epoxide (9) (200 mg.) followed by the 6α-benzoate (7) (100 mg.), m. p. 180—181°, these products being identified by infrared examination. Elution with benzene–ether (4 : 1) afforded 9α-lumista-7,22-diene-3β,5β,6α-triol (8) (350 mg.), m. p. 180—181°, identified by comparison with authentic material described below.

9α-*Lumista*-7,22-*diene*-3β,5β,6β-*triol* (10).—The 6β-benzoate (6) (700 mg.) was refluxed for 3 hr. with 5% methanolic potassium hydroxide (50 ml.). Standard manipulation gave the 3β,5β,6β-*triol* (540 mg.), m. p. 183—184° (from aqueous acetone), $[\alpha]_D$ +113° (*c* 0·6) (Found: C, 78·2; H, 10·65. $C_{28}H_{46}O_3$ requires C, 78·1; H, 10·75%).

Acetylation with acetic anhydride–pyridine at 20° gave the 3β,6β-*diacetate*, m. p. 113—114° (from aqueous acetone), $[\alpha]_D$ +45° (*c* 0·5) (Found: C, 74·4; H, 9·6. $C_{32}H_{50}O_5$ requires C, 74·65; H, 9·8%), $\nu_{max.}$ 3590, 1739, and 1240 cm.$^{-1}$.

[9] G. H. Alt and D. H. R. Barton, *J.*, 1954, 1356.
[10] A. Windaus and A. Lüttringhaus, *Annalen*, 1930, **481**, 119.

9α-*Lumista*-7,22-*diene*-3β,5β,6α-*triol* (8).—The 6α-benzoate (7) (100 mg.) was refluxed in ether (20 ml.) with lithium aluminium hydride (200 mg.) for 2 hr. Standard treatment gave the 3β,5β,6α-*triol* (65 mg.), m. p. 180—181°, $[\alpha]_D$ +158° (*c* 0·5) (Found: C, 78·0; H, 10·6. $C_{28}H_{46}O_3$ requires C, 78·1; H, 10·75%). Acetylation at 20° afforded the 3β,6α-*diacetate*, m. p. 148—149° (from aqueous acetone), $[\alpha]_D$ +230° (*c* 0·6) (Found: C, 74·65; H, 10·05. $C_{32}H_{50}O_5$ requires C, 74·65; H, 9·8%), $\nu_{max.}$ 3600, 1740, and 1239 cm.$^{-1}$.

Alkaline Hydrolysis of 3β,5β-*Dihydroxy*-9α-*lumista*-7,22-*diene*-6α-*yl Benzoate* (7).—The benzoate (320 mg.) was refluxed with 5% methanolic potassium hydroxide (25 ml.) for 3 hr. Working up gave a mixture of triols (260 mg.), m. p. 128—142° (from aqueous acetone), $[\alpha]_D$ +198° (*c* 0·5) (Found: C, 78·1; H, 10·85. $C_{28}H_{46}O_3$ requires C, 78·1; H, 10·75%).

Acetylation of the mixture (140 mg.) at 20° for 72 hr. followed by chromatography on alumina (20 g.) and elution with light petroleum–benzene (1 : 1) gave material (60 mg.), m. p. 113—115° (from aqueous acetone), $[\alpha]_D$ +186° (*c* 0·5). Infrared examination (ε 700 for the acetate peak at 1740 cm.$^{-1}$) suggested that the material was a mixture of a mono- and a di-acetate [Found: C, 75·6; H, 9·85. $C_{32}H_{50}O_5$ (diacetate) requires C, 74·65; H, 9·8; and $C_{30}H_{48}O_4$ (monoacetate) requires C, 76·2; H, 10·25%].

Oxidation of the triol mixture (100 mg.) in acetone (10 ml.) with 8N-chromic acid, followed by chromatography on deactivated alumina (10 g.) and elution with benzene–ether (9 : 1) afforded 5β-hydroxy-9α-lumista-7,22-diene-3,6-dione (11) (27 mg.), m. p. 194—197°, identical with authentic material described below.

5β-*Hydroxy*-9α-*lumista*-7,22-*diene*-3,6-*dione* (11).—A solution of the 3β,5β,6β-triol (10) (130 mg.) in acetone (10 ml.) was treated with 8N-chromic acid (0·2 ml.). After 15 sec. at 20° water was added and the mixture extracted with ether to give a product (110 mg.) which was chromatographed on deactivated alumina (10 g.). Elution with benzene–ether (9 : 1) gave the *hydroxy-dione* (78 mg.), m. p. 194—197° (from aqueous acetone), $[\alpha]_D$ +210° (*c* 0·6) (Found: C, 78·5; H, 10·0. $C_{28}H_{42}O_3$ requires C, 78·8; H, 9·9%), $\lambda_{max.}$ 249 mμ (ε 15,800), $\nu_{max.}$ (Nujol) 3400, 1714, 1686, and 1639 cm.$^{-1}$.

The 3β,5β,6α-triol (8) (120 mg.) similarly gave the hydroxy-dione (75 mg.) m. p. and mixed m. p. 193—196°.

5β,6β-*Isopropylidenedioxy*-9α-*lumista*-7,22-*dien*-3β-*ol* (12).—The 3β,5β,6β-triol (10) (400 mg.) in dry acetone (100 ml.) was refluxed with anhydrous copper sulphate (3 g.) for 3 days and the product chromatographed on deactivated alumina (20 g.). Elution with benzene gave the *hydroxy-acetonide* (121 mg.), m. p. 157—158° (from aqueous acetone), $[\alpha]_D$ +138° (*c* 0·6) (Found: C, 79·2; H, 10·55. $C_{31}H_{50}O_3$ requires C, 79·1; H, 10·7%), $\nu_{max.}$ 3600 and 1021 cm.$^{-1}$. Elution with ether gave unchanged triol (10) 235 mg.), m. p. and mixed m. p. 183—184°.

The hydroxy-acetonide (75 mg.) in acetone (10 ml.) was oxidised with 8N-chromic acid (0·18 ml.) for 30 sec. at 20°. Standard manipulation gave 5β,6β-*isopropylidenedioxy*-9α-*lumista*-7,22-*dien*-3-*one* (14) (64 mg.), m. p. 181—182° (from aqueous acetone), $[\alpha]_D$ +121° (*c* 0·8) (Found: C, 79·15; H, 10·15. $C_{31}H_{48}O_3$ requires C, 79·45; H, 10·3%), $\nu_{max.}$ 1716 and 1023 cm.$^{-1}$, no selective absorption between 210 and 260 mμ before or after treatment with alkali.

9α-*Lumista*-7,22-*diene*-3β,5β-*diol* (15).—The triol 6β-benzoate (6) (1 g.) and ethylamine (50 ml.) were shaken with lithium wire (800 mg.) in a stoppered flask until a blue colour persisted and then for a further 30 min. Solid ammonium chloride was added, the mixture was diluted cautiously with water, and then extracted with ether. The material (950 mg.) so obtained was chromatographed on deactivated alumina (50 g.). Elution with light petroleum–benzene (3 : 1) gave material (150 mg.), m. p. 120—137° (from aqueous acetone), $[\alpha]_D$ −5° (*c* 0·6) (Found: C, 83·9; H. 12·0 Calc. for $C_{28}H_{46}O$: C, 84·35; H, 11·65%). Comparison with reference compounds [7] (including infrared examination) showed that this material is probably a mixture of 9α-lumista-5,22-dien-3β-ol (16) and 5β,9α-lumista-7,22-dien-3β-ol, the former compound predominating.

Elution with benzene gave 9α-*lumista*-7,22-*diene*-3β,5β-diol (15) (300 mg.), m.p. 169—170° (from aqueous acetone), $[\alpha]_D$ +122° (*c* 0·6) (Found: C, 80·9; H, 11·05. $C_{28}H_{46}O_2$ requires C, 81·1; H, 11·2%), and elution with benzene–ether (2 : 3) afforded the 3β,5β,6β-triol (10) (130 mg.), m. p. and mixed m. p. 182—184°.

Acetylation of the diol (15) at 20° gave 5β-*hydroxy*-9α-*lumista*-7,22-*diem*-3β-*yl acetate*, m. p. 136—137° (from aqueous acetone), $[\alpha]_D$ +104° (*c* 0·6) (Found: C, 78·5; H, 10·6. $C_{30}H_{48}O_3$ requires C, 78·9; H, 10·6%).

9α-*Lumista*-7,22-*dien*-3β,5β-*ylene Carbonate* (13).—Toluene (50 ml.), saturated with carbonyl

chloride at 5°, was added to the 3β,5β-diol (15) (95 mg.) in a mixture of pyridine (3 ml.) and chloroform (20 ml.). After being kept at 20° for 2 days the mixture was washed successively with sodium hydrogen carbonate solution (to remove excess of carbonyl chloride), 3N-hydrochloric acid, sodium hydrogen carbonate solution, and water. Evaporation of the dried solution, chromatography of the residue on deactivated alumina (15 g.), and elution with light petroleum–benzene gave the *carbonate* (50 mg.), m. p. 264—267° (from aqueous acetone), $[\alpha]_D$ +105° (*c* 0·5) [Found: C, 78·6; H, 10·0%; *M* (Rast), 432. $C_{29}H_{44}O$ requires C, 79·0; H, 10·1%; *M*, 441], $\nu_{max.}$ (Nujol) 1727 (C=O; value in CCl_4, 1762), 1236, 1191, 1100, and 1060 cm.$^{-1}$.

Oxidation of 9α-Lumisteryl Acetate (17) *with Perbenzoic Acid.*—An 0·19M-solution of perbenzoic acid in dry benzene (28 ml.) was added at 5° to a solution of the acetate (2·3 g.) in benzene (22 ml.), and after 22 hr. the mixture was poured on to deactivated alumina (200 g.). Elution with benzene gave dehydrolumisteryl acetate (18) (170 mg.), m. p. and mixed m. p. 139—140°, $[\alpha]_D$ +241° (*c* 0·6) (lit.,[7] m. p. 142—142·5°, $[\alpha]_D$ +227°).

Elution with benzene–ether (9 : 1) gave a compound formulated as 5α-*hydroxy*-9α-*lumista*-6,8,22-*trien*-3β-*yl acetate* (19) (2·1 g.), m. p. 165—166° (from aqueous acetone), $[\alpha]_D$ +217° (*c* 0·7) (Found: C, 79·2; H, 10·1. $C_{30}H_{46}O_3$ requires C, 79·25; H, 10·2%), $\lambda_{max.}$ 275 mμ (ε 4360), $\nu_{max.}$ (in dilute CCl_4 solution) 3619 and 3604 (5α-OH), and 1737 cm.$^{-1}$ (3-OAc not involved in hydrogen bonding).

Refluxing this compound (250 mg.) with 5% methanolic potassium hydroxide (50 ml.) for 3 hr., followed by acetylation and chromatography of the product, yielded dehydrolumisteryl acetate (50 mg.), m. p. and mixed m. p. 140—141°.

Reaction of 9α-Lumisterol with Osmium Tetroxide.—Osmium tetroxide (300 mg.) was added to 9α-lumisterol (500 mg.) in dry ether (20 ml.) containing pyridine (2 ml.) and the mixture kept at 20° for 3 days and evaporated. The residue was refluxed for 1 hr. with a solution of potassium hydroxide (10 g.) and mannitol (10 g.) in a mixture of water (20 ml.), methanol (100 ml.), ethanol (40 ml.), and benzene (300 ml.). Hot water (500 ml.) was added, the mixture was cooled, and the layers were separated. After extraction of the aqueous layer with ether–chloroform (9 : 1; 3 × 100), the organic layers were combined, washed with water, dried, and evaporated. The product (490 mg., shown to contain at least three components by examination on chromatoplates) was acetylated and chromatographed on alumina (30 g., grade H). Light petroleum–benzene (1 : 3) eluted 9α-lumisteryl acetate (110 mg.), m. p. and mixed m. p. 78—79°. Further elution with light petroleum–benzene (1 : 3) gave product (A) (100 mg.), m. p. 188—189° (from aqueous acetone), $[\alpha]_D$ +26° (*c* 0·5) (Found: C, 73·9; H, 9·75%; *M*, 454), $\nu_{max.}$ 3600 (weak) and 1740 cm.$^{-1}$ (very strong). Elution with benzene–ether (1 : 1) gave product (B) (70 mg.), m. p. 78—84° (from aqueous acetone), $[\alpha]_D$ +109° (*c* 0·5) (Found: C, 72·95; H, 9·95%; *M*, 454), $\nu_{max.}$ 3600 (weak) and 1740 cm.$^{-1}$ (very strong).

In another experiment osmium tetroxide (250 mg.) in dry ether (20 ml.) was added under nitrogen during 15 min. to a stirred solution of 9α-lumisterol (500 mg.) in ether (50 ml.) and pyridine (2 ml.). After a further 30 min. the mixture was worked up to give 9α-lumisteryl acetate and products (A) and (B) (105, 90, and 80 mg., respectively). It was established that 9α-lumisterol is unchanged by standing for 4 days in ether and pyridine.

Oxidation of 9β-Ergosterol (20) *with Perbenzoic Acid.*—After oxidation of 9β-ergosterol[8] (2·6 g.) for 22 hr. as described above for 9α-lumisterol, the benzene solution was poured on to deactivated alumina (200 g.). Elution with benzene gave dehydroergosterol (24) (530 mg.), m. p. and mixed m. p. 143—144°, $[\alpha]_D$ +147° (*c* 0·5) (lit.,[11] m. p. 142—144°, $[\alpha]_D$ +150°). Further elution with benzene gave a mixture (2·1 g.) which was rechromatographed on alumina (100 g.). In graded development, elution with benzene–ether (1 : 4) afforded 3β,5α-*dihydroxy*-9β-*ergosta*-7,22-*dien*-6α-*yl benzoate* (22) (1·2 g.), m. p. 153—154° (from aqueous acetone), $[\alpha]_D$ −89° (*c* 0·7) (Found: C, 78·35; H, 9·45. $C_{35}H_{50}O_4$ requires C, 78·6; H, 9·4%), $\nu_{max.}$ 1722 cm.$^{-1}$.

An experiment (similar to that described for 9α-lumisterol) showed that in the presence of perbenzoic acid at 5° the absorbance of 9β-ergosterol at 287 mμ decreased by 50% in 15 sec.

Chromatography of the products obtained in a 10 min. oxidation of 9β-ergosterol (2 g.) afforded dehydroergosterol (140 mg.), m. p. 143—144°, and the triol 6α-benzoate (22) (700 mg.), m. p. 153—154°. Elution with ether–methanol (9 : 1) gave more-polar material (450 mg.) which was not investigated.

9β-*Ergosta*-7,22-*diene*-3β,5α,6α-*triol* (27).—(*a*) *From the dihydroxy*-6α-*benzoate* (22) *and the*

[11] D. H. R. Barton and J. D. Cox, *J.*, 1949, 219.

acetoxy-hydroxy-6α-benzoate (23). The dihydroxy-6α-benzoate (22) (230 mg.) was refluxed for 3 hr. with 5% methanolic potassium hydroxide (20 ml.). Standard manipulation gave the *triol* (180 mg.), m. p. 216—217° (from acetone), $[\alpha]_D$ −47° (*c* 0·6) (Found: C, 78·0; H, 10·85. $C_{28}H_{46}O_3$ requires C, 78·1; H, 10·75%). The triol (220 mg.), m. p. 214—215° was also obtained by refluxing the dihydroxy-6α-benzoate (300 mg.) in ether (20 ml.) with lithium aluminium hydride (200 mg.) followed by the usual work-up. Similar reduction of the acetoxy-hydroxy-6α-benzoate (23) (250 mg.) afforded the triol (160 mg.), m. p. 215—216°.

With acetic anhydride–pyridine at 20° the triol gave the 3β,6α-*diacetate*, m. p. 128—129° (from aqueous acetone), $[\alpha]_D$ −106° (*c* 0·7) (Found: C, 74·25; H, 9·6. $C_{32}H_{50}O_5$ requires C, 74·65; H, 9·8%), $\nu_{max.}$ 3580, 1740, and 1240 cm.$^{-1}$.

(*b*) *By osmium tetroxide oxidation of* 9β-*ergosterol* (20). A solution of osmium tetroxide (1·7 g.) and 9β-ergosterol (2·6 g.) in ether (100 ml.)–pyridine (10 ml.) was kept at 20° for 3 days. Working up as described for 9α-lumisterol afforded the triol (27) (2·5 g.), m. p. 216—217°, identified by comparison with authentic material.

3β,5α-*Dihydroxy*-9β-*ergosta*-7,22-*dien*-6-*one* (31).—A solution of the above triol (200 mg.) in dry pyridine (6 ml.) was added to a slurry prepared by adding chromic oxide (400 mg.) in small portions to pyridine (4 ml.) and the mixture kept at 20° for 12 hr. Dilution with water and extraction with ether afforded the *dihydroxy-ketone* (160 mg.), m. p. 184—187° (from aqueous acetone), $[\alpha]_D$ −31° (*c* 0·5) (Found: C, 75·3; H, 10·15. $C_{28}H_{44}O_3,H_2O$ requires C, 75·3; H, 10·4%), $\lambda_{max.}$ 252 mμ (ε 12,400), $\nu_{max.}$ 1669 cm.$^{-1}$.

This material was recovered unchanged after further treatment with chromic oxide–pyridine at 20° for 24 hr.

5α-*Hydroxy*-9β-*ergosta*-7,22-*diene*-3,6-*dione* (32).—Oxidation of the preceding dihydroxy-ketone (140 mg.) in acetone (10 ml.) with 8N-chromic acid (0·2 ml.) for 30 sec. afforded the hydroxy-diketone (70 mg.), m. p. 198—201° (from aqueous acetone), $[\alpha]_D$ +2° (*c* 0·9) (Found: C, 78·95; H, 10·0. $C_{28}H_{43}O_3$ requires C, 78·8; H, 9·9%), $\lambda_{max.}$ 250 mμ (ε 15,400), $\nu_{max.}$ 1721 and 1668 cm.$^{-1}$.

5α,6α-*Isopropylidenedioxy*-9β-*ergosta*-7,22-*dien*-3β-*ol* (29).—The 3β,5α,6α-triol (27) (190 mg.) in dry acetone (50 ml.) was refluxed with anhydrous copper sulphate (1·5 g.) for 4 days and the product chromatographed on deactivated alumina (30 g.). Elution with benzene–ether (1 : 1) gave the *hydroxy-acetonide* (95 mg.), m. p. 88—89° (from aqueous acetone), $[\alpha]_D$ +9° (*c* 0·8) (Found: C, 76·1; H, 10·7. $C_{31}H_{50}O_3,H_2O$ requires C, 76·2; H, 10·7%), $\nu_{max.}$ 3600 and 1018 cm.$^{-1}$.

Oxidation of the hydroxy-acetonide (40 mg.) with 8N-chromic acid for 30 sec. gave 5α,6α-*isopropylidenedioxy*-9β-*ergosta*-7,22-*dien*-3-*one* (30) (27 mg.), m. p. 160—161° (from aqueous acetone), $[\alpha]_D$ +39° (*c* 0·6) (Found: C, 79·0; H, 10·35. $C_{31}H_{48}O_3$ requires C, 79·45; H, 10·3%), $\nu_{max.}$ 1718 and 1018 cm.$^{-1}$, no selective absorption between 210 and 260 mμ before or after treatment with alkali.

Reduction of 3β,5α-*Dihydroxy*-9β-*ergosta*-7,22-*dien*-6α-*yl Benzoate* (22).—The benzoate (700 mg.) was reduced with lithium (600 mg.) and ethylamine (40 ml.), and the mixture worked up as described for the similar 9α-lumisterol derivative (6). The part (60 mg.) of the product which was insoluble in benzene was shown to be the 3β,5α,6α-triol (27), m. p. 213—214° (after crystallisation), by comparison with an authentic specimen. The part which was soluble in benzene was chromatographed on deactivated alumina (30 g.). Elution with benzene gave 5β,9β-ergosta-7,22-dien-3β-ol (28) (200 mg.), m. p. 131—132° (from aqueous acetone), $[\alpha]_D$ +13° (*c* 0·5) identified by comparison of its infrared spectrum with that of authentic material.[8]

The same products were obtained in varying proportions when the triol monobenzoate (22) was reduced with lithium and ammonia, sodium in ammonia, and sodium in propan-2-ol.

Oxidation of 9β-*Ergosteryl Acetate* (21) *with Perbenzoic Acid.*—After oxidation of the acetate (2·5 g.) in benzene (25 ml.) for 22 hr. at 5° with 0·17M-perbenzoic acid in benzene (38 ml.), the solution was diluted with light petroleum (65 ml.) and chromatographed on silica (200 g.). Elution with light petroleum–benzene (2 : 3) gave material (1 g.) which was rechromatographed (see below). Elution with benzene–ether gave 3β-*acetoxy*-6α-*benzoyloxy*-9β-*ergosta*-7,22-*dien*-5α-*ol* (23) (1·0 g.), m. p. 164—165° (from aqueous acetone), $[\alpha]_D$ −113° (c 0·7) (Found: C, 76·9; H, 8·9. $C_{37}H_{52}O_5$ requires C, 77·05; H, 9·1%), $\nu_{max.}$ 3590, 1740, and 1726 cm.$^{-1}$.

The above material (1 g.) was chromatographed on deactivated alumina (100 g.). Elution with benzene gave dehydroergosteryl acetate (25) (200 mg.), m. p. and mixed m. p. 145—146°, $[\alpha]_D$ +200° (*c* 0·6) (lit.,[11] m. p. 145—146°, $[\alpha]_D$ +195°). Further elution with benzene gave a

mixture (600 mg.) in which the main ultraviolet maxima were at 278 (ε 3900) and 325 mμ (ε 630). These absorptions are assigned to the $\Delta^{6,8}$-diene (26) and dehydroergosteryl acetate, respectively.

5β,6α-*Dihydroxylumista*-7,22-*dien*-3β-*yl Acetate* (4).—The epoxy-acetate (2; R = Ac)[1] (300 mg.) was adsorbed from benzene on to alumina (30 g., grade H). After 3 hr., elution with benzene–ether (1 : 1) afforded the triol monoacetate (210 mg.), m. p. 182—188°, $[\alpha]_D$ +54° (*c* 0·6), identified by comparison with authentic material (lit.,[1] m. p. 181—188°, $[\alpha]_D$ +56°).

5α,6α-*Isopropylidenedioxyergosta*-7,22-*dien*-3-*one*.—Oxidation of the 3β-hydroxy-5α,6α-acetonide (100 mg.)[12] with 8N-chromic acid afforded the *oxo-acetonide* (70 mg.), m. p. 212—213°, $[\alpha]_D$ −35° (*c* 1·0) (Found: C, 79·45; H, 10·0. $C_{31}H_{48}O_3$ requires C, 79·45; H, 10·3%).

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[12] M. Fieser, A. Quilico, A. Nickon, W. E. Rosen, E. J. Tarlton, and L. F. Fieser, *J. Amer. Chem. Soc.*, 1953, **75**, 4066.