229. *Steroids of Unnatural Configuration. Part I. The Stereochemistry of Lumisterol and 9α-Lumisterol (Pyrocalciferol).**

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Lumisterol and pyrocalciferol are two of the four ergosterol stereoisomers involving variations in the configurations at the 9- and the 10-position. The preparation from these two sterols of pairs of $C_{(9)}$-epimers and comparison of their molecular rotations indicated that the accepted structures should be reversed, *i.e.*, that lumisterol has the 9β- and pyrocalciferol the 9α-configuration.

This proposal has been confirmed by a procedure involving the elimination of the stereochemical differences at position 10. Degradation of lumisterol yields a tricyclic ketone (XIII, ii) identical with that derived from *iso*pyrocalciferol (9β-ergosterol).

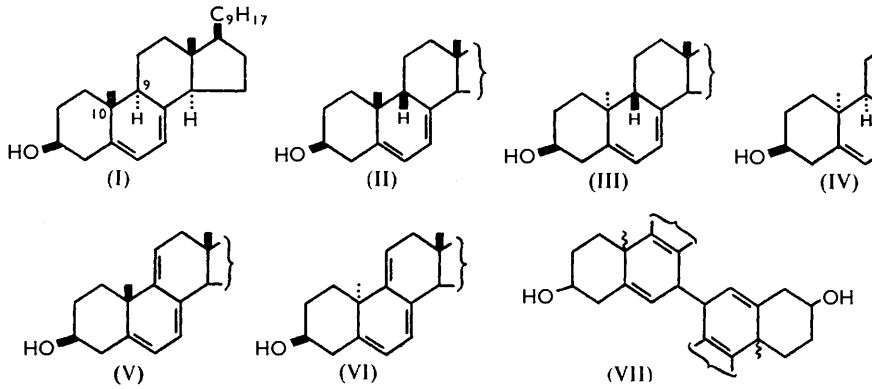
The use of the more systematic names 9β-ergosterol and 9α-lumisterol in place of *iso*pyro- and pyro-calciferol respectively is now desirable.

WHILE studying routes to cortisone from ergosterol we encountered compounds with the unnatural 9β-configuration and made some studies of their properties.[1] One of the most unexpected observations was that a 7 : 8-double bond in such compounds could be hydrogenated without difficulty, whereas in the natural (9α) steroids this linkage is very inert and cannot be reduced directly. This observation emphasised that much of the behaviour regarded as characteristic of steroids is dependent upon the common stereochemistry of the group. It led on to the suggestion that investigations on unnatural steroids might reveal significant chemical and biological variations. Although many such steroids have been prepared, either from naturally occurring compounds or in synthetic work, there has been as yet little systematic study of their properties. Inversion of configuration at position 8, 9, or 10 produces a profound effect on molecular shape, and series of suitable compounds are available from the multifarious transformation reactions of the readily accessible ergosterol. Working with ergosterol stereoisomers has the

* A preliminary account of this work appeared in *Proc. Chem. Soc.*, 1958, 7.

[1] Bladon, Henbest, Jones, Lovell, Wood, and Woods; Elks, Evans, Hathway, Oughton, and Thomas, *J.*, 1953, 2921.

additional advantage that the results obtained can be considered in the light of the extensively known chemistry of the parent " normal " sterol.



Studies have been made [2] of the many partial and complete reduction products of the 9- and the 10-isomers (II—IV) of ergosterol (I), during which doubts accumulated concerning the accepted configurations of lumisterol and pyrocalciferol at position 9. It is therefore necessary at the outset to consider the structures of the parent substances, lumisterol, one of the irradiation products of ergosterol, and pyro- and *iso*pyro-calciferol, formed by thermal cyclisation of calciferol (vitamin D_2).

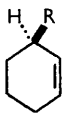
It is rigorously established [3] that all three are stereoisomers of ergosterol (I), differing only in the configurations at one or both of the centres 9 and 10. The orientations at $C_{(10)}$ are revealed by dehydrogenation of ergosterol and *iso*pyrocalciferol by mercuric acetate to dehydroergosterol (V), and of lumisterol and pyrocalciferol to dehydrolumisterol [4] (VI). Since ergosterol is certainly the 10β-Me : 9α-H-isomer, *iso*pyrocalciferol is unequivocally represented as the 9β-epimer (II).

Lumisterol and pyrocalciferol are thus shown to have 10α-methyl groups but formulation of the former as the 9α-compound (IV) and pyrocalciferol as the 9β-isomer (III) was based in a tenuous way on the behaviour of ergosterol and its companions towards irradiation (in the presence of eosin) in the absence of oxygen.[5] Only two (ergosterol and pyrocalciferol) of the four isomers underwent dehydrogenation to give " pinacols " (VII), and on the assumption that an *anti*-arrangement of the 10-methyl group and the 9-hydrogen atom was the necessary condition for pinacol formation, the currently accepted structures were proposed. Although a mechanism for this and similar reactions has been suggested recently [6] the structural features governing pinacol formation are not yet apparent. An *X*-ray analysis [7] of lumisteryl 4-iodo-3-nitrobenzoate was interpreted (electron-density projections only were calculated) as supporting the 9α-representation but the results are not incompatible with a 9β-configuration.[8]

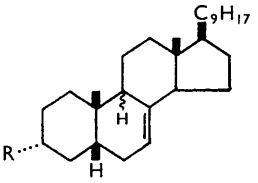
As our studies advanced it became possible to compare the molecular rotations of pairs of compounds, derived from lumisterol and pyrocalciferol, differing only in their configurations at position 9. The preparation of these substances (see Table) will be described in later papers: it is stressed here that all structural details (except the $C_{(9)}$-orientations) were unambiguously determined by methods independent of the stereochemistry at position 9.

[2] Ph.D. theses of Castells, Fletcher, and Ridley, Manchester, 1955—56.
[3] See summary of evidence in Fieser and Fieser, " Natural Products related to Phenanthrene," 3rd edn., Reinhold Publ. Co., New York, 1949.
[4] Windaus and Dimroth, *Ber.*, 1937, **70**, 376.
[5] Kennedy and Spring, *J.*, 1939, 250.
[6] Owades, *Experientia*, 1950, **6**, 258.
[7] Hodgkin and Sayre, *J.*, 1952, 4561.
[8] Personal communication from Dr. D. M. Hodgkin.

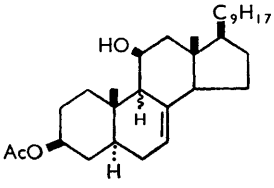
Type (A) Type (B)



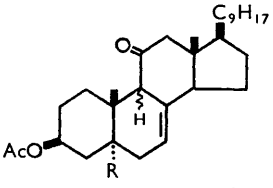

10β-Methyl steroids



Ref.	$[M]_D$	R	Ref.	$[M]_D$	ΔM_D (A − B)
9α-Compounds			9β-Compounds		
a	+171°	OH	*b*	+24°	+147°
a	+260	OAc	*b*	+31	+229

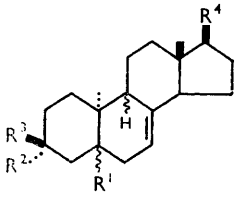


c	−96°		*d*	−395°	+299°



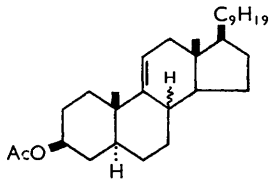
e	+113°	H	*e*	−867°	+980°
e	+430	OAc	*e*	−251	+681

10α-Methyl steroids

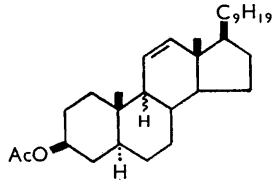


Pyrocalciferol derivs. (9α)			Lumisterol derivs. (9β)	
$[M]_D$	R^2	R^3	$[M]_D$	ΔM_D (A − B)
	R^1 = 5β-H; R^4 = C_9H_{17}			
+605°	H	OH	+131°	+474°
+585	H	OAc	+97	+488
+426	H	DNB	+18	+408
+648	OH	H	+179	+469
+689	3-Oxo		+137	+552
	R^1 = 5β-H; R^4 = C_9H_{19}			
+617	3-Oxo		+147	+470
	R^1 = 5α-H; R^4 = C_9H_{19}			
+296	H	OH	−24	+320
+203	H	OAc	−141	+344
+125	H	DNB	−220	+345

Type (A) Type (B)

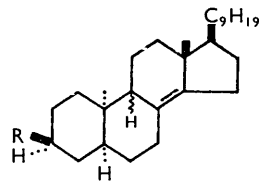



Ref.	$[M]_D$	Ref.	$[M]_D$	ΔM_D (A − B)
9α-Compound		9β-Compound		
c	+110°	*c*	−287°	+397°



8α-Compound		8β-Compound		
f	+477°	*g*	+75°	+402°

[a] Barton and Miller, *J.*, 1949, 337. [b] First prepared by Busse, *Z. physiol. Chem.*, 1933, **214**, 211, but $[M]_D$ values taken from unpublished work by Jones, Henbest, and Ridley. [c] Crawshaw, Henbest, Jones, and Wagland, *J.*, 1955, 3420. [d] Deduced from 11α-OH-compound [c] by applying standard correction (Barton and Klyne, *Chem. and Ind.*, 1948, 755). [e] Bladon, Henbest, Jones, Lovell, Wood, Woods, Elks, Evans, Hathway, Oughton, and Thomas, *J.*, 1953, 2921. [f] Bream, Eaton, and Henbest, *J.*, 1957, 1974. [g] Crawshaw, Henbest, and Jones, *J.*, 1954, 731.



Lumisterol derivs. (9β)		Pyrocalciferol derivs. (9α)	
$[M]_D$	R	$[M]_D$	ΔM_D (A − B)
+80°	OH	−252°	+332°
−22	OAc	−305	+283
−59	DNB	−238	+179

DNB = 3 : 5-$(NO_2)_2C_6H_3$·CO·O.

Comparisons were based on Mills's generalisation [9] that, in terpenes with asymmetry at allylic positions, compounds of type (A) have more positive rotations than their enantiomers (B). This relation has been used for structural diagnosis at position 9 in tetracyclic triterpenoids having 7 : 8-double bonds (see the comparison [10] between derivatives of butyrospermol [11] and euphol, and the allocation of configuration to position 9 in masticadienonic acid [12]), and its validity with 10β-methyl steroids is demonstrated by the data in the upper part of the annexed Table of molecular rotations for compounds of established constitution. [The allylic centre is at $C_{(9)}$ in six of the pairs and at $C_{(8)}$ in the remaining pair. Throughout the Table the compounds are arranged to show their relations to structures (A) and (B).]

The ΔM_D values of the 10β-methyl steroids are of the expected (positive) sign, and average about 450 units. (The exceptionally high values of the fourth and fifth pairs probably arise from the presence of oxo-groups adjacent to the allylic centres.) To produce a corresponding result (positive differences, average 390 units) with the lumisterol and pyrocalciferol derivatives it is necessary to adopt a 9β-structure (III) for lumisterol and the 9α-formulation (IV) for pyrocalciferol, *i.e.*, to reverse the previously accepted values. [The appearance of lumisterol (and pyrocalciferol) derivatives on both sides of the Table arises from the differing positions of the olefinic bonds which alter the relations to structures (A) and (B).]

In spite of the strong indication from the molecular-rotation data it seemed desirable to produce independent evidence. This has now been obtained in the manner indicated in the formula scheme (VIII—XIII), all four isomers having been degraded by the same procedure to stereoisomeric tricyclic ketones (XIII), those derived from lumisterol (VIIIc) and *iso*pyrocalciferol (VIIIb) proving identical. Since no opportunities for inversion at the 9-position exist in the series of transformations effected (see below), it follows that lumisterol, having now the same 9-configuration as *iso*pyrocalciferol, must be represented by (III).

Nomenclature (in concurrence with the Editors).—Now that structural certainty has been achieved in this group the names 9β-ergosterol and 9α-lumisterol are to be preferred to *iso*pyro- and pyro-calciferol respectively. The four stereoisomers with their names are therefore:

(I)	(II)	(III)	(IV)
Ergosterol	9β-Ergosterol (formerly *iso*pyrocalciferol)	Lumisterol	9α-Lumisterol (formerly pyrocalciferol)

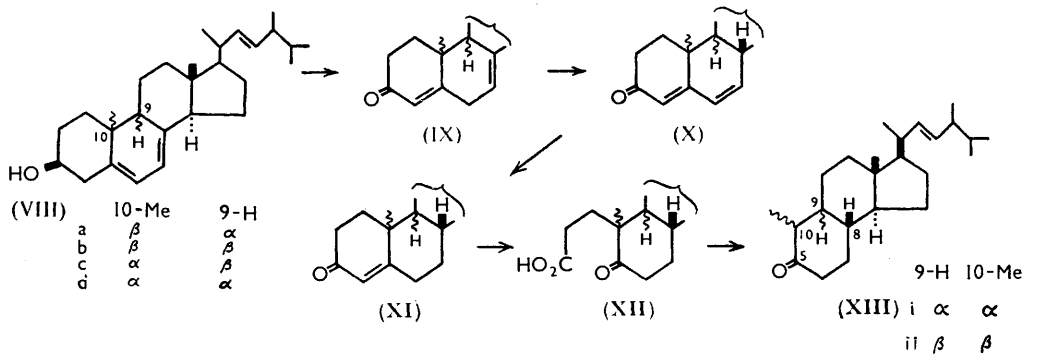
The series of reactions involved in the degradations to the tricyclic ketones were essentially similar with all four sterols and only notable differences will be discussed. The first three stages of Oppenauer oxidation, isomerisation to the 4 : 6-dienone (X), and selective hydrogenation of the 6 : 7-ethylenic bond were carried out mainly according to the highly developed procedure of Ott and his co-workers.[13]

Oppenauer oxidation of 9β-ergosterol (*iso*pyrocalciferol) (VIIIb) gave both the αβ-unsaturated ketone (IXb), and the unisomerised 5 : 7 : 22-trien-3-one (XIV), the mixture being resolved chromatographically. Isomerisation of the homocyclic diene-ketone (XIV) into the αβ-unsaturated isomer (IXb) seemed to proceed only slowly under the normal oxidation conditions.

The ketone (IXd) derived from 9α-lumisterol (pyrocalciferol) (VIIId) did not crystallise although it showed ultraviolet absorption of the expected intensity. Fortunately a

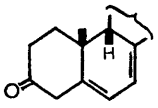
[9] Mills, *J.*, 1952, 4976.
[10] Dawson, Halsall, Jones, Meakins, and Phillips, *J.*, 1956, 3172.
[11] Lawrie, Hamilton, Spring, and Watson, *J.*, 1956, 3272.
[12] Barton and Seoane, *J.*, 1956, 4150; Barton, Head, and May, *J.*, 1957, 935.
[13] Shepherd, Donia, Campbell, Johnson, Holysz, Slomp, Stafford, Pederson, and Ott, *J. Amer. Chem. Soc.*, 1955, **77**, 1212.

crystalline conjugated dienone (Xd) was obtained after isomerisation with hydrogen bromide in chloroform–acetic acid. The conjugated dienones (Xb and Xc) derived from 9β-ergosterol and lumisterol were formed along with isomeric ketones (infrared band at



1712 cm.$^{-1}$ indicating unconjugated carbonyl) showing maxima in the region of 2520 Å (ε ~20,000) and obviously containing conjugated diene systems in the B–C–D-ring region [*e.g.*, 8(9) : 14].

It is of course vital that in the processes involved in going from (VIII) to the tricyclic ketones (XIII) no alteration in the stereochemistry at position 9 should occur. This might be the case if the unconjugated dienones (*e.g.*, XIV) or related species were in equilibrium, during the acid-induced isomerisations, with the mono- and fully conjugated di-enones. Such behaviour would be unlikely with normal steroids but could not be ruled out with these " unnatural " isomers. Proof that such equilibria do not exist is available, however, since 9β-lumista- and 9α-lumista-4 : 7 : 22-trienone (IXc and IXd) yield different 4 : 6 : 22-trienones (Xc and Xd); identical products would have resulted if intermediates containing an 8 : 9-double bond had been involved.



(XIV)

Ozonolysis [14] of the unsaturated ketones (XI), the side-chain double bond being protected by bromination, gave the keto-acids (XII). Originally the conversion of the latter into the tricyclic ketones (XIII) was effected in rather poor yields by heating the barium salts,[15] but later pyrolysis of solutions of the sodium salts in molten sodium phenylacetate was employed most advantageously. (We are much indebted to Professor R. B. Woodward for informing us of this improved procedure, worked out in collaboration with Dr. Weesner.) In the case of the keto-acid derived from ergosterol, only one ketone seemed to be formed; no isomerisation could be brought about by acid- or alkali-treatment [16] and it could reasonably be concluded that the 10-methyl group in (XIII, i) must be in the more stable 10α-configuration (*i.e.*, equatorial). Moreover, the pyrolysis of the sodium salt can be regarded as a reversed Michael reaction; [17] the 5 : 10-enolate anion of (XIII) first produced would ketonise to give the 10α-methyl isomer.

From the acids (XIIb and c) derived from 9β-ergosterol (*iso*pyrocalciferol) (VIIIb) and lumisterol (VIIIc) respectively, good yields of the same low-melting tricyclic ketone (XIII, ii) were obtained. Identity was proved by mixed m. p. determinations with both ketones and oximes, by infrared spectra, and by determination of the optical rotatory dispersion curves.* These were identical in form and in the direction of the Cotton effect to that of

* These determinations and many others during this work were made at Wayne University through the good offices of Professor Carl Djerassi. We are much indebted to him for his willing collaboration.

[14] Cf. Turner, *J. Amer. Chem. Soc.*, 1950, **72**, 579.

[15] Turner, *ibid.*, 1954, **76**, 1390.

[16] Cf. the behaviour of 4-methyl-3-oxo-steroids (Sondheimer and Mazur, *J. Amer. Chem. Soc.*, 1958, **80**, 5220).

[17] Cf. Julia, Eschenmoser, Heusser, and Tarköy, *Helv. Chim. Acta*, 1953, **36**, 1885 and references given there.

the mirror image of the coprostanone curve,[18] thus providing additional confirmation of the *cis*-fusion of rings B/C and the β-configurations at positions 8 and 9 in (XIII, ii).

Further evidence supporting the assignment of the equatorial conformation to the 10-methyl group in the ketones (XIII, i and ii) can be derived from the solvent effects on their rotations. Sondheimer and Mazur[16] have drawn attention to the fact that the rotations of epimerisable (and hence normally axial) α-methyl ketones are considerably lower in methanol or dioxan than in chloroform, whereas little or no solvent effect is observed with the equatorial compounds. The differences with the ketones (XIII) are indicated in the accompanying Table from which it is clear that they support the equatorial assignment.

	1, $[\alpha]_D$ in MeOH	2, $[\alpha]_D$ in $CHCl_3$	Δ(1 − 2)
(XIII, i)	−25° *	−30°	+5°
(XIII, ii)	−44	−48	+4
2α-Methylcholestan-3-one	+31	+32	−1
4α-Methylcholestan-3-one	+25	+26	−1
2β-Methylcholestan-3-one	+67	+86	−19
4β-Methylcholestan-3-one	+16	+36	−20

* In dioxan because of low solubility in MeOH.

The configuration at position 8 in the final products is determined during the isomerisation of the dienone (IX) to the fully conjugated compound (X), and variations would have vitiated the final comparisons between the tricyclic ketones (XIII). Inspection of models indicated that if protonation at $C_{(8)}$ led to the more stable configuration, this would certainly be 8β in three cases; with the ketone derived from 9α-lumisterol (pyrocalciferol) however, no clear indication could be obtained in this way. It is established that 8β-addition occurs in the ergosterol series since progesterone has been obtained[13] from the ketone (XIa) produced by isomerisation. The tricyclic ketone (m. p. 85·5—87°, $[\alpha]_D$ +51·5°) obtained from 9α-lumisterol (pyrocalciferol) was different from that (XIII, i) (m. p. 101—103°, $[\alpha]_D$ −30°) derived from ergosterol. Also the optical rotatory dispersion curve of the former ketone was rather anomalous. We are inclined to suspect stereochemical variation rather at $C_{(8)}$ than at $C_{(10)}$ but, in view of the identity of the ketones from (VIIIb) and (VIIIc) and the attainment of the objective of the investigation, the nature of the difference between the other pair of final products was not further pursued.

With the structures of the parent compounds established it is now possible to interpret the results of a detailed study of the partial and complete reduction products from lumisterol, pyrocalciferol, and *iso*pyrocalciferol. The work will be described in subsequent communications.

Experimental

Rotations were measured for chloroform solutions at room temperature. M. p.s were determined on a Kofler block and are corrected. Ultraviolet light absorption data were obtained with alcoholic solutions. Alumina used for chromatography was Spence's Grade " H; " deactivated alumina refers to Grade " H " to which 5% of 10% acetic acid had been added according to the method of Farrar, Hamlet, Henbest, and Jones.[19] Light petroleum refers to the fraction of b. p. 60—80°.

22 : 23-*Dibromoergost*-4-*en*-3-*one*.—A solution of ergosta-4 : 22-dien-3-one[13] (1·29 g.) in chloroform (250 c.c.) was cooled to −80° and bromine in chloroform (9 c.c.; 2% v/v) was added dropwise with stirring. Triethylamine was added, solvent removed under reduced pressure, and the residue dissolved in ether and filtered; removal of the ether and crystallisation of the residue from ethanol afforded 22 : 23-*dibromoergost*-4-*en*-3-*one* (1·36 g.), m. p. 195—198°, $[\alpha]_D$ +38° (*c* 0·9) (Found: C, 60·35; H, 8·0; Br, 28·7. $C_{28}H_{44}OBr_2$ requires C, 60·45; H, 7·95; Br, 28·7%), $\lambda_{max.}$ 2390 Å (ε 15,600), $\nu_{max.}$ (Nujol mull) 1681 (conjugated carbonyl) and 1620 (conjugated double bond), no peak at *ca.* 970 (*trans*-CH=CH−) cm.$^{-1}$. Debromination with activated zinc dust gave a high yield of the parent ergosta-4 : 22-dien-3-one, m. p. and mixed m. p. 127—131°, $[\alpha]_D$ +39° (*c* 1·1), $\lambda_{max.}$ 2410 Å (ε 16,800).

[18] Djerassi and Closson, *J. Amer. Chem. Soc.*, 1956, **78**, 3761.
[19] Farrar, Hamlet, Henbest, and Jones, *J.*, 1952, 2657.

5-*Oxo*-3 : 5-seco-A-*norergost*-22-*en*-3-*oic Acid* (XIIa).—A stream of ozonised oxygen (10 l./hr.; 2%) was passed into 22 : 23-dibromoergost-4-en-3-one (1·45 g.) in " AnalaR " ethyl acetate (15 c.c.) at −80° until a permanent blue colour was produced (50 min.). Nitrogen was then passed in, the solution being allowed to warm to room temperature. " AnalaR " acetic acid (150 c.c.), water (50 c.c.) and 26% hydrogen peroxide (6 c.c.) were added and, after 40 hr., isolation *via* ether furnished an oil which was treated with activated zinc dust (2 g.) in refluxing 1 : 1 ether–methanol (100 c.c.) for 2 hr. More zinc dust (1 g.) was added and refluxing was continued for 1 hr. The hot solution was filtered, the solid being washed with hot methanol and ether; removal of solvents gave an oil to which were added dilute hydrochloric acid and ether. The ethereal layer yielded an oil which was chromatographed on silica gel (80 g.). Benzene and benzene–ether (1—2% of ether) eluted an intractable gum (203 mg.). Material eluted with benzene–ether (5—10%) afforded, after crystallisation from light petroleum, 5-*oxo*-3 : 5-seco-A-*norergost*-22-*en*-3-*oic acid* (295 mg.), m. p. 141—143°, $[\alpha]_D$ −4° (*c* 1·0) (Found: C, 78·2; H, 10·8. $C_{27}H_{44}O_3$ requires C, 77·85; H, 10·65%), $\nu_{max.}$ (in CS_2) 1706 (carbonyl) and 966 (*trans*-CH=CH–) cm.$^{-1}$.

22 : 23-*Dibromo*-5-*oxo*-3 : 5-seco-A-*norergostan*-3-*oic Acid*.—This was obtained by a similar reaction in which the debromination procedure was omitted. It had m. p. 196—199°, $[\alpha]_D$ +11° (*c* 1·2) (Found: C, 56·6; H, 7·9; Br, 27·5. $C_{27}H_{44}O_3Br_2$ requires C, 56·3; H, 7·7; Br, 27·7%).

De-A-*ergost*-22-*en*-5-*one* (XIII, i).—The sodium salt of 5-oxo-3 : 5-*seco*-A-norergost-22-en-3-oic acid was prepared by titrating a solution of the acid in methanol with methanolic 0·5N-sodium methoxide to phenolphthalein. One drop of very dilute hydrochloric acid was added and solvent removed, to furnish the sodium salt, which was thoroughly dried. The salt (780 mg.) and sodium phenylacetate (3·6 g.) were heated at 295—300°/0·02—0·05 mm. for 3 hr. The semi-crystalline distillate (638 mg.) was chromatographed on alumina (200 g.). Light petroleum–benzene (10 : 3) eluted material, m. p. 95—103°, which, after two crystallisations from methanol, afforded *de*-A-*ergost*-22-*en*-5-*one* (404 mg.), m. p. 101—103°, $[\alpha]_D$ −30° (*c* 1·4) (Found: C, 83·4; H, 11·7. $C_{24}H_{40}O$ requires C, 83·65; H, 11·7%), $\nu_{max.}$ (in CS_2) 1715 (carbonyl) cm.$^{-1}$.

9α-*Lumista*-4 : 6 : 22-*trien*-3-*one* (Xd).—A solution of 9α-lumista-5 : 7 : 22-trien-3β-ol (pyrocalciferol) (9·5 g.) in xylene (200 c.c.) and *cyclo*hexanone (100 c.c.) was dried by azeotropic distillation. Aluminium *iso*propoxide (7 g.) in dry xylene was added to the boiling solution and distillation was continued for 15 min. The solution was then refluxed for 40 min.; acetic acid (200 c.c.) and tetralin (500 c.c.) were added and solvents removed at 140°/17 mm. and finally at 140°/0·01 mm. More tetralin (50 c.c.) was again added and the solvent removed. The residue was taken up in dilute hydrochloric acid and ether, and the ethereal layer was worked up to yield an oil which was dissolved in chloroform (200 c.c.) and treated with a 50% w/v solution (4 c.c.) of hydrogen bromide in acetic acid for 45 min. at 20°. Pyridine was added and solvent was removed under reduced pressure. The residue in light petroleum was chromatographed on deactivated alumina (250 g.). Material eluted with light petroleum–benzene (10 : 1 ⟶ 2 : 1) crystallised from methanol, to give 9α-*lumista*-4 : 6 : 22-*trien*-3-*one* (3·56 g.), m. p. 87—91°, $[\alpha]_D$ +68° (*c* 1·0) (Found: C, 85·4; H, 10·9. $C_{28}H_{42}O$ requires C, 85·2; H, 10·75%), $\lambda_{max.}$ 2880 Å (ε 24,000), $\nu_{max.}$ (Nujol mull) 1667 (doubly conjugated carbonyl), 1613 and 1582 (conjugated double bonds), and 970 (*trans*-CH=CH–) cm.$^{-1}$. [Note: it was not possible to obtain crystalline 9α-lumista-4 : 7 : 22-trien-3-one; the crude product of Oppenauer oxidation was therefore directly isomerised.]

9α-*Lumista*-4 : 22-*dien*-3-*one* (cf. ref. 13) (XId).—The above trienone (3·32 g.) in ethanol (150 c.c.) containing potassium hydroxide (15 mg.) was hydrogenated in the presence of 5% palladium–norite (750 mg.) until 1·03 mol. of hydrogen had been absorbed. Acetic acid (0·1 c.c.) and pyridine (0·1 c.c.) were added, the catalyst was filtered off, and the solvent removed under reduced pressure. A sample (70 mg.) of the crude product was chromatographed on deactivated alumina (5 g.). Elution with light petroleum–benzene (2 : 1) afforded material which crystallised from ethanol to give 9α-*lumista*-4 : 22-*dien*-3-*one* (30 mg.), m. p. 140—143°, $[\alpha]_D$ −53° (*c* 0·9) (Found: C, 84·4; H, 11·0. $C_{28}H_{44}O$ requires C, 84·8; H, 11·2%), $\lambda_{max.}$ 2420 Å (ε 14,900).

22 : 23-*Dibromo*-9α-*lumist*-4-*en*-3-*one*.—The remainder of the above crude product (3·15 g.) in chloroform (700 c.c.; alcohol-free) was cooled to −80° and a 2% v/v solution of bromine in chloroform (27·5 c.c.) added dropwise with stirring. Triethylamine (0·5 c.c.) was added, solvent

removed under reduced pressure, and the residue, in light petroleum–benzene (50 : 1), chromatographed on silica gel (275 g.). Material eluted with benzene and benzene–ether (100 : 1) crystallised from ethanol, to give 22 : 23-*dibromo*-9α-*lumist*-4-*en*-3-*one* (1·89 g.), m. p. 190—192°, $[\alpha]_D$ −37° (*c* 1·2) (Found: C, 60·1; H, 7·8. $C_{28}H_{44}OBr_2$ requires C, 60·45; H, 7·95%), $\lambda_{max.}$ 2410 Å (ε 15,000).

5-*Oxo*-3 : 5-seco-A-*nor*-9α-*lumist*-22-*en*-3-*oic Acid* (XIId).—A solution of 22 : 23-dibromo-9α-lumist-4-en-3-one (1·79 g.) in " AnalaR " ethyl acetate (150 c.c.) was ozonised and treated with acetic acid and hydrogen peroxide, and after 40 hr., isolation *via* ether gave an oil which was treated with activated zinc dust (2 g.) in ether–methanol, as above. The semi-crystalline product was taken up in dilute hydrochloric acid and ether. The ethereal layer was extracted with 2N-sodium hydroxide, and the alkaline washings were acidified with 2N-hydrochloric acid, to furnish, after isolation with ether and crystallisation from ethanol, 5-*oxo*-3 : 5-seco-A-*nor*-9α-*lumist*-22-*en*-3-*oic acid* (513 mg.), m. p. 190—197°, $[\alpha]_D$ −43·5° (*c* 1·1) (Found: C, 77·55; H, 10·6. $C_{27}H_{44}O_3$ requires C, 77·85; H, 10·65%).

Tricyclic Ketone from 9α-*Lumisterol.*—Aqueous 0·061N-barium hydroxide (9 c.c.) was added dropwise to the above acid (226 mg.) in dioxan (6 c.c.). Solvent was removed under reduced pressure and the residue dried and then heated at 300—350°/0·001 mm. The sublimate (149 mg.) in light petroleum, was chromatographed on alumina (13 g.). Material eluted with light petroleum–benzene (5 : 1 → 10 : 3) crystallised from methanol, to give the *ketone* (19 mg.), m. p. 85·5—87°, $[\alpha]_D$ +51·5° (*c* 0·9) (Found: C, 83·6; H, 11·6. $C_{24}H_{40}O$ requires C, 83·65; H, 11·7%), $\nu_{max.}$ (in CS_2) 1715 (carbonyl) and 967 (*trans*-CH=CH–) cm.$^{-1}$. The m. p. of the above ketone was strongly depressed on admixture with de-A-ergost-22-en-5-one.

Lumista-4 : 6 : 22-*trien*-3-*one* (Xc).—A 50% w/v solution (9 c.c.) of hydrogen bromide in acetic acid was added to lumista-4 : 7 : 22-trien-3-one [20] (8·8 g.) in chloroform (700 c.c.; alcohol-free). The reaction was stopped after 40 min. by addition of pyridine; solvent was removed under reduced pressure, and the oily residue taken up in light petroleum and filtered. The filtrate was reduced to small volume and adsorbed on deactivated alumina (400 g.). Next morning, when unchanged lumisterone was transformed into non-elutable material, elution with light petroleum–benzene (2 : 1) afforded material (1·7 g.) which, after two crystallisations from acetone, had m. p. 177·5—180·5°, $\lambda_{max.}$ 2490 Å [ε (*M*, 394·6) 21,300], $\nu_{max.}$ (Nujol mull) 1712 (carbonyl) cm.$^{-1}$. This unconjugated ketone was not investigated further.

Elution with light petroleum–benzene (2 : 1) and crystallisation from acetone–methanol yielded *lumista*-4 : 6 : 22-*trien*-3-*one* (3·4 g.), m. p. 99—101°, $[\alpha]_D$ −629° (*c* 1·2) (Found: C, 84·95; H, 10·6. $C_{28}H_{42}O$ requires C, 85·2; H, 10·75%), $\lambda_{max.}$ 2870 Å (ε 25,100), $\nu_{max.}$ (Nujol mull) 1660 (doubly conjugated carbonyl), 1624 and 1588 (conjugated double bonds) and 973 (*trans*-CH=CH–) cm.$^{-1}$.

Lumista-4 : 22-*dien*-3-*one* (XIc) (cf. ref. 13).—The above trienone (4·8 g.) in ethanol (250 c.c.) containing potassium hydroxide (22 mg.) was hydrogenated in the presence of 5% palladium–norite (760 mg.) until 1·1 mol. of hydrogen had been absorbed (the ratio $\varepsilon_{2420} : \varepsilon_{2860}$ was then 36). Acetic acid (1 drop) and pyridine (1 drop) were added, the catalyst was removed, and the solvent evaporated under reduced pressure. The residue in light petroleum–benzene (20 : 1) was adsorbed on deactivated alumina (200 g.). Elution with light petroleum–benzene (10 : 1) gave material (930 mg.) (probably lumist-22-en-3-one) with no intense ultraviolet absorption. Further elution with the same mixture and with light petroleum–benzene (5 : 1) gave, after crystallisation from ethanol, *lumista*-4 : 22-*dien*-3-*one* (3·5 g.), m. p. 124—125·5°, $[\alpha]_D$ −170° (*c* 1·05) (Found: C, 84·9; H, 11·3. $C_{28}H_{44}O$ requires C, 84·8; H, 11·2%), $\lambda_{max.}$ 2420 Å (ε 16,700), $\nu_{max.}$ (Nujol mull) 1675 (conjugated carbonyl), 1623 (conjugated double bond) and 983 (*trans*-CH=CH–) cm.$^{-1}$.

22 : 23-*Dibromolumist*-4-*en*-3-*one*.—A solution of lumista-4 : 22-dien-3-one (4·01 g.) in chloroform (800 c.c.; alcohol-free) was cooled to −80° and 2% v/v bromine–chloroform (28 c.c.) was added dropwise with stirring. Triethylamine was added, solvent was removed under reduced pressure, and the residue taken up in ether and filtered; removal of the ether and crystallisation from acetone–methanol furnished 22 : 23-*dibromolumist*-4-*en*-3-*one* (4·3 g.), m. p. 169—172°, $[\alpha]_D$ −102° (*c* 0·8) (Found: C, 60·2; H, 7·9. $C_{28}H_{44}OBr_2$ requires C, 60·45; H, 7·95%), $\lambda_{max.}$ 2410 Å (ε 15,900), $\nu_{max.}$ (Nujol mull) 1681 (conjugated carbonyl) and 1618 (conjugated double bond), no peak at *ca.* 970 (*trans*-CH=CH–) cm.$^{-1}$. Debromination with activated zinc dust gave,

[20] Prepared in 45% yield by using the procedure of Shepherd *et al.*[13]; the constants agreed with those given by Heilbron, Kennedy, Spring, and Swain, *J.*, 1938, 869.

in high yield, the parent lumista-4 : 22-dien-3-one, m. p. and mixed m. p. 122—125°, with light absorption properties identical with those quoted above.

22 : 23-*Dibromo*-5-*oxo*-3 : 5-seco-A-*nor*-*lumistan*-3-*oic Acid*.—A solution of 22 : 23-dibromolumist-4-en-3-one (3·95 g.) in ethyl acetate (385 c.c.) was ozonised and treated with hydrogen peroxide as above. The mixture was rapidly stirred until the initial white precipitate had dissolved (41 hr.). The solution was extracted with ether, and the ethereal layer washed with water and extracted with 2N-sodium hydroxide. Acidification of the aqueous layer with 2N-hydrochloric acid and ether-extraction furnished, after drying and removal of solvent, an oil (3·78 g.) which yielded crystals from acetone–light petroleum of 22 : 23-*dibromo*-5-*oxo*-3 : 5-seco-A-*nor*-*lumistan*-3-*oic acid* (1·32 g.), m. p. 177·5—179°, $[\alpha]_D$ +6° (*c* 1·1) (Found: C, 56·4; H, 7·6; Br, 27·5. $C_{27}H_{44}O_3Br_2$ requires C, 56·3; H, 7·7; Br, 27·7%), $\nu_{max.}$ (Nujol mull) 1715 (carbonyl) cm.$^{-1}$.

De-A-*lumist*-22-*en*-5-*one* (XIII, ii).—The above acid (1·26 g.) was treated with activated zinc dust (2·5 g.) in refluxing 1 : 1 ether–methanol (120 c.c.) for 2 hr. More zinc dust (1 g.) was added and refluxing was continued for 1 hr. The usual working up furnished an oil which from light petroleum gave 5-oxo-3 : 5-*seco*-A-norlumist-22-en-3-oic acid (800 mg.), m. p. 78—80°, $[\alpha]_D$ −11° (*c* 0·9) (no satisfactory analyses were obtained; the methyl ester was an oil), $\nu_{max.}$ (in CS_2) 1712 (carbonyl) and 967 (*trans*-CH=CH–) cm.$^{-1}$.

The sodium salt (600 mg.) of the above acid was heated with sodium phenylacetate (3 g.) at 290—300°/0·05 mm. for 3¼ hr. The oily distillate (435 mg.) was chromatographed on alumina (120 g.). Light petroleum–benzene (10 : 1 ⟶ 20 : 3) eluted *de*-A-*lumist*-22-*en*-5-*one* (355 mg.), m. p. 28—34°, $[\alpha]_D$ −48° (*c* 0·96) (Found: C, 83·8; H, 11·65. $C_{24}H_{40}O$ requires C, 83·65; H, 11·7%), $\nu_{max.}$ (in CS_2) 1715 (carbonyl) and 968 (*trans*-CH=CH–) cm.$^{-1}$.

The ketone was wax-like and could not be crystallised by conventional methods but when pure it solidified spontaneously and had the m. p. recorded above. It was recovered unchanged after treatment with 0·2N-sodium methoxide in refluxing methanol and with toluene-*p*-sulphonic acid (0·5%) in acetic acid–benzene (1 : 1) at room temperature. The *oxime* had m. p. 134·5—137°, $[\alpha]_D$ −94° (*c* 0·7) (Found: C, 80·4; H, 11·2; N, 4·1. $C_{24}H_{41}ON$ requires C, 80·2; H, 11·5; N, 3·9%).

9β-*Ergosta*-4 : 7 : 22-*trien*-3-*one* * (IXb).—A solution of 9β-ergosterol (*iso*pyrocalciferol) (41 g.) in toluene (900 c.c.) and *cyclo*hexanone (165 c.c.) was dried by azeotropic distillation in the dark. Aluminium *iso*propoxide (25 g.) in dry distillate (140 c.c.) was added and the solution refluxed for 5 hr. under nitrogen. The solution was then cooled and poured into dilute hydrochloric acid containing ice. Material isolated with ether was dried under reduced pressure and chromatographed on deactivated alumina (1 kg.). Light petroleum eluted oily condensation products (25·4 g.). Further elution with light petroleum and light petroleum–benzene (10 : 1 ⟶ 5 : 1) afforded an oil (9·7 g.) (A). Light petroleum–benzene (5 : 1 ⟶ 1 : 1) eluted semi-crystalline material (7·6 g.) (B). Material (A) was chromatographed on deactivated alumina (1 kg.). Light petroleum–benzene (10 : 1 ⟶ 5 : 1) eluted material which crystallised from methanol, to give 9β-ergosta-5 : 7 : 22-trien-3-one (XIV) (273 mg.), m. p. 123—131°, $[\alpha]_D$ +521° (*c* 0·9), $\lambda_{max.}$ 2720 (ε 9400), 2820 Å (ε 10,000), $\nu_{max.}$ (Nujol mull) 1712 (carbonyl) cm.$^{-1}$. Elution with light petroleum–benzene (5 : 1 ⟶ 10 : 3) yielded semi-crystalline material (6·2 g.) which was combined with (B) and crystallised from methanol, to give 9β-*ergosta*-4 : 7 : 22-*trien*-3-*one* (10·3 g.), m. p. 110—112·5°, $[\alpha]_D$ +185° (*c* 1·3) (Found: C, 85·4; H, 10·8. $C_{28}H_{42}O$ requires C, 85·2; H, 10·75%), $\lambda_{max.}$ 2420 Å (ε 14,200), $\nu_{max.}$ (Nujol mull) 1667 (conjugated carbonyl), 1626 (conjugated double bond) and 970 (*trans*-CH=CH–) cm.$^{-1}$.

9β-*Ergosta*-4 : 6 : 22-*trien*-3-*one* (cf. ref. 13) (Xb).—Concentrated hydrochloric acid (2 c.c.) was added to 9β-ergosta-4 : 7 : 22-trien-3-one (1·033 g.) in methanol (40 c.c.), and the solution refluxed for 45 min., then cooled, diluted with ether, and treated with excess of solid sodium hydrogen carbonate. The mixture was filtered, the solid being washed with ether. Material isolated from the filtrate was chromatographed on deactivated alumina (150 g.). Light petroleum–benzene (20 : 1 ⟶ 10 : 1) eluted semi-crystalline material (549 mg.) from which no homogeneous compound was obtained; repeated crystallisation from acetone–methanol afforded a solid, m. p. 105—117°, $\lambda_{max.}$ 2540 Å [ε (*M*, 394·6) 19,300], $\nu_{max.}$ (Nujol mull) 1712 (carbonyl) cm.$^{-1}$. This ketone was not investigated further.

Elution with light petroleum–benzene (5 : 1 ⟶ 10 : 3) afforded a solid which crystallised

* The original experimental work on this oxidation was carried out by Miss S. Ridley, Ph.D. Thesis, Manchester 1955.

from acetone–methanol to give 9β-*ergosta*-4 : 6 : 22-*trien*-3-*one* (280 mg.), m. p. 161—164°, $[\alpha]_D$ −14° (*c* 0·98) (Found: C, 85·5; H, 10·65. $C_{28}H_{42}O$ requires C, 85·2; H, 10·75%), $\lambda_{max.}$ 2840 Å (ε 26,200), $\nu_{max.}$ (Nujol mull) 1656 (doubly conjugated carbonyl), 1623 and 1587 (conjugated double bonds), and 965 (*trans*-CH=CH–) cm.$^{-1}$.

9β-*Ergosta*-4 : 22-*dien*-3-*one* (XIb).—9β-Ergosta-4 : 6 : 22-trien-3-one (2·4 g.) in ethanol (370 c.c.) containing potassium hydroxide (11 mg.) was hydrogenated in the presence of 5% palladium–norite (380 mg.) until 1·1 mol. of hydrogen had been absorbed. Acetic acid (2 drops) and pyridine (2 drops) were added, the catalyst was removed, and solvent evaporated under reduced pressure. The residue (which had a ratio $\varepsilon_{2440} : \varepsilon_{2860} = 11$) was chromatographed on deactivated alumina (130 g.). Light petroleum–benzene (20 : 1 ⟶ 20 : 3) eluted material (377 mg.) (probably 9β-ergost-22-en-3-one) with no intense ultraviolet absorption. Elution with light petroleum–benzene (20 : 3 ⟶ 2 : 1) afforded a product which after two crystallisations from acetone–methanol gave 9β-*ergosta*-4 : 22-*dien*-3-*one* (1·54 g.), m. p. 155—157°, $[\alpha]_D$ +25° (*c* 1·0) (Found: C, 85·0; H, 11·1. $C_{28}H_{44}O$ requires C, 84·8; H, 11·2%), $\lambda_{max.}$ 2440 Å (ε 14,500), $\nu_{max.}$ (Nujol mull) 1667 (conjugated carbonyl), 1616 (conjugated double bond) and 965 (*trans*-CH=CH–) cm.$^{-1}$.

22 : 23-*Dibromo*-9β-*ergost*-4-*en*-3-*one*.—A solution of 9β-ergosta-4 : 22-dien-3-one (1·41 g.) in chloroform (300 c.c.; alcohol-free) was cooled to −80° and bromine in chloroform (9·8 c.c.; 2% v/v) was added dropwise with stirring. Triethylamine was added, solvent removed under reduced pressure, and the residue dissolved in ether and filtered. Removal of the ether and crystallisation of the residue from acetone–ethanol afforded 22 : 23-*dibromo*-9β-*ergost*-4-*en*-3-*one* (1·4 g.), m. p. 203·5—206°, $[\alpha]_D$ +39° (*c* 0·9) (Found: C, 60·6; H, 7·8; Br, 28·3. $C_{28}H_{44}OBr_2$ requires C, 60·45; H, 7·95; Br, 28·7%), $\lambda_{max.}$ 2440 Å (ε 16,700), $\nu_{max.}$ (Nujol mull) 1669 (conjugated carbonyl) and 1621 (conjugated double bond), no peak at *ca.* 970 (*trans*-CH=CH–) cm.$^{-1}$. Debromination with activated zinc dust gave a high yield of the parent 9β-ergosta-4 : 22-dien-3-one, m. p. and mixed m. p. 155—157°, $[\alpha]_D$ +25° (*c* 1·0), $\lambda_{max.}$ 2440 Å (ε 14,500).

22 : 23-*Dibromo*-5-*oxo*-3 : 5-seco-A-*nor*-9β-*ergostan*-3-*oic Acid*.—22 : 23-Dibromo-9β-ergost-4-en-3-one (1·15 g.) in ethyl acetate (300 c.c.) was ozonised as above. Isolation of the acid as before gave semi-solid material which crystallised from acetone–light petroleum, affording 22 : 23-*dibromo*-5-*oxo*-3 : 5-seco-A-*nor*-9β-*ergostan*-3-*oic acid* (760 mg.), m. p. 178—182°, $[\alpha]_D$ −9° (*c* 0·95) (Found: C, 56·65; H, 7·45; Br, 27·9. $C_{27}H_{44}O_3Br_2$ requires C, 56·3; H, 7·7; Br, 27·7%), $\nu_{max.}$ (in CS_2) 1712 (carbonyl) cm.$^{-1}$.

Des-A-*lumist*-22-*en*-5-*one* (XIII, ii) *from the Acid of the* 9β-*Ergosterol Series*.—The above acid (690 mg.) was treated with activated zinc dust (1·3 g.) in refluxing 1 : 1 ether–methanol (64 c.c.) for 2 hr. More zinc dust (530 mg.) was added and refluxing was continued for 1 hr. The usual working up afforded a solid (480 mg.) which readily formed gels with a variety of solvents and was not obtained crystalline. The gel formed from light petroleum was cooled to −80°, filtered, and dried under reduced pressure, to give 5-*oxo*-3 : 5-seco-A-*nor*-9β-*ergost*-22-*en*-3-*oic acid* as an amorphous solid, m. p. 92—98°, $[\alpha]_D$ −39° (*c* 1·0), $\nu_{max.}$ (in CS_2) 1712 (carbonyl) and 967 (*trans*-CH=CH–) cm.$^{-1}$. (No satisfactory analysis could be obtained.)

The sodium salt (395 mg.) of the above acid, and sodium phenylacetate (1·2 g.), were heated at 290—295°/0·02—0·05 mm. for 2½ hr. The oily distillate (257 mg.), $[\alpha]_D$ −44° (*c* 0·8), was chromatographed on alumina (50 g.). Light petroleum–benzene (4 : 1 ⟶ 2 : 1) eluted an oil (225 mg.) which solidified slowly, then having m. p. (alone or mixed with de-A-lumist-22-en-5-one) 28—34°, $[\alpha]_D$ −48° (*c* 1·0) (Found: C, 83·35; H, 11·4. Calc. for $C_{24}H_{40}O$: C, 83·65; H, 11·7%), $\nu_{max.}$ (in CS_2) 1715 (carbonyl) and 968 (trisubstituted double bond) cm.$^{-1}$. The oxime had m. p. (alone or mixed with de-A-lumist-22-en-5-one oxime) 134—137°, $[\alpha]_D$ −96° (*c* 0·85).

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