88. *Studies in the Sterol Group. Part XXXI. The Structure of Lumisterol.*

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In Parts XXI and XXX (Heilbron, Spring, and Stewart, J., 1935, 1221; Burawoy, preceding paper) it has been established that lumisterol is a stereoisomeride of ergosterol. In further support of the structural identity of the two sterols we now find that lumisteryl acetate gives an *adduct* with maleic anhydride which, as in the case of the corresponding adduct from ergosteryl acetate, can be thermally decomposed with regeneration of its components.

In the hope of obtaining more precise information concerning the stereo-relationship of the two sterols we have now examined the fully saturated lumistanol. Lumistanyl acetate has previously been prepared by the catalytic hydrogenation of dihydrolumisteryl acetate (Ahrens, Fernholz, and Stoll, *Annalen*, 1933, **500**, 109), itself obtained by the reduction of lumisterol with sodium and ethyl alcohol (Windaus, Dittmar, and Fernholz, *Annalen*, 1932, **493**, 259). Although the direct catalytic hydrogenation of lumisteryl acetate was stated by the former authors to give an inseparable mixture, we have now succeeded in isolating both *lumistenyl acetate* (tetrahydrolumisteryl acetate), m. p. 179°, and lumistanyl acetate from the reaction mixture. Oxidation of lumistanol with chromic acid gives *lumistanone*, m. p. 121—122°, together with *lumistanedicarboxylic acid*, m. p. 208—210°. Although lumistanone is not identical with ergostanone (Reindel and Walter, *Annalen*, 1928, **460**, 222), it cannot be concluded that stereochemical differences other than the orien-

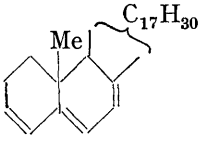
tation of the groups associated with C_3 exist between the two sterols, since it is conceivable that this alone would influence the spatial arrangement assumed by the ingoing hydrogens at the potentially asymmetric centres, C_5 and C_8.

In view of this complication it is apparent that a comparative study of ergosterol and lumisterol would be more profitably pursued by reference to their respective unsaturated derivatives.

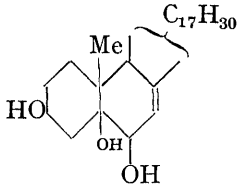
We have previously shown that dehydration of lumisterol with phosphorus oxychloride gives a lumistatetraene which differs from either of the ergostatetraenes A and B (Heilbron, Spring, and Stewart, *loc. cit.*). Lumistatetraene exhibits a well-defined absorption band at 3140 A., which by comparison with the principal band of lumisterol (max. 2790 A.) establishes the presence of three conjugated ethenoid linkages in the hydrocarbon, the structure of which must be (I). On the other hand, both the ergostatetraenes show a single-banded absorption spectrum with a maximum at 2600 A., showing that these hydrocarbons no longer contain the conjugated system of ergosterol.

In order to test the hypothesis that lumisterol is simply *epi*ergosterol, we attempted to oxidise the former to its ketone, reduction of which under specified conditions would lead to *epi*lumisterol. Although the oxidation of lumisterol with copper-bronze gives a *ketone*, m. p. 156—157°, this fails to exhibit the selective absorption characteristic either of lumisterol or of an αβ-unsaturated ketone which would result if the oxidation was accompanied by migration of the Δ^5-ethenoid linkage to the Δ^4-position (cf. cholestenone). A possible explanation of this anomaly is that the oxidation has been accompanied by a simultaneous saturation of the Δ^5-ethylenic linkage. The oxidation of dihydrolumisterol with copper-bronze, however, yields a *lumistadienone*, m. p. 175—176°, which differs from the ketone derived from lumisterol.

We next directed our attention to lumistadiene-3 : 5 : 6-triol-I (Heilbron, Spring, and Stewart, *loc. cit.*) and lumistadiene-3 : 5 : 6-triol-II (Dimroth, *Ber.*, 1936, **69**, 1123) (II). Each was oxidised with chromic acid, whereby one and the same *lumistadiene*-3 : 6-*dion*-5-*ol* (III), m. p. 182—183°, was obtained, a result which incidentally proves that the two triols are stereoisomeric, differing solely in the orientation of the C_6 hydroxyl groups.



(I.)



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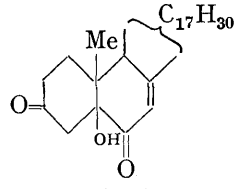
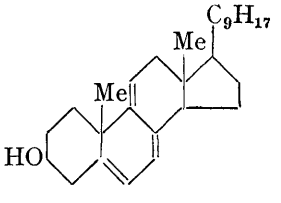
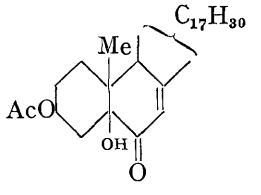


(III.)

The fact that lumistadiene-3 : 6-dion-5-ol differs from ergostadiene-3 : 6-dion-5-ol establishes either that asymmetric differences other than around C_3 exist between the two sterols, or that the two diketo-alcohols differ in the orientation of the hydroxyl group attached to C_5. The latter alternative is considered untenable in the light of the following spectrographic observations.

(IV.)





(V.)

Ergosterol and lumisterol, the chromophores of which are structurally the same, differ considerably in the intensity of their ultra-violet absorption. A similar difference is observed between dehydroergosterol and dehydrolumisterol (IV), such differences being common among stereoisomers ("Stereochemie," p. 746, Wassermann–Freudenberg, Leipzig, 1933). That these effects are not to be ascribed to the alternative orientation of

the C_3-hydroxyl group may be adduced from the observation that ergostadiene-3 : 6-dion-5-ol and lumistadiene-3 : 6-dion-5-ol (III) show the same persistent spectrographic differences in the low-intensity band, although in these diketones the C_3-centre of asymmetry has been removed. Further, the fact that lumistadiene-3 : 6-dion-5-ol and 3-acetoxylumistadien-6-on-5-ol (V) (Burawoy, *loc. cit.*) are spectroscopically indistinguishable, as are also the corresponding derivatives of the ergosterol series, demonstrates that neither the nature nor the orientation of the groups attached to C_3 contributes to the characteristic absorption of these αβ-unsaturated ketones.

	Ergo-		Lumi-	
	λ, max., A.	ε, max.	λ, max., A.	ε, max.
-sterol	2,815	12,000	2,790	8,500
Dehydro- -sterol (IV)	3,235	11,400	3,200	7,500
-stadienedionol (III)	2,525	13,450	2,530	13,500
	3,325	160	3,250	100
-stadienonediol monoacetate (V)	2,520	13,570	2,520	13,430
	3,330	155	3,230	105

The stereoisomerism responsible for the observed spectrographic differences between dehydrolumisterol and dehydroergosterol must therefore have its seat in C_{10}, which is directly adjacent to the chromophore. This difference in configuration around C_{10} must likewise exist between ergosterol and lumisterol. No information is available concerning the relative orientation of the hydroxyl groups and the C_9-hydrogen atoms of the two sterols.

Experimental.

Lumisteryl Acetate–Maleic Anhydride Adduct.—A mixture of lumisteryl acetate (0·5 g.) and maleic anhydride (0·3 g.) was heated for 30 minutes in an evacuated tube at 170—180°. The product was crystallised repeatedly from acetic anhydride, from which the *adduct* separated in plates, m. p. 176—177°, $[\alpha]_D^{22°}$ + 28·2° (l = 1, c = 2·13 in chloroform) (Found : C, 75·8; H, 9·1. $C_{34}H_{48}O_5$ requires C, 76·1; H, 9·0%); it distilled unchanged at 180°/3 × 10^{-4} mm., but on heating at 240°/15 mm. for 2 hours, followed by vacuum sublimation of the residue, lumisteryl acetate was obtained quantitatively.

Lumistenol.—A solution of lumisteryl acetate (5 g.) in glacial acetic acid (120 c.c.) was shaken with hydrogen and platinum oxide (0·5 g.) at 70—80°. After 5 hours the catalyst was removed, the solution cooled, and the separated solid collected and washed with acetic acid. Repeated crystallisation from alcohol gave *lumistenyl acetate* in plates, m. p. 178—179°, $[\alpha]_D^{20°}$ − 33·1° (l = 1, c = 1·51 in chloroform). Titration with perbenzoic acid proved the presence of one ethenoid linkage (Found : C, 81·5; H, 11·1. $C_{30}H_{50}O_2$ requires C, 81·4; H, 11·4%). Hydrolysis of the acetate with 5% methyl-alcoholic potash gave *lumistenol*, separating from aqueous alcohol in needles, m. p. 114—116°, $[\alpha]_D^{20°}$ − 0·5° (l = 1, c = 5·42 in chloroform) (Found : C, 83·8; H, 12·2. $C_{28}H_{48}O$ requires C, 83·9; H, 12·1%).

Lumistanol.—The acetic acid mother-liquor of lumistenyl acetate was diluted with water (30 c.c.), the solution clarified by warming, and slowly cooled, whereupon a second crop of impure lumistenyl acetate separated. Repetition of this process gave a third crop of lumistenyl acetate; further dilution of the mother-liquor with water resulted in the separation of oils. The solution was completely precipitated with water, extracted with ether, and the residue obtained after removal of the ether was hydrolysed by refluxing with 3% methyl-alcoholic potash (100 c.c.) for 2 hours. The product, isolated by means of ether, was repeatedly crystallised from acetone–alcohol (1 : 1), lumistanol being obtained in needles, m. p. 126—127°, $[\alpha]_D^{20°}$ + 8·2° (l = 1, c = 2·43 in chloroform).

Lumistanone and Lumistanedicarboxylic Acid.—A solution of lumistanol (1·5 g.) in glacial acetic acid (75 c.c.) was oxidised at 70° with a solution of chromic acid (1·0 g.) in acetic acid (85%; 8 c.c.), added during 1½ hours. The solution was stirred for a further 2 hours at the same temperature, and largely diluted with water. The product, isolated by means of ether, was separated into an acid and a neutral fraction by means of aqueous sodium carbonate. The neutral fraction (0·5 g.) separated in micro-needles from alcohol, and after repeated crystallisation from the same solvent had m. p. 121—122°, $[\alpha]_D^{22°}$ − 17·5° (l = 1, c = 3·97 in chloroform) (Found : C, 83·9; H, 11·8. $C_{28}H_{48}O$ requires C, 83·9; H, 12·1%). The *oxime* separated from alcohol in needles, m. p. 165—166° (Found : C, 80·8; H, 12·0; N, 3·4. $C_{28}H_{49}ON$ requires C, 80·9; H, 11·9; N, 3·4%).

The acid fraction from the oxidation mixture was crystallised repeatedly from dilute methyl alcohol, from which *lumistanedicarboxylic acid* separated in clusters of needles, m. p. 208—210°, $[\alpha]_D^{22°}$ + 24·6° (l = 1, c = 1·04 in chloroform) (Found: C, 74·7; H, 11·0. $C_{28}H_{48}O_4$ requires C, 74·9; H, 10·8%). The *dimethyl* ester, prepared by means of diazomethane, separated from dilute methyl alcohol in clusters of needles, m. p. 48—49° (Found: C, 75·2; H, 10·7. $C_{30}H_{52}O_4$ requires C, 75·6; H, 11·0%).

Lumistatetraene.—According as ergosterol is dehydrated with phosphorus oxychloride or with *p*-toluenesulphonyl chloride, two different hydrocarbons, ergostatetraene A and B, are obtained (Rygh, *Z. physiol. Chem.*, 1929, **185**, 99; Stoll, *ibid.*, 1931, **202**, 233). Furthermore, on refluxing with acetic anhydride, the tetraene A is isomerised to tetraene B. In the case of lumisterol, however, we find that dehydration by the latter reagent gives the same lumistatetraene as dehydration by the former reagent (Heilbron, Spring, and Stewart, *loc. cit.*). Lumisterol (1·0 g.) in pyridine (20 c.c.) was heated with *p*-toluenesulphonyl chloride (2·0 g.) for 30 minutes on the steam-bath. The cold mixture was diluted with water and extracted with ether, and the extract washed successively with dilute acetic acid, aqueous sodium carbonate, and water. The residue obtained by removal of the solvent from the dried solution was repeatedly crystallised from ether–methyl alcohol, from which lumistatetraene separated in long needles, m. p. 88°, either alone or in admixture with the phosphorus oxychloride preparation.

Lumistadiene-3 : 6-dion-5-ol.—A solution of lumistadiene-3 : 5 : 6-triol-I or -II (2·5 g.) in glacial acetic acid (190 c.c.) was treated with chromic anhydride (1·5 g.) in acetic acid (20 c.c.) and water (2 c.c.), added during an hour at room temperature with stirring. The stirring was continued for a further 1½ hours; the solution was then diluted with water and extracted with ether. The neutral fraction of the product, isolated in the usual manner, was crystallised from methyl alcohol, *lumistadiene-3 : 6-dion-5-ol* separating in clusters of needles, m. p. 182—183° (Found: C, 78·3; H, 10·2. $C_{28}H_{42}O_3$ requires C, 78·8; H, 9·9%).

Oxidation of Lumisterol with Copper-bronze.—An intimate mixture of lumisterol (5·6 g.) and copper-bronze was distilled under 5—6 mm. The fraction distilling at 245° (bath temp. 300—310°) (4 g.) solidified, and after repeated crystallisation from acetone the *ketone* separated in prisms, m. p. 156—157°, $[\alpha]_D^{22°}$ + 5·5° (l = 1, c = 1·37 in chloroform) (Found: C, 85·3; H, 11·3. $C_{28}H_{42}O$ requires C, 85·2; H, 10·7. $C_{28}H_{44}O$ requires C, 84·8; H, 11·2%). The 2 : 4-*dinitrophenylhydrazone* separated from alcohol in orange needles, m. p. 204—205° (Found: C, 71·2; H, 8·3; N, 9·65. $C_{34}H_{46}O_4N_4$ requires C, 71·0; H, 8·1; N, 9·7%. $C_{34}H_{48}O_4N_4$ requires C, 70·7; H, 8·4; N, 9·7%). The *oxime* separated from alcohol in plates, m. p. 168—169° (Found: C, 81·8; H, 11·0; N, 3·4. $C_{28}H_{43}ON$ requires C, 82·0; H, 10·6; N, 3·4%. $C_{28}H_{45}ON$ requires C, 81·7; H, 11·0; N, 3·4%).

Lumistadienone.—A mixture of dihydrolumisterol (3·0 g.) and copper-bronze (2·3 g.) was distilled at 3—5 mm. The main fraction distilled at 220—240°, and after repeated crystallisation of this from acetone, *lumistadienone* was obtained in plates, m. p. 175—176°, $[\alpha]_D^{24°}$ + 31·6° (l = 1, c = 1·64 in chloroform) (Found: C, 85·3; H, 11·3. $C_{28}H_{44}O$ requires C, 84·8; H, 11·2%). The *oxime* separated from aqueous alcohol in needles, m. p. 210—212° (decomp.) (Found: C, 81·8; H, 11·2; N, 3·4. $C_{28}H_{45}ON$ requires C, 81·7; H, 11·0; N, 3·4%).

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