289. *Studies in the Sterol Group. Part XXI. Lumisterol.*

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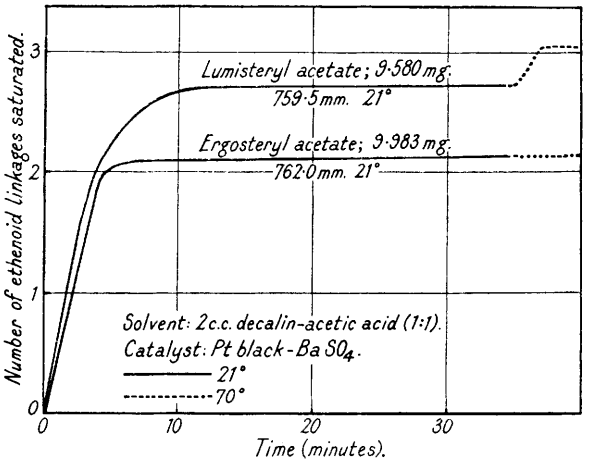
The photochemical conversion of ergosterol into calciferol proceeds according to the scheme :

Ergosterol —→ Lumisterol —→ Tachysterol —→ Calciferol

and further irradiation of the last results in the formation of the suprasterols-I and -II [compare Setz, (bibl.) *Z. physiol. Chem.*, 1933, **215**, 183].

A preliminary study of the constitution of calciferol has revealed that it differs profoundly from the tetracyclic triethenoid ergosterol, $C_{28}H_{44}O$, with which it is isomeric in that it has *four ethylene linkages* and possesses a *tricyclic* system consequent upon the rupture of ring B of ergosterol (Heilbron, Samant, and Spring, *Nature*, 1935, **135**, 1072). Calciferol thus resembles tachysterol, which Lettré (*Annalen*, 1934, **511**, 280) has also shown to be tricyclic.

As a result of these findings, we have now begun an investigation of lumisterol in order to ascertain whether ring B is intact at this stage in the photochemical transformation. In view of the fact that calciferol had previously been reported to possess only three ethenoid linkages (Windaus, Linsert, Lüttringhaus, and Weidlich, *Annalen*, 1932, **492**, 226; Askew *et al.*, *Proc. Roy. Soc.*, 1932, *B*, **109**, 468), we deemed it advisable to redetermine the degree of unsaturation of lumisterol. We find, in accord with previous investigations (Windaus, Dithmar, and Fernholz, *Annalen*, 1932, **493**, 259; Dimroth, *Ber.*, 1935, **68**, 539), that it contains three ethylene linkages, as shown both by the method of perbenzoic acid titration and by that of quantitative hydrogenation. The tetracyclic nature of lumisterol is further demonstrated by the fact that it yields methylbenzenetetracarboxylic acid on oxidation with concentrated nitric acid (Inhoffen, *Annalen*, 1932, **494**, 122) and gives Diels's hydrocarbon ($C_{18}H_{16}$) on dehydrogenation with selenium (Dimroth, *loc. cit.*). We deduce from the former observation that, as in the case of ergosterol, lumisterol contains two ethenoid linkages in ring B; its third ethylenic linkage is located in the side-chain ($\Delta^{22:23}$), since it gives methyl*iso*propylacetaldehyde on ozonolysis (Guiteras, *Annalen*, 1932, **494**, 117). Thus it would appear that the three ethenoid linkages of lumisterol are in substantially the same positions as are those of ergosterol.



Lumisterol differs from ergosterol in that it fails to form an insoluble digitonide and is strongly dextrorotatory. Both of these differences would be accounted for if lumisterol were simply *epi*ergosterol. With a view to test this hypothesis, we have dehydrated lumisterol with phosphorus oxychloride; the product, *lumistatetraene*, is, however, not identical with Rygh's ergostatetraene (*Z. physiol. Chem.*, 1929, **185**, 99), thus proving that, apart from possible epimerisation, other changes must have occurred during the photochemical transformation.

With the object of determining the precise position of the two nuclear ethenoid linkages of lumisterol, we have oxidised it with perbenzoic acid, obtaining *lumistadienetriol monobenzoate*, m. p. 185—186°. Hydrolysis of this gives *lumistadienetriol*, m. p. 180—181°, which yields only a *diacetate*, m. p. 128—130°. Titration of both lumistadienetriol monobenzoate and lumistadienetriol with lead tetra-acetate indicates that the former *is not* and

the latter *is* an α-glycol. As a completely analogous series of reactions has been observed with ergosterol resulting in the formation of ergostadiene-3 : 5 : 6-triol (Windaus and Lüttringhaus, *Annalen*, 1930, **481**, 119; Heilbron, Morrison, and Simpson, J., 1933, 302), it appears almost certain that lumisterol likewise contains a $\Delta^{5:6}$-ethenoid linkage. A detailed investigation of the new triol and its derivatives is in progress with a view to confirm this deduction.

A further analogy between ergosterol and lumisterol is to be seen in the facile dehydrogenation of lumisteryl acetate by mercuric acetate, *dehydrolumisteryl acetate*, m. p. 142—143°, being obtained (compare Windaus and Brünken, *Annalen*, 1928, **460**, 225).

Experimental.

Lumisteryl 3 : 5-*Dinitrobenzoate*.—Repeated crystallisation of crude material from methyl alcohol–acetone (1 : 1) gave the *ester* as pale yellow needles, m. p. 140—141°, $[\alpha]_D^{24°}$ + 24° (l = 1, c = 1·1 in benzene) (Askew *et al.*, *loc. cit.*, give m. p. 139—141° and $[\alpha]_{5461}^{20°}$ + 24° for the 3 : 5-dinitrobenzoate of their " sterol x " without, however, analysing this ester) (Found : C, 71·1; H, 7·7; N, 4·65. $C_{35}H_{46}O_6N_2$ requires C, 71·1; H, 7·85; N, 4·7%)

Lumisterol.—A suspension of lumisteryl 3 : 5-dinitrobenzoate (10 g.) in boiling methyl alcohol (120 c.c.) was treated with aqueous sodium hydroxide (12 c.c.; 2*N*), added during 20 minutes, and the solution refluxed for a further hour. Precipitation with water and crystallisation of the collected solid from methyl alcohol gave lumisterol in needles, m. p. 116—117°, $[\alpha]_D^{23°}$ + 177·6° (l = 1, c = 1·17 in acetone) (Askew *et al.*, *loc. cit.*, give m. p. 116·5—118·5°, $[\alpha]_D^{20°}$ + 176° in ethyl alcohol; Windaus, Dithmar, and Fernholz, *loc. cit.*, give m. p. 118°, $[\alpha]_D^{19°}$ + 191·5° in acetone). Lumisteryl acetate separates from methyl alcohol in long needles, m. p. 99—100°, $[\alpha]_D^{23°}$ + 129·6° (l = 1, c = 0·83 in acetone) (Windaus, Dithmar, and Fernholz, *loc. cit.*, give m. p. 100°, $[\alpha]_D^{19°}$ + 130·5° in acetone).

Titration of Lumisterol with Perbenzoic Acid.—A solution of lumisterol (0·609 g.) in chloroform (20 c.c.) was set aside at 0° with a solution of perbenzoic acid in chloroform (50 c.c.) containing 0·157 g. of active oxygen :

Time (hours)	70	95	124	192
Atoms of O absorbed	2·91	3·0	3·1	3·04

Micro-hydrogenation of Lumisterol.—A comparison of the velocity of hydrogenation of lumisterol and ergosterol is shown in the fig. It is noteworthy that under the standard conditions employed, whereas with ergosterol the absorption ceases, even after heating, at the tetrahydro-stage, yet with lumisterol absorption proceeds until the equivalent of 3 mols. of hydrogen is absorbed.

Lumistadienetriol Monobenzoate.—A solution of perbenzoic acid in chloroform (300 c.c. containing 0·82 g. active oxygen) was added slowly (1 hr.) and with constant stirring to a solution of lumisterol (18·7 g.) in chloroform (350 c.c.) at 0°, and the whole maintained at this temperature for a further 20 hrs. After removal of the solvent, an ethereal solution of the residual solid was washed with aqueous sodium carbonate, dried, and the ether removed. The crude solid was crystallised first from methyl alcohol–ether and then repeatedly from acetone, *lumistadienetriol monobenzoate* (11·2 g.) separating in prismatic needles, m. p. 185—186°, $[\alpha]_D^{24°}$ − 68·0° (l = 1, c = 1·81 in chloroform) (Found : C, 78·7; H, 9·4. $C_{35}H_{50}O_4$ requires C, 78·6; H, 9·4%). With antimony trichloride it gives a very intense carmine coloration, which slowly changes to brown and finally to green.

Lumistadienetriol.—A solution of the benzoate (10·7 g.) in methyl-alcoholic potash (540 c.c.; 5%) was refluxed for 3 hours. After concentration to half-bulk, the solution was diluted with water, and the precipitated solid crystallised repeatedly from methyl alcohol, *lumistadienetriol* separating in clusters of needles, m. p. 180—181°, $[\alpha]_D^{20°}$ − 8·7° (l = 1, c = 5·763 in chloroform) (Found : C, 78·3; H, 10·9. $C_{28}H_{46}O_3$ requires C, 78·1; H, 10·8%).

The triol (0·30 g.) was set aside with a chloroform solution of perbenzoic acid (35 c.c.) containing 0·597 g. of active oxygen :

Time (hours)	25	48	75
Atoms of O absorbed	1·91	1·96	1·96

The *diacetate*, prepared by the acetic anhydride–pyridine method, separated from methyl alcohol in stout prisms, m. p. 128—130°, $[\alpha]_D^{24°}$ − 48·4° (l = 1, c = 1·551 in acetone) (Found : C, 74·5; H, 9·8. $C_{32}H_{50}O_5$ requires C, 74·65; H, 9·8%). It is extremely soluble in common organic solvents.

Lumistatetraene.—Lumisterol (2·5 g.) was added quickly to a mixture of phosphorus oxychloride (1 g.) and pyridine (20 c.c.), and the solution boiled for 2 minutes. The resultant turbidity was removed by addition of methyl alcohol, and after refrigeration over-night the separated crystalline solid was collected, washed with water, and dried. After several crystallisations from methyl alcohol–ether, *lumistatetraene* was obtained as clusters of long needles, m. p. 88—90°, $[\alpha]_D^{24°}$ + 298·9° (l = 1, c = 1·097 in chloroform) (Found: C, 88·5; H, 11·2. $C_{28}H_{42}$ requires C, 88·8; H, 11·2%). With antimony trichloride in chloroform the following colour changes were observed: crimson ⟶ dark red ⟶ purple ⟶ violet ⟶ green.

Dehydrolumisteryl Acetate.—Lumisteryl acetate (4 g.) in chloroform (50 c.c.) was added to a solution of mercuric acetate (9·2 g.) in glacial acetic acid (100 c.c.), and the mixture shaken at room temperature for 3½ days. The separated mercurous acetate was removed, and the filtrate diluted with water and extracted with ether. The extract was washed with sodium carbonate solution and dried; after removal of solvent it gave an oil which slowly crystallised from methyl alcohol, giving *dehydrolumisteryl acetate* in long needles, m. p. 142—143°, $[\alpha]_D^{20°}$ + 226·4° (l = 1, c = 1·02 in chloroform) (Found: C, 82·5; H, 10·3. $C_{30}H_{44}O_2$ requires C, 82·5; H, 10·2%).

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