VI.—*Studies in the Sterol Group. Part I. The Absorption Spectra of Some Cholesterol Derivatives.*

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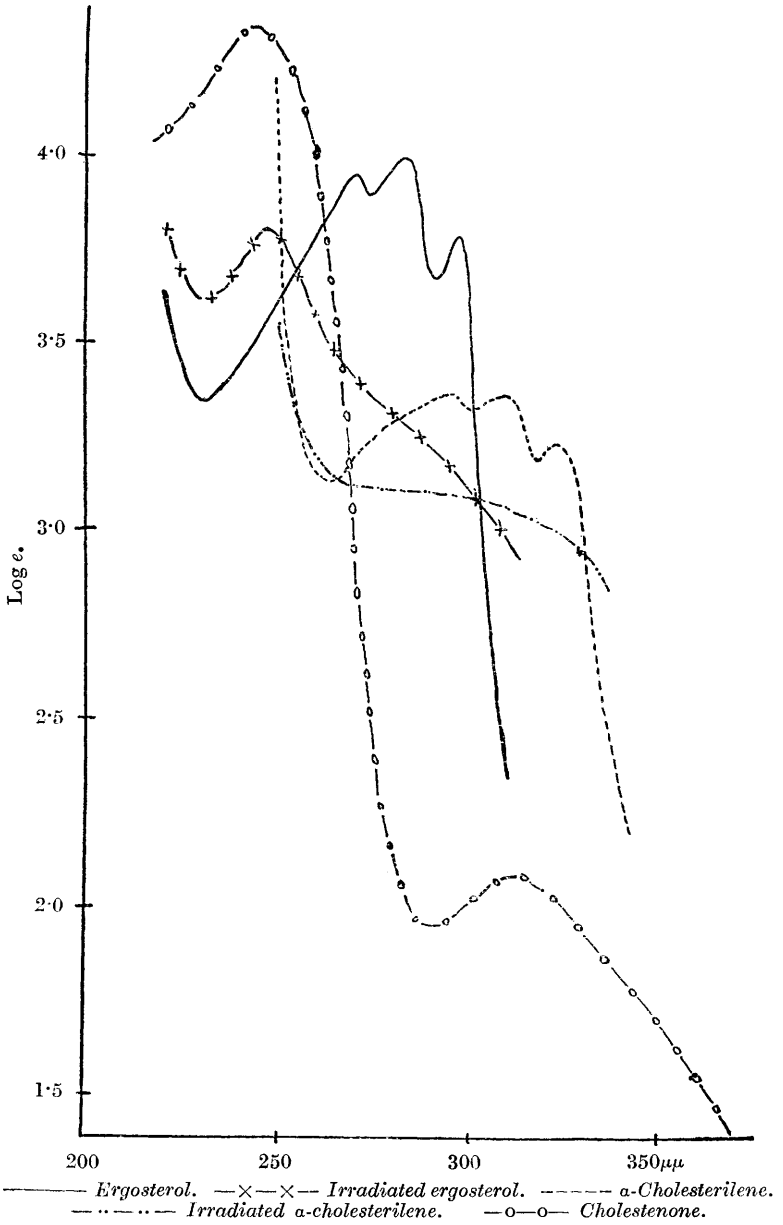
The pro-vitamin ergosterol, characterised by its well-defined absorption bands at 293·5 μμ, 281·5 μμ, and 270 μμ, is converted on irradiation into a photochemically unstable substance (vitamin D) giving an absorption band at 247 μμ (Morton, Heilbron, and Kamm, J., 1927, 2000). The work now to be described was undertaken with the view of correlating, if possible, unsaturation and ultra-violet absorption in the sterol group and obtaining some idea of the type of compound with which the vitamin may be classed. As previously mentioned (Morton, Heilbron, and Kamm, *loc. cit.*), it is very doubtful whether the formation of the vitamin is due to polymeris-

FIG. 1.


4·0
3·5
3·0
2·5
2·0
1·5
Log ε.
200
250
300
350μμ
Ergosterol. —×—×— Irradiated ergosterol. ----- α-Cholesterilene.
— ·· — ·· — Irradiated α-cholesterilene. —o—o— Cholestenone.

ation of the ergosterol, and it is also unlikely, in view of the total alteration of the type of selective absorption, that the reaction is due simply to stereochemical changes in the molecule, for *cis*- and *trans*-isomerides usually differ in extinction rather than the general shape of the absorption curve (Henri and Errera, *Compt. rend.*, 1925, **180**, 2049; **181**, 548).

The following cholesterol derivatives have been examined spectrographically :

Compound.	Double bonds.	Types of absorption.
Cholesteryl chloride	1	No marked selective absorption.
Cholesteryl acetate	,,	,, ,, ,,
Cholesterol (pure)	,,	,, ,, ,,
Cholestene	,,	,, ,, ,,
ψ-Cholestene	,,	,, ,, ,,
Dicholesteryl ether	2	,, ,, ,,
Cholestenone	,, (one being C:O)	Bands at 243 $\mu\mu$ and 312 $\mu\mu$.
Cholesterilene	2	Bands well-defined at 294 $\mu\mu$, 304 $\mu\mu$, and 321 $\mu\mu$.
Oxycholesterylene	3 (one being C:O)	Band at 277 $\mu\mu$.
Ergosterol	3	Bands at 270 $\mu\mu$, 281·5 $\mu\mu$, and 293·5 $\mu\mu$.

Selective absorption is only shown when at least two double bonds are present in the single molecule. In comparison with Mauthner's cholesterilene the maximum absorption for each of the three bands of ergosterol is some 250 Å. units further in the ultra-violet :

Maxima for cholesterilene	3210	3040	2940
,, ,, ergosterol	2935	2817	2690
Differences	275	223	250

The molecular extinction coefficients are of the order : ergosterol, 10,000; cholesterilene, 2400. The shifting of the absorption bands in the direction of the ultra-violet region and the greatly increased extinction coefficient of the former compound, due to the introduction of an additional double bond, are quite in harmony with general views on absorption spectra. It seems reasonable to infer from these results that of the three double bonds in ergosterol two occupy the same positions as in cholesterilene.

The case of cholestenone is of interest. Whereas the shallow band at 312 $\mu\mu$ is probably an ordinary ketone band, that at 243 $\mu\mu$ is strikingly similar to the well-defined band 247 $\mu\mu$ of vitamin D. The irradiation of cholestenone in alcoholic solution resembles that of the vitamin in that the absorption band at 243 $\mu\mu$ disappears rapidly; the ketone band disappears less rapidly. On irradiation, no new bands are shown either by cholestenone or by any of the cholesterol derivatives showing selective absorption, although in all cases photochemical decomposition occurs. It is a well-established

fact that cholesterol readily undergoes intramolecular rearrangement, the type of compound isolated depending upon the conditions. The fundamental reaction, however, would appear to be dehydrogenation, leading to cholestenone formation (unpublished work), and it may well be that the passage of ergosterol into the vitamin follows a somewhat similar course, giving rise to ergostantrienone or a partly hydrogenated ketone derived from this. Although no band has been observed in the case of the vitamin in the region corresponding to the 312 μμ band of cholestenone, this does not preclude its possible occurrence, for owing to the incomplete transformation of ergosterol into vitamin a band in this region would be masked. Its presence may, however, be inferred from the fact that the photolabile vitamin continues to decompose even when screened by vita glass (Heilbron, Kamm, and Morton, *Nature*, October 29th, 1927).

Rosenheim and Webster (*Lancet*, September 17th, 1927) have stated that ergosteryl acetate and benzoate can be rendered antirachitic on irradiation, but this in no way refutes our arguments, for photochemical hydrolysis could readily precede photo-oxidation. That ergosterol is, indeed, extraordinarily sensitive to oxidative processes has recently been shown by Windaus, Borgeaud, and Brunken (*Nachr. Gess. Wiss. Göttingen*, 1927), who have found that ergosterol is both readily dehydrogenated to a pinacol and photo-oxidised to a peroxide by sunlight in presence of suitable catalysts.

Experimental.

Cholesterol was obtained from cod-liver oil and repeatedly crystallised from alcohol; it had m. p. 148·5°, and $[\alpha]_D^{20°}$ −39·08° in chloroform ($c = 2·124$).

The derivatives were all prepared by the standard methods described in the literature. Cholesteryl chloride, m. p. 96°, was made by the action of thionyl chloride on cholesterol (Diels and Blümberg, *Ber.*, 1911, **44**, 2848). Cholesterilene and dicholesteryl ether were prepared by heating cholesterol with anhydrous copper sulphate (Mauthner and Suida, *Monatsh.*, 1896, **17**, 29). The cholesterilene was repeatedly crystallised from ether–alcohol until the m. p. (78°) remained constant; it had $[\alpha]_D^{20°}$ − 102·1° in chloroform ($c = 4·000$). Mauthner and Suida give m. p. 79—80° and $[\alpha]_D$ − 81·63°. The dicholesteryl ether, after twice crystallising from ethyl acetate containing a little benzene, melted at 194—195°.

Cholestenone was prepared by oxidation of cholesterol dibromide and subsequent debromination (Windaus, *Ber.*, 1906, **39**, 518). Recrystallisation from methyl alcohol gave a product melting sharply at 79° (Windaus gives 81—82°), which was characterised by the preparation of its semicarbazone, m. p. 234°. Oxycholesterylene

was prepared by oxidation of cholesteryl acetate (Mauthner and Suida, *Monatsh.*, 1896, **17**, 579). After repeated crystallisation from 80% alcohol, it had a constant m. p. of 110° (Mauthner and Suida give 112°).

Cholestene was prepared by the action of sodium and amyl alcohol upon cholesteryl chloride (von Fürth and Felsenreich, *Biochem. Z.*, 1915, **69**, 416). After repeated crystallisation from ether–alcohol it melted at 90—91°; the specific rotation, $[\alpha]_D$ — 53·05° (c = 3·544), was not affected by further crystallisation (von Fürth and Felsenreich give — 55·5°). It was further characterised by means of its isomeric α- and β-dibromides, m. p. 140° and 106°, respectively (Mauthner, *Monatsh.*, 1894, **15**, 85). ψ-Cholestene was prepared from cholestene hydrochloride (Mauthner and Suida, *Monatsh.*, 1907, **28**, 1113). After repeated crystallisation from ether–alcohol it melted at 78—79° and gave the dibromide, m. p. 116°, as described. It had $[\alpha]_D$ + 60·13° in chloroform (c = 3·176). Mauthner and Suida give + 64·86° for the same concentration, whereas Dr. A. Triebs of Münich finds + 57·3° (private communication). It is clear that the whole question of optical rotations in the cholesterol group of compounds is an extraordinarily complex one, and the specific rotations give no sure criteria of purity; *e.g.*, values of $[\alpha]_D$ ranging — 61·55° to — 116·2° have been found for cholesterilene, prepared in different ways (Windaus, *Z. physiol. Chem.*, 1921, **117**, 156). It is obvious that these anomalous results must arise from the complex nature of the cholesterol molecule, which contains numerous asymmetric carbon atoms, in any one of which racemisation may occur.

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