

207. Studies in the Sterol Group. Part XXIV. The Constitution of Calciferol.*

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THE experiments recorded in this communication were commenced in 1934 with a view to the elucidation of the nature of the photochemical changes effected during the irradiation of ergosterol and particularly with the object of investigating the constitution of calciferol.

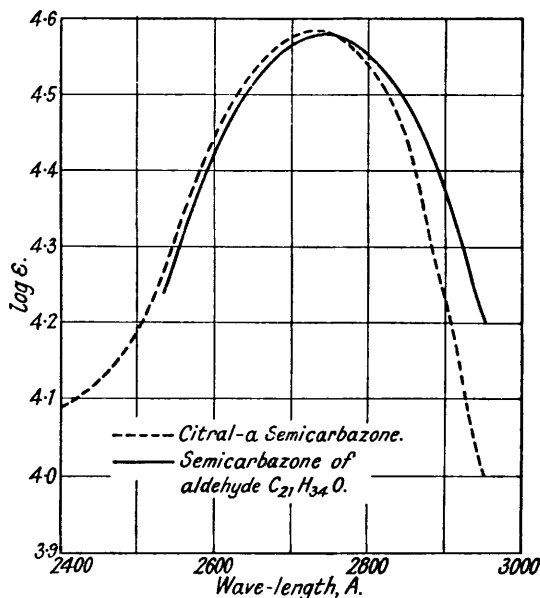
That calciferol, in contradistinction to ergosterol, contains four ethylenic linkages appeared probable to us from a quantitative study of the action of perbenzoic acid upon the vitamin. Values of 3.6—3.7 atoms of oxygen absorbed per mol. of calciferol were observed in agreement with the microcatalytic hydrogenation result of Kuhn and Möller (*Angew. Chem.*, 1934, **47**, 145; cf., however, Windaus, Linsert, Lüttringhaus, and Weidlich, *Annalen*, 1932, **492**, 226; Angus, Askew, *et alia*, *Proc. Roy. Soc.*, 1931, *B*, **108**, 340). Since there is no doubt that calciferol is isomeric with ergosterol (Windaus, Werder, and Gschaider, *Ber.*, 1932, **65**, 1006), this result led us to the conclusion that the former must be tricyclic, a fact abundantly confirmed by subsequent experience (Müller, *Z. physiol. Chem.*, 1935, **233**, 222; Windaus and Thiele, *Annalen*, 1935, **521**, 160; Reichel and Deppe, *Z. physiol. Chem.*, 1936, **239**, 143).

Oxidation of calciferol with either chromic anhydride or potassium permanganate yields an oily aldehyde characterised by its *semicarbazone*, m. p. 242°, numerous analyses of which established the formula $C_{21}H_{34}O$ for the aldehyde. Spectroscopic examination of the semicarbazone, m. p. 242°, the absorption curve of which is reproduced together with that of citral semicarbazone in the accompanying diagram, shows that the parent

* Preliminary notes on the results of this investigation have been published (Heilbron, Samant, and Spring, *Nature*, 1935, **135**, 1072; Heilbron and Spring, *J. Soc. Chem. Ind.*, 1935, **54**, 795). We use the designation calciferol for the antirachitic vitamin obtained from ergosterol in preference to "vitamin D," since the latter term clearly includes a number of homologues and to avoid ambiguity needs qualifying by such terms as "from ergosterol."

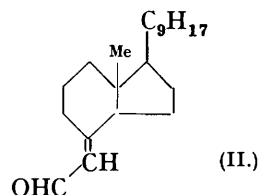
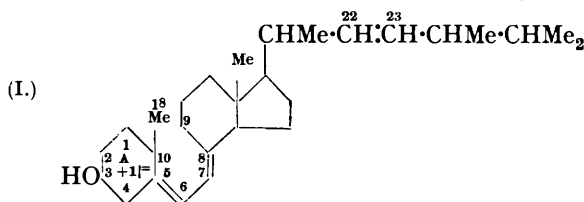
aldehyde is $\alpha\beta$ -unsaturated (compare Menschick, Page, and Bossert, *Annalen*, 1932, **495**, 225).*

The partial formulation of calciferol previously derived by us (Heilbron and Spring, *loc. cit.*) must therefore be expanded to (I), in which a third ethylenic linkage is situated between C_7-C_8 , the aldehyde $C_{21}H_{34}O$ being represented by (II).

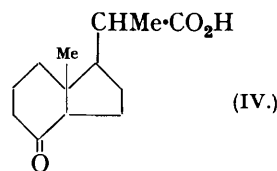
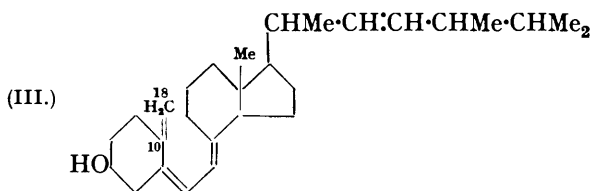


Windaus and Thiele (*loc. cit.*) have confirmed this partial structure by an independent method and furthermore they have adduced evidence that the unlocated ethylenic linkage is situated between $C_{10}-C_{18}$ (III). This decision depends upon an assumption that, during the drastic pyrogenic reaction employed, no rearrangement has occurred.

The presence of this exocyclic methylene group has now been confirmed by the ozone degradation of calciferol, whereby formaldehyde was obtained and characterised as its dimedon derivative. From the acid fraction of the decomposed ozonide, a keto-acid, $C_{13}H_{20}O_3$, was isolated and characterised by its semicarbazone, m. p. 219° . This can only be represented by (IV) and must be derived by fission of the calciferol molecule at the $C_{22}-C_{23}$ (cf. Guiteras, *Annalen*,



1932, **494**, 117) and the C_7-C_8 ethylenic linkages. Its isolation provides independent and convincing proof that calciferol is correctly formulated by (III).



EXPERIMENTAL.

Oxidation of Calciferol with Chromic Anhydride.—A solution of calciferol (5 g.) in glacial acetic acid (125 c.c.) was treated with chromic anhydride (2.5 g.) in water (2.5 c.c.), added during 1 hour with mechanical stirring, and the whole was maintained at room temperature for a further period of 3 hours. The reaction mixture was largely diluted with water and extracted with ether, and the product separated into neutral and acid fractions. The former was a thick brown oil (4.8 g.) easily soluble in the common organic solvents with the exception of methyl alcohol. It had a penetrating agreeable odour and exhibited pronounced aldehydic properties

* We note that the semicarbazone, m. p. 242° , has also been spectroscopically examined in the Göttingen laboratories with the same results (Über Sterine, Gallensäuren, u.s.w., p. 303, Lettré and Inhoffen, Stuttgart, 1936).

as evidenced by the facile reduction of ammoniacal silver oxide and Fehling's solutions and by its positive reaction with Schiff's reagent.

Semicarbazone of the Aldehyde $C_{21}H_{34}O$.—An alcoholic solution of the neutral oil was treated with excess of an alcoholic solution of semicarbazide acetate. After 2 days the deposited solid was collected and purified by crystallisation from alcohol. The melting point was soon raised to 235° (four recrystallisations), but the pure *semicarbazone*, m. p. 242° , was only obtained by fourteen subsequent recrystallisations (Found: C, 73.5, 73.5, 73.6; H, 10.5, 10.5, 10.3; N, 11.55, 11.8; *M*, 389, 388. $C_{22}H_{37}ON_3$ requires C, 73.45; H, 10.4; N, 11.7%; *M*, 359. $C_{22}H_{35}ON_3$ requires C, 73.9; H, 9.8; N, 11.8%; *M*, 357. $C_{21}H_{35}ON_3$ requires C, 72.9; H, 10.2; N, 12.2%; *M*, 345. $C_{21}H_{33}ON_3$ requires C, 73.4; H, 9.7; N, 12.2%; *M*, 343). When the oxidation was effected at 40° and 70° the same semicarbazone was obtained but in diminished yield.

Oxidation of Calciferol with Potassium Permanganate.—Calciferol (2 g.) in benzene (25 c.c.) was added to a solution of potassium permanganate (2 g.) in water (100 c.c.) and sulphuric acid (1.1 c.c.) and shaken at room temperature for 7 hours. The mixture was clarified with sulphur dioxide and extracted with ether. The neutral portion (1.4 g.), isolated in the usual manner, formed a pale yellow oil with aldehydic properties, the semicarbazone of which after repeated crystallisation from methyl alcohol had m. p. 235° , not depressed on admixture with the semicarbazone of the chromic anhydride oxidation product (Found: C, 73.3; H, 10.3; N, 11.75. $C_{22}H_{37}ON_3$ requires C, 73.45; H, 10.4; N, 11.7%).

Ozonolysis of Calciferol.—A solution of calciferol (5 g.) in carefully purified chloroform (50 c.c.) was treated with a slow stream of ozone (6%) and oxygen at 0° for 6 hours, the issuing gases being washed with ice-cold water. The subsequent procedure for the estimation of the formaldehyde produced was as described by Clemo and Macdonald (J., 1935, 1294), the weight of dimedon derivative being 263 mg. (7.2%), m. p. $186-187^{\circ}$, showing no depression on admixture with an authentic specimen (Found: C, 69.7; H, 7.5. Calc. for $C_{17}H_{24}O_4$: C, 69.8; H, 8.3%).

A comparative ozonolysis of lumisterol under identical conditions gave no weighable quantity of formaldehyde dimedon derivative.

Semicarbazone of the Keto-acid $C_{13}H_{20}O_3$.—After distillation in steam of the decomposed ozonide from 10 g. of calciferol, the non-volatile residue was isolated by means of ether and separated into acid and neutral fractions by extraction with sodium carbonate solution. The neutral fraction (1.1 g.) was a pale yellow oil, from which no crystalline semicarbazone could be obtained. The acid fraction, a brown oil (2.8 g.), was treated with excess of semicarbazide acetate in alcohol and kept at room temperature for 2 days. The solution (30 c.c.) was diluted with water (70 c.c.), giving a brown precipitate which could not be purified. Further dilution of the filtrate with water (400 c.c.) and long standing gave a colourless solid, which after two crystallisations from methyl alcohol separated in plates, of the constant m. p. 219° . The *semicarbazone* is sparingly soluble in methyl alcohol, chloroform, and ethyl acetate (Found: C, 59.8; H, 8.4; N, 14.8. $C_{14}H_{23}O_3N_3$ requires C, 59.8; H, 8.2; N, 14.9%).

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