villosa (V). In studying the hybrids *Tr. turgidum* × *Secale cereale* (*i.e.*, ABS) and *Tr. vulgare* × *Secale cereale* (*i.e.*, ABCS) we obtained data which showed that S gene is not homologous with genes A, B and C of *Triticum*. Similar data were reported by other students (Lebedeff, 1932; Müntzing, 1935, etc.). The relation between the *Secale* (S) gene, and β-gene of *Tr. Timopheevi* was not however studied. Kihara and Lilienfeld's attempts to cross *Tr. Timopheevi* with *Secale cereale* were unsuccessful. We raised in 1935 two hybrids *Tr. Timopheevi* × *Secale cereale* (*i.e.*, AβS). It was possible to study cytologically one of them. The absence of bivalents in the I metaphase of PMC's in the hybrid and the appearance of only one or sometimes of two bivalents with one terminal chiasma showed that β-gene is not homologous with S gene.

But what is then the relation between V gene (of *Haynaldia villosa*), and S gene (of *Secale cereale*)? Numerous attempts were made to cross *Secale cereale* with *Haynaldia villosa* but they were always unsuccessful. We produced, in 1931, only one hybrid and it died at an early stage of development. Recently, we followed another way in combining the gene of *Secale cereale* (S) with that of *Haynaldia villosa* (V), namely, by using a bridge species. *Tr. dicoccum* served as one such. In crossing the hybrid *Tr. dicoccum* × *Haynaldia villosa* (*i.e.*, ABV) with *Secale cereale* (S) a trigeneric triple hybrid with 28 chromosomes (ABVS) was produced which contained all A, B, V, and S genes. In studying the meiosis of this hybrid we found usually 28 univalent chromosomes, or 1 bivalent and 26 univalents. Such a behaviour of the chromosomes during the I metaphase of the trigeneric hybrid shows that gene S is not homologous with gene V. (Detailed description will be given elsewhere by Kostoff and Arutinnova.)

Kihara, H., *Mem. Coll. Sci. Kyoto Imp. Univer. Ser.*, 1924, B, 1.

Kihara, H., *Genomanalyse bei* Triticum und Aegilips. V; Lilienfeld, F., und Kihara, H., *Cytologia*, **1934, 6,** 87–122.

Kostoff, D., *Chromosome behaviour in* Triticum *hybrids and allied genera*, **1936**, I, II, and III (In press).

Müntzing, A., *Heriditas*, 1935, **20**, 137–160.

Sax, K., *Genetics*, 1921, 6.

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The Photoisomerides of Ergosterol.

By I. M. Heilbron and F. S. Spring.

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IT is not the intention of this resumé to trace the history of the recognition and characterisation of vitamin D (the antirachitic accessory food factor),[1] but rather to deal with the chemistry of calciferol. It is necessary, however, at the outset to emphasise that abundant evidence has accumulated which shows that vitamin D of cod-liver oil and other natural sources is not identical with calciferol, the antirachitic photoisomeride of ergosterol.[2]

That ergosterol on irradiation is converted into a material with strong antirachitic activity was demonstrated independently by Rosenheim and Webster,[3] and by Windaus and Hess.[4] The first crystalline photoisomerides of ergosterol to be isolated were the suprasterols I and II formed by prolonged irradiation of a solution of ergosterol[5]; they are physiologically inactive and no longer exhibit selective absorption in the ultraviolet region of the spectrum. If however, ergosterol be carefully irradiated to give a product of maximum physiological activity, unchanged ergosterol can be removed by taking advantage of the fact that the irradiation products, in contrast to ergosterol, are not precipitated by digitonin. Bourdillon *et alia*,[6] isolated a crystalline product with a high antirachitic potency by high vacuum sublimation of the resin so obtained; this product was later shown to be a mixture of calciferol, m.p. 114–117°, possessing an enhanced antirachitic activity, and a physiologically inactive pyrocalciferol.[7] Almost

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[1] For such a summary see Heilbron, *J. Soc. Chem. Ind.*, 1936, **55**, 1219.

[2] Steenbock, *et alia*, *J. Biol. Chem.*, **1932, 97**, 249; Ender, *Z. Vitaminforsch.*, **1933, 2, 241**; Rygh, *Nature*, **1935, 136,** 396.

[3] *Biochem. J.*, **1927, 21,** 389.

[4] *Nachr. Ges. Wiss. Göttingen*, **1927**, 175.

[5] Windaus, Gaede, Köser and Stein, *Annalen*, 1930, **483,** 17.

[6] *Proc. Roy. Soc.*, B, 1930, **107,** 76.

[7] Askew, Bruce, Callow, Philpot and Webster, *Proc. Roy. Soc.*, B, **1932, 109,** 488.

simultaneously, Windaus and Deppe[8] observed that the ergosterol free-resin obtained by irradiation with "long-wave" light (the unfiltered light of the mercury arc) on treatment with citraconic anhydride at room temperature followed by removal of the acidic fraction, gave a crystalline compound, "vitamin D_1" which possesses a high antirachitic activity. Later,[9] it was observed that if the unfiltered light of the magnesium arc be used (main emission at 278–280$m\mu$) for the irradiation process, the ergosterol-free resin so obtained after treatment with citraconic anhydride gave a crystalline antirachitic factor, vitamin D_2, identical with the calciferol of Askew, Bourdillon, *et alia*[7]; it possesses a physiological activity considerably greater than that of vitamin D_1.

The problem then resolved itself into whether irradiation of ergosterol gives more than one antirachitic factor or whether vitamin D_1 is a mixture. The latter was found to be the case when vitamin D_1 was resolved by fractionation of its dinitrobenzoate into calciferol (vitamin D_2) and a physiologically inactive isomer to which the name lumisterol was given.[10] Yet another photoisomeride of ergosterol was obtained by examination of the fraction of the resinous irradiation product from ergosterol which reacts with citraconic anhydride.[11] Because of the ease with which it combines with citraconic anhydride this new isomeride is called tachysterol; it is antirachitically inactive and toxic, producing calcification in the kidney.

Thus although irradiation of ergosterol produces a series of photoisomerides, only calciferol (vitamin D_2) has the property of controlling normal calcification. In order to facilitate a chemical study of the constitution of calciferol it is clearly important to establish whether the various isomerides are formed simultaneously, representing individual reactions, or whether they are stages in a continuous transformation. Irradiation of lumisterol has been shown to give tachysterol, calciferol and the suprasterols I and II, whilst irradiation of tachysterol gives calciferol and the suprasterols I and II. Further irradiation of calciferol gives only the suprasterols I and II, which are unchanged by further irradiation.[12] Thus the photochemical process may be represented by the scheme:—

Ergosterol → Lumisterol → Tachysterol → Calciferol → { Suprasterols I and II. / Toxisterol.

In the "short-wave" irradiation of ergosterol, lumisterol is not formed; toxisterol is the name given to a probable photoisomeride of ergosterol, the existence of which is inferred from the appearance of high toxicity and a band at 240 $m\mu$ during the over-irradiation of calciferol.

Chemical Investigation.—For details of the methods by which the constitution of ergosterol (I) has been established the reader must be referred to the original literature.[13] The photoisomerides of ergosterol all resemble the latter in possessing a side chain ethylenic linkage since each gives methyl*iso*propylacetaldehyde on ozonolysis.[14] The constitution of lumisterol has been, to a large measure, established by the observations that it is tetracyclic, containing three ethenoid linkages,[15] and that it gives 3-methyl-*cyclo*pentenophenanthrene on dehydrogenation with selenium[16]; thus it must contain the normal sterol condensed ring system. In addition to an ethylenic linkage between C_{22} and C_{23} a second ethylenic linkage has been located between C_5 and C_6, an observation which automatically locates the remaining unsaturated centre at C_7–C_8 since lumisteryl acetate gives an adduct with maleic anhydride and must therefore contain a conjugated system of ethenoid linkages.[17] Since lumisterol is not precipitated by digitonin, it is highly probable that in contrast to ergosterol the hydroxyl group has the *epi*-configuration; lumisterol (II) probably differs from ergosterol in the arrangement of the groups associated with C_3 and C_9 (and/or C_{10}).

Calciferol and Tachysterol.—That tachysterol in contrast to ergosterol and lumisterol contains four ethylenic linkages was demonstrated by the method of perbenzoic acid

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[8] *Annalen*, 1931, **489**, 252.

[9] Windaus, Linsert, Lüttringhaus and Weidlich, *Annalen*, 1932, **492**, 226.

[10] Windaus and Linsert, *Annalen*, 1931, **489**, 269.

[11] Windaus, Werder and Lüttringhaus, *Annalen*, 1932, **499**, 188.

[12] Setz, *Z. Physiol. Chem.*, 1933, **215**, 183.

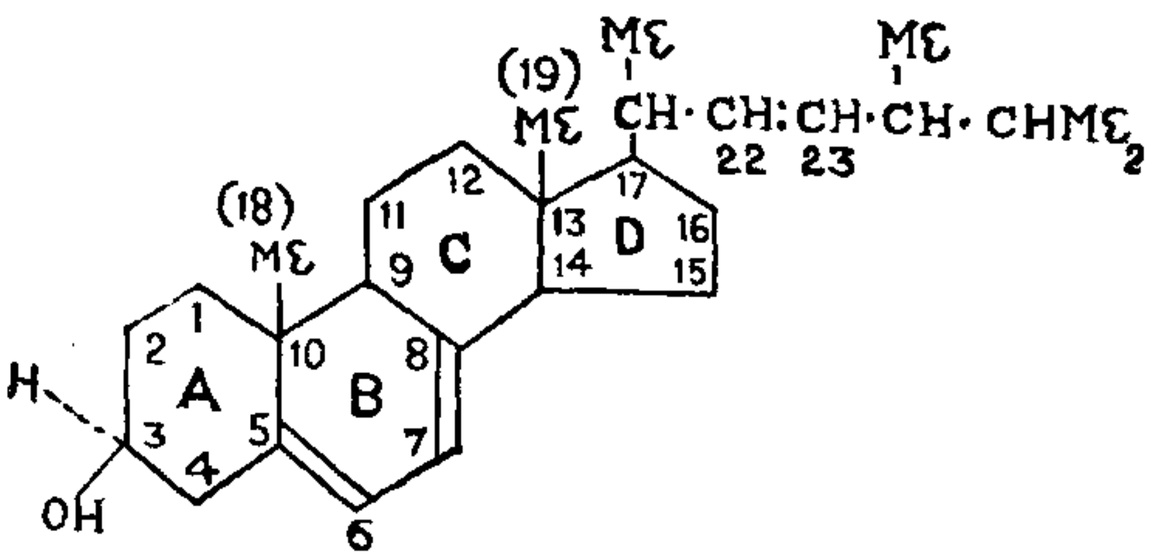
[13] Windaus, Inhoffen and Reichel, *Annalen*, 1934, **510**, 248; Dunn, Heilbron, Phipers, Samant and Spring, *J. Chem. Soc.*, 1934, 1576.

[14] Guiteras, *Annalen*, 1932, **494**, 116.

[15] Heilbron, Spring and Stewart, *J. Chem. Soc.*, 1935, **1221**.

[16] Dimroth, *Ber.*, 1935, **68**, 539.

[17] Heilbron, Moffet and Spring, (unpublished observation).



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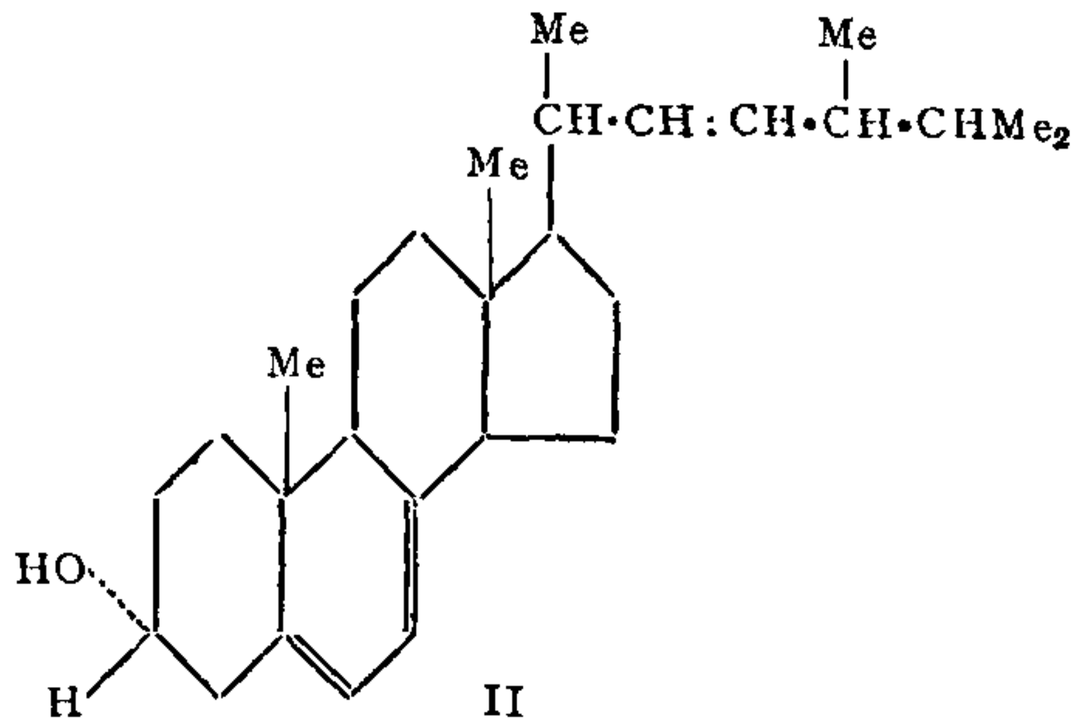


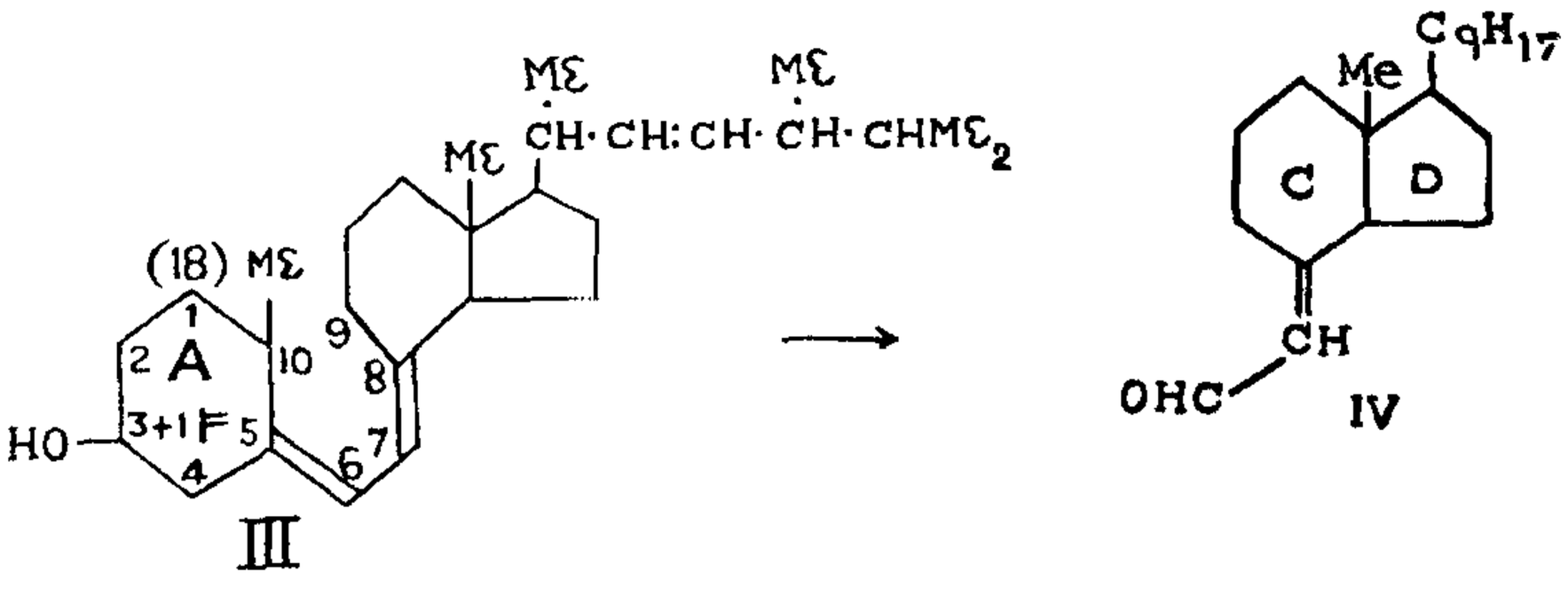
II

titration.[18] It was subsequently shown that calciferol is likewise tetraethenoid both by perbenzoic titration[19] and by catalytic hydrogenation.[20] Calciferol and tachysterol must possess the same nuclear structure since each on reduction with sodium and alcohol give one and the same dihydro-derivative.[21]

Since both ergosterol and lumisterol are tetracyclic it follows that their transformation into tachysterol and calciferol must be accompanied by the rupture of a ring with the consequent appearance of a fourth ethylenic linkage. It is clear that the conjugated system of ethylenic linkages in both ergosterol and lumisterol is the most probable seat of the photochemical change, thus leading to six possible nuclear structures for calciferol and tachysterol according to the mode of rupture of ring B.[19]

formation of this aldehyde (IV) clearly shows that calciferol has the structure (III), in which the remaining ethylenic linkage must be associated with ring A.

This constitution has been confirmed and extended by an independent method.[23] Calciferol gives two isomeric adducts with maleic anhydride, which on partial hydrogenation give dihydro-derivatives. Ozonolysis and selenium dehydrogenation of these derivatives give respectively a ketone, $C_{19}H_{34}O$, and 2 : 3-dimethylnaphthalene respectively. These reactions can only be interpreted if structure (III) for calciferol be expanded to (V) in which the ring A ethylenic linkage is situated between C_{10} and C_{18}. The presence of such an exocyclic methylene group has been demonstrated by ozonolysis of calciferol when formaldehyde is obtained together



III

IV

The first evidence concerning the nature of this tricyclic nucleus was obtained by oxidation of calciferol when an $\alpha\beta$-unsaturated aldehyde $C_{21}H_{34}O$, was isolated.[22] The with a keto-acid, $C_{13}H_{20}O_3$ (V*a*).[24] There can thus be no manner of doubt that calciferol is correctly represented by (V).

It is probable that if a sterol contains a conjugated system of ethylenic linkages in positions C_5–C_6 and C_7–C_8 it can be transformed into an antirachitic factor by

[18] Lettré, *Annalen*, 1934, **511**, 280.

[19] Heilbron and Spring, *J. Soc. Chem. Ind.*, 1935, **54**, 795.

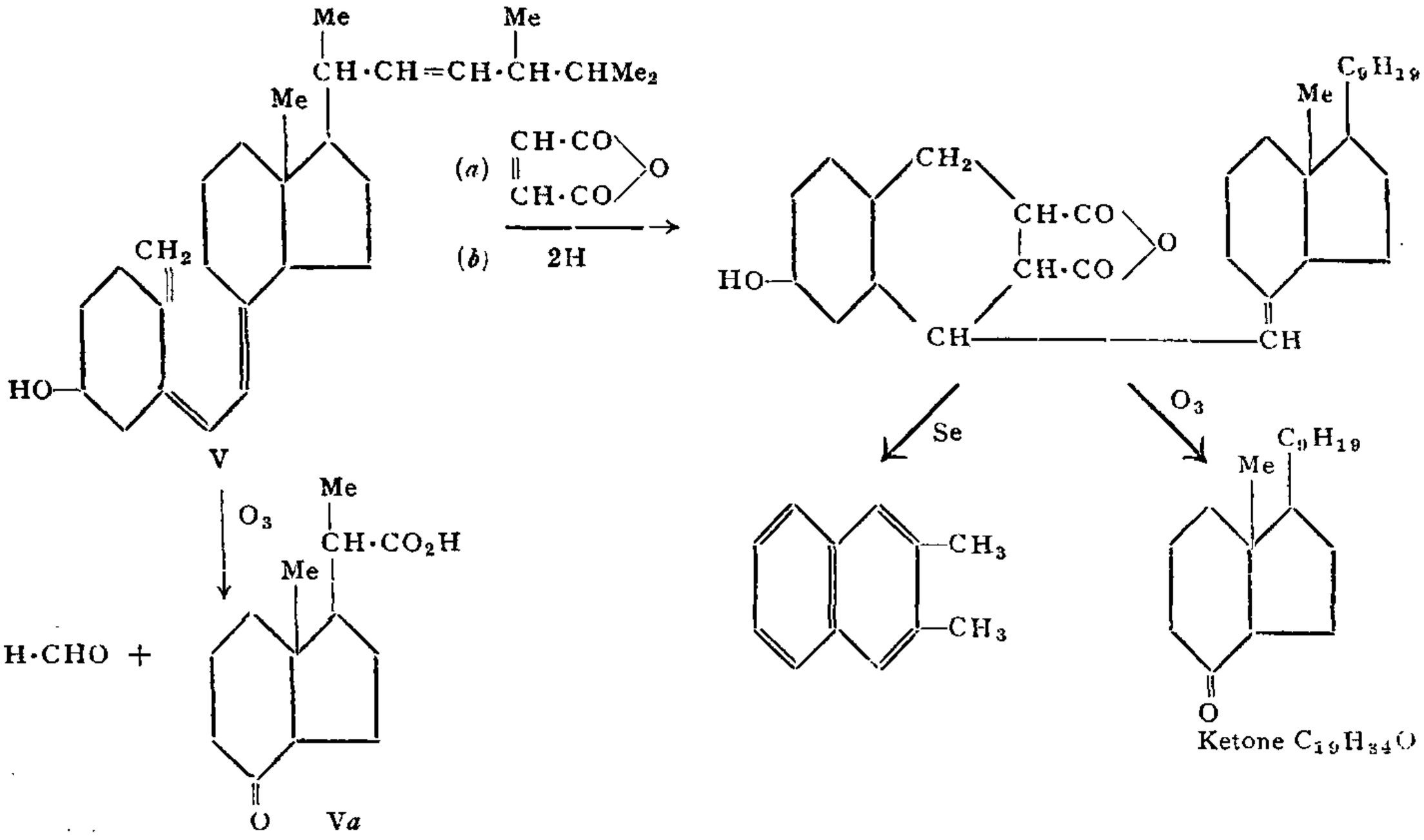
[20] Kuhn and Möller, *Z. angew. Chem.*, 1934, **47**, 145.

[21] Müller, *Z. physiol. Chem.*, 1935, **233**, 222.

[22] Heilbron, Samant and Spring, *Nature*, 1935, **135**, 1072; *J. Chem. Soc.*, 1936 (in press).

[23] Windaus and Thiele, *Annalen*, 1935, **521**, 160.

[24] Heilbron, Jones and Spring, (unpublished observation).



irradiation with ultra-violet light. Thus the synthetic 7-dehydrocholesterol (VII)[25] is converted into a highly antirachitic product on irradiation, whereas the synthetic 7-methylene cholesterol (VIII) is antirachitically inactive after similar treatment.[26] It has been reported however, that 7-dehydrostigmasterol is comparatively inactive after irradiation.[27]

VII

VIII

[25] Windaus, Lettré and Schenck, *Annalen*, 1935, **520**, 98.

[26] Bann, Heilbron and Spring, (unpublished observations).

[27] Lettré and Inhoffen, Sterine, Gallensauren, etc., *Stuttgart*, 1936, p. 312.