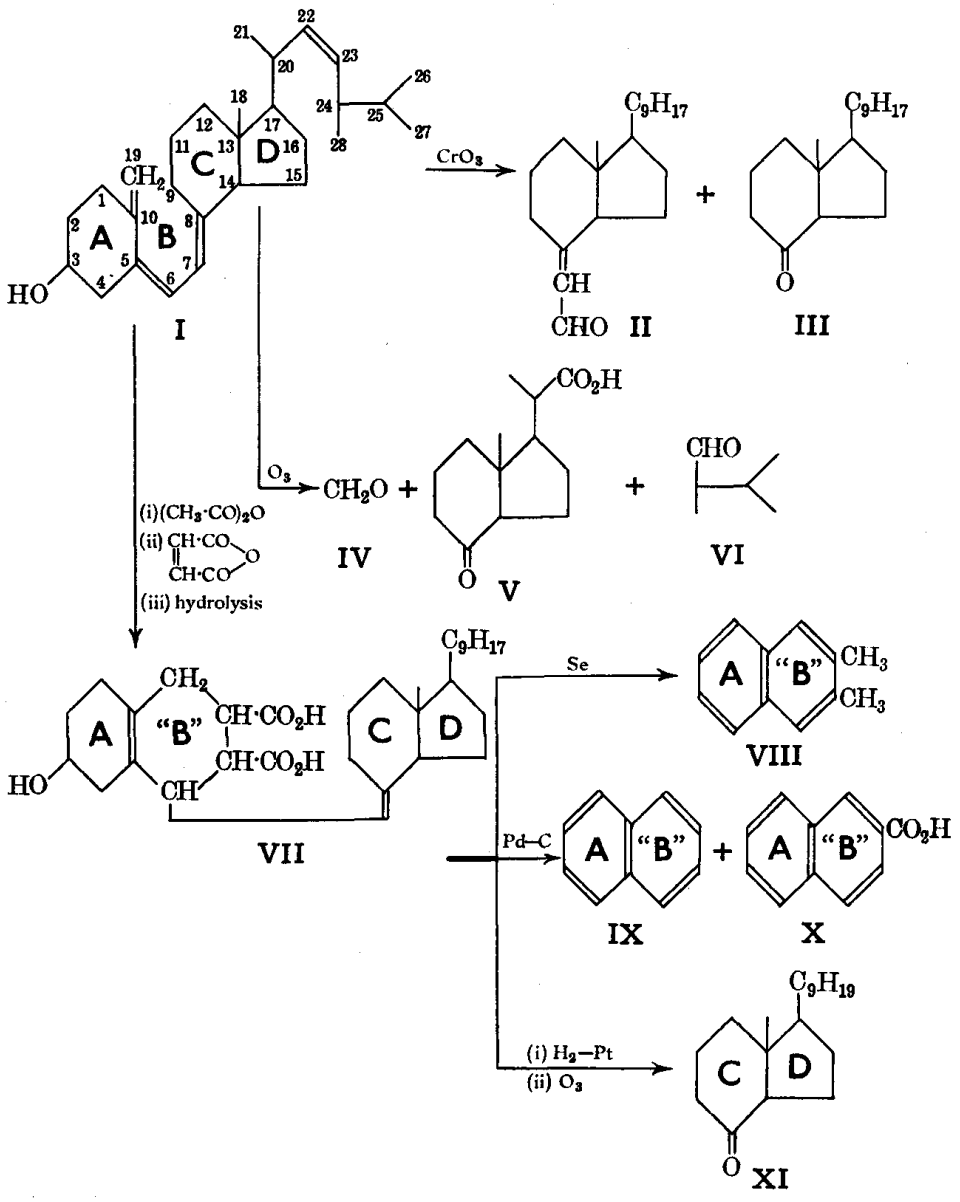
Velluz *et al.* (1949) isolated the pre-ergocalciferol (P) by irradiation of ergosterol at 20°, and showed that it formed ergocalciferol (E) on heating (see also below). Velluz *et al.* (1955) and Havinga *et al.* (1955) showed that pre-ergocalciferol is the 6 : 7-*cis*-isomer of tachysterol (T), and the interconversion of these two compounds has been studied by Inhoffen *et al.* (1959) and Havinga *et al.* (1959). Lumisterol (L) is converted directly into pre-ergocalciferol (Rappoldt, 1960). It should be noted that tachysterol and lumisterol are formed in a side reaction from pre-ergocalciferol and are *not* directly involved in the formation of ergocalciferol as postulated in the original scheme of Windaus *et al.*, who carried out the irradiation in solution and allowed the temperature to rise to 50°:

$$\text{Ergosterol} \xrightarrow{h\nu} \text{L} \xrightarrow{h\nu} \text{T} \xrightarrow{h\nu} \text{E}$$

§6a. Ergocalciferol (calciferol, vitamin D_2) is an optically active crystalline solid, m.p. 115–117°. Its molecular formula is $C_{28}H_{44}O$, and since it forms esters, the oxygen is present as a hydroxyl group. Furthermore, since ergocalciferol gives a ketone on oxidation, this hydroxyl group is a secondary alcoholic group. Ozonolysis of ergocalciferol produces, among other products, methyl*iso*propylacetaldehyde. Thus the side-chain in ergocalciferol is the same as that in ergosterol. Catalytic hydrogenation converts ergocalciferol into the fully saturated compound octahydroergocalciferol, $C_{28}H_{52}O$. This shows that there are four double bonds present, and since one is in the side-chain, three are therefore in the nucleus. The parent hydrocarbon of ergocalciferol is $C_{28}H_{52}$, and since this corresponds to the general formula C_nH_{2n-4}, the molecule therefore is *tricyclic*. Furthermore, ergocalciferol does not give Diels' hydrocarbon when distilled with selenium. These facts indicate that ergocalciferol does not contain the four-ring system of ergosterol. The problem is thus to ascertain which of the rings in ergosterol has been opened in the formation of ergocalciferol. The following reactions of ergocalciferol are readily explained on the assumption that its structure is I. The absorption spectrum of the semicarbazone of II ($C_{21}H_{34}O$) was shown to be characteristic of $\alpha : \beta$-unsaturated aldehydes. The absence of the hydroxyl group and the carbon content of II indicate the *absence* of ring A. These facts suggest that in ergocalciferol " ring B " is open between C_9 and C_{10}, and that II arises by scission of the molecule at a double bond in position 5 : 6, and can be an $\alpha : \beta$-unsaturated aldehyde only if there is a double bond at 7 : 8 (these double bonds are also present in ergosterol). The isolation of the ketone III ($C_{19}H_{32}O$) confirms the presence of the double bond at 7 : 8 (Heilbron *et al.*, 1935).

The isolation of formaldehyde (IV) shows the presence of an exocyclic methylene group, and the presence of this group at C_{10} is in keeping with the opening of ring B at 9 : 10. The formation of V ($C_{13}H_{20}O_3$), a keto-acid, suggests that ring B is open at 9 : 10, and that there are two double bonds at 7 : 8 and 22 : 23. The position of the latter double bond is confirmed by the isolation of methyl*iso*propylacetaldehyde, VI (Heilbron *et al.*, 1936).

Structure I for ergocalciferol is also supported by the formation of VII, the structure of which is shown by the products VIII, IX, X and XI (Windaus *et al.*, 1936). The production of 2 : 3-dimethylnaphthalene (VIII) is in keeping with the fact that carboxyl groups sometimes give rise to methyl groups on selenium dehydrogenation (*cf.* §2 vii. X). Similarly, the formation of naphthalene, IX, and naphthalene-2-carboxylic acid, X, shows the presence of rings A and " B " in VII. Catalytic reduction of VII (to reduce the double bond in the *side-chain* only), followed by ozonolysis, gives XI. Thus the formation of these compounds VIII–XI establishes the structure of VII, and shows that the double bonds are at 5 : 6, 10 : 19 and 7 : 8.



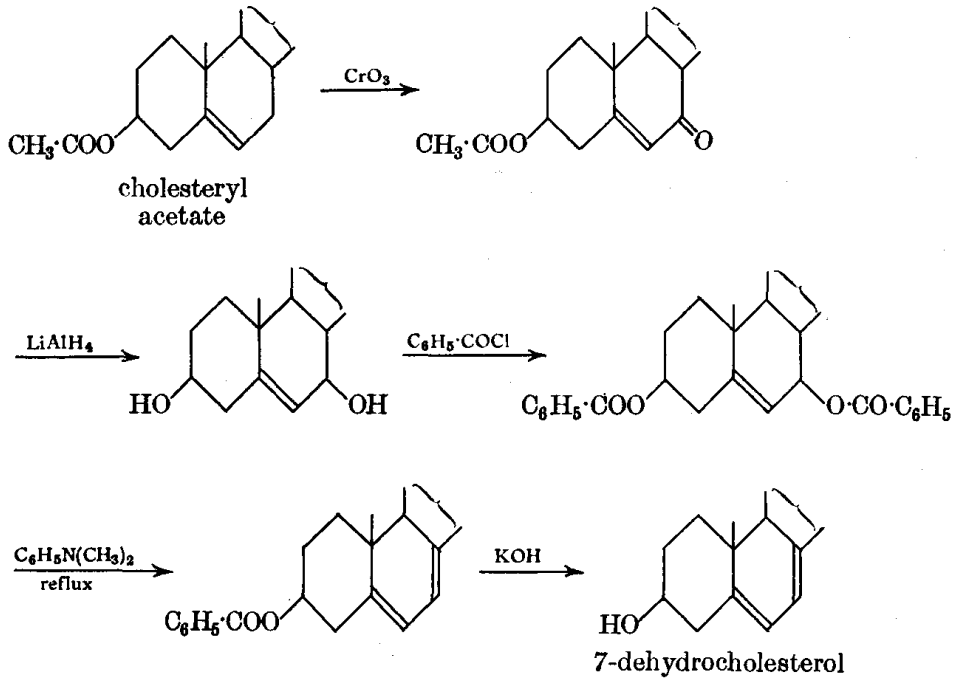
X-ray analysis studies of the 4-iodo-3-nitrobenzoate of ergocalciferol confirm structure I for ergocalciferol (Crowfoot *et al.*, 1948).

The presence of the two double bonds 5 : 6 and 7 : 8 gives rise to the possibility of various geometrical isomeric forms for ergocalciferol. Ultraviolet spectroscopic studies (Braude *et al.*, 1955) and other work (§6) have led to the conclusion that ergocalciferol has the configuration shown in the chart in §6. This is further supported by the work of Crowfoot *et al.* (1957) who, from calculations of electron densities in the ester crystal (the 4-iodo-3-nitrobenzoate), have shown that their results agree with the configuration given in the chart.

Lythgoe *et al.* (1957) have carried out a partial synthesis of ergocalciferol from the aldehyde II.

§6b. Vitamins D_3 and D_4. A detailed biological investigation has shown that the vitamin D in cod-liver oil is not identical with ergocalciferol, and that vitamin D activity could be conferred on cholesterol, or on some

impurity in cholesterol other than ergosterol. Windaus (1935) therefore suggested that natural vitamin D (in cod-liver oil) is derived from 7-dehydrocholesterol. The following chart shows the method of preparing 7-dehydrocholesterol (originated by Windaus, 1935, and improved by Buser, 1947, and by Fieser *et al.*, 1950).

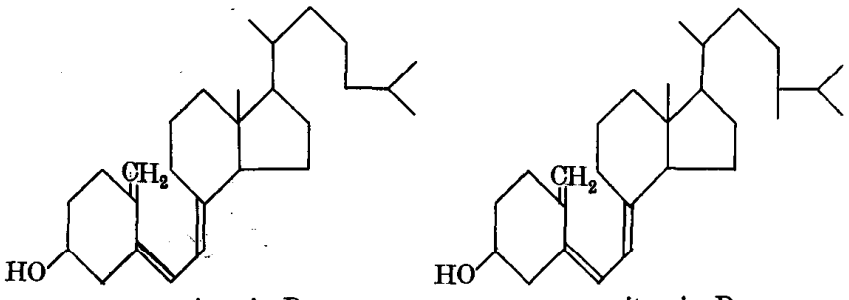


cholesteryl acetate

7-dehydrocholesterol

7-Dehydrocholesterol, on irradiation with ultraviolet light, gives a product that is about as active as ergocalciferol (vitamin D_2). This product was shown to be impure, and the pure active constituent was isolated as the 3 : 5-dinitrobenzoate (Windaus *et al.*, 1936). This vitamin D with a cholesterol side-chain is named **vitamin D_3**, and has been shown to be identical with the natural vitamin that is isolated from tunny-liver oil (Brockman, 1937). Vitamin D_3 has also been isolated from other fish-liver oils, *e.g.*, halibut. The *Chemical Society* (1951) has proposed the name **cholecalciferol** for vitamin D_3. It has now been shown that the irradiation of 7-dehydrocholesterol (at low temperature) first produces the previtamin D_3, and this, on gentle heating, is converted into the vitamin itself (*cf.* ergocalciferol, §6).

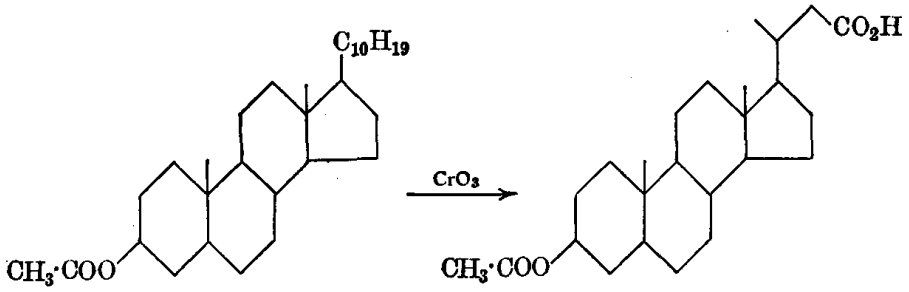
Irradiation of 22 : 23-dihydroergosterol gives a compound with antirachitic properties (Windaus *et al.*, 1937); this is known as **vitamin D_4**.



vitamin D_3 vitamin D_4

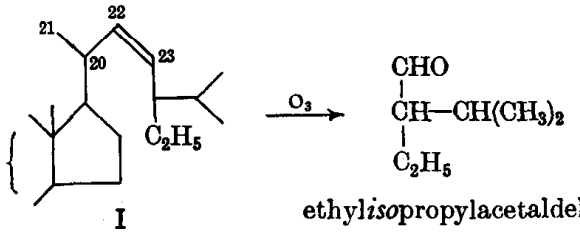
§7. Stigmasterol, $C_{29}H_{48}O$, m.p. 170°, is best obtained from soya bean oil. Since stigmasterol forms an acetate, etc., a hydroxyl group is therefore

present. Stigmasterol also forms a tetrabromide; thus it contains two double bonds. Hydrogenation of stigmasterol produces stigmastanol, $C_{29}H_{52}O$, and since the acetate of this gives the acetate of 3β-hydroxynor-*allo*cholanic acid on oxidation with chromium trioxide, it follows that stigmastanol differs from cholestanol only in the *nature* of the side-chain (Fernholz



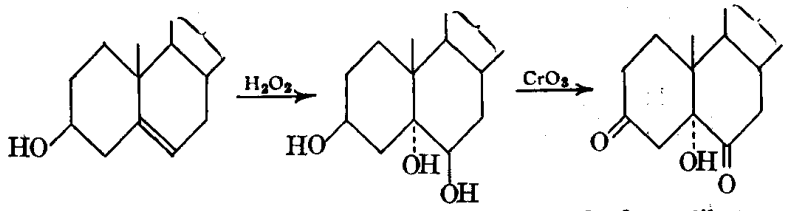
stigmastanyl acetate — acetate of 3β-hydroxynor-*allo*cholanic acid

et al., 1934; *cf.* ergosterol, §5). Ozonolysis of stigmasterol gives, among other products, ethyl*iso*propylacetaldehyde (Guiteras, 1933). This suggests that the side-chain is as shown in I, with a double bond at 22 : 23.

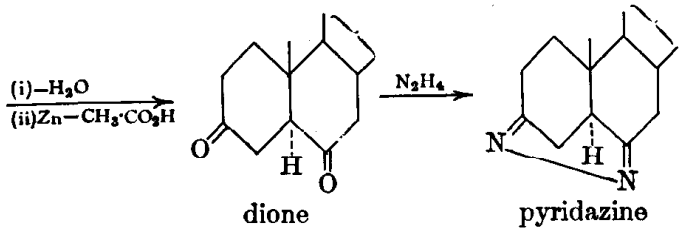


I — ethyl*iso*propylacetaldehyde

Thus the final problem is to ascertain the position of the second double bond in stigmasterol. This has been shown to be 5 : 6 by the method used for cholesterol (Fernholz, 1934). Stigmasterol, on hydroxylation with hydrogen peroxide in acetic acid, gives a triol which, on oxidation with chromium trioxide, forms a hydroxydiketone. This, on dehydration followed by reduction, forms a dione which combines with hydrazine to form a pyridazine derivative. These reactions can be explained as follows (*cf.* cholesterol, §3 ii):

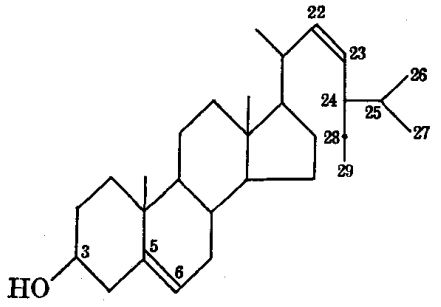


stigmasterol — triol — hydroxydiketone



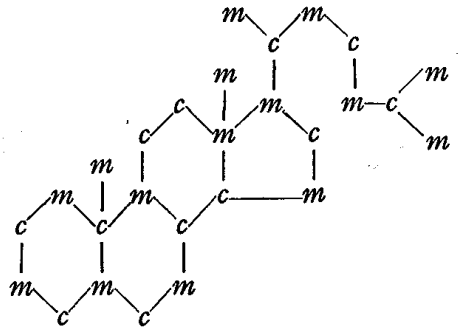
dione — pyridazine

This position for the nuclear double bond is supported by other evidence; thus stigmasterol is:



stigmasterol

§7a. Biosynthesis of sterols. It has long been known that animals can synthesise cholesterol, but the possible pathways were unknown until biosynthetic cholesterol was prepared from acetic acid labelled isotopically (with ^{14}C) in either the methyl (*m*) or the carboxyl (*c*) group, or labelled in both groups ($^{13}CH_3 \cdot ^{14}CO_2H$). These tracer studies were carried out mainly by Bloch *et al.* (1942–) and by Cornforth *et al.* (1953–), and the results established that the distribution of the carbon atoms is as shown in I. Thus



I

acetic acid can be regarded as the fundamental unit. Evidence was also obtained that *iso*valeric acid can serve as a precursor for cholesterol, and then Tavormina *et al.* (1956), using labelled mevalonic acid (MVA), showed that this is converted almost completely into cholesterol by rat liver; the route from acetic acid to MVA has been described in §32a. VIII. The problem now is to discover the route whereby MVA is converted into cholesterol. As far back as 1926 Heilbron *et al.* suggested that squalene (§32. VIII) is a precursor of cholesterol, and Robinson (1934) proposed a scheme for the cyclisation of the squalene molecule with the loss of three methyl groups. Woodward *et al.* (1953), however, suggested that squalene is first cyclised to lanosterol, and then this loses three methyl groups to give cholesterol. Bloch *et al.* (1952) showed that squalene is a precursor of cholesterol in the intact animal. Furthermore, Bloch *et al.* (1955) showed that lanosterol is converted into cholesterol in rats, and in 1956 carried out the biosynthesis of lanosterol from labelled acetate. Thus we have evidence for the suggested route from squalene to cholesterol. As mentioned above, Woodward *et al.* (1953) suggested that squalene ring-closes to form lanosterol, and proposed a 1,3-shift of the methyl group at C_8 to C_{13} (the squalene molecule is numbered to give the numbering in the closed-ring system in the steroid). On the other hand, Ruzicka *et al.* (1955) and Bloch *et al.* (1957) proposed a 1,2-shift of the methyl group from C_{14} to C_{13} and another 1,2-shift from C_8 to C_{14}. Further work by Bloch *et al.* (1958) showed that the 1,2-shifts were correct; this is also supported by the work of Cornforth *et al.* (1958).