[CONTRIBUTIONS FROM THE STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY]

ERGOSTEROL F[1]

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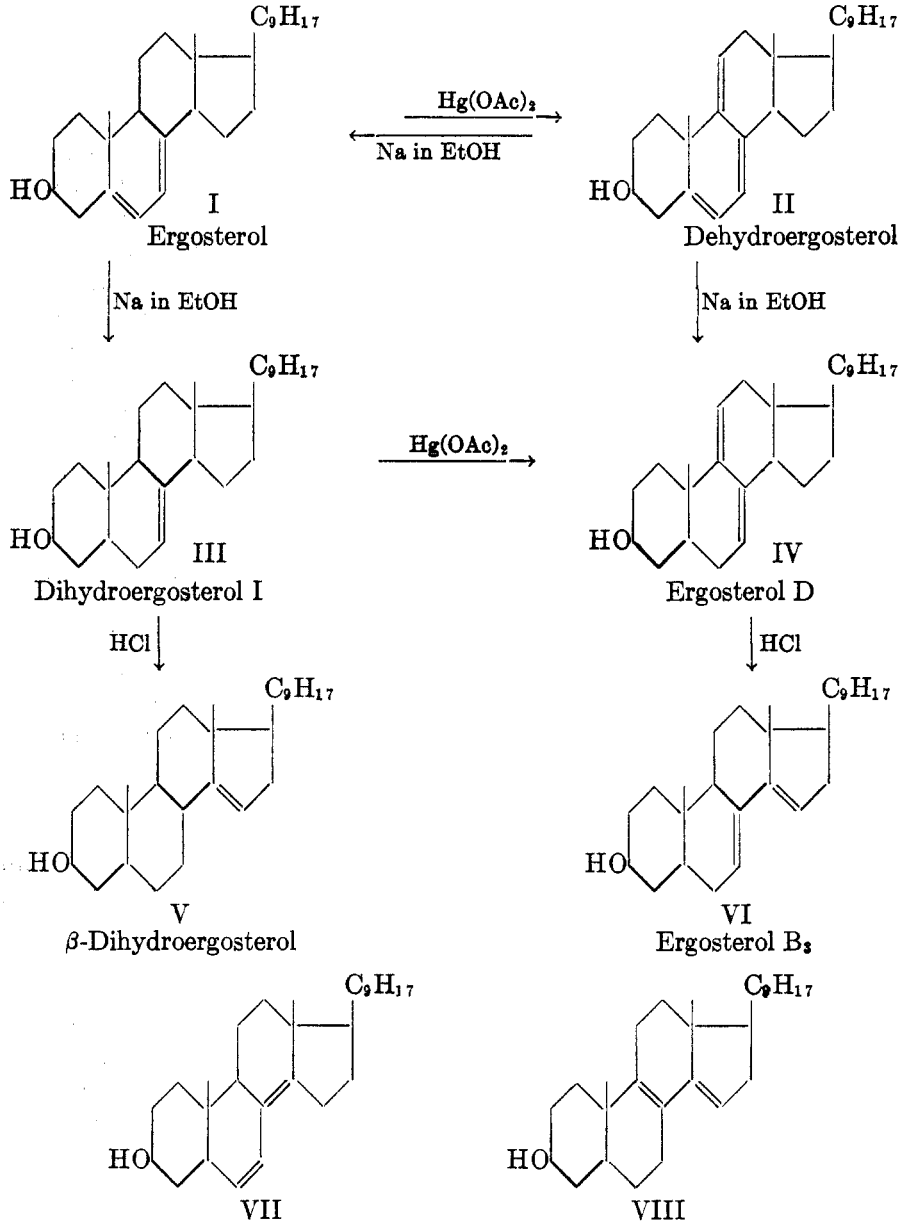
In connection with the studies on the conversion of ergosterol (I) to adrenal cortical hormones, which have been discussed in the preceding paper (1), a search was made for derivatives of ergosterol which possess unsaturation at C-11 and which do not require protection by maleic anhydride during the alteration of the molecule. One of the more promising isomers of ergosterol appeared to be ergosterol F which had been prepared by Windaus and collaborators (2) by the reduction of dehydroergosterol (II) with sodium in ethanol. After it had become evident that certain "isomers" of ergosterol lack uniformity (3), Nakamiya, working in Windaus' laboratory, reinvestigated ergosterol F (4). He found that heating this sterol with maleic anhydride gave about forty per cent of a complex reaction product. The unreacted material possessed physical properties identical with those of the starting material, a fact which Nakamiya interpreted as proof for the uniformity of ergosterol F.

Without giving specific reasons, it has been suggested by Sobotka (5) that ergosterol F possesses structure VII. The present authors, however, regarded it as more likely that the cyclic double bonds of this sterol were at 7,8 and 9,11 (IV), and that the sterol was formed from dehydroergosterol (II) by the addition of hydrogen to the 5,6-double bond. Such preferential reductions, seemingly unaccompanied by migration of the unreacted double bonds are well known in this series. Thus ergosterol (I) upon reduction with sodium in ethanol affords dihydroergosterol I (III) and with a catalyst γ-ergosterol (6).

The physical properties of ergosterol F and its derivatives prepared in this laboratory by Windaus' method (2) agreed with those reported in the literature. In the course of a preliminary study of the chemistry of ergosterol F, doubts arose once more concerning its homogeneity. They were strengthened by a critical analysis of certain statements made in the literature in regard to this compound. Thus the reported maxima of the absorption spectrum of ergosterol F (2) while at the expected wave length are of such low intensity as to indicate that the absorption is due to the presence of an impurity. It was also found difficult to understand that dehydrogenation of ergosterol followed by hydrogenation should give ergosterol F, while hydrogenation followed by dehydrogenation affords a different isomer, ergosterol D (7). Since the experimental conditions in both sequences of reactions are quite similar, the formation of identical final products might be expected.

[1] The work described in this paper was done under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Yale University.

In the course of testing the homogeneity of ergosterol F recourse was taken to reactions which are known to be typical of certain systems of unsaturation.



Thus steroids, which like ergosterol (I) possess a system of conjugated double bonds in ring B, are readily dehydrogenated by eosin in the presence of light to give difficultly soluble, high melting "bi-steroids" (9, 10, 11, 12). When sub-

jected to such dehydrogenation, ergosterol F gave about ten per cent of a bi-compound which like the corresponding derivative of ergosterol melted at 202–203° (9) and gave neoergosterol m.p. 151°; $[\alpha]_D^{22}$ −12.5°, upon heating (13). This evidence therefore established the presence in ergosterol F of about ten per cent of ergosterol. It is supported by the absorption spectrum of ergosterol F (Figure 1) which shows the presence of about six per cent of ergosterol.

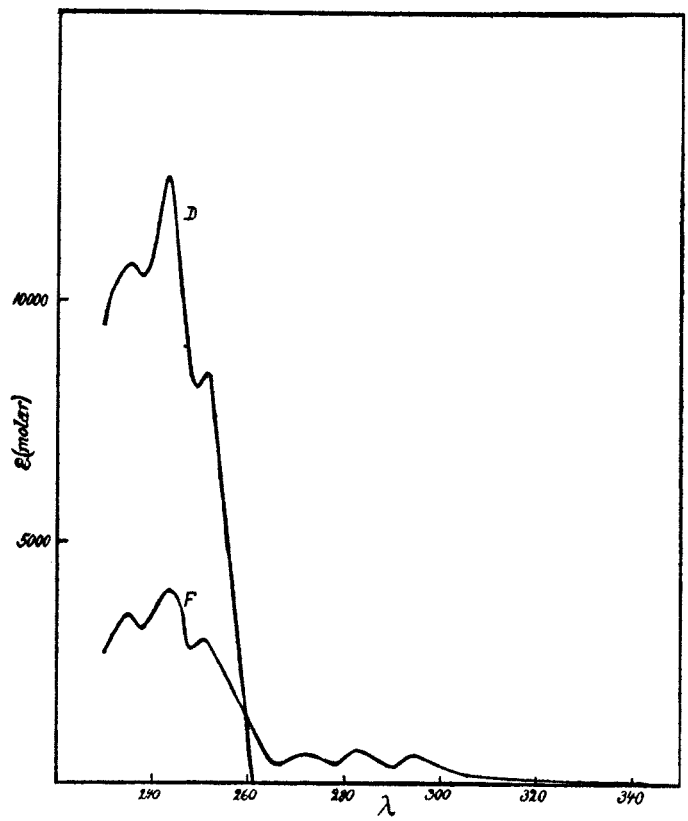


FIG. 1. ABSORPTION SPECTRA OF ERGOSTEROL-F (F) AND ERGOSTERYL-D-ACETATE (D)

Frequent recrystallizations of the acetate of the soluble, unreacted fraction from the photodehydrogenation of ergosterol F eventually gave a product, the physical properties of which, m.p. 169°; $[\alpha]_D$ +22°, showed closest similarity to those of ergosteryl-D acetate.

A comparison of the absorption spectrum (Figure 1) of the present acetate with that of ergosterol D (7, 8) demonstrated the identity of the two compounds. Since the absorption spectra of ergosterols F and D are closely similar with the exception of the maxima which are unusually low in the former, it may now be concluded that the absorption of ergosterol F (Figure 1) is due to the presence of ergosterol D, which represents about thirty-five per cent of the mixture.

Taking into consideration that ergosterol F contains beside ergosterol D also

about ten per cent ergosterol, it follows that the remainder of the mixture is represented by one or more compounds devoid of absorption in the ultraviolet region. Successful isolation of such a product was eventually achieved by repeated recrystallizations of ergosteryl-F benzoate, saponification of the final fraction and further recrystallizations of the sterol. The compound thus obtained no longer showed absorption in the ultraviolet and proved to be identical with 5,6-dihydroergosterol (dihydroergosterol I), m.p. 170°; $[\alpha]_D$ −18°.

The assumption that ergosterol F is unsaturated at 7,8 and 9,11 had been the starting point of the present investigation. After it had been demonstrated that this sterol is not an isomer of ergosterol but a mixture consisting principally of dihydroergosterol and ergosterol D, it appeared reasonable to assign to ergosterol D the structure (IV) which originally had been assumed to be that of ergosterol F. This formulation explains the observed experimental facts better than the one (VIII) originally proposed by Callow (14). A similar conclusion based substantially on molecular rotation differences has recently been arrived at by Barton (15). For analogous reasons that same author has also questioned the uniformity of ergosterol F.

It now appears that the reduction of dehydroergosterol (II) proceeds along two different routes. In the one the over-all effect is the addition of one mole of hydrogen to the 5,6-double bond to give ergosterol D (IV). Since this is a sterol with conjugated double bonds extending over two rings it is inert to the action of sodium in alcohol (16, 17), and therefore remains unchanged under the conditions of the reaction. In the second route the first steps involve the disappearance of the 9,11-double bond of dehydroergosterol (II) with the formation of ergosterol (I), small amounts of which may be detected in the reaction mixture. The bulk of the ergosterol, however, undergoes further hydrogenation in the well known manner to give 5,6-dihydroergosterol, for which formula (III) has been definitely established (6, 15). It is of interest to note in this connection, that according to Windaus and collaborators (3) heating dehydroergosterol with sodium ethoxide at 185° affords a fraction precipitable with digitonin, which contains ergosterol D and a compound similar to dihydroergosterol. Windaus and collaborators (2) have also reported that the hydrogenation of dehydroergosterol (II) with sodium in isopropanol affords dihydroergosterol II. The fact that this rather ill-defined sterol shows absorption in the ultraviolet at once demonstrated the presence of some material with conjugated cyclic bonds. On the basis of experiences gained with ergosterol F, it is now safe to conclude that dihydroergosterol II is also a mixture consisting principally of ergosterol D and 5,6-dihydroergosterol. The reported absorption spectrum for dihydroergosterol II (2) shows maxima at the same wave lengths as those of ergosterol D, and of an intensity corresponding to the presence in the mixture of about forty per cent of this sterol.

According to Nakamiya, treatment of ergosterol F with hydrochloric acid leads to the well characterized ergosterol B_3 (VI) and the new ergosterol G. It now appears certain that the progenitor of the former has been the ergosterol D present in the mixture. Ergosterol G, which is devoid of ultraviolet absorp-

tion, might conceivably be regarded as the rearrangement product of the 5,6-dihydroergosterol present in ergosterol F. Its reported physical properties, however, contra-indicate its identity with the expected β-dihydroergosterol (V) (8). It seems at present most likely that ergosterol G is a mixture, as has already been suggested by Barton (15).

CONCLUSIONS

It has been demonstrated that ergosterol F is a mixture consisting of approximately equal parts of ergosterol D and dihydroergosterol and small amounts of ergosterol.

It has been suggested that dihydroergosterol II is a mixture of similar composition and that ergosterol G lacks homogeneity.

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