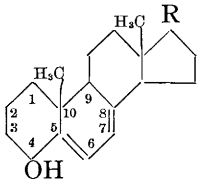
344. *Studies in the Sterol Group. Part XIX. Observations on the Constitution of Ergosterol.*

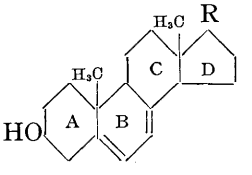
By J. L. Dunn, I. M. Heilbron, R. F. Phipers, K. M. Samant, and F. S. Spring.

Rosenheim and King (*J. Soc. Chem. Ind.*, 1934, **53**, 196) and Windaus, Inhoffen, and Reichel (*Annalen*, 1934, **510**, 248) have respectively suggested the formulæ (I) and (II) for ergosterol, which differ from one another solely in the location of the hydroxyl group.

(I.)



(R = C_9H_{17})



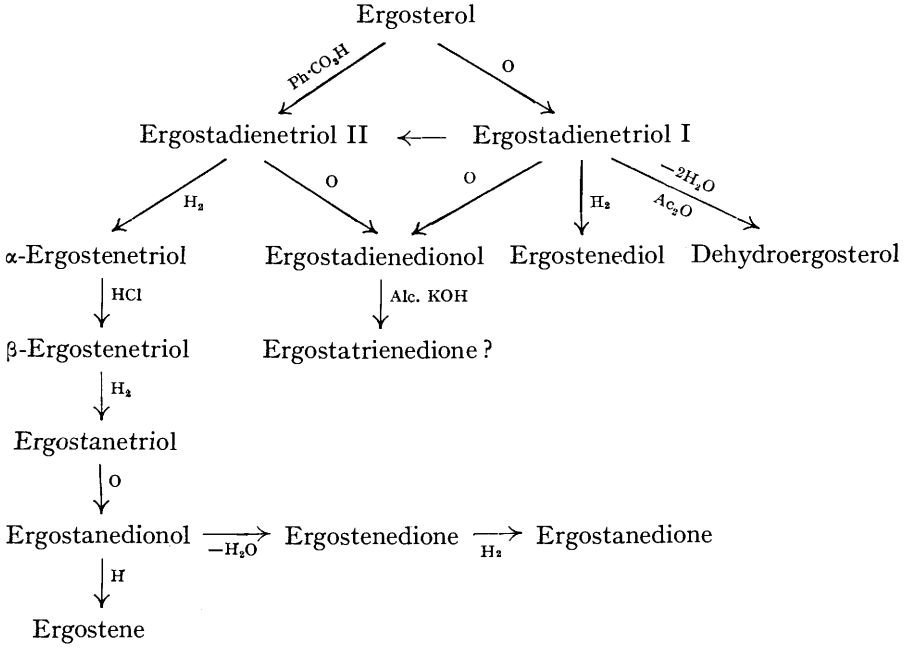
(II.)

In support of (I) the former authors claim that it accords best with the absorption spectrum, unimolecular-film measurements, and *X*-ray analysis, and also with the experimental evidence advanced by Heilbron, Samant, and Simpson (J., 1933, 1410), who demonstrated that the chloro*allo*norcholanic acid derived from ergosterol is different from that obtained from cholesterol. In view of its importance we have repeated and confirmed the latter observation, which, however, as already pointed out by Heilbron, Samant, and Simpson (*loc. cit.*) (compare also Windaus, Inhoffen, and Reichel, *loc. cit.*), does not necessarily preclude attachment of the hydroxyl group to C_3, as the two acids may be stereoisomerides. In the hope of gaining further information on this point, we have attempted to convert methoxycholestane and methoxyergostane into the corresponding methoxy*allo*norcholanic acids. Unfortunately, oxidation of methoxycholestane simply occasioned rupture of ring A with the production of the dicarboxylic acid, $C_{27}H_{46}O_4$, m. p. 195°, obtained by Windaus and Uibrig (*Ber.*, 1914, **47**, 2384) from β-cholestanol. We have also attempted to locate the position of the hydroxyl group by conversion of ergostanol into *methyl ergostanyl-xanthate*, from which on distillation an *ergostene*, m. p. 86—87°, was obtained. On the assumption that the hydroxyl group is attached to C_4 it is reasonable to anticipate that the introduced ethylene linkage would be situated between C_4 and C_5, and that oxidation would give a 5-keto-4-carboxylic acid, $C_{28}H_{48}O_3$. Actually, however, a dicarboxylic acid, $C_{28}H_{48}O_4$, m. p. 218—219°, identical with that described by Reindel (*Annalen*, 1928, **466**,

131) was isolated. This result, while not wholly precluding the possibility of hydroxyl being on C_4, nevertheless favours its union to C_3.

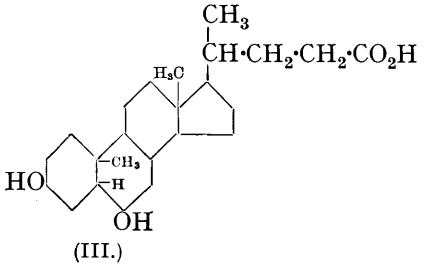
If formula (I) correctly represents the constitution of ergosterol, then the ergostadienetriol monobenzoate (triol II) formed by addition of perbenzoic acid to the C_5—C_6 ethylene linkage (Windaus and Lüttringhaus, *Annalen*, 1930, **481**, 119) must contain vicinal hydroxyl groups and consequently will react with lead tetra-acetate causing fission of ring A (Criegee, *Ber.*, 1932, **65**, 1770). That no such oxidation occurs was first demonstrated by Heilbron, Morrison, and Simpson (J., 1933, 302); a repetition of this work shows that the triol monobenzoate is unchanged after fourteen days' treatment at room temperature. Further, Windaus, Inhoffen, and Reichel (*loc. cit.*) oxidised ergostanetriol with lead tetra-acetate and found that only one atom of oxygen was absorbed. Again, it is impossible to interpret the formation of dehydroergosterol by loss of water from a 4 : 5 : 6-trihydroxy-system. These results definitely exclude the possibility of ergostanetriol and ergostadienetriol possessing the 4 : 5 : 6-triol group demanded by formula (I), and prove that ergosterol cannot contain the system ·CH(OH)·C:CH–. They further demonstrate that deductions made from ultra-violet absorption data (Lowry, *J. Soc. Chem. Ind.*, 1933, **52**, 10) in such complex molecules as the sterols must be applied with reserve (compare Morton and de Gouveia, this vol., p. 916).

The major point of evidence which has led Windaus, Inhoffen, and Reichel (*loc. cit.*) to suggest constitution (II) for ergosterol is the close analogy observed in the reactions of cholestane-3 : 6-dione and ergostanedione. During the past year we have approached the structural problem from a closely similar angle, but have found, in contrast to the above observations, that a very marked difference in behaviour exists between cholestane-3 : 5 : 6-triol and ergostanetriol. Whereas Westphalen (*Ber.*, 1915, **48**, 1064) has shown that the diacetate of the former on treatment with acetic anhydride and sulphuric acid is readily dehydrated to cholestene-3 : 6-diol diacetate, similar treatment of ergostanetriol diacetate fails to bring about dehydration. Furthermore, prolonged treatment of this diacetate with phosphoric oxide is without effect. In view of this result we were led to re-examine the two isomeric ergostadienetriols derived from ergosterol, *viz.*, triol II formed by hydrolysis of its monobenzoate referred to above, and triol I prepared by the reduction of ergosterol peroxide (Windaus and Linsert, *Annalen*, 1928, **465**, 148). Whereas the former distils unchanged in a high vacuum, similar treatment of the latter yields dehydroergosterol with

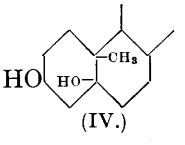


loss of two molecules of water. We have now ascertained that this dehydration is effected simply by treatment of triol I with acetic anhydride and that even repeated crystallisation in the presence of acetic acid gives dehydroergosterol. The ease of dehydration of triol I is further exemplified by hydrogenation of the compound, when, in place of the anticipated ergostenetriol, an ergostenediol is actually obtained (Windaus, Bergmann, and Lüttringhaus, *Annalen*, 1929, **472**, 195). That the two triols are actually stereo- and not position-isomerides has been demonstrated by Achtermann (*Z. physiol. Chem.*, 1933, **217**, 281) by direct conversion of triol I into triol II. We have confirmed the deductions of this author by oxidising triol I to an ergostadienedionol identical with that previously obtained by Heilbron, Morrison, and Simpson (*loc. cit.*) from triol II. Prolonged oxidation of triol I gives on the other hand a *compound*, m. p. 204—205°, analysis of which indicates the formula $C_{28}H_{40}O_4$. Ergostadienedionol reacts with alcoholic potash to give in small yield a substance, apparently an *ergostatrienedione*. The table on. p. 1577 shows the various transformations of the triols discussed above.

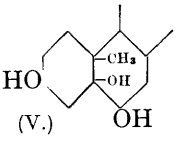
Whereas both ergostadienetriol I and ergostanedionol are readily dehydrated on treatment with hydrogen chloride, α-ergostenetriol only isomerises under prolonged treatment with this reagent. This inability of members of the triol II series to dehydrate can only be attributed to a specific spatial configuration of the tertiary hydroxyl group. The fact that ergostanedionol, apparently a member of the triol II series, is labile suggests that during its formation by oxidation of ergostanetriol (Windaus, Inhoffen, and Reichel, *loc. cit.*) an inversion of the hydroxyl group around C_5 has occurred. The labile character of ergostanedionol is also exemplified by its reduction by the Clemmensen method, an *ergostene*, m. p. 78°, being obtained. An identical inversion is known to occur in derivatives of hyodeoxycholic acid (III) (Windaus, *Z. angew. Chem.*, 1923, **36**, 309; Wieland and Dane, *Z. physiol. Chem.*, 1932, **212**, 41) in which rings A and B are of the *cis*-decalin (coprostane) type; it is effected under very mild catalytic conditions, when a carbonyl group is situated at C_6, and gives rise to derivatives of the cholestane series, in which rings A and B now have the *trans*-decalin configuration. As a consequence the easily dehydrated triol I must be the *trans*-decalin (IV) and the unreactive triol II the *cis*-decalin derivative (V). These considerations provide independent proof that the reactive double bond of ergosterol is actually between C_5 and C_6, a position originally postulated by one of us (*J. Soc. Chem. Ind.*, 1932, **51**, 1061).



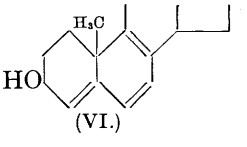
(III.)



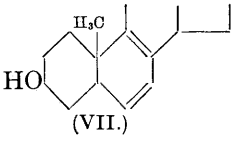
(IV.)



(V.)



(VI.)



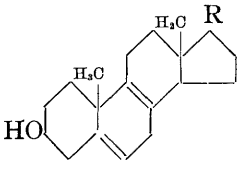
(VII.)

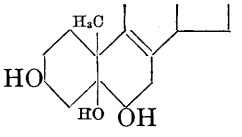
The position of the second nuclear ethenoid linkage still remains to be unambiguously determined. It is abundantly clear that it must be in the same ring as C_5—C_6 (ring B) (Windaus, *Nachr. Ges. Wiss. Göttingen*, 1933, 100; Honigmann, *Annalen*, 1934, **511**, 292). There are, therefore, two possible formulations for ergosterol, the one containing a C_7—C_8 linkage (II) and the other a C_8—C_9 linkage (VIII) (compare Heilbron, Morrison, and Simpson, *loc. cit.*). Its allocation at C_7—C_8 (II) assumes its direct conjugation with the C_5—C_6 bond, but this arrangement fails to give a satisfactory interpretation of some important and repeatedly observed reactions of ergosterol. For instance, the dehydration of triol I (IV) to dehydroergosterol cannot occur unless it be assumed that during the reaction the C_7—C_8 ethylene linkage migrates to the C_8—C_9 position, in which case dehydroergosterol must be (VI). Such a constitution cannot, however, be reconciled with formula (II), *since the hydrogenated adducts of these two sterols (acetates) with maleic anhydride are identical* (Honigmann, *Annalen*, 1933, **508**, 89). This point was clearly appreciated by this

author, who was forced to employ constitutions (VI) and (VII) for the respective sterols (compare Windaus and Langer, *Annalen*, 1933, **508**, 105).

It thus follows that formula (II), and any other representation of the constitution of ergosterol in which the nuclear double bonds are conjugated, fails to account for these experimental results. Again, this formula tacitly assumes that during the hydrogenation of ergosterol to α-ergostenol, a migration of the C_7—C_8 ethylene bond to the C_8—C_9 position occurs in order to account for the presence in the latter of an " inert " ethenoid linkage (compare Rosenheim and King, *loc. cit.*). The characteristic sensitivity of both ergosterol and α-ergostenol to the Tortelli–Jaffe reagent (Heilbron and Spring, *Biochem. J.*, 1930, **24**, 133) indicates, however, that the double bond of the latter is also present in the parent sterol. The evidence adduced by Rosenheim and King (*loc. cit.*) for the existence of a C_7—C_8 ethylene linkage to a large extent depends upon the non-identity of α-ergostenetriol (Heilbron, Morrison, and Simpson, *loc. cit.*) and *iso*ergostenetriol (Windaus and Langer, *loc. cit.*). It must be observed, however, that these two isomerides are not comparable, as the first is obtained by hydrogenation of the *cis*-triol II, whilst the second is prepared from 22-dihydroergosterol *via* its peroxide, a reaction which we have shown gives rise to the *trans*-isomeride. In so far as the position of the ethylene linkage is concerned this argument is therefore invalid. The fact that the catalytic hydrogenation of ergosterol gives a dihydro-derivative identical with that obtained by the reduction of this sterol with sodium and alcohol also invalidates the suggestion made by these authors that platinum or palladium catalysts effect a migration of an ethylene linkage in the ergosterol series. Although the reduction of ergosterol with sodium is difficult when ethyl alcohol is used, it proceeds readily with amyl alcohol to give, in addition to dihydroergosterol, the epi-isomeride (Heilbron, Johnstone, and Spring, J., 1929, 2248; Lettré, *Z. physiol. Chem.*, 1930, **189**, 1) in small amount. In view of this we consider that the recently described γ-ergostenol of Windaus and Langer (*loc. cit.*) requires further investigation.

(VIII.)





(IX.)

The allocation of the second nuclear ethenoid linkage to the C_7—C_8 position thus fails to satisfy the necessary experimental evidence. The only remaining position for this linkage is between C_8—C_9 to give the constitution (VIII) for ergosterol first advanced by one of us (*J. Soc. Chem. Ind.*, 1932, **51**, 1061). This constitution offers the only logical interpretation of the reactions of ergosterol together with the formation and reactions of dehydroergosterol; the ergostadienetriols will thus be (IX) and dehydroergosterol formed from (IX) by loss of two molecules of water will be (VI). In agreement with the constitution (VIII), it is known that the addition of maleic anhydride to ergosterol requires a considerably higher reaction temperature than is normal in diene syntheses or than that required for dehydroergosterol, although addition occurs ultimately at the same carbon atoms (see p. 1578). This previously unexplained discrepancy is satisfactorily accounted for by constitution (VIII), in which migration of the $C_5 : C_6$ bond to the unstable $C_6 : C_7$ position must precede addition, to give the conjugated system already present in dehydroergosterol.

Experimental.

Methoxycholestane.—This compound has been previously described by Wagner-Jauregg and Werner (*Z. physiol. Chem.*, 1932, **213**, 123), but it is more conveniently prepared by methylating cholestanol by the method described by Heilbron and Simpson (J., 1932, 268), the product thus obtained crystallising from acetone in plates, m. p. 82—83°.

Oxidation of Methoxycholestane.—Methoxycholestane (5 g.) in glacial acetic acid (200 c.c.) was treated with a solution of chromic anhydride (15 g.) in acetic acid (45 c.c. of 66%), added slowly during 3½ hours with stirring, the temperature throughout being maintained at 80°. The excess of chromic anhydride was decomposed with sulphur dioxide, and the solvent removed

under reduced pressure. The residue was dissolved in ether and the ethereal solution, after being washed with water, was extracted with dilute sodium hydroxide solution. The alkaline extract was acidified, and the precipitated acid extracted with ether and crystallised first from acetic acid and finally from ether–light petroleum; it then separated in leaflets, m. p. 195—196° (yield, 25%) (Found : C, 74·8; H, 10·6. Calc. for $C_{27}H_{46}O_4$: C, 74·6; H, 10·6%).

Methyl Ergostanylxanthate.—A solution of ergostanol (5 g.) in dry benzene (30 c.c.) was added to emulsified potassium (1·5 g.) in benzene (30 c.c.), and the mixture heated under reflux for 2 hours. The potassium ergostanyl oxide was converted, in an exactly analogous manner to that employed by Bose and Doran (J., 1929, 2244) for the preparation of methyl cholesterylxanthate, into *methyl ergostanylxanthate*, which crystallised from benzene–alcohol in prisms, m. p. 109—110°, $[\alpha]_D^{21°}$ — 3·6°, readily soluble in ether, benzene, and chloroform, but only sparingly soluble in alcohol (Found : C, 73·2; H, 10·4; S, 12·8. $C_{30}H_{52}OS_2$ requires C, 73·2; H, 10·6; S, 13·0%).

Ergostene, m. p. 86—87°.—Methyl ergostanylxanthate (15 g.) was decomposed by heating at 200—210° under reduced pressure, and the residue crystallised first from ether containing a small quantity of alcoholic potash (compare Bose and Doran, *loc. cit.*) and finally from ether–alcohol; the hydrocarbon was then obtained in plates. It gave no coloration either with antimony trichloride or with the Tortelli–Jaffe reagent, but responded to the Liebermann–Burchard test (Found : C, 87·6; H, 12·5. $C_{28}H_{48}$ requires C, 87·5; H, 12·5%). The *ergostene* was oxidised with chromic anhydride as described for the oxidation of methoxycholestane; the resultant crude acid crystallised from ether–light petroleum in plates, m. p. 218—219°, giving no depression on admixture with the acid prepared by Reindel (*loc. cit.*) from ergostanol (Found : C, 74·5; H, 10·6. Calc. for $C_{28}H_{48}O_4$: C, 75·0; H, 10·7%). The dimethyl ester, prepared by means of diazomethane, crystallised from aqueous methyl alcohol in needles, m. p. 82—83° (Reindel gives 81—83°) (Found : C, 75·6; H, 10·9. Calc. for $C_{30}H_{52}O_4$: C, 75·7; H, 10·9%).

Cholestenediol Diacetate.—This compound was first prepared by Westphalen (*loc. cit.*), who, however, gives no experimental details. The following method provides satisfactory results : cholestanetriol diacetate (2 g.) (Pickard and Yates, J., 1908, **93**, 1680) was gently refluxed with acetic anhydride (8 c.c.) and concentrated sulphuric acid (1 drop) for 1 hour. The cold solution was diluted with water and extracted with ether, and the ethereal solution successively washed with dilute sodium carbonate solution and water. After removal of solvent the residual oil was crystallised from methyl alcohol, cholestenediol diacetate separating in stout needles, m. p. 124—125°.

Attempted Dehydration of Ergostanetriol Diacetate.—(a) Ergostanetriol diacetate was treated exactly as described above; the only substance isolated in crystalline form from the residual oil was the unchanged triol diacetate in similar yield to the cholestenediol diacetate obtained above (*ca.* 20%). (b) Ergostanetriol diacetate (2 g.) in dry xylene (200 c.c.) was refluxed with phosphoric oxide (2·5 g.) for 4 hours, the whole being mechanically stirred. The reaction mixture was poured into water, ether-extracted, and steam-distilled to remove solvents. The residue was extracted with ether and crystallised from methyl alcohol–ethyl acetate, the unchanged triol diacetate being quantitatively recovered.

Dehydroergosteryl Acetate.—In attempts to crystallise ergostadienetriol I from ethyl acetate containing a small quantity of acetic acid the m. p. continuously fell until a constant value of 140° was reached. The compound of this m. p. appeared to be identical with dehydroergosterol, giving no depression on admixture with an authentic specimen. In order to investigate this apparently facile loss of water the triol (1 g.) was heated on a steam-bath with acetic anhydride (50 c.c.) for 3 hours. The suspended triol slowly went into solution with the development of a reddish-yellow colour. The whole was poured into dilute sodium carbonate solution and ether-extracted, and the concentrated ethereal solution treated with methyl alcohol. The yellow gum was repeatedly crystallised from alcohol–ethyl acetate, finally furnishing dehydroergosteryl acetate, m. p. 142°, $[\alpha]_D^{22·5°}$ + 177·6° (dehydroergosteryl acetate has m. p. 146°, $[\alpha]_D^{15°}$ + 192°; Windaus and Linsert, *loc. cit.*).

Reduction of Ergostanedionol.—A solution of the keto-alcohol (1 g.) in glacial acetic acid (300 c.c.) was heated under reflux with amalgamated zinc (20 g.), and concentrated hydrochloric acid added hourly in 10 c.c. portions during 48 hours. The reaction product was diluted with water, extracted with ether, washed with sodium carbonate, and dried. The residue was repeatedly crystallised from ether–alcohol, from which the new *ergostene* separated in small plates, m. p. 78° (Found : C, 87·7; H, 12·2. $C_{28}H_{48}$ requires C, 87·5; H, 12·5%). Hydrogenation of this hydrocarbon gave ergostane, m. p. 82°, unchanged on admixture with an authentic specimen.

Oxidation of Ergostadienetriol I.—A solution of chromic anhydride (2·4 g.) in acetic acid

(40 c.c.) and water (3·5 c.c.) was added during 5 hours at room temperature to a well-stirred solution of ergostadienetriol I (2 g.) in acetic acid (150 c.c.). The ketone crystallised from ethyl acetate–alcohol in plates, m. p. 248° (decomp.), unchanged on admixture with the ergostadienedionol, m. p. 249°, obtained from triol II (Heilbron, Morrison, and Simpson, *loc. cit.*). Similarly the monoxime, m. p. 232°, gave no depression with the oxime of ergostadienedionol from triol II.

Substance $C_{28}H_{40}O_4$.—When ergostadienetriol I was oxidised as described above, but the reaction period increased to 15 hours, the product obtained after several recrystallisations from ethyl acetate–alcohol separated in needles, m. p. 204—205° (Found : C, 76·8; H, 9·1. $C_{28}H_{40}O_4$ requires C, 76·4; H, 9·1%). This *substance*, which has not so far been further investigated, gives a *monoxime* crystallising from absolute alcohol in shining needles, m. p. 165—167° (Found : N, 3·2. $C_{28}H_{41}O_4N$ requires N, 3·1%).

Ergostatrienedione (?).—Ergostadienedionol (0·4 g.) was warmed with methyl-alcoholic potash (50 c.c.) until it was completely dissolved. The deep red solution was diluted with water and acidified with hydrochloric acid; the colour was then discharged. The resultant greenish-yellow solid was extracted with ether and well washed with water. The residual dark yellow solid obtained on removal of solvent from the dried solution was repeatedly crystallised, yielding lemon-yellow needles, m. p. 145—146° (Found : C, 81·8; H, 10·3. $C_{28}H_{40}O_2$ requires C, 82·3; H, 10·0%). The yield of this product was extremely small and precluded further attempts at purification and investigation.

Note :—Action of Lead Tetra-acetate on Methoxyergostadienediol.—We find that the product of this reaction (compare Heilbron, Morrison, and Simpson, *loc. cit.*) has m. p. 130—131° and not 105—106° as previously reported (Found : C, 78·8; H, 10·3. Calc. for $C_{29}H_{46}O_3$: C, 78·7; H, 10·4%).

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