85. *Studies in the Sterol Group. Part XVII. The Unsaturated Centres in Ergosterol.*

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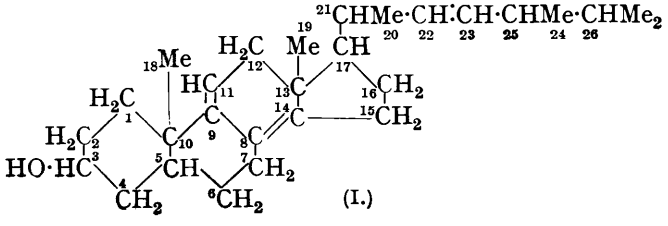
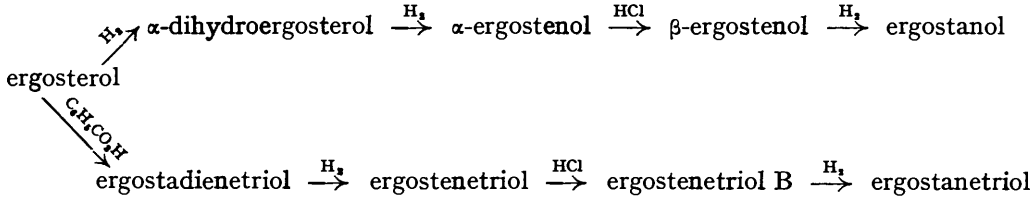
During the past few years many derivatives of ergosterol have been described, in the formation of which one or more of its three ethenoid linkages have been involved. Until recently, however, no precise information regarding the position in the molecule of any one of the unsaturated centres was available, despite the fact that they undoubtedly differ very widely in character.

The first step towards the elucidation of this problem was made by Reindel and Kipphan (*Annalen*, 1932, **493**, 181), who showed that ergosterol on ozonisation yielded methyl*iso*propylacetaldehyde, thus proving the presence of an ethylenic linkage in the side chain between C_{22}–C_{23} (I). Following upon this, Guiteras, Nakamiya, and Inhoffen (*Annalen*, 1932, **494**, 116) found that α-dihydroergosterol also gave the above aldehyde on ozonisation but that α-ergostenol (tetrahydroergosterol) failed to do so, a result indicating that in the hydrogenation of ergosterol one of the two ethenoid linkages in the nucleus first becomes saturated and hydrogen is then added to the side-chain unsaturated centre.



(I.)

We have now carried out a detailed study of the ergostadienetriol, first described by Windaus and Lüttringhaus (*Annalen*, 1930, **481**, 119), and have ascertained, in the first place, that all hydroxyl groups are contained in the nucleus, for ozonisation of the triol diacetate yields methyl*iso*propylacetaldehyde. Further, although the addition of hydrogen to ergostadienetriol ceases when two atoms of hydrogen have been absorbed, the ergostenetriol thus formed (Windaus and Lüttringhaus, *loc. cit.*) undergoes isomerisation with hydrogen chloride in chloroform solution, giving an isomeric *ergostenetriol* B, m. p. 191—193° (diacetate, m. p. 209—210°), which can now be further hydrogenated to the saturated *ergostanetriol*, m. p. 246—247°. These changes, as indicated in the annexed scheme, are wholly analogous to those involved in the hydrogenation of ergosterol, and provide adequate proof that the mechanism of reaction must be identical in both cases. From this it follows (*a*) that both ergostenetriol and α-ergostenol contain the same inert double bond, and (*b*) that the same nuclear double bond is involved in the formation of α-dihydroergosterol and ergostadienetriol.



Windaus and Lüttringhaus (*loc. cit.*) have observed that both ergostadienetriol and ergostenetriol form a series of di-esters only. Ergostanetriol reacts similarly, furnishing a *diacetate*, m. p. 190—191°, while methoxyergostatriene (Heilbron and Simpson, J., 1932, 268) reacts with perbenzoic acid, yielding a *methoxyergostadienediol monobenzoate*, m. p. 158—159°, which resists further benzoylation. The *methoxyergostadienediol*, m. p. 174—175°, formed by hydrolysis again forms the monobenzoate on treatment with benzoyl chloride. It is obvious from these results that one of the hydroxyls introduced must be present as a tertiary carbinol, a conclusion supported by the fact that only diketone-alcohols are formed on oxidation of any of the triols with chromic anhydride (see experimental portion). The action of lead tetra-acetate upon both *ergostadienetriol* and *methoxyergostadienediol* has been examined and products (presumably keto-aldehydes) melting respectively at 155—156° and 105—106° isolated.

These results prove (*a*) that the formation of ergostadienetriol (and consequently also of α-dihydroergosterol) has involved direct 1 : 2-addition to the reactive nuclear double bond (cf. Criegee, *Ber.*, 1931, **64**, 260), (*b*) that this double bond must be present as a >C═CH– group, (*c*) that the inert double bond in α-ergostenol * and ergostenetriol must also be present in ergosterol and in α-dihydroergosterol, and (*d*) that, provided, as seems likely from the evidence of Diels and Karstens (*Annalen*, 1930, **478**, 129), that the nuclear skeleton of ergosterol is identical with that of cholesterol, the only possible positions for the reactive nuclear ethylenic linkage must be between C_5–C_6 or C_9–C_{11}. Of these the 5 : 6-position was arbitrarily suggested (Heilbron, *J. Soc. Chem. Ind.*, 1932, **51**, 1061) in view of the close analogy of ergostanetriol to cholestanetriol (Pickard and Yates, J., 1908, **93**, 1678), which can only be represented as cholestane-3 : 5 : 6-triol (Windaus, *Z. physiol. Chem.*, 1932, **213**, 149). A difficulty is here encountered, for, arising from the work of Inhoffen (*Annalen*, 1932, **497**, 130) and of Guiteras, Nakamiya, and Inhoffen (*loc. cit.*), it would appear that the two nuclear double bonds are contained in one ring, and for these Rosenheim and King (*J. Soc. Chem. Ind.*, 1932, **51**, 954) have suggested positions C_5–C_6 and C_7–C_8. Although the reactions of ergosterol, as shown by the formation of a maleic anhydride addition product and by its reduction with sodium and alcohol (Windaus and Brunken, *Annalen*, 1928, **460**, 225), certainly point to the presence of a conjugated system, the evidence now advanced shows that such a system is unlikely to be of the above normal

* Professor Sugden has kindly examined the parachor of this substance which is in agreement with that required by an alcohol containing an ethylenic link and not a bridged ring.

type. Observations by Windaus (*Nach. Ges. Wiss. Gött.*, 1929, **159**; *Ber.*, 1931, **64**, 850) on the reaction between ergosterol and maleic anhydride had also previously indicated that ergosterol did not contain a normal conjugated system in one ring.

A careful scrutiny of the whole of the experimental data leads us to the view that it is possible adequately to represent the whole of the observed facts by a formulation containing both double bonds in Ring B only if these are in positions C_5–C_6 and C_8–C_9. In this event " conjugative " reactions must be preceded by the shift of the reactive nuclear bond to C_6–C_7. On the other hand, if the reactive ethenoid linkage be situated between C_9–C_{11} and the inert double bond between C_8–C_{14}, an abnormal conjugated system containing both the double bonds in one ring is provided which will satisfactorily explain the known reactions of ergosterol (I) without necessitating the assumption of any isomeric change to account for its reaction with maleic anhydride and with sodium and alcohol.

β-*Dihydroergosterol.*—Heilbron, Johnstone, and Spring (J., 1929, 2248) have described the conversion of α-dihydroergosterol into the isomeric β-dihydroergosterol, which compound was stated to furnish α-ergostenol on hydrogenation with a palladium catalyst (see also Dithmar and Achtermann, *Z. physiol. Chem.*, 1932, **205**, 55). We have now found that ozonisation of β-dihydroergosterol yields methyl*iso*propylacetaldehyde. Since α-dihydroergosterol contains the side-chain and inert nuclear double bonds, its transformation to the β-compound must accordingly involve either (*a*) *cis-trans*-isomerism round the side chain ethylenic linkage C_{22}–C_{23} or (*b*) shift of the inert nuclear double bond, as in the change α-ergostenol ⟶ β-ergostenol (Heilbron and Wilkinson, J., 1932, 1708). Of these two hypotheses, (*b*) was considered the more probable, and the hydrogenation of β-dihydroergosterol was re-examined, since, if (*b*) be correct, ergostanol should result. Actually, it has been found that hydrogenation in acetic acid solution with a platinum oxide catalyst produces both α-ergostenol and ergostanol. We therefore consider it probable that pure β-dihydroergosterol has not so far been isolated, and that the compound, m. p. 123—124°, represents a eutectic mixture of α- and β-isomerides, as in the case of the isomeric ergostenols (Heilbron and Wilkinson, *loc. cit.*). This involves the question whether ergosterol E (Heilbron, Johnstone, and Spring, *loc. cit.*), which is certainly anomalous in failing to show selective absorption, is a pure compound, and this point is being reinvestigated.

The action of acetic acid (cold or hot) and platinum oxide produces no isomerisation of the inert nuclear double bond, since ergosteryl acetate has been found by us under these conditions to give exclusively α-ergostenyl acetate on hydrogenation. We believe that instances in the literature in which complete or partial hydrogenation of ergosterol to ergostanol is reported are due to isomerisation *in situ* produced by traces of hydrogen ion, since Dithmar and Achtermann (*loc. cit.*) have recorded the saturation of certain derivatives, which must contain the inert nuclear double bond, by hydrogenation in presence of hydrogen chloride.

The Double Bond in β-*Ergostenol.*—Rosenheim and King (*J. Soc. Chem. Ind.*, 1932, **51**, 464) state that experimental evidence indicates the presence in dihydroxycholenic acid of an ethenoid linkage between C_{22}–C_{23} (cf. I). Arising from the work of Morrison and Simpson (J., 1932, 1710) it would appear that β-ergostenol contains the same double bond as this acid, and therefore on treatment with ozone should yield methyl*iso*propylacetaldehyde. No trace of this substance was, however, observed on ozonisation either of this alcohol or of dehydroergostenol.

Experimental.

Ergostadienetriol monobenzoate was prepared by treatment of ergosterol with perbenzoic acid as described by Windaus and Lüttringhaus (*loc. cit.*) and was isolated in better yield (40%) by the following procedure. The gelatinous residue left after distillation of the $CHCl_3$ under reduced press. was digested first with light petroleum and then with hot MeOH. After one recrystn. from Et_2O–MeOH or from AcOEt, pure ergostadienetriol monobenzoate, m. p. 194°, was obtained. Ergostadienetriol monobenzoate gives an immediate pink coloration with a $CHCl_3$ solution of $SbCl_3$.

Ergostadienediolone Monobenzoate.—A solution of CrO_3 (2 g.) in AcOH (60 c.c.) and H_2O (2 c.c.) was added during ¾ hr. to a well-stirred solution of ergostadienetriol monobenzoate

(5 g.) in AcOH (300 c.c.), the temp. being kept between 35—40° during the addition and maintained for a further ½ hr. The reaction mixture was diluted with H_2O, and the pptd. ketone crystallised from EtOAc (yield, 70%). *Ergostadienediolone monobenzoate* formed needles, m. p. 217—218°, sol. in $CHCl_3$ but sparingly sol. in other solvents. On heating with dil. alc. KOH, the solution turned dark brown almost immediately, but no cryst. product could be isolated (Found : C, 78·3; H, 8·9. $C_{35}H_{48}O_4$ requires C, 78·9; H, 9·0%). The 2 : 4-*dinitrophenylhydrazone*, prepared by Allen's method (*J. Amer. Chem. Soc.*, 1930, **52**, 2955), formed red needles (from EtOAc), m. p. 232° (decomp.) (Found : N, 8·9. $C_{41}H_{52}O_7N_4$ requires N, 7·9%).

Ergostadienedionol was prepared from ergostadienetriol (5 g.) as described above, CrO_3 (4 g.) in AcOH (60 c.c.) and H_2O (5 c.c.) being used. Heating was continued for a further ½ hr. The diketone crystallised from $CHCl_3$–MeOH in plates, m. p. 249° (decomp.), sparingly sol. in cold $CHCl_3$ and almost insol. in other solvents (Found : C, 78·8; H, 10·2. $C_{28}H_{44}O_3$ requires C, 78·5; H, 10·3%). The *monoxime*, prepared in the usual manner, separated from EtOH in needles, m. p. 232—233° (decomp.) after softening at 225° (Found : N, 3·3. $C_{28}H_{45}O_3N$ requires N, 3·2%).

Methoxyergostadienediol Monobenzoate.—A solution of perbenzoic acid in $CHCl_3$, containing 0·22 g. active O_2, was added slowly with stirring to one of methoxyergostatriene (5 g.) in $CHCl_3$ (100 c.c.) at − 15°. After standing over-night in the ice-chest, the solution was washed with Na_2CO_3 aq. and with H_2O, and dried. The $CHCl_3$ was removed under reduced press., and the residual oil was dissolved in low-boiling petroleum (20 c.c.), from which it slowly crystallised. *Methoxyergostadienediol monobenzoate* separated from MeOH in needles, m. p. 158—159°, and gave a pink coloration with $SbCl_3$ (Found : C, 78·4; H, 9·3. $C_{36}H_{52}O_4$ requires C, 78·8; H, 9·5%). On hydrolysis with alc. KOH *methoxyergostadienediol* was obtained, which crystallised from aq. MeOH in long prisms, m. p. 174—175° (Found : C, 78·3; H, 10·9. $C_{29}H_{48}O_3$ requires C, 78·4; H, 10·8%). Benzoylation yielded the original monobenzoate, m. p. 158—159°.

Ozonisation of Ergostadienetriol Diacetate, m. p. 181°.—This was carried out by the method of Guiteras, Nakamiya, and Inhoffen (*loc. cit.*). The ozonide was decomposed in the usual way, the aldehyde removed in steam, and the semicarbazone formed. After two recrystns. from H_2O it had m. p. 129—130°, alone or mixed with methyl*iso*propylacetaldehydesemicarbazone obtained from ergosterol.

Action of Lead Tetra-acetate on Ergostadienetriol and Methoxyergostadienediol.—Ergostadienetriol (1 g.) was shaken with a solution of lead tetra-acetate in AcOH (75 c.c. of *N*/10) until it dissolved, and left for 2 hr. at room temp. Excess of lead tetra-acetate was reduced by aq. KI–AcONa, and the free I_2 dissolved by shaking with *N*/10-$Na_2S_2O_3$. The product was extracted with Et_2O, washed free of AcOH, dried, and recrystallised from 80% MeOH until the m. p. remained const. at 155—156°. The *keto-aldehyde* crystallised in needles, very sol. in EtOH (Found : C, 78·0; H, 10·2. $C_{28}H_{44}O_3$ requires C, 78·5; H, 10·3%). Ergostadienetriol monobenzoate is unaffected by lead tetra-acetate.

Methoxyergostadienediol was treated with lead tetra-acetate solution in the same manner and yielded a *product*, m. p. 105—106° (Found : C, 78·3; H, 10·6. $C_{29}H_{46}O_3$ requires C, 78·7; H, 10·4%).

Ergostenetriol B.—Dry HCl was passed for 3 hr. into a 5—6% solution of ergostenetriol diacetate (m. p. 171—173°) in dry $CHCl_3$ maintained at − 5°. The solution was washed successively with dil. Na_2CO_3 aq. and H_2O, and dried, and solvent removed. The residual oil crystallised immediately from warm EtOH, in which it was sparingly sol. Pure *ergostenetriol* B *diacetate* separates from C_6H_6–EtOH in rhombs, m. p. 209—210° (yield, 20—25%) (Found : C, 74·3; H, 10·0. $C_{34}H_{52}O_5$ requires C, 74·4; H, 10·1%). Hydrolysis of the diacetate with 2% alc. KOH yielded the free triol, which crystallised from EtOH in soft plates, m. p. 191—193°.

Ergostanetriol Diacetate.—Ergostenetriol B diacetate was hydrogenated at 2 atm. press. in AcOH–AcOEt (5 : 2) with Adams's PtO_2 at room temp. The solution was filtered from catalyst, concentrated under reduced press. to small bulk, and pptd. with H_2O. Pure *ergostanetriol diacetate* crystallised from EtOH, in which it was moderately easily sol., in hard cubes, m. p. 191° (Found : C, 74·0; H, 10·7. $C_{32}H_{54}O_5$ requires C, 74·1; H, 10·4%). The free ergostanetriol obtained by hydrolysis with alc. KOH separated from EtOH in prismatic needles, m. p. 246—247°, from which the original diacetate was regenerated on heating with Ac_2O.

Ozonisation of β*-Dihydroergosterol.*—A suspension of the acetate (1·9 g.) in AcOH (19 c.c.) was treated with ozonised O. After solution was complete (about 1 hr.) the liquid was diluted with H_2O and distilled until no more oily drops passed over. On working up the distillate by Guiteras, Nakamiya, and Inhoffen's method (*loc. cit.*), methyl*iso*propylacetaldehydesemi-

carbazone (0·1 g.) was obtained, which after one recrystn. from H_2O had m. p. 130° alone or mixed with an authentic specimen.

Under the same conditions no trace of aldehyde could be obtained from either β-ergostenol or dehydroergostenol.

Hydrogenation of β-Dihydroergosterol.—The acetate (1·5 g.) in AcOH (150 c.c.) was shaken with H at room temp. in presence of Adams's PtO_2 (0·3 g.). After reactivation of the catalyst and repeated agitation, the solution was filtered and concentrated under reduced press. The residue after 3 crystns. from abs. EtOH yielded the characteristic sparingly sol. prisms of ergostanyl acetate (m. p. 143°), giving no depression in m. p. on admixture with an authentic specimen (yield of pure product, 0·4 g.). From the combined mother-liquors a further crop of impure ergostanyl acetate was obtained, and finally a small amount (0·15 g.) of α-ergostenyl acetate was isolated, m. p. 105—106°, which did not depress the m. p. of pure α-ergostenyl acetate (109—110°).

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