

227. *Studies in the Sterol Group. Part XII. The Oxidation of α -Ergosterol and its Derivatives.*

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OXIDATION of α -ergosterol by means of chromic anhydride yields according to Reindel (*Annalen*, 1928, **466**, 131) the corresponding ketone, α -ergosterone, together with a second product, m. p. 219—221°, for which analytical values in agreement with the formula $C_{26}H_{42}O_3$ were obtained. In attempting to repeat the oxidation of α -ergosterol under the precise conditions described by Reindel (*loc. cit.*) we have obtained α -ergosterone only in comparatively small amount, the main product being a *substance* (designated A) which after purification was obtained in pale lemon-yellow needles, m. p. 185°. The acidic products of oxidation (15—20% of the whole) formed a viscous oil, from which a minute amount of a crystalline acid (m. p. 214—216°), $C_{22}H_{34}O_4$ or $C_{23}H_{36}O_4$, was isolated *via* the *methyl* ester. Although α -ergosterone is not readily obtainable

by the above method, it can be prepared in good yield by high-vacuum distillation of the alcohol in presence of copper-bronze. Oxidation of α -ergostenol with the Beckmann reagent also gives rise to α -ergostenone in moderate yield, without production of substance A, but here acidic products predominate. Although these have not so far been examined in detail, a crystalline acid, identical with that obtained by the chromic anhydride oxidation of α -ergostenol, has been isolated.

Substance A.—Analytical values for the vacuum-dried compound show that molecular degradation has occurred, the results pointing to either the formula $C_{22}H_{34}O_2$ or $C_{21}H_{32}O_2$. Regarding the nature of the two oxygen atoms in the molecule, it seems unlikely that hydroxyl groups are present, as, apart from the fact that the yellow colour is removed, the original substance is recovered otherwise unchanged after treatment with acetic anhydride. A similar phenomenon is cited by Windaus and Auhagen (*Annalen*, 1929, 472, 185), who find that the yellow colours of both ergostadienone and ergostatrienone D are only removed after warming with acetic anhydride. Treatment of substance A with semicarbazide acetate produces immediately a very sparingly soluble product, m. p. 275°, which, however, does not appear to be a true semicarbazone. Similarly with hydroxylamine acetate a crystalline substance, m. p. 196—198°, has been isolated with much difficulty, but here again the analytical data are not in accordance with the values for either a mono- or a di-oxime. The substance fails to react with alkaline hypobromite. The possibility of an oxide linkage has been considered, but this seems to be precluded by the fact that the compound is recovered unchanged after refluxing with alcoholic sulphuric acid. Reduction of A by Clemmensen's method yields a *hydrocarbon*, m. p. 85—86°, which gives positive Liebermann-Burchard and Tortelli-Jaffe reactions but is completely resistant to catalytic hydrogenation and thus apparently retains the inert unsaturated linkage of the original α -ergostenol. It would thus seem plausible to assume that this same linkage is also present in the unreduced product, the unsaturated nature of which is indicated by the deep purple colour given with the Liebermann-Burchard reagent.

The oxidation of α -ergostenyl acetate gives rise almost entirely to a mixture of neutral products. Although these have not been quantitatively separated, three compounds comparable with those obtained by the chromic anhydride oxidation of cholesteryl acetate (Mauthner and Suida, *Monatsh.*, 1896, 17, 594) have been isolated in small yield. Of these, α -ergostenonyl acetate, m. p. 172°, separates first and is present in largest amount. It readily forms a *semicarbazone* and on hydrolysis is converted into the free keto-alcohol,

α -ergostenonol, $C_{27}H_{44}O_2$, m. p. 155—156°, which on further oxidation with chromic anhydride yields α -ergostenedione, m. p. 183°, together with a trace of a crystalline acid, m. p. 225—230°.

From the original filtrate after removal of the α -ergostenonyl acetate two other compounds melting respectively at 214—215° and 134—135° have been obtained, both of which appear to have the same molecular formula $C_{29}H_{46}O_4$ or $C_{29}H_{44}O_4$: the amounts were too small to allow of further investigation of their structures.

In addition to the above crystalline substances the oxidation of α -ergostenyl acetate yielded a large quantity of neutral viscous oil, from which a small amount of volatile material, which readily formed a *semicarbazone*, m. p. 156—158°, was removed in steam. Further quantities of this substance became available on re-oxidation of the non-volatile residue with chromic anhydride. Its formation is reminiscent of the isolation of methyl *isohexyl* ketone by Windaus and Resau (*Ber.*, 1913, 46, 1246) on oxidation of cholesteryl acetate with chromic anhydride. That, however, it is not identical with the latter ketone is shown by analysis of the *semicarbazone*, which fixes the formula of the free carbonyl compound as $C_9H_{18}O$.*

The oxidation of α -ergostenyl chloride with chromic anhydride gives rise, as in the case of α -ergostenyl acetate, to a neutral oil, from which about 25% of solid material separated from methyl alcohol solution. By repeated fractional crystallisation of the solid from alcohol a substance, m. p. 217°, giving analytical data corresponding to $C_{27}H_{43}O_2Cl$ has been isolated in small amount. It readily forms a *monoxime*, m. p. 190°, but does not appear to contain a hydroxyl group and is probably a ketone-oxide. The alcoholic filtrates on concentration supplied further crystalline material of lower melting point, from which, however, no other definite compound could be isolated.

EXPERIMENTAL.

Oxidation of α -Ergostenol with Chromic Anhydride.—Into a stirred solution of pure α -ergostenol (4 g., m. p. 131—132°) in glacial acetic acid (80 c.c.), maintained at 67—70°, a solution of chromic anhydride (4 g.) in glacial acetic acid (28 c.c.) and water (2 c.c.) was run during 70 minutes. After an hour, the mixture was cooled, diluted with water, and extracted with ether, and the extract separated into acid (Y) and neutral fractions.

Isolation of substance A. The thick brown oil (3 g.) obtained from the dried neutral extract was crystallised from ether-alcohol

* While this paper was in the press a paper appeared (Guiteras, Nakamiya, and Inhoffen, *Annalen*, 1932, 494, 116) identifying this as a dihydrothujaketone.

(1 : 3). The crude material (m. p. 145—155°) was washed free from oil with cold methyl alcohol, and thrice crystallised from methyl alcohol—light petroleum and finally from alcohol (charcoal), from which it separated in lemon-yellow needles, m. p. 185°, $[\alpha]_D^{25} + 87^\circ$ ($c = 2.32$ in chloroform), very sparingly soluble in light petroleum, moderately soluble in alcohol, and easily soluble in acetone (Found : C, 79.6, 79.8, 79.7; H, 10.3, 10.4, 10.1. $C_{21}H_{32}O_2$ requires C, 79.8; H, 10.1%. $C_{22}H_{34}O_2$ requires C, 80.0; H, 10.3%).

α -Ergosterone having the properties ascribed to it by Reindel (*loc. cit.*) was isolated from the first methyl alcohol—light petroleum filtrate.

Oxidation of α -ergosterol with chromic anhydride both at 50° and also at room temperature produced similar results. The further oxidation of α -ergosterone with chromic anhydride also yields A in small amount.

Clemmensen Reduction of Substance A.—The compound (3 g., m. p. 184—185°) was boiled for 36 hours under reflux with hydrochloric acid (300 c.c. of equal volumes of concentrated acid and water) and amalgamated zinc (45 g.), all crystalline material slowly disappearing. The cold solution was extracted with ether, and the extract washed, dried, and evaporated. The residual oil was taken up in ether—methyl alcohol, and the solid which slowly separated in the ice-chest was repeatedly crystallised from ether—methyl alcohol and finally from acetone, from which the *hydrocarbon* separated in prisms, m. p. 85—86° (Found : C, 87.2, 87.0; H, 12.5, 12.7. $C_{22}H_{38}$ requires C, 87.4; H, 12.6%. $C_{21}H_{36}$ requires C, 87.5; H, 12.5%).

Catalytic Oxidation of α -Ergosterol.— α -Ergosterol (9 g.) was refluxed for 1 hour with clean copper-bronze (9 g.) in a high vacuum, and then distilled. The distillate (6 g.), b. p. 240—260°/0.1 mm., solidified almost completely on cooling, and was crystallised from ether—methyl alcohol and from acetone, α -ergosterone being obtained in colourless needles, m. p. 130° (benzylidene derivative, m. p. 161—162°).

Acid Products from Chromic Anhydride Oxidation of α -Ergosterol.—The oil separating from the sodium carbonate solution (Y, p. 1701) on acidification would not crystallise and was therefore esterified with methyl alcohol and sulphuric acid. The ester was isolated as an oil, a methyl-alcoholic solution of which gradually deposited crystals at -10° . The pure *ester* separated, after repeated crystallisation from methyl alcohol, in needles, m. p. 211—213°, $[\alpha]_D^{25} + 161^\circ$ ($c = 1.93$ in chloroform) (Found : C, 73.5, 73.7; H, 9.2, 9.4. $C_{24}H_{38}O_4$ requires C, 73.8; H, 9.7%. $C_{25}H_{40}O_4$ requires C, 74.2; H, 9.9%).

Oxidation of α -Ergosterol with the Beckmann Reagent.—Finely powdered α -ergosterol (20 g.) was shaken mechanically with the Beckmann reagent (500 c.c.) for 100 hours: carbon dioxide was generated and the pressure was periodically released. The product, which formed a semi-solid mass suspended in the solution, was diluted with an equal volume of water and repeatedly extracted with ether, the extract subsequently being separated into acid and neutral fractions by means of sodium carbonate solution. Much semi-solid material remained in the aqueous layer and gradually hardened. The material appears to be a complex chromium salt, but has not yet been fully examined.

Acid fraction. Acidification of the sodium carbonate solution gave an oil, from which on esterification the same methyl ester, m. p. 212—213°, was obtained as from the chromic anhydride oxidation.

Neutral fraction. Evaporation of the dried ethereal solution left a semi-solid residue, from which α -ergosterone (20% yield) was readily obtained after two crystallisations from acetone.

Oxidation of α -Ergosterenyl Acetate.—A solution of chromic anhydride (7.5 g.) in glacial acetic acid (50 c.c.) and water (5 c.c.) was added during 1½ hours to a well-stirred solution of α -ergosterenyl acetate (10 g.) in glacial acetic acid (200 c.c.) at 60°. After a further ½ hour's heating, the solution was largely diluted with water and extracted with ether, and the extract washed successively with sodium carbonate and water. (The acidic product of oxidation consisted of a very small amount of oily acid which was not examined further.) Removal of solvent from the dried ethereal solution gave a viscous oil (8 g.), which partly crystallised when diluted with methyl alcohol (20 c.c.), yielding a pale yellow solid (2 g.), m. p. 122—126°. A solution of this product (13 g., collected from various experiments) in hot alcohol (100 c.c.) deposited, on cooling, crop A (8 g.), m. p. 122—132°; the filtrate after concentration gave crop B (4.5 g.), m. p. 115—120°.

α -Ergosterenyl acetate was obtained, by repeated crystallisation of crop A from alcohol, in pale yellow plates, m. p. 170—171°, sparingly soluble in alcohol (Found: C, 78.8; H, 10.3. $C_{29}H_{46}O_3$ requires C, 78.7; H, 10.4%). The *semicarbazone*, which was obtained after long standing with semicarbazide acetate in ether-alcohol, separated from alcohol in colourless needles, m. p. 199—200° (Found: C, 71.9; H, 10.0; N, 8.3. $C_{30}H_{49}O_3N_3$ requires C, 72.1; H, 9.8; N, 8.4%).

Hydrolysis with methyl-alcoholic sodium hydroxide yielded *α -ergosterenol*, which crystallised from alcohol in colourless needles, m. p. 155—156° (Found: C, 80.6; H, 11.0. $C_{27}H_{44}O_2$ requires

C, 81.0; H, 11.0%). Reacetylation with acetic anhydride gave the acetate in a colourless form having the same melting point as the original yellow modification.

α-Ergostenedione.—Oxidation of *α*-ergostenonol (0.3 g.) with chromic anhydride, the product being worked up in the usual manner, gave an oil, from a hot methyl-alcoholic solution of which *α*-ergostenedione separated. The pure ketone crystallised from alcohol in long plates, m. p. 183° (Found: C, 81.8; H, 10.8. $C_{27}H_{42}O_2$ requires C, 81.4; H, 10.6%).

Substance, m. p. 134—135°.—The accumulated filtrates from which *α*-ergostenonyl acetate had been separated were concentrated and on standing at 0° deposited crystalline material (0.5 g.). Repeated crystallisation from alcohol yielded the pure *substance* in prisms, readily soluble in most organic solvents (Found: C, 76.0, 76.2; H, 10.5, 10.2. $C_{29}H_{44}O_4$ requires C, 76.3; H, 9.7%. $C_{29}H_{46}O_4$ requires C, 76.0; H, 10.0%).

Substance, m. p. 214—215°.—This *product* was isolated as shining plates in minute amount from crop B by repeated crystallisation from alcohol (Found: C, 76.2, 76.2; H, 10.0, 10.1. $C_{29}H_{44}O_4$ requires C, 76.3; H, 9.7%. $C_{29}H_{46}O_4$ requires C, 76.0; H, 10.0%).

The yield of pure substances from crops A and B represented only a very small proportion of the total materials, but a more complete separation could not be effected. Attempts to separate pure substances in the form of their semicarbazones also proved ineffective.

Volatile Product, $C_9H_{18}O$.—The neutral oil remaining after removal of solid material was subjected to steam distillation, whereby a small quantity of volatile oil was obtained. Further oxidation of the residue with chromic anhydride, followed by steam distillation, yielded a further quantity of this material, which readily formed a *semicarbazone* crystallising from methyl alcohol in needles, m. p. 156—158° (Found: C, 60.4; H, 10.4; N, 21.8. $C_{10}H_{21}ON_3$ requires C, 60.3; H, 10.6; N, 21.1%).

Oxidation of α-Ergostenyl Chloride.—A well-stirred suspension of *α*-ergostenyl chloride (6.5 g.) in glacial acetic acid (100 c.c.) was oxidised with chromic anhydride as described in the case of *α*-ergostenyl acetate. The viscous oil (6 g.) obtained on evaporation of the dried ethereal extract was dissolved in methyl alcohol (25 c.c.), yielding a solid (1.6 g.), from which, after repeated crystallisation from alcohol, a *substance*, m. p. 217°, was obtained in shining plates (0.3 g.), very sparingly soluble in most organic solvents (Found: C, 74.7; H, 10.1; Cl, 8.2. $C_{27}H_{43}O_2Cl$ requires C, 74.6; H, 9.9; Cl, 8.2%). The *oxime*, prepared by refluxing the ketone with hydroxylamine acetate in alcoholic solution for 5 hours, separated

from alcohol in needles, m. p. 190° (Found: C, 72.2; H, 10.0; N, 3.3. $C_{27}H_{44}O_2NCl$ requires C, 72.1; H, 9.8; N, 3.1%).

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