103. *The Action of Selenium Dioxide on Sterols and Bile Acids. Part I. Ergosterol and Dihydroergosterol.*

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THE introduction of selenium dioxide as an oxidising agent for organic compounds by Riley, Morley, and Friend (J., 1932, 1875) suggested an investigation of its action on sterols and bile acids. Montignie (*Bull. Soc. chim.*, 1932, **51**, 144) had previously stated that the precipitation of selenium from a hot alcoholic solution of selenium dioxide was a reaction characteristic of ergosterol and "γ-dihydroergosterol." A preliminary examination has now been made of the behaviour of fifty representatives of this group towards selenium dioxide. In aqueous-alcoholic solution marked differences in reactivity are observed. The separation of selenium at room temperature takes place with ergosterol, dihydroergosterol, lumisterol, calciferol, and *apo*cholic acid. In a number of other cases, mostly ergosterol derivatives, reaction takes place readily on the water-bath. A third class, which includes nearly all the derivatives of cholesterol examined, does not react under these conditions, but reaction generally takes place in acetic acid or nitrobenzene at 100°. It is notable that the compounds which have been assumed to contain an ethenoid or bridge linkage resistant to catalytic hydrogenation are among the more reactive compounds, and comparison of the behaviour of α- and β-ergostenols and *apo*cholic and dihydroxycholenic acids confirms in another particular the analogy between the reactions of these two pairs of compounds to which attention has been drawn by Rosenheim and King (*Chem. and Ind.*, 1932, **464**, 954) and by Morrison and Simpson (J., 1932, 1710).

The formation of a ketone, which might have been expected in analogy to the specific oxidising action of selenium dioxide on other unsaturated compounds (Riley and Friend, J., 1932, 2342; Schwenk and Borgwardt, *Ber.*, 1932, **65**, 1601), has not been observed in the cases in which the products have been examined. In these the main reaction is either a partial dehydrogenation (ergosterol, α-ergostenol, and *apo*cholic acid), or the formation of an oxide (dihydroergosterol and cholesterol).

The reaction of ergosterol with selenium dioxide is a complex one, but the main product formed at room temperature is dehydroergosterol, previously obtained by the dehydrogenating action of mercuric acetate on ergosterol in boiling alcohol (Windaus and Linsert, *Annalen*, 1928, **465**, 157). Dihydroergosterol yields at room temperature mainly *dihydro-*

ergosterol oxide, accompanied by some ergosterol-*D*. The latter compound has been obtained previously by dehydrogenation of dihydroergosterol by mercuric acetate (Windaus and Auhagen, *Annalen*, 1929, **472**, 185; Heilbron, Johnstone, and Spring, J., 1929, 2248) or by perbenzoic acid (Windaus and Lüttringhaus, *Annalen*, 1930, **481**, 119). Ergosterol-*D* does not appear to be formed from the oxide by way of a di-tertiary glycol and loss of water, but rather by an alternative reaction directly from dihydroergosterol. In presence of a little sulphuric acid dihydroergosterol oxide acetate actually yields ergosterol-B_3 acetate, which contains two conjugated double linkages and reacts readily with maleic anhydride (Windaus, Dithmar, Murke, and Suckfüll, *Annalen*, 1931, **488**, 100). The acetate of ergosterol-*D*, which does not react with maleic anhydride (Windaus and Lüttringhaus, *Ber.*, 1931, **64**, 850), does not isomerise under these conditions, although it yields the acetate of ergosterol-B_3 with hydrogen chloride in chloroform (Windaus, Dithmar, Murke, and Suckfüll, *loc. cit.*, p. 98).

Investigation of the other reactions mentioned in the experimental part is in progress, and an interpretation of the results on the basis of the new cholane formula (Rosenheim and King, *loc. cit.*) will be given in the next communication.

Experimental.

The following procedure was used for testing reactivity with SeO_2. The substance (5—10 mg.) and an equal amount of SeO_2 in a test-tube were shaken with 2—3 c.c. of the solvent, and, when reaction did not take place at room temp., the contents of the tube were warmed gently until solution was complete, and then heated on the water-bath. In the case of those substances which are described as reacting readily a pink ppt. of Se appeared within 5 min., sometimes preceded by a turbidity. In some cases, in which at most a slight yellowing or a mere trace of Se was observed, even after prolonged boiling, the reaction was considered to be negative. The following results were obtained:

(i) *Cholesterol and its derivatives.* Cholesterol, cholesteryl acetate, cholesteryl chloride, cholesteryl bromide, *allo*cholesterol, ψ-cholesterol, α-cholestantriol, cholesteryl ether, α-cholesterol oxide, ψ-cholestane, cholestene, ψ-cholestene, cholesterilene, cholestan-6-one, cholestenone, cholestan-6-on-3-ol acetate, cholestan-3 : 6-dion-5-ol, oxycholesterylene, oxycholestenone, and coprosterol did not react with SeO_2 in boiling 90% EtOH. Reaction took place in AcOH at 100° with all these substances with the exception of α-cholestantriol, ψ-cholestane, cholestan-6-on-3-ol acetate, and oxycholesterylene. Cholestan-3 : 6-dione and coprostanone reacted both in 90% EtOH and in AcOH.

(ii) *Phytosterols.* Sitosterol and stigmasterol did not react in 90% EtOH, but reacted in AcOH at 100°.

(iii) *Mycosterols.* Ergosterol, dihydroergosterol, lumisterol, and calciferol reacted at room temp. in 90% EtOH. Ergosteryl benzoate, dehydroergosterol, ergosterol peroxide, ergosterol-B_3, ergosterol-*D*, α-ergostenol, α-ergostene, α-ergostenone, and dihydroergosterol oxide reacted in boiling 90% EtOH. β-Ergostenol and dehydroergostenol reacted only in AcOH at 100°. Zymosterol reacted slightly in boiling 90% EtOH (admixture of ergosterol?).

(iv) *Bile acids.* *apo*Cholic acid reacted in 80% EtOH at room temp., whilst methyl *apo*cholate, methyl dihydroxycholenate, choladienic acid, and cholatrienic acid reacted in boiling 90% EtOH, and dihydroxycholadienic acid reacted only in AcOH at 100°. Cholic, deoxycholic, 12-ketocholanic, and dehydrodeoxycholic acids did not react in EtOH or in AcOH at 100°.

Of substances related to the sterols, β-amyrin benzoate reacted in AcOH at 100°, but not in aq. EtOH; abietic acid reacted readily in boiling 90% EtOH; ketohydroxyœstrin reacted slowly in AcOH at 100°; digitoxigenin and gitoxigenin reacted in AcOH at 100°, whilst digitaligenin did not react either in boiling aq. EtOH or in AcOH at 100°.

Ergosterol and SeO_2.—The reaction takes place in dil. EtOH solution at room temp., but is most conveniently carried out in a mixture of C_6H_6 and EtOH, in which the ergosterol is more sol. To ergosterol ($[\alpha]_{5461}$ — 165° in $CHCl_3$; 5 g.) in 250 c.c. of C_6H_6 and 50 c.c. of 95% EtOH was added a solution of SeO_2 (5 g.) in 25 c.c. of 95% EtOH, and the mixture was kept for 19 hr. at about 18°. The pptd. Se (0·65 g.) was removed, H_2O added, and the C_6H_6 layer washed with H_2O, dried over Na_2SO_4, and evaporated. The residue was extracted with MeOH (100 c.c. in two portions). The crude product which separated (1·9 g., m. p. 85—120°) was recrystallised from MeOH (charcoal), and the dehydroergosterol acetylated by boiling

with Ac_2O. Recryst. from EtOAc, the acetate had m. p. 145—147°, $[\alpha]_D^{20°}$ + 162°, $[\alpha]_{5461}^{20°}$ + 201° in $CHCl_3$. The identity of the product was further confirmed by preparing the 3 : 5-dinitrobenzoate from the crude sterol by treatment with 3 : 5-dinitrobenzoyl chloride in C_5H_5N at 100° for 20 min. Recryst. from EtOAc, *dehydroergosteryl* 3 : 5-*dinitrobenzoate* formed crimson leaflets, m. p. 185—189° (colour changes to yellow at 170°). The ester from dehydroergosterol prepared from ergosterol and $Hg(OAc)_2$ had m. p. 186—189° (colour change at 179°), $[\alpha]_{5461}^{20°}$ + 126° in $CHCl_3$ [Found (micro.): C, 71·4; H, 7·6. $C_{35}H_{44}O_6N_2$ requires C, 71·4; H, 7·5%]. A mixture of the two specimens had m. p. 185—189°.

When a solution of ergosterol (10 g.) and SeO_2 (10 g.) in 95% EtOH (250 c.c.) was refluxed for 30 min., 2·9 g. of Se, corresponding to the consumption of 1·5 mol. SeO_2, were pptd. The product was a red-brown tar, from which crystals could not be isolated. Treatment of a portion with semicarbazide yielded a trace of cryst. product which was not further investigated.

Preparation of Dihydroergosterol.—It was not found possible to reduce ergosterol completely by the action of Na in EtOH (Windaus and Brunken, *Annalen*, 1928, **460**, 232; cf. Heilbron, Johnstone, and Spring, J., 1932, 926). The desired product was obtained conveniently by hydrogenation of ergosterol (10 g.) in Et_2O (750 c.c.) in presence of palladised charcoal (10 g. from 1 g. $PdCl_2$). After absorption of 750—800 c.c. of H (1·5 mols.) the product no longer gave a blue colour with $CCl_3{\cdot}CO_2H$. After recrystn. from EtOH the product was treated with BzCl in C_5H_5N. The benzoate, m. p. 190—194°, from EtOAc, yielded dihydroergosterol, m. p. 173—175°. *Dihydroergosteryl* 3 : 5-*dinitrobenzoate*, colourless needles from EtOAc, m. p. 206·5—207·5°, $[\alpha]_D^{20°}$ − 1·8°, $[\alpha]_{5461}^{20°}$ − 2·0° in C_6H_6, was prepared in the usual way [Found (micro.): C, 71·0; H, 8·0. $C_{35}H_{48}O_6N_2$ requires C, 70·9; H, 8·2%].

Dihydroergosterol and SeO_2.—Dihydroergosterol (3·6 g.) and SeO_2 (3·6 g.), dissolved in a mixture of C_6H_6 (80 c.c.) and 95% EtOH (240 c.c.), were allowed to react at 37° for 24 hr. The Se (0·35 g.) was separated, the filtrate poured into 500 c.c. of H_2O, and the C_6H_6 layer separated, washed with H_2O, dried, and evaporated. The residue was treated with 3 : 5-dinitrobenzoyl chloride (4 g.) in C_5H_5N. The crude ester (4 g.), recryst. once from C_6H_6–MeOH, had m. p. 178—182°. After 5 recrystns. from EtOAc it formed brownish-yellow needles, m. p. 196—198° (decomp.). It was not pure, as shown by the presence of a somewhat high proportion of C [Found (micro.): C, 69·6, 69·5. Calc., 69·0%], and a low optical rotation as compared with the pure product described below. Hydrolysis of the ester by NaOH in EtOH and pptn. of the product by H_2O yielded *dihydroergosterol oxide*, which, recryst. from MeOH, formed soft needles, m. p. 99° when rapidly heated, containing solvent of crystn. (Found: Loss at 95°/0·1 mm. over P_2O_5, 3·2. Calc. for $C_{28}H_{46}O_2$,0·5MeOH, 3·7%). After drying over P_2O_5 at room temp., it lost the solvent and had m. p. 110—111°, $[\alpha]_D^{20°}$ − 44·3°, $[\alpha]_{5461}^{20°}$ − 53·1° in $CHCl_3$ [Found: No loss at 95°/0·1 mm., and (micro.) C, 80·9; H, 11·2. $C_{28}H_{46}O_2$ requires C, 81·1; H, 11·2%]. The absorption spectrum in EtOH showed a faint band at 242 mμ which could be accounted for by the presence of 1·5% of ergosterol-*D*. The colour reactions were as follows: Salkowski, $CHCl_3$ layer yellow, acid red; Liebermann–Burchard, purple, then blue, finally green; $CCl_3{\cdot}CO_2H$, pink, with green fluorescence; $Hg(OAc)_2$ in HNO_3 added to $CHCl_3$ solution, $CHCl_3$ layer blue, acid yellow; $SbCl_3$, pink; Tortelli–Jaffe, green. The digitonide is readily sol. in EtOH. No ppt. is produced by a 1% solution of digitonin in 90% EtOH added to a conc. solution of the oxide, but the digitonide is pptd. by excess of H_2O.

Dihydroergosterol oxide acetate, needles from MeOH, m. p. 95—105° (with solvent of crystn.), and m. p. 107—109° (dried over P_2O_5 in vac.), $[\alpha]_D^{20°}$ − 47°, $[\alpha]_{5461}^{20°}$ − 58° in $CHCl_3$, was prepared by treating the alcohol with Ac_2O in C_5H_5N at 100° for 30 min., followed by pptn. of the product by addition of H_2O [Found (micro.): C, 79·1; H, 10·7. $C_{30}H_{48}O_3$ requires C, 78·9; H, 10·6%]. The *benzoate*, needles from aq. EtOH, m. p. 118—120°, $[\alpha]_D^{20°}$ − 36°, $[\alpha]_{5461}^{20°}$ − 43° in $CHCl_3$, was prepared from the alcohol by treatment with BzCl in C_5H_5N [Found (micro.): C, 80·8; H, 9·8. $C_{35}H_{50}O_3$ requires C, 81·0; H, 9·7%]. Dihydroergosterol oxide was obtained by hydrolysis of the acetate or benzoate in EtOH with NaOH. The 3 : 5-*dinitrobenzoate*, pale yellow needles from EtOAc, m. p. 198° (decomp.), $[\alpha]_D^{20°}$ − 25°, $[\alpha]_{5461}^{20°}$ − 30° in C_6H_6, was prepared from the alcohol in the usual way [Found (micro.): C, 69·2; H, 8·1. $C_{35}H_{48}O_7N_2$ requires C, 69·0; H, 8·0%].

Separation of Ergosterol-D.—The crude product from dihydroergosterol and SeO_2 was refluxed with Ac_2O for 10 min.; an oil separated, on cooling, which slowly solidified (yield, 3 g., m. p. 75—105°, from 4 g. of dihydroergosterol). Recryst. from EtOH, this yielded 0·6 g. of a product, m. p. 146—154°, which, after being four times recrystallised from acetone, had m. p. 170—173°, $[\alpha]_D^{20°}$ + 11°, $[\alpha]_{5461}^{20°}$ + 13° in $CHCl_3$. Hydrolysis with NaOH in EtOH yielded the sterol, leaflets from EtOH, m. p. 162—164°, $[\alpha]_D^{20°}$ + 17°, $[\alpha]_{5461}^{20°}$ + 20° in $CHCl_3$. The

absorption spectrum in EtOH showed a sharp max. at 242 mμ ($\varepsilon/c = 54$) with subsidiary bands at 238 and 252 mμ. The correspondence in physical properties with those recorded in the literature for ergosterol-*D* is fairly close.

Ergosterol-B_3 *from Dihydroergosterol Oxide.*—When dihydroergosterol oxide was refluxed with Ac_2O for a few min., or, in presence of excess of anhyd. NaOAc, for 15 min., the product was the acetate of the oxide described above. When, however, the boiling with Ac_2O alone was prolonged for 20 min., a further change occurred. In the light of subsequent experience this is to be attributed to the presence of traces of mineral acid. In one such case, after 12 hr., crystals separated, m. p. 134—138°. Recryst. four times from EtOH, the compound formed thin leaflets, m. p. 139—141°, $[\alpha]_D^{20°}$ — 196°, $[\alpha]_{5461}^{20°}$ — 241° in $CHCl_3$ [Found (micro.): C, 81·8; H, 10·1. $C_{30}H_{46}O_2$ requires C, 82·1; H, 10·6%]. Hydrolysis by NaOH in EtOH yielded the sterol, leaflets from aq. EtOH, m. p. 136—137·5°, $[\alpha]_D^{20°}$ — 207°, $[\alpha]_{5461}^{20°}$ — 253° in $CHCl_3$. The absorption spectrum in EtOH showed a sharp max. at 242 mμ ($\varepsilon/c = 25·5$). The physical properties agree with those recorded for ergosterol-B_3.

The formation of ergosterol-B_3 also occurred when dihydroergosterol oxide acetate (50 mg.) was dissolved in AcOH (1 c.c.), and 1 drop of conc. H_2SO_4 added. Crystals separated immediately, which were collected and washed with MeOH. They had m. p. 137—139°, not depressed by admixture with ergosterol-B_3 acetate. Also, when the oxide acetate (50 mg.) in MeOH (10 c.c.) with 3 drops of 2*N*-H_2SO_4 was refluxed for 10 min., leaflets separated on cooling, m. p. 133—135°, not depressed by admixture with ergosterol-B_3 acetate. A similar expt. with ergosterol-*D* acetate yielded unchanged initial material, m. p. 170—173°.

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