350. *Studies in the Sterol Group. Part XVI.* (a) *The Molecular Formula of Ergosterol.* (b) *The Oxidation of Ergosterol and of* α*-Dihydroergosterol with Manganese Dioxide and Sulphuric Acid.*

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(*a*) The hitherto generally accepted view that cholesterol, sitosterol, and ergosterol each contain twenty-seven carbon atoms has recently

been shown to be incorrect by Sandqvist and Bengtsson (*Ber.*, 1931, **54**, 2167), who, from acetyl estimations of sitosteryl acetate, conclude that this sterol has actually the formula $C_{29}H_{50}O$, a result now confirmed by Windaus, Werder, and Gschaider (*Ber.*, 1932, **65**, 1006). Similarly, Windaus and Lüttringhaus (*Nach. Ges. Wiss. Gött.*, 1932, 4) have suggested from the analytical data of various derivatives of ergosterol, especially the dinitrobenzoate and allophanate, that this sterol is more probably better represented as $C_{28}H_{44}O$ than $C_{27}H_{42}O$ (compare also Windaus, Werder, and Gschaider, *loc. cit.*). We have now succeeded in obtaining *bromoergostanone*, m. p. 191°, and *bromodehydroergostenone*, m. p. 178—179°, from both ergostanone and β-ergostenone by treatment respectively with slightly more than one and two molecules of bromine. That the bromination of β-ergostenone is accompanied by dehydrogenation and is in this respect similar to the action of bromine on β-ergostenol (Morrison and Simpson, this vol., p. 1710) has been proved by the reduction of the bromo-ketone with zinc dust and acetic acid to *dehydroergostenone*, $C_{28}H_{44}O$, m. p. 148°, which is identical with the ketone derived from the distillation of dehydroergostenol with copper–bronze. With the isolation of these bromo-ketones, compounds became available which have enabled us to reach definite conclusions regarding the number of carbon atoms in the ergosterol molecule. The analytical data given below show that the molecular formula of ergosterol cannot possibly be $C_{27}H_{42}O$, but must be represented as $C_{28}H_{44}O$ (or less probably $C_{29}H_{46}O$), thus confirming the views of Windaus and Lüttringhaus (*loc. cit.*). The analyses were carried out by Dr. A. Schoeller, Berlin, and in each case two different preparations of the bromo-ketones were submitted.

Bromoergostanone

$C_{27}H_{45}OBr$ requires	C, 69·7;	H, 9·7;	Br, 17·2%
$C_{28}H_{47}OBr$,,	70·2;	9·8;	16·7%
$C_{29}H_{49}OBr$,,	70·6;	9·9;	16·2%
$C_{30}H_{51}OBr$,,	71·0;	10·1;	15·8%
Found : Preparation A	70·6;	10·0;	16·6%
,, ,, B	70·3;	9·9;	16·5%
	70·3;	9·9;	16·6%

Bromodehydroergostenone

$C_{27}H_{41}OBr$ requires	C, 70·3;	H, 8·9;	Br, 17·4%
$C_{28}H_{43}OBr$,,	70·7;	9·05;	16·85%
$C_{29}H_{45}OBr$,,	71·2;	9·2;	16·4%
$C_{30}H_{47}OBr$,,	71·6;	9·3;	15·9%
Found : Preparation A	70·5;	9·3;	16·6%
,, ,, B	70·6;	9·3;	16·6%
	70·5;	9·1;	16·6%

The bromination of α-ergostenone has also been attempted, but no bromine-containing substance could be isolated. Further work on these substances is now being carried out.

(*b*) The oxidation of ergosterol with nitric acid was examined by Reindel and Niederländer (*Annalen*, 1930, **482**, 264), who isolated a polycarboxylic acid which they considered to be a *cyclo*pentadienetricarboxylic acid, but Guiteras, Nakamiya, and Inhoffen have proved to be a methylbenzenetetracarboxylic acid by further oxidation to the closely related benzenepentacarboxylic acid (*Annalen*, 1932, **494**, 115). We have ourselves obtained the latter acid in the form of its pentamethyl ester from both ergosterol and α-dihydroergosterol by oxidation with manganese dioxide and 57% sulphuric acid. In each case a complex mixture of acids was formed, but whereas from ergosterol the yield of pure pentamethyl ester was 4% of the ergosterol used, from α-dihydroergosterol only 1½% was obtained, despite the fact that the percentage amount of total acids from the latter was almost twice as great as that from ergosterol. This result is in accord with that of Reindel and Niederlander and Guiteras, Nakamiya, and Inhoffen (*loc. cit.*), who find that the production of methylbenzenetetracarboxylic acid is practically specific to ergosterol, and that the acid is not formed from the *iso*-ergosterols, α- and β-ergostenols, ergostanol, or other sterols such as cholesterol and sitosterol. We have ourselves found that α-dihydroergosterol also fails to yield this acid.

Although evidence obtained by drastic oxidation methods such as have been employed here must be applied with extreme caution to problems relating to the elucidation of chemical structure (compare Ruzicka, Schinz, and Meyer, *Helv. Chim. Acta*, 1923, **6**, 1077), we believe that, taken in conjunction with the results of Guiteras, Nakamiya, and Inhoffen (*loc. cit.*), our results assume a not inconsiderable significance and do, in fact, support the hypothesis that, in attempting to arrive at a structural formula for ergosterol, an unsaturated ring system allowing of the formation of a methylbenzenetetracarboxylic acid must be taken into consideration.

EXPERIMENTAL.

Bromoergostanone.—To a solution of ergostanone (0·5 g.) in $CHCl_3$ (5 c.c.) at room temp., Br (5 g. in 100 c.c. $CHCl_3$; slightly more than 1 mol.) was added during 20 mins. The solution was then boiled for 1 min., cooled, and washed successively with $Na_2S_2O_3$ aq. and H_2O. The cryst. residue left after removal of solvent from the dried solution was recrystallised from $CHCl_3$–EtOH and then from acetone. The *bromo-ketone*, m. p. 191° (decomp.), formed long needle clusters, sparingly sol. in Et_2O and EtOH, moderately in acetone, and readily in $CHCl_3$.

Bromodehydroergostenone.—A solution of β-ergostenone (1 g.) in dry $CHCl_3$ was treated as above with 18 c.c. of the Br solution during 10—15 mins., at — 15°. The solvent was removed from the reddish-brown fluorescent solution under reduced press. and the residual dark solid was crystallised from acetone and then repeatedly from $CHCl_3$–EtOH. *Bromodehydroergostenone* separated

in thin prisms, m. p. 178—179° (decomp.), sparingly sol. in EtOH, moderately in acetone, and readily in $CHCl_3$.

Dehydroergostenone.—(a) A solution of bromodehydroergostenone (0·3 g.) in AcOH (24 c.c.) was heated for 2 hrs. with Zn dust (0·8 g.). The hot solution was diluted with H_2O and the solid which separated at 0° was recrystallised from EtOH. *Dehydroergostenone* formed plates, m. p. 147—148°, readily sol. in hot EtOH (Found : C, 84·8; H, 11·3. $C_{28}H_{44}O$ requires C, 84·8; H, 11·1%). The *oxime* crystallised from EtOH in prisms, m. p. 212—213° (Found : N, 3·7. $C_{28}H_{45}ON$ requires N, 3·4%).

(b) Dehydroergostenol was heated for 1 hr. at 0·1 mm. with an equal wt. of copper–bronze and then distilled. The distillate, which solidified on cooling, was crystallised from EtOH, giving dehydroergostenone, m. p. 148°, identical with that obtained by the above method.

Oxidation of Ergosterol with Manganese Dioxide and Sulphuric Acid.—A suspension of ergosterol (25 g.), conc. H_2SO_4 (500 c.c.), and H_2O (600 c.c.) was heated to 90°, and MnO_2 (100 g.) added. The whole was boiled under reflux for 24 hrs., 270 g. of MnO_2 being added in five equal portions during the first 4 hrs. The cooled reaction mixture was diluted with an equal vol. of H_2O, the residue after filtration washed with boiling H_2O, and the filtrate and washings continuously extracted for 48 hrs. with Et_2O. The semi-solid mass left after removal of solvent was dissolved in H_2O, and the filtered solution neutralised with NH_3 aq. and treated with $AgNO_3$. The dried mixture of Ag salts (10 g.) was treated with MeI, a vigorous initial reaction occurring; esterification was completed by heating under reflux for 1 hr. After removal of the excess of MeI the residue was twice crystallised from MeOH, methyl benzenepentacarboxylate (1 g.) being obtained in prisms, m. p. 148° (Ruzicka, Shinz, and Meyer, *loc. cit.*, give m. p. 146—147°; Guiteras, Nakamiya, and Inhoffen, *loc. cit.*, m. p. 147—148°) (Found : C, 52·5; H, 4·4; OMe, 41·7. Calc. for $C_{16}H_{16}O_{10}$: C, 52·2; H, 4·4; OMe, 42·1%).

Oxidation of α-Dihydroergosterol.—This was carried out as described above. Methylation of the mixed Ag salts (15 g. from 22 g. of α-dihydroergosterol) yielded a complex mixture of esters from which no single substance could be isolated by fractional crystn. The whole (4·8 g.) was therefore distilled at 0·3 mm., yielding the following fractions : (a) 140—170°, 0·4 g.; (b) 170—195°, 1·6 g.; (c) 195—220°, 1·4 g. After three recrystns. of fraction (c) from MeOH, 0·4 g. of methyl benzenepentacarboxylate was isolated. No individual could be obtained from fraction (b), and this was reoxidised with hot alkaline $KMnO_4$ aq. in the hope of converting any methylbenzenetetracarboxylic ester present into potassium benzenepentacarboxylate, but no evidence of the presence of the former acid was obtained.

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