36. *Studies in the Sterol Group. Part XI. The Formation of Ethers of Ergosterol and its Derivatives.*

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Although many esters of ergosterol have been prepared (compare Reindel, Walter, and Rauch, *Annalen*, 1927, **452**, 34; Windaus and Rygh, *Nach. Ges. Wiss. Göttingen*, 1928, 202; Bills and Honeywell, *J. Biol. Chem.*, 1928, **80**, 15), its ether oxides have not hitherto been described. In the case of cholesterol Diels and Blumberg (*Ber.*, 1911, **44**, 2847) were able to prepare various ethers by the action of magnesium alkoxides on cholesteryl chloride, but this method cannot be applied to ergosterol owing to inability to isolate the corresponding ergosteryl chloride. Other methods had consequently to be sought. Attempts to prepare *methoxyergostatriene* by the usual methylation process with methyl sulphate and alkali proved unsuccessful. With a large excess of methyl iodide and with silver oxide prepared by the aid of baryta and well washed, partial methylation was effected after 70 hours' heating under reflux. The process was improved to give high yields of methoxyergostatriene by the use of " activated " silver oxide (compare Haworth, Hirst, and Miller, J., 1929, 2469) together with a small quantity of added sodium hydroxide. Employing this procedure, we have also succeeded in obtaining *ethoxyergostatriene*, *methoxy-α-ergostadiene* from α-dihydroergosterol, and *methoxy-α-ergostene* from α-ergostenol.

A second method giving satisfactory results was an adaptation of that used by Obermüller, who obtained benzyloxycholestene (cholesteryl benzyl ether) by the action of benzyl chloride upon sodium cholesteryloxide (*Z. physiol. Chem.*, 1891, **15**, 44). By acting upon potassium ergosteryloxide, which was prepared by a method similar to that used by Bose and Doran (J., 1929, 2244) for the preparation of potassium cholesteryloxide, with methyl iodide, methoxyergostatriene, identical in properties with the compound prepared by direct alkylation, was readily obtained. The latter method has the advantage of being much quicker than the silver oxide one, but

the yields are somewhat lower. The interaction of hot benzyl chloride with potassium ergosteryloxide leads smoothly to the formation of *benzyloxyergostatriene*, m. p. 134—135°.

Methoxyergostatriene.—The pure ether, m. p. 151—152°, gives the same colour reactions with antimony trichloride and also with the Rosenheim and Tortelli–Jaffé reagents as ergosterol itself. On treatment with dry hydrogen chloride an isomeric methoxyergostatriene, m. p. 116°, is produced which, unlike the *iso*ergosterol prepared in this manner from ergosterol (Reindel, Walter, and Rauch, *loc. cit.*), appears from its preliminary examination to be a single substance (compare Heilbron and Spring, J., 1929, 2807; Windaus, Dithmar, Murke, and Suckfüll, *Annalen*, 1931, **488**, 91). On treatment with mercuric acetate, methoxyergostatriene is smoothly dehydrogenated to *methoxydehydroergostatriene*, m. p. 106°, $[\alpha]_D^{20°}$ + 166°, a reaction which proves that in the conversion of ergosterol into dehydroergosterol (Windaus and Linsert, *Annalen*, 1928, **465**, 148) the >CH·OH group cannot be intermediately involved.

The absorption spectrum of methoxyergostatriene is practically indistinguishable from that of pure ergosterol, maxima at 293, 281·5, 271 and 262 mμ being clearly observed. Irradiation of the material in alcohol has been carried out under the same conditions as those employed by Morton, Heilbron, and Kamm (J., 1927, 2001) in the case of ergosterol, whereby the same changes in absorption occur. After 5 hours a clear maximum at about 247 mμ is observed, while the much over-irradiated solution shows only feeble general absorption.

In view of these results it seemed a matter of importance to ascertain whether methoxyergostatriene acquired antirachitic properties on irradiation. Samples of the pure material were submitted for physiological test to both Professor J. C. Drummond and Mr. A. L. Bacharach, to whom we desire to express our thanks. Comparison of the degree of activation was made against ergosterol irradiated under exactly the same conditions. The results obtained by Professor Drummond show that with 0·1γ of the irradiated ether no antirachitic potency is developed, whereas irradiated ergosterol in this dose gives almost complete cure. At higher dosage (5γ) Professor Drummond, using the *X*-ray test, reports that there is some evidence of partial cure, a result possibly attributable to slight breaking down of the ether during irradiation with loss of methoxyl. Mr. Bacharach, employing the methyl, ethyl, and benzyl ethers and using both the line-test and also the method of bone analysis, reports that at all the doses tested (0·05γ, 0·1γ, and 0·15γ) the ethers have developed little, if any, antirachitic activity on irradiation. It is thus clear that, compared with the antirachitic potency of ergo-

sterol, that of the methoxy-derivative is negligible. These results are in agreement with the observations of Windaus and Rygh (*loc. cit.*), who have shown that the irradiated esters of ergosterol are inactive at normal dosage. As, however, the sterol obtained on hydrolysis of the irradiated esters was found to be highly active, these authors conclude that the secondary alcohol group must be present unimpaired in vitamin-*D* itself, a result also borne out by the present investigations.

Methylation of α-ergostenol with activated silver oxide and methyl iodide gives *methoxy-α-ergostene*, m. p. 56°, which with dry hydrogen chloride isomerises to *methoxy-β-ergostene*, m. p. 100°.

Work is at present being undertaken on the oxidation of these ethers in the hope of elucidating the position of the ethenoid linkages.

Experimental.

Methoxyergostatriene.—Silver oxide method. A solution of ergosterol (20 g.) in methyl iodide (150 c.c.) was heated under reflux with silver oxide (prepared with sodium hydroxide, 60 g.) together with added powdered sodium hydroxide (0·2 g.) for 70 hours. The excess of methyl iodide was removed, and the residue repeatedly extracted with chloroform. After concentration of the chloroform solution to 25—30 c.c. methoxyergostatriene was precipitated by addition of methyl alcohol and purified by recrystallisation from ethyl acetate–alcohol, from which it separated in large laminæ, m. p. 151—152°, $[\alpha]_D^{22°} - 114°$ ($c = 2·4$ in chloroform). It is insoluble in methyl alcohol, sparingly soluble in cold ethyl alcohol and acetone, and readily soluble in chloroform and hot benzene.

Potassium salt method. A solution of ergosterol (5 g.) in benzene (35 c.c.) was added to potassium (1·2 g.) emulsified in benzene (30 c.c.), and the mixture continuously shaken in the warm for 2 hours to ensure complete formation of the potassium ergosteryloxide. Methyl iodide (20 c.c.) was then added and the brown semi-solid mass was gently refluxed for 3 hours, during which period the dark colour gradually disappeared with formation of a precipitate of potassium iodide. The cold mixture was diluted with ether and shaken with water, and the separated benzene–ether layer dried and concentrated; crude *methoxyergostatriene* then separated (m. p. 147—148°) and was purified as described above [Found (micro): C, 84·8; H, 11·2; OMe, 7·8. $C_{28}H_{44}O$ requires C, 84·8; H, 11·1; OMe, 7·8%].

Benzyloxyergostatriene.—Potassium ergosteryloxide (5 g. of ergosterol), prepared as described above, was refluxed on the water-bath for 7 hours with benzyl chloride (30 c.c.). The mixture was diluted

with its own volume of ether, washed with water, concentrated to small bulk, and mixed with 2—3 volumes of alcohol. After standing over-night at 0°, the separated crude material (m. p. 126—129°) was crystallised first from ethyl acetate and then from acetone. *Benzyloxyergostatriene* forms fine silky needles, m. p. 134—135°, $[\alpha]_D^{19°}$ — 61° (c = 1·59 in chloroform), and is readily soluble in chloroform, moderately so in alcohol, acetone, and ethyl acetate. Its colour reaction with antimony trichloride is similar to that of the free sterol, as is also its absorption spectrum [Found (micro): C, 86·3; H, 10·6. $C_{34}H_{48}O$ requires C, 86·4; H, 10·2%].

Ethoxyergostatriene.—Ergosterol (7 g.) was dissolved in ethyl iodide (90 c.c.) and refluxed for 70 hours with silver oxide (26 g.) and powdered sodium hydroxide (0·2 g.). The reaction mixture was then worked up as described in the case of the methyl ether. Pure *ethoxyergostatriene* crystallises from acetone in microscopic needles, m. p. 123—124°, $[\alpha]_D^{21°}$ — 111° (c = 1·43 in chloroform), which are rather more soluble in organic solvents than the methoxy-derivative. With antimony trichloride, it gives the same colour reaction as ergosterol itself [Found (micro): C, 84·9; H, 11·4 OEt, 11·1. $C_{29}H_{46}O$ requires C, 84·9; H, 11·2; OEt, 11·0%].

Methoxydehydroergostatriene.—Methoxyergostatriene (5 g.), dissolved in hot absolute alcohol (600 c.c.), was heated for 3 hours under reflux with a solution of mercuric acetate (12 g.) in methyl alcohol (75 c.c.) and glacial acetic acid (10 c.c.). The yellow solution was filtered to remove precipitated material, and the filtrate concentrated under reduced pressure; the dehydro-ether then separated. After repeated crystallisation first from ethyl acetate–methyl alcohol and finally from benzene–alcohol *methoxydehydroergostatriene* was obtained in thick needles, m. p. 106°, $[\alpha]_D^{20°}$ + 166° (c = 1·57 in chloroform), which acquire only a slight yellow colour after long standing with antimony trichloride [Found (micro): C, 85·2; H, 10·6; OMe, 7·5. $C_{28}H_{42}O$ requires C, 85·3; H, 10·7; OMe, 7·9%].

*Methoxy*isoergostatriene.—A solution of methoxyergostatriene (3 g.) in dry chloroform (60 c.c.) was treated with a rapid stream of dry hydrogen chloride for 1 hour, the temperature being maintained throughout at 0°. The solution was concentrated to 15 c.c. and mixed with an equal volume of methyl alcohol: the solid material then deposited was repeatedly crystallised from acetone until the melting point remained constant. *Methoxy*isoergostatriene separates from acetone in plates, m. p. 116°, $[\alpha]_D^{20°}$ — 66° (c = 1·95 in chloroform), and shows an absorption band with its head at 247mμ. Preliminary attempts to separate this compound into isomerides by the method of fractional crystallisation from benzene–alcohol at 0°

as carried out by Heilbron and Spring (*loc. cit.*) with crude *iso*ergosterol failed to bring about any separation [Found (micro) : C, 84·7; H, 11·2; OMe, 7·9. $C_{28}H_{44}O$ requires C, 84·8; H, 11·1; OMe, 7·8%].

Methoxy-α-ergostadiene.—α-Dihydroergosterol (3 g.) was methylated in the usual manner with methyl iodide (40 c.c.), silver oxide (12 g.), and powdered sodium hydroxide (0·1 g.). After removal of the excess of methyl iodide from the filtered solution the crude ether was purified by recrystallisation first from ethyl acetate–methyl alcohol and finally from acetone. *Methoxy-α-ergostadiene* forms large laminæ, m. p. 148°, $[\alpha]_D^{22°}$ − 22·8° (c = 2·59 in chloroform), readily soluble in chloroform but only very sparingly soluble in cold methyl and ethyl alcohols, ethyl acetate, and acetone [Found (micro) : C, 84·3; H, 11·8; OMe, 7·8. $C_{28}H_{46}O$ requires C, 84·4; H, 11·6; OMe, 7·8%].

Methoxy-α-ergostene.—α-Ergostenol was methylated by the activated silver oxide method. The residue remaining after removal of the methyl iodide by distillation was thoroughly extracted with ether, and the extract, after concentration to small bulk, was mixed with an equal volume of industrial alcohol; an oil then separated which slowly crystallised in the ice-chest. *Methoxy-α-ergostene* crystallises from ether–methyl alcohol in colourless plates with a pearly lustre, m. p. 56°, $[\alpha]_D^{20°}$ + 6·3° (c = 4·75 in chloroform). It is sparingly soluble in methyl alcohol and cold ethyl alcohol, readily soluble in other organic solvents, and clearly shows the modified Tortelli–Jaffé reaction (Heilbron and Spring, *Biochem. J.*, 1930, **24**, 133) [Found (micro) : C, 84·1; H, 11·8; OMe, 7·6. $C_{28}H_{48}O$ requires C, 84·0; H, 12·0; OMe, 7·8%].

When a rapid stream of dry hydrogen chloride was passed into an ice-cold chloroform solution of methoxy-α-ergostene, partial isomerisation occurred. After removal of the solvent the residual oil was mixed with ethyl alcohol and after standing over-night at 0° the solid (m. p. 74—79°) which had separated was repeatedly crystallised from ether–methyl alcohol, yielding a product melting sharply at 100°, $[\alpha]_D^{22°}$ + 15·3° (c = 2·74 in chloroform). Despite the fact that further crystallisation failed to raise the melting point, the substance is in all probability still contaminated with a small amount of the original α-ether, since it gives the modified Tortelli–Jaffé reaction in very concentrated solution. The isomerising reaction whereby methoxy-β-ergostene is formed only proceeds to the extent of about 50%, unchanged α-isomeride always being recovered in the filtrates. An increase in the time of treatment with hydrogen chloride failed to increase the extent of isomerisation. *Methoxy-β-ergostene* crystallises from alcohol in plates and from ether–methyl alcohol in trans-

lucent needles [Found (micro): C, 84·1; H, 12·2; OMe, 7·6. $C_{28}H_{48}O$ requires C, 84·0; H, 12·0; OMe, 7·8%].

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