CCXCI.—*Studies in the Sterol Group. Part VII. Preliminary Note on the Isolation of Zymosterol.*

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The discovery of a dextrorotatory sterol, to which the name zymosterol has been given, in yeast is due to Smedley-Maclean (*Biochem. J.*, 1928, **22**, 22), who concluded from the results of analysis and the iodine values that the sterol has the formula $C_{27}H_{42}O|^{=}_{3}$ and is thus an isomeride of ergosterol.

More recently (*Chem. and Ind.*, 1929, **48**, 295) the same author has announced that at least two dextrorotatory sterols, (*a*) zymosterol, m. p. 110—116°, and (*b*) a sterol melting at 120—124°, are present in yeast.

The yeast sterols have also been investigated by Wieland and Asano (*Annalen*, 1929, **473**, 300), who, by fractional crystallisation of the mixed benzoates, have succeeded in isolating, in addition to ergosterol, the following four individuals : (*a*) Neosterol, $C_{27}H_{44}O|^{=}_{2}$, m. p. 164—165°, $[\alpha]_D$ −105°; (*b*) faecosterol, $C_{27}H_{46}O|^{=}$, m. p. 161—163°, $[\alpha]_D$ +42·1°; (*c*) ascosterol, $C_{27}H_{46}O|^{=}$, m. p. 141—142°, $[\alpha]_D$ +45°; and (*d*) zymosterol, $C_{27}H_{44}O|^{=}_{2}$, m. p. 108—110°, $[\alpha]_D$ +47·3°.

According to these authors, zymosterol contains, contrary to Smedley-Maclean's contention, two more hydrogen atoms than ergosterol.

We also have been engaged in this laboratory with the examination of the yeast sterols, a detailed account of which will be given later. In the meantime we desire to record the isolation of a sterol agreeing closely in properties with the zymosterol of Wieland and Asano (*loc. cit.*). Our attack on the problem has been through the bromine addition compounds, and we have found that, if the crude sterol mixture, after removal of the greater part of the ergosterol, is

treated in ethereal solution with bromine in glacial acetic acid, insoluble bromides are precipitated which can be separated by fractional crystallisation. Of these the least soluble, m. p. 168°, yields on debromination a sterol, m. p. 108—110°, giving an acetate, m. p. 106—107°; these values are identical with those recorded by Wieland and Asano (*loc. cit.*) for zymosterol. In agreement with these authors we find that the sterol itself crystallises from methyl alcohol in a hydrated form; we have consequently analysed the anhydrous acetate and obtained figures in excellent agreement with the formula $C_{27}H_{43}\cdot OH|\frac{=}{2}$.

On hydrogenation of the sterol in ethereal solution absorption of hydrogen ceases after one ethenoid linkage is saturated, *dihydrozymosterol*, m. p. 115—116°, being formed which, as shown by the Liebermann–Burchard reaction, is still unsaturated. The hydrogenation of zymosterol is thus similar to that of α-dihydroergosterol (preceding paper), which, despite the presence of two ethenoid linkages in the molecule, takes up only two atoms of hydrogen in presence of palladium at room temperature.

Experimental.

The crude sterol mixture employed in these experiments contained the yeast sterols left after separation of ergosterol; it was obtained from Boots Pure Drug Co., Ltd., to whom we desire to express our thanks.* 5 G. were dissolved in dry ether (100 c.c.) and to the ice-cold solution a solution of bromine in glacial acetic acid (30 c.c. of 10%) was added. After 10 minutes the precipitate was collected and quickly washed with ether–acetic acid. The crude bromide (1·5 g.) was repeatedly crystallised from chloroform–ethyl alcohol (1 : 3), from which the pure compound separated in colourless crystals, m. p. 168°.

Isolation of Zymosterol.—A solution of the bromide (1·5 g.) in hot absolute alcohol (150 c.c.) was treated with zinc dust (2 g., carefully freed from oxide), and the whole boiled for 10 minutes. After removal of inorganic material, the filtrate was diluted with water until faintly cloudy; the sterol, which then separated in quantitative yield, was obtained after three crystallisations from methyl alcohol, in flaky plates, m. p. 108—110°, $[\alpha]_{5461}^{22°}$ +38·6° (c = 0·8 in chloroform), a value somewhat lower than that given by Wieland and Asano (*loc. cit.*).

The acetate, which was prepared by boiling the sterol with acetic anhydride for 15 minutes, separated after repeated crystallisation

* We are also examining a sterol mixture, kindly given us by Messrs. Böehringer und Söhne, which appears to react similarly. This is probably the same as that employed by Wieland and Asano (*loc. cit.*).

from ethyl alcohol in transparent leaflets, m. p. 106—107° (Wieland and Asano give m. p. 104—106°) (Found: C, 81·7, 81·6; H, 11·1, 11·2; *M*, 399. Calc. for $C_{29}H_{46}O_2|\overline{=}_2$: C, 81·6; H, 10·9%; *M*, 426. Calc. for $C_{29}H_{44}O_2|\overline{=}_3$: C, 82·0; H, 10·45%; *M*, 424).

Both zymosterol and its acetate give a green coloration with bromine (Tortelli–Jaffe reaction, *Chem.-Ztg.*, 1915, **39**, 14).

Dihydrozymosterol.—Zymosterol was hydrogenated in ethereal solution in the presence of twice its weight of palladium until all absorption of hydrogen had ceased. The ethereal solution was filtered, concentrated to small bulk, and, after the addition of methyl alcohol, left to crystallise; pure *dihydrozymosterol* then separated in long white plates, m. p. 115—116°; $[\alpha]_{5461}^{22°}$ + 28·9° (*c* = 1·8 in chloroform). The sterol gives negative reactions both with antimony trichloride and with the Rosenheim reagent, but a green colour is produced with the Tortelli–Jaffe reagent and a deep purple in the Liebermann–Burchard reaction (Found: H_2O, 4·4. $C_{27}H_{46}O,H_2O$ requires H_2O, 4·5%. The water-free sterol gives C, 84·0, 83·9; H, 12·2, 12·1. $C_{27}H_{46}O|^{=}$ requires C, 83·9; H, 11·9%. $C_{27}H_{44}O|\overline{=}_2$ requires C, 84·3; H, 11·5%).

The *acetate* separates from ether–methyl alcohol in glistening leaflets, m. p. 83—84° (Found: C, 81·5, 81·4; H, 11·5, 11·5. $C_{29}H_{48}O_2$ requires C, 81·3; H, 11·2%).

In conclusion we desire to thank Mr. F. S. Spring for his help with this investigation.

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