THE ISOLATION OF ERGOSTEROL FROM *STACHYBOTRYS ATRA* CORDA*

By R. THOMAS†

During an investigation of the cellulose metabolism of the mould *Stachybotrys atra* Corda, a colourless crystalline solid with properties closely resembling those of ergosterol was isolated in low yield from the mycelium of a cellulose culture. A further quantity of the same product was isolated from a sample of mycelium grown on starch medium. Repeated crystallization yielded a pure product, the melting point of which was not depressed on admixture with an authentic sample of ergosterol. This identity was confirmed by comparing their specific optical rotations and infra-red spectra.

No metabolic product of the genus *Stachybotrys* has been previously described; however, the demonstration of ergosterol as a mycelial constituent

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† Biochemistry Unit, Wool Textile Research Laboratory, C.S.I.R.O., Melbourne.

of *S. atra* is in accordance with its previous isolation from many yeasts and lower fungi (Bills and Honeywell 1928; Birkinshaw, Callow, and Fischmann 1931; Ottke 1949).

Experimental

The melting points are uncorrected.

The mould was grown at 28 °C in submerged culture on a modified Waksman-Carey medium (Jermyn 1953); biotin was an essential growth factor.

After 12 days' growth on a 1% cellulose medium, the cellulose-mycelium residue (*c.* 20% mycelium) was washed, dried in a forced draught oven at 50 °C, and 28 g of the finely ground mixture extracted with light petroleum (b.p. 40–60 °C) in a Soxhlet apparatus for 24 hr. It was then further extracted with ether for 20 hr. The light petroleum extract yielded an orange waxy product (0·37 g) containing a crystalline residue. The crystals were separated by washing with light petroleum; the residue consisted of colourless needles (0·040 g), m.p. 135–148 °C, which were insoluble in hot 2N sodium hydroxide, but soluble in hot benzene, chloroform, and ethanol. In concentrated sulphuric acid they dissolved forming a bright orange solution. The ether Soxhlet extract on concentration yielded a pale yellow gum (0·68 g) which could not be induced to crystallize.

The mycelium from a 5 day culture on 1% starch medium was washed, dried, and extracted with light petroleum and ether as above. The light petroleum extract yielded an oil (0·70 g from 40 g of mycelium) which partially crystallized on trituration with light petroleum, yielding colourless needles (0·110 g), m.p. 150–153 °C with properties closely parallel to those of the corresponding product from the cellulose-mycelium mixture. The ether extract consisted of a yellow oil (0·41 g) containing some crystalline material. The crystals were isolated and proved to be identical with those of the light petroleum extract (0·045 g). The combined yield was 0·155 g from 40 g of mycelium, that is, 0·4%. Purification was effected by repeated crystallization from ethanol-benzene mixture (2 : 1); the final product consisted of colourless plates (0·012 g), m.p. 158–159 °C; $[\alpha]_D^{20}$ —129° (*c*, 2·387 in $CHCl_3$).

An authentic sample of ergosterol recrystallized in an identical manner; $[\alpha]_D^{20}$ —128° (*c*, 2·010 in $CHCl_3$) and m.p. 158 °C not depressed on admixture with the mycelium product. Both substances gave identical colour sequences in chloroform on addition of glacial acetic acid and concentrated sulphuric acid (Liebermann-Burchard reaction). When examined in a Perkin-Elmer spectrometer (model 12C) as a "Nujol"-mull, the two specimens possessed identical infra-red spectra over the range 650 to 1350 cm^{-1}.

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