The extract was concentrated and gave a ppt. of the dimer (360 mg). The supernatant was subjected to CC over Si gel and on elution with petrol–EtOAc (49:1) gave **1** (100 mg; found: M^+ 290. 2595; $C_{20}H_{34}O$ requires 290.2610) followed by more dimer (200 mg). Further elution with petrol–EtOAc (9:1) gave **3** (20 mg; found: M^+ 316.2029; $C_{20}H_{28}O_3$ requires 316.2038) followed by **4** (17 mg; found: M^+ 360.2326; $C_{22}H_{32}O_4$ requires 360.2300). Finally elution with petrol–EtOAc (4:1) gave a mixture which on prep. TLC (Si gel: solvent, toluene–EtOAc–HOAc 40:9:1) yielded **5** (R_f = 0.23, 5 mg; found: M^+ 320.2359; $C_{20}H_{30}O_0$ requires 320.2351).

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ERGOSTEROL, THE UNUSUAL DOMINANT STEROL OF THE PYTHIACEOUS FUNGUS *ZOOPHAGUS INSIDIANS*

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Key Word Index—*Zoophagus insidians*; Pythiaceae; fungi; ergosterol; sterols.

Abstract—The pythiaceous fungus, *Zoophagus insidians*, synthesizes ergosterol as its dominant sterol. This observation is unusual because the Pythiaceae were previously throught to be incapable of sterol synthesis and the pantonemic fungi in general to not produce ergosterol.

INTRODUCTION

Fungi may be described as either pantonemic or non-pantonemic. The former group consists of the Oomycetes and Hyphochytriomycetes, which are considered by most mycologists to have a phylogenetic ancestry different from all other fungi[1]. Members of these fungal classes usually possess cholesterol and the 24-alkylidene cholesterols as dominant sterols[2–6] although a few may possess 24-alkyl sterols[7]. In contrast, the non-pantonemic fungi generally possess ergosterol and its precursors as their major sterols[2–4, 8, 9], although some may possess sterols of the stigmastane[10, 11] or cholestane[7, 12] series. Some of the Oomycetes, such as *Lagenidium giganteum* and the pythiaceous fungi *Pythium* and *Phytophthora*, are unable to synthesize sterols and must depend upon an exogenous source to fulfill metabolic requirements[4, 6, 13].

The pantonemic fungi are considered by some to have arisen from pantonemic algal ancestors as they

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possess many morphological and metabolic features normally present in plants[1, 14]. These observations have prompted us to undertake a comparative study to ascertain if the bifurcation of the cyclization of 2,3-oxidosqualene to lanosterol or cycloartenol[15] may be a further metabolic similarity[6]. When the Oomycete *Zoophagus insidians* Sommerstorff, a highly specialized parasite of certain rotifers, was examined it was observed to possess an atypical sterol composition, which is described in this report.

RESULTS AND DISCUSSION

The major sterol comprised 96% of the total neutral sterols at an absolute concentration of 3 μg/mg dry wt. This sterol was analysed by GC, UV and GC/EIMS and identified as ergosterol (5,7,22 - ergostatrienol). This is the first report of this compound in a pantonemic fungus and the first reported *de novo* synthesis of sterols by a member of the Pythiaceae. This finding lends support to the suggestion that *Zoophagus* spp. may be more closely allied with another order of the Oomycetes[16] which is capable of sterol synthesis, although none of these organisms has been reported to synthesize ergosterol[2].

Five minor sterols comprised *ca* 0.8% each of the remaining neutral sterols. These sterols were analysed by GC, UV, and GC/EIMS and four were identified as 7,14,22-ergostatrienol, 7,24(28) - ergostadienol, 4α - methyl - 8(19),14 - ergostadienol, and lanosterol. All but lanosterol have not been observed in other pantonemic fungi [2,3,5,7; Warner, S.A. and Domnas, A.J., unpublished results]. The fifth sterol, which could not be identified, exhibited GC relative retention times (to cholesterol) of 1.97 (SE-30), 1.77 (QF-1), 1.87 (Hi-Eff 8BP) and 2.70 (PMPE) and GC/EIMS *m/z* (rel. int.): 426 $[M]^+$ (82), 411 $[M-Me]^+$ (18), 408 $[M-H_2O]^+$ (3), 393 $[M-H_2O-Me]^+$ (7), 299 [M − acyl chain $-H_2O]^+$ (9), 285 (2), 283 (3), 281 (3), 273 (4), 272 (4), 259 (5), 257 (3), 255 (8).

EXPERIMENTAL

Zoophagus insidians, isolate 65-5, was obtained from the culture collection of the University of California, Berkeley, courtesy of Dr. R. Berman. Cultures for sterol analysis were grown as described previously[17] except that 1 cm^2 pieces of agar stock culture were used for inocula.

Vegetative mycelia were harvested after 14 days of growth and the unsaponifiable lipids obtained as described previously[6]. The sterols were fractionated by HPLC with a 10 μm C_{18} column (4.6 × 250 mm) using a Waters instrument (eluent, MeOH–H_2O, 96:4, 2 ml/min[18], detection at 210 nm).

Sterols were identified by GC with an FID instrument. Columns employed were 3% SE-30, N_2 flow rate, 20 ml/min; 1% QF-1, N_2 flow rate, 20 ml/min; 3% Hi-Eff 8BP, N_2 flow rate, 30 ml/min; and 2% PMPE, N_2 flow rate, 30 ml/min[19]. All columns were 2 m × 2 mm glass. Oven temp. was 240° with detector and injector temps. of 260°.

UV and GC/EIMS (70 eV) were performed and the spectra compared to those of authentic standards wherever possible. A 15 m flexible fused Si capillary column coated with OV-101 and programmed from 160 to 260° at 10°/min with an initial 3 min hold was used for GC/EIMS.

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