case of callus tissues and regenerated shoots and roots for each phase of growth) was hydrolysed with 2 5 l of HCl (5%) under reflux for 6 hr, the volume of the mixture was maintained constant by adding H_2O from time to time After cooling, the mixture was filtered and the residue washed thoroughly with H_2O (to free from acid) and then dried at 60° The dried mass was then extracted in a Soxhlet apparatus with *n*-hexane for 10 hr, after which the extract was distilled (to remove solvent) on a waterbath and then chromatographed over aluminium oxide (neutral grade I, 20 g of Al_2O_3 per g extract) The fractions were monitored on TLC for diosgenin A fraction of *n*-hexane–benzene (1 1) eluates showed the presence of only diosgenin (compared by co-TLC with reference diosgenin) The residue from these eluates were combined and crystallized from Me_2CO Quantitation of diosgenin from callus and regenerated shoots and roots in culture was performed by IR and HPLC analysis [8]

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STEROLS FROM *CANDIDA LIPOLYTICA* YEAST GROWN ON *n*-ALKANES

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Key Word Index—*Candida lipolytica*, Ascomycetes, fungi, sterols

Abstract—The sterols of *Candida lipolytica* grown on *n*-alkanes were isolated by reverse phase HPLC and found to be mainly ergosterol, with small quantities of ergost-7-en-3β-ol, ergosta-7,22-dien-3β-ol, ergosta-7,24(28)-dien-3β-ol and ergosta-5,7,9(11),22-tetraen-3β-ol

Recently much attention has been given to the yeasts *Candida tropicalis* and *Candida lipolytica*, which, being capable of utilizing aliphatic hydrocarbons as the sole carbon source, attracted commercial interest for production of microbial proteins which may be utilized as components in animal feeds [1] In continuing our work on sterols from fungi [2, 3], we examined the sterol composition of *C lipolytica* grown on *n*-alkanes

The residue from the chloroform–methanol extract of *C lipolytica* upon saponification followed by column chromatography, gave the 4-demethylsterol mixture, which was acetylated The preliminary GC of the steryl acetates showed four small peaks beside the major peak identical with that of standard ergosteryl acetate The sterol acetate mixture, subjected to reverse phase HPLC, yielded ergosta-5,7,9(11),22-tetraen-3β-yl acetate, ergosteryl acetate, ergosta-7,24(28)-dien-3β-yl acetate, ergosta-7,22-dien-3β-yl acetate and ergost-7-en-3β-yl acetate The sterols were identified on the basis of their mass, UV and ^{1}H NMR spectra The percent composition of the sterols in *C lipolytica* and the chromatographic mobility data are summarized in Table 1 A previous investigation of the sterol mixture of *C lipolytica* grown on *n*-alkanes revealed the presence of ergosterol [4]

EXPERIMENTAL

C lipolytica was grown on *n*-alkanes by the industrial process of Italproteine HPLC was on a Waters instrument equipped with a differential refractometer and Whatman Partisil 5/25 ODS-3 column (4 6 mm × 25 cm), ^{1}H NMR, 270 MHz, $CDCl_3$, TMS as internal standard, UV, MeOH, GC, DB-1 fused silica capillary column (30 m × 0 25 mm) at 265°, MS, 70 eV

Extraction and separation of sterols C lipolytica (58 g) was extracted × 3 at room temp with $CHCl_3$–MeOH (1 1) The solvents were evapd to give a viscous oil (8 4 g), which was

Table 1 Sterol composition of *C lipolytica*

Compound	Composition (%)	GC RR_t*	HPLC RR_t*
Ergosta-5,7,9(11),22-tetraen-3β-ol	3	1 12	0 61
Ergosterol	81	1 20	0 82
Ergosta-7,24(28)-dien-3β-ol	6	1 41	0 87
Ergosta-7,22-dien-3β-ol	5	1 23	0 95
Ergost-7-en-3β-ol	5	1 45	1 13

*Retention time of acetate derivatives relative to cholesteryl acetate used as the standard (1 00) for both GC RR_t (on a DB-1 capillary column, 265°) and HPLC RR_t (on a Partisil 5/25 ODS-3 column and methanol as eluent)

saponified in 5% methanolic KOH under reflux for 1 hr The unsaponifiable lipid fraction (540 mg) was chromatographed on a silica gel column, eluted with CH_2Cl_2 The 4-demethylsterol fraction (194 mg) was acetylated (Ac_2O–pyridine, 16 hr at room temp) and the steryl acetates were purified on a silica gel column eluted with petrol–Et_2O (95 5) The five steryl acetates were separated by reverse phase HPLC with a Partisil 5/25 ODS-3 column and absolute MeOH as the mobile phase

(22E)-*Ergosta*-5,7,9(11),22-*tetraen*-3β-*yl acetate* MS *m/z* (rel int) 436 $[M]^+$ (12), 421 (2), 376 $[M-HOAc]^+$ (100), 361 (12), 333 $[M-HOAc-43]^+$ (4), 291 (5), 277 (4), 263 (3), 251 [M − HOAc and side chain]$^+$ (62), 249 (13), 237 (10), 235 (15), 224 (11), 209 [M − HOAc and ring D fission]$^+$ (31), 207 (14), ^{1}H NMR ($CDCl_3$) δ0 574 (3H, *s*, H-18), 0 820 (3H, *d*, *J* = 6 5 Hz, H-27), 0 835 (3H, *d*, *J* = 6 7 Hz, H-26), 0 915 (3H, *d*, *J* = 6 8 Hz, H-28), 1 016 (3H, *d*, *J* = 6 5 Hz, H-21), 1 252 (3H, *s*, H-19), 2 031 (3H, *s*, acetate), 4 64 (1H, *m*, H-3), 5 19 (2H, *m*, H-22 and H-23), 5 40 (1H, *m*, H-6), 5 51 (1H, *m*, H-7), 5 70 (1H, *m*, H-11)

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THE MAJOR STEROLS FROM THREE SPECIES OF POLYPORACEAE

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Key Word Index—*Ganoderma applanatum*, *Ganoderma lucidum*, *Polyporus sulfureus*, Polyporaceae, fungi, sterols

Abstract—The free sterols of the fungi *Ganoderma applanatum*, *Ganoderma lucidum* and *Polyporus sulfureus* were isolated and characterized by means of GC and GC/MS techniques 24-Methylcholesta-7,22-dien-3β-ol was the main component of the sterol mixtures while 24-methylcholesta-5,7,22-trien-3β-ol (ergosterol) and 24-methylcholest-7-en-3β-ol were also present although in lower amounts *P sulfureus*, besides the mentioned sterols, also contained 24-ethylcholestan-3β-ol

The fungi *Ganoderma applanatum* (Pers ex Fr), *Ganoderma lucidum* (Lyss ex Fr) Karst and *Polyporus sulfureus* (Bull ex Fr), especially the last one, infect different trees, mainly oaks, rotting their wood and being therefore of economical importance to the wood industry

Continuing with our research on sterols from natural sources [1], the main sterol components of the above mentioned fungi were investigated There are several reports about the chemical composition of these three species [2–4] and 24-methylcholesta-7,22-dien-3β-ol and