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

Table 1 Mass spectra of identified sterols

| Sterol | Identification | Mass spectrum *m/z* (rel int) |
|---|---|---|
| 1 | 24-Methylcholesta-5,7,22-trien-3β-ol (ergosterol) | 396 $[M]^+$ (56), 381 (10), 378 (35), 363 (100), 337 (28), 271 (20), 269 (18), 253 (95) |
| 2 | 24-Methylcholesta-7,22-dien-3β-ol | 398 $[M]^+$ (40), 383 (18), 273 (44), 271 (100) 255 (56), 246 (33), 229 (32), 213 (23) |
| | as TMS derivative | 470 (26), 455 (20), 380 (7), 365 (11), 345 (20), 343 (55), 318 (15), 255 (82), 229 (41), 69 (100) |
| 3 | 24-Methylcholest-7-en-3β-ol | 400 $[M]^+$ (100), 385 (32), 382 (3), 367 (7), 273 (24), 271 (12), 255 (90), 231 (33) |
| | as TMS derivative | 472 (100), 457 (25), 382 (11), 367 (22), 345 (8), 343 (5), 255 (97) |
| 4 | 24-Ethylcholestan-3β-ol | 416 $[M]^+$ (63), 401 (22), 398 (5), 383 (15), 290 (14), 257 (5), 248 (16), 234 (58), 233 (100) |
| | as TMS derivative | 488 (44), 473 (100), 398 (30), 383 (52), 305 (47), 257 (14), 215 (33) |

24-methylcholesta-7,22-dien-3-one have been reported in *G applanatum* [5] In this fungus the presence of 24-methylcholesta-4,6,8(14),22-tetraen-3-one has been reported [6] The sterol components of other mushrooms have also been analysed [7]

The fungi were extracted and the extracts were saponified leading to the isolation of the sterol fraction as described in the Experimental section These fractions were analysed by GC and GC/MS techniques as free sterols and as their trimethylsilyl ether derivatives

The *G lucidum* extract showed two main components (**1** and **2**) and another minor sterol (**3**) while *G applanatum* showed mainly compound **2** and traces of **1** and **3** *P sulfureus* presented three main components (**1–3**) and a minor one (**4**) The mass spectra of compounds **1–4** are shown in Table 1 and were characterized as indicated in Table 1 by comparison with the mass spectra of known standards stored in the data system library [8–11]

Our results (Table 2) are in accordance with those reported by Ripperger and Budzikiewicz [5] for *G applanatum* except for the keto-steroids, but in disagreement with the results of Strigina *et al* [4] who reported the presence of a $\Delta^{16}$ sterol To the best of our knowledge, the sterol composition of the other two fungi has not been previously reported

## EXPERIMENTAL

*General* Preparative TLC was performed on silica gel G of 2 mm in thickness activated at 120° for 1 hr, and eluted with $CHCl_3$–EtOH (95 5) Analytical GC was conducted with a fused silica capillary column (12 m × 0 02 mm) coated with methyl silicone fluid (Hewlett–Packard) The sterol fractions were chromatographed between 200 and 290° at a rate of 10°/min with helium as the carrier gas The identities of the major sterols were assigned by GC/MS using a Varian-MAT CH7-A mass spectrometer coupled to a Varian 1440 gas chromatograph and interfaced to a Varian-MAT Data System 166 computer Trimethylsilyl ether derivatives were prepared using hexamethyldisilazane–trimethylchlorosilane–pyridine (3 3 10)

*Extraction and purification of sterols* Specimens of each fungus, collected near Montevideo, Uruguay, were cut into small pieces and dried in an oven at a temp below 60° The material was kept under EtOH at 18° for 3 days The extracts were saponified by refluxing them with 10% methanolic KOH for 3 hr The unsaponifiable fractions were extracted with $Et_2O$, washed, dried and evaporated The crude sterol mixtures were fractionated by preparative TLC and analysed by GC and GC/MS as free sterols and as their TMS-derivatives

Table 2 Percentages of sterols in the fungi *G applanatum* (*G a*), *G lucidum* (*G l*) and *P sulfureus* (*P s*)

| Sterols | *G a* | *G l* | *P s* |
|---|---|---|---|
| 24-Methylcholesta-5,7,22-trien-3β-ol | 1 1* | 34 2 | 10 5 |
| 24-Methylcholesta-7,22-dien-3β-ol | 96 3 | 52 4 | 64 5 |
| 24-Methylcholest-7-en-3β-ol | 2 5 | 13 3 | 20 1 |
| 24-Ethylcholestan-3β-ol | — | — | 4 6* |

*Estimated by single ion detection method

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