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THE STEROIDS AND FATTY ACIDS OF THE BASIDIOMYCETE *SCLERODERMA POLYRHIZUM*

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Key Word Index—*Scleroderma polyrhizum*; Basidiomycete; sterols; fatty acids; ergosta-4,6,8(14),22-tetraen-3-one; 5α,8α-epidioxyergosta-6,22-dien-3β-ol; palmitic acid; oleic acid.

Abstract—The fruit bodies of the Basidiomycete *Scleroderma polyrhizum* have been shown to contain the steroids ergosta-4,6,8(14),22-tetraen-3-one and 5α,8α-epidoxyergosta-6,22-dien-3β-ol and also palmitic and oleic acids.

INTRODUCTION

The importance of sterol metabolites in chemotaxonomy is becoming increasingly apparent. However, while it is doubtful that sterols will emerge as an important character of distinguishing fungal species and organizing fungal taxa at higher taxonomic levels, they may provide additional characters to aid in the distinction of closely related species or subspecies which cannot be readily delimited on a morphological basis [1].

We report here the isolation and identification of the sterol constituents of the fresh fruit bodies of a basidiomycete mushroom, *Scleroderma polyrhizum*, collected along the borders of the cattle tracks in the north of La Palma (Canary Islands).

RESULTS

The components of the ethanol extract of the fruit bodies were separated by chromatography on Si gel. The less polar fraction represented a steroidal compound, $C_{28}H_{40}O$, mp 115–116° and was identified as ergosta-4,6,8(14),22-tetraen-3-one by analysis of its ^1H NMR spectrum. This product has been obtained from a luminous mushroom *Lampteromyces japonicus* [2] and from a luminous bacteria [3], hence it has been related to the bioluminiscence displayed by these organisms [4]. However, it has also been found in nonluminous fungi, such as *Fomes officinalis* [5], *Ganoderma applanatum* [4] and now by us in *Scleroderma polyrhizum*.

The next fraction was a mixture of palmitic and oleic acids, which were identified by mass spectrometry and GLC of their methyl esters.

A more polar steroid, $C_{28}H_{44}O_3$, mp 178–180°, was shown to be the 5α,8α-epidioxyergosta-6,22-dien-3β-ol (ergosterol peroxide) by analysis of its ^1H NMR spectrum. This compound gave with acetic anhydride and pyridine a monoacetate identical with that described in the literature. Ergosterol peroxide is also frequently obtained from fungi and it has been suggested that it is an artefact [6–8]; it has also been suggested that it is an important metabolite in the biosynthesis of ergosterol [11].

Both steroids isolated have been obtained by us starting from ergosterol. Thus, Oppenauer oxidation with aluminium *tert*-butoxide and *p*-benzoquinone [9] led to ergosta-4,6,8(14),22-tetra-en-3-one, identical in all respects (NMR, IR, TLC) with that obtained directly from the fungus. Ergosterol peroxide was obtained by photosensitized oxygenation with eosin as described in ref. [10].

EXPERIMENTAL

Mps were measured on a Kofler block and all mps are uncorr. Optical rotations were measured in $CHCl_3$, IR spectra in films and $CHCl_3$. High resolution MS were measured at 70 eV and ^1H NMR spectra on a 60 MHz instrument in $CDCl_3$ with TMS as int. ref. GC analyses: 1.5% QF-1, temp. programme: 120–250° at 4°/min, N_2 30 ml/min.

Extraction. Fresh fruit bodies of *S. polyrhizum* Pers. (2.5 kg) collected in January on the Monte de Los Sauces (La Palma) were disintegrated in EtOH and the extract was concd. The concentrate was distributed between H_2O and EtOAc. The organic

layer was dried on Na_2SO_4, evaporated and the residue (4 g) repeatedly chromatographed on Si gel using hexane to hexane–EtOAc (7:3).

Ergosta-4,6,8(14),22-tetraen-3-one. Elution with hexane–EtOAc (95:5) gave a yellow oil. This product showed a green chemiluminescence in the Liebermann–Burchard reaction conditions and with alkaline H_2O_2. This compound was purified by chromatography on Si gel; elution with C_6H_6 gave a crystalline product which was recrystallized from MeOH yielding yellow plates (54 mg), mp 115°, $[\alpha]_D^{20}$ + 570° ($CHCl_3$; *c* 2.7) (lit. [12] mp 114–115°, $[\alpha]_D$ + 590°). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1655, 1640, 1585 (trienone), 975 (Δ^{22}-*trans*) and 880; UV λ_{max}^{EtOH} nm (log ε): 350 (4.5); ^1H NMR: δ0.81, 0.90, 0.97, 1.00, 1.13 (18H, Me-6), 1.40–2.60 (17H, *m*), 5.28 (2H, *m*, C-22 and C-23), 5.78 (1H, *s*, C-4), 6.08 (1H, *d*, *J* = 10 Hz, C-6), 6.66 (1H, *d*, *J* = 10 Hz, C-7); MS *m/z*: 392.3074, $[M]^+$ (calculated for $C_{28}H_{40}O$: 392.3079), 267.1758 $[M - C_9H_{17}]^+$ requires 267.1749).

Fatty acids. Further elution with hexane–EtOAc (95:5) gave a semicrystalline gum (2.92 g). IR ν_{max}^{film} cm^{-1}: 3600–2400, 1710 and 725; MS *m/z*: 282., 256 $[M]^+$ (oleic and palmitic acids). Treatment with CH_2N_2 resulted in an oil shown by GC analysis to be a mixture of methyl oleate (40%) and methyl palmitate (46%).

5α,8α-Epidioxy-ergosta-6,22-dien-3β-ol (ergosterol peroxide). Elution with hexane–EtOAc (85:15) gave a solid (410 mg), mp 178–180° (MeOH), $[\alpha]_D^{20}$ − 25° ($CHCl_3$; *c* 1.24) (lit. [7] mp 180.5°, $[\alpha]_D$ − 29°). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3450, 1603, 1380 and 973 (Δ^{22}-*trans*); ^1H NMR: δ0.79, 0.83, 0.88, 0.97, 1.07 (18H, Me-6), 3.94 (1H, *m*, C-3), 5.24 (2H, *m*, C-22 and C-23), 6.29 (1H, *d*, *J* = 8.7 Hz, C-7), 6.55 (1H, *d*, *J* = 8.7 Hz, C-6); MS *m/z*: 428.3289 $[M]^+$ ($C_{28}H_{44}O_3$ requires 428.3291), 410, 396, 363 and 337. Acetylation of 100 mg (Ac_2O–C_5H_5N, 24 hr, room temp.) yielded 100 mg of a crystalline monoacetate, mp 200–202° (MeOH) (lit. [7] mp 202–205°). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1255 and 975 (Δ^{22}-*trans*). ^1H NMR: δ0.50, 0.77, 0.81, 0.87, 0.95, 1.03 (18H, Me-6), 2.00 (3H, *s*, –OCOMe), 5.22 (2H, *m*, C-22 and C-23), 6.26 (1H, *d*, *J* = 8.7 Hz, C-7), 6.54 (1H, *d*, *J* = 8.7 Hz, C-6). MS *m/z*: 470.3387 $[M]^+$ ($C_{30}H_{46}O_4$ requires 470.3395), 410 $[M - HOAc]^+$, 378 $[M - HOAc - O_2]^+$ and 285 $[M - HOAc - C_9H_{17}]^+$.

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