Pergamon

PII: S0031-9422(97)00249-5

Phytochemistry, Vol. 45, No. 8, pp. 1669–1671, 1997

0031-9422/97 $17.00+0.00

ERGOSTA-4,6,8,22-TETRAEN-3-ONE FROM THE EDIBLE FUNGUS, *PLEUROTUS OSTREATUS* (OYSTER FUNGUS)

VLADIMIR CHOBOT, LUBOMÍR OPLETAL, LUDĚK JAHODÁŘ, ASMITA V. PATEL,* CHRISTOPHER G. DACKE* and GERALD BLUNDEN*†

Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic; * School of Pharmacy and Biomedical Science, University of Portsmouth, St Michael's Building, White Swan Road, Portsmouth, Hampshire PO1 2DT, U.K.

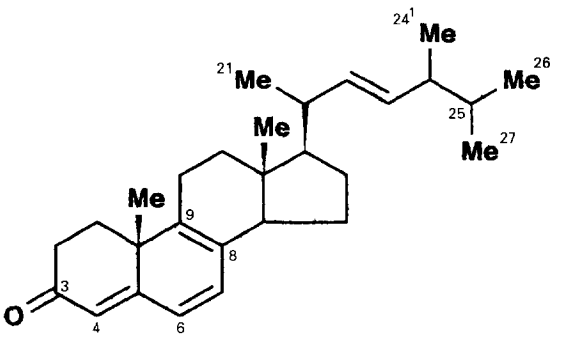
(*Received* 28 *October* 1996)

Key Word Index—*Pleurotus ostreatus*; Basidiomycota; sterols; ergosta-4,6,8,22-tetraen-3-one; ergosterol; fatty acid esters of ergosterol.

Abstract—From extracts of the dried oyster fungus, *Pleurotus ostreatus*, three steroidal fractions were obtained. The major fraction was proved to be ergosterol and the second a mixture of two fatty acid esters of ergosterol, which could not be fully resolved. The minor fraction contained ergosta-4,6,8,22-tetraen-3-one, which has not been recorded previously. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The oyster fungus, *Pleurotus ostreatus* (Jacq. ex Fr.) Kumm., has been used as a food in Europe for many years and is now commonly cultivated [1]. The fungus, when fed to experimental rodents along with a simultaneous supply of dietary cholesterol, led to lower plasma cholesterol levels than those of animals which had not received the fungal supplement [2]. As a result, the constituents of the fungus are being investigated and in this communication we report the isolation and characterization of the sterols, which include ergosta-4,6,8,22-tetraen-3-one which, to our knowledge, is a novel compound.



2

RESULTS AND DISCUSSION

Dry *Pleurotus ostreatus* was extracted with dichloromethane, and the extract examined by TLC. Under UV light, followed by spraying with a solution of vanillin in sulphuric acid, three components were detected which were believed to be sterols. One (**1**) predominated and the other two (**2**, **3**) were minor. The *P. ostreatus* extract was dissolved in hot petrol and on cooling gave a white precipitate (**1**), which was shown by TLC and the NMR and mass spectra to be mainly ergosterol, which has been reported previously for *P. ostreatus* [3]. TLC examination of the remaining petrol solution showed the presence of **2**, which under UV light was detected as a turquoise-blue spot, which became yellow-brown after spraying with a solution of vanillin in sulphuric acid. This compound was isolated as a yellow, non-crystalline material.

The EI mass spectrum of **2** gave a molecular ion $[M]^+$ at *m/z* 392.4321 (calculated for $C_{28}H_{40}O$, 392.3154). In comparison with ergosterol, these data indicated two extra double bonds in **2**. The ^1H NMR spectrum of **2** exhibited the two proton multiplet at δ 5.20 (H-22 and H-23) which was comparable to that in **1**, but the H-6 and H-7 methine signals (δ 5.38, $J_{6,4ax} = 2.8$, $J_{6,4eq} = 2.8$, $J_{6,7} = 5.7$ Hz and δ 5.57, $J_{7,14} = 2.8$, $J_{7,6} = 5.7$ Hz, respectively) observed in the spectrum of **1** were further downfield (δ 6.03, $J_{6,7} = 9.5$ Hz and δ 6.61, $J_{7,6} = 9.5$ Hz) in the spectrum of **2**. In addition, the spectrum of **2** displayed an extra singlet at δ 5.74 (H-4) and the characteristic C3-HOH multiplet of **1** (δ 3.64) was absent.

The assignment of the ^{13}C NMR absorptions (Table 1) confirmed the mass spectral analysis showing that

† Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR spectral details (in $CDCl_3$) of ergosterol (**1**) and ergosta-4,6,8,22-tetraen-3-one (**2**)

Carbon:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	24[1]
1	38.4	32.0	70.4	40.8	141.4	119.6	116.3	139.8	46.3	37.0	21.1	39.1	42.8	54.5	23.0	28.3	55.7	12.1	16.3	40.4	19.7	135.6	132.0	42.8	33.1	21.1	20.0	17.6
2	34.1	34.1	199.5	123.0	164.4	124.5*	134.0*	156.1*	131.1*	36.8	29.7*	35.6	44.0	44.3	25.4	27.7*	55.7	16.7*	19.0*	39.3	19.7	135.0	132.5	42.9	33.1	21.2	20.0	17.6

* Respective figures may be interchanged.

the nine carbon side chain was comparable to that of **1**. The lack of a methine absorption (DEPT spectrum) at about δ 70.4 (C3-OH) and the presence of a quaternary carbon at δ 199.5 was suggestive of a C-3 keto group in conjunction with a double bond [4]. The remaining double bond can then be located between C-8 and C-9 on the basis of the chemical shifts of the carbons of rings A, C and D. Compound **2** was thus ergosta-4,6,8,22-tetraen-3-one, which has not been recorded previously. It was detected by TLC in the crude dichloromethane extract of the fungus and so was considered to be a true constituent of the dry plant material, which is what is used as a food. However, the possibility that **2** is an autoxidation product formed on drying the fungal material cannot be ruled out, although drying was carefully carried out in a desiccator.

The ^1H NMR spectrum of **3** showed that the absorptions for the methine protons bonded to unsaturated carbons (δ 116.3, 120.1, 132.0, 135.6, 138.7, 141.5 Hz) were highly comparable to those of **1**. However, the C-3-HX multiplet absorbed further downfield at δ 4.71 compared to that of **1** (δ 3.64). The ^{13}C NMR spectrum of **3** clearly showed that this was a mixture of two closely related compounds. The chemical shifts for the ergosterol moiety were easily identifiable, with the signal for C-3 at δ 72.5. The additional presence of two quaternary absorptions (DEPT spectrum) at δ 173.5 and 173.4 (approximately in the ratio of 3:1), and a number of overlapping methylene (between δ 20–42) and methyl signals (δ 14.1, 14.1, 17.6, 21.4) were indicative of fatty acids esterified to the C-3 hydroxyl group of ergosterol. Attempts to separate the two compounds by GC/mass spectrometry were unsuccessful. The molecular ions could not be identified in the mass spectrum, but a base peak (100%) at *m/z* 378 was detected, resulting from elimination of the fatty acid chains along with one molecule of water.

EXPERIMENTAL

Plant material. Pleurotus ostreatus (Jacq. ex Fr.) Kumm., obtained from the Czech Collection of Microorganisms, Brno (clone number 3019), was cultured on a lignocellulose substrate and harvested when at full maturity. The fungal material was dried in a desiccator and powdered.

Chromatography. For TLC, Silufol UV_{254} layers (Kavalier, Votice, Czech Republic) were used with $CHCl_3$–EtOH (95:5) as the development solvent, unless otherwise stated. The sterols were detected under UV light (λ 366 nm) as turquoise-blue spots which, after spraying with H_2SO_4-vanillin, became yellowish-brown. For CC, silica gel L. 100–200 μm (Lachema, Brno, Czech Republic), deactivated with 10% H_2O, was used.

Extraction and purification of compounds. Dried, powdered *P. ostreatus* (16.5 kg) was extracted for 4 hr with CH_2Cl_2 in a Soxhlet extractor, under N_2. The

extract was concd to dryness, the residue (308 g) mixed with 300 ml petrol (50–70°) and heated at 40°. On cooling and leaving for several hr, a white ppt was removed by filtration (46 g, **1**). The petrol soln was concd to dryness. The residue, containing **2** and **3** (TLC), was redissolved in 2 l petrol (50–70°) and fractionated by passage through a column of silica gel L (6 × 116 cm) by eluting first with petrol (50–70°) (250 ml frs, total 4500 ml), followed by 95% EtOH (7250 ml). Frs 2–6 of the petrol eluate (containing **3**) were evapd to dryness to give an orange coloured oil. After storage for several days at 4°, a waxy material sepd from the oil. From this wax, **3** was isolated by prep. TLC using Silufol UV_{254} layers, petrol–$CHCl_3$ (3:1) as the development solvent and detection under UV light (λ 366 nm). After elution of the bands from the TLC layers with $CHCl_3$, concn to dryness and crystallization from EtOH, **3** was obtained as a white, waxy material (0.138 g, mp 97–104°). The EtOH eluate (containing **2**) was concd to dryness (156 g), dissolved in 95% EtOH containing 62.5 g KOH (1200 ml) and heated, under N_2, on a H_2O bath for 2 hr at 80°. After evapn to dryness at 30°, the residue was mixed with H_2O (3200 ml) and extd × 3 with 800 ml Et_2O. The combined Et_2O extracts were washed with 0.1 M aq. KOH, dried (Na_2SO_4) and concd to dryness. The residue (15.1 g) was fractionated by CC using silica gel L (470 g, 5 × 84 cm) and elution with $CHCl_3$. Each fr. (200 ml) was tested by TLC: **2** was detected in frs 4 and 5, which were combined (980 mg) and further fractionated by CC using silica gel L (2 × 52 cm; 20 ml frs) and petrol (50–70°) with increasing proportions of CH_2Cl_2. Frs 80–102 (petrol–CH_2Cl_2, 1:1; 816 mg) contained **2**.

Compound **2** was isolated by prep. TLC, first by using Silufol UV_{254} layers and development × 2 with $CHCl_3$. The compound, located under UV light, was scraped from the plates and eluted with $CHCl_3$. Final purification was achieved by prep. TLC using silica gel GF 254 (Merck) layers and development × 2 with $CHCl_3$. After elution of the bands detected under UV light, 18 mg **2** was obtained.

^{1}H NMR spectra were obtained in $CDCl_3$ using a 270.6 MHz machine with TMS as int. standard. ^{13}C NMR spectra were recorded on the same instrument (67.80 MHz).

Ergosta-4,6,8,22-*tetraen*-3-*one* (**2**). $C_{28}H_{40}O$, UV λ_{max}^{EtOH}, (log ε): 349 (4.13). EI MS (probe) 70 eV *m/z* (rel. int.): 392.4321 $[M]^+$ (56.6), 268 $[M-C_9H_{16}]^-$ (100), 253 $[268-Me_3]$ (15.0). ^{1}H NMR ($CDCl_3$) δ ppm: 0.83, 0.85, 0.93, 1.06 (4 × Me, *d*, J = 6.6 Hz; Me-21, -26, -27 and -24'), 0.96, 1.00 (2 × Me, *s*; Me-18 and -19), 5.15–5.31 (2H, *m*; H-22 and H-23); 5.74 (1H, *s*; H-4), 6.03 (1H, *d*, J = 9.5 Hz; H-6 or H-7); 6.61 (1H, *d*, J = 9.5 Hz; H-7 or H-6). ^{13}C NMR (Table 1).

REFERENCES

1. Ginterová, A. and Gallon, J., *Biochemistry Society Transactions*, 1979, **7**, 1293.
2. Bobek, P., Ginter, E., Kuniak, L., Babala, N., Jurčovičová, M., Ozdin, L. and Červen, J., *Nutrition (Burbank Calif)*, 1991, **7**, 105.
3. Yokokawa, H., *Yukagaku*, 1970, **19**, 496; from *Chemical Abstracts*, 1970, **73**, 97597 t.
4. Blunt, J. W. and Stothers, J. B., *Organic Magnetic Resonance*, 1977, **9**, 439.