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SOME LIPIDS OF THE OIDIA OF *PLEUROTUS OSTREATUS*

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Abstract—The oidia of *Pleurotus ostreatus* were shown to contain the terpenoids squalene, ergosterol [free and esterified] and ubiquinone [UQ_7]. The major neutral lipid, which constituted 29% of the dry weight, was found to be triglyceride in nature, the major fatty acid being oleic acid with lesser amounts of palmitic and linoleic.

INTRODUCTION

THE lipid fractions from fungal mycelia have been actively investigated and the results are well documented for nearly all aspects of the broad field; yet the corresponding fractions from fungal spores have received little attention apart from studies on the gross composition. The present investigation was carried out to identify the major neutral lipid constituents of the oidia of the Basidiomycete, *Pleurotus ostreatus*. This organism, from the sub-class Holobasidiomycetes, order Agaricales, family Agaricaceae (the family of the gill fungi), has been reported growing on the limbs or trunks of living or dead trees and is of cosmopolitan distribution. Apart from identifying the major neutral lipid the oidia were also examined for the presence of the terpenoids squalene, ergosterol and ubiquinone normally found in moulds. (The distribution of ubiquinone in the Eumycetes has been reviewed in a previous paper.[1])

RESULTS AND DISCUSSION

Pleurotus ostreatus was cultured on a solid agar medium dispensed as slopes; sufficient oidia for harvesting were obtained after a 3-month incubation period at 30°. The oidia were harvested by adding a small amount of water to the culture, shaking and filtering the suspension through muslin of sufficient porosity to allow the oidia but not the mycelium to pass through. The oidia were then washed free of slime and ingredients of the medium by suspending in cold distilled water and reharvesting by high speed centrifugation. Microscopic examination showed that the preparation was free from hyphae.

Various techniques were used in an attempt to extract the lipid; sonication and alternate freezing and thawing were found to be ineffective as a means of rupturing the spores. The procedure eventually adopted was to grind the oidia with acid-washed alumina (Woelm) and sufficient acetone to form a magma. The acetone-extract was collected by filtration and the residue washed with acetone. The alumina-oidia residue was then ground successively with ethanol and ether. All the extracts and washings were bulked and washed with water to remove the acetone and ethanol; the ethereal layer containing neutral lipids was then dried

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[1] H. M. YUSEF, D. R. THRELFALL and T. W. GOODWIN, *Phytochem.* 4, 551 (1965).

over anhydrous Na_2SO_4 and taken to dryness. 1·05 g. dry weight of oidia yielded 306 mg (29%) of lipid extract.

The lipid extract was chromatographed on a 15 g column of Brockmann grade III acid-washed alumina (Woelm), Table 1. The 2, 5 and 20% ether/light petroleum fractions all exhibited selective absorption in the u.v. region. The most striking feature of the chromatography was the high percentage (89·5%) recovery of lipid off the column; especially its association with the early chromatographic fractions, i.e. 2 and 5% ether light petroleum fractions.

TABLE 1. CHROMATOGRAPHY OF THE LIPID EXTRACT FROM OIDIA OF *Pleurotus ostreatus*

Fraction No.	Ether in light petroleum (%)	Wt. of fraction (mg)	λ_{max} (mμ) and $E^{1\%}_{1cm}$* of fractions†
1	0	2·3	no selective absorption
2	2	150·8	272 (0·68), 283 (0·64), 295 (0·37) shoul. 263 (0·54)
3	5	107·8	275 (0·38)
4	10	4·0	no selective absorption
5	20	11·3	272 (15·5), 283 (15·1), 295 (9·3) should. 263 (12·9)
	Recovered	273·9, 89·5%	

305·8 mg lipid from 1·045 g, dry weight of Oidia. Chromatographed on 15 g Brockmann grade III alumina (Woelm) 5 150 ml fractions of petroleum ether containing increasing amounts of ether collected.

* $E^{1\%}_{1cm}$ in brackets after absorption peak.

† All determined in cyclohexane except for Fraction 3 whose spectrum was determined in ethanol.

The light petroleum fraction was examined for the presence of squalene by thin-layer chromatography (T.L.C., see Experimental to previous paper) and on iodine staining a spot was observed which ran with and was not separated from authentic squalene.

The 2 and 5% ether/light petroleum fractions apart from accounting for the bulk of the recovered lipid, also contained minor amounts of u.v. absorbing material. Thus the 2%, in common with the 20% ether light petroleum fraction, had selective absorption peaks at λ_{max} 272, 283 and 295 mμ which were attributed to the presence of an ester and the free form of ergosterol respectively. The 5% ether/light petroleum fraction had a small peak at 275 mμ (in ethanol) which on treatment with $NaBH_4$ declined and a new peak appeared at 291 mμ; the difference spectrum ($E_{oxidized} - E_{reduced} \nu\lambda m\mu$) was identical to that of ubiquinone. The levels of esterified ergosterol, free ergosterol and ubiquinone in the fractions were estimated as being 0·268 mg, 0·402 mg ,and 0·114 μmole/g dry weight, respectively.

Before the identification of the ergosterol (esterified and free) and ubiquinone was carried further the 2 and 5% ether light petroleum fractions were examined by infra-red spectroscopy and both showed absorption bands typical of a triglyceride, containing oleic acid as the major fatty acid. The 5% fraction was then run quantitatively on thin layers of "Kieselgel G" with benzene-chloroform (1:1 v/v) as developing solvent to resolve the ubiquinone from the triglyceride [UQ, R_f = 0·52, triglyceride R_f = 1].

The triglyceride recovered off the thin layer chromatogram was bulked with the 2% ether/light petroleum fraction and saponified. The saponification mixture was extracted with ether and the ethereal layer containing 'ergosterol' from the hydrolysis of the ester was examined by gas-liquid chromatography (see below). The alkaline aqueous phase was acidified (pH 2) with conc. HCl and re-extracted with ether to remove the fatty acids. This ethereal extract, after washing with distilled water, was taken to dryness and the methyl esters of the fatty acids prepared using diazomethane (a suitable scaled down method employing nitrosomethylurea as described by Vogel[2] was used for the methylation). The methyl ester(s) on infra-red analysis had a spectrum identical to methyl oleate. In order to complete the identification the sample was subjected to gas-liquid chromatography (Pye Argon Chromatograph) on a 4-ft column of polyethyleneglycol adipate at a temperature of 148° with a flow rate of 530 ml Argon/min. The sample gave one major peak with two minor peaks, the retention times of which were identical to those recorded for authentic methyl oleate, methyl palmitate and methyl linoleate using the same instrument; from the areas under the peaks the mixture was calculated to contain 79·4% oleate, 14·3% palmitate and 6·3% of linoleate by weight.

The ergosterol recovered from the saponification of the ester (see above) was identified as ergosterol by gas-liquid chromatography (A. Dennis, unpublished observations). From the u.v. spectroscopic studies it was seen that the ergosterol in fraction 5 (20% ether/light petroleum) only accounted for some 3·5% of the weight in this fraction. That ergosterol was the only major sterol occurring in the fraction was confirmed by carrying out a quantitative Lieberman-Burchard reaction,[3] when it was found that the colour response of this reagent showed the presence of only a $\Delta^{5,7}$-stenol at the same concentration as the ergosterol previously determined by u.v. spectroscopy. The material in fraction 5 was not investigated further, and the sterol was assumed to be ergosterol—since this is the most common mould sterol.

The ubiquinone fraction recovered off the plates after quantitative thin-layer chromatography of fraction 3, was run on a reversed phase thin layer chromatogram against authentic ubiquinone samples. The quinone behaved as the -35 homologue (UQ_7.)

The results show that the oidia of *P. ostreatus* contain, in terms of dry weight, a high percentage of neutral lipid which presumably is one of the major storage sources of energy for the spores. It is of interest that the major fatty acid constituent of the triglyceride is oleic with lesser amounts of palmitic, since these are the major fatty acids produced by many moulds, e.g. *Aspergillus nidulans*[4] 40% oleic, 21% palmitic; Penicillium sopii,[5] 45% oleic, 22% palmitic; *P. lilacinum*,[6] 39% oleic, 32% palmitic. Linoleic acid which was found in smaller amounts (6%) appears to be in general the third major fatty acid of fats in moulds. Therefore the oidia as regards fatty acid make up show the typical distribution found in many fungal hyphae.

The occurrence of the terpenoids squalene, ergosterol (esterified and free) was not unexpected, although the percentage of ergosterol esterified (40%) was higher than normally encountered in the mycelia where it rarely seems to exceed ~ 25% total sterol. The concentration of ubiquinone in the oidia (0·114 μmole/g dry wt.) was similar to values recorded for

[2] A. VOGEL, *A Textbook of Practical Organic Chemistry* (3rd Ed.), Longmans, Green, London (1956).
[3] W. E. DAVIES, Ph.D. Dissertation, U.C.W. Aberystwyth (1963).
[4] J. SINGH, T. K. WALKER and M. L. MEARA, *Biochem. J.* **61**, 85 (1955).
[5] J. SINGH, S. E. PHILLIP and T. K. WALKER, *J. Sci. Food Ag.* **8**, 697 (1957).
[6] J. SINGH, S. SHAH and T. K. WALKER, *Biochem. J.* **62**, 222 (1956).

various fungal hyphae and was higher than anticipated. Since, it had seemed reasonable to expect that in a relatively metabolically inert structure such as an oidia, that ubiquinone, which in terms of concentration generally parallels metabolic activity, would be present in greatly reduced amounts.

EXPERIMENTAL

Organism

Pleurotus ostreatus was cultured on dextrose peptone agar by inoculating the medium with a small piece of mycelium from the interior of a basidocarp growing on a willow tree in Alexandria (Egypt). On the medium the fungus produces black heads on upright stalks. Each head is an aggregation of oidia in a slimy material. The fungus was maintained on the agar slopes at room temperature.

Cultural Conditions

The organism was cultured on an agar medium of the following composition: KH_2PO_4, 1g; $MgSO_4.7H_2O$, 0·5 g; peptone (Evans), 5g; beef extract (Oxoid), 3g; agar, 15g and tap water to 1 l. The medium was dispensed as slopes in lemonade and syrup bottles.

METHODS

The methods of column and thin-layer chromatography, the saponification procedure, and the estimation of ubiquinone and ergosterol are described in the previous paper.[1]

INFRA-RED SPECTROSCOPY

The i.r. spectra were determined as films on rock-salt discs using the Perkin-Elmer 137E Infracord.

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