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Indole compounds in fruiting bodies of some selected *Macromycetes* species and in their mycelia cultured *in vitro*

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Methanol extracts of fruiting bodies of two purportedly edible species of higher fungi (*Macromycetes*)- *Tricholoma equestre* (L.: Fr.) Kumm. and *Xerocomus badius* (Fr.) Kühn.ex Gilb., collected from a natural environment and of biomass obtained from *in vitro* cultures were assayed for contents of nine indole compounds. Common indole compounds in extracts from fruiting bodies of both species were: tryptophan, tryptamine and serotonin, in mycelial extracts from *in vitro* cultures: tryptophan and tryptamine. Contents of the compounds investigated varied widely, from 0.182 to 2.851 mg/100g d.w. in fruiting bodies and from 0.322 to 2.096 mg/100g d.w. in biomass from *in vitro* cultures, respectively.

Among indole compounds, indole alkaloids and hallucinogenic compounds of fungal origin have been the most frequent focus of interest (Somei and Yamada 2003; Wurst et al. 2002). Studies of nonhallucinogenic indole compounds have concentrated, for example, on tryptophan in *Basidiomycetes* (Turner and Aldridge 1983). Tryptamine is relatively frequent, although in some dangerous species e.g. in *Panaeolus genius, Coprinus genius* and *Inocybe hirsuta.* Serotonin has been identified in some *Panaeolus* species and 5-hydroxytryptophan has been found only in *Panaeolus shinctrinus* (Grzybek and Kohlmünzer 1972). Recently, Muszyńska et al. (2007) have identified several indole compounds, apart from tryptophan and tryptamine, melatonin, IAA and indole in *Lactarius deterrimus* fruiting bodies (this fungus species is edible with caution.)

The present studies were conducted on two species of purportedly edible fungi *Tricholoma equestre* (L.: Fr.) Kumm. and *Xerocomus badius* (Fr.) Kühn.ex Gilb. (*Macromycetes*). They are common in European coniferous and mixed forests, but occur also in other geographical regions, e.g. in Japan and the USA. The chemical composition of both species is known only fragmentarily. *T. equestre* fruiting bodies have been reported to contain fragrant compounds and dyes (Glouchoff et al. 1972). The most detailed studies of *X. badius* have focused on its metal content: cesium and potassium, and dyes (Schmidt 1988). There are no reports in the available literature about indole compounds in the species investigated in the present study, and in mycelia cultured *in vitro* derived from fruiting bodies.

Fruiting bodies of the two species under study were found to contain tryptophan, tryptamine and serotonin. *T. equestre* fruiting bodies contained in addition a serotonin precursor, 5-hydroxytryptophan, while *X. badius* fruiting bodies contained tryptophan degradation products: kynurenic acid and kynurenine sulfate.

Contents of indole compounds in *T. equestre* fruiting bodies ranged from 0.182 to 2.851 mg/100 g d.w., and in *X. badius* from 0.468 to 1.964 mg/100 g d.w. (Table). Respective values in *L. deterrimus* fruiting bodies were of the same order of magnitude and varied from 0.176 to 2.676 mg/100 g d.w. (Muszyńska et al. 2007).

Tryptophan contents in both species were significant and amounted to 2.851 mg/100 g d.w. in *T. equestre*, and 0.679 mg/100 g d.w. in *X. badius*. This amino acid, exogenous for humans, is a biogenetic precursor of all indole compounds. When consumed at high doses with food, it can damage the nervous system and contribute to inducing cancer of the urinary bladder (Stone et al. 2003). It is controversial whether *T. equestre* is an edible species, since there are reports from France about rhabdomyolysis following its ingestion (Bedry et al. 2001). However, those authors did not identify the chemical compounds that may be the cause of this serious condition. Results of the present study provide further evidence that *T. equestre* is not a safe species. Fruiting bodies collected in Poland were demonstrated for the first time to contain several indole compounds that are not harmless to human physiological processes, being tissue hormones and neurotransmitters. Serotonin and tryptamine contents in *T. equestre* fruiting bodies were different and totalled to 0.182 mg/100 g d.w.

Table: Contents (mg/100 g d.w.) of indole compounds under study in extracts of fruiting bodies and of mycelia from *in vitro* cultures of *T. equestre* and *X. badius*

Indole compounds*	Tricholoma equestre		Xerocomus badius	
	Fruiting bodies	Mycelium	Fruiting bodies	Mycelium
L-Tryptophan	2.851 +/−0.242	1.036 +/−0.093	0.679 +/−0.047	0.827 +/−0.049
5-Hydroxytryptophan	0.586 +/−0.041	0.344 +/−0.031	– **	– **
Serotonin	0.182 +/−0.013	0.598 +/−0.049	0.524 +/−0.042	– **
Melatonin	– **	0.322 +/−0.026	– **	– **
Tryptamine	2.011 +/−0.141	0.598 +/−0.048	0.468 +/−0.045	0.409 +/−0.045
Kynurenic acid	– **	– **	1.569 +/−1.142	– **
Kynurenine sulfate	– **	– **	1.964 +/−0.157	2.096 +/−0.126

Data presented as mean of 3 series ± SE.
* Indole and indoleacetamide were not included in the table because they were not present in any extract under study
** Content lower than 0.001 mg/100 g d.w.

and to 2.011 mg/100 g d.w. respectively while their concentrations in *X. badius* fruiting bodies were 0.524 and 0.468 mg/100 g d.w., respectively. Extracts of *T. equestre* mycelium from *in vitro* cultures were demonstrated to contain the five indole metabolites: L-tryptophan, 5-hydroxytryptophan, serotonin, melatonin and tryptamine. The spectrum of indole compounds in *X. badius* cultures was narrower and comprised three compounds: tryptophan, tryptamine and kynurenine sulfate. The content of indole compounds in mycelia cultured *in vitro* ranged from 0.322 to a maximum of 1.036 mg/100 g d.w. in *T. equestre* and from 0.409 to 2.096 mg/100 g d.w. in *X. badius*, and was comparable with their contents in fruiting bodies (Tab.).

The similar contents of indole compounds in mycelia from *in vitro* cultures and fruiting bodies collected from natural conditions indicates that it may be possible to use *in vitro* cultures as a model for studies on the biosynthesis and accumulation of the above compounds. Large amounts of metabolites with physiological activity in the human body in both species justify the need for further studies of their chemical composition.

Experimental

1. Origin of fruiting bodies

The studies were conducted on *Tricholoma equestre* (L.: Fr.) and *Xerocomus badius* (Fr.) Kühn.ex Gilb. fruiting bodies collected in mixed forests in southern Poland in September 2004 (deposited in the Department of Pharmaceutical Botany, Jagiellonian University, Collegium Medicum, Kraków, Poland).

2. Establishment of in vitro cultures

Mycelial cultures were derived in 2004 from explants originating from the hymenial part of *T. equestre* and *X. badius* fruiting bodies. Fruiting body fragments were sterilized with 70% ethyl alcohol and placed on Petri dishes with modified Oddoux (1960) medium.

Cultures were incubated at a temperature of $25 \pm 2\,^{\circ}C$ under 16-h light (900 lx)/8 h dark, and were subcultured every second week.

3. Experimental in vitro cultures

The agitated cultures of *T. equestre* and stationary liquid cultures of *X. badius* were maintained in Erlenmayer flasks (500 mL), containing 250 mL of medium (initial biomass was 0.1 g). The flasks with *T. equestre* mycelium were shaken at a rate of 140 rpm. The cultures (three culture series) were maintained for two weeks. After two weeks the biomass was separated from the medium, and the fresh biomass was frozen and lyophilized.

4. Extraction

Lyophilized fruiting bodies and mycelia from *in vitro* cultures (approximately 3 g) were extracted in a percolator with petroleum ether in order to remove the lipid fraction according to the procedure developed in our laboratory (Muszyńska et al. 2007). After petroleum ether extraction, the study material was dried and extracted with methanol (10 portions/ 300 mL) in a percolator for 24 h. Extracts were combined and evaporated to dryness. The residues were quantitatively dissolved in 2 mL of methanol and analyzed by HPLC.

5. Chromatographic analysis

Contents of indole, indoleacetamide, 5-hydroxytryptophan, kynurenic acid, L-tryptophan, melatonin, serotonin, kynurenine sulfate, and tryptamine were determined according to the procedure developed by Kysilka and Wurst (1985) with our modifications. Briefly, the analytical conditions were as follows: HPLC apparatus-Hitachi; pump-L-7100; column-Purospher® RP-18 (4×200 mm, 5 μm); solvent system-methanol/water/ammonium acetate (15 : 14 : 1 v/v/v); flow rate- 1 ml/min; detector UV-λ = 280 nm; standards-manufactured by Sigma-Aldrich.

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