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Note

Analysis of indole compounds from the fruiting bodies and the culture mycelia of *Sarcodon imbricatus*

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ABSTRACT

Amounts of non-hallucinogenic indole compounds were determined in methanolic extracts from the fruiting bodies and biomass of *Sarcodon imbricatus* cultured in vitro. In both extracts there were non-hallucinogenic indole compounds present, L-tryptophan, tryptamine and serotonin. Additionally, melatonin was found also in the fruiting bodies. The total amount of indole compounds was 89.38 mg/100 g d.w. in the fruiting bodies and 8.45 mg/100 g d.w. in the cultured mycelia. The leading compound in the fruiting bodies and the mycelium was serotonin (52.02 mg/100 g d.w. and 3.03 mg/100 g d.w., respectively). This main indole compound was isolated and identified using spectral methods.

Chemical evaluation of Basidiomycota species has been an object of many investigations in different aspects as metabolism, nutrition and medicine (Wasser and Weis 1999). The genus *Sarcodon* belongs to the order Aphyllophorales. Numerous species of these fungi are a source of anti-cancer and immunomodulating compounds (Wasser 2002). In Poland, the genus *Sarcodon* is mainly represented by two species: *S. imbricatus* (L.) P. Karst. and *S. squamosus* (Schaff.) Quel. The subject of this study was *S. imbricatus* (the scaly hedgehog) occurring in spruce forests in the northern hemisphere. It is common in Central Europe and in North America, but is especially common in the Rocky Mountains, where it grows under Engelmann spruce and subalpine fir during the monsoon season and can attain astounding cap sizes (e.g., up to 25–30 cm in diameter). In Poland, *S. imbricatus* is classified as an endangered species and protected by law. In the earlier study, from mycelial biomass of in vitro cultures of *S. imbricatus* two fractions of polysaccharides, fucogalactan and fucoglucan, were isolated (Sułkowska-Ziaja et al. 2011). Both these fractions showed biological activities, i.e., antiviral (anti-Human Papilloma Virus) and antibacterial (against some gram-negative as well as some gram-positive bacterial strains) (Sułkowska-Ziaja et al. 2011). Moreover, non-hallucinogenic indole compounds, such as serotonin or melatonin, play the role of neurotransmitters or their precursors. They also exhibit anti-oxidant, anti-cancer, anti-inflammatory, analgesic and anti-aging properties (Ueda et al. 1996; Hardeland et al. 2009). These features encouraged further research on the chemical composition of this species.

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The aim of the present study was to examine the accumulation of non-hallucinogenic indole derivatives in the methanolic extracts from the fruiting bodies and mycelia from in vitro cultures. This is the first report on the quantitative analysis of indole compounds in *S. imbricatus* in vitro cultures.

Mature fruiting bodies were harvested in spruce forests of Southern Poland in 2005–2008. After taxonomic identification (Hrouda 2005a,b), mixture of stipe and pileus of fruiting bodies were frozen, lyophilized and powdered in a mortar. In vitro cultures of *S. imbricatus* was grown in 500 ml flasks containing 150 ml of basal medium describing by Turło (Turło et al. 2004) with modifications. A medium composed of 5% glucose, 1% yeast extract, 1% casein hydrolyzate, 0.3% KH_2PO_4. The initial pH value was set at 6.0 and incubation was carried out at 30 °C, which value was optimal for mycelial growth of this fungus. Voucher specimens UJCMBF-51FB was deposited in the Department of Pharmaceutical Botany, Jagiellonian University, Medical College in Kraków. The strain UJCMBF-51MC (PCM 2719) was deposited in the Polish Collection of Microorganisms, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland (PCM).

The lyophilized fruiting bodies (10 g) and mycelia from in vitro cultures (10 g) were placed in a percolator and extracted with petroleum ether to remove the lipid material. Then, the defatted material was dried and further extracted with methanol for 24 h (percolator, 10 portions/300 ml). The extracts were then combined and evaporated to dryness. The residues were dissolved in methanol and applied onto silica gel column (50 cm × 2.5 cm, Kiesel 60, Merck, Darmstad, Germany). The column was eluted sequentially with *n*-hexane, *n*-hexane and chloroform at 1:1 (v/v), chloroform, chloroform and methanol at 10:1 (v/v), chloroform and methanol at 10:2.5 (v/v), chloroform and methanol at 10:5 (v/v), chloroform and methanol at 10:10 (v/v), and methanol. The eluents were switched depending upon the changes in the color of the eluate. Samples of 1.5 ml were collected from the eluant fraction and analyzed for non-hallucinogenic indole compounds by chromatography methods (TLC, HPLC). After preliminary purification by column chromatography, the obtained fractions which showed a positive reaction of the DAB reagent (a mixture of 10% *p*-3,3′-diaminobenzidine in conc. HCl, diluted before use with acetone 1:4) were used for HPLC analysis. Indole compounds were isolated from the methanol extracts by preparative silica gel 60 (Merck, Art. 1.055540001) using *n*-butanol/acetic acid/water 12:3:5 (v/v/v) as developing solvent. Indole compounds were detected under UV light at 280 nm and eluted from the silica gel with methanol. After evaporation to dryness at 22 °C, the residues were quantitatively dissolved in 1.0 ml of methanol and subjected to HPLC and spectral analysis.

Quantitative analysis of indole compounds was performed using the HPLC procedure developed by Kysilka et al. 1985, modified by the authors (Muszyńska et al. 2009). In brief, the analytical conditions were as follows: HPLC apparatus – Hitachi (Merck); pump – L-7100; Purospher® column – RP-18e (250 mm × 4 mm, 5 μm) termostated at 22 °C; detector: UV–Vis (L-7400) $\lambda = 280$ nm, solvent system was methanol/water/ammonium acetate 15:14:1 (v/v/v), flow rate – 1 ml/min. The reagents used for the HPLC analysis were: HPLC grade methanol and ammonium acetate (Merck). Eleven standards of non-hallucinogenic indole compounds were used: L-tryptophan, 5-hydroxytryptophan, 5-methyltryptophan, tryptamine, 5-methylotryptamine, serotonin, melatonin, indoleacetic acid, indoleacetonitrile, indole and indoleacetamide (Sigma–Aldrich, St Louis, USA). All the analyses were carried out in triplicate. The results were expressed as mean values with standard deviation (SD).

Serotonin, one of the non-hallucinogenic indole compound, from the fruiting bodies and mycelial culture was isolated by using TLC plates (Merck, Art. 1.11844). The ^1H NMR spectra were recorded using a Mercury 300-BRÜKER (^1H, 300.08; MHz) (Bruker, MA, USA) apparatus in the NMR Spectroscopy Laboratory of the Department of Organic Chemistry at UJ CM in Kraków.

The biomass growth, obtained during 3-week growth cycles amounted to 10.2 g d.w./l of the medium. The achieved increase in biomass and the dynamics of mycelium growth did not differ from the results that we had obtained for *Xerocomus badius* and *Tricholoma equestre* cultures studied earlier (Muszyńska et al. 2009). In that case, biomass increments averaged from 8.9 to 9.6 g d.w./l of the medium respectively (Muszyńska et al. 2009).

The fruiting bodies of Basidiomycota species also produce numerous compounds with an indole structure. These compounds can be divided into hallucinogenic tryptamine derivatives and bio-active indole derivatives, which do not demonstrate hallucinogenic properties (Kohlmunzer et al. 2001). Our studies have confirmed the presence of indole compounds, and allowed, for the first time, to quantify them in the fruiting bodies and, cultured mycelia of *S. imbricatus*. The chemical composition of indole compounds in the fruiting bodies and cultured mycelia are presented in Table 1. The analysis performed for 14 indole compounds from the fruiting bodies resulted in the identification of four compounds: L-tryptophan, tryptamine, serotonin and melatonin. The amounts of these compounds varied widely: serotonin – 52.02 mg/100 g d.w., L-tryptophan – 22.12 mg/100 g d.w., tryptamine – 13.01 mg/100 g d.w. and melatonin – 2.13 mg/100 g d.w. The representative chromatogram is presented in Fig. 1. In the methanolic extract from in vitro cultures the presence of 3 compounds: L-tryptophan, tryptamine and serotonin were confirmed. Their amounts were much lower than in the extracts from fruiting bodies: 1.31, 4.11, and 3.03 mg/100 g d.w., respectively. The presence of melatonin was not confirmed. The present study is the first attempt to

Table 1 – Contents of indole compounds from fruiting bodies and mycelium from in vitro cultures of *Sarcodon imbricatus* [mg/100 g d.w.].

Indole compounds	Extract of fruiting bodies	Extract of mycelium from in vitro cultures
L-Tryptophan	13.01 ± 0.01	1.31 ± 0.6
Tryptamine	22.12 ± 0.03	4.11 ± 0.26
Serotonin	52.02 ± 0.4	3.03 ± 0.8
Melatonin	2.13 ± 0.3	n.d.

n.d.: Not detected; each value represents mean ± S.D. of triplicate ($n = 3$).