Biotransformation of Indole Derivatives by Mycelial Cultures

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Biotransformation of tryptophan to tryptamine and 3-methyl-indole by *Psilocybe coprophila* was performed. On the other hand, *Aspergillus niger* was able to transform tryptophan to 5-hydroxy-tryptophan. *P. coprophila* biotransformed 5-hydroxy-tryptophan to 5-hydroxy-tryptamine. These results prove once more that fungi are good tools to establish hydroxy-indole derivatives.

Key words: Biotransformation, *Psilocybe coprophila*, *Aspergillus niger*

Introduction

Many alkaloids have a complex polycyclic nature and the presence of diverse moieties and functional groups induces many difficulties in their synthesis processes that consume much time and materials and often produce low yields. Microbial transformations offer the use of enzymes with high stereospecificity, eliminating the need to protect and de-protect exposed functional groups. Those bioprocesses are very attractive for synthetic chemists, since they are operating at non-extreme pH value and temperature with low levels of toxicity.

The hydroxy-indole derivative is an important building block in the synthesis of pharmaceuticals, dye compounds, and chemicals (Fujii *et al*., 2001; Gartz, 1989). Gathergood and Scamel (2003), and Bartoli *et al*. (1989) reported the synthesis of 4-hydroxy-tryptamine scaffold and 7-hydroxy-tryptamine, respectively. However, this method is not amenable for the direct preparation of the desired indole moiety present in tryptamine.

We have previously reported the biotransformation of tryptophan by *P. coprophila* (Alarcón *et al.*, 2006). In this study, the biotransformation of indolic compounds, tryptophan and 5-hydroxy-tryptophan, by *Psilocybe coprophila* and *Aspergillus niger* was analyzed. The structures of the metabolites obtained were elucidated by spectroscopy experiments and comparison with authentic samples.

Experimental

Organism collection

Fruiting bodies of P. coprophila were collected in the rain forest of Southern Chile (Región del Bío-Bío), growing on horse or cow dung. Mycelia cultures of the strain were derived from the spore print of the fruiting bodies. A voucher specimen of the mushroom is deposited in the herbarium of Departamento de Ciencias Básicas de la Universidad del Bío-Bío, Chillán, Chile. *Aspergillus niger* ATCC 5142 was obtained from the American Type Culture Collection, Rockville, MD, USA.

Fungal strain and culture conditions

Stock cultures of *P. coprophila* (PCUBB-001) and *A. niger* (ATCC 5142) were maintained on potato dextrose agar (PDA) under refrigeration. Small sections of this agar were transferred to Erlenmeyer flasks containing a liquid medium (250 ml/flask) comprised of: 0.05 g/l $CaCl_2 \cdot 2H_2O$ (Merck), 0.025 g/l KH_2PO_4 (Merck), 0.25 g/l $(NH_4)_2HPO_4$ (Merck), 0.15 g/l $MgSO_4 \cdot 7H_2O$ (Merck), 1.3 ml 1% $FeCl_3$ (Merck), 3.0 g/l malt extract (Merck) and 10 g/l glucose (Merck) in distilled water; the pH value was adjusted to 6.5 with a solution of aqueous HCl (2 M) or KOH (2 M). The cultures were incubated under magnetic stirring (5 d for *P. coprophila* and 2 d for *A. niger*). 125 ml of well-grown culture were used as inoculum. Cells (125 ml portions) were transferred to

a new flask and, in both cases, reached abundant growth after 24 h when the substrates were added (tryptophan to *P. coprophila* and *A. niger*, and 5-hydroxy-tryptophan to *P. coprophila*) as an ethanolic solution (100 mg/ml). The fermentation with *P. coprophila* was stopped after 30 d and the *A. niger* fermentation after 7 d.

Indole compound isolation

Culture filtrate (2000 ml) obtained by filtration was acidified to pH 3 with 0.1 M HCl and extracted with diethyl ether (3×200 ml). The combined extracts were dried (Na_2SO_4) and concentrated to a final volume of 5 ml. The mycelia were washed with 0.05 M HCl and stirred at room temperature for 1 h, then filtered and, after acidification, extracted with diethyl ether (3×100 ml) for 1 h under stirring. The extract was dried (Na_2SO_4) and concentrated under vacuum to a final volume of 5 ml. Then the acidic solution was alkalinized to pH 13 with 0.1 M NaOH, stirred at room temperature for 1 h and extracted with diethyl ether. The extract was dried (Na_2SO_4) and concentrated under vacuum to a final volume of 5 ml.

Results and Discussion

The biotransformation of tryptophan and 5-hydroxy-tryptophan (5-HTP) by *P. coprophila* led to the formation of a mixture of compounds that gave a positive Dragendorff test. The compounds were identified as 3-methyl-indole, tryptamine, 5-hydroxy-3-methyl-indole and 5-hydroxy-tryptamine (5-HT) (Fig. 1, Tables I and II). Interestingly, when 3-indoleacetic acid was fed to *Pseudomonas* sp. (Kieslich, 1976) and 3-indolylacetonitrile was fed to *Beauveria bassiana* (Boaventura *et al.*, 2004) 3-methyl-indole was also obtained. The fact that microorganisms can metabolize different indole derivatives to 3-methyl-indole suggests that the synthesis of this compound by using a biological reagent can be successfully addressed and even improved. This observation can also be useful for biosynthesis studies of interesting naturally occurring indole compounds, especially those of fungal origin (King *et al.*, 1998)

When tryptophan was fed to *A. niger*, 5-hydroxy-tryptophan (Tables I and II) was recovered by chromatography procedures. Boaventura *et al.* (2004) reported that tryptamine was transformed with *A. niger* into 5-hydroxy-indole-3-acetamide. Interestingly, these fungi were able to perform both reduction and oxidation of the indole compound. Other studies reported the microbial hydroxylation of indole to 7-hydroxy-indole by *Acinetobacter calcoaceticus* (Sugimori *et al.*, 2004).

Our result shows that liquid fungus cultures are excellent tools to establish hydroxy-indole deriva-

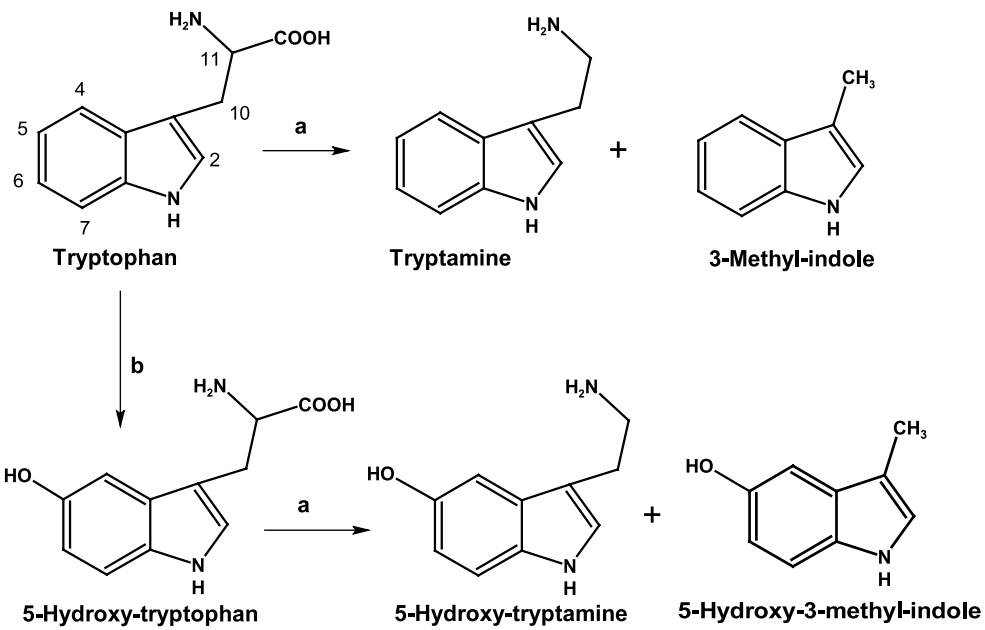

Fig. 1. Pathway of biotransformation of indole derivatives by (a) *P. coprophila* and (b) *A. niger*.

Table I. ^{1}H NMR data (250 MHz, *J* in Hz in parentheses) for biotransformation products.

Position	3-Methyl-indole[a]	Tryptamine[a]	5-HTP[b]	5-HT[a]
H-1	8.65 (br s)	9.2 (br s)	7.98 (br s)	8.43 (br s)
H-2	7.22 (m)	7.72 (d, 2.1)	7.13 (s)	7.71 (d, 2.2)
H-4	8.22 (d, 7.80)	8.24 (d, 7.81)	7.06 (d, 2.4)	7.03 (s)
H-5	7.82 (t, 7.25)	7.45 (t, 7.29)	–	–
H-6	7.15 (t, 7.56)	7.22 (t, 7.42)	6.73 (dd, 2.4, 8.8)	7.15 (t, 7.35)
H-7	8.05 (d, 8.0)	8.14 (d, 8.10)	7.22 (d, 8.8)	8.09 (d, 8.0)
H-10	2.65 (s)	1.75 (t, 6.33)	3.46–3.09 (dd, 15.2, 3.8)	1.82 (t, 6.35)
H-11	–	1.25 (t, 6.34)	3.83 (dd, 9.6, 4.0)	1.28 (t, 6.23)
NH_2	–	0.95 (br s)	1.85 (br s)	0.98 (br s)

[a] $CDCl_3$ was used as solvent.
[b] D_2O was used as solvent.

Table II. ^{13}C NMR data (65 MHz) for biotransformation products.

Position	3-Methyl-indole[a]	Tryptamine[a]	5-HTP[b]	5-HT[a]
C-2	112.91	122.13	125.9	123.8
C-3	121.81	113.26	108.7	107.9
C-4	121.81	118.72	103.5	102.4
C-5	120.09	119.03	151.5	148.6
C-6	128.26	121.77	112.9	112.1
C-7	138.61	111.14	112.8	112.4
C-8	111.54	127.36	133.2	132.6
C-9	140.16	136.40	129.1	128.5
C-10	141.76	29.29	28.5	29.5
C-11	20.30	42.17	56.5	42.32
C-12	–	–	174.7	–

[a] $CDCl_3$ was used as solvent.
[b] D_2O was used as solvent.

tives. The position for hydroxylation depends on the fungal species used, as demonstrated by literature.

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