

MICROORGANISMS AS TOOLS IN MODERN CHEMISTRY: THE BIOTRANSFORMATION OF 3-INDOLYLACETONITRILE AND TRYPTAMINE BY FUNGI

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ABSTRACT

Chemical reactions performed by microorganisms have been used as a modern tool in chemistry. In this work, the ability of the fungi *Beauveria bassiana* and *Aspergillus niger* to modify the chemical structure of indole compounds was studied. *Beauveria bassiana* was able to transform 3-indolylacetonitrile into 3-methylindole while *Aspergillus niger* transformed tryptamine into 5-hydroxyindole-3-acetamide. Interestingly, these fungi were able to perform both reduction and oxidation of the indole compounds fed, the oxidation occurring with improved levels of oxygen uptake. The results prove once more the versatility of microorganisms to perform a wide range of industrially attractive chemical reactions.

Key words: Biotransformations, *Beauveria bassiana*, *Aspergillus niger*, 3-indolylacetonitrile, tryptamine

INTRODUCTION

The use of microorganisms in chemistry is not a new issue. Bacteria and fungi have been used to produce chemicals, pharmaceuticals and perfumes for decades (1,8). Microorganisms can also be successfully used for pollutants degradation and recovery of the environment contaminated by chemicals (7). Their use for modifying the chemical structures of natural products is more recent but very well documented (4). However, the use of this methodology by the industry is still modest, considering the great availability of useful microorganisms and the large scope of useful reactions that can be accomplished by them. Considering the modern requirements for a more safe and ecological chemistry (3), the use of reactions performed by microorganisms tends to an increase in the near future. Such reactions, usually referred as biotransformations, have some advantages over the conventional chemical reactions. Biotransformations are usually carried out in aqueous systems and at a neutral pH, avoiding the use of harmful solvents common in conventional syntheses. In addition to this, microorganisms are able to perform a large variety of reactions, including some nearly inaccessible by chemical means.

Description of new useful reactions is, therefore, of interest. The biotransformations of indole compounds are of special interest because of the large scope of their biological activities. They can be used to treat many diseases, such as arthritis, skin diseases, headaches and glaucoma (2). Tryptamine, for example, has a well-described action on the central nervous system and can also be used as anti-feeding and anti-attractive compounds over *Drosophila melanogaster* (9,12). 5-Hydroxytryptamine is a very powerful vasoconstrictor agent called serotonin. The synthetic use of microorganisms to perform reactions in the indole nucleus is of industrial interest as a way to synthesize active indole derivatives and this area has attracted great attention (4). Our group is interested on useful reactions that can be performed by microorganisms, and we have been presenting many options towards the conventional chemistry in this area (11).

In this way, we report herein the action of *Beauveria bassiana* and *Aspergillus niger* over 3-indolylacetonitrile (1) and tryptamine (3), respectively.

MATERIALS AND METHODS

Solvents (ethyl acetate, ethanol, methanol, petrol ether) from Merck were of analytical grade. Silica gel 60 and 60G were

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purchased from Sigma-Aldrich (Milwaukee, WI, USA). Sephadex LH-20 was purchased from Sigma (St. Louis, MO, USA). Reagents to prepare the culture medium were purchased from Difco (Sparks, MD, USA).

Stock cultures of *Beauveria bassiana* (CCT 3161) and *Aspergillus niger* (NRRL 4026) were maintained on malt agar under refrigeration. Cultures were donated by Coleção de Culturas Tropical (Campinas, SP, Brazil) and **National Center for Agricultural Utilization Research** (Peoria, IL, USA). Small sections of this agar were transferred to Erlenmeyer's flasks containing a liquid medium (200 mL/flask) comprised of (g/L): glucose (20.0), peptone (5.0), yeast extract (3.0), potassium phosphate (5.0) in distilled water and pH was adjusted to 6.0 with a solution of aqueous NaOH (1mol/L). The culture was incubated over magnetic stirring (72 h for *B. bassiana* and 40 h for *A. niger*) to reach stage I. Cells (15 mL portions) on stage I were transferred to new flasks and, in both cases, reached stage II (abundant growth) after 24 hours when the substrates (1mL/flask) were added [3-indolylacetonitrile (**1**) to *B. bassiana* and tryptamine (**3**) to *A. niger*] as a solution in ethanol (50 mg/mL). After 13 days, the flasks' contents were extracted with ethyl acetate and the solvent was taken out under vacuum. Residue (326 mg) from the experiment with *B. bassiana* was chromatographed over silica gel (petrol ether/ethyl acetate/methanol). Fractions eluted from petrol ether:ethyl acetate 8:2 furnished a solid (23 mg) identified by spectroscopic means as 3-methylindol (**2**). From the experiment with *A. niger*, the residue (680 mg) was then dissolved in methanol and repeatedly chromatographed over Sephadex LH-20 using methanol as eluent to furnish a solid (12 mg) identified as 5-hydroxyindole-3-acetamide (**4**).

RESULTS AND DISCUSSION

The biotransformation of 3-indolylacetonitrile (**1**) by *B. bassiana* led to the formation of a solid less polar than the starting material. This compound was identified as 3-methylindole (**2**) by using ¹H and ¹³C NMR techniques. The presence of a new methyl group in the molecule was confirmed in the ¹H NMR spectrum due to a singlet at δ 2.33 integrating for 3 hydrogens. The corresponding carbon signal was observed at δ 9.86 in the ¹³C NMR spectrum. The disappearing of the signal at δ 118.38 of the starting material was also observed and this is in accordance with the cleavage of the CN group from the starting material. Full ¹H and ¹³C NMR spectra assignment for compound **2** can be found in Tables 1 and 2, respectively. Coupling constants (*J* values) of aromatic hydrogens are, according to the expected values, showing that no alterations on the aromatic ring substitution pattern has occurred. ¹³C NMR data for compound **1** (starting material) is also presented for comparison purposes. Absence of CN group at C-3 changed the chemical shift of this carbon from

Table 1. ¹H NMR data (D₂O, 200 MHz) for biotransformation products 3-methylindole (**2**) and 5-hydroxyindole-3-acetamide (**4**).

H	2 (δ)	4 (δ)
1	7.86 (br s)	8.24 (br s)
2	6.94-6.95 (m)	6.95-7.05 (m)
4	7.58 (dd, <i>J</i> =0.9 and 8.0 Hz)	7.53 (dd, <i>J</i> =1.4 and 7.2 Hz)
5	7.18 (dt, <i>J</i> =1.2, 8.0 and 8.0 Hz)	7.21 (dt, <i>J</i> =1.4, 7.0 and 7.2 Hz)
6	7.11 (dt, <i>J</i> =0.9, 8.0 and 8.0 Hz)	7.01 (dt, <i>J</i> =1.4, 7.0 and 7.2 Hz)
7	7.31 (ld, <i>J</i> =8.0 Hz)	7.31 (dd, <i>J</i> =1.4 and 7.0 Hz)
10	2.33 (s)	3.68 (s)

s = singlet; m = multiplet, dd= double doublet; dt= double triplet; l=large; *J*= coupling constant.

δ c 103.97 to δ c 112.19, while C-10 shifted from δ c 14.03 to 9.86. Interestingly, 3-methylindole (**2**) was also obtained when 3-indolacetic acid was fed to *Pseudomonas* sp (**4**). The fact that microorganisms can metabolize different indole derivatives into 3-methylindole suggests that the synthesis of this compound by using biological reagents can be successfully addressed and even improved. This observation can also be useful for biosynthesis studies of interesting naturally occurring indol compounds, especially those of fungal origin (**5**).

When tryptamine (**3**) was fed to *A. niger*, compound **4** was recovered after chromatography step. Analysis of **4** by ¹H NMR showed, in the aromatic region, signals for 4 aromatic hydrogens. Therefore, a new group replaced one hydrogen from the aromatic ring. Careful analysis of the aromatic hydrogens region profile showed that the substitution occurred at C-5. Analysis of the ¹³C NMR spectra showed the presence of a new signal at δ 148.72 and the disappearing of C-5 signal, both good indications that C-5 was hydroxylated. The ¹³C resonance of C-11 was not

Table 2. Full ¹³C NMR assignment (δ , D₂O, 50 MHz) for 3-indolylacetonitrile (**1**), 3-methylindole (**2**), tryptamine (**3**) and 5-hydroxyindole-3-acetamide (**4**).

C	1	2	3	4
2	122.83	122.12	122.13	125.60
3	103.97	112.19	113.26	115.50
4	117.78	119.18	118.72	111.60
5	122.51	121.81	119.03	148.72
6	119.88	119.18	121.77	112.60
7	111.49	111.18	111.14	102.62
8	125.57	128.81	127.36	127.37
9	136.05	136.82	136.40	131.35
10	14.03	9.86	29.29	31.09
11	118.38	—	42.17	177.96

observed, but a new signal was observed at δ 177.96, indicating the oxidation of this carbon. Table 2 shows the complete ^{13}C NMR data assignment for compound **4**, in comparison to data of the starting material tryptamine (**3**). From the best of our knowledge, NMR data for compound **4** is not reported in the literature (6). Hydroxylation of an indole nucleus (3-indoleacetic acid) at C-5 by *Aspergillus niger* is reported (4).

Both experiments were carried out with and without orbital shaking. Interestingly, in the biotransformations of 3-indolylacetonitrile by *B. bassiana*, the product (3-methylindole) was only observed for the experiment without shaking. 3-methylindole, formed by reduction of the precursor 3-indolylacetonitrile, was neither isolated nor detected by TLC when the cells were shaken. On the other hand, in the biotransformations of tryptamine by *A. niger*, the product 5-hydroxyindole-3-acetamide results from the oxidation of the substrate. In this case, it was only produced when the experiment was shaken. These observations show the importance of an adequate oxygen uptake for the synthesis of oxidative products, especially hydroxylated derivatives (10).

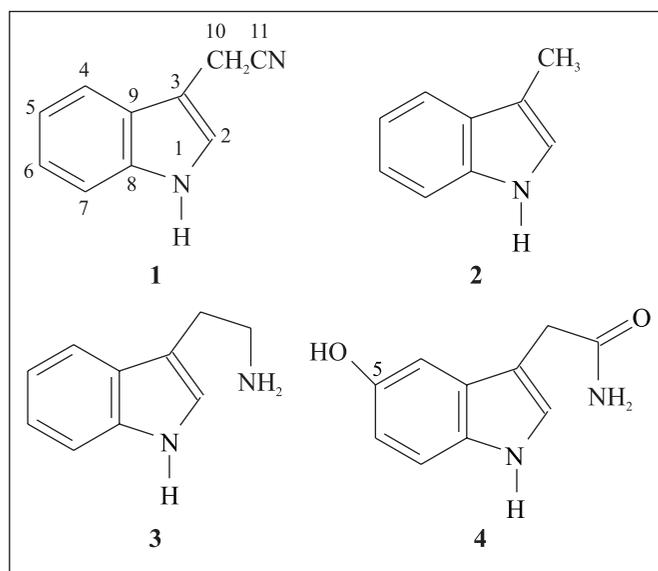


Figure 1. Chemical structures of 3-indolylacetonitrile (**1**), 3-methylindole (**2**), tryptamine (**3**) and 5-hydroxyindole-3-acetamide (**4**).

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RESUMO

Microrganismos como ferramentas na química moderna: a biotransformação da 3-indolilacetonitrila e triptamina por fungos

O uso de microrganismos para a realização de reações químicas tem sido explorado como uma ferramenta moderna em química. Neste trabalho explorou-se a habilidade dos fungos *Beauveria bassiana* e *Aspergillus niger* de modificarem quimicamente a estrutura de compostos indólicos, uma classe de substâncias com diversas atividades biológicas relatadas. A 3-indolilacetonitrila foi reduzida, com formação do 3-metilindol por *Beauveria bassiana*, enquanto *Aspergillus niger* converteu a triptamina em um derivado oxidado, a 5-hidroxiindol-3-acetamida. A oxidação ocorreu somente no experimento sob agitação e provavelmente está relacionada ao alto grau de oxigenação da reação. A ocorrência de redução e oxidação de derivados indólicos ilustra bem a versatilidade do uso de microrganismos para a condução de uma grande variedade de reações químicas de interesse industrial.

Palavras-chave: biotransformações, *Beauveria bassiana*, *Aspergillus niger*, 3-indolilacetonitrila, triptamina

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