



A Comparative Study on the Biotransformation of Selected Steroids and Steroid Drugs Using Different *Aspergillus* Species

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ABSTRACT

In nature, it can be said that there are many types of stimulants. including those being in bacteria, plants, fungi, and animals. It is a scientific fact that microbial transformation plays an important role in the biotransformation of steroids, enabling the production of stereoselective products. Our primary goal in this research is to explain and clarify the concept of microbial biotransformation and its importance in producing molecules with properties deemed useful in the pharmaceutical industry.

We also aim to highlight the importance of biotransformation in generating novel metabolites. Furthermore, we will shed light on the traits and features of some *Aspergillus fungi*.

We have demonstrated the biotransformation of some steroids such as DHAE and steroid drugs by some *Aspergillus* species such as *Aspergillus albicans* and *Aspergillus niger*. In order to clarify the results and how these fungi metabolize their substrates, we will use a previously conducted experiment and then compare the results of the experiment with the results of other previous experiments.

In conclusion, we hope that this work will be of benefit to students and researchers in the field of biochemistry.

Keywords: Microbial transformation, Beclomethasone, dipropionate (BDP), *Aspergillus glaucus*, Steroids

INTRODUCTION

"Biotransformation" as a term can be scientifically defined as the process of using biological systems to bring about structural development in chemical compounds that do not constitute their natural substrates. It is a metabolic process by which microorganisms convert chemicals or radionuclides by changing their oxidation states. (Tembeni, 2024)

It should be noted here that one of the most effective applications in the field of biotransformation, which is used in the synthesis of many biologically active compounds, is the process of microbial transformation of steroids (Zappaterra F, 2021).

The word steroids are derived from the Greek word "stereos," which means solid or solid alcohols and can be found in abundance in many animals and plants (Gupta, 2019). All steroids share a distinctive molecular structure consisting of 17 carbon atoms arranged in four rings, commonly referred to as rings A, B, C, and D, which are bonded to 28 hydrogen atoms, Fig. (1). Illustrate Steroid structure. This basic structure can be modified in numerous ways by removing, substituting, or adding individual atoms. Over the past decades, vast number of steroids has been extracted from plants and animals, and many of them have been created by chemically processing natural steroids or by synthesizing them from elementary compounds (Timothy J. Cole, 2019).

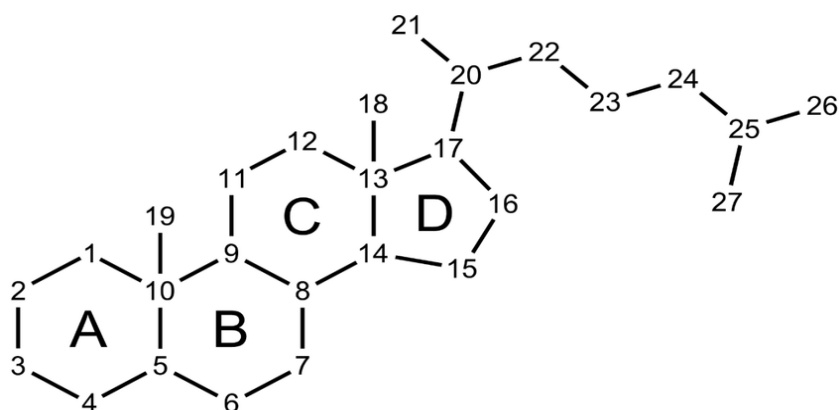


Fig. 1: Steroid Structure (Dodan, 2021).

Steroids in nature and their diversity

In the fields of biology and medicine, steroids have played a fundamental and effective aspect. Efforts are underway to discover new steroids from land and marine sources, including the discovery of new compounds and the exploration of their newly discovered biological activities. The structural diversity of these steroids contributes to their broad range of biological activities, including, for example, anticancer and antimicrobial properties. Steroids derived from marine invertebrates, fungi, and medicinal plants have demonstrated promising therapeutic potential. In the field of finding effective solutions to global health issues, including those associated to cancer research or antibiotics, the need and importance of steroids have increased, helping to discover new treatments (Hajdaś G, 2025).

Steroidal natural products are essential for drug discovery due to their unique structural properties and biological activity. However, the limited availability of steroid samples from natural sources has hampered a comprehensive assessment of their biological effects. The chemical synthesis of these compounds has become a fundamental and practical approach to producing them in sufficient quantities. For decades, chemists have focused on the effective synthesis of steroids, leading to significant advances over the past few decades. Methods for isolating steroids depend on their chemical nature, size, and intended use. Steroids are often extracted from natural sources using organic solvents.

One of the natural steroids used as essential raw materials in the pharmaceutical industry for the synthesis of steroid drugs is sapogenins. An example is diosgenin, a sapogenin abundant in the genus *Polygonatum*. Microbial bioconversion of diosgenin is an emerging approach for producing novel steroids with therapeutic benefits. However, the chemical conversion of sapogenins into usable steroid compounds remains challenging due to high production costs and the need for complex, multi-step synthesis processes (Li, 2023).

Sterols are essential components of cell membranes, contributing significantly to membrane fluidity, cell differentiation, and proliferation. Cholesterol is an example of an animal sterol, while sitosterol, stigmasterol, campesterol, and brassicasterol are common plant sterols (Marina Donova, 2012).

Microbial biotransformation

The potential of microbial steroids for biotransformation and the results achieved from them are not new, but have been known for decades, and their use can provide many advantages when compared to chemical synthesis. It allows spatial or regional functionalization of molecules at sites not always accessible to chemical agents, through a single step involving multiple sequential reactions, and is environmentally friendly, particularly in aqueous media and mild reaction conditions (Sanchez-Yañez, 2025).

Microbial biotransformation is a powerful and essential tool for generating molecules with pharmaceutical, nutritional, and cosmetic potential. This process requires three key components: A parent molecule, a transforming microorganism, and a suitable fermentation system. Shake flasks are the most widely used fermentation vessels for initial screening and small-scale experiments due to their simplicity and low cost. However, despite their widespread use, shake flasks face several limitations, such as inconsistent oxygen transfer, limited parameter control, and scalability issues, which can affect the reproducibility and reliability of results. Multi-parameter bioreactors allow precise control of environmental conditions, resulting in more consistent and scalable biotransformation results. However, these systems are expensive, and the use of multiple bioreactors for parallel screening is often not feasible for smaller research groups due to financial and logistical constraints (de Oliveira, 2023).

Dehydroepiandrosterone (DHEA)

Dehydroepiandrosterone (DHEA) is one of the most well-known and important steroids. The adrenal cortex produces the main steroid hormone, C19 sulfonate. Small amounts are also produced in the gonads and brain. Most DHEA is converted to dehydroepiandrosterone sulfate ester (DHEAS), due to its long plasma half-life. It is stored and, when needed, converted to specific hormones. As an important pharmacological steroid, its hydroxylation at different sites exhibits diverse biological activities. For example, the 9 α /16 α hydroxylation is essential for the biosynthetic activities of glucocorticoids. The 11 α hydroxylation is essential for anti-inflammatory activities. The biotransformation of DHEA-like steroids produces a diverse spectrum of metabolites, as they are the precursors to all steroid hormones (Fedotcheva TA, 2024).

The production of the hormone dehydroepiandrosterone (DHEA) occurs through the adrenal gland, which is primarily responsible for its production (Song, 2023).

Biocatalytic reactions have a wide scope in the synthesis of countless compounds that pose significant challenges in organic synthesis. This was also due to the avoidance of using toxic chemicals and organic solvents to reach the desired target compounds.

Steroidal compounds are attractive substrates for biotransformation due to their deactivated carbons and extensive biological activities that facilitate complex chemical transformations. The most common classes of steroids are glucocorticoids, which include betamethasone, methylprednisolone, dexamethasone, and prednisolone.

Among the steroid hormone groups are glucocorticoids, which perform numerous metabolic and homeostatic functions, including growth, apoptosis, and developmental behavior, all of which are essential for life. Prednisolone, dexamethasone, and other corticosteroid analogues are used to treat various medical conditions associated with glucocorticoid receptors (Sana R., 2024).

The genus *Aspergillus*:

In research and experiments related to microbial steroid conversion, fungi are widely used. This is due to the diversity of their enzymes, which have the ability to convert a large number of steroids thanks to their multiple functions (Wang, 2023).

Given their diverse range of medicinal properties, particularly their anticancer and antioxidant activities, fungi of all species considered as an important resource for drug discovery (Cicero, 2025).

The diversity of the genus *Aspergillus* has had a significant impact on the economy and societies. *Aspergillus* species can be found in various environments and locations around the world, and are classified as pathogenic to humans and sometimes animals. These fungi play a role in food spoilage through the production of mycotoxins (Olivia L. Riedling, 2024).

The genus *Aspergillus* has numerous species with diverse morphological, biological, and ecological characteristics, making them highly versatile. In agricultural environments, *Aspergillus* are widespread because they grow in soil and have the ability to decompose organic matter. For example, *Aspergillus niger*, which is common in a variety of soil types, can survive in soils that are highly alkaline to highly acidic (Atallah, 2022).

The ability to produce a variety of metabolites that characterize the genus *Aspergillus* has made it a subject of great research interest (Simões, 2023).

For example, *Aspergillus* cyanobacterium, which can cause mild disease in humans, is drought-resistant and, due to its physiological adaptations, can grow in a variety of environments (Cene Gostinčar, 2022).

Six metabolites were produced by incubating DHEA with *Aspergillus candidus* MRC 200634 for five consecutive days in a previously conducted experiment. DHEA was hydroxylated at C-6 β , C-7 β , C-7 α , C-11 α , and C-15 α (Yildirim, 2020).

As a result of the biotransformation of 7-oxo-dehydroepiandrosterone (1) by *Fusicum amygdala* AM258 resulted in the formation of a single metabolite. Preliminary analysis by mass spectrometry (MS) indicated that this product had a molecular weight 16 units higher than that of the parent material. Furthermore, in the ¹H NMR spectrum of the compound, no significant changes were observed, except for downward shifts of the methyl groups, especially at C-18, compared to the parent material (Paulina Łyczko, 2021).

In the pharmaceutical industry, lupin seeds play a significant role, as they are used to produce protein isolates with important functional and nutritional properties, as these isolates are important sources of protein used in human and animal nutrition. The fungal metabolite produced by *A. nilotica*. Spectroscopic analysis revealed bioconversion to flavonoids (3',4',7'-trimethoxyquercetin) and monoterpenes (4-methyl-p-menth-8-n-3-one), as well as increased production rates of active compounds that have applications in the pharmaceutical industry (Gamel, 2023).

Among the strains used for dehydroepiandrosterone (DHEA) bioconversion are *B. bassiana* strains KCh BBT and KCh J1. *B. bassiana* strain KCh J1 has been shown to selectively hydroxylate DHEA at the 7 α and 11 α positions, performing a hydroxylation at the C-7 position. *Beauveria caledonica* strains KCh J3.3 and KCh J3.4 have been used for DHEA bioconversion. Hydroxylate DHEA done efficiently by these strains in positions 7 α , 7 β , and 11 α also oxidize the hydroxyl group at position C-7 (Kozłowska E, 2020).

Van Tieghem is considered the first to describe *Aspergillus niger* in 1867. *Aspergillus niger* is an aerobic organism, requiring oxygen for growth and can grow on a wide variety of substrates. Its ability to produce various enzymes outside the cell has made it of great industrial importance.

Aspergillus niger is characterized by its versatility and the ability to convert a wide range of terpenoids (Igor A. Parshikov, 2014). One of the fungi commonly used in the experimental and industrial bioconversion of various organic compounds is *Aspergillus niger*, due to its ability to convert steroids at the 16 β position. For the bioconversion of dehydroepiandrosterone (DHEA),

androstenediol, and testosterone, *Aspergillus niger* strain KCH910 was used. According to previous studies, in vitro bioconversion of synthetic 5-ene steroids occurs through two metabolic pathways:

The first is oxidation by 3β -HSD to 4-ene steroids, and the second is minor allylic hydroxylation to 7-epimeric alcohols. Further conversion of 3-oxo-4-ene metabolites results in the non-selective conversion of 16-hydroxylation to non-selective 16-hydroxylation. *Aspergillus niger* also has the ability to introduce both 16β - and 16α -hydroxylation into steroids (Alina Świzdor, 2017).

Diterpenoids of various structures, including abitanane, atisane, pyran, chlorodane, curane, labdan, pimarane, stemodane, taxane, and triclopan, were investigated for their biotransformation. Specific biotransformation of the diterpenoid molinane was carried out using the fungi *Aspergillus flavus* and *Mucor circinelloides*. The resulting *Mucor circinelloides* culture yielded two new metabolites, identified as molin-11,13-diene-16,20-dioic acid (3) and 7a,13b-dihydroxy-molin-11-en-20-oic acid (4), based on their spectroscopic data (Stephanie G; Herrera-Canché, 2019).

Beclomethasone dipropionate (BDP)

Enzymes found in microorganisms, animal and plant cells, and other sources have been used to carry out reactions in chemically inaccessible sites of organic compounds. Numerous studies on the microbial transformation of steroids have also yielded the production of various hydroxylated derivatives, and they have also been used in the synthesis of steroid drugs.

Beclomethasone dipropionate (BDP) can be described as a corticosteroid medication containing steroids. Doctors prescribe it to treat bronchial asthma in patients requiring continuous treatment, and it is also prescribed to treat seasonal rhinitis. This medication can be administered orally, by nasal inhalation, or sometimes topically (Singh, 2016). The great importance of glucocorticoids, including BDP, in the pharmaceutical industry stems from their potent anti-inflammatory properties (S. Noetzlin, 2022).

Laboratory experiment

In this work, we will explain the results obtained from an experiment using the fungus *Aspergillus niger* to biotransform the steroid beclomethasone dipropionate. The focus will be on the microorganisms' metabolism of the drug and the identification of the chemical metabolites produced. Using techniques such as chromatography and spectroscopy, the study isolates and characterizes the resulting metabolites. These findings contribute to our understanding of the microbial transformation pathways of compounds and may aid in the production of new pharmaceutical derivatives or the improvement of drug metabolism properties (Madni, 2014).

Through the results of this experiment, we will clarify the metabolites resulting from this biotransformation and compare them with the results of biotransformation experiments for some steroids that were previously conducted.

MATERIALS AND METHODS

Beclomethasone dipropionate (1) was obtained from a commercial supplier. Thin layer chromatography (TLC) was conducted for analysis. Column chromatography (CC) was carried out using silica gel as the stationary phase. The ^1H and ^{13}C NMR spectra were recorded in CDCl_3 using a nuclear magnetic resonance (NMR) spectrometer. Chemical shifts (δ values) are measured in parts per million (ppm), and coupling constants are expressed. EI-MS data were collected using a JMS-600H mass spectrometer and are presented in mass-to-charge ratio (m/z).

The *Aspergillus niger* strain FCBP-764 was provided by institutions specializing in fungal cultivation. The medium required for the cultivation of *Aspergillus niger* was prepared by dissolving glucose (40 g), yeast extract (20 g), peptone (20 g), sodium chloride (20 g), KH_2PO_4 (20 g), and glycerol (20 ml) in four liters of distilled water (Hewa O. HAMAD, 2015). This mixture was then distributed into forty 250 ml conical flasks, placing 100 ml of the medium in each flask.

The fermentation process was employed during the second stage to investigate microbial transformation. In the first stage, using three 250-ml conical flasks, each containing 100 ml of sterile medium, liquid cultures were initiated by inoculating fungal spores from the mycelium onto Sabouraud dextrose agar. The cotton-lined flasks were then incubated using a rotating vibrator at 114 rpm for 6

to 9 days to stimulate fungal growth. In the subsequent second stage, small portions of the first-stage fungi were transferred to 40 new conical flasks, each containing 100 ml of sterile medium, where they were incubated for three days with the cotton-covered lids in place (Hao Cao, 2024). All purified samples were subjected to nuclear magnetic resonance (NMR) analysis to elucidate their structures.

RESULTS AND DISCUSSION

Four compounds (2-5) were produced by fermentation of beclomethasone dipropionate with *Aspergillus niger* for 12 days. (Figure 2) shows the structures of the intelligible biotransformation products of beclomethasone dipropionate (1) by *Aspergillus niger*.

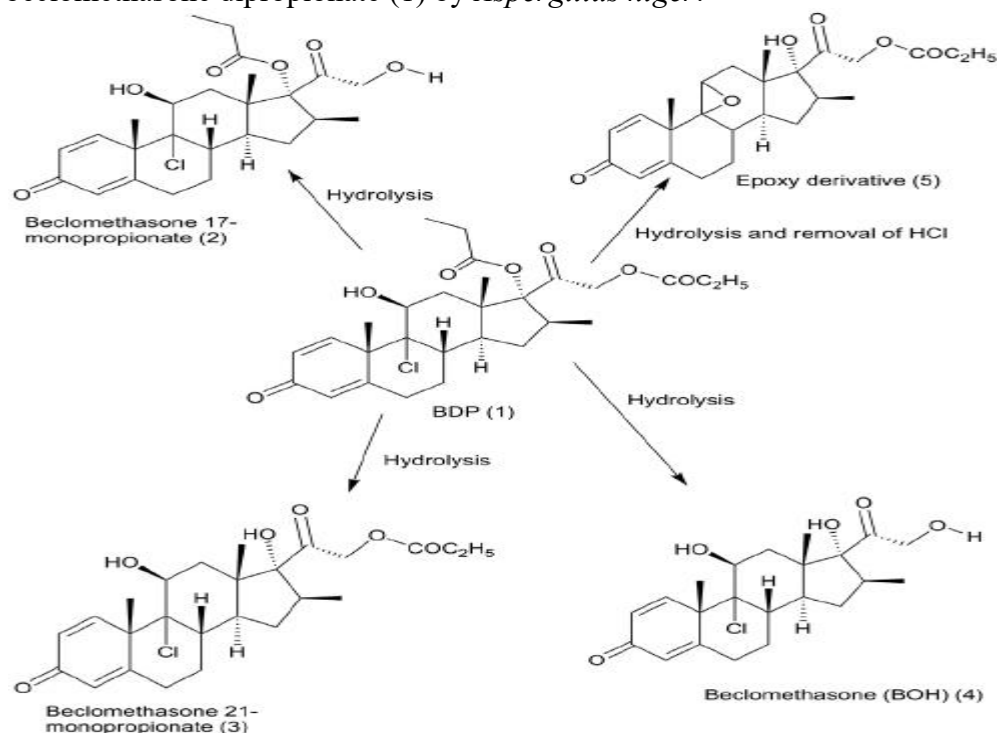


Fig. 2: Outcomes of the biotransformation of beclomethasone dipropionate (1) using *Aspergillus niger* (Madni, 2014).

Beclomethasone 17-Monopropionate (2), a colorless, amorphous solid with the molecular formula $C_{25}H_{33}ClO_6$.

A colorless, amorphous solid, beclomethasone 21-monopropionate (3), with the molecular formula $C_{25}H_{33}ClO_6$, was also produced. A colorless, amorphous solid, beclomethasone (BOH) (4), with the molecular formula $C_{22}H_{29}ClO_5$, was produced.

9 β ,11 β -Epoxy-17,21-Dihydroxy-16 β -Methylpregna-1,4-Diene-3,20-Dione 21-Propionate (5). By using preparative thin layer chromatography (TLC), this compound was purified and appeared as a colorless solid. Its molecular formula is $C_{25}H_{32}O_6$. Metabolite (5) has been identified as 9 β ,11 β -epoxy-17,21-dihydroxy-16 β -methylpregna-1,4-diene-3,20-dione-21-propionate.

In this biotransformation, multiple reactions were carried out in a single vessel, ensuring moderate conditions, even at room temperature. This process is described as green chemistry because no hazardous materials are used in the production of these metabolites. Due to the presence of hydrolase enzymes in the enzyme system of *Aspergillus niger*, the compounds can be degraded. During the synthesis, two reactions occurred: the ester was hydrolyzed and hydrochloric acid was lost to form an epoxide. Only compounds (2), (3), and (4) have been documented to have been metabolized by humans. The four resulting compounds were two monomers, beclomethasone 17-monopropionate (2) and beclomethasone 21-monopropionate (3), one epoxy compound (5), and one dipropionate derivative (4). Compound (4) was previously reported to be pharmacologically

inactive, (2) to be pharmacologically active, and (3) to be pharmacologically active. The resulting new metabolites are expected to have better anti-inflammatory activity and therapeutic properties, a higher level of safety and efficacy, and may be used as effectively as the parent compound in the treatment of asthma.

Numerous new bioactive fungal metabolites have been identified, exhibiting broad therapeutic properties, including anti-inflammatory, antibiotic, antioxidant, and antitumor effects. Drug discovery research has grown interest in fungal chemicals and their extracts, thanks to an integrated strategy combining computational, pharmacological, and molecular approaches (Shah, 2022).

The above experience highlights the efforts and capabilities that support the drug metabolism pathway while striving to discover promising new drug therapies.

In (Table 1) below we show the results of the biotransformation of some of the experiments that were conducted previously, which can be compared with the results of the experiment mentioned in this research.

Table (1): Some results of the biotransformation of steroids and steroid medications using *Aspergillus* species

Substrate	<i>Aspergillus</i>	Metabolite
DHEA	fungal cell culture of <i>Cephalosporium aphidicola</i> 143	3 β -hydroxy androst-4-en-17-one (144) (14%) 3 β , 4 β -dihydroxyandrost-5-en-17-one (145) (17.3%) (Mahwish Siddiqui, 2023).
DHEA	<i>Fusicoccum amygdali</i> (AM258)	3 β -hydroxy-17 α -oxa-D-homo-androst-5-en-7,17-dione (Paulina Łyczko, 2021).
DHEA	<i>Aspergillus candidus</i> MRC 200634	Dihydroxyandroste-5-ene (3 β ,17 β) β -Dihydroxyandroste-4-N-3-one (6 β ,17 β) -5-N-17-one 3 β ,11 α -Dihydroxyandroste 5-N-17-one 3 β ,7 β -Dihydroxyandroste 5-N-17-one 3 β ,7 α -Dihydroxyandroste β -Dihydroxyandroste-4-N-3-one,15 α ,17 (Kudret Yildirim, 2015).
DHEA	<i>Aspergillus versicolor</i> TJ1 MRC	6 β -hydroxyandrost-4-ene-3,17-dione 6 β hydroxyandrost-1,4-diene-3,17-dione (6 β OH-ADD) (Ewa Kozłowska, 2017)
Beclomethasone dipropionate	<i>Aspergillus niger</i>	3 β -hydroxy androst-4-en-17-one (144) (14%) 3 β , 4 β -dihydroxyandrost-5-en-17-one (145) (17.3%) (Mahwish Siddiqui, 2023)
DHEA	strain <i>Aspergillus niger</i> KCH910	5-ene and 4-ene steroidal molecules (Alina Świzdor, 2017).
A. nilotica	<i>Subolivaceus flavonoid</i>	(3',4',7'-trimethoxyquercetin) and a monoterpene (4-methyl P-menth-8-en-3-one) (Gamel, 2023)
mulin-11,13-dien-20-oic acid	<i>Aspergillus alliaceus</i> UI 315 and <i>Mucor circinelloides</i> ATCC	molin-11,13-diene-16,20-dioic acid (3). 7 α ,13 β -dihydroxy-molin-11-en-20-oic acid (4) (Stephanie G. Herrera-Canché, 2019).

The results shown in Table 1 emphasize the necessity for further research and experimentation to identify new metabolites. Four compounds were produced from the fermentation of beclomethasone dipropionate with *Aspergillus niger*. These findings reveal the potential of drug biotransformation as a viable alternative to traditional metabolite research, offering a pathway to develop new drug candidates more cost-effectively and with greater production efficiency. The high biotransformation capacity of *A. niger* strains positions them as suitable candidates for experimental studies, increasing the potential for developing new strains for biotransformation in fungal species.

Other findings, listed in (Table 1) above, highlight the importance of metabolomics in providing important insights into cellular pathways, biological mechanisms, and physiological states, making it a foundation for biomarker discovery and disease diagnosis. Advances in bioinformatics tools and technologies, along with advances in chromatography methodologies, have fostered innovation and the detection of large numbers of metabolites.

These results also highlight the critical importance of carefully selecting environmental conditions and strains when designing experiments involving secondary metabolism. The expression of secondary metabolites can be significantly affected by subtle changes in medium volume, light, temperature, pH, and nutrients. Furthermore, different strains of the same species do not always produce the same secondary metabolites, so it is important to know the growth conditions and commonly used media that are suitable for the fungus of choice. These results highlight the importance of adopting an appropriate strategy for studying secondary metabolites in fungi, plants, or bacteria, including a chemical approach, which identifies the full spectrum of metabolites produced to discover targets of interest, and a screening process to identify fractions of the metabolic extract that exhibit a specific biological activity.

CONCLUSIONS

Biotransformation of steroids is a key process for preparing new steroid derivatives that may have important pharmacological activities due to their high regional and spatial selectivity. In the experiment mentioned above, microbial biotransformation of beclomethasone dipropionate by *Aspergillus niger* led to the production of four metabolites. This experiment also highlighted the enzymatic potential of *Aspergillus niger*. Metabolite (5) was identified as 9 β ,11 β -epoxy-17, 21-dihydroxy-16 β -methylpregna-1,4-diene-3,20-dione-21-propionate after the purification of the compound. Given the importance of biotransformation processes, international regulatory frameworks exist for assessing a substance's potential for biotransformation in in vitro studies before licensing. Accredited international organizations have developed these guidelines. To date, several shortcomings have been identified in some studies, particularly regarding the variability and highly variable nature of results, which are often non-reproducible. A comprehensive understanding of the relevant transformation processes and specific changes still requires further research and development.

Therefore, the extensive and ongoing studies and efforts undertaken by researchers in this field are directed towards enhancing the biotransformation efficiency of steroids, believing that these efforts may lead to the development of metabolites beneficial to humanity.

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دراسة مقارنة حول التحول الحيوي لبعض الستيرويدات والأدوية الستيرويدية باستخدام أنواع مختلفة من فطر الرشاشيات

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الملخص

في الطبيعة، يُمكن القول بوجود أنواع عديدة من المُنتِجات، بما في ذلك تلك الموجودة في البكتيريا والنباتات والفطريات والحيوانات. ومن الحقائق العلمية أن التحول الميكروبي يلعب دورًا هامًا في التحول الحيوي للستيرويدات، مما يُتيح إنتاج منتجات انتقائية فراغياً. هدفنا الرئيسي في هذا البحث هو شرح وتوضيح مفهوم التحول الحيوي الميكروبي وأهميته في إنتاج جزيئات ذات خصائص تُعتبر مفيدة في صناعة الأدوية. كما نهدف إلى تسليط الضوء على أهمية التحول الحيوي في توليد مُستقلبات جديدة. علاوة على ذلك، سنُسلط الضوء على سمات وخصائص بعض فطريات الرشاشيات. لقد برهننا على التحول الحيوي لبعض الستيرويدات مثل DHAE والأدوية الستيرويدية بواسطة بعض أنواع الرشاشيات مثل الرشاشيات البيضاء والرشاشيات السوداء. ولتوضيح النتائج وكيفية استقلاب هذه الفطريات لركائزها، سنستخدم تجربة أُجريت سابقاً، ثم سنُقارن نتائج التجربة بنتائج تجارب أخرى سابقة. وفي الختام نأمل أن يكون هذا العمل مفيداً للطلبة والباحثين في مجال الكيمياء الحيوية.

الكلمات الدالة: التحول الميكروبي؛ الستيرويدات؛ ديبروبيونات بيكلوميثازون (PDB)؛ الرشاشيات الزرقاء؛ الرشاشيات السوداء.