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**Review Article**

# The Importance and Techniques of Steroids Biotransformation: A Review

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**ABSTRACT**

Steroids are important compounds in living systems, and widely available in nature. These compounds are made up of various sterols, bile acids, and steroid hormones. Steroid compounds are among the most widely marketed pharmaceutical products. The benefits of biotransformation include the synthesis of novel compounds that cannot be synthesized using chemical tools, high material recovery and yield, low energy consumption, and eco-friendly processes. Microbial transformation techniques are increasingly being used to structurally modify natural and synthetic compounds. Since the early 1950s, several steroid metabolic transformations have been identified, along with the first-time identification of microbial hydroxylation. This work aims to provide an overview of steroid biotransformation, including the various types. This theoretical research will be valuable to students and researchers in the field of steroid biotransformation.

**Keywords:** Steroids biotransformations, microbial biotransformation, biotransformation techniques.

## INTRODUCTION

The natural world is filled with steroids, steroids are present in animals, plants, fungi, and some bacteria. These small organic molecules are vital for the normal growth and development of living organisms. Natural steroids, such as cholesterol, are important components of cell membranes and significantly contribute to their fluidity and integrity. Additionally, steroids play a crucial role as valuable hormones (Zhao *et al*., 2023).

Intensive efforts in this field began in 1950, starting with the discovery of the pharmacological effects of the hormone's cortisol and progesterone. Both of these hormones are considered natural stimulants, and their findings were published. In addition, identification of the 11-hydroxylation actions of a species of black bread mold *Rhizopus* was considered a crucial step in the practical synthesis of steroids with useful biological activity. Since then, many microbial biotransformations of steroids and sterols have been reported, focusing on the hydroxylation of steroids and the dehydrogenation and cleavage of the sterol side chain (Fernandes *et al*., 2003).

At the present time, industrialists, researchers, and specialists can produce large and diverse quantities of steroids and their by-product using various biotransformation reactions and integrating them with chemical processes. The demand for isolating, purifying, and settling pure enzymes has been largely eliminated, as it has become unnecessary. Furthermore, the cost and effort involved in manufacturing steroids using whole cells have decreased (Nassiri-Koopaei and Faramarzi, 2015).

In studies of microbial steroid transformation, fungi are extensively used. For instance, a diverse set of their multipurpose enzymes can convert a wide range of steroid (Hosseinabadi *et al*., 2015).

## MATERIALS AND METHODS

To demonstrate related concepts using an experience, dehydroepiandrosterone, also known as (DHEA), it was incubated with Aspergillus glaucus MRC 200914 to investigate how this fungus metabolizes substrate. The medium was prepared for the fungus in 1L of distilled water. The intermediate was uniformly distributed in 10 Erlenmeyer flasks and sterilized using an autoclaving. After sterilization, these water flasks were inoculated with the fungus. The flasks were incubated for a five-dad period. After incubation, the fungal mycelium was separated from the broth by filtration under vacuum. The fungus was washed with ethyl acetate. Then, chromatography was conducted on silica gel 60. As a result of this experiment, 3β,11α-dihydroxyandrost-5-ene-17-one and 3β,7α-dihydroxyandrost-5-ene-17-one were obtained by chromatography. In addition, the structure of these compounds was confirmed by comparing their melting points, NMR spectra, and infrared spectra. The description of this experiment reflects the concepts, definitions, and methodologies that will be discussed later in this research.

## RESULTS AND DISCUSSION

Based on the results of the experiment mentioned above, it is essential to define and clarify the following concepts related to the biotransformation of steroids. These concepts represent the inputs, processes, and outputs of the experiment.

**Steroids**

One of the key facts about to steroid compounds is their bountiful presence in living organisms and has a basic role in the vital activities of these organisms (Silva and Salvador, 2020). The basic structure of steroids depends on the four-ring cyclopentanoperhydrophenanthrene nucleus (steroid nucleus; Fig. (1)) (You, 2004).

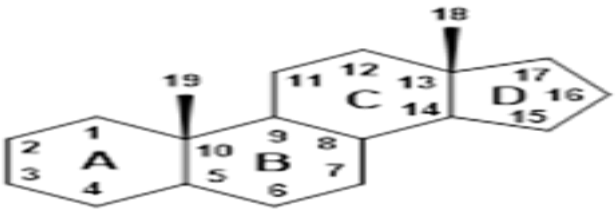


**Fig. 1: Cyclopentanoperhydrophenantrene (Greaves *et al*., 2014).**

All steroids share a unique molecular structure, as it consists of 17 carbon atoms organized in four rings linked to 28 hydrogen atoms. In general, it has been agreed to use the letters A, B, C, and D to denote these rings. The methyl groups attached at positions C13 and C10 are labeled 18-CH3 and 19-CH3, consecutively, the alkyl substituents at C17 mode the side chain for steroid (Wang and Abe, 2024). As the 18- and 19-methyl groups are positioned above the plane of the steroid skeleton, they unite to form the β-configuration. Thus, the substituents or atoms placed above this plane also have β-configuration, while those below it has α-configuration (Silva and Salvador, 2020). Natural steroids, e.g., cholesterol, handle as imperative components of cell membranes, contributing to their fluidity and stability. Additionally, steroids function as escential hormones, encompassing sex hormones in mammals, growth governors such as brassinolide in plants, and ecdysone in insects (Zhao *et al*., 2023). It is also necessary to point out here the importance of cholesterol as a raw material that plays a major role in the production of well-known biological products, including bile acids, steroid hormones, and vitamins D (Kim *et al*., 2006).

Since steroids are known to have special properties because of their interactions with enzymes and receptors, even minor alterations in their structure can have a big impact on biology. Many diseases require the use of natural and synthetic steroids as essential treatments. Because of these numerous qualities, including their anti-inflammatory, anti-allergic, anti-tumor, anti-viral, and anti-bacterial steroids are one of the most familiar medication classes (Kozłowska *et al*., 2017).

**Biotransformations**

Microorganisms have been very important socially and economically throughout human history. Even before ancient humans aware of their existence, they were utilized in the production of beverages and foods. Historical sources have mentioned that beer was made in the Sumerian and Babylonian civilizations since 6000 BC and references to winemaking and also found in the book of genesis, where some references were made in this regard. However, although the knowledge of producing chemicals such as alcohol and organic acids through fermentation is relatively recent, the ancient Egyptians are known to have used yeast to bake bread (Vasic-Racki, 2006).

Chapman showcased various early industrial fermentation processes for organic chemicals in 1921. Over time, he found that microorganisms could be used to alter specific compounds using simple, well-defined chemical reactions catalyzed by enzymes. These processes are now known as "biotransformations" (Vasic-Racki, 2006).

When studying the biotransformation of steroids, it was noted that the capability to degrade the steroid structure is not restricted to a specific group of microorganisms, but previous reports indicated the presence of this feature in bacteria, yeasts, algae, actinomycetes, and even protozoa (El-Menoufy *et al*., 2024).

**Microbial biotransformation**

The synthesis of new drugs and various intermediates has greatly benefited from the microbiological transformations of steroids. These transformations allow chemical functions such as hydroxylation, reduction, condensation, Baeyer–Villiger oxidation, Michael addition, and condensation-to be performed at various sites along the steroid structure, a phenomenon which was extremely difficult to conventional chemical techniques. It is now possible to selectively hydroxylate the steroid structure (Arturo *et al*., 2019).

The microbial transformation processes have several important traits. First, since the reaction conditions are moderate and the microbial transformation conditions are external and suitable for the growth and metabolism of microbes, the best temperature and pH are chosen during the conversion process. Secondly, the microbial transformation process is selective, and an enzyme's substrate specificity means that it can only interact with one matrix and be selective for the spatial structure. Third, the biotransformation process follows a concise reaction cycle due to the autotrophic metabolism of microorganisms that are fast-growing and have a short lifespan (Hailin, 2019).

Fourth, the low cost and relatively environmentally friendly results of the process are advantageous. Additionally, the product is completely specific and does not need to be separated, extracted and purified repeatedly. The need for heating, cooling, and pH correction is reduced during the microbial conversion process. This means that compared to other chemical conversion processes, microbial conversion technology reduces production costs and environmental pollution (Hegazy *et al*., 2015).

**Types of microbial transformations of steroids**

Active microorganisms form enzymes that facilitate the microbial transformation of steroids. These enzymes alter the steroid nucleus in several ways (El-Menoufy *et al*., 2024). The major sites of biotransformation reactions on a steroid molecule are shown in Fig. (2).

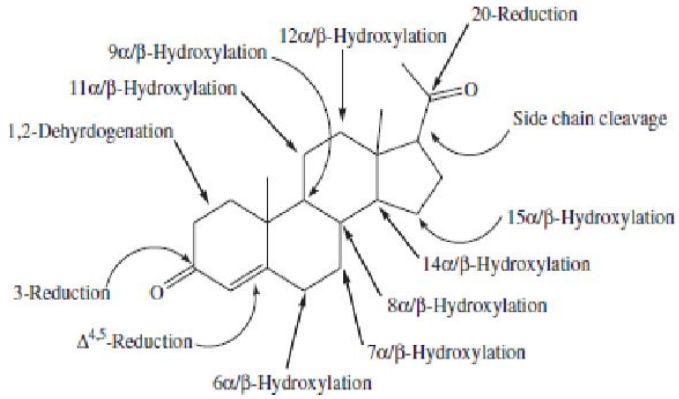


**Fig. 2: Major sites of biotransformation reactions (El-Menoufy *et al*., 2024).**

**Hydroxylation**

One of the most common reactions that involved in microbial steroid conversion is hydroxylation. This enzymatic process has been widely used to produce intermediates for many steroid drugs. Peterson and Morey first described this process of microbial conversion in 1952. It offers the potential to significantly reduce the cost of steroid drug production by replacing multiple complex chemical synthesis steps (Tong and Dong, 2009). Microorganisms such as aspergillus and rhizopus are frequently employed for hydroxylation (Sedlaczek and Smith, 1988).

The biological activity of hydroxylated steroids is typically greater than that of their non-hydroxylated counterparts, which are less polar. For example, when the 7α-hydroxy derivatives of DHEA were compared with non-hydroxylated DHEA, they showed significantly greater immunomodulatory and immunoregulatory properties (Shah *et al*., 2013).

**Dehydrogenation**

Vischer and Wettstein noted Fusaria's role in the dehydrogenation of steroid ring A at C1-2 position for the first time in the 1953s (Singh *et al*., 1963). Dehydrogenation is a process that reduces the number of hydrogen atoms in organic molecules, and it is considered one of the most common processes in biotransformation.

**Reduction**

17 β-reductase catalyzes a significant and widespread reaction in microbial steroid metabolism. It is involved many microbial steroid transformations and plays a key role in regulating the equivalent reduction pool and in detoxifying exogenous steroids (Donova *et al*., 2005).

**Side-chain cleavage**

Compared to unsaturated sterols, removing side chains from saturated sterols by chemical means, is more difficult and hence the great interest in the microbial method (Jabbar *et al*., 2017).

**Biotransformation techniques**

In biotransformation, whole-cell systems and isolated enzymes are the most common methods used. In using whole cells instead of isolated enzymes for biocatalytic purposes, there is no need for cell lysis or enzyme purification, which naturally reduces cost (Lin and Tao, 2017).

For these processes, integrated cells are used, and it is believed that fungi are commonly utilized to obtain natural metabolic products and initiate biotransformation reactions. Isolated enzymes are used to convert molecules in many industrial fields, as they are best suited for simple catalytic procedures (Bianchini *et al*., 2015).

**The importance of steroid biotransformation**

Many endocrine and cardiovascular diseases have been treated using extracts of animal glands, this was achieved in the early twentieth century. Historically, the discovery large scale production of steroids is considered two of the most important medical developments of the last century (Zhao *et al*., 2023). Several hypotheses have been proposed, including one suggesting that the chemical components derived from animal glands possess significant physiological and pharmacological properties. Additionally, efforts have been made to extract steroid hormones efficiently from animal tissues. The significant contributions of German scientists during that era of discovery were pioneering in the field of determining the activities of bile acids, vitamin D, and sex hormones. Consequently, the demand for more efficient and eco-friendly steroid manufacturing way is increasing day by day.

In discussing the sectors of the pharmaceutical industry, it is clear that steroids have become one of the major sectors in this field. The great demand for steroid compounds with biological activity, along with the limitations of the availability of their sources, has led to the enhancement of efforts and research to find effective, low-cost ways to synthesize them. It is important to note that in the 1950s and 1960s, several microorganisms that catalyze the hydroxylation, hydrogenation, dehydrogenation, and carbon-carbon bond cleavage of steroids were discovered, many of which were subsequently incorporated into industrial processes; hydroxylation at C-11 position was particularly of great commercial importance (Žnidaršič-Plazl and Plazl, 2010).

## CONCLUSIONS

This theoretical research is aimed to emphasize the importance of microbial steroid biotransformation as a valuable biochemical tool for producing novel and environmentally friendly derivatives for research and industrial purposes. Microbial steroid transformation is increasingly being used as a practical method for structurally modifying both natural and synthetic compounds.

## ACKNOWLEDGEMENT

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# أهمية وتقنيات التحول الحيوي للستيرويدات: مراجعة

**رغدة محمود عبد الغفور العاني**

ماجستير كيمياء حيوية/ جامعة سكاريا/ معهد العلوم الطبيعية/ سكاريا/ تركيا

**الملخص**

الستيرويدات هي مركبات مهمة في الأنظمة الحية، ومتوفرة على نطاق واسع في الطبيعة. تتكون هذه المركبات من أنواع مختلفة من الستيرولات، والأحماض الصفراوية، والهرمونات الستيرويدية. تعد المركبات الستيرويدية من بين المنتجات الصيدلانية الأكثر انتشارًا في السوق. تشمل فوائد التحول الحيوي تخليق مركبات جديدة لا يمكن تصنيعها باستخدام أدوات كيميائية، واستعادة المواد العالية والعائد، وانخفاض استهلاك الطاقة، والعمليات الصديقة للبيئة. تُستخدم تقنيات التحول الميكروبي بشكل متزايد لتعديل المركبات الطبيعية والاصطناعية هيكليًا. منذ أوائل الخمسينيات من القرن العشرين، تم تحديد العديد من التحولات الأيضية الستيرويدية، جنبًا إلى جنب مع التعرف لأول مرة على الهيدروكسيل الميكروبي. يهدف هذا العمل إلى تقديم نظرة عامة على التحول الحيوي الستيرويدي، بما في ذلك الأنواع المختلفة. سيكون هذا البحث النظري قيماً للطلاب والباحثين في مجال التحول الحيوي الستيرويدي.

**الكلمات الدالة:** التحولات الحيوية للستيرويدات، التحول الحيوي الميكروبي، تقنيات التحول الحيوي.