Application of Steroidal Bio-Biotransformation in the Pharmaceutical Industry

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Abstract: Steroidal compounds have important physiological and pharmaceutical effects, and the market demand is huge. The steroidal compounds and their key steroids are prepared by microbial transformation, and are gradually applied to chemical synthesis methods with regional stereoselectivity, shortening synthesis steps, shortening production cycle, increasing yield and eco-friendliness. The catabolic mechanism needs to be further explored and determined. This paper summarizes the structure types and main sources of steroids, physiological functions, microbial transformation and catabolism mechanism, focusing on the key enzymes in the catabolism of steroids and their molecular mechanisms. Provide reference for the development of strains and the construction of engineering bacteria and the development of microbial transformation industrial production processes.

1. Introduction

The market demand for steroids in the pharmaceutical industry is second only to antibiotics. The global total output value has reached 40 billion yuan. More than 400 licensed drugs are in circulation and future demand will increase [1]. Most steroidal drugs are structurally modified and chemically synthesized using natural products with a steroidal backbone. As an important hormonal drug, steroidal drugs play an important role in regulating the body and have developed into anesthetics and antiarrhythmic drugs. Antibacterial, anticholinesterase, anticoagulant, antifungal, antitumor, antibiotic, bile secretion, diagnosis, neuromodulation blocker, gallstone dispersant, hemostatic agent, calcium regulator, lipid regulator, neurological agent , laxatives and sedatives [2]. Steroids are initially extracted from the adrenal glands of animals, and it is clear that the actual application is not important. Later, some researchers tried to explore the chemical synthesis process using small organic molecules as raw materials. Although theoretically feasible, due to the long synthetic route, low yield, poor reactivity, difficult processing of by-products, and environmental pollution problems, the value of industrial production is seriously insufficient [3]. Industrial production of steroidal drugs mainly includes chemical synthesis methods and microbial transformation methods. Microbial transformation refers to the use of enzymes produced by microorganisms to act on a group of compounds and convert them into new, structurally similar and valuable products. This reaction is essentially an enzymatic reaction with strong properties. Uniformity and chiral selectivity can make up for the lack of chemical synthesis.

2. Microbial Transformation Technology and Application

Microbial transformation refers to the process of converting a particular substrate to another specific product using an enzyme system of the microbial cell. For example, resveratrol (product) can be prepared by microbial transformation of resveratrol (substrate), and can be converted to dihydrocurcumin and tetrahydrocurcumin by microorganisms using curcumin as a substrate. The process by which a microorganism converts a substrate into a product utilizes the life activities of the microbial cell itself, primarily a biocatalytic microbial cell or one or a series of enzymes outside the cell. Microbial transformation is the branching direction of large-scale organic chemical synthesis, and its application in the modification of natural product structures is more and more extensive. The microbial transformation method has the following characteristics:

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First, the reaction conditions are mild, efficient, environmentally friendly, three-dimensional selection, mild reaction conditions, and simple and easy to operate equipment. In general, microorganisms undergo metabolic activities under normal temperature and atmospheric conditions. The conditions for microbial transformation are external conditions suitable for microbial growth and metabolism, so the optimal temperature and pH chosen during the conversion process will be mild. At the same time, relatively mild reaction conditions do not easily cause chemical bond cleavage of the target product, and the complex structure of the natural compound molecules can maintain chemical structural stability during the conversion process.

Second, the microbial transformation reaction is specific, and the substrate specificity of the enzyme plays a role in the microbial transformation process, that is, it has a reaction to only one or one matrix and is selective to the stereostructure. The group and the reaction zone. Therefore, there is no need to protect and deprotect the substrate.

The third is that microbial transformation utilizes the self-metabolism of microorganisms, and the reaction cycle of biotransformation is very short. Generally, microorganisms grow rapidly and have a short life span. The target substance conversion reaction is carried out during the growth cycle of a certain stage of the microorganism, so that when the microbial cells age or die, the conversion reaction is substantially completed.

The fourth is lower cost and relatively friendly to the environment. The product is also relatively specific and does not require repeated separation, extraction and purification of the product. During the microbial conversion process, operations such as heating, cooling, and pH adjustment are reduced. Therefore, microbial conversion technology can reduce environmental pollution and reduce production costs compared to other chemical conversion processes.

3. Microbial Transformation and Catabolic Mechanism of Terpenoids

3.1. Indole compound conversion microorganisms

Steroid microbial transformation is the use of microbial cell enzymes to chemically react specific parts of a steroidal compound to produce a new product. Microorganisms are likely to convert atoms or groups at many points in the steroidal compound[4]. The types of reactions involved are oxidation, reduction, hydrolysis, esterification, condensation, isomerization, rearrangement and side chain degradation. The main sites of the hydroxylation reaction are C9α, C11α, C11β, C15α, C16α, C16β, C17α, C19, C26. The dehydrogenation reaction usually occurs between C1, 2 and C4, 5 of the A ring, the aromatization reaction mainly occurs on the A ring, and the epoxidation reaction generally occurs between C9, 11 and C14, position 15. The double bond reduction reaction generally occurs between the A ring C1, 2, C4, 5 and B ring C5, 6 positions. Keto group reduction is mainly at the C3, C17 and C20 positions, and common sites for the oxidation of hydroxyl groups to ketone groups are C3α, C3β and C17α. Most studies on the conversion of steroids involve hydroxylation, dehydrogenation and side chain degradation. Steroid-converting microorganisms include bacteria, actinomycetes, yeasts and molds.

3.2. Steroidal biotransformation type

3.2.1 Hydroxylation

Hydroxylation refers to the reaction of introducing a hydroxyl group into an organic molecule, and modifying the steroid composition by hydroxylation generally affects its pharmacological activity. Conventional organic chemical synthesis methods are difficult to carry out such direct hydroxylation reactions. However, in biotransformation, hydroxylation of this C-H bond is a very common and important biotransformation reaction. Microbial hydroxylation of compounds with steroid nucleus such as estradiol, pregnane and androstane usually occurs at specific locations. Hydroxylation is often achieved by microorganisms such as Rhizopus, Aspergillus, and Streptomyces[5]. Modification of steroids by microbial hydroxylation of different sites in the steroid nucleus produces a variety of steroid derivatives that can also alter the biological activity of steroids.

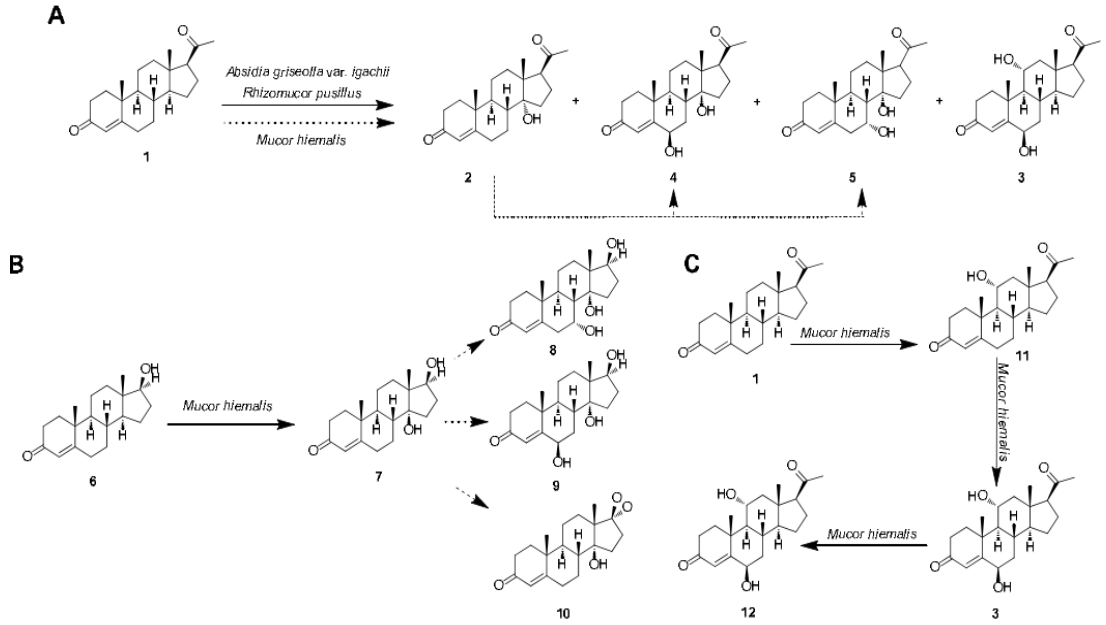

Fig.1. Biotransformation of progesterone and testosterone

3.2.2 Carbonylation

Carbonylation The reaction of introducing a carbonyl group into a complex organic molecule. Although it is also a very common biotransformation reaction, it is less in steroid compounds and is involved in the biotransformation of steroids. In the material process, the carbonylation reaction is usually accompanied by a hydroxylation reaction. Biotransformation of metestosterone using Macrophomina phaseolina produces a new metabolite, 1α-methyl-17β-hydroxy-5α-androst-3,6-dione (50), such as figure 2.

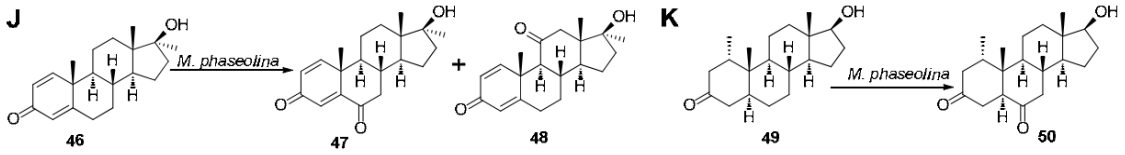

Fig.2. Biotransformation of methyl androstenone and metestosterone

3.2.3 Hydrogenation

Hydrogenation This is a reduction reaction that is the reaction of organic compounds with hydrogen molecules and is of great value in biotransformation research. In the microbial transformation, a substrate having a carbonyl group or a double carbon-carbon bond structure can be subjected to a hydrogenation reaction by a microorganism. Microbial hydrogenation can be reduced in the ketone group to the hydroxyl group, mainly to reduce the ketone group in the national C-3, C-20 and C17, while the simultaneous hydrogenation of the steroid double bond usually occurs in the ring to reduce the double bond C of A. -5, C -6 rings C-1, C-2 and C-4, C-5 and B differ from the hydroxylation of steroids by biotransformation, and studies on biotransformation rarely achieve hydrogenation of steroid structures. However, as another important means of structural modification, the modification of hydrogenation is also important.

3.2.4 Dehydrogenation

Dehydrogenation, in contrast to hydrogenation, is a process that reduces the number of hydrogen atoms in organic molecules, which is common in chemical processes but is also common in biotransformation processes. Microbial dehydrogenation of steroids usually occurs between C-1, C-2 and C-4, C-5 ring A, and small amounts of dehydrogenation can also occur in the hydroxyl group, while steroid dehydrogenation helps to improve and improve pharmacology. Reduction in activity

and toxic side effects [6].

3.2.5 Baeyer-Villiger oxidation and bromination

The steroidal compound is oxidized by Baeyer-Villiger, and after the oxygen atom is placed near the carbonyl group, the corresponding azlactone is produced. Steroids have been shown to possess a variety of anti-cancer biological activities, while they inhibit androgen-dependent diseases by blocking the testosterone pathway in 5α-dihydrotestosterone to treat androgen-dependent diseases[7]. Steroid testosterone inhibits steroid aromatase and can be used to treat breast cancer[8]. Regarding the bromination reaction, steroid biotransformation is small, and the main application is also focused on the degradation of harmful substances.

4. Application of Microbial Transformation in New Drug Fields

Since the 1950s, with the advancement of science and technology, detection methods including mass spectrometry, nuclear magnetic resonance and infrared ultraviolet light have been significantly improved, and the application of microbial transformation technology in new drug molecules has begun[9]. Research on microbial transformation technology has received increasing attention. Microbial transformation technology can effectively solve the problem of new structural changes of drug molecules. Microbial transformation technology has become an important way to change the molecular structure of drugs. Active natural compounds such as antibiotics, steroids, hormonal drugs and alkaloids are particularly suitable. There are three aspects to implementing microbial transformation techniques to change the molecular structure of a drug:

4.1. Develop new molecular structure drugs to enhance resistance to drug resistance

The use of microbial transformation technology to acquire new natural products is critical to the development of new drugs. Under the action of Penicillium simplissimum, artemisinin can be converted to 9-acetoxy artemisinin, the first new artemisinin analog construct. In addition, Rhizopus chinensis can convert progesterone to hydroxycortisone, which can be as high as 90%[10].

In addition, increasing drug resistance is one of the main goals of changing the structure of drugs. The reason for drug resistance is that due to the long-term effects of the same drugs, pathogenic bacteria, viruses and tumor cells in the body will have a significant reduction in the efficacy of the corresponding drugs having the same structure. For example, one of the main factors in the failure of cancer treatment is that cancer cells are resistant to drugs such as taxis. Zou and others used microbial transformation of the taxa and found that Ginkgo biloba can oxidize the taxonomic group, and the resulting polyoxane reduces the resistance of tumor cells[11].

4.2. Reduce toxic side effects

One of the difficulties in clinical application of drugs is that their toxic side effects are very large. Modification of certain drug groups by chemical modification is a common method of reducing side effects, but chemical modifications are often not specific and require detention. The group that you do not want to modify after the modification is completed, the protected group is deleted, and the steps are serious. Microbial transformation technology makes drug reorganization easier. For example, a broad-spectrum antibiotic combination has strong antibacterial activity and has kanamycin and gentamicin activity, but the ototoxic damage to the human body is much lower than that of gentamicin. The mixed mode was obtained by transforming the structural modification of Micromonospora and using kanamycin as a substrate. As another example, Tripterygium wilfordii is a natural ingredient used in the treatment of rheumatoid disease, but its renal toxicity is limited, which limits its clinical application. Zhao et al. They used the fashionable Xiaoyan Yinhan to convert Tripterygium into lower nephrotoxic Leiden tree, which promoted clinical application[12].

4.3. Improve water solubility and bioavailability

Many clinical drugs fail to exert their original pharmacodynamic activities, and their activity is affected by their low bioavailability. Water composition is a major factor affecting the bioavailability

of drugs. Therefore, one of the main problems in the development of new drugs is the increased water solubility of the drugs, thereby increasing their bioabsorbability. Hydroxylation and glycosylation of drug structures are the most important ways to increase their solubility in water. For example, the strain streptomyces ambofaciens is capable of acetylating tylosin molecules, and the acetylated product has improved water solubility, thereby increasing the bioavailability of tilosin. Natural macromolecules have a variety of biological activities, but low water solubility limits the development and utilization of active molecules. Yang Yabo et al. used the galactosidase of Escherichia coli to modify the glycosylation of paclitaxel, and the obtained product not only retained the original pharmacodynamic activity, but also greatly improved the water solubility. For example, when the polyphenol composition is contacted by a human body, it is decomposed by an enzyme produced by the human intestinal flora, and can be converted into a small molecular substance that is most easily absorbed by the human body, such as phenolic acid, that is, the bioavailability of the polyphenol is improved. .

5. Conclusion

Microorganisms play an important role in the process of steroids, introducing advanced bio-production concepts in steroid manufacturing to improve resource use, reduce energy consumption, and achieve sustainable green development. Therefore, further revealing the key enzymes of genomics, proteomics and transcriptomics and their catalytic mechanisms not only have important theoretical significance, but also have important industrial value. In one aspect, further studies and steroid-based HMP catabolic pathways, 4-AD pathways demonstrate previous studies of microbial degradation, ADD trails, road 9 jurisdiction, HMP road slopes and enzymes, and genetic means by engineering other bond mechanisms, In addition to the production of knockouts from the construction of new intermediate bacterial engineering drug steroids from a single gene, reaching 4-AD, ADD, 9 jurisdiction, DHEA, HMP and other key steroid drug products, the conversion rate is high, and the separation of cracked products is achieved. Cleansing shrinkage, almost all steroid hormones can be based on 4-AD or ADD for synthesis, new enzymes or chemical process development. On the other hand, a new system for expressing a transformed microbial sequence gene and a clone constructing engineered strain by transcriptome analysis and proteomic analysis by using the existing structure and function of the transformed microorganism or enzyme is further used to further use an enzyme having high catalytic activity. Mutations show that enzymatic engineering techniques and enzymatic conversion of steroid drugs result in incomplete chemical synthesis reactions and overcome the lack of chemical synthesis zones and by-products of stereoselective production of level-shifted microbial cells, drug steroid growth, whereas Residual activity of harmful chemicals in the human body.

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