[P-C.11]

Combined chemical and microbiological synthesis of exemestane from sitosterol

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Steroid hormones obtained by enzymatic conversion of cholesterol are natural regulators of many essential physiological processes. These hormones are hydrophobic compounds which are capable of crossing membrane barriers of the cell and affecting transcription of various genes, related to physiologically optimal or pathological processes, by binding to the corresponding receptors Levina, 1998

Some synthetic analogs of the endogenous steroid hormones, obtained by chemical or microbiological transformation of natural phytosterols, can also act in the same way. Therefore the study of the fundamental nature of the relationships between functional activity of steroid drugs and their structure are extremely actual.

Hormone-dependent malignant tumors such as breast or ovary cancers are caused by the disturbance of sex hormone synthesis leading to the excessive accumulation of estrogens. This process is caused by the excessive activity of enzymatic complex (aromatase) which catalyzes multistep conversion of Δ^4-3-keto androgen–androst-4-ene-3,17-dione (AD) to phenolic estrogens.

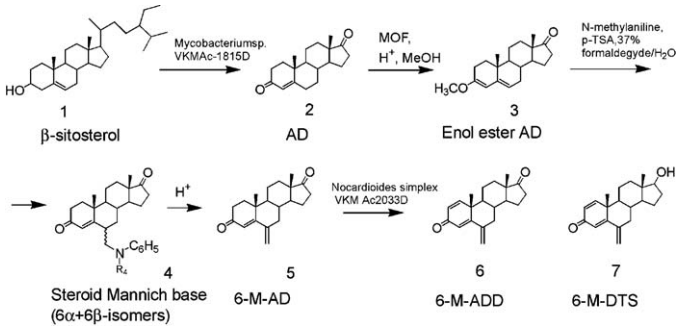
The most prominent irreversible aromatase inhibitor is 6-methyleneandrosta-1,4-diene-3,17-dione (6-M-ADD) which is used as anticancer drug under the trade name Aromazine (INN: exemestane) Giudici et al., 1988.

Exemestane is usually obtained by the 1,2-dehydration of 6-methyleneandrost-4-ene-3,17-dione (6-M-AD) by chemical dehydrogenation agents or microorganisms acting as biocatalysts.

Here we present the preparation of exemestane from sitosterol by the combined chemical and microbiological process (Scheme 1) which includes:

1. microbiological oxidative degradation of sterol side chain yielding AD;
2. chemical C_6-methylenathion of AD;
3. microbiological 1,2-dehydrogenation of 6-M-AD.

Oxidative cleavage of sitosterol (1) to AD (2) was mediated by *Mycobacterium* sp. VKM Ac-1815D by the known method Olga et al., 2002. Introduction of methylene group to C_6-position of AD was performed by the known reaction sequence Longo and Lombardi, 1991 including the acid-catalyzed condensation of AD enol ether (3) with formaldehyde and N-methylaniline. The reaction was conducted in aqueous aliphatic alcohol with 37% aq. formaldehyde solution and p-toluenesulfonic acid as catalyst. The obtained unseparated mixture of 6α- and 6β-epimers (4) was transformed to 6-M-AD (5) and deaminated by conc. mineral acid. The intermediates in the process were isolated and 6-M-AD (5) was obtained with 80-82% yield from AD.



Scheme 1. Combined chemical and microbiological synthesis of exemestane from sitosterol.

The peculiarities of 6-M-AD 1,2-dehydrogenation with the formation of 6-M-ADD were studied. The culture of *Nocardioides simplex* VKM Ac-2033D with 4-ene-3-oxosteroid:(acceptor)-1-ene-oxidoreductase (3-KSD, EC 1.3.99.4) Fokina et al., 2003 was used as biocatalyst.

We also tested the effect of the composition of growth and transformation media, cultivation conditions of *N. simplex*, induction of enzyme synthesis, and the method of substrate introduction. Selective (92-95%) accumulation of 6-M-ADD with substrate loads up to 100 g/L was achieved under optimized conditions. The content of the product with C_{17}-keto reduced group: 6-methylene-1,2-dehydrotestosterone (6-M-DTS, 7) was less than 1-3%.

The proposed microbiological method of exemestane (6-M-ADD) preparation is a significant improvement over the known method which is based on the two-phase system, containing water-immiscible organic solvent Krook and Hewitt, 2001.

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Production and purification of chitinase by *Metarhizium anisopliae* Isolated from soil

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The use and production of enzymes have become an area of major interest of the biotechnology industry due to the search for the replacement of conventional chemical processes by enzymatic processes, the irreversible trend of prevalence of environmental policies. Chitin is a biopolymer found in the cuticle of insects and crustaceans and in cell walls of fungi. The enzyme responsible for hydrolysis of chitin is the chitinase (EC 3.2.1.14) that is involved in a series of biological mechanisms of defense, digestion of nutrients among others. Among the bacteria that produce the chitinase *Metarhizium anisopliae* has been used on industrial scale. This study