🕮 BIOCONVERSION OF 6-(N-METHYL-N-PHENYL)-AMINOMETHYL-ANDROSTANE STEROIDS BY THE NOCARDIOFORM ACTINOBACTERIAL STRAIN

Galina Sukhodolskaya[1], Victoria Fokina[1*], Andrei Shutov[1], Vera Nikolayeva[1], Tatiana Savinova[2], Yuri Grishin[2], Alexey Kazantsev[2], Nikolay Lukashev[2], and Marina Donova[1]

[1] G.K. Skryabin Institute of Biochemistry & Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow region, Russian Federation, [2] Faculty of Chemistry, M.V. Lomonosov Moscow State University, Moscow, Russian Federation
E-mail: vvfokina@rambler.ru

The N-methyl-N-phenyl-aminomethyl functionality is one of the most efficient substituents which significantly changes steroid features and may influence different physiological activities. Except for some aminosteroids (1), nitrogen-containing androstanes have not been strongly investigated as the substrates for bioconversion.

Nocardioform actinobacterial strain Nocardioides simplex VKM Ac-2033D is an effective microbial catalyst capable of 1-dehydrogenation of a variety of 3-ketosteroids. We report here an application of N. simplex cells for 1-dehydrogenation of newly synthesized α/β-diastereomers of 6-(N-methyl-N-phenyl)-aminomethyl-androst-4-ene-3,17-dione (6-MPhAM-AD) and 6-(N-methyl-N-phenyl)-aminomethyl-androst-4-en-17β-ol-3-one (6-MPhAM-T) in comparison with their unsubstituted analogs, - androst-4-ene-3,17-dione (AD) and androst-4-en-17β-ol-3-one (T).

N. simplex cells actively perform 1-dehydrogenation of 6-MPhAM-AD and 6-MPhAM-T as well as AD and T. 1-Dehydroderivatives were identified as major bioconversion products from all the substrates tested (Fig.1). The structures of steroids were confirmed using TLC, HPLC, MS, ^{1}H-and ^{13}C-NMR and elemental analysis data.

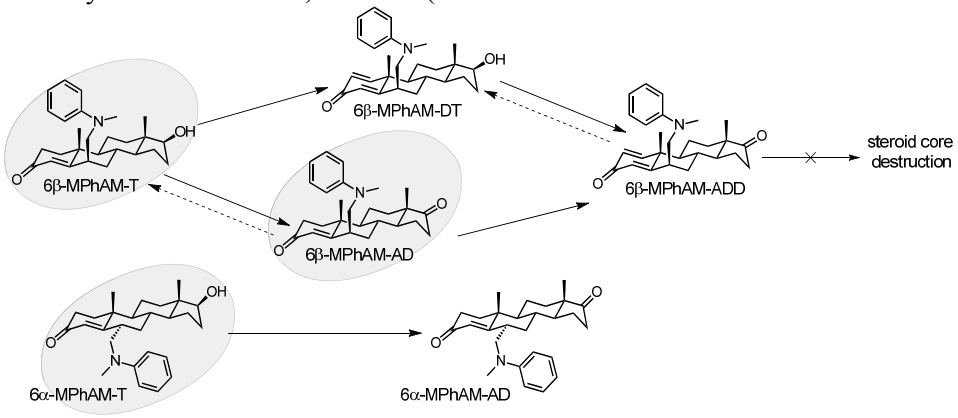

Fig.1. Bioconversion of 6α/β-MPhAM-derivatives of AD and T.

Along with 1-dehydrogenation, N. simplex oxidized hydroxyl group at C-17 of 6-MPhAM-T. Both α- and β-isomeric forms of 6-MPhAM-T were transformed to the corresponding 17-keto derivatives. In general, the rate of conversion of the unsubstituted androstanes was by over ten times higher as compared with that of their 6β-MPhAM-substituted analogs. As one of the reasons, higher hydrophobicity of the substituted substrates and its hindered transport into the cells was proposed. Application of DMSO as a co-solvent partly enhanced the conversion thus confirming this suggestion. No steroid core destruction was observed at the conversion of 6-substituted androstanes, while it was significant when the strain incubating with the unsubstituted AD and T.

The known bacterial degradative pathway proceeds via 9α-hydroxylation after 1(2)-double bond introduction into C_{19} 3,17-dioxosteroid with unstable intermediate formation followed by spontaneous cleavage of the C9–C10 bond and further degradation. In accordance with the current knowledge on

3-ketosteroid dehydrogenase (KstD) active site, the α-face of the 3-oxosteroid is in close proximity to the isoalloxazine ring of FAD (Fig.2).

1-Dehydroderivatives with an axial orientation of the substituents (6β-MPhAM-ADD and 6β-MPhAM-DT) were accumulated as major products, while no any 1-dehydrogenated α-isomers were formed. Probably, the equatorial (α) position of the bulky substituent prevents substrate interaction with KstD. Accordingly, an increase of β-stereoisomer content in the substrate α/β-stereoisomer mixtures resulted in higher yields of their 1-dehydrogenated derivatives. The lower KstD activity towards 6β-substituted androstanes in comparison with AD or T could also be explained by increased size of the complicated substituent at the axial 6β-position.

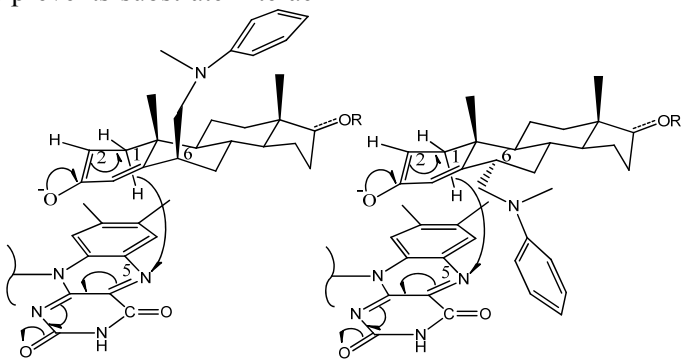

Fig. 2. Proposed effect of α/β-orientation of the (*N*-methyl-*N*-phenyl)-aminomethyl functionality at C-6 of AD or T (R=0, AD; R=H, T) on the 1-dehydrogenation mechanism catalyzed by KstD (adopted from (2)).

The permissible size was formulated earlier as no more than two carbon atoms in the chain of a β-face axial substituent (3). However, bulkier 6β-MPhAM-AD and 6β-MPhAM-T selectively converted by the strain to 6β-MPhAM-ADD. For the 6β-substituted substrates it can be proposed that this orientation of the large, partially hydrophobic and locally polar branch spatially screens the active site of the KstD (2). As shown earlier for KstD from Arthrobacter simplex, the presence of bulky substituents in the middle part of the α-face of the substrate and especially at the A-ring area may prevent 1(2)-dehydrogenation (3).

High regio-specificity of 3-ketosteroid 9α-hydroxylase (9α-KsH) as one of many Rieske oxygenases is explained by the characteristic O_2-binding to the metal center at the core of the catalytic domain (4) and accounted for by the shape of a substrate-binding pocket and a position of active site channel of the oxygenase unit of 9α-KsH (KshA), fit for the steroid substrates (5). The known KshAs amino acid residues predicted to interact with steroid substrate bound in the active site are conserved (5). So, the presence of multi-structured substituent at position C-6 (both in α- and β-orientation) appears to negatively affect the active site of the KshA in N. simplex thus preventing 9α-hydroxylation and making impossible further degradative pathways. Noteworthy, the strain did not hydrolyze, or somehow modify the (N-methyl-N-phenyl)-aminomethyl moieties at C-6, and provided only steroid core modifications at the rings A and D.

The results evidence high potential of N. simplex VKM Ac-2033D at the bioconversion of synthetic steroids.

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Russian Science Foundation is gratefully acknowledged for a support of the work (Grant No. 14-24-00169).
AVK, TSS, YuKG, NVL acknowledge support from M.V. Lomonosov Moscow State University Program of Development.