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Testosterone Degradation induced by *Rhodococcus eryth.* and *Comamonas test.*

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Introduction

Former studies have shown that 1,2-dehydrogenation of steroids is a potential secondary metabolism of bacteria. Boldenone metabolites, presumably resulting from microbial activity, were detected in some routine doping control samples at very low concentration [1,2]. This phenomenon has to be taken into consideration for the evaluation of doping control samples. Boldenone has also been the subject of a heated debate because of ongoing confusion about its endogenous or exogenous origin when detected in one of its forms in faecal or urine samples from cattle [3].

It is a matter of fact that several steroids are substrates for microorganisms [4]. Two bacterial strains which are known to produce Δ^1–steroid dehydrogenase (DH) were chosen as reference model to investigate the conversion of steroids.

Experimental

Rhodococcus eryth. and *Comamonas test.* cultures grown on agar plate "Mueller-Hinton" were utilised. The experiments were carried out in a blank urine of a male infant spiked with 100 ng/mL of testosterone. Two aliquots of each sample were prepared, one blank without addition and a positive control with addition of bacteria solution. The incubation kinetics at 30°C were monitored for 48 hours. The solution was adjusted to pH 9 ($NaHCO_3$ / KCO_3) and extracted by n-pentane/ methanol (24:1). After evaporation to dryness the samples were derivatised with a mixture of MSTFA/ ammonium iodide/ n-propanethiole. Metandienone was utilised as internal standard.

All samples were analysed by GC-MS to detect testosterone as well as 4-androstene-3,17-dione, 1,4-androstadiene-3,17-dione (boldione) and 1,4-androstadiene-17β-hydroxy-3-one

(boldenone) formed as result of the bacterial induced degradation. The measurement was carried out in SIM modus and focused on the following ions: 432 (testosterone); 430/ 206 (boldenone); 430/ 234 (4-androstene-3,17-dione) and 428 (boldione), 444 (methandienone).

Results

► An almost complete transformation of testosterone was observed in case of *Rhodococcus eryth.* within the incubation period (see figure 1). The process of degradation seems to be initiated by dehydrogenation of the 17β-hydroxy group. The formed androstendienone (4-androstene-3,17-dione) then undergoes a Δ^1 –dehydrogenation to boldione (1,4-androstadiene-3,17-dione) (see figure 2).

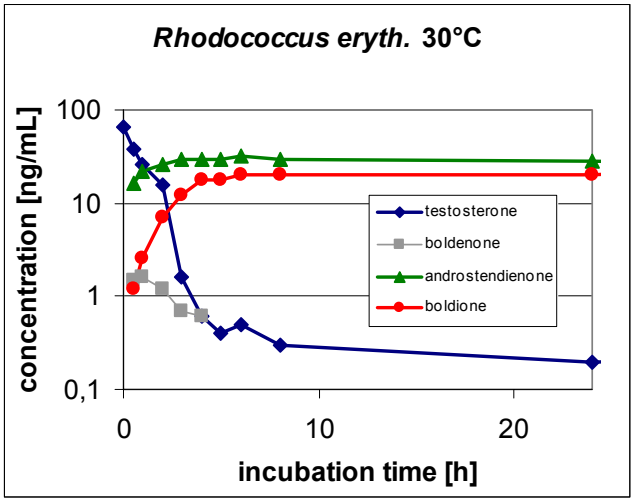


figure 1: kinetics of testosterone degradation with *Rhodococcus eryth*. at 30°C

An additional kinetic experiment carried out with 4-androstene-3,17-dione as substrate confirmed this direct transformation.

► The Δ^1-dehydrogenation of testosterone itself occurred in a small extent and the corresponding boldenone was only detected within the first hours of incubation (see figures 1 and 2). The diminished formation of boldenone appears to be caused by limited activity of Δ^1-dehydrogenase enzyme and by concurrent production of 4-androstene-3,17-dione and boldione, respectively.

► *Comamonas test.* caused a slower degradation process compared to the *Rodococcus eryth.* yielding the same compounds.

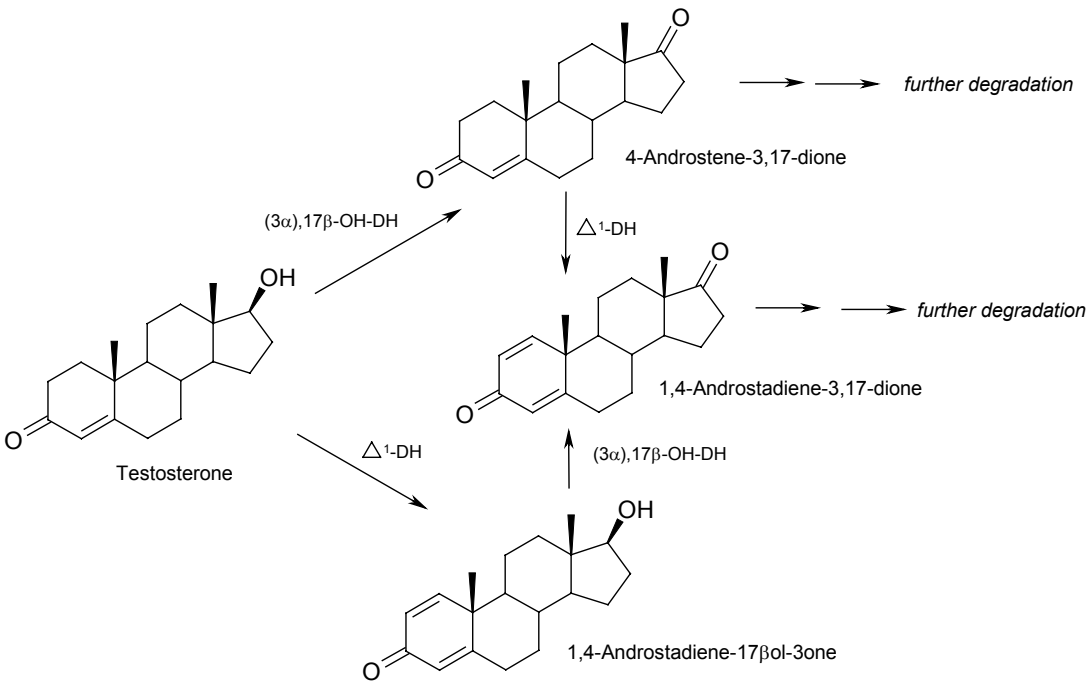
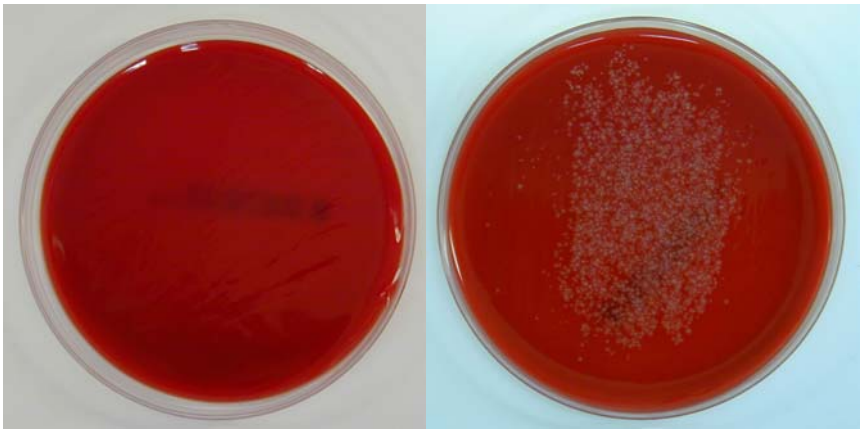


figure 2: proposed pathway of degradation of testosterone in presence of *Rhodococcus eryth.* and *Comamonas test.*

► Urine samples with and without *Rhodococcus eryth.* incubation were diluted 100-fold and cultivated at 30°C for 48 hours on an agar plate. Growth of bacterial cells were observed in the *Rhodococcus eryth.* containing sample indicating the survival in an urine medium (see below) .

blank urine urine + *Rhodococcus eryth.*



Conclusions

Our objective was the investigation of presumably endogenous production of boldenone from testosterone caused by microorganisms. Both selected bacteria strains *Rhodococcus eryth.* and

Comamonas test. are able to bio-transform testosterone. The mechanism by which testosterone is degraded comprises different enzyme catalysed reactions. The formation of 4-androstene-3,17-dione, boldione and boldenone, respectively, were confirmed. Consequentially, an endogenous formation of 1,2-dehydro-steroids has to be taken into consideration to avoid adverse analytical findings.

Reference

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