Synthesis of 5α-andrstan-3,16-dione and its biocatalysis by the fungus *Corynespora cassiicola.*

Introduction

Synthesis of complex molecules, large or small can be time and resources consuming, to synthesise the 5α-andrstan-3,16-dione from dehydroisoandrosterone can take 7 steps to simply reach the desired molecular structure of hydroxylation at the carbon 16-position. The synthesis takes several steps as well as days with additional characterisation of the produced molecule to confirm its structure. The fungi *C. cassiicola* is expected to make a change to the 5α-andrstan-3,16-dione yet not possible to execute in a generic laboratory or to costly for standard step by step synthesis. This transformation will also reveal important information about the metabolic pathways in this organism.

Rational

Determine the effect of different steroidal structural architecture on initiation of biocatalytic trans-esterification reactions. This project involves steroid synthesis, fungal biocatalysis and determination of steroidal metabolic fate to reveal the mechanistic basis behind a unique trans-esterification reaction. Recently we have found that hydroxylation at the 14α-position of steroids that contain a ring-D lactone undergo an enzyme catalysed trans esterification (Hunter et al., 2017). This two-step reaction generates a spiro-carbon centered 5-ring lactone, in high yield, from the initial 6-ring lactone ring D of the steroid.

In order to probe and subsequently define the mechanistic basis of the trans-esterification mechanism architectural deconstruction of the lactone is being investigated. The steroid 5α-androstan-3,16-dione retains structural parity with the previously investigated lactone but is devoid of the ethereal oxygen in ring-D. Biocatlaysis of this compound will determine if the ethereal oxygen is essential for 14α-hydroxylation of the steroid. This is of importance, as it is the first step in the mechanism to trans-esterification of the steroidal lactone previously reported (Hunter et al., 2017). If hydroxylation does not occur at carbon 14, this will confirm that the heteroatom is an essential component in the steroidal structural architecture, with the converse being true in the absence of oxidation at this position.

Discussion

The generation of the 5-membered ring lactone though the green fungal biocatalyst *C. cassiicola* is a completely unique observation. In order to understand the molecular architectural requirements for the reaction we have had to design a synthetic pathway to the 16-ene using a multi-step synthetic methodology. From the 3,16-diketosteroid generated we will be able to determine the role of the C-16 Ketone. This will reveal important mechanistic understanding be the result attack at C-14 or uptake into a completely different metabolic pathway.

References

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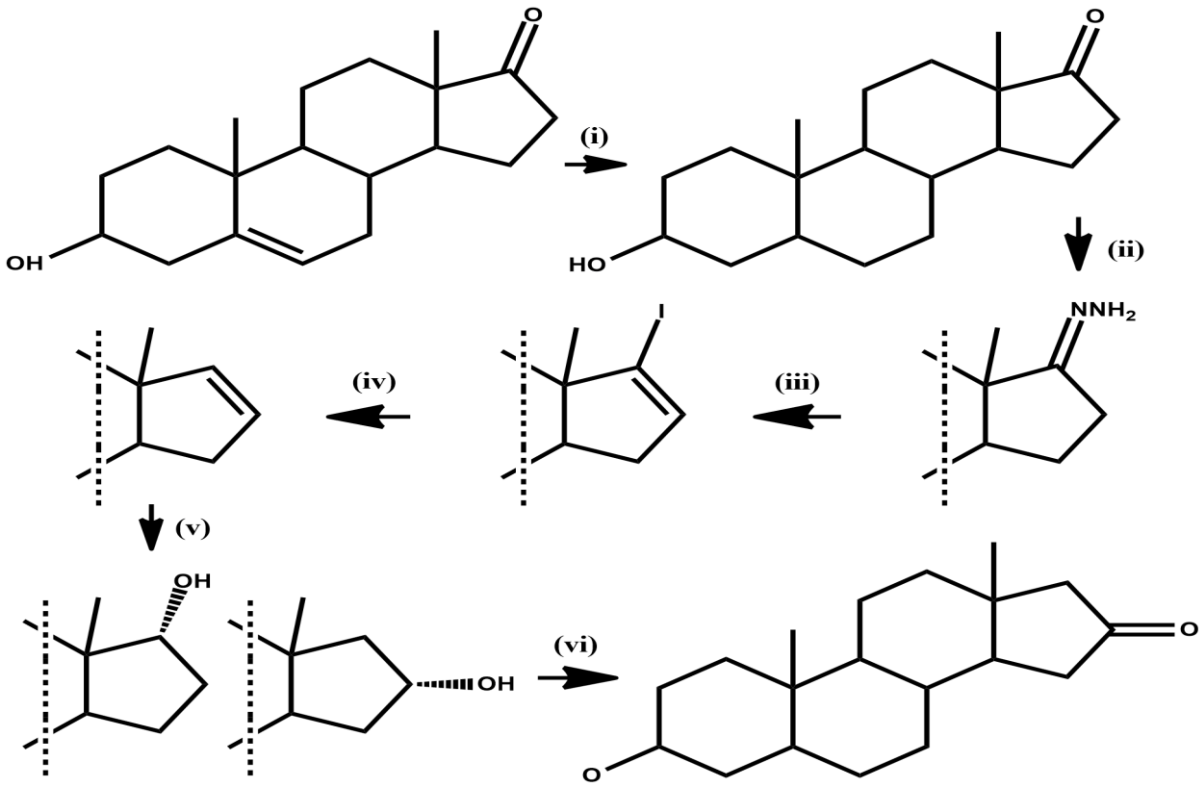
Synthesis Pathway From Dehydroiosoandrosterone To 5α-andrstan-3,16-dione



(i) Hydrogenation (ii) Hydrazone formation (iii) Vinyl Iodide formation (iv) 16-ene (v) Hydroboration (vi) Jones oxidation

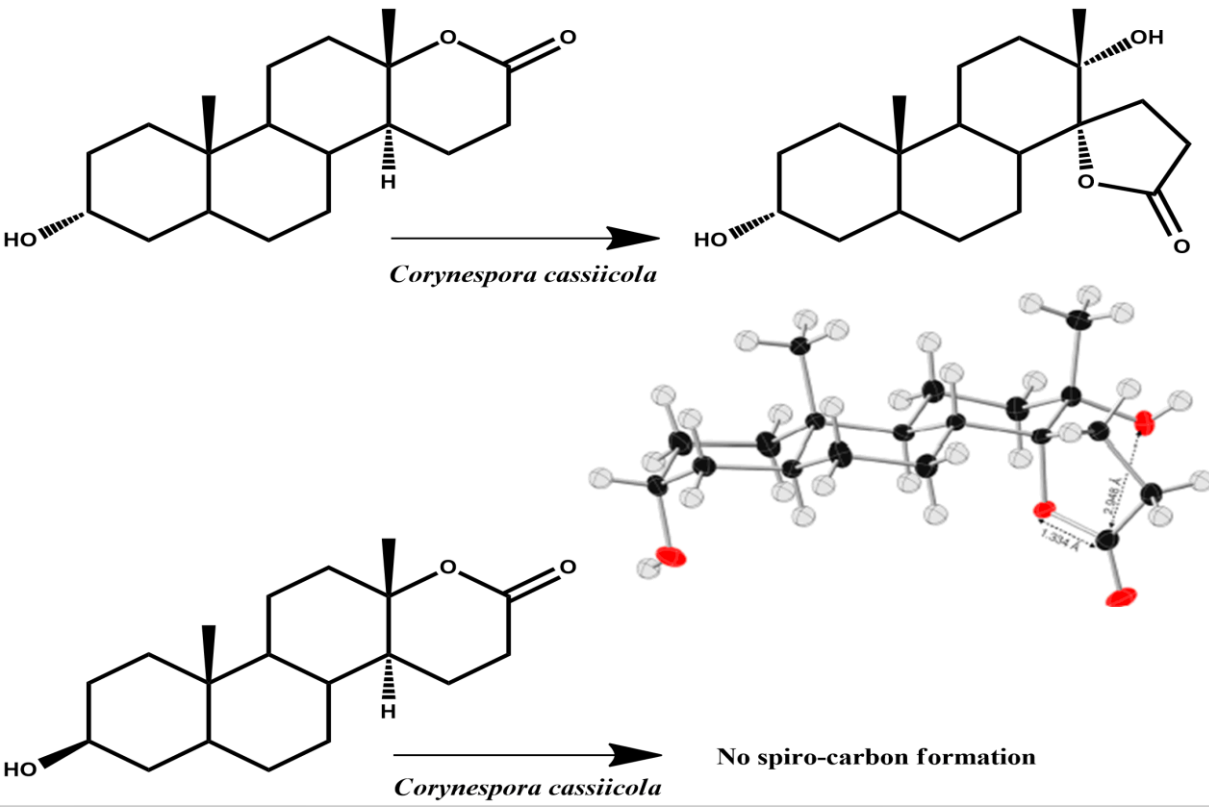
Method

Stock cultures of *C. cassiicola* grown on potato dextrose agar slopes for 3 days at 4°C. The steroid transformation was then carried out in 3% malt extract medium.

Spores transferred into 500mL Erlenmeyer flask containing 300mL of sterile media and were incubated for 72h at 25°C and shaken at 180rpm

Steroid dissolved in dimethylformamide was distributed between the flasks at 1mg/mL and incubated for additional 5 day, then the metabolites were extracted from the broth, further methods were carried out in (Hunter et al., 2017).

The extracted metabolites were furtherer characterised by the use of ^{1}H and ^{13}C NMR spectra and DEPT analysis in combination with the primary compounds were used to further identify the metabolites.



Graphic abstract of 3α,13α-dihydroxy-nor-15,16,17-5α-androst-14α-carbolactone with X-ray crystal structure after bio catalysis with the *C. cassiicola.* The similarity to the 5α-andrstan-3,16-dione although the lactone is absent, it also posses a ketone functional group makingthe 5α-andrstan-3,16-dione a primary candidate for the study.

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