

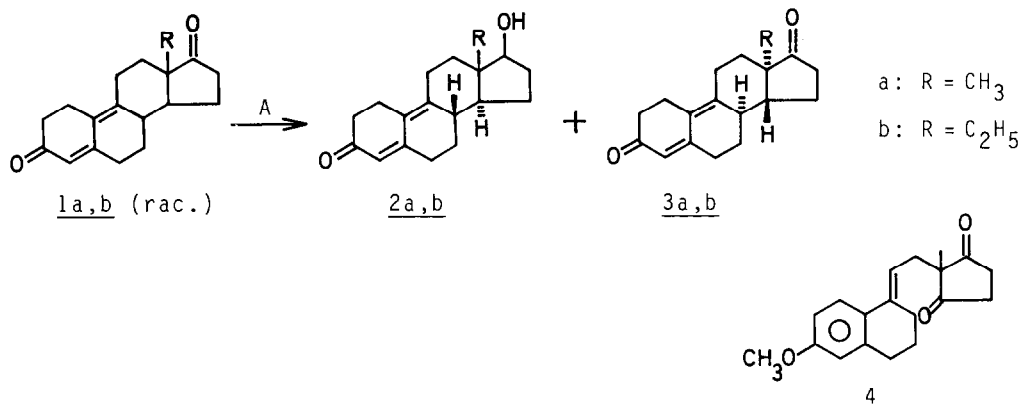
A MICROBIOLOGICAL APPROACH TO KINETIC RESOLUTION OF RACEMIC ESTRA-4,9-DIENES

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Summary: Resolution of rac.-estra-4,9-diene-3,17-dione is achieved by enantioselective, microbiological reduction.

Estradienes of type 1 are important intermediates for the synthesis of anti-progestins, a recently discovered class of steroidal anti-hormones¹⁾. In the course of structure-activity studies we became interested in a convenient access to the enantio-derivatives of 1. Although microbiological methodology exists to reduce prochiral Torgov-intermediate 4 to either enantiomer with perfect asymmetric induction²⁾, we attempted to avoid the lengthy sequence of total synthesis via compound 4³⁾.



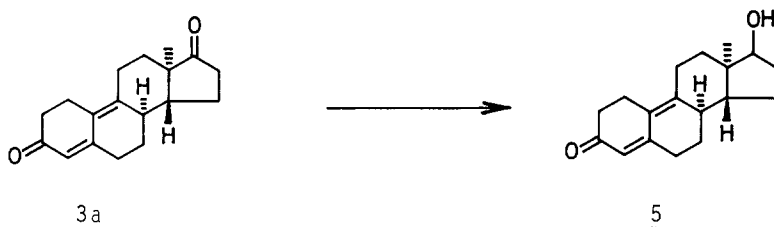
Procedure A: *Hansenula capsulata* (IFO 984), substrate concentration 200 mg/L culture broth, fermentation time 48 h, yield of 2a 42%, mp 189-190°C, $[\alpha]_D^{20} = -310.6$ (CHCl₃, c=0.5); yield of 3a 45.7%, mp 139-141°C, $[\alpha]_D^{20} = +196.4$ (CHCl₃, c=0.5); yield of 2b 41.2%, mp 156-157°C, $[\alpha]_D^{20} = -315.2$ (CHCl₃, c=0.5); yield of 3b 43.6%, mp 143-145°C, $[\alpha]_D^{20} = +195.7$ (CHCl₃, c=0.5).

A screening programme comprising 50 microorganisms known to effect C-17-keto

reduction resulted in the discovery of two yeast strains well suited for the desired purpose: *Hansenula capsulata* (IFO 984) and *Saccharomyces chevalieri* (NCYC 91).

Both microorganisms rapidly reduced the naturally configured enantiomers, whereas the unnaturally configured fraction remained unchanged. Chromatographic separation was easily performed giving optically pure 17-hydroxy compounds 2a,b of natural configuration and 17-keto derivatives 3a,b belonging to the unnaturally configured series. The isolated yields were close to theory being in the range of 41-46% for either component. Most of the other microorganisms investigated in our screening programme were found to reduce both enantiomers of 1a,b unselectively to form racemic 2a,b. Chemical yields were slightly better with *Hansenula capsulata* than with *Saccharomyces chevalieri*.

In the case of *Hansenula* another favorable aspect deserves a comment: Prolonged fermentation (>40 h) of 1a resulted in a slow transformation of the unnatural enantiomer 3a with formation of hydroxy derivative 5 [mp 139-140°C, $[\alpha]_D^{20} = +354.1$ (CHCl₃, c=0.5)], a diastereomer with respect to the initially formed product 2a.



The formation of compound 5, therefore, was not associated with a decrease in optical purity for reduction product 2a. Furthermore, the appearance of small amounts of 5 in the fermentation mixture indicated that all of the natural enantiomer had been consumed so that fermentation could be terminated. In conclusion, the reported process is an illustrative example of a self-indicative kinetic resolution.

References:

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2. Kosmol, H., Kieslich, K., Vössing, R., Koch, H.-J., Petzoldt, K., and Gibian, H., Liebigs Ann. Chem. 701, 198 (1967).
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