Unusual Stereoselectivity in the Reduction of Bicyclo[3.3.0]oct-2-en-8-one by *Thermoanaerobium brockii* Alcohol Dehydrogenase

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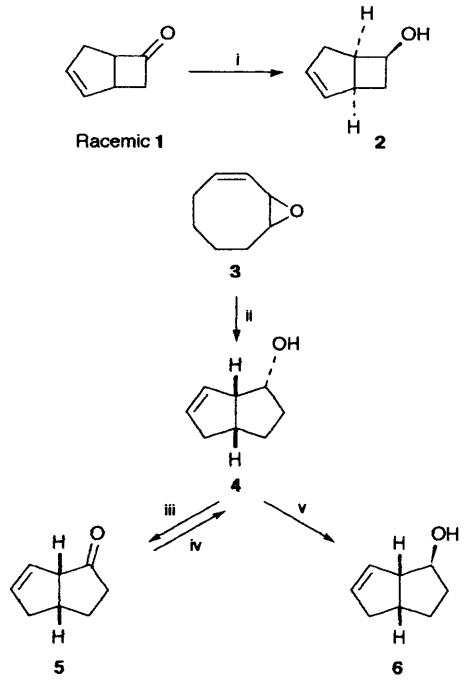
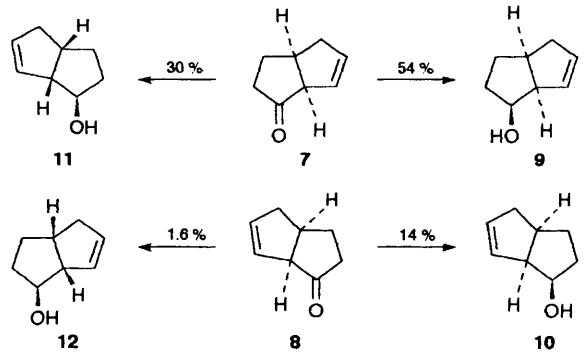
The *Thermoanaerobium brockii* alcohol dehydrogenase (TBADH) catalysed reduction of the racemic bicyclic ketone **5** gives epimeric alcohols derived from the same enantiomeric series, the reduction is enantioselective but not diastereoselective, an unusual result for a single enzyme.

The usefulness of enzymes for the preparation of chiral synthons has been amply demonstrated[1] but nevertheless there is still a need for new enzymes or microorganisms which have improved selectivity. The commercially available alcohol dehydrogenase (TBADH, EC 1.1.1.2) from the thermophilic bacterium *Thermoanaerobium brockii*, is a good candidate for exploitation because of its thermal stability.[2] TBADH reduces short chain aliphatic ketones on the *Si*-face[3] whereas long chain analogues,[4] acetyl furans[5] and the bicyclic ketone **1** are reduced on the *Re*-face.[6]

Treatment of 3,4-epoxycyclooctene **3** (Scheme 1) with lithium diethylamide yields the bicyclic *endo*-alcohol **4** by intra-annular carbene insertion.[7] Jones' oxidation gave bicyclo[3.3.0]oct-2-en-8-one **5** and Mitsunobu inversion[8] followed



Scheme 1 *Reagents*: i, TBADH, NADPH, Pr^{i}OH; ii, $LiNEt_2$, Et_2O; iii, Jones' reagent; iv, $NaBH_4$, EtOH; v, (*a*) Ph_3P, DEAD, AcOH, (*b*) MeOH, KOBu^t (catalytic); (DEAD = diethyl azodicarboxylate)



Scheme 2

by ester cleavage gave the *exo*-alcohol **6**. Reduction of the ketone **5** with sodium borohydride gave the *endo*-alcohol **4** [94% diastereomeric excess (d.e.)], which securely confirmed the stereochemical assignments.†

Racemic ketone **5** (1.008 g) was dissolved in TRIS [tris(hydroxymethyl)aminomethane] buffer (50 ml, 50 mmol dm^{-3}, pH 8) and propan-2-ol (10 ml, 17% v/v), which acts as co-solvent and ultimate hydrogen source. To this was added NADPH (52 mg), mercaptoethanol (10 μl) and TABDH (20 mg). The reaction was maintained at 31 °C for 214 h and monitored by GLC which indicated the gradual increase of the two alcohols **4**, **6** and decrease in amount of the starting ketone **5**. The epimeric alcohols were inseparable from each other but could be separated from the ketone by preparative column chromatography. The proportion of each stereoisomeric alcohol was determined by 360 MHz ^{1}H NMR spectroscopy after derivatisation as Mosher's esters using (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride.[9] Similarly the recovered ketone was reduced with sodium borohydride to give the *endo*-alcohol **4** and converted to the Mosher's ester.

† The diagnostic ^{1}H NMR signals for 8-H were as follows, *endo*-alcohol **4** δ 4.22, q, 6 Hz, NOE from 8-H to 1-H 5%, from 1-H to 8-H 2.5%, *exo*-alcohol **6** δ 4.05, broad singlet, NOE < 2% for either irradiation.

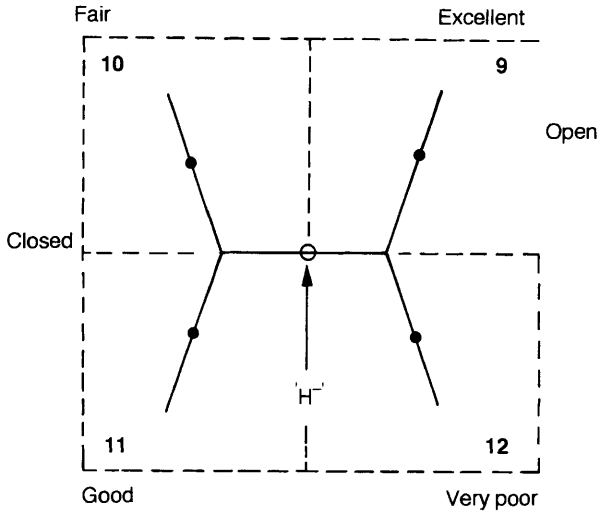


Fig. 1

The products were found to be (1*R*,5*S*,8*S*)-*endo*-bicyclo[3.3.0]octen-8-ol **4** [247.5 mg, 24%, 60% enantiomeric excess (e.e.)], (1*R*,5*S*,8*R*)-*exo*-bicyclo[3.3.0]octen-8-ol **6** (116.5 mg, 11%, > 90% e.e.) and (1*S*,5*R*)-bicyclo[3.3.0]-octen-8-one **5** (426.5 mg, 42%, 46% e.e.).

The enantiomeric excesses of the alcohols and their yields enable a calculation to be made of the expected enantiomeric enrichment of the recovered ketone. If the alcohols **4** and **6** are derived from the same series the ketone **5** is calculated to have a 58% e.e. whereas if they are derived from different series a 10% e.e. is to be expected. The assignment of enantiomeric series was confirmed by oxidation of the mixture of alcohols **4** and **6** with Jones' reagent, followed by reduction with sodium borohydride. The Mosher's esters of the *endo*-alcohol **4** so formed had an e.e. of 72% in excellent agreement with the calculated value (70%), which again assumes the predominant enantiomers of the alcohols are derived from the same enantiomeric series. The *endo*-alcohol **4** was also treated with (−)-camphanoyl chloride to yield the ester which had a disappointingly low optical rotation of $[\alpha]_D^{21}$ −49.8° (*c* 1.06, ethanol) indicating a 40% e.e.,‡ based on an $[\alpha]_D^{20}$ of +129.3° (*c* 1.05, ethanol) for the (1*R*,5*R*,8*R*)-camphanic ester.[10] Nevertheless this was sufficient to assign the absolute configuration.

If we examine the percentage transformation flux (Scheme 2), the majority of the reaction proceeds *via* a reduction (**7** to **9**) with the same *Re* stereoselectivity as the bicycloheptanone **1**.[6] However, the same enantiomer **7** is also reduced to the epimeric *exo*-alcohol **11**. In contrast the enantiomer **8** is reduced at 19% of the rate of the enantiomer **7**, but with a diastereoisomeric ratio of almost 10 : 1.

This type of selectivity is unusual for a single enzyme, although it has been observed with the multienzyme system found in yeast.[11] For example, Jones obtained *exo* and *endo* alcohols derived from the same enantiomeric series in the reduction of *cis*-8-oxabicyclo[4.3.0]nonan-3-one by horse liver alcohol dehydrogenase, but in this case more than 75% of the transformation flux resulted in a single product.[12]

In order to rationalise this selectivity in terms of a rigid active site model, four regions must be defined. These are shown in Fig. 1 in a view directly facing down the carbonyl double bond. The products which arise from binding in each of the possible orientations are noted in the corner of each quadrant. The products in Scheme 2 are shown in the same relative orientation such that the 'hydride ion' is donated from

‡ We do not consider this to be a serious difference because the rotations were measured under slightly different conditions and our material contains 3% of the *exo*-epimer.

the bottom face. The acyclic and monocyclic substrates used in the prior work[3] lack the rigidity present in the ketones **2**, **7** and **8**, and hence could only distinguish a two site model. The lefthand quadrants efficiently bind small alkyl groups (isopropyl or smaller) but are limited in size; the top righthand quadrant binds less efficiently but is not limited by size.

This model explains the stereochemistry of all reported reductions of ketones by TBADH; however, we cannot exclude the possibility that selectivity could also come about by the presence of multiple active sites in the enzyme, different conformational states of the same active site§ or by an active site in which molecular recognition is divorced from the reduction step.

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