

Enzymatic Reduction of Ketones to Optically Active Secondary Alcohols

R.A. Bawa*, F. Ajjabou and E. Shalfooh

Department of Chemistry, Faculty of Science, University of 7th October,
P.O. Box 875, Misurata, Libya

*Corresponding author: ramadanali70@yahoo.com

Abstract: A number of unsymmetrical ketones such as ethyl acetoacetate, 4-hydroxy acetophenone, 4-methylacetophenone, 4-acetylpyridine and pyruvic acid were reduced to the corresponding optically active secondary alcohols. The reduction reaction was monitored by Uv-Vis spectrophotometer and a complete conversion was observed in all cases within 24 to 48 h. The procedure was optimized in order to accelerate the reduction process and reduce the reaction time. This was achieved by increasing the temperature to 40°C.

Keywords: ketones, secondary alcohols, enzymatic reduction

1. INTRODUCTION

Enantioselective reduction of ketones to optically active secondary alcohols is one of the most interesting areas of research for a number of research groups.¹⁻⁵ Enzymes have been widely used in converting ketones to the corresponding optically active secondary alcohols. This technique has shown good to excellent levels of enantiomeric excess.¹⁻³ An alcohol dehydrogenase from the hyperthermophilic archaeon *Pyrococcus furiosus* has been used to catalyze the reduction of a variety of aliphatic ketones, aryl ketones, α - and β -ketoesters. Aryl ketones, α - and β -ketoesters that contain phenyl substituents were reported to be reduced to the corresponding enantiomerically pure chiral alcohols, whereas the reduction of aliphatic ketones gave a moderate levels of enantioselectivity. This indicates that the presence of a phenyl group adjacent to the carbonyl group could be an important factor for obtaining high levels of enantioselectivity.¹ Voss et al. used a practical approach for inverting (*R*)-alcohols to the (*S*)- counterparts *via* an oxidation/reduction biochemical process using lyophilised cells of *Rhodococcus*. (*S*)-2-decanol with 92% ee was obtained when a racemic mixture of 2-decanol was subjected to this biocatalytic oxidation/reduction transformation.²

2. EXPERIMENTAL

Baker's yeast was purchased from the supermarket and used as such. Ethyl acetoacetate 99% and absolute ethyl alcohol 99.8% were purchased from Riedel–Deehaen, 4-hydroxyacetophenone 98%, 4-acetylpyridine 98%, pyruvic acid 98% and sucrose 99% were purchased from PARK Scientific Limited. 4-methylacetophenone 98% was purchased from Schuchardt-Munche, disodium hydrogen phosphate 99% was purchased from Merck while barium hydroxide 97% was purchased from T-Baker Lab Chemicals. Chemicals were used without further purification. Shimadzu Uv-Vis spectrophotometer model 1240 was used to monitor the reaction progress.

2.1 General Procedure for the Enzymatic Reduction

Sucrose 116.96 mmol and disodium hydrogen phosphate 1.76 mmol were placed in 500 cm³ Erlenmeyer's flask and dissolved in warm (40°C) tap water (75 cm³). Dry active baker's yeast (8.0 g) was added to the reaction mixture. The anaerobic fermentation set-up was installed. The reaction mixture was stirred vigorously for 1 h at 40°C and then was allowed to cool to room temperature. The prochiral ketone (21.55 mmol) was added and the reaction mixture was stirred vigorously for 24–48 h at room temperature unless otherwise mentioned. The reaction mixture was filtered with the help of a pad of cotton and the filtrate was saturated with solid sodium chloride. The mixture was extracted with chloroform (3 × 30 cm³) and the organic layers were combined, dried over sodium sulphate, filtered and the solvent was evaporated to give the desired product. Wavelengths were recorded for the ketones and the resulting secondary alcohols are as follows: Ketones **I**, **II**, **III**, **IV** and **V**; λ_{\max} 322.5 nm (CHCl₃), λ_{\max} 365.5 nm (H₂O), λ_{\max} 364.0 nm (H₂O), λ_{\max} 382.5 nm (H₂O) and λ_{\max} 373.0 nm (H₂O), respectively for secondary alcohols **VI**, **VII**, **VIII**, **IX** and **X**; λ_{\max} 315.5 nm (CHCl₃), λ_{\max} 334.0 nm (CHCl₃), λ_{\max} 296.5 nm (EtOH), λ_{\max} 343.5 nm (CHCl₃), 271.5 nm and 336.5 nm (CHCl₃), respectively. The enantiomeric excess values were measured by comparing the $[\alpha]_D$ values of the products with the specific rotation of single and highly enantiomeric enriched enantiomers.

3. RESULTS AND DISCUSSION

Attempts were made to apply the enzymatic reduction to convert a number of prochiral ketones **I–V** (Fig. 1) to the corresponding optically active secondary alcohols. The ketoreductase enzyme that was involved in the reduction process was generated in the reaction using baker's yeast at 40°C. This enzyme has been reported to distinguish between the two faces of the carbonyl group.⁶

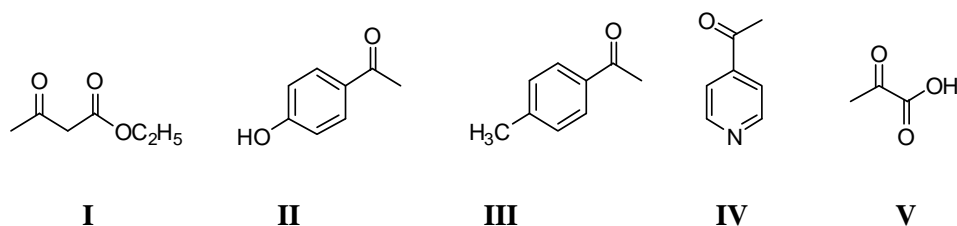
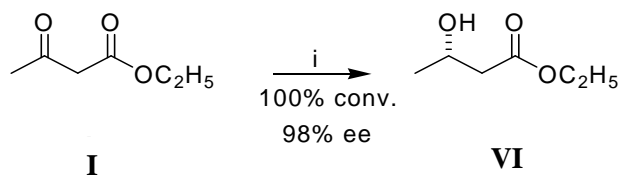


Figure 1: Prochiral ketones **I** – **V** being converted to optically active secondary alcohols.

The enzyme brings the ketone and the reduced coenzyme NADH together by which the hydride is transferred to the carbonyl group leading to the formation of (*S*)-enantiomer in excess.^{6,7} This reaction has been reported to give levels of enantiomeric excesses ranging from 70% to 97%.⁶

Ethyl acetoacetate **I** was reduced successfully to the corresponding optically active secondary alcohol **VI**. The reaction mixture was stirred for 48 h at 30°C to room temperature giving a complete transformation and 98% ee. However, increasing the temperature that was required for the fermentation process, to 40°C led to a complete conversion after 24 h. The reaction progress was monitored using an Uv-Vis spectroscopic technique. This slight increase in the temperature during the fermentation step accelerated the generation of the ketoreductase enzyme and its cofactor in which the reaction time was reduced. As an attempt to further accelerate this process, a catalytic amount of ethyl alcohol (~8%) was added to the reaction mixture. However, no complete conversion was observed after 20 h (Fig. 2).

Phenyl-substituted ketones such as **II** and **III** were subjected to the same reduction reaction to form the corresponding enantiomerically enriched secondary chiral alcohols **VII** and **VIII** with enantiomeric excesses of 62% ee and 92% ee, respectively (Fig. 3). A complete conversion was obtained within 24 to 48 h stirring at 40°C to room temperature. The presence of a phenyl ring adjacent to the carbonyl group has been found to enhance the enantioselectivity.¹



Conditions: (i) Baker's yeast, sucrose, H₂O, 40°C to RT, 24 h

Figure 2: Enzymatic reduction of ethyl acetoacetate.

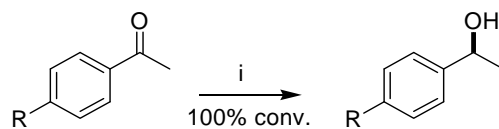
R = OH; (**II**)R = CH₃; (**III**)R = OH; (**VII**) 62% eeR = CH₃; (**VIII**) 92% eeConditions: (i) Baker's yeast, sucrose, H₂O, 40°C to RT, 24 h

Figure 3: Enzymatic reduction of acetophenone derivatives.

4-acetylpyridine **IV** and the pyruvic acid **V** were also reduced to the corresponding chiral secondary alcohols **IX** and **X** with 67% ee and 80% ee, respectively (Fig. 4). The reduction process of the ketone **IV** went to completion after 48 h at 40°C to room temperature yielding chiral alcohol **IX**, whereas the reduction of pyruvic acid **V** to the lactic acid **X** required conducting the reaction (after the fermentation step and before the addition of the pyruvic acid) at temperature lower than the room temperature, as the pyruvic acid **V** is rather sensitive to heat.

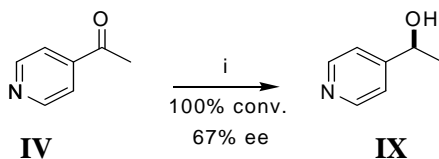
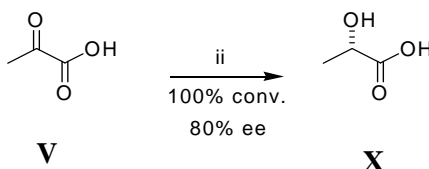
Conditions: (i) Baker's yeast, sucrose, H₂O, 40°C to RT, 24 hConditions: (ii) Baker's yeast, sucrose, H₂O, 40°C to 10°C, 48 h

Figure 4: Enzymatic reduction of 4-acetylpyridine and pyruvic acid.

4. CONCLUSION

The enzymatic reduction of a number of ketones has been optimized in which the time required for such process has been reduced dramatically by increasing the temperature of the fermentation step to 40°C. This finding is encouraging to apply such technique in asymmetric synthesis. However, the addition of catalytic amount of ethyl alcohol to the reaction mixture showed no effect on the reaction time.

5. ACKNOWLEDGEMENT

Authors would like to thank the Faculty of Science, University of 7th October, Misurata, Libya.

6. REFERENCES

1. Zhu, D., Malik, H.T. & Hua, L. (2006). Asymmetric ketone reduction by a hyperthermophilic alcohol dehydrogenase. The substrate specificity, enantioselectivity and tolerance of organic solvents. *Tet. Asymm.*, 17(21), 3010–3014.
2. Voss, C.V., Gruber, C.C. & Kroutil, W. (2007). A biocatalytic one-pot oxidation/reduction sequence for the deracemisation of a sec-alcohol. *Tet. Asymm.*, 18(2), 276–281.
3. Zhu, D., Ankati, H., Mukherjee, C., Yang, Y., Biehl, E.R. & Hua, L. (2007). Asymmetric reduction of beta-ketonitriles with a recombinant carbonyl reductase and enzymatic transformation to optically pure beta-hydroxy carboxylic acids. *Org. Lett.*, 9(13), 2561–2563.
4. Grau, B.T., Devine, P.N., DiMichele, L.N. & Kosjek, B. (2007). Chemo- and enantioselective routes to chiral fluorinated hydroxyketones using ketoreductases. *Org. Lett.*, 9(24), 4951–4954.
5. Busto, E., Gotor-Fernandez, V. & Gotor, V. (2006). Enantioselective synthesis of 4-(dimethylamino)pyridines through a chemical oxidation-enzymatic reduction sequence. Application in asymmetric catalysis. *Adv. Syn. & Cat.*, 348(18), 2626–2632.
6. Gilbert, J.C. & Martin, S.F. (2002). *Experimental organic chemistry*, 3rd ed. Orlando, USA: Harcourt College Publishers, 544–547.
7. Morrison, R.T. & Boyd, R.N. (1983). *Organic chemistry*, 4th ed. Massachusetts, USA: Allyn and Bacon, Inc., 504–509.