International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN : 0974-4290
Vol.4, No.1, pp 203-207, Jan-Mar 2012

Biocatalytic Preparative Method of Asymmetric Alcohols Using *Lycopersicumesculentum* (Tomato)

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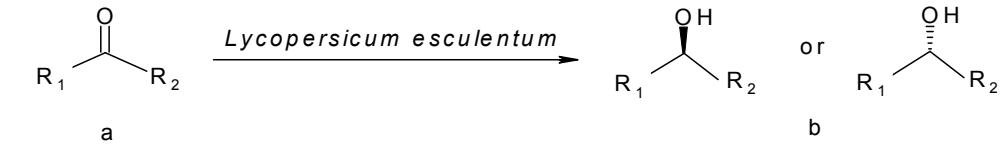
Abstract: A general, efficient and simple methodolgy for biocatalytic reduction of a diverse set of carbonyl compounds at room temperature using freshly cut ripen fruit of Lycopersicumesculentum (tomato) in aqueous medium has been reported. It was found that in addition to reduction of simple carbonyls to the corresponding primary and secondary alcohols, the prochiral ketones,both cyclic and acyclic, could be reduced to chiral secondary alcohols in a generalized way. Very high enantioselectivity (92-99%) with moderate to excellent chemical yields (70-95%) were observed. Most of the reductions were completed within 25-35 h. The regioselectivity of the biocatalyst is well established by the reduced product of cinnamaldehyde where the C = C bond was not reduced in the present investigation. The synthesized products were characterized by melting points, TLC, FT-IR and ^{1}H NMR data. Mild reaction condition, simple operation, and easy availability of tomato fruit reveal this protocol as an attractive and alternative eco-friendly option for general reduction of all types of carbonyl compounds.
Key words: Enantioselective reduction, biocatalysis, carbonyls, alcohols, *Lycopersicumesculentum.*

Introduction

The biochemical potential of plant cell cultures to produce specific secondary metabolites such as drugs, flavours, pigments and agrochemicals is of considerable interest in connection with their biotechnological utilization[1]. A wide variety of chemical compounds including aromatics, steroids, alkaloids, coumarins and terpenoids can undergo biotransformation using plant cells, organ cultures and enzymes.

In recent years, great attention has been paid to asymmetric synthesis of chiral synthons, the demand for which is increasing as precursors in the development of modern drugs and agrochemicals. A recent market study predicts that in 2010, 60% of the fine chemical products will be prepared by a biotechnology method (16% in 2001). Chiral alcohols are well known synthons and can be obtained from the corresponding prochiral ketones by asymmetric reduction. Though numerous chemical[2] and biocatalytic[3] reductions are reported in the literature, difficulties still remain in attaining high chemical and optical yield. Asymmetric reduction by means of chemical methods involves the use of expensive chiral reagents, and environmentally hazardous heavy metals are often employed[4]. Baker's yeast is by far the most widely used microorganism for the reduction of prochiral ketones yielding the corresponding optically active alcohols with fair to excellent enantioselectivity. Unfortunately[5], recovery of the desired product might not be straight forward. Moreover, use of enzymes often needs costly cofactors. Recently Baldassare*et al.*successfully used freshly cut root of carrot (Daucuscarota L) in the reduction of ketones[6]. J. S.



a b

R_1, R_2 = aliphatic, aromatic, unsaturated, saturated, cyclic etc.

Scheme 1

Yadav and his group[7] also reported the chiral reduction of various substrates containing the keto functionality with *Daucuscarota* root as biocatalyst. Nicolas Blanchard and Pierre van de Weghe[8] have explained the use of *Daucuscarota* root in the chemoselective and stereoselectivebioreduction of ketonic functionality present in different multifunctional substrates.

We present here a few general, simple, eco-friendly and inexpensive methods for some important organic functional group transformations using readily available biomaterials. The potential of ripen tomato fruit (*Lycopersicumesculentum*) soaked in deionised water as a biocatalysts for the asymmetric reduction of prochiral ketones as well as simple ketones has been investigated for the first time. Reduction of simple aldehydes and ketones including unsaturated carbonyl compound in addition to the prochiral ketones were also carried out to investigate the potential of this biomaterial towards the redox process on multifunctional organic compounds.(Scheme 1).

Experimental

Materials and Methods

All the chemicals used in the present research work were of analytical grade and were used without further purification. *Lycopersicumesculentum* (tomato) was collected from local market. Deionized water was used throughout the work. The reactions were monitored by TLC for completion. Melting points were determined in a WISWO digital melting point apparatus .The infrared (IR) spectra were recorded on *Shimadzu IR-435* spectrophotometer in KBr pallets and ^{1}HNMR spectra were recorded on a *Bruker 400 MHz* spectrometer in $CDCl_3$ solvent. Optical activity of the chiral products were determined using *Thorlab IPM5300*polarimeter.

Culture method of *Lycopersicumesculentum* (tomato)

The inner portion of the rippen tomato fruit with seeds and the thin external part were removed and the rest were cut carefully into about 2 mm thin and 1cm long slices with an ordinary stainless steel blade. This handling avoids the increase in temperature during cutting that could denature the cellular protein. The slices were them soaked in deionized water for 18 hours and then used as the biocatalytic system.

General method for enantioselective reduction of ketones with *Lycopersicumesculentum* (tomato) as biocatalyst:

The carbonyl compound (0.5mmol) was added to 15 g of cultured tomato fruit suspension in 50 mL deionized water and the mixture was stirred on a magnetic stirrer at room temperature from 30 - 40 hours (TLC monitored). The tomato pieces were then removed by filtration, washed with deionized water and the filtrate was extracted with petroleum ether (3×100 mL). The petroleum ether fraction was dried over anhydrous Na_2SO_4 and the solvent was evaporated to get the product. The products were identified by comparing with the authentic samples on TLC, by IR and ^{1}HNMR spectra. The presence of alcoholic group in the final product was chemically confirmed by acetyl chloride test.

Determination of optical activity of chiral products:

Optical properties of the products obtained from the prochiral were studied with the help of a Thorlab IPM 5300 polarimeter using the following method.

1% solution of the chiral alcohols in a suitable solvent (methanol or chloroform) was prepared and introduced it to the polarimetertube.The optical rotation values were determined individually for the product of each of the prochiral substrates. Specific rotation values are then calculated using the relation (eq. 1).

$$\text{Specific rotation} = \frac{\alpha}{l \times c} \qquad (1)$$

Where,

α = angle of rotation

l = length of polarimeter tube

c = concentration of the solution.

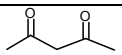
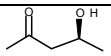
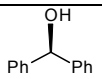
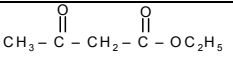
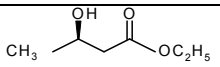
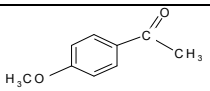
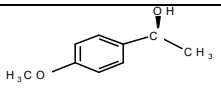
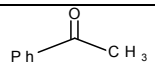
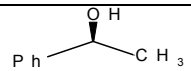
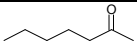
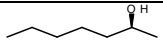
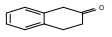
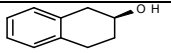
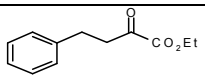
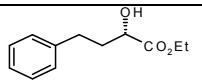
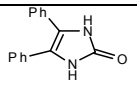
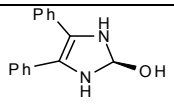
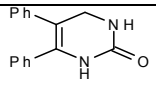
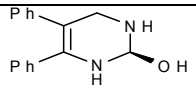
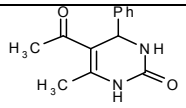
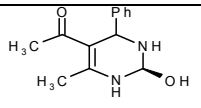
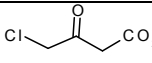
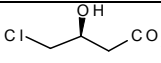
Further enantiomeric excess values of the chiral products are determined by the equation (eq. 2)

$$\% \text{ ee} = \frac{\text{Observed specific rotation}}{\text{Specific rotation of pure enantiomer}} \times 100 \qquad \text{.....(2)}$$

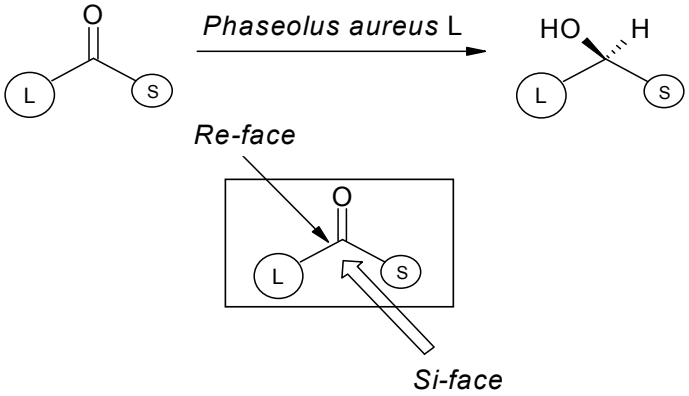
Result and discussion

The results of the reduction of ketones using *Lycopersiumesculentum* L fruit as biocatalyst are summarized in Table 1. In all the cases, excellent chemical yield (70 – 95%) and optical purity (> 99%) was observed. It was observed that the presence of electron donating substituents in the aromatic ring ($–CH_3$, $–OCH_3$) slows down the reaction rate. Reduction of β-ketoester is probably the most extensively studied small molecule microbial transformation leading to chiral intermediates in asymmetric synthesis. Previously some discrepancies regarding the ee and chemical yield[13] have been reported for the yeast mediated reduction of β-ketoesters. Later on Yadav and his workers[7] reported that using *Daucuscarota* root as biocatalyst, the reduction of β-ketoesters were completed within 55-70 hours yielding the products in high chemical and optical yield. Similar results are obtained in our present investigation where we used *Lycopersicumesculentum* fruit as biocatalyst. The reaction time was reduced in the present work (**Table 1**).

Table 1 Reduction of carbonyl compounds by *Lycopersicumesculentum*(tomato) fruit at room-temperature[a]

Entry	Substrate, a	Time[b], h	Product, b	B/S[c]	Yield(%)[d]	ee%	Confign.
1		35		10	72	-	-
2		38		12	70	98	S
3		30		10	85	-	-
4		35		11	85	96	S
5		30		9	92	-	-
6	$C_6H_5CH = CHCHO$	35	$C_6H_5CH = CHCH_2OH$	10	80	-	-
7		40		12	70	99	S
8		35		20	95	97	S
9		38		15	80	95	S
10		38		9	70	98	S
11		40		10	74	98	S
12		35		10	89	92	S
13		29		11	90	95	S
14		30		11	85	96	S
15		32		12	85	94	S
16		20		9	86	97	S

[a]All the reactions use 0.5mmol of the substrate, [b]as monitored by TLC, [c]Biocatalyst to substrate ratio (dry weight), [d]on the basis of conversion



Scheme 2

A major innovative idea laid down in the present work is the reduction of carbonyl present in imidazolinones and pyrimidinones using a plant material directly for the first time. A diverse set (Table 1, entries 13, 14 and 15) of such compounds are reduced successfully using ripen fruit of *Lycopersicumesculentum*with very good chemical yield and enantiomeric excess. Interestingly the ring carbonyl was reduced easily keeping the aliphatic side chain carbonyl intact (Table 1, entry 15). The regioselectivity of the biocatalyst is well understood from the reduction product of cinnamaldehyde where carbon-carbon double bond was not reduced in the present investigation (Table 1, entry 6).

It is difficult to obtain pure simple aliphatic secondary alcohols by reduction of corresponding ketones by chemical methods despite their utility as chiral building blocks. The present investigation demonstrated that the open chian aliphatic ketones can be reduced with *Lycopersicumesculentum* fruit as a biocatalyst. However in some case (Table1, entries1, 2, 7, 10 and 11) chemical yield was comparatively low and reduction time was longer (Table 1).

Hydride delivery occurs from the *re*-face of the carbonyl group, following Prelog's rule (Scheme 2) resulting the corresponding S-alcohols. These easy-to-perform asymmetric LE bioreductions are accompanied by several limitations, concerning both the conversion and the stereoselectivity. Long reaction times and large biocatalyst to substrate (B/S) ratios are sometimes required since the organic substrate might modify or disrupt the cellular system, thus impeding a synthetically useful conversion. A drop in conversion is usually observed when the recovered biomaterial is subjected to a second reduction reaction.[7]Recent efforts to overcome this limitation have been reported by Chˆenevert in 2005 using *Daucuscarota*highly branched roots, obtained by natural genetic transformation.[17]The corresponding intact cells showed a higher biochemical stability allowing six consecutive reuses in the reduction of acetophenone without erosion of the enantioselectivity (>98%). Moreover, the B/S ratio could be lowered to 4, a very low figure compared to Baker's yeast mediated reductions. Substrate specificity is also more pronounced with *Lycopersicumesculentum*than with Baker's yeast. *Para*-substitution of acetophenone with an electron donating group can slow down dramatically the reduction rate, highlighting the sensitivity of bioreduction kinetics to electronic effects.[16,18]Moreover, *ortho*-substituted acetophenones are badly recognized and poor conversions are generally obtained.[15,18] On the stereoselectivity standpoint, two main limitations have appeared. The Preloglike selectivity usually observed in these bioreductions could be problematic if the other absolute configuration is required. Moreover, several oxidoreductases of *Lycopersicumesculentum*might come into play, with their own intrinsic selectivities, thus complicating the prediction of the stereochemical outcome of the reduction reaction.[14]To date, no applications of *Lycopersicumesculentum*mediated reductions to the synthesis of biologically active compounds have been disclosed. The reported work is an addendum to the search for biocatalytic alternatives of the conventional organic transformations in the context of green chemistry.

Conclusion

The main advantages of this reduction over the traditional yeast mediated reductions are easy isolation of the products, elimination of the need of costly cofactors, easy availability and cheapness of tomato fruits. The whole method is environmentally benign. The selectivity of the biocatalyst is well understood from the reduction product of cinnamaldehyde where carbon carbon double bond was not reduced in the present investigation. Intact cells from cut portions of

plants can mediate useful asymmetric transformations. For example, *Lycopersicumesculentum*L. bioreduction of prochiral ketones offers new possibilities to the synthetic organic chemist in terms of simplicity and efficiency. This emerging methodology could also simplify environmental issues raised by the traditional use of borane or metal-mediated asymmetric reduction reactions. Future work in this area should be devoted to the current limitations concerning conversion and stereoselectivity. A better understanding of the underlying biochemical mechanism would also be of interest.

References

1. Bruni R., Fantin G., Pedrinl P., Sacchetti G., Plants in organic synthesis: An alternative to Baker's Yeast; Tetrahedron lett., 2002, 43, 3377- 3379.
2. Itsuno S., EnantioselectiveReuction of Ketones. In Organic Reactions; John Wiley &Sons : New York, 1998, 52, 395-576.
3. Kroutil W., Mang H., Edegyer K., Faber K. Recent Advances in Biocatalytic Reduction of Ketones and Oxidation of Alcohols, J. Mol. Catalysis, 2004, 8, 120- 126.
4. Noyori R. Okumura T., Ooka H., Hashiguchi S., Ikaria T. , Asymmetric Catalysis in Organic Synthesis, J. Am. Chem. Soc., 1995, 117, 2675.
5. Choudhari S.S., Akamanchi K.G.,Deoximation using Des Martin Peroxidane: Regeneration of Ketones from Ketoximes, Tetrahedron Lett., 1998. 39, 3209.
6. Baldassare F., Bertoni G., Chiappe C. and Marioni F., Preparative Synthesis of Chiral Alcohols by Enantioselective Reduction with *Daucuscarota* Root as Biocatalyst, J. Mol. Catal. B : Enzymatic, 2000, 11(1), 55-58.
7. Yadav J. S., Nanda S., Reddy P. T. and Rao A. B., Efficient Enantioselective Reduction of Ketones with *Daucascarota*Root, J. Org. Chem., 2002, 67, 3900-3903.
8. Blanchard N. and Weghe P. van de,*Daucuscarota* L Mediated Bioreduction of Prochiral Ketones, J. of the Royal Society of Chem., 2006, 4, 2348-2353.
9. Ahmed K., Rao M. V. and Meshram H. M., Enzymatic Deoximation of Oximes by Ultrasonically Simulated Baker's Yeast, J. Chem. Soc. Perkin Trans.1, 1991, 2056-2057.
10. UedaM., SekiK., Imai Y., S- and N-acyl Derivatives of 2-mercaptobenzoxazole : New Highly Reactive Acylating Agents for Synthesis of Amides and Amine, Synthesis, 1981, 991-953.
11. KumaraswamyG. and RameshS. Soaked *Phaseolousaureus L*: An Efficient Biocatalyst for Asymmetric Reduction of Prochiral Aromatic Ketones, Green Chem. 2003, 5, 306-308.
12. PardhasarthiM., KumaraswamyG., RameshS., SnehalathaN.C.K. and Das A. K., Ecofriendly Process for the Preparation of Chiral Alcohols by Asymmetric Reduction of Prochiral Ketones in Water Using Soaked *Phaseolousaureus L* (Green Gram), US Patent, 2007, No. 7179624.
13. Seebach D., Sutter M. A., Weber R. H., Zuger M. F., Org. Synth., 1985, 63, 1.
14. In intact cells, several oxidoreductases can compete for a substrate. Numerous parameters have to be considered: apparent kinetic constants (*v*max and *K*), number, relative concentration and compartmentalization of enzymes, effective co-enzyme concentration, permeability factor. Thus, the Prelog rule has to be carefully used in whole cell bioreduction of prochiral ketones; *cf.* SihC. J. and ChenC.S., *Angew. Chem., Int.Ed. Engl.*, 1984, 23, 570–578.
15. ComassetoJ. V., OmoriA. T., PortoA. L. M. and AndradeL. H., *Tetrahedron Lett.*, 2004, 45, 473–476.
16. ScarpiD., OcchiatoE. G. and Guarna A., *Tetrahedron: Asymmetry*, 2005, 16, 1479–1483.
17. CaronD., CoughlanA. P., SimardM., BernierJ., Pich´eY. and Chˆenevertr., *Biotechnol. Lett.*, 2005, 27, 713–716.
18. Maczka W. K. and MironowiczA., *Tetrahedron: Asymmetry*, 2004, 15, 1965–1967. For cases where electronic effect does not seem to play a role, see: BaskarB., GaneshS., LokeswariT. S. and ChadhaA., *J.Mol.Catal. B: Enzym.*, 2004, 27, 13–17.

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