BIOCATALYTIC REDUCTION OF IMIDAZOL-2-ONES AND PYRIMIDIN-2-ONES MEDIATED BY *LYCOPERSICUM ESCULENTUM* L (TOMATO)

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ABSTRACT

The ripen fruit of *Lycopersicum esculentum* L (tomato) has been successfully used for enantioselective reduction of Imidazol-2-ones and Pyrimidin-2-ones to the corresponding nitrogenous heterocyclic alcohols. Significantly without using any chemical reducing agents aqueous suspension of ripen tomato fruits alone results in almost complete reduction of a set of five and six membered heterocyclic ketones within 24-35 hrs at room temperature giving corresponding heterocyclic *S*-alcohols in excellent chemical yields and enantiomeric excess. The present work reveals that chemical transformations using direct plant cells as biocatalysts have now become an attractive and alternative eco-friendly route for synthesis of a variety of commercially significant compounds.

**KEYWORDS:** Biocatalytic reduction, imidazol-2-ones, pyrimidin-2-ones, *Lycopersicum esculentum* L.

INTRODUCTION

Aromatic and aliphatic heterocyclic compounds are frequent structural motifs in nature's molecules and in man–made chemical active substances such as pharmaceuticals and agrochemicals. Asymmetric reduction of prochiral ketones is one of the most important, fundamental and practical reactions for producing chiral alcohols. Enantiomerically pure secondary alcohols are important synthons/intermediates for the synthesis of numerous pharmaceuticals, agrochemicals, flavors, fragrances and industrial fine chemicals.[1-3] There are numbers of chemical and biological methodologies available to obtain chiral molecules; of these biocatalysts has proven to be useful supplementary technology, allowing in some

cases reactions which are not easily conducted by classical organic synthesis or in other cases reactions which take several chemical steps, can be carried out in a single step using biocatalyst under highly selective and mild conditions. Thus the biocatalytic process (both whole cells and isolated enzymes) continues to remain an area of intensive research, with a desire to develop alternative green routes to the synthesis of fine chemicals.[4-6] Heterocyclic aromatic compounds containing nitrogen, oxygen or sulfur in the heterocyclic ring are important core groups found in natural and synthetic products of biological interest. There are many reports describing synthesis of chiral secondary alcohols using biocatalysts obtained from microbial/different parts of plant tissues.[7-9] The asymmetric reductions of heteroaryl methyl ketones is a straight forward approach and a large number of chemical and biological methodologies (microbial and plant) are known to produce heterocyclic asymmetric alcohols of biological interest.[10-12] However, most of these processes have limitation in commercial application due to long incubation time, low substrate loading, poor isolated yields and enantioselectivity.[13,14] Unfortunately practically no literature is available for the reduction of the carbonyl group of heterocyclic ketones till date. In continuation of our interest towards synthesis of chiral alcohols of biological importance using biocatalysts, we here in present the process for production of chiral aromatic heterocyclic secondary alcohols by bioreduction of simple and substituted nitrogenous heterocyclic ketones using ripen *Lycopersicum esculentum* L fruits.

In one of our earlier works, the biocatalytic activity of ripen tomato fruits was esatablished by the successful reduction of simple carbonyl compounds to the corresponding alcohols.[15] This diverted our mind to apply the methodology towards the reduction of carbonyl groups of imidazol-2-ones and pyrimidin-2-ones that were synthesized as described in another piece of work in our laboratory.[16] Reductions of cyclic ketones are of great importance in synthetic organic chemistry yet very few methods are available in the literature for this conversion. We have not found any literature is involving biocatalytic methodology for the reduction of cyclic ketones.

A set of ten different five and six membered two nitrogen containing heterocyclic ketones were successfully reduced to the corresponding alcohols using the soaked tomato fruits *(Scheme 1)*.

Heterocyclic ketone   **Heterocyclic alcohol**
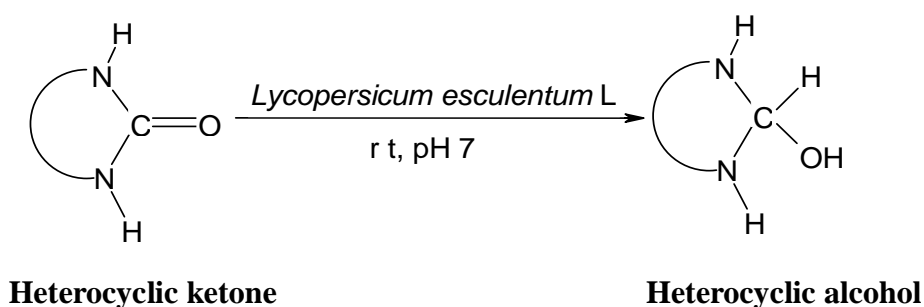
Scheme 1 **Bioreduction of Heterocyclic Ketones Using Tomato**

EXPERIMENTAL

Preparation of the Biocatalytic System of *Lycopersicum esculentum* (tomato): The inner portion of the ripen 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 then soaked in deionized water for 18 hours and then used as the biocatalytic system.

Determination of Optical Activity of Chiral Products Obtained by Reduction of Prochiral Ketones: The optical properties of the products obtained from the prochiral ketones were studied with the help of a Thorlab IPM 5300 polarimeter. 1% solution of the chiral alcohols in a suitable solvent (methanol or chloroform) was prepared and introducing it to the polarimeter tube the optical rotation values are determined individually for each of the prochiral substrates. Specific rotation values are than calculated using the relation *(eq. 1)*

$$\text{Specific Rotation} = \frac{100\,\alpha}{l \times c} \qquad (1)$$

Where, α = observed angle of rotation

l = length of polarimeter tube (2dm)

c = percentage concentration of the solution

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

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

General Method for Reduction of Imidazol-2-ones and Pyrimidin-2-ones: 2-5 mmol of the heterocyclic ketone was added to 5 g of tomato fruit suspension in 50 mL deionized water and the mixture was stirred on a magnetic stirrer at room temperature from 24-35 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 crude product. Purification by column chromatography [silica gel, 15-20% MeOH-DCM] afforded the heterocyclic alcoholic products The products were identified by comparing with the authentic samples on TLC, by IR and ^{1}H NMR spectra.

Typical Method for Reduction of 1*H*, 2*H*-4,5-diphenylimidazol-2-one : 0.5g (2.12mmol) of 1*H*,2*H*,4,5-diphenylimidazol-2-one was added to 5 g of tomato fruit suspension in 50 mL deionized water and the mixture was stirred on a magnetic stirrer at room temperature from 20-30 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 crude product. Purification by column chromatography [silica gel, 20% MeOH-DCM] afforded the product 1*H*,2*H*,4,5-diphenylimidazol-2-ol, 0.107g (90% yield) in 29 h time as white crystals, mpt. 203[0]C.

1*H*,2*H*,4,5-Diphenylimidazol-2-one   1*H*,2*H*,4,5-Diphenylimidazol-2-ol
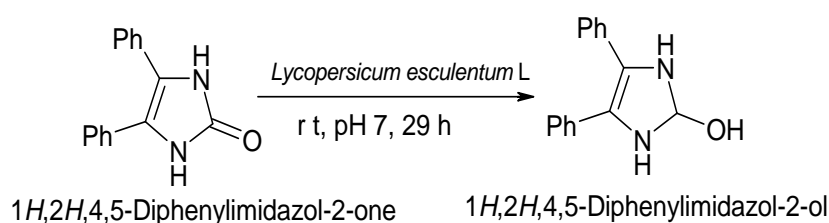
Scheme 2 **Reduction of 1*H*,2*H*,4,5-diphenylimidazol-2-one**

Compound 2 *(Table 1),* **1*H*,2*H*,4,5-diphenylimidazol-2-ol**

White crystals, mpt. 203[0]C, IR (KBr) : ν, cm[−1] 3450(N-H$_{str}$), 3334 (C-H$_{str}$, Ar-H), 2924(O-H$_{str}$), 993(C-N$_{str}$) ; ^{1}H NMR (CDCl$_3$) : δ, d ppm 1.3 (m,1H, C-H), 3.5 (d, 1H, N-H), 3.2 (d, 1H, N-H), 7.2-7.4(m, 10H, Ph-H), 9.0 (d, 1H, O-H)**.**

Reduction of 1*H*, 3*H*-4,5,6-triphenylpyrimidin-2-one: 0.5g (1.53mmol) of 1*H*,3*H*-4,5,6-triphenylpyrimidin-2-one was added to 5 g of tomato fruit suspension in 50 mL deionized

water and the mixture was stirred on a magnetic stirrer at room temperature from 20 - 30 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. Purification by column chromatography [silica gel, 20% MeOH-DCM] afforded the product 1*H*,3*H*-4,5,6-triphenylpyrimidin-2-ol, (0.141g, 86%) in 25 h time as an amorphous solid, mpt. 248[0]C.
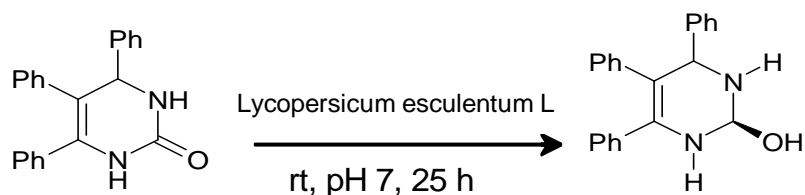
1*H*,3*H*-4,5,6-triphenylpyrimidin-2-one   1*H*,3*H*-4,5,6-triphenylpyrimidin-2-ol

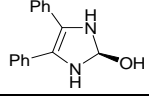*Scheme 3* **Reduction of 1*H*,3*H*-4,5,6-Triphenylpyrimidin-2-one**
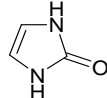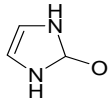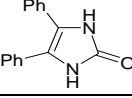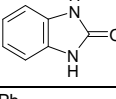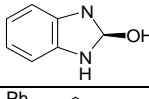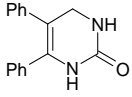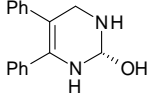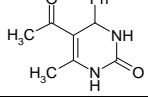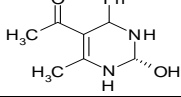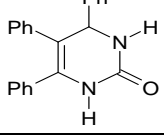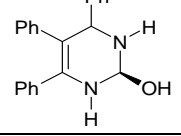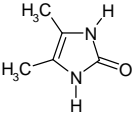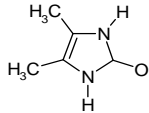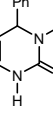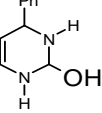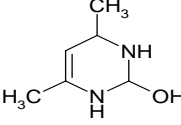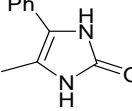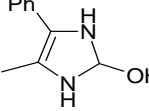
Compound 6 *(Table 1),* **(+)1*H*,3*H*,4,5,6-triphenylpyrimidin-2*S*-ol**

$$[\alpha]_D^{26} = +40.2^0 \quad C = 1\,(Chloroform), \quad ee = 97.5\%$$

Optically active, white crystals at room temperature, mpt. 248[0]C,   IR (KBr) : ν,   cm[−1] 3492(N-H$_{str}$), 3312 (C-H$_{str}$, Ar-H), 2986(O-H$_{str}$), 1412(C-N$_{str}$); ^{1}H NMR (CDCl$_3$) : δ, ppm 1.9 (d,1H, C-H), 2.2 (d, 1H, C-H), 3.6 (d, 2H, N-H), 4.9 (d, 1H, O-H)7.1-7.3 (m, 10H, Ph-H), 7.5 (m, 5H, Ph-H).

Optical Study of the Chiral Heterocyclic Alcohols: 1% solution of the chiral heterocyclic alcohols was prepared in a suitable solvent (methanol or chloroform) and introducing it to the polarimeter tube the optical rotation values are determined individually. Specific rotation values are than calculated using *eq. (1).* Further enantiomeric excess and absolute configuration of the chiral alcohols 4-6, 8-10 were determined by HPLC using chira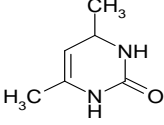l columns. The optically pure chiral heterocyclic alcohols (**Table 1**, Entries 4-6, 8-10) show exclusively (*S*) configuration thus following common Prelog's rule.[17]

Table 1. Reduction of Heterocyclic Ketones by Lycopersicum esculentum (tomato) Fruit

Entry	Substrate	Product	Time	% Yield	Optical rotation	Abs. Conf.	ee (%)
1			24	89	-	-	-
2			29	90	-	-	-
3			35	91	-	-	-
4			30	90	$+28.4^0$	*S*	96.1
5			32	85	-36.8^0	*S*	96.4
6			30	91	$+40.2^0$	*S*	97.5
7			28	90	$+24.6^0$	-	-
8			29	87	$+20.4^0$	*S*	94.1
9			30	88	-39.8^0	*S*	98.0
10			28	91	27.4^0	*S*	99.0

RESULTS AND DISCUSSION

Reduction of a set of 1*H*,2*H*-imidazol-2-ones and 1*H*,2*H*-pyrimidin-2-ones (Entries 1-10, *Table 1*) were studied using *Lycopersicum escolentum* (tomato). The results obtained highlight enantioselective synthesis of heterocyclic secondary alcohols (Entries 1-10) using *Lycopersicum escolentum* (tomato). It was demonstrated that the enzyme dehydrogenases present in the *Lycopersicum escolentum* selectively reduced substituted aromatic heterocyclic ketones to the corresponding single chiral secondary alcohols in good isolated yields and high enantioselectivity (only single isomer was obtained), as conformed through HPLC using chiral column (no second isomer was present in the reaction medium, the unreacted starting

compound was recovered). It was observed that the enzymes responsible for chiral reduction of both six and five membered heterocyclic ketones have shown broad enzyme specificity and enantioselectivity. The results also highlight the enzyme dehydrogenase enantioselectivity towards the ketonic group present in the ring moiety of the heterocyclic N-containing aromatic compounds compared to the ketonic group present in the side aliphatic chain (Entry 5, *Table 1*). It is well known that the N-atom present in the heterocyclic aromatic ring increased the affinity of the substrate towards the enzyme when compared to the side ketonic group, which show low selectivity and poor yields, this may be due to steric binding factors of the com- pounds towards enzyme active sight.[18,19] When compared to earlier reports[20-23], the present study demonstrates that the dehydrogenase enzymes present in L. esculentum has reduced keto groups in five member and six heterocyclic compounds with good enantioselectivity, thus demonstrating the broad specificity of the enzyme dehydrogenase present in the tomato. The present process developed has the advantage over the currently known chemical and biological methodologies in obtaining value added chiral aromatic hetero alcohols in higher yields and enantioselectivity.[24,25] The main advantage of the methodology developed is to obtain optically pure heterocyclic secondary alcohols with (*S*) configuration in a mild, inexpensive and eco-friendly environment. Further commercial application of this methodology and characterization of the enzyme responsible for selective reduction process is in progress.

In brief, add 5 grams of cut pieces (1 cm - 1.5 cm long slices) of ripen tomato fruits, in 200 ml of 0.1 mM sodium phosphate buffer pH 6.8. To this add 200 mg of different heterocyclic ketones (1-10) and the reaction mixture was incubated at 37°C in an orbital shaker. The progress of the reaction was monitored at regular intervals by TLC/HPLC analysis (RP-18 column using mobile phase acetonitrile: water 80: 20, 254 nm). The products obtained were isolated, purified through silica gel column chromatography and the chiral alcohols obtained were confirmed by HPLC using chiral columns Daicel CHIRALCEL OD columns (25 cm - 4.6 mm I.D.) UV detector at 210 nm, varying the mobile phase conditions depending on the specific substrate/product. The structures of the products were determined by mass, infrared spectra, ^{1}H and ^{13}C NMR spectroscopic studies and also confirmed by the spectral data of the products obtained through chemical synthesis of the compounds by $NaBH_4$ reduction and compared with literature values. The enantiomeric excess (*ee*) and absolute configuration of chiral compounds 4-6, 8-10 were determined by the sign of specific rotation or by HPLC using chiral columns.

A major innovative idea laid down in the present work is the reduction of carbonyl present in imidazol-2-ones and pyrimidin-2-ones using a plant material *i.e*., tomato directly for the first time with very good chemical yield and enantiomeric excess.

CONCLUSION

In conclusion, this study highlights a practical and efficient process of bioreduction of heterocyclic ketones to respective heterocyclic secondary alcohols by alcohol dehydro-genase enzyme present in *L. esculentum* L. The results confirm that the plant cell membrane bound enzyme alcohol dehydrogenase show broad substrate specificity and chiral selectivity. The bioreduction of different prochiral heterocycles to corresponding optically active chiral alcohols has shown exclusively (*S*) configuration thus following common *Prelog*'s rule. Thus, this study demonstrates a simple, inexpensive approach in synthesis of optically pure (*S*)- heterocyclic secondary alcohols of biological importance in an eco-friendly environment.

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