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Asymmetric Reduction of Heteroaryl Methyl Ketones Using *Daucus carota*

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Abstract

Asymmetric reduction of the heteroaryl prochiral ketones to corresponding chiral alcohols by *Daucus carota* was studied. The study highlights selective bioreduction of different substituted heteroaryl ketones (**1a - 1j**) to their respective chiral alcohols (**2a - 2j**) using plant dehydrogenase enzymes present in *Daucus carota in* good yields (60% - 95%) and enantioselectivity (76% - 99%) with *S*-form configuration. The results obtained confirm that the membrane bound dehydrogenase enzyme has broad substrate specificity and selectivity in catalyzing both six and five membered heteroaryl methyl ketones. The present methodology demonstrates promising and alternative green route in the synthesis secondary chiral alcohols of biologically importance in a simple, inexpensive and eco-friendly process.

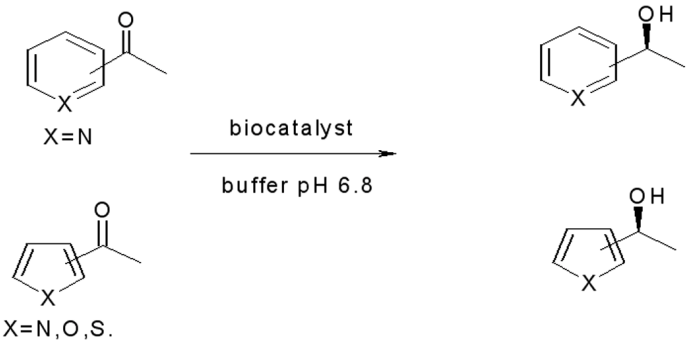
Keywords: *Daucus carota*, Bio-Reduction, Chiral Alcohols, Enantioselectivity

1. 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 number 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. Acetyl-pyridines (2 - 4) are known as aromatic components of foods, perfumes and smoking suppressants. Chiral heteroaryl alcohols have numerous applications as important intermediates in the synthesis of biological active molecules and also act as chiral ligands/auxiliaries in a number of asymmetric addition reactions [7-9]. There are many reports describing synthesis of chiral secondary alcohols using biocatalysts obtained from microbial/different parts of plant tissues [10-12].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 chiral alcohols of biological interest [13-15]. However, most of these processes have limitation in commercial application due to long incubation time, low substrate loading, poor isolated yields and enantioselectivity [16,17]. In continuation of our interest towards synthesis of chiral alcohols of biological importance using biocatalysts [18,19], we here in present, the process for production of chiral aromatic heterocyclic secondary alcohols by bioreduction of substituted heteroaryl ketones using *D.carota.*

2. Results and Discussion

Chiral reduction of substituted heteroaryl prochiral ketones (**1a - 1j**) **Scheme 1** were studied using *D carota.* (carrot). The results obtained **Table 1,** high lights enan-



Compound 1a-j

(**1a**) 2-acetyl pyridine
(**1b**) 3-acetyl pyridine
(**1c**) 4-acetyl pyridine
(**1d**) 2-acetyl-6-chloro pyridine
(**1e**) 2-acetyl-6 bromo pyridine
(**1f**) 2-acetyl-4-methyl pyridine
(**1g**) 2- propionyl pyridine
(**1h**) 2-acetyl pyrrole
(**1i**) 2- acetyl furan
(**1j**) 2- acetyl thiophene

Compound 2a-j

(**2a**) 2-(1-hydroxyethyl) pyridine
(**2b**) 3-(1-hydroxyethyl)pyridine
(**2c**) 4-(1-hydroxyethyl)pyridine
(**2d**) 2-(1-hydroxyethyl)-6-chloro pyridine
(**2e**) 2-(1-hydroxyethyl)-6-bromo pyridine
(**2f**) 2-(1-hydroxyethyl)-4-methyl pyridine
(**2g**) 2-(1-hydroxypropyl)pyridine
(**2h**) 2-(1-hydroxyethyl)pyrrole
(**2i**) 2-(1-hydroxyethyl) furan
(**2j**) 2-(1-hydroxyethyl)thiophene

Scheme 1. Chiral reduction of substituted heteroaryl pro chiral ketones by *Daucus carota*.

tioselective synthesis of heterocyclic secondary alcohols (**2a - 2j**) using *D.carota* (carrot). It was demonstrated that the enzyme dehydrogenases present in the *D.carota* selectively reduced substituted aromatic heterocyclic methyl ketones (**1a - 1j**) to the corresponding single chiral secondary alcohols (**2a - 2j**) 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 un-reacted starting compound was recovered). It was observed that the enzymes responsible for chiral reduction of both six and five membered heteroaryl ketones (**1a - 1j**)**,** has shown broad enzyme specificity and enantioselectivity. The results also highlights the enzyme dehydrogenase enantioselectivity towards six member heterocyclic N-containing aromatic compounds, when compared to five membered aromatic heterocyclic compounds containing "N", "O" and "S" atoms. 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 other three compounds (**1h - 1j**), which show low selectivity and poor yields, this may be due to steric binding factors of the compounds towards enzyme active sight. [20,21]. When compared to earlier reports [22-25], the present study demonstrates that the dehydrogenase enzymes present in *D.carota* has reduced keto groups attached to five member heteroaryl compounds (**1h - 1j**) (pyrrole, furan and thiophene) with good enantio-selectivity. thus demonstrating the broad specificity of the dehydrogenase enzyme present in the carrot. The obtained optically pure chiral pyridyl alcohols (**2a - 2h**) show exclusively (*S*) configuration thus following common *Prelog*' s rule [26]. Whereas biocatalytic reduction of thermodynamically unstable 2-acetyl furan and 2-acetyl thiophene (**1i - 1j**) gave respective chiral alcohols with (*R)* configuration [27]. 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 [28,29]. To study the steric-orientation of the enzyme active site in enantioselective reduction of the heteroaryl methyl ketones to respective chiral alcohols, it is planned to carry out chemical additive studies like allyl bromide and ethylenediamine tetra acetic acid (EDTA) that influence the stereoselectity in enzymatic bioreduction processes [30-32]. On addition of allyl bromide (0.2 mM solution) to the incubation medium the process of bio-reduction of pyridyl ketones to chiral alcohols using *D.carota,* was found increased by 20% - 40%, whereas no difference was observed in the enzyme dehydrogenase activity on addition of EDTA (0.5 mM) (results not presented). Thus, from the enzyme inhibition studies, it was confirmed that the cell membrane bound enzyme alcohol dehydrogenase has shown broad substrate specificity and enantioselectivity in bioreduction of substituted heteroaryl methyl ketones. The main advantage of the methodology developed is to obtain optically pure heterocyclic secondary alcohols with (*S*) configuretion 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.

Table 1. Asymmetric reduction of substituted hetreoaryl ketones (1a-1j) by *Daucus carota.*

Entry	Product	Time in Hours	Conversion%	Isolated yield%	ee%	Conf.
2a	2-(1-hydroxyethyl) pyridine	48	100	95	99	*S*
2b	3-(1-hydroxyethyl)pyridine	56	90	94	98	*S*
2c	4-(1-hydroxyethyl)pyridine	52	100	94	92	*S*
2d	2-(1-hydroxyethyl)-6-chloro pyridine	60	80	74	89	*S*
2e	2-(1-hydroxyethyl)-6-bromo pyridine	65	75	78	92	*S*
2f	2-(1-hydroxyethyl)-4-methyl pyridine	55	95	80	96	*S*
2g	2-(1-hydroxypropyl)pyridine	65	95	63	90	*S*
2h	2-(1-hydroxyethyl)pyrrole	72	65	65	90	*S*
2i	2-(1-hydroxyethyl) furan	76	55	60	76	*R*
2j	2-(1-hydroxyethyl)thiophene	70	50	55	78	*R*

Values were mean of the three independent experiments. The specific activity of the enzyme dehydrogenase present in *D.carota* was 255 mmol min^{-1} mg^{-1} protein (protein—25 µg/ml). Spectral data for the products (2a - 2j) obtained: 2a. (*S*)-*2-(1-hydroxyethyl) pyridine* **(C_7H_9NO),** liquid; optical rotation $[\alpha]^{25}_{D}$ = –30.2° (c 1, methanol), 99% ee (HPLC, Diacel Chiralcel OJH) [lit.[32 - 34] $[\alpha]^{25}_{D}$ -29.6 (c7.7 $CHCl_3$) for 99% ee]; IR (cm^{-1}) 3395 (OH), 2923 (CH_3); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.48 (d, 3H, *J* = 6.04 Hz),3.59(br s,1H), 4.84 (q, 1H, *J* = 6.04 Hz), 7.13 - 7.34 (m, 2H), 7.66 (t, 1H, *J* = 7.55 Hz), 8.53 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): 21.4 (CH_3), 66.5 (C-OH), 121.4 `36.1, 142.3, 164.1 (C1); GC/MS: 123 M^+. 2b. (*S*)-3-*(1-hydroxyethyl)pyridine* (C_7H_9NO**)**, liquid; optical rotation $[\alpha]^{25}_{D}$ = –44.9°, (c1, methanol), 98% ee (HPLC, Diacel (Chiralcel OJH) [lit. [32 - 34] $[\alpha]^{25}_{D}$ –38.9 (c1.0 ethanol) or 96% ee]; IR(cm^{-1}) 3259 (OH), 2972 (CH3); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.43 (d, 3H, *J* = 6.04 Hz), 3.46 (br s, 1H), 4.83 (q, 1H, J = 6.043 Hz), 7.19 (s, 1H), 7.65 (d, 1H, J = 7.554 Hz), 8.29 - 8.48 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): 23.2 (-CH_3) , 67.8 (C-OH), 134.5, 140.4, 122.5, 149.5; GC/MS- 123 M^+. 2c. *(S)-4-(1-hydroxyethyl)pyridine* (C_7H_9N O), solid, mp 64°C - 65°C; optical rotation $[\alpha]^{25}_{D}$ = –58.0°, (c1, methanol) 92% ee (HPLC Diacel Chiralcel OJH) [lit. [32-34] $[\alpha]^{25}_{D}$ –43.0 (c5.0 methanol) for 93% ee]; IR (cm^{-1}) 3313 (OH), 2924(CH_3); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.53 (d, 3H, J = 6.61 Hz), 3.06 (br s, 1H), 4.90 (q, 1H, J = 6.61 Hz), 7.28 - 7.40 (m, 2H), 8.44 - 8.55 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.9 (C4-CH_3), 68.2 (C-OH), 120.5, 149.0, 155.8 ppm; GC/MS: 123 M^+. 2d.(*S*)-2-(1-*hydroxyethyl*) 6-*chloro pyridine* (C_7H_8ClNO), colorless oil, optical rotation $[\alpha]_D^{20}$ -13.2(c1 chloroform), 89% ee (HPLC Dialcel Chiralcel OD); IR(cm^{-1}) 3350 (OH); ^{1}H-NMR (200 MHz): δ = 1.61(d, J = 6.6 Hz, 3H, CH_3), 3.59 (br s, 1H), 4.60 (d, q, J = 6.6, 3.6 Hz, 1H, CHOH), 7.33(d, J = 8.3 Hz, 1H; aromatic) 7.73 (dd, J = 8.4, 2.6Hz.1H; aromatic, H); ^{13}C-NMR(400 MHz): δ24.8 (CH_3), 66.8, 123.2,134.6, 140.2, 148.0, 152.1; EI-MS (m/z): 157 M^+. 2e.(*S*)-2-(1-*hydroxyethyl*)6-*bromo pyridine* (C_7H_8 BrNO), colorless oil; Optical rotation $[\alpha]_D^{25}$ –12.1 (c2, chloroform), 92% ee (HPLC Diacel Chiralcel OJH); IR (cm^{-1}) (film) 3390 (OH), 1742; ^{1}HNMR (200MHz) δ = 1.43 (d, J-6.6Hz,3H,CH3), 4.02 (br, d, J = 3.4Hz, 1H, OH), 4.94 (d, q, J = 6.6, 3.6 Hz, 1H, CHOH),7.43 (d, J = 8.2 Hz, 1H, aromatic), 8.44 (d,J=2.6Hz,1H, aromatic); ^{13}C NMR (400 MHz) δ28.0 (CH_3), 67.2 (CH), 129 (CH), 136.4 (CH), 140.8 (C) 148.0 (CH); = MS (EI, 70 ev) = m/z = 203 M^+.(lit.[38-39]). 2f. (*S*)-2-(1-*hydroxyethyl*)4-*methyl pyridine* (C_8 $H_{11}NO$), color less liquid; optical rotation $[\alpha]^{25}_{D}$–60.2° (c 1, chloroform), 96% ee (HPLC Diacel Chiralcel OJH) ; IR (cm^{-1}) 3416 (OH), 2923 (CH_3); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.46 (d, 3H, *J* = 6.42 HzCH_3), 2.37 (s, CH3 at C-4), 4.78 (q, brs, 1H, *J* = 6.42 Hz), 6.98 (d, 1H, *J* = 5.66 Hz), 7.03 (s, 1H), 8.35 (d, 1H, J = 4.91 Hz); ^{13}C NMR (400 MHz, $CDCL_3$) δ 20.8 (C4-CH_3), 21.6 (C2-CH_3), 66.5 (C-OH), 119.2 (C3), 121.8, 140.4, 151.2, 164.7 (C1); GC/MS: 137 M^+. 2g (*S*)-2-(1-*hydroxypropyl*)*pyridine* (C_8 $H_{12}NO$), colorless oil; optical rotation $[\alpha]_D^{20}$ –56.5 (c2, methanol), 90%ee (HPLC Diacel Chiralcel OJH); IR (cm^{-1}) 3404 cm^{-1} (OH);.^{1}H NMR (200MHz, CD Cl_3) δ = 0.93 (d, 3H, *J* = 6.42 Hz ,CH_3), 4.66 (1H, dd, J = 13.9, 7.2 Hz), 7.63 (1H, ddm, J = 7.6,4.6Hz), 8.32 (1H, dm J = 4.8 Hz); ^{13}C NMR (400 MHz), $CDCl_3$) δ = 8.9 (CH_3),29.2 (CH_2), 73.6, 119, 123.1, 138.2, 150.1, 165.0; MS (FAB) m/z = 138. 2h. (*S*)-2-(1-*hydroxyethyl*)*pyrrole* (C_6H_9 NO) colorless oil; optical rotation $[\alpha]_D^{25}$ –37.8 (c2, chloroform) 80% ee (HPLC, Diacel, Chiral ODH); IR (cm^{-1}) 3332 (OH), 2872 (CH_3); ^{1}H NMR (200 MHz, $CDCl_3$) δ = 1.41 (d, J = 6.4 Hz, 3H, CH_3), 4.66 (OH), 6.05 (OH) ,5.52 (dd, J = 3.3, 0.8 Hz, 1H), 5.64 (dt, J = 3.3, 0.7 Hz, 1H), 7.00(dd, J = 3.0, 0.6Hz, N-H); ^{13}C NMR (400MHzCD Cl_3) δ 21.6 (CH_3), 69.8, 104.2, 109.5, 144.2 MS m/z 112. 2i. (*R*)-2-(1-*hydroxyethyl*)*furan* ($C_6H_8O_2$), colorless liquid; optical rotation $[\alpha]_D^{25}$ +22.8 (c1.0,chloroform), 76% ee (HPLC Diacel, Chiralcel OJH) [lit.[36-37] $[\alpha]^{25}_{D}$ +23.2 (neat) for 96 % ee]; IR (cm^{-1}) 3334 (OH) ,2979 (CH_3); ^{1}H NMR (200 MHz) δ 1.43 (d, J = 6.6 Hz, 3H, Me), 3.06 (br.s, 1H, OH), 4.80(q, J = 6.6 Hz CHMe), 6.19 (dt, J = 3.3, 0.7 Hz 1H, 7.23 (dd, J = 3.3, 0.8 Hz, 1 H); ^{13}C NMR (400MHzCD Cl_3) δ 22.5 (CH_3), 64.8, 104.2, 139.5, 156.2; MS m/z 112. 2j. (*R*)-2-(1-*hydroxyethyl*)*thiophene* (C_6H_8OS) colorless oil; optical rotation $[\alpha]_D^{25}$ +22.1 (c3.0, chloroform), 78% ee (HPLC Diacel, Chiralcel OJH) [lit.[36-37] $[\alpha]^{25}_{D}$ +21.9 (neat) for 91% ee]; IR (cm-1) 3343 (OH), 2973 (CH_3), 842(C-S); ^{1}HNMR (200 MHz, $CDCl_3$) δ1.56 (d, J = 6.6Hz,3H, CH_3), 2.90 (br.s, 1H, OH) 5.12 (q, 1H J = 6.4Hz, $CHCH_3$); 6.92 - 7.1 (m, 2H, thienyl H3 and H5 and 7.15 - 7.20 (m, 1H thienyl H4 ppm); ^{13}CNMR (400MHz, $CDCl_3$) δ 25.0 (CH_3), 64.0, 119.2, 124.3, 124.6, 149.0; MS m/z 128.

3. Experimental

Daucus carota were purchased from local super market. The heterocyclic compounds used in the study were obtained from M/S.Alkali Metals Ltd; Hyderabad India and Aldrich Chemicals USA, all other reagents and chemicals used in the study were of analytical grade obtained locally.Bioreduction of substituted heteroaryl prochiral ketones (**1a - j**) was carried using fresh *Daucus carota*. In brief, add 5 grams of cut pieces(1 cm - 1.5 cm long slices) of *D.carota* tubers roots, in 200 ml of 0.1 mM sodium phosphate buffer pH 6.8, except compounds **1i - 1j** suspended in phosphate buffer pH 8.2, containing 0.2 mM $MgCl_2$. To this add 200 mg of different heteroaryl prochiral ketones (**1a - 1j**) and the reaction mixture was incubated under nitrogen atmosphere at 37°C in an orbital shaker. The progress of the reaction was monitored at regular intervals by TLC/HPLC analysis (RP-18 co-

lumn 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 or OJH or ODH 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 (**1a - 1g**) by $NaBH_4$ reduction and compared with literature values [33-40]. The enantiomeric excess (ee) and absolute configuration of chiral compounds **2a - 2j** were determined by the sign of specific rotation or by HPLC using chiral columns. Protein concentrations of *D.carota* homogenates determined by Bradford method and were found to be 25 µg/ml (bovine serum albumin as a standard protein) [41].

4. Conclusions

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

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