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Tetrahedron: *Asymmetry* 16 (2005) 1479–1483

Selectivity of *Daucus carota* roots and baker's yeast in the enantioselective reduction of γ-nitroketones

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Received 13 January 2005; accepted 1 March 2005
Available online 23 March 2005

Abstract—The enantioselective reduction of a series of aromatic γ-nitroketones was achieved by using *Daucus carota* roots in water, which afforded the corresponding (*S*)-alcohols with ees ranging from 73% to 100%. A comparison of these results with the data obtained by reducing the same substrates with baker's yeast resulted in *D. carota* always being more enantioselective than baker's yeast, although a lower number of substrates were reduced. The possible influence of the aromatic ring substituents on the reaction outcome is also discussed.

1. Introduction

The synthetic usefulness of enantiopure δ-nitroalcohols as precursors in the synthesis of natural substances has been well established.[1–4] Previously, we reported that alkaloids such as pyrrolidines,[5] indolizines[6] and pyrrolizidines,[7] pheromones, such as spiroketals[8,9] and lactones[5] can all be easily prepared starting from δ-nitroalcohols and δ-nitrodiols. The synthesis of enantiopure δ-nitroalcohols has been based mainly on the reduction of the corresponding γ-nitroketones, in particular by reduction using baker's[5,10] and other yeasts,[11,12] and chemical reduction by diisopinocamphenylchloroborane (DIP-Cl™).[9] The latter procedure allows the enantioselective synthesis of both enantiomers but the reagent is quite expensive. The reduction with baker's yeast is much more convenient although some of the γ-nitroketones are not reduced at all and the product selectivity with these substrates is often poor.[5] Thus, for finding an inexpensive alternative procedure for the synthesis of enantiopure δ-nitroalcohols, we decided to evaluate the reduction of a series of γ-nitroketones by *Daucus carota* roots. Recently, *D. carota* has been shown to reduce acetophenones[13–17] and other ketones,[13,18,19] with excellent selectivity. It has some advantages compared to baker's yeast; for instance the very low cost, the availability of the material and the ease of the reaction work-up and product recovery.[13,18] Moreover, it should be highlighted that the use of *D. carota* roots as reducing system applied to the organic synthesis has not been widely explored and very recently have a few articles been published,[13–19] which disclosed the potential for the enantioselective reductions of carbonyl compounds.

2. Results and discussion

Herein we report on the bio-reduction of a series of aromatic γ-nitroketones that have already been used as substrates for baker's yeast reductions.[5] Furthermore, three new ketones have been synthesised to better explore the effects of the substituents on the aromatic ring on the enzyme selectivity.

All substrates **3a**–**f** were synthesised (Scheme 1) as reported starting from the corresponding acetophenones **1a**–**f**,[20] by treatment of the intermediate Mannich bases with CH_3NO_2,[5] with the exception of **3g** (R = *p*-Cl) which was prepared from the commercially available β-chloropropiophenone **2** and CH_3NO_2 under basic conditions.[21]

The reduction of these carbonyl compounds was carried out according to the experimental procedures reported in the literature: one[13] employs cut roots in water, while the other[15] involves minced roots in 0.1 M phosphate buffer at pH 6.5. The results are reported in Table 1 together with the data obtained by baker's yeast reduction of the same substrates for comparison.

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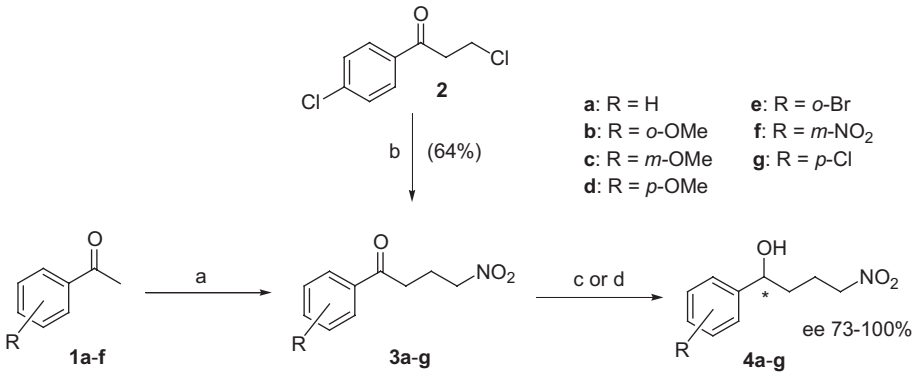
doi:10.1016/j.tetasy.2005.03.002



Scheme 1. Reagents and conditions: (a) (i) $(HCHO)_n$, $Me_2NH{\cdot}HCl$, EtOH, H^+, reflux, 2 h; (ii) NaOH aq; (iii) CH_3NO_2, TRITON B, reflux, 2 h; (b) CH_3NO_2, NaOH, MeOH–H_2O, rt, 1.5 h; (c) *Daucus carota* root, H_2O, rt, 10 days; (d) *Daucus carota* root, 0.1 M phosphate buffer, pH 6.5, rt, 10 days.

Table 1.

Entry	Substrate	Product	Conditions[a]	*Daucus carota*			Baker's yeast[5]		
				Conv.[b] (%)	Ee (%)[c]	Conf.[d]	Conv.[b] % (time)	Ee (%)[c]	Conf.[d]
1	**3a**	**4a**	A	67	>99	*S*	89 (7d)	78	*S*
2	**3a**	**4a**	B	97[e]	53	*S*			
3	**3b**	**4b**	A or B	0	—	—	64 (7d)	>99	*S*
4	**3c**	**4c**	A	100	>99	*S*	67 (7d)	76	*S*
5	**3c**	**4c**	B	82[f]	90	*S*			
6	**3d**	**4d**	A or B	0	—	—	82 (7d)	76	*S*
7	**3e**	**4e**	A or B	0	—	—	0	—	—
8	**3f**	**4f**	A	53	73	*R*	85 (9d)	58	*R*
9	**3g**	**4g**	A	73	94	*S*	50 (8d)	71	*S*
10	**6**		A or B	0	—	—	0	—	—
11	**7**	**8**	A	0	—	—	58 (7d)	95	*S*,*S*
12	**7**	**8**	B	76	58	*S*,*S*			

[a] Reductions were carried out at room temperature over 10 days either in water (A) or in 0.1 M phosphate buffer pH 6.5 (B).
[b] Determined by ^{1}H NMR of the crude reaction mixture.
[c] Determined by ^{1}H NMR analysis of the Mosher esters.[5,27]
[d] Determined by the sign of the specific rotation and by ^{1}H NMR analysis of the Mosher esters.[5,27]
[e] **4a** (68%) and **5a** (29%).
[f] **4c** (57%) and **5c** (25%).

The reductions by *D. carota* roots were quite slow under both conditions and were all stopped after 10 days. With the exception of *m*-NO_2 substituted ketone **3f** (entry 8), reduction by *D. carota* of the aromatic nitroketones afforded the corresponding alcohols with (*S*) absolute configuration. In all cases the roots proved more enantioselective than baker's yeast. For example, alcohols **4a** (R = H), **4c** (R = *m*-OMe) and **4g** (R = *p*-Cl) were all obtained with ee >94% when the reduction was carried out with *D. carota* in water (entries 1, 4 and 9), whereas the yeast afforded the same products with ee ranging from 71% to 78%. Conversely the range of substrates accepted by *D. carota* seems more limited as the reduction of **3b** (R = *o*-OMe) and **3d** (R = *p*-OMe) was not achieved (entries 3 and 6). It should be noted that in these two ketones, the substituent on the aromatic ring is the electron donor methoxy group in *ortho* and *para* positions, which could account for the decrease in the reduction rate of the carbonyl group by *D. carota*. Instead, as in the case of substrate **3c**, the same electronic effect could not be exerted by the *m*-OMe substituent and thus the reduction to the corresponding alcohol **4c** is achieved. It must be pointed out, however, that in the case of **3b**, the steric hindrance due to the *o*-OMe group, as a cause of the lack of reactivity, cannot be excluded. In fact substrate **3e**, bearing an *o*-Br group, was not reduced at all either by *D. carota* or baker's yeast (entry 7). Two more γ-nitroketones, bearing electron withdrawing substituents on the ring, that is, *m*-NO_2 **3f** and *p*-Cl **3g** were subjected to reduction. Since in the reduction of **3a** and **c** the use of the buffer decreased the enantioselectivity, the reduction of ketones **3f** and **g** was performed only in water. Both ketones were converted into the corresponding alcohols by *D. carota* (entries 8 and 9), thus suggesting that electronic effects do play a role in determining the reduction rate. Interestingly, baker's yeast seems less sensitive to the same electronic effects since all methoxy-substituted substrates were reduced to **4b**–**d**.

Reduction of ketone **3f** (R = *m*-NO_2) by *D. carota* afforded alcohol **4f** in good enantiomeric excess (73%, entry 8) and, surprisingly, with an (*R*)-configuration. Also the reduction by baker's yeast of **3f** afforded the

(*R*)-alcohol albeit, as in the other cases discussed so far, with a lower enantiomeric excess (58%). We cannot explain, at the moment, this change of facial selectivity in the reduction of **3f** by both systems.

It is interesting to note at this point the consistency of our results to those obtained by other authors in the reduction of simple acetophenones **1a**–**e** and **g**,[13,14] which suggests that the effects of the ring substituents on the reduction of aromatic ketones by *D. carota* are quite general. First, the corresponding (*S*)-alcohols were obtained in all cases, with enantiomeric excesses ranging from 78% to 100%. More significantly, methoxy-substituted substrates **1b** and **d** and *o*-bromo-substituted compound **1e** were only slowly converted in buffer to the corresponding alcohol with conversions never exceeding 12%.[14,22] By contrast, as in the case of nitroketone **3g**, *p*-chloro-substituted ketone **1g** was easily converted to the corresponding alcohol in 76% yield and 95% ee.[13]

Interestingly, in the reduction of **3a** (entry 2) and **3c** (entry 5) only, when carried out in buffer, the formation of chiral γ-lactones **5a** and **c** (Fig. 1) as byproducts was observed. These compounds could derive from a Nef reaction[23] underwent by the corresponding nitroalcohols[24] **4a** and **c**. However, treating the isolated alcohols with buffer did not yield even traces of the lactones after 10 days, while the same experiment carried out on **4c** in the presence of *D. carota* roots afforded a 2.5:1 mixture of the starting alcohol and lactone **5c**. Thus *D. carota* must play a role in the Nef reaction undergone by the alcohols.[25]

Finally, since *D. carota* proved such an efficient reduction system, we tested its ability on diketone **6** (Fig. 1), a precursor in the synthesis of chiral pyrrolizidines.[7] However, like baker's yeast, *D. carota* failed in the reduction of this substrate (entry 10), most probably because of its very low solubility in aqueous medium. When the analogous dialkyl compound **7** (Scheme 2)

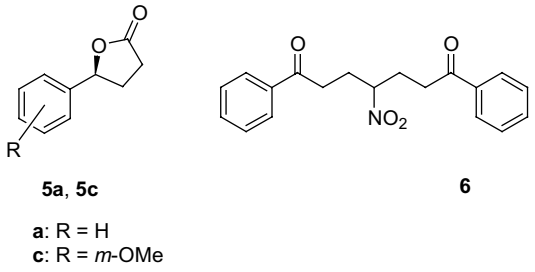
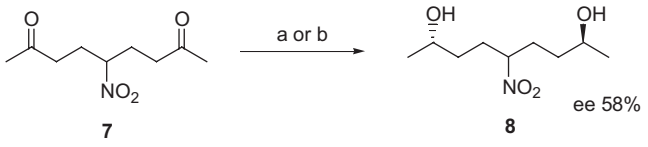


Figure 1.



Scheme 2. Reagents and conditions: (a) *Daucus carota* root, H_2O, rt, 10 days; (b) *D. carota* root, 0.1 M phosphate buffer, pH 6.5, rt, 10 days.

was used, the corresponding (*S*,*S*)-diol **8** was obtained, but in lower enantiomeric excess (58%) than with baker's yeast.

3. Conclusion

In conclusion, the enantioselective reduction of a series of aromatic γ-nitroketones was achieved by using *D. carota* roots in water, which afforded the corresponding (*S*)-alcohols with ees ranging from 73% to 100%. A comparison of these results to the data obtained by reducing the same substrates with baker's yeast resulted in *D. carota* always being more enantioselective than baker's yeast, although a lower number of substrates were reduced. The stereoselectivity of the reduction seems to be influenced by the reaction conditions and, for the substrates presented herein, reductions in plain water gave the best results. The possibility of using *D. carota* as an alternative to baker's yeast in the enantioselective reduction of γ-nitroketones increases the range of enantiopure δ-nitroalcohols which can be used as precursors in the synthesis of natural products. The availability of the enzymatic system, its low cost and the ease of the work-up and product recovery all are advantages that cannot be neglected.

4. Experimental

Melting points are uncorrected. Chromatographic separations were performed under pressure on silica gel by flash-column techniques; R_f values refer to TLC carried out on 25 mm silica gel plates (Merck F254), with the same eluent as indicated for the column chromatography. ^{1}H NMR (200 MHz) and ^{13}C NMR (50.33 MHz) spectra were recorded with a Varian XL 200 instrument in $CDCl_3$ solution. Mass spectra were carried out by EI at 70 eV, unless otherwise stated, on QMD 1000 Carlo Erba instruments. Microanalyses were carried out with a Perkin-Elmer 2400/2 elemental analyser. Optical rotations were determined with a JASCO DIP-370 instrument.

4-Nitro-1-phenylbutan-1-one **3a**,[5] 1-(2′-methoxyphenyl)-4-nitrobutan-1-one **3b**,[5] 1-(3′-methoxyphenyl)-4-nitrobutan-1-one **3c**,[5] 1-(4′-methoxyphenyl)-4-nitrobutan-1-one **3d**,[5] 4-nitro-1,7-diphenylheptane-1,7-dione **6**,[9] and 5-nitrononane-2,8-dione **7**[26] were synthesised as reported. Baker's yeast (YSC2, Sigma) reductions were performed under the already reported conditions[5] on 100 mg of the substrate.

4.1. 1-(2′-Bromophenyl)-4-nitrobutan-1-one 3e

The compound was prepared according to the already reported procedure,[5,20] starting from 2′-bromoacetophenone **1e**. Compound **3e**: R_f = 0.16 (eluent: EtOAc–petroleum ether, 1:12). ^{1}H NMR δ (ppm): 7.61 (d, *J* = 8.8 Hz, 1H), 7.39–7.24 (m, 3H), 4.53 (t, *J* = 6.6 Hz, 2H), 3.08 (t, *J* = 6.6 Hz, 2H), 2.43 (quint, *J* = 6.6 Hz, 2H). ^{13}C NMR δ (ppm): 202.0 (s), 167.9 (s), 133.8 (d), 131.9 (d), 128.3 (d), 127.6 (d), 74.4 (t), 38.7 (t), 21.5 (t). MS *m*/*z* (%) 271–273 (M^+, 1/1), 183–185 (95/87), 155–157 (35/35).

Anal. Calcd for $C_{10}H_{10}BrNO_3$: C, 44.14; H, 3.70; N, 5.15. Found: C, 44.37; H, 3.87; N, 5.27.

4.2. 4-Nitro-1-(3′-nitrophenyl)butan-1-one 3f

The compound was prepared accordingly to the already reported procedure,[5,20] starting from 3′-nitroacetophenone **1f**. Compound **3f**: $R_f = 0.21$ (eluent: EtOAc–petroleum ether, 1:4). ^{1}H NMR δ (ppm): 8.77 (s, 1H), 8.45 (d, $J = 8.0$ Hz, 1H), 8.29 (d, $J = 7.8$ Hz, 1H), 7.71 (t, $J = 8.0$ Hz, 1H), 4.57 (t, $J = 6.6$ Hz, 2H), 3.23 (t, $J = 6.6$ Hz, 2H), 2.49 (quint, $J = 6.6$ Hz, 2H). ^{13}C NMR δ (ppm): 195.8 (s), 148.4 (s), 137.5 (s), 133.5 (d), 130.1 (d), 127.7 (d), 122.8 (d), 74.3 (t), 34.9 (t), 21.2 (t). MS *m*/*z* (%) 192 ($M^+ - NO_2$, 1), 150 ($M^+ - CH_2CH_2CH_2NO_2$, 100), 104 (31), 76 (30). Anal. Calcd for $C_{10}H_{10}N_2O_5$: C, 50.42; H, 4.23; N, 11.76. Found: C, 50.29; H, 4.21; N, 11.57.

4.3. 1-(4′-Chlorophenyl)-4-nitrobutan-1-one 3g

To a stirred solution of CH_3NO_2 (1.4 mL, 26.0 mmol) in MeOH (26 mL), cooled at 0 °C, was slowly added a 2.0 M NaOH aqueous solution (13 mL, 26.0 mmol), keeping the temperature under 5 °C during the addition. After 20 min, 3,4′-dichloropropiophenone **2** (1.0 g, 4.92 mmol) was slowly added and the resulting solution stirred at first for 30 min at 0 °C and then at room temperature until TLC revealed that the reaction was complete (1.5 h). After cooling at 0 °C, glacial CH_3COOH (3 mL) and H_2O (40 mL) were added, the product extracted with DCM (3×30 mL) and the combined organic phases dried over Na_2SO_4. After filtration and evaporation of the solvent, crude **3g** was obtained. Purification by chromatography (eluent: EtOAc–petroleum ether, 1:5, $R_f = 0.38$) afforded pure **3g** (721 mg, 64%) as a yellow oil. Compound **3g**: ^{1}H NMR δ (ppm): 7.89 (d, $J = 8.8$ Hz, 2H), 7.45 (d, $J = 8.8$ Hz, 2H), 4.54 (t, $J = 6.6$ Hz, 2H), 3.12 (t, $J = 6.6$ Hz, 2H), 2.44 (quint, $J = 6.6$ Hz, 2H). ^{13}C NMR δ (ppm): 196.7 (s), 140.0 (s), 134.6 (s), 129.4 (d, 2C), 129.1 (d, 2C), 74.6 (t), 34.5 (t), 21.4 (t). MS *m*/*z* (%) 227 (M^+, 2), 139–141 (100/34), 111–113 (30/10). Anal. Calcd for $C_{10}H_{10}ClNO_3$: C, 52.76; H, 4.43; N, 6.15. Found: C, 52.40; H, 4.46; N, 5.85.

4.4. General procedure for *D. carota* root reduction in water

To a suspension of healthy and freshly cut roots (10 g) in water (70 mL), kept under vigorous stirring, a solution of the substrate (100 mg) in abs. EtOH (0.5 mL) was slowly added. The resulting mixture was stirred at room temperature. After 5 days, a second portion of freshly cut roots (5 g) was added and after 10 days the reaction was stopped by filtration over adsorbent cotton. The product was extracted with EtOAc and, after filtration and evaporation of the solvent, crude alcohol was purified by chromatography.

4.5. (*R*)-4-Nitro-1-(3′-nitrophenyl)butan-1-ol 4f

Compound **4f**: $R_f = 0.07$ (eluent: EtOAc–petroleum ether, 1:5). $[\alpha]_D^{25} = +24.7$ (*c* 0.64, $CHCl_3$). ^{1}H NMR δ (ppm): 8.24 (s, 1H), 8.16 (d, $J = 8.2$ Hz, 1H), 7.69 (d, $J = 8.2$ Hz, 1H), 7.55 (t, $J = 7.7$ Hz, 1H), 4.91 (t, $J = 6.2$ Hz, 1H), 4.46 (td, $J = 7.0$, 2.6 Hz, 2H), 2.26–2.04 (m, 2H), 1.90–1.79 (m, 2H). ^{13}C NMR δ (ppm): 146.1 (s), 131.6 (d), 130.0 (s), 129.5 (d), 122.7 (d), 120.5 (d), 75.2 (t), 72.5 (d), 35.6 (t), 23.7 (t). MS *m*/*z* (%) 240 (M^+, 1), 239 ($M^+ - 1$, 6), 150 (34), 104 (15). Anal. Calcd for $C_{10}H_{12}N_2O_5$: C, 50.00; H, 5.04; N, 11.66. Found: C, 49.92; H, 5.23; N, 11.42.

4.6. (*S*)-1-(4′-Chlorophenyl)-4-nitrobutan-1-ol 4g

Compound **4g**: $R_f = 0.20$ (eluent: EtOAc–petroleum ether, 1:3). $[\alpha]_D^{24} = -43.1$ (*c* 0.95, $CHCl_3$). ^{1}H NMR δ (ppm): 7.36–7.24 (m, 4H), 4.74 (dd, $J = 7.4$, 5.4 Hz, 1H), 4.43 (td, $J = 7.0$, 2.6 Hz, 2H), 2.25–1.98 (m, 2H), 1.95–1.73 (m, 2H). ^{13}C NMR δ (ppm): 142.1 (s), 133.4 (s), 128.6 (d, 2C), 126.9 (d, 2C), 75.3 (t), 72.9 (d), 35.3 (t), 23.8 (t). MS *m*/*z* (%) 183 ($M^+ - NO_2$, 2), 142–140 (7.3), 141–139 (84.27). Anal. Calcd for $C_{10}H_{12}ClNO_3$: C, 52.30; H, 5.27; N, 6.10. Found: C, 52.24; H, 5.36; N, 6.07.

4.7. General procedure for *D. carota* root reduction in buffer

Healthy and freshly cut roots were minced in an electric mixer for 2 min and the vegetable pulp (70 mL) suspended in 0.1 M phosphate buffer pH 6.5 (170 mL). A solution of the substrate (100 mg) in acetone (1 mL) was then added and the resulting mixture stirred at room temperature. After 5 days, a second portion of minced roots was added (35 mL) and after 10 days, the reaction was stopped by filtration over absorbent cotton. The product was extracted with EtOAc and, after filtration and evaporation of the solvent, crude alcohol was purified by chromatography.

Acknowledgements

We thank MIUR and Università di Firenze for financial support. Mr. Maurizio Passaponti and Mrs. Brunella Innocenti are acknowledged for their technical support. Dr. Daniela Stranges is acknowledged for carrying out some experiments. Ente Cassa di Risparmio di Firenze is acknowledged for granting a 400 MHz NMR spectrometer.

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