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A Novel Baker's Yeast Catalysed Hydride Reduction of an Epoxide Moiety

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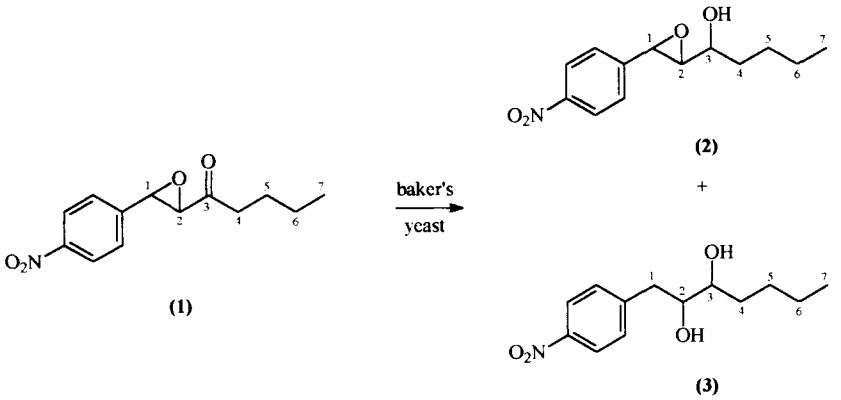
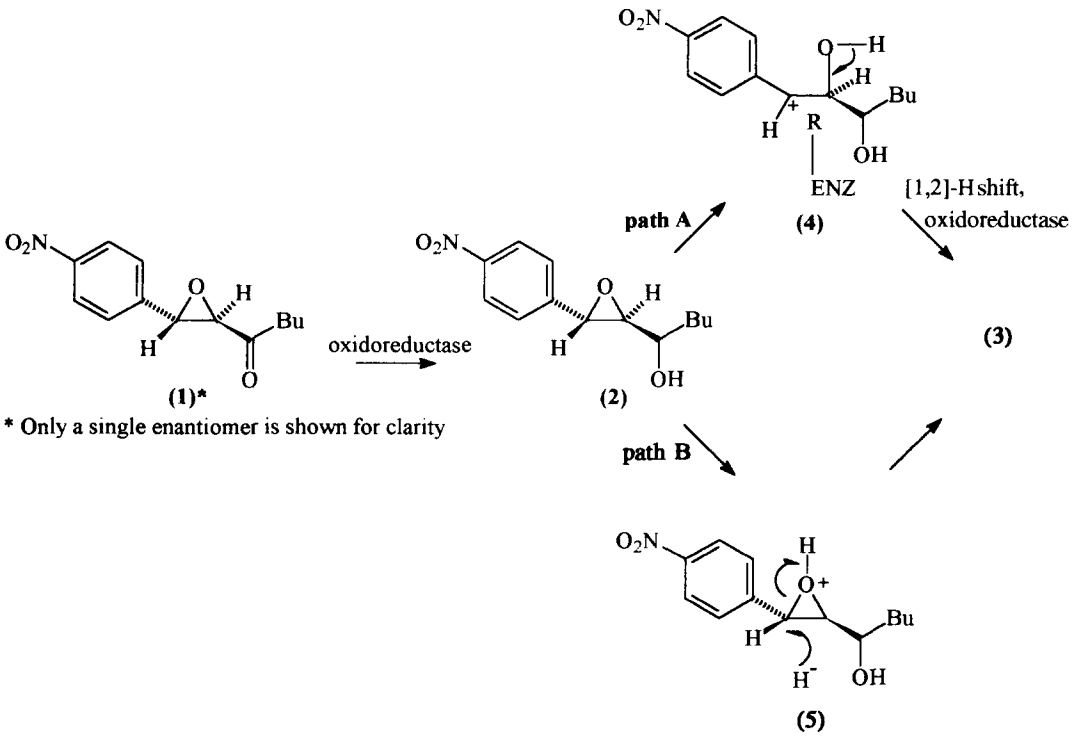
Abstract: The preparation of 2-[^{2}H]-1-(p-nitrophenyl)-1,2-epoxyheptan-3-one and its subsequent reduction by baker's yeast to the 2,3-diol is described. The mechanism of the reduction of the epoxide was determined using the β-^{2}H isotope shift in ^{13}C n.m.r. and mass spectroscopy and appears to be due to a novel enzyme catalysed hydride transfer from cofactors such as NADH or NADPH.

The ability of baker's yeast to achieve asymmetric reduction of ketones is well documented.[1] We have previously reported that the baker's yeast catalysed reduction of α,β-epoxy ketones with small β substituents eg. H, Me, gives α,β-epoxy alchohols.[2] Aromatic substituents result in the formation of 1,2,3-triols which are racemic, but single diastereomers, formed via the highly unusual *syn* ring opening of the epoxide.[2,3] While investigating the mechanism of this reduction we observed that the baker's yeast catalysed reduction of the *p*-nitrophenyl epoxy ketone **1** gave the epoxy alchohol **2** as well as the unexpected 2,3-diol **3**, as a single diastereomer of a racemate, (see Scheme 1).



Scheme 1

We were intrigued by the mechanistic pathway for the 2,3-diol formation and considered the two mechanisms: The oxidoreductase catalysed asymmetric reduction of the ketone of each enantiomer of compound **1** proceeds with complete enantiofacial selectivity to give the 1,2-epoxy alchohol **2** (see Scheme 2). Regiospecific enzyme catalysed opening of the epoxide ring can then generate a benzylic carbocation **4** which would be destablised by the electron withdrawing nitro substituent (Scheme 2, path A). The positive charge is subsequently quenched by an intramolecular [1,2]-hydride shift before nucleophilic attack of a water molecule can occur.

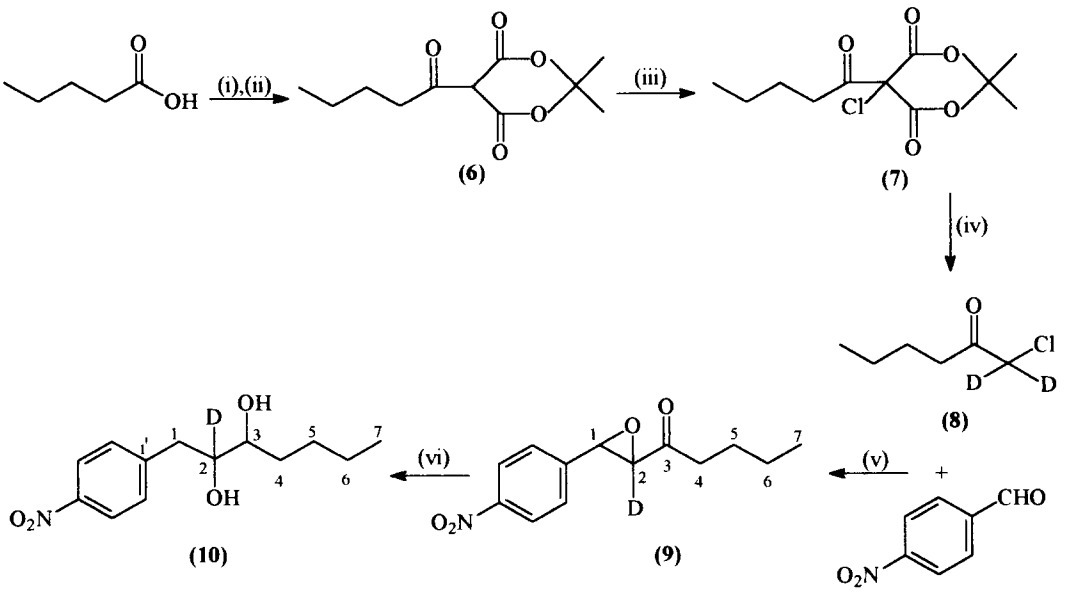


* Only a single enantiomer is shown for clarity

Scheme 2

An alternative mechanism, outlined in scheme 2, path B, involves the formal reduction of the oxirane **5** by a novel baker's yeast catalysed hydride transfer from NADH or NADPH to the epoxide yielding the diol **3**. A convenient method to establish whether the proposed 1,2 hydride shift occurs is to carry out the baker's yeast catalysed reduction on the epoxy ketone, isotopically labelled with deuterium at C-2, (compound **9**). The location of deuterium in the resulting 2,3 diol as indicated by ^{13}C n.m.r. and mass spectrometry will distinguish between the two proposed pathways.

The preparation of the required substrate, 2-[^{2}H]-1-(*p*-nitrophenyl)-2,3-epoxyheptan-3-one **9** is outlined in Scheme 3 and required the synthesis of the deuterium labelled 1-chlorohexanone **8**. This was successfully prepared by the coupling[4] of valeric acid chloride to Meldrum's acid to yield the C-acyl derivative **6**. Compound **6** was treated with SO_2Cl_2 yielding the chloro acyl derivative of Meldrum's acid **7**. Deuterium incorporation was achieved by reacting compound **7** with ^2H_2O and acetic anhydride to give 1-[$^2H_2$]-1-chlorohexa-2-one **8**; shown by ^{1}H n.m.r. spectroscopy to contain greater than 90 atom % deuterium. The labelled *p*-nitro epoxy ketone was obtained by potassium-*t*-butoxide catalysed condensation[2] of *p*-nitrobenzaldehyde with chlorohexanone **8**. The mass spectrum of the epoxy ketone **9** showed it to contain 28 atom % deuterium at position C-2. The loss of deuterium is ascribed to enolisation of the chlorohexanone **8** during the base catalysed condensation.[5]



Reagents: (i) $SOCl_2$, 70%, (ii) Meldrum's acid, pyridine, 80%, (iii) SO_2Cl_2, 65%, (iv) D_2O, Ac_2O, 20%, (v) K-t-butoxide, 20%, (vi) Baker's yeast, 12%.

Scheme 3

The deuterium labelled epoxyketone **9** was treated with baker's yeast and the diol **10** isolated. The β-isotope shifts[6] for C-1 and C-3 observed in the ^{13}C proton noise decoupled spectrum of diol **10** (see Table 1) unambiguously fix the deuterium position at C-2. No β-isotope shifts were observed for the C-2 and C-1′ resonances.

Table 1: ß-isotope shifts observed in the proton noise decoupled ^{13}C n.m.r. spectrum of 1-(*p*-nitrophenyl)-2,3-dihydroxyheptanone (**10**) obtained from baker's yeast reduction of 2-[^2H]-1-(*p*-nitrophenyl)-2,3-epoxyheptanone (**9**).

Carbon	δ_C*	$\Delta\delta_C$*
1	37.65	-0.10
2	75.05	-
3	74.39	-0.06
1′	115.46	-

* The unambiguous assignment of the ^{13}C n.m.r. signals of the revelant nuclei has been published.[2]

The position of the deuterium atom of diol **10** was further proven by mass spectrometry. A peak in the mass

spectrum at *m/z* 118 (29% of that at 117 for the non-deuterated species), characteristic of the ion $CH_3(CH_2)_3CHOHC^2HOH^+$, and no peak at *m/z* 138 ($NO_2PhCH^2H^+$) further supports the argument for the label being located at C-2 of the diol **10**.

The possible formation of the diol through a 1,2 hydride migration (Scheme 2) can therefore be ruled out and the reaction mechanism appears to be one in which baker's yeast catalyses a hydride transfer from cofactors such as NADH or NADPH to the epoxide as outlined in Scheme II. To our knowledge no example of an enzyme catalysed hydride opening of an epoxide has been reported in the literature. The stereochemical implications of the hydride reduction is currently under investigation.

A typical procedure for the baker's yeast reaction was carried as follows: The epoxy ketone (0.3 g) was added to actively fermenting yeast (4.0 g), sucrose (6.0 g) and water (30 ml) at 30 C. After 24 h a further aliquot of sucrose was added and after 48h the total mass was continuously extracted with chloroform.

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