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Biotransformations with baker's yeast: pH effects on diastereoselectivity during α-hydroxy-β-ketoester reductions and carbon–carbon bond cleavages †

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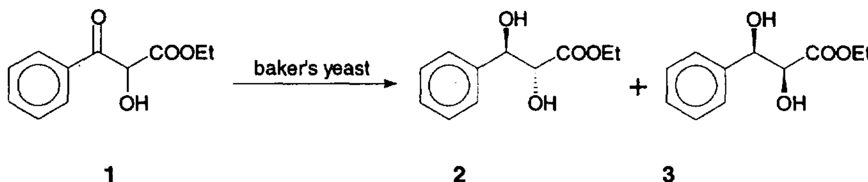
Abstract: Baker's yeast mediated reduction of α-substituted-β-ketoesters lead to reduction of the carbonyl group with high enantiospecificity and diastereoselectivity at low pH (4.0–5.0, e.e.>99%, d.e.>90%) but cleavage of the C–C bond is observed at higher pH (>8.0). Similar carbon–carbon bond cleavages are observed in the reactions of α-acetamido-β-ketoesters and acetamidocinnamic acid. © 1997 Elsevier Science Ltd

Enantiomerically pure β-hydroxy esters form the chiral building blocks in natural product synthesis[1,2] and their synthesis via enantiospecific reduction of β-ketoesters with baker's yeast (*Saccharomyces cerevisiae* NCIM 3305) is now a well-known method.[3] It is generally observed that the stereoselectivity in baker's yeast mediated reductions can be predicted by Prelog's rule,[4] and thus, depending upon the substrate, the stereochemical outcome can be controlled by several methods such as changing the size of the ester group,[5] adding cosolvents,[6] controlling pH etc. We have earlier demonstrated the effect of pH on the enantiomeric purity of the hydroxy ester during reduction of β-ketoester with immobilized baker's yeast.[7] In this communication, we wish to report that the pH of the medium affects not only the enantioselectivity but also the diastereoselectivity during the reduction process, and in some cases it can even lead to carbon–carbon bond cleavage.

Reduction of ethyl-2-hydroxy-3-oxo-3-phenylpropanoate **1** with baker's yeast (*Saccharomyces cerevisiae*) immobilized in calcium alginate beads predominantly gives ethyl (2R,3R)-2,3-dihydroxy-3-phenylpropanoate **2a** (Scheme 1), an important intermediate in the total synthesis of goniobutenolide, a potential anticancer drug.[8]



Scheme 1.

When the reaction is carried out under controlled pH, it is observed that changing the pH of the medium causes a significant change in the enantiomeric as well as diastereomeric purity of the product (Table 1).

The results described in Table 1 are very interesting. Even if one assumes a high (S)-stereoselectivity during enzymatic reduction of the carbonyl group by baker's yeast, one would expect two products **2** (2R,3R) and **3** (2S,3R) in 1:1 ratio since the starting material is racemic. However, we observe a much higher ratio (10:1) at low pH which gradually reaches the expected value at pH 7. One possibility is

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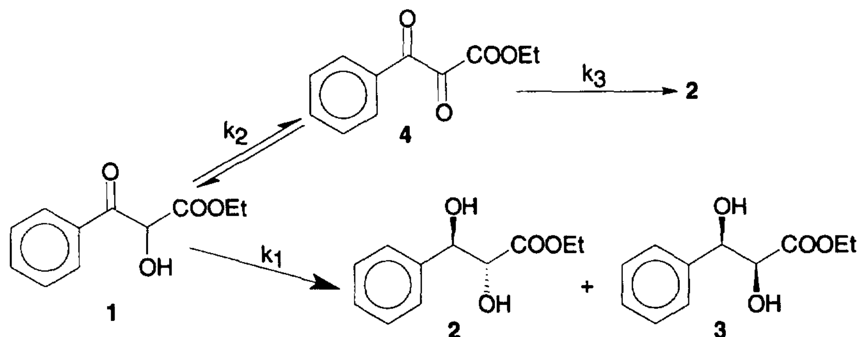
Table 1. Effect of pH on reduction of ethyl-2-hydroxy-3-oxo-3-phenylpropanoate **1** with immobilized baker's yeast

pH	anti / syn[a]	%e.e (anti) **2** (2R,3R)	% e.e (syn) **3** (2S,3R)	Yield (%)
4.0	10:1	99	99	82
5.0	7:2	96	95	70
6.0	5:3	95	90	64
7.0	1:1	91	87	45

a)The relative stereochemistry of *syn* and *anti* was determined by ^1H NMR after converting the dihydroxy ester to corresponding acetonide[9] and enantiomeric excess of the crude dihydroxy ester was determined by chiral HPLC.

that, one of the 2-hydroxy-3-oxo isomer is selectively reduced to the 2R,3R-dihydroxy ester while the substrate undergoes a dynamic kinetic resolution due to epimerization occuring at C_2 via the enol form. The pH of the medium will have an effect upon the rate of epimerization at the 2-position and this will determine the diastereomeric ratio of the product. A similar diastereomeric excess has also been observed in cases of baker's yeast mediated reduction of 2-substituted 3-oxoesters where an alkyl group is present in place of a hydroxy;[10] the diastereomeric excess found, however, was rather modest (30–50%). Although this possibility can not be ruled out, d.e.s as high as observed in Table 1 are too high to be expected on the basis of such a mechanism, especially because the pH changes are not really drastic. It is well known that baker's yeast possesses several alcohol dehydrogenases with varying substrate selectivities and even opposing enantioselectivities[11] and presence of such a mixture of enzymes acting simultaneously could be the reason for pH dependent diastereoselectivity observed in our studies. We have carefully monitored the progress of the reaction and have observed the formation of diketo intermediate **4**. This intermediate is also formed on reaction of **1** with pure alcohol dehydrogenase from baker's yeast and NADH/NAD^+, both at pH 4 and at pH 7. Interestingly, under identical reaction conditions quantitative conversion of **1** to **4** is observed at pH 4 while at pH 7 almost 85% of starting material remains unreacted; more interestingly, formation of the dihydroxy product is not observed. When **4** is reduced with baker's yeast at pH 4, exclusively **2** is formed while at pH 8 decomposition of **4** to benzoic acid is observed.[12] This indicates that at low pH the 2-hydroxy-3-oxo ester **1** first undergoes oxidation to 2,3-dioxo ester **4** (reaction 2) which then gets enantioselectively reduced to 2,3-dihydroxy ester **2** (reaction 3) but not by the same enzyme (Scheme 2).

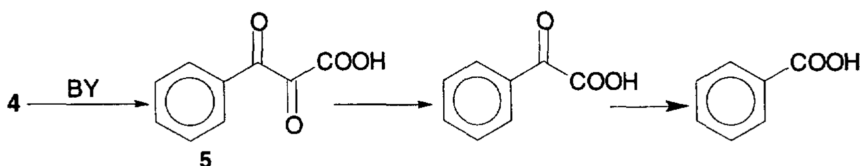


Scheme 2.

The equilibrium of reaction 2 shifts from right (dioxo ester **4**) to left (hydroxy ester **1**) as the pH increases. Simultaneously, other alcohol dehydrogenases also act to reduce **4** to **2** (reaction 3),

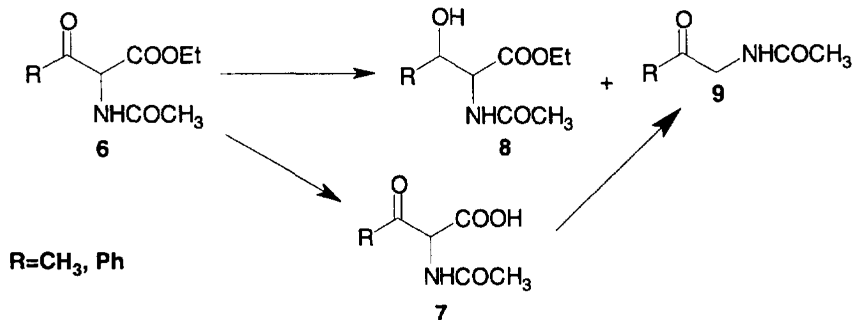
and **1** to **2** and **3** (reaction 1). The diastereomeric composition of the final dihydroxy product then depends upon the rates of various reactions occuring simultaneously in the reactor. Apparantly, at pH 4, $k_2>k_3>k_1$ providing product with high diastereomeric excess. As the pH increases, the rate pattern changes causing a shift in diastereoselectivity, probably due to changes in relative activities of different alcohol dehydrogenases present in baker's yeast.

The results in Table 1 also indicate that the product yield too is strongly affected by a change in pH, especially at pH higher than 6. At pH 8, there is a significant cleavage of C–C bond with formation of more than 75% benzoic acid. This can be explained on the basis of hydrolysis **4** to corresponding acid by the action of esterases present in baker's yeast.[13] This acid readily decarboxylates to benzoylformic acid which in turn decarboxylates to benzoic acid with an apparant manifestation of C–C bond cleavage during baker's yeast reduction (Scheme 3).



Scheme 3.

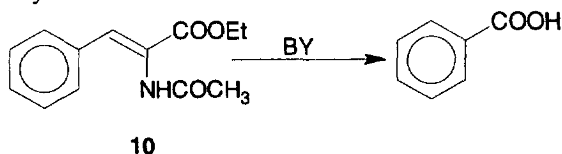
Such a decomposition of the reagent with a C–C bond cleavage is also observed when α-acetamido-β-ketoesters **6** are reduced with baker's yeast. Most of the starting material decomposes into unidentified products and only a small amount (10%) of the corresponding reduced product **8** and traces of **9** are obtained[14] at low pH (<5.5). At pH 8, cleavage product **9** is obtained in almost quantitative yield (Scheme 4).



Scheme 4.

This reaction also apparantly occurs through hydrolysis of ester **6** to acid **7** and subsequent decarboxylation to form **9** (Scheme 4).

Reaction of acetamido cinnamic acid or its corresponding ethyl ester **10** with baker's yeast in aqueous buffer in pH range of 5–8 also leads to C=C cleavage and formation of benzoic acid in very high yields instead of phenylalanine.



10

Although acid catalyzed conversion of **10** to phenylpyruvic acid is well known[15] its decarboxylation would lead to phenylacetic acid and not benzoic acid. The substrate is quite stable in the reaction medium without baker's yeast and it is unlikely that the formation of benzoic acid would occur by a

non-enzymatic process. Reduction of carbon–carbon double bonds by baker's yeast has been reported by several authors[3] but exclusive C=C cleavages are not reported. It appears that the presence of a nitrogen at α-position facilitates the action of oxidative enzymes such as cytochrome P-450 present in baker's yeast[16] and the substrate undergoes oxidative decomposition to benzoic acid. Work is in progress to explore this possibility and understand the phenomenon.

Experimental

Varian Gemini 200 MHz spectrometer was used to obtain ^{1}H-NMR with $CDCl_3$ as solvent, (TMS as internal standard), Mass spectra were recorded on "VG Auto Spec" LSIMS spectrometer. Optical rotations were measured on JASCO DIP-370 digital polarimeter. HPLC analysis was done on Shimadzu LC 9A. Elemental analysis was carried out on a Perkin–Elmer 240B apparatus. Ethyl-2-hydroxy-3-oxo-3-phenylpropanoate **1** was prepared by the method of Karrer and coworkers[17] and α-acetamido-β-ketoesters **3** were prepared as reported in literature.[18]

*Ethyl (2R,3R)-2,3-dihydroxy-3-phenylpropanoate (**2a**) and ethyl (2S,3R)-2,3-dihydroxy-3-phenyl propanoate (**2b**)*

In a typical procedure, commercial baker's yeast was immobilized (5 g) in calcium alginate as described earlier.[7] The beads were suspended in citrate buffer (250 ml, pH 4) containing glucose (10 g) and stirred with magnetic bead at room temperature. After activation of the yeast for 2 h, pH of the medium was adjusted with 10% ammonia to desired value and the substrate **1** (500 mg in 5 ml ethanol) was added slowly over 10 h with stirring. Reactants were stirred for another 48 hours with addition of glucose (2 g) every 6 h. The pH of the solution was measured every 15 minutes and maintained by addition of 10% ammonia solution in the initial stages and then every half an hour. Reaction was followed by TLC and after complete reduction, the beads were filtered and washed with chloroform. The reaction mixture was extracted with chloroform and combined organic extracts were evaporated. The residue was purified by column chromotography [hexane:diethylether,10:1] to obtain a mixture of unseparable diastereomers. **2a** and **2b**: ^{1}H-NMR ($CDCl_3$) δ 7.4 (m, 5H), 4.98–4.90 (d, 1H), 4.45–4.30 (d, 1H), 4.25–4.10 (q, 2H), 1.3–1.15 (t, 3H); Mass spectrum (LSIMS): (210).

Reaction of 1 with alcohol dehydrogenase from baker's yeast

Alcohol dehydrogenase (15 mg), dissolved in citrate buffer (0.05 M, pH 4.0), substrate **1** (52 mg, 0.25 mmol), NADH (89 mg, 0.125 mmol) and NAD^+ (83 mg, 0.125 mmol) were stirred at room temperature and the reaction was monitored by TLC. When the starting material was consumed (24 h), the reaction mixture was extracted with ethyl acetate, washed with water and dried over sodium sulfate. The solvent was evoporated under vacuum to obtain **4**. ^{1}H-NMR ($CDCl_3$) δ 7.4 (m, 5H), 4.2–4.3 (q, 2H), 1.1–1.3 (t, 3H); IR: 2990, 1750, 1715, 1705. Analysis: Calc. for $C_{11}H_{10}O_4$: C,64.15; H, 4.89%. Found: C,64.24; H, 4.92% .

Determination of enantiomeric excess

Optical purity was determined by using Chiralcel O.J. column (25×0.46 cm, Daicel, Japan; solvent system 10% isopropanol in hexane; flow rate 0.6 ml/min; Detection 254 nm; Retention times of ethyl-2,3-dihydroxy-3-phenylpropanoate; (2R,3S), (2S,3R), (2S,3S) and (2R,3R) were 22.1, 23.4, 27.4 and 29.7 min respectively.

Determination of diastereomeric execss

The diastereomeric mixture of the dihydroxy ester was converted into the corresponding acetonide by reaction with 2,2-dimethoxy propane in presence of p-toluene sulfonic acid. Ethyl (2R,3R)-2,3-isopropylidenyloxy-3-phenylpropanoate: ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5H), 5.4 (d, 1H), 4.7 (d, 1H), 3.5 (q, 2H), 1.5 (s, 3H), 0.8 (t, 3H); $[\alpha]_D^{23}$=16.9 (c 1.0, dichloromethane) {lit.[9] $[\alpha]_D^{20}$=16.3, c 1.0 dichloromethane}. Ethyl (2S,3R)-2,3-isopropylidenyloxy-3-phenylpropanoate: ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5H), 5.1 (d, 1H), 4.25 (d, 1H), 3.65 (q, 2H), 1.8 (s, 3H), 1.3 (t, 3H); $[\alpha]_D^{23}$=−65.9 (c 1.0,

dichloromethane) {lit.[9] $[\alpha]_D^{20}$=−67.3 dichloromethane}; diastereomeric excess was determined by comparing the relative areas for the singlet proton at 1.5 ppm for **2a** and 1.8 ppm for **2b** and the configuration was assigned on the basis of optical rotation cited in literature.

Acknowledgements

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References

1. a) Mori, K.; Katsurada, M. *Justus Liebigs Ann. Chem.*, **1984**, 157. b) Hirama, M.; Uei, M. *J. Am. Chem. Soc.*, **1982**, *104*, 4251.
2. Frater, G. *Helv. Chim. Acta*, **1979**, *62*, 2825.
3. a)Servi, S. *Synthesis*, **1990**, 1. b) Csuk, R.; Glanzer, B. *Chem. Rev.* **1991**, *91*, 49.
4. Sih, C.J.; Chen, C.S. *Angew. Chem., Int. Ed. Eng.*, **1984**, *23*, 570.
5. a) Shieh, W.R.; Gopalan, A.S.; C. Sih, J. *J. Am. Chem. Soc.*, **1995**, *107*, 2993. b) Hirama, M.; Shimizu, M.; Iwashita, M. *J. Chem. Soc., Chem. Commun.*, **1983**, 599.
6. Nakamura, K.; Konda, S.; Kanai, Y.; Hida, K.; Ohno, A. *Tetrahedron: Asymmetry*, **1996**, *7*, 409.
7. Bhalerao, U.T.; ChandraPrakash, Y.; Luke Babu, R.; Fadnavis, N.W. *Synth. Commun*, **1993**, 1201.
8. a.)Surivet, J.P.; Vatele, J.M. *Tetrahedron Lett.*, **1996**, *37*, 4373. b) Yang, Z.; Zhou, W. *Tetrahedron*, **1995**, *51*, 1429.
9. Effenberger, F.; Hopf, M.; Ziegler, T.; Hudelmayer, J. *Chem. Ber.*, **1991**, *124*, 1651.
10. a) Sato, T.; Tsurumaki, M.; Fujisawa, T. *Chem. Lett.*, **1986**, 1367. b) Fujisawa, T.; Itoh, T.; Sato, T. *Tetrahedron Lett.*, **1984**, *25*, 5083. c) Nakamura, K.; Miyai, T.; Nagar, A; Oka, S.; Ohno, A. *Bull. Chem. Soc., Japan*, **1988**, *61*, 2089.
11. Heidlas, I.J.; Engel, K.H.; Tressi, R. *Eur. J. Biochem.*, **1988**, *172*, 633.
12. Control experiments without baker's yeast under identical conditions showed that compounds **1–4**, **6**, **8** and **10** were stable; discounting the possibility of formation of cleavage products due to simple decomposition in aqueous solutions.
13. Fadnavis, N.W.; Prabhakar Reddy, N.; Bhalerao, U.T. *J. Org. Chem.*, **1989**, *54*, 3218.
14. Due to insignificant yields, we did not find the stereochemistry and optical purity of the products.
15. *Vogel's Text book of Practial Organic Chemistry*, Longman Singapore Publishers Ptc. Ltd., 1989, p 741.
16. Armstrong, R.N., CRC *Critical Review in Biochemistry*, **1987**, *22*, 39.
17. Karrer, P.V.; Kebrle, J.; Thakkar, R.M. *Helv. Chim. Acta.*, **1950**, 220.
18. Adkins, A.; Reeve, E.W. *J. Am. Chem. Soc.*, **1938**, *60*, 1329.

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