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Short Communication

Preparation of Enantiomerically Pure Methyl (*R*)-α-Hydroxy(2-furyl)acetate by Baker's Yeast Reduction in Multiphase Systems

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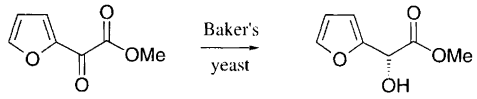
Furanoid compounds are important building blocks in organic synthesis. functioning either as masked 1,4-diketones or cyclopentenones[1] or as masked acids.[2] In addition, chiral compounds containing both a furan moiety and a dienophilic group are valuable synthetic intermediates in intramolecular Diels–Alder reactions.[3] Enantiomerically pure α-hydroxy acid derivatives are also important building blocks,[4] and have been obtained via enzyme-catalysed kinetic resolution of racemates,[5] or by enantioselective asymmetric reduction of α-substituted α-keto acid derivatives using chiral catalysts, isolated enzymes or micro-organisms.[6]

Microbial enantioselective reductions of carbonyl compounds, especially β-dicarbonyl compounds, are well known and baker's yeast (*Saccharomyces cerevisiae*, BY) is often the reagent of choice.[7] However, only a few α-hydroxy acid derivatives have been obtained in satisfactory ee via BY-reduction.[8] We have previously studied such a reduction of α-oxo(2-furyl)acetic acid derivatives and found that the α-oxo amide affords the α-hydroxy amide in 45% yield and 98% ee, when the reaction is carried out in water in the presence of 2-propanol.[9] However, under similar conditions, methyl α-oxo-(2-furyl)acetate (**1**) furnishes (−)-methyl (*R*)-α-hydroxy-(2-furyl)acetate (**2**, see Scheme 1) of only 35% ee.[9] Since hydrolysis of amides requires harsh conditions, which may lead to racemisation or decomposition, we wanted to improve the BY reduction of **1** in order to obtain the enantiomerically pure methyl ester of α-hydroxy-(2-furyl)acetic acid, which should constitute a more versatile chiral building block than the amide.



1 (-)-(*R*)-**2**

Scheme 1. Baker's yeast reduction of methyl α-oxo(2-furyl)-acetate (**1**) to give (−)-methyl (*R*)-α-hydroxy(2-furyl)acetate (**2**).

Whole cell systems like BY produce many reducing enzymes with opposite or low enantioselectivities.[10] In order to have stereochemical control in BY reductions, i.e., utilising only the desired enzyme activity, three different strategies are available: (1) substrate modification, (2) control of the enzymatic activities by selectively depressing or enhancing a specific activity by means of different additives, e.g. metal ions[11] or inhibitors,[10c,12] or (3) control of the substrate concentration by using solvents or absorbing resins in what could be defined as extractive biocatalysis.[13] Multiphase systems containing water-immiscible solvents have been considered in biocatalysis using whole cells.[8e,14]

In this communication we address the problem of increasing the enantiomeric excess of methyl (*R*)-α-hydroxy(2-furyl)acetate (**2**), the product of the BY reduction of methyl α-oxo(2-furyl)acetate (**1**, Scheme 1), by means of a rational approach to a combination of the above-mentioned strategies.

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Results and discussion

When performed essentially as described previously[9] (water at 35 °C with fermenting BY and glucose), the biotransformation depicted in Scheme 1 furnished fair yields of (−)-methyl (*R*)-α-hydroxy(2-furyl)acetate (**2**) of 30–35% ee in several experiments. The use of pre-fermented yeast in water in the absence of glucose gave (−)-(*R*)-**2** in 54% ee.

Various compounds, including ethyl chloroacetate, allyl alcohol, allyl bromide and methyl vinyl ketone, have been employed extensively as additives or inhibitors in order to increase ee in BY reductions of β-keto esters.[10c,12b–d,13] Other compounds, for instance oxamic acid[12a] and allicin[15] (obtained *in situ* from garlic), are known for their ability to inhibit specific reductases but have not been used before for stereochemical control of yeast reductions.

We found that BY reductions of **1** performed in the presence of oxamic acid (0.1–10 mM) or ethyl chloroacetate (25 or 67 mM) either with or without added glucose gave no significant improvement to the ee of isolated **2** (<45% ee). Reactions in the presence of allyl alcohol or methyl vinyl ketone (both 1 g^{-1}) gave a surplus of (*S*)-**2** (ee_{max} = 15%) within 7 h and (ee_{max} = 15%) within 17 h, respectively. Apparently both compounds inhibited the enzyme providing the desired (*R*)-enantiomer, the activity of which we wished to single out. When the biotransformation (Scheme 1) was performed in the presence of crushed garlic cloves, (*R*)-**2** of only 15% ee was obtained, indicating that the inhibitory effect of garlic is similar to that displayed by allyl alcohol and methyl vinyl ketone.

We and others have previously shown that the addition of 1- or 2-propanol to some substrates can result in increased ee in BY reductions.[9,14a] In our hands, although 2-propanol was ineffective, 1-propanol used as the solvent for the substrate in the reference reaction gave the product in 43% ee.

Work by some of us has recently demonstrated that the ee in the BY reduction of ethyl β-oxobutanoate can be substantially improved by adsorption of the substrate on a resin before the reaction.[16] This method also proved to be successful with substrate **1** which on BY reduction in the presence of 1-propanol furnished compound **2** partitioned approximately 2:1 between the BY–water phase (90% ee) and the resin phase (92% ee) in a 50% yield.

Ethyl β-oxobutanoate can be successfully reduced in a two-phase system (water–organic solvent, 1:1) by crude BY in high enantiomeric excess.[17] When the reference reaction was tried with subtrate **1** under vigorous agitation with an equal volume of hexane, no significant improvement in the ee of the product **2** was observed. However, since only a few layers of water molecules are required to hydrate enzymes fully, their activity should be preserved even when only very small amounts of water are present in the system. A reduction of the water content is easy to achieve if dry baker's yeast (DBY) is used.

Some aliphatic α-oxo esters can be reduced in benzene to chiral α-hydroxy esters with satisfactory ee of the produced α-hydroxy esters using DBY and a small amount of water.[8e] Therefore reductions of **1** using 1.6 ml H_2O per/g of DBY and an added solvent were studied. Two bulk solvents were tested, benzene and hexane. Small amounts of ethanol or 1-propanol were used to dissolve **1**. In addition, different buffers (pH 7.1; 6.15; 4.94; 3.94) were tested against pure water. The results are presented in Table 1.

Hexane was the best of the two solvents, giving both superior ee and superior yield within 25–29 h. The benefits of using 1-propanol instead of ethanol were now evident. Although the ee:s were not significantly higher than those obtained using pure water, the reaction at pH 7.1 gave more reproducible results. A plot of the ee of the product **2** versus reaction time is shown in Fig. 1.

For experiments on a larger scale, we selected the conditions that gave 93% ee at 98% conversion. In order to achieve high conversions and enantiomeric excesses, the large scale reactions required longer reaction times, probably due to the multiphase system used, in which the efficiency of the stirring was crucial.

One of the puzzling facts that emerged from this study and which warrants further investigation was the observed increase of the ee of the product (*R*)-**2** with the time of reaction (see Fig. 1). Similar results have been noted before and have been suggested to be due to enantioselective hydrolysis of the produced hydroxy esters by the action of hydrolytic enzymes present in the yeast.[8d] In our experiments, however, the mass balances did not support the possibility that one enantiomer of the product was consumed in an enantioselective degradation process. An alternative explanation of the

Table 1. Reduction of methyl α-oxo(2-furyl)acetate (0.1 g) in a system consisting of dry baker's yeast (3 g), water (5 ml) and an organic solvent (30 ml; Hx = hexane, Bz = benzene) with vigorous mechanical shaking. (RT = reaction time; c = conversion; ee = enantiomeric excess).

	Hx			Bz		
pH	RT (h)	C (%)	ee[d] (%)	RT (h)	C (%)	ee[a,c,f] (%)
[a,b]	26	98	77	26	8	—
[a,c]	29	93	92	26	17	8
7.1[b]	26	100	75	26	11	—
7.1[c]	29	98	93	26	16	6
6.2[e]	29	94	89	29	2	—
4.9[c]	29	86	87	29	8	—
3.9[c]	29	<1	—[f]	29	0	—

[a]Unbuffered water phase. [b]The substrate was dissolved in absolute ethanol (0.6 ml). [c]The substrate was dissolved in 1-propanol (0.6 ml). [d]The ee was determined by GLC on capillary columns with derivatised cyclodextrin phases. [e]See Fig. 1. for ee vs. time graphs. [f]At low conversions (<15%) the product ee:s were not determined owing to experimental difficulties.

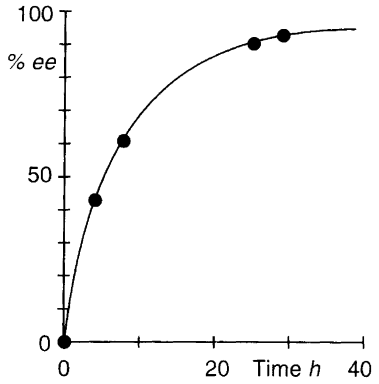

Fig. 1. Enantiomeric excess vs. reaction time of methyl (*R*)-α-hydroxy(2-furyl)acetate (**2**) produced from the small scale reaction of methyl α-oxo(2-furyl)acetate (**1**) dissolved in 1-propanol using DBY in a pH 7.1 aqueous buffer solution suspended in hexane (see Table 1).

observed increase in the ee of **2** with time is that this compound is subject to a redox equilibrium and that two enzymes with opposite enantiomeric preferences are unevenly destroyed (or produced) during the course of the yeast reduction. Experiments with labelled **2** are in progress.

This work demonstrates that excellent results can be obtained in whole cell biotransformations by medium engineering.

Experimental

*(−)-Methyl (*R*)-α-hydroxy(2-furyl)acetate* (**2**). DBY (62 g) and hexane (625 ml) were mixed with an aqueous phosphate buffer solution (100 ml, pH 7.1, 30 mM), and the substrate **1** (2 g) in 1-propanol (12 ml) was added. The mixture was stirred vigorously with an efficient mechanical stirrer for 90 h. The hexane was separated from the yeast–water mixture and the latter was extracted several times with ethyl acetate. The combined organic phases were dried and concentrated to give an oil, which, after chromatography on silica gel, furnished 0.94 g of (−)-methyl (*R*)-α-hydroxy(2-furyl)acetate (**2**, 47%, 95% ee) as an oil, which crystallised on standing. Recrystallisation from pentane gave colourless needles of >99% ee (by GC on permethylated cyclodextrin liquid phase), m.p. 46.5–47 °C, $[\alpha]_D^{21}$ −135 (*c* 1, $CHCl_3$) {lit.[5b] $[\alpha]_D^{22}$ −128.5 (*c* 1, $CHCl_3$)}. Although the yield of **2** was fairly constant, about 50%, shorter reaction times on the same scale gave lower ee:s: 28 h, 51%, 77% ee; 48 h, 44%, 90% ee. Less efficient stirring had the same effect: shaking for 48 h, 44%, 69% ee.

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References

1. Piancatelli, G., D'Auria, M. and D'Onofrio, F. *Synthesis* (1994) 867.
2. Fuganti, C., Graselli, P., Servi, S. and Högberg, H.-E. *J. Chem. Soc., Perkin Trans. 1* (1988) 3061.
3. Kappe, C. O., Murphee, S. S. and Padwa, A. *Tetrahedron 42* (1997) 14179.
4. Hanessian, S. *Total Synthesis of Natural Products: The Chiron Approach*, Pergamon, New York 1983.
5. (a) Fukuda, Y., Harada T. and Izumi, Y. *J. Ferment. Technol. 51* (1973) 393; (b) Waldmann, H. *Tetrahedron: Asymmetry 30* (1989) 3057.
6. (a) Carpentier, J.-F. and Mortreux, A. *Tetrahedron: Asymmetry 8* (1997) 1083; (b) Gottwald, M. In: Helmchen, G., Hoffmann, R. W., Multzer, J. and Schaumann, E., Eds., *Houben-Weyl. Methods of Organic Chemistry, Stereoselective Synthesis*, Thieme, Stuttgart 1995, Vol. E21d, 4143; (c) Simon, E. S., Plante, R. and Whitesides, G. M. *Appl. Biochem. Biotechnol. 22* (1989) 169; (d) Sheldon, R. A. *Chirotechnology*, Marcel Dekker, New York 1993.
7. (a) Czuk, R. and Glänzer, B. I. *Chem. Rev. 91* (1991) 49; (b) Faber, K. *Biotransformations in Organic Chemistry*, 3rd ed., Springer, Berlin 1997; (c) Servi, S. *Synthesis* (1990) 1; (d) Jones, J. B.. In: Trost, B. M. and Fleming, I., Eds., *Comprehensive Organic Synthesis*, Pergamon, Oxford 1991, Vol. 8, p. 183; (e) Ward, O. P. and Young, C. S. *Enzyme Microb. Technol. 12* (1990) 482.
8. (a) Barton, D. H. R., Brown, B. D., Ridley, D. D., Widdowson, D. A., Keys, A. J. and Leaver, C. J. *J. Chem. Soc., Perkin Trans. 1* (1975) 2069; (b) Deol, B. S., Ridley, D. D. and Simpson, G. W. *Aust. J. Chem. 29* (1976) 2459; (c) Iriuchijima, S. and Ogawa, M. *Synthesis* (1982) 41; (d) Nakamura, K., Inoue, K., Ushio, K., Oka, S. and Ohno, A. *J. Org. Chem. 53* (1988) 2589; (e) Nakamura, K., Kondo, S.-I., Kawai, Y. and Ohno, A. *Tetrahedron Lett. 32* (1991) 7075.
9. Pedrocchi-Fantoni, G., Redaelli, S., Servi, S. and Högberg, H.-E. *Gazz. Chim. Ital. 122* (1992) 499.
10. (a) Chen, C.-S., Zhou, B., Girdaukas, G., Shieh, W.-R., Van Middlesworth, F., Gopalan, A. S. and Sih, C. J. *Bioorganic Chemistry 12* (1984) 98; (b) Nakamura, K., Higaki, M., Ushio, K., Oka, S. and Ohno, A. *Tetrahedron Lett. 26* (1985) 4213; (c) Nakamura, K., Kawai, Y., Nakajima, N. and Ohno, A. *J. Org. Chem. 56* (1991) 4778; (d) Nakamura, K. In: Servi, S., Ed., *Microbial Reagents in Organic Synthesis*, Kluwer, Dordrecht 1992, p. 389.
11. Nakamura, K., Kawai, Y., Oka, S. and Ohno, A. *Tetrahedron Lett. 30* (1989) 2245.
12. (a) Wilkinson, J. H. and Walter, S. J. *Enzyme 13* (1972) 170; (b) Nakamura, K., Inoue, K., Ushio, K., Oka, S. and Ohno, A. *Chem. Lett.* (1987) 679; (c) Nakamura, K., Kawai, Y., Oka, S. and Ohno, A. *Bull. Chem. Soc. Jpn. 62* (1989) 875; (d) Nakamura, K., Kawai, Y. and Ohno, A. *Tetrahedron Lett. 31* (1990) 267.
13. (a) Servi, S. In: Rehm, H.-J. and Reed, G., Eds., *Yeast in Biotechnology, II*, Wiley–VCH, Weinheim 1998, Vol. 8a, 363; (b) Mattiasson, B. and Holst, O. *Extractive Bioconversion*, Marcel Dekker, New York 1991.
14. (a) Nakamura, K., Inoue, K. and Ohno, A. *Tetrahedron Lett. 36* (1995) 265; (b) Naoshima, Y., Maeda, J. and Munakata, Y. *J. Chem. Soc., Perkin. Trans. 1* (1992) 659.
15. (a) Wills, E. D. *Biochem. J. 63* (1956) 514; (b) Rabinkov, A., Miron, T., Konstantimovski, L., Wilchek, M., Mirelman, D. and Weiner, L. *Biophys. Acta 1379* (1998) 233.
16. D'Arrigo, P., Pedrocchi-Fantoni, G. and Servi, S. *Tetrahedron: Asymmetry 8* (1997) 2375.
17. Jayasinghe, L. Y., Smallridge, A. J. and Trewhella, M. A. *Tetrahedron Lett. 34* (1993) 3949.

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