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Enantioselective reduction of β-keto amides by the fungus *Mortierella isabellina*

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Abstract: Incubations of the fungus *Mortierella isabellina* NRRL 1757 with 3-oxo-3-phenylpropanamide, 3-oxobutanamide and with some of their *N*-alkyl derivatives afford the corresponding (*S*)-3-hydroxyamides, usually in high chemical yields and enantiomeric excesses. © 1997 Elsevier Science Ltd

Although the stereoselective reduction of β-keto esters with baker's yeast is well known, both in aqueous media[1] and in organic solvents,[2] the biocatalytic reduction of β-keto amides remains almost unexplored. In this context, only some very specific examples of reduction have been reported, namely those of 4-chloro-, 4-azido- and 4-(acetylamino)acetoacetamide derived from ethyl 2-aminoacetate with baker's yeast,[3] as well as these of *N*-ethyl- and *N*-phenylacetoacetamide in the presence of immobilised baker's yeast.[4]

We have recently shown that the *Candida antarctica* lipase-catalysed aminolysis and ammonolysis of β-keto esters are convenient ways to prepare a notable variety of β-keto amides.[5] The enantioselective reductions of these compounds would afford optically active β-hydroxy amides which, if adequately substituted, would be suitable precursors for the chemical synthesis of, for instance, the antidepressants fluoxetine, tomoxetine and nisoxetine,[6] or their *N*-alkyl analogs, in optically active forms. We have previously developed an alternative route to optically active β-hydroxy amides by enzymatic aminolysis of the corresponding racemic β-hydroxy esters;[7] however, this methodology fails when applied to relatively bulky substrates, such as (±)-3-aryl-3-hydroxypropanoic acid esters. Thus, we felt it would be of interest to widen the scope of the barely explored stereoselective bioreduction of β-keto amides; the first results of this work are presented in this communication.

Taking into account the previously reported unsatisfactory findings with 3-oxobutanamides,[8] as well as our own preliminary work with 3-oxo-3-phenylpropanamide,[9] we discarded the use of baker's yeast in this study. Since we wished to include here several 'bulky' β-keto amides, such as the aforementioned 3-oxo-3-phenylpropanamide, we decided to test the ability of the fungus *Mortierella isabellina* NRRL 1757[10] in order to achieve the desired enantioselective reductions.

All the β-keto amides **1** employed were prepared as previously described;[5b] however, *N*-methyl-3-oxo-3-phenylpropanamide (**1c**; R=Me, R′=Ph; see Scheme 1) required a slight modification of the method.[11] 3-Oxo-3-phenylpropanamide, **1a**, was chosen as a test substrate to optimize the enantioselectivity of the processes. Thus, **1a** was added to different cultures of *M. isabellina* after growing times ranging from 24 to 96 hours, and then biotransformations were carried out until disappearance of the substrate. Since the best enantiomeric excess (92% *S*) was obtained with a 24 h old culture,[12] this growing time was standardized for the remaining substrates, all of which were transformed into their corresponding (*S*)-β-hydroxy amides (*S*)-**2** after 15–96 hours of incubation. The *N*-unsubstituted β-keto amides **1a** and **1d** were by far the most slowly reduced substrates (see Scheme 1 and Table 1).

Although the crude products (*S*)-**2** already were essentially pure (as shown by their ^{1}H- and ^{13}C-NMR spectra), they were subsequently submitted to flash column chromatography to give very good isolated

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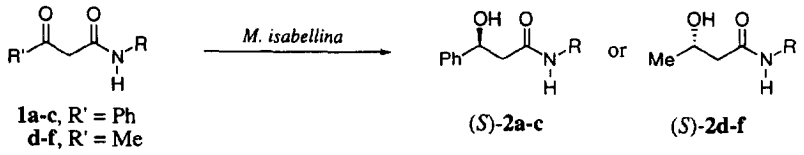

Scheme 1.

Table 1. Metabolites **2** from the incubation of *Mortierella isabellina* with the β-keto amides **1**

Product	R	Reaction time (h)[a]	Yield[b] (%)	eec (%)	$[\alpha]_D^{20}$ (conc., solvent)
2a	H	89	83	92	–33.7 (0.90, EtOH)
2b	$PhCH_2$	15	82	92	–45.7 (0.96, $CHCl_3$)
2c	Me	48	86	43	–37.0 (0.91, $CHCl_3$)
2d	H	96	82	>99	+27.6 (0.59, EtOH)
2e	$PhCH_2$	24	83	89	+26.6 (0.61, $CHCl_3$)
2f	$CH_2CH{=}CH_2$	24	90	98	+38.4 (0.90, $CHCl_3$)

[a] Until disappearance of the substrate (TLC). [b] After column chromatography. [c] The predominant enantiomer is always the corresponding (*S*)-**2**.

yields (see Table 1).[13,14] It should also be pointed out that the enantiomeric excesses of the products (except **2c**) are high or very high; this is particularly interesting in the cases of the *N*-unsubstituted (*S*)-β-hydroxy amides **2a** and **2d**,[15] which are potential precursors of optically active 1,2-amino alcohols, well-known as important chiral auxiliaries, building blocks, and ligands in transition metal catalysed reactions.[16] Moreover, with regard to the above mentioned alternative approach to optically active β-hydroxy amides, it is noteworthy that the method described here shows the complementary enantioselectivity (indeed, **2e,f** were obtained as the *R*-enantiomers by the *Candida antarctica* lipase-catalysed aminolysis of their corresponding racemic β-hydroxy esters).[7]

The enantiomeric excesses were determined as follows. For products **2a–d**, they were measured by HPLC analysis using a Chiralcel-OD column:[17] **2b** as the compound itself; **2a,c** after conversion into their *O*-acetyl derivatives; **2d** after transformation into its *O*-triphenylsilyl derivative. In the cases of products **2e,f**, they were transformed with (*R*)-MTPA-Cl into their MTPA-esters derivatives,[18] and these subsequently analysed by ^{19}F-NMR.[19]

The absolute configurations of the obtained (*S*)-β-hydroxy amides were established in the following ways. By comparison of the signs of their specific rotations (see Table 1) with the corresponding literature data, those of **2b,e,f**[7b] and **2c**.[6b] In the case of **2a**, it was first necessary to have available ethyl (*R*)-3-hydroxy-3-phenylpropanoate,[20] and then to subject it to ammonolysis (saturated solution of ammonia in methanol, 4°C, 4 days); the sign of the specific rotation of the resulting (*R*)-3-hydroxy-3-phenylpropanamide[21a] was compared with that of **2a**. Finally, for **2d** a similar ammonolysis procedure was followed, but starting from the commercially available ethyl (*S*)-3-hydroxybutanoate.[21b]

Reductions by *M. isabellina* NRRL 1757 have usually been reported to obey[10,22a,b] Prelog's rule,[23] even though examples are also known in which this fungus displays the opposite discrimination of the substrate's enantiotopic faces.[22b,c] Five (**1a,c–f**) out of our six substrates have been clearly reduced in the sense of this rule; the case of **1b** may be doubtful, but it could be assumed that the large size of the carbonyl-attached phenyl substituent is sufficient argument to also include it into Prelog's group.

In summary, we have developed a very simple and efficient method for the enantioselective reduction of β-keto amides with the fungus *Mortierella isabellina*. Further investigations on the diastereoselective activity of this microorganism in this kind of process, as well as on the synthetic applicability of the resulting optically active β-hydroxy amides, are currently in progress.

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8. See reference cited in note 4.
9. Using an aqueous suspension of dry baker's yeast, no 3-hydroxy-3-phenylpropanamide was detected (^{1}H-NMR analysis) after 110 hours of incubation.
10. *Mortierella isabellina* NRRL 1757 transforms, *inter alia*, acetophenone into (*S*)-1-phenylethanol with moderate ee (54%), and also chroman-4-one and thiochroman-4-one into their respective enantiopure (*S*)-alcohols. See: a) Holland, H. L.; Bergen, E. J.; Chenchaiah, P. C.; Khan, S. H.; Munoz, B.; Ninniss, R.; Richards, D. *Can. J. Chem.* **1987**, *65*, 502–507. b) Holland, H. L.; Manoharan, T. S.; Schweizer, F. *Tetrahedron: Asymmetry* **1991**, *2*, 335–338.
11. Saturation of 1,4-dioxane with gaseous methylamine coming from heating a mixture of methylammonium chloride and solid sodium hydroxide; then, *Candida antarctica* lipase-catalysed aminolysis of ethyl 3-oxo-3-phenylpropanoate.
12. Shorter growing times require too long reaction times.
13. The experimental procedure is as follows. *M. isabellina* is precultured and cultured as previously described,[14] in a growing medium prepared by dissolving 30 g of tryptic soy broth (Difco) and 17.5 g of D-glucose in 1 l of distilled water. After 24 hours of culture (350 ml, in a 1 l Erlenmeyer flask), the mycelium is harvested by filtration and resuspended in 350 ml of distilled water. Substrate is added as an ethanolic solution (*ca.* 0.1% w/v) to reach a concentration of 1 mg/ml. Incubation is then carried out (rotary shaker, 200 rpm, 28°C) until disappearance of the substrate (TLC monitoring). The mycelium is filtered out, washed with distilled water, and the combined aqueous phases continuously extracted with AcOEt (24 hours). After drying and elimination of the solvent, the crude residue is purified by flash column chromatography [silica gel; eluent, hexane:AcOEt 1:1 (except for **2d**, which requires $CHCl_3$:Et_2O:MeOH 4:1:0.1, and then AcOEt)] to afford the corresponding (*S*)-β-hydroxy amide **2**.
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17. Data and conditions for chiral HPLC analyses. (*S*)-*O*-Acetyl-**2a**, t_R 13.5 min.; (±)-*O*-acetyl-**2a**, two peaks, t_R 12.0 and 13.6 min.; Rs 1.5 (hexane:Pr^{i}OH 85:15, 1.0 mL/min.). (*S*)-**2b**, t_R 13.7 min.; (±)-**2b**, two peaks, t_R 13.8 and 16.8 min.; Rs 2.0 (hexane:Pr^{i}OH 90:10, 1.0 mL/min.). (*S*)-*O*-Acetyl-**2c**, t_R 10.2 min.; (±)-*O*-acetyl-**2c**, two peaks, t_R 7.3 and 10.3 min.; Rs 3.2 (hexane:Pr^{i}OH 85:15, 1.0 mL/min.). (*S*)-*O*-(Triphenylsilyl)-**2d**, t_R 27.4 min.; (±)-*O*-(triphenylsilyl)-**2d**, two peaks, t_R 27.1 and 31.2 min.; Rs 1.8 (hexane:Pr^{i}OH 95:5, 0.8 mL/min.).
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