COMMUNICATION

Biotransformations of benzoylacetonitrile with the fungus *Curvularia lunata*: highly diastereo- and enantioselective synthesis of α-alkyl β-hydroxy nitriles and γ-amino alcohols

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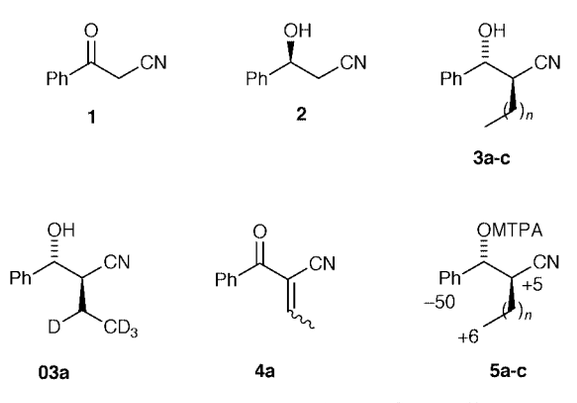
Incubation of alcoholic solutions of benzoylacetonitrile, 1, with a suitable aqueous culture of *C. lunata* results in highly diastereo- and enantioselective reactions leading to α-alkyl β-hydroxy nitriles 3, which are easily reduced to the corresponding γ-amino alcohols 6.

The importance of optically active β-hydroxy acids and their derivatives (especially β-hydroxy esters, α-substituted or not) as building blocks for the synthesis of biologically active compounds, agricultural chemicals, and natural products is well established.[1] This has led to the development of bioreduction processes of β-keto acids and their derivatives as a powerful methodology for obtaining the above mentioned optically active building blocks.[2]

With our ongoing interest in preparing optically active β- or γ-amino alcohols,[3] useful as chiral auxiliaries in asymmetric syntheses,[4] and present as structural elements in biologically active compounds,[1e,1f,5] we envisaged that the bioreduction of β-keto amides or -nitriles (much less studied than other β-keto acid derivatives' bioreductions[6,7]) could be an adequate approach to this class of product. Indeed, we have recently reported our first results on the highly stereoselective reduction of 2-oxocyclopentanecarboxamides with the fungus *Mortierella isabellina*.[3a] Herein we describe our somewhat unexpected initial findings in the microbial reductions of benzoylacetonitrile (3-oxo-3-phenylpropanenitrile, **1**).



(a, $n = 1$; b, $n = 2$; c, $n = 3$)

At first, we assayed several yeasts and fungi useful in the microbial reduction of β-keto acid derivatives, but only two strains were able to give significant amounts of the corresponding 3-hydroxy-3-phenylpropanenitrile, **2**. Thus, *Mortierella isabellina* NRRL 1757 gave a 90% chemical yield of **2**, but with a moderate ee (70%), whereas a first experiment with *Curvularia lunata* CECT 2130[8] (cells grown during two days) affords an almost equimolar mixture of **2** and the unexpected 2-(1-hydroxy-1-phenylmethyl)butanenitrile, **3a**. To our knowledge, only one example of carbonyl reduction with concomitant formal α-ethylation has been described during the incubation of 3-oxobutanenitrile with baker's yeast (BY; Itoh *et al.*[7c]). Two other formal α-ethylation, non-reductive processes have also been reported during the exposure of BY to ethyl cyanoacetate (aqueous medium; Fuganti *et al.*[9]), and to **1** (in petrol–water 10 : 1, and in the presence of two equivalents of acetaldehyde).[10] No formal α-alkylation other than ethylation has been described. A common feature of our work and those of Itoh[7c] and Fuganti[9] is the use of ethanol as cosolvent in aqueous media.

As product **3a** can be seen as a precursor of interesting amino alcohols bearing two stereogenic centres, our efforts were immediately addressed to optimize its chemical and optical yields.[11] Thus, the best chemical yield for this product was reached after the study of the effects of the *C. lunata* culture age on the product distribution. In fact, using cells grown during four days (when glucose from the culture medium has just been consumed), we observed a maximum **3a**–**2** molar ratio of 7 : 1,[12] compound **3a** being obtained with very high de and ee (see Table 1). From these results we assume that, at this time, enzymes able to metabolize molecules smaller than glucose have to be biosynthesized by the fungus, and that such enzymes have also an enhanced ability to oxidize the ethanol added as cosolvent to acetaldehyde, which is involved in the formation of **3a** (see below).

Fuganti[9] has supplied some evidence for a reasonable α-ethylation pathway in the above mentioned BY biotransformations of ethyl cyanoacetate and 3-oxobutanenitrile. This pathway consists of three steps: (1) BY-mediated oxidation of ethanol to acetaldehyde; (2) non-enzymatic aldol-type condensation between the aldehyde and the active methylene compound; (3) yeast reduction of the new C=C double bond[13] (prior to the final bioreduction of the keto group).

In order to check the validity of this pathway for our biotransformation with *C. lunata*, we performed several experiments. (i) Since the step (2) should be favoured with increasing pH, we carried out incubations with cells grown during two days at pH values ranging from 6 to 10; as expected, the **3a**–**2** products molar ratio rose from 0.8 : 1 to 2.3 : 1. (ii) We prepared, by Knoevenagel condensation between **1** and acetaldehyde, the supposed intermediate 2-benzoylbut-2-enenitrile, **4a**, and subjected it to *C. lunata* under our standard conditions; in this way, product **3a** showing identical de and ee as that obtained during the conventional biotransformation was reached. (iii) Finally, in order to determine the source of the ethyl group in **3a**, we substituted hexadeuteroethanol for ethanol as cosolvent, which led to the tetradeuterated product **03a**;[14] thus, it is clear that ethanol is the ethyl source. As a whole, these three experiments also support the above three-step pathway.

Bearing this in mind, we decided to test the potential of *C. lunata* to promote other unknown formal α-alkylations.[15] We therefore used other alcohols instead of ethanol under the same previously optimized conditions. The results summarized in Table 1 show that, indeed, *C. lunata* is able to perform this task, although with decreasing effectiveness as the alcohol chain increases; this can probably be related to the substrate specificity of the enzymes responsible for the oxidation of alcohols

Table 1 Products **3** obtained in biotransformations of benzoylacetonitrile **1** with the fungus *Curvularia lunata*[a]

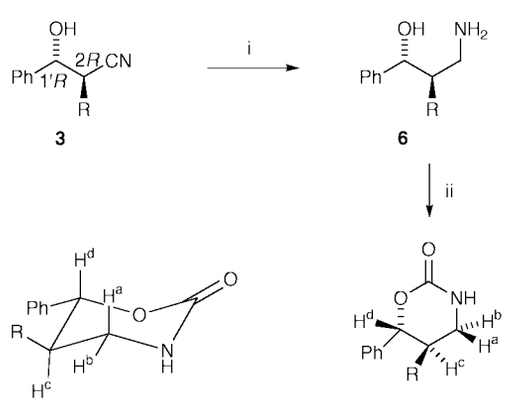
Product	R[b]	Yield (%)[c]	De (%)	Ee (%)	$[a]_D^{25}$ (EtOH)/ 10^{-1} deg cm^2 g^{-1}
3a	Et	69	96	98	+16.8 (*c* 1.1)
3b	Pr	38	97	98	+12.8 (*c* 1.7)
3c	Bu	13	86	86	+7.3 (*c* 2.8)

[a] In the presence of the corresponding alcohol ROH as cosolvent (see Experimental). [b] See **3** in Scheme 1. [c] After purification by column chromatography.

to aldehydes. The isobutyl group is also introduced (14% yield), but the de and ee of the product have not been determined yet. Interestingly, the fungus fails to insert methyl and isopropyl substituents. The latter could be seen as an indirect support of the Fuganti's pathway, since it is well known that aldol-type equilibria with ketones are strongly shifted towards their left-hand sides.

As shown in Table 1, the de and ee of the β-hydroxy nitriles, especially those of **3a** and **3b**, are very high. The des and ees were determined by treatment of products **3** with both (*R*)- and (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride (MTPA-Cl), and the resulting (*S*)- and (*R*)-MTPA esters[16] **5**, respectively were analysed by GC.[17] Through a comparative analysis, de and ee values of **3** can be unambiguously established.

The *R* absolute configuration of the benzyl carbon atom (C-1′) in the products **3** was determined from the $\Delta\delta$ values ($\delta_{S\text{-MTPA}} - \delta_{R\text{-MTPA}}$)[18] measured in the ^{1}H-NMR spectra of the MTPA esters **5** (the average magnitudes in Hz are placed beside the corresponding ^{1}H nuclei in structure **5**). Furthermore, a $\Delta\delta$ of +50 Hz observed for the methoxy protons of the MTPA moiety also corroborates this assignment.[19]



Scheme 1 *Reagents and conditions*: i, $LiAlH_4$, Et_2O, rt (86–90%); ii, CDI, CH_2Cl_2, rt (63–75%).

In order to establish the absolute configuration of C-2 (C-α), it was necessary to perform the transformations outlined in Scheme 1. LAH reduction[20] of **3** gave very good yields of γ-amino alcohols **6**, which, in turn, were treated with *N*,*N*′-carbonyldiimidazole[21] and thus easily converted into 1,3-oxazinan-2-ones **7**. From the ^{1}H-NMR spectra of these heterocycles, coupling constants of *ca.* 8.7 Hz are observed between protons H^c and H^d, which implies an *anti* arrangement and, consequently, the *R* configuration for the alkyl substituted chiral centre. This geometry is consistent with the coupling constants of proton H^c with H^a (*ca.* 9.5 Hz) and with H^b (*ca.* 5.2 Hz). As it can be derived from the Scheme 1 reactions, both chiral centres on the β-hydroxy nitriles **3** and the γ-amino alcohols **6** also show *R* absolute configurations.

In conclusion, a previously unknown ability of any microbial strain to formally introduce alkyl chains other than ethyl into β-keto nitriles, with concomitant carbonyl group reduction, has been shown with the fungus *C. lunata*. The alkyl group originates from the primary alcohol used as cosolvent. The process is highly diastereo- and enantioselective, and furnishes α-alkyl β-hydroxy nitriles easily transformable into almost homochiral γ-amino alcohols. Further study concerning the scope and applicability of this methodology is currently under way.

Experimental

General procedures for growing *C. lunata* and for biotransformations

A loop of a solid culture of *C. lunata*, from an agar plate, was sowed on to a test tube containing 3 ml of sterilized Sabouraud's liquid medium [composed of bactopeptone (10 g), D-glucose (20 g) in distilled water (1.0 l); pH adjusted to 5.8]. After growing over 72 h (rotary shaker, 200 rpm, 28 °C), this initial culture (0.5 ml) was used to inoculate another sterilized medium (75 ml, in a 250 ml Erlenmeyer flask), identical to that used for fungi in ref. 22. After 96 h of incubation (same conditions as above), a solution of **1** (75.0 mg, 0.517 mmol) in the corresponding alcohol (750 μl) was added. Biotransformation was continued until disappearance of the substrate (*ca.* 12 h, TLC monitoring). The mycelia were then filtered off, washed with aqueous 0.8% NaCl, and the combined aqueous phases continuously extracted with ethyl acetate (24 h). After drying, the solvent was eliminated and the crude residue purified by silica gel flash column chromatography (eluent: hexane–ethyl acetate 5:1) to obtain the corresponding 2-alkyl-3-hydroxynitrile **3**.

Selected analytical data

(2*R*,1′*R*)-2-(1-Hydroxy-1-phenylmethyl)butanenitrile, 3a. δ_H (200 MHz, $CDCl_3$) 1.05 (t, *J* 7.4, 3H), 1.42–1.66 (m, 2H), 2.66–2.76 (m, 1H), 3.34 (br s, 1H), 4.73 (d, *J* 6.4, 1H), 7.36 (br s, 5H); δ_C (75 MHz, $CDCl_3$) 11.3, 22.0, 42.3, 73.0, 120.0, 125.9, 128.1, 128.3, 140.3; *m*/*z* (EI) 175 (M^+, <1%), 107 (100%), 79 (39%); HRMS calc. for $C_{11}H_{13}NO$ 175.0997, found 175.0999.

3,4,4,4-Tetradeutero-2-(1-hydroxy-1-phenylmethyl)butanenitrile, 03a. δ_H (200 MHz, $CDCl_3$) 1.54 (d, *J* 10.0, 1H), 2.72 (dd, *J* 10.0, 6.6, 1H), 3.00 (br s, 1H), 4.75 (d, *J* 6.6, 1H), 7.37 (br s, 5H); δ_C (75 MHz, $CDCl_3$) 10.5 (m, *C*D_3), 21.7 (t, *C*HD), 42.4, 73.5, 120.2, 126.1, 128.4, 128.6, 140.3; *m*/*z* (EI) 179 (M^+, 1%), 107 (100%), 79 (92%).

(1*R*,2*R*)-2-(Aminomethyl)-1-phenylbutan-1-ol, 6a. δ_H (300 MHz, $CDCl_3$) 0.86 (t, *J* 7.4, 3H), 1.15–1.55 (m, 3H), 2.77 (dd, *J* 12.3, 7.0, 1H), 3.00 (dd, *J* 12.3, 2.8, 1H), 3.28 (br s, 3H), 4.69 (d, *J* 6.5, 1H), 7.20–7.45 (m, 5H); δ_C (75 MHz, $CDCl_3$) 11.4, 21.9, 43.0, 46.6, 79.0, 126.3, 126.7, 127.9, 144.7; *m*/*z* (EI) 179 (M^+, 4%), 132 (80%), 117 (100%); HRMS calc. for $C_{11}H_{17}NO$ 179.1310, found 179.1307.

Acknowledgements

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