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Lactone synthesis via biotransformations of γ-hydroxyamides

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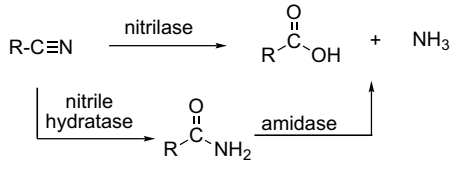
Abstract—An enzyme was expressed in *Escherichia coli* from a cloned amidase gene. When characterized, it was more enantioselective than commercial amidases. Three pheromones were made using this biotransformation chemistry.

1. Introduction

The use of biotransformations offers significant advantages over chemical hydrolyses of compounds that normally require harsh hydrolytic conditions (e.g., nitriles[1,2] and amides[2]). For example, the hydrolysis of the nitrile 4-hydroxydodecanenitrile required reflux for 12 h in 3.5 M KOH (3:1 ethanol/H_2O).[3] The same reaction was completed via a biotransformation in just 2 h at pH 6 and 37 °C.[1] Another attractive feature of these reactions is that they are usually enantioselective.[4,5] The biotransformation routes to hydrolyze nitriles[5–7] are illustrated below. A direct hydrolysis to an acid is possible, but the nitrile can also be partially hydrolyzed to an amide, followed by hydrolysis of the amide to the corresponding carboxylic acid.[5]



The hydrolyses of γ-hydroxy amides and γ-hydroxy nitriles offer a significant attraction since if enantioselectively hydrolyzed, the resulting γ-hydroxy acids could esterify to form a chiral γ-lactone, many of which are insect pheromones[8,9] (see Table 1 for the synthesis of three pheromones by this method). We are pursuing both approaches.

2. Results and discussion

γ-Hydroxy amide hydrolysis was attempted with commercially available amidases; five of the amidases from two screening[10] kits did indeed hydrolyze the amide, but not very enantioselectively. For example, penicillin acylase, subtilisin Carlsberg, pig kidney acylase, PGA 450, and SP409 all gave a hydrolysis product, but with only 0–10% ee at 40–50% conversion.

Consequently, a model amidase was sought through cloning and expression experiments. Three microorganisms were grown, and the amidase from one, the *Brevibacterium* species,[6,7] was cloned and expressed.[†] This was enantioselective, and the major products of the

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[†] The amidase gene of *Brevibacterium* R312 was amplified using primers TGACCTTTTCTTATGTGGGCTGATCATGTGTGAATC and CGATCCGGAAACAGTACTTCGGCAGCTTGCCACGAC. The 1.8 Kb PCR product was cloned into pGEM-T Easy (Wisconsin, Promega, Inc.) and the recombinant plasmid electroporated into *Escherichia coli* XL-1 Blue. The resulting recombinant strain was used. The recombinant plasmid was subjected to restriction enzyme digestion and sequencing to further confirm that the amidase was successfully cloned. Subsequent bacterial cultures were grown in a rich nutrient broth for 24 h at 30 °C or for 18 h at 24 °C. Cells were centrifuged at 10,000 rpm, and the bacterial pellet was resuspended in phosphate buffer for use in subsequent reactions.

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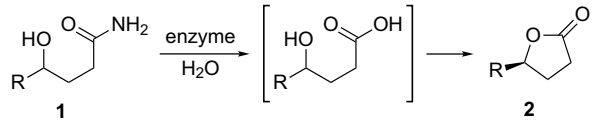
Table 1.



Entry	Substrate R=	Conversion (%)	*R*:*S* ratio[a] **2a–e**	Isolated yield (%), **2a–e**	*E*	Pheromone
1a	Ethyl	45	90:10	20 (46)[b]	19	*Trogoderma* beetle
1b	Propyl	57	83:17		14	
1c	Butyl	65	75:25	45	9	*Fopius arisanaus* wasp
1c	Butyl	11	91:9	—	11	*Fopius arisanaus* wasp
1d	Pentyl	No reaction		None	—	Rice weevil
1e	Octyl[c]	38	75:25	20	4	Rove beetle
1e	Octyl[c]	13	90:10		10	Rove beetle

[a] Which were determined by chiral GC on a β-cyclodextrin column. The (*R*)-enantiomer eluted first.
[b] Only 43% of the product could be extracted from the medium, which indicates a 46% yield. Some product was lost during extraction.
[c] Must be dissolved in MeOH before adding buffer.

hydrolyses led to the pheromones[8,9] (Table 1). For some reason, hydrolysis of the pentyl-substituted compound **1d** did not occur to yield the fourth pheromone.

These reactions[‡] are kinetic resolutions. However, the precursors to these reactions can be made in nearly quantitative yields.[11] We are now working on a method to achieve ~100% conversion and minimize the limitation of this kinetic resolution. The precursor hydroxyamide is highly polar, and can easily be separated from the less polar lactone product. For example, the product mixture (lactone+hydroxyamide) can be passed through a plug of silica gel with 1:3 hexane/ethyl acetate. The lactone [the (*R*)-enantiomer] will come through quickly, but the hydroxyamide stays on top of the silica plug. The hydroxyamide [the (*S*)-enantiomer] can then be easily washed off the plug with ethyl acetate containing 10–20% ethanol. It can be inverted through the Mitsunobu[12] reaction and then transformed into the (*R*)-lactone product.

The two reactions shown for **1e** (Table 1) have different *E* values. Higher *E* values in the reaction were obtained when liquid cultures were grown from freshly prepared bacterial plates. Freshly grown cultures are important for consistency in reaction results. Specific rotations for all of the lactones have been reported,[8] and our data is based on chiral GC and spectral comparison to samples obtained by independent synthesis of the enantiomers.[8,13]

3. Conclusion

The *Brevibacterium* amidase was obtained by standard molecular biological and biochemical techniques. This enzyme yielded better results than the commercial amidases that were evaluated. This highlights the occasional necessity of seeking enzymes not commercially available to effect desired enzymatic transformations.

[‡] A representative reaction: A 20 mg sample of **1a** was suspended in 0.7 g microbial clone paste and 3–5 mL of pH 6 buffer solution. After rotary shaking for 27 h at 30 °C, the mixture was adjusted to pH 3 with 1 M HCl, saturated with NaCl, and extracted with ethyl acetate three times. The residue left after evaporation was run through a plug of Florisil with excess ethyl acetate, concentrated by rotary evaporation, and analyzed by NMR. The pure lactone could be obtained by running the product through a plug of silica gel with 1:1 hexane/ethyl acetate.

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