Conversion of Hydroxy Nitriles to Lactones Using *Rhodococcus rhodochrous* Whole Cells

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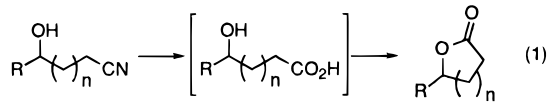
Nitriles are one of the most synthetically versatile functional groups in organic synthesis. Their ease of preparation from varied precursors and their subsequent reactions, particularly those of their enolates, allow rapid assembly of structurally and stereochemically complex compounds.[1] Moreover, γ-butyro- and δ-valerolactones are prominent moieties in biologically active compounds. They are components of natural flavors and odors, pheromones of several species of insects, and intermediates in the synthesis of natural products.[2] Hydrolysis of 4- and 5-hydroxy nitriles and subsequent lactonization of an intermediate hydroxy acid would seem an attractive, general route to these important molecules (eq 1).



(1)

Unfortunately, chemical conversion of a nitrile to its corresponding carboxylic acid is often difficult, requiring harsh conditions of concentrated acid or base and extended reaction times at elevated temperature.[3] Such vigorous conditions are not desirable for the preparation of structurally elaborate and/or sensitive substrates. Therefore, the hydrolysis of a hydroxynitrile to the corresponding lactone by way of the intermediate hydroxy acid has not been a transformation of choice for the construction of substituted lactones.[4] Herein we report our preliminary results in the facile microbial hydrolysis and lactonization of hydroxynitriles using *Rhodococcus rhodochrous* whole cells. This high-yielding transformation takes place at *pH 6 in a matter of minutes at 30* °C. This method paves the way to a milder general preparation of substituted lactones from nitrile precursors.

Several reports of microbial hydrolysis of nitriles to either their corresponding amides or carboxylic acids have appeared recently, and many of the studies have focused on chemo-, regio-, and enantioselectivity.[5−7] The proposed mechanisms of enzyme-catalyzed nitrile hydrolysis has been discussed, and several species of microorganisms have been identified to possess this activity.[8] In this paper we describe our initial efforts to extend microbial hydrolysis of nitriles to substrates containing a hydroxyl group in the 4- and 5-position of the substrate such that a γ-butyrolactone or δ-valerolactone is formed from the presumed hydroxy acid intermediate.

R. rhodochrous IFO 15564[9] was incubated at 30 °C in a culture medium containing glycerol as a carbon source and ϵ-caprolactam as the source of nitrogen blending the methods of Ohta[5a] and Mayaux.[10] The harvested wet cells were suspended in a phosphate buffer, the hydroxy nitrile substrate **1**[11,12] was introduced, and the reaction was monitored by TLC. After workup, the products were purified by chromatography on silica and characterized by GC, IR, NMR, and chiral GC.[13] Compounds **2a**,[4a] **2b**,[4a] **2c**,[2a,14] **2d**,[2a,14] **2e**,[15] **2f**,[16] **2g**,[17] **2h**,[18] and **2i**[4a] have been reported, and their identity was confirmed by comparison with authentic standards and/or literature data.

As illustrated in Table 1, microbial hydrolysis of the hydroxy nitriles **1** provides good yields of lactones **2**.[19] Younger, actively growing cell cultures (2−3 days) gave modest enantioselectivity in the formation of **2** (Table 1, entries 1 and 2), but at the expense of the chemical yield. In these experiments a low mass recovery of crude material was obtained, possibly as a result of further metabolism of the substrate by the microbe. Older, resting cells (6−10 days) provided very good yields of the various lactones **2**, although with little or no enantioselectivity (Table 1, entries 3−8). Occasionally, traces of an amide were seen in the ^{1}H NMR spectrum of the crude product mixture from the hydrolysis of **1a**,[20] but this was not consistently reproducible, and similar evidence of

† Camille and Henry Dreyfus Scholar/Fellow 1995−1997.

(1) (a) *The Chemistry of the Cyano Group*; Rappoport, Z., Ed.; Interscience: New York, 1970. (b) Arseniyadis, S.; Kyler, K. S.; Watt, D. S. In *Organic Reactions*; Dauben, W. G., Ed.; Wiley: New York, 1984; Vol. 31, Chapter 1.

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(4) For representative examples of this transformation, see: (a) Gopalan, A.; Lucero, R.; Jacobs, H.; Berryman, K. *Synth. Commun.* **1991**, *21*, 1321. (b) Matsuda, I.; Murata, S.; Ishii, Y. *J. Chem. Soc., Perkin Trans. 1* **1979**, 26.

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(11) Substrates **1a**−**h** were prepared by opening the appropriate epoxide with lithioacetonitrile. Preparation of these and other hydroxy nitriles by this method, including examination of the diastereoselectivity of this reaction will be described elsewhere. Compound **1i** was prepared by $NaBH_4$ reduction of commercially available 5-oxohexanenitrile.

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(13) See Supporting Information for typical experimental procedure.

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(18) Alonso, D.; Font, J.; Ortuño, R. M. *J. Org. Chem.* **1991**, *56*, 5567.

(19) As a control, substrate **1a** was placed in the pH 6 buffer at 30 °C for 18 h. No **2a** was detected by TLC or GC, and **1a** was recovered quantitatively.

(20) Determined by comparison to the ^{1}H NMR spectrum of 4-hydroxyhexanamide (**3**), which was prepared by partial chemical hydrolysis of **1a** using the method of Rao: Rao, C. G. *Synth. Commun.* **1982**, *12*, 177.

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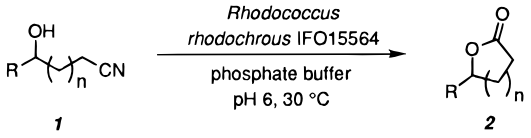
Table 1. Microbial Hydrolysis of Hydroxynitriles

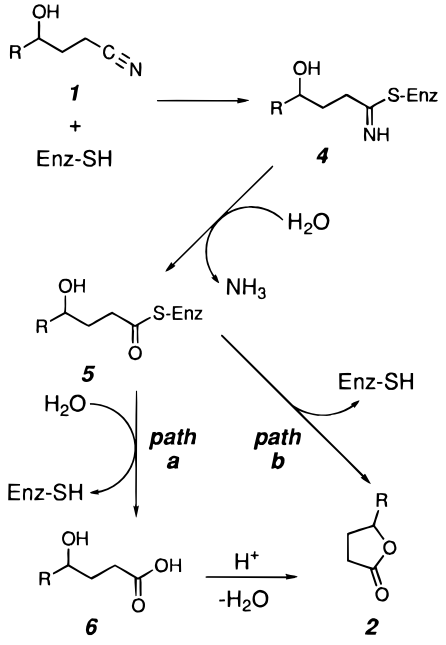


1 2

entry	nitrile	R	n	product	yield[a] (%)	ee (%)
1	**1a**	Et	1	**2a**	9	62
2[b]	**1a**	Et	1	**2a**	14	56
3	**1a**	Et	1	**2a**	79	0
4	**1b**	C_5H_{11}	1	**2b**	82	12
5	**1c**	*t*-Bu	1	**2c**	37	6
6	**1d**	Ph	1	**2d**	68	4
7	**1e**	5-hexenyl	1	**2e**	40	0
8	**1f**	3-butenyl	1	**2f**	53	8
9	**1g**	C_8H_{17}	1	**2g**	40	c
10	**1h**	*c*-C_5H_{10}	1	**2h**	79	
11	**1i**	Me	2	**2i**	16[d]	c

[a] Unoptimized isolated yields of material that has been chromatographed on silica gel. [b] Glucose was used in place of glycerol in the culture. [c]Not determined. [d] Crude yield; see text regarding isolation problems.

Scheme 1



amide formation was not observed with other substrates.[21] Reaction times ranged from 30 min to 3 h for most substrates.

There are two possible pathways by which the lactone **2** could be formed within the framework of the proposed mechanism of nitrilase enzymes (Scheme 1).[8] The proposed acyl-enzyme species **5** could be cleaved by water (path a) to produce the hydroxy acid **6**. Acid-catalyzed dehydration of **6** would produce **2**. Alternatively, the tethered hydroxyl in **5** could attack to produce **2** directly (path b). A hydroxy acid intermediate (cf. **6** in Scheme 1) was observed by TLC and IR analysis of the crude reaction mixture in some runs.[22]

This suggests that path a is operational, but does not rule out some lactonization by path b. Hydroxy acid formation was especially prevalent in substrates that lead to δ-valerolactones (see below). In those cases where a hydroxy acid was observed, acidification of the reaction mixture prior to extraction promoted lactonization and allowed good recovery of **2**. It is an open question whether the modest enantiodifferentiation of the substrate occurs prior or subsequent to (via path b) the formation of **5**.[21]

Comparison with an authentic standard showed that the major enantiomer of **2a** produced (Table 1, entries 1 and 2) had the 4*R* configuration, which is the natural enantiomer of the *Trogoderma* beetle pheromone.[24] Lactone **2b** is an attractant for the rice weevil,[2f] and compound **2g** is a pheromone for the rove beetle.[17]

The microbial hydrolysis proceeds rapidly on 5-hydroxy nitriles as well, although large amounts of a hydroxy acid intermediate are usually observed prior to acidification of the reaction mixture.[23,24] Workup and recovery of the lactone **2i** (Table 1, entry 12) is complicated by a relatively slow lactonization and competing oligomerization of the hydroxyacid intermediate. This is responsible for the low yield of **2i**, which we feel will be remedied in the optimization phase of this work.

In summary, we have demonstrated that hydroxy nitriles can be efficiently converted to the corresponding lactones in one step using the microorganism *R. rhodochrous* under very mild conditions. Current studies include defining the scope of acceptable substrates, optimizing workup procedures and yields, immobilizing the microorganism to facilitate product isolation, and improving the enantioselectivity of the hydrolyses. Our immediate future plans include refining the proposed mechanism of this transformation and isolating the enzyme(s) responsible for this activity.

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Supporting Information Available: Typical experimental procedures and copies of ^{1}H NMR spectra for compounds **2a**−**c**,**e**−**h** and **3** and copies of ^{13}C NMR spectra for **2c**,**e**−**g** as representative examples of product purity (13 pages).

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(21) Various microorganisms including *Rhodococcus* sp. have been shown to possess hydratase/amidase activity, nitrilase activity, or both (see refs 5−8). Although we cannot rule out the two-step hydratase/amidase mechanism, we have chosen to present our results within the context of a nitrilase mechanism.

(22) Evidence for the hydroxy acid included an unsymmetrical very broad OH stretch (3500−3000 cm^{-1}) and an acid carbonyl stretch (1715 cm^{-1}) in the IR and a very polar spot by TLC that dissappeared upon treatment with acid.

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