Enzymatic Hydrolysis of Nitriles and Dinitriles

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An immobilized enzyme system derived from *Rhodococcus sp.* catalyses under neutral conditions the hydrolysis of nitriles (1)—(6) and dinitriles (7) and (8) into the corresponding acids (1a)—(6a) and monocyano carboxylic acids (7a) and (8a), respectively.

Although attempts have been made to develop mild procedures for the hydrolysis of nitriles,¹ the chemical hydrolysis of these molecules to carboxylic acids usually requires rather drastic, basic or acidic conditions,² often incompatible with molecules carrying sensitive functionalities. Moreover, especially in larger scale transformations, considerable quantities of inorganic salts are always produced as byproducts with unfavourable ecological consequences.

Clearly the hydrolysis of nitriles to carboxylic acids at room temperature and under neutral conditions would be highly attractive as an alternative synthetic tool.

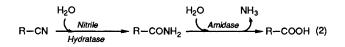
In view of the amazing properties of enzymes in this respect

Table 1. Enzymatic hydrolysis of nitriles and dinitriles (1)-(8).

Substrate ^a	Product ^b	Reaction time/h	Conversion /%	Yield ^d /%
(1)	(1 a)	435	44	98
(2)	(2a)	435	22	81
(3)	(3a)	120	63	82
(4)	(4a)	120	60	70
(5)	(5a)	482	34	81
(6)	(6a)	71	100	76
(7)	(7a)	72	92°	79
(8)	(8a)	239	82°	99

^a See text for experimental conditions. ^b Carboxylic acids (1a)—(6a), monocyano carboxylic acids (7a) and (8a). ^c Hydrolysis of one nitrile group corresponds to 100%. ^d Isolated yields, based on converted substrate.



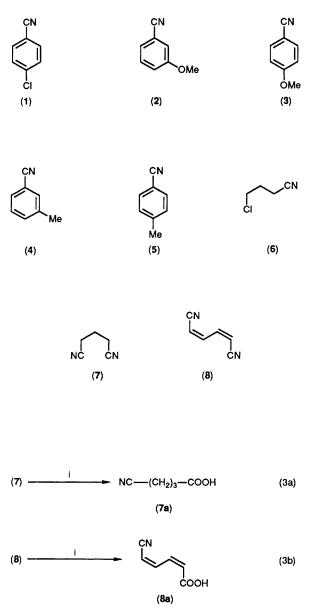


and the fact that micro-organisms have been known for some time to convert organic nitriles into amides and carboxylic acids³⁻¹³ we tried to develop on this basis a laboratory procedure suitable for synthetic organic applications.

While the so-called nitrilases convert nitriles directly to carboxylic acids, equation (1), the immobilized strain of Rhodococcus sp. CH 5 used in this work contains two enzymes, nitrile hydratase and amidase, equation (2), thus allowing the conversion of both nitriles and amides with the same enzyme preparation. With the intention to develop a synthetic method for organic chemists, the substrate tolerance of the enzyme system was tested with a large number of structurally different substrates, both nitriles and amides. Although it turned out that the substrate tolerance was indeed quite broad, we found that only a few compounds, exemplified by (1)—(8) showed acceptable rates of transformation suitable for preparative scale applications.

In typical experiments, 20 mmol of (1)–(8) and 1 g of the activated[†] enzyme system were suspended under shaking (200 r.p.m.) in 200 ml phosphate buffer (0.1 M, pH 7), the reaction progress being monitored by the release of ammonia.¹⁴ After removal of the immobilized biocatalyst and adjusting the pH of the remaining solution to pH 8 by addition of solid NaHCO₃, the unreacted substrates were removed by continuous extraction with ether. The solution was acidified to pH 2, then the liberated acids (1a)–(8a) were again isolated by continuous extraction with ether and purified by distillation or recrystallisation. The results are summarized in Table 1.

With the given enzyme concentrations, conversions of 20-100% were achieved, the yields of isolated products being good to excellent. Interestingly and paralleling the behaviour



Scheme 1. Reagents and conditions: i, nitrile hydratase, amidase, buffer, pH 7, room temp.

of diesters,¹⁵ the hydrolysis of dinitriles (7) and (8) terminates after the hydrolysis of only one nitrile function, leading to 4-cyano-butyric acid (7a) and (Z,Z)-5-cyanopenta-2,4-dienoic acid (8a), respectively, equations (3a) and (3b) (Scheme 1). The advantage of an enzymic procedure is particularly obvious in these cases; selectivities (*i.e.*, yields) of this kind cannot be achieved using conventional chemical methods for the hydrolysis of nitriles. Compounds (1a)–(8a) were characterized spectroscopically, (1a)–(7a) are known compounds.

From this preliminary report it is obvious that enzyme systems of this kind are indeed capable of achieving the hydrolysis of selected nitriles on a preparative scale and under mild conditions. The method may develop in the future into an attractive synthetic alternative to more conventional procedures.

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^{\dagger} The dried preparation of immobilized *Rhodococcus sp.* strain CH 5 is activated by stirring in phosphate buffer (0.1 m, pH 7) for 1 h at room temperature.

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