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SELECTIVE HYDROLYSIS OF NITRILES UNDER MILD CONDITIONS BY AN ENZYME.

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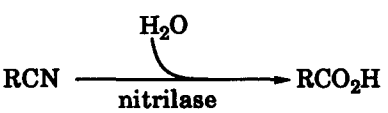
Abstract: A wide range of aromatic/aliphatic nitriles and dinitriles have been selectively hydrolysed using a commercially available enzyme preparation from a *Rhodococcus* sp.

Nitrile containing compounds are useful intermediates in organic synthesis owing to their ease of preparation *via* a number of methods. These include addition of cyanide ion (or its equivalent) to alkyl halides,[1] the reaction of aryl halides and copper cyanide,[2] dehydration of amides,[3] reaction of ketones with tosylmethyl isocyanide,[4] and the Sandmeyer reaction.[5] The ability of enzymes to hydrolyse nitriles is well known,[6] selective hydrolysis has been demonstrated in some instances[7] and the mechanism of some of these enzymes has been extensively studied.[8] Two distinct pathways have been recognised,[6b] namely the stepwise conversion of nitriles to amides (*via* a hydratase) followed by hydrolysis of the amide to a carboxylic acid (*via* an amidase) (Eqn. 1), or the direct conversion of nitriles to carboxylic acids (*via* a nitrilase) (Eqn. 2).

Eqn. 1



Eqn. 2

An immobilised enzyme system prepared from a *Rhodococcus* sp. is commercially available.[9] This enzyme system contains all three enzymes involved in nitrile hydrolysis and has been previously shown to transform a selected range of substrates,[7b,10] In order to provide useful predictive guidelines for the use of this catalyst in synthesis we set out to examine specific facets of the hydrolysis of nitriles, namely i) the selective mono-hydrolysis of aromatic dinitriles, ii) the hydrolysis of nitriles containing an acid/base sensitive functionality and iii) the relationship between structure of the nitrile and conversion to amide and/or carboxylic acid. The results of testing a wide range of substrates are described herein.

For all the following reactions the substrate was dissolved (or suspended) in potassium phosphate buffer (100 mM, pH 7, 50-200 ml) giving a final concentration of 5-100 mM,

depending on the substrate. Immobilised nitrile hydratase SP361[9] (1g/100 ml buffer) was added and the reactions shaken at 220 rpm, 30°C. The reactions were monitored by t.l.c., work up involved removal of the enzyme by filtration (through a celite pad), basification (pH 9, NaOH) of the filtrate and extraction with ethyl acetate or ether to recover unreacted starting material or amide product. The aqueous portion was then acidified (pH 2, HCl) and extracted with ethyl acetate or ether to recover the acid product. Purification was by recrystallisation or distillation as appropriate.

Table 1

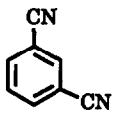
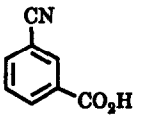
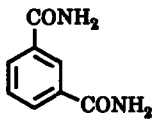
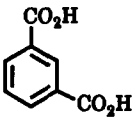
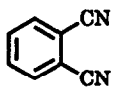
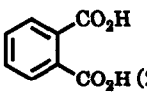
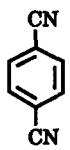
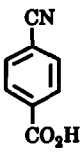
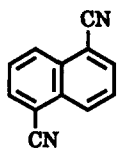
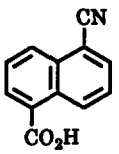
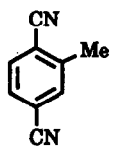
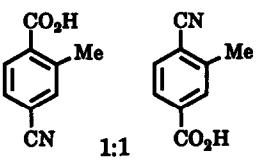
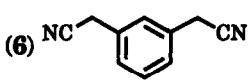
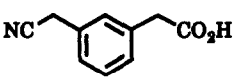
Substrate	Conc^{n} (mM)	Reaction Time (h)	Product(s)	Yield (%)
(1) 	100	48		91
(1a) 	10	22.5		62
(2) 	40	168		45 (26% recovered sm)
(3) 	25	15		62
(4) 	10	552		13 (52% recovered sm)
(5) 	30	48	 1:1	53
(6) 	50	21		48

Table 1 shows the results using aromatic dinitriles. (1)[7a] and (4) gave exclusive monohydrolysis, (2) gave the diacid, although this reaction often gave variable results and never good yields. An attempt was made to detect intermediate nitrile-amide formation during the reaction of (1). Aliquots were removed from the reaction at intervals and

examined by ^1H n.m.r (250 MHz). No significant accumulation (<5%) of the intermediate amide could be detected and at the end of the reaction only the nitrile-acid could be detected. The reaction of (1a) clearly shows that the selectivity of (1) occurs at the level of nitrile hydrolysis rather than amide hydrolysis. (3) and (6) gave the monoacid but unlike (1) gave the diacid on extended hydrolysis; some amide was observed with (3). The non-symmetrical dinitrile (5) was hydrolysed to nitrile-acid, but no regioselectivity was observed. In some cases *e.g.* (2) *ortho*-substitution clearly effects the reaction (*n.b. ortho*-substituted aromatic nitriles have been reported to be substrates for some nitrile hydrolysing enzymes[6a,6c]).

Table 2

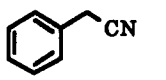
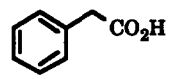
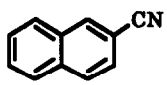
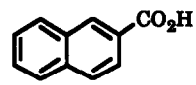
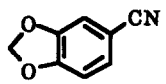
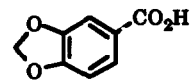
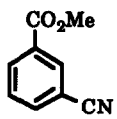
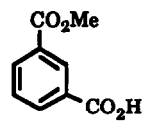
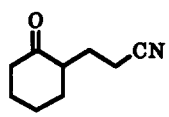
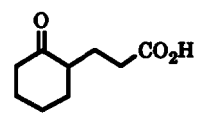
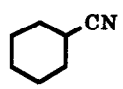
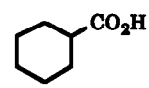
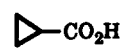
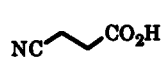
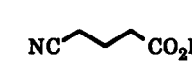
	Substrate	Conc^n (mM)	Reaction Time (h)	Product(s)	Yield (%)
(7)		100	23		71
(8)		10	67		80
(9)		25	26		86
(10)		100	12		92
(11)		100	14		75
(12)		25	168		90
(13)		100	27		90
(14)		10	24		92

Table 2 indicates the tolerance of SP361 for a wide range of substrates. It should be noted that many substrates (*e.g.* (1), (4), (7), (8)) do not have appreciable solubility in the buffer but still react. Entries (9) and (10) indicate the selectivity of enzymatic compared to chemical hydrolysis. Entries (13) and (14) indicate that cyclic secondary nitriles are amenable to hydrolysis, a class of substrates that has not previously been transformed. Table 3 indicates that monohydrolysis of aliphatic dinitriles can occur. However, dihydrolysis also occurs (after only 18 h in the case of (17)[7b]) and the nitrile-amide can be easily isolated from (15), indicating that careful monitoring of the reactions is required.

Table 3

	Substrate	Product(s)		Reaction Time (h)	Yield (%)
(15)			(15a)	5	9% (15), 38% (15a), 44% (15b
			(15b)	24	25% (15a), 71% (15b)
				72	19% (15a), 45% (15b)
(16)				71	65%
(17)			(17a)	18	71% (17a)
			(17b)	40	70% (17a), 9% (17b), 8:1.
				72	71% (17a), 7% (17b), 5.5:1

In conclusion we have shown that immobilised SP361 nitrilase is a useful catalyst for the selective hydrolysis of nitriles under mild conditions. In particular, the catalyst can be used to achieve:

i) The selective conversion of dinitriles to mononitrile-carboxylic acids (and/or mono nitrile-amide in the case of (15)), albeit without regioselectivity.

ii) Conversion of amides to carboxylic acids.

iii) Hydrolysis of nitriles in the presence of acid/base labile groups.

Currently we are investigating further aspects of this transformation, particularly the enantioselective hydrolysis of racemic and prochiral nitriles.

Acknowledgements

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References and Notes

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