

Rationalisation of the regioselective hydrolysis of aliphatic dinitriles with *Rhodococcus rhodochrous* AJ270

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Aliphatic dinitriles undergo regioselective hydrolysis with the title organism to give monoacids with up to four methylenes between the nitrile functions (optimally 2–3) or when either an oxygen is placed β , γ or δ to the nitrile (δ -placement being optimal) or β or γ (optimally γ) but not δ sulfur substituents are present; nitrogen substituents appear to behave as for oxygen but suffer a steric limitation of the size of the nitrogen substituent.

We have demonstrated¹ that the title organism is a powerful, general nitrile hydrolysing organism, involving two enzymes effecting nitrile-to-amide and amide-to-acid conversions respectively. As a prelude to studying the hydrolysis of prochiral dinitriles we have made a broad study of the effect of structure on the regioselectivity of hydrolysis of aliphatic dinitriles. The literature contains a number of papers on this topic but the overall background picture is decidedly unclear.

In 1980 Yamada showed that a *Fusarium* strain transformed glutaronitrile to 4-cyanobutyric acid² and 1,3,6-tricyanohexane to a mixture of diacids,³ the former transformation also being accomplished by a *Pseudomonas* organism. Gutman⁴ observed that the series $\text{NC}[\text{CH}_2]_n\text{CN}$ with $n = 3\text{--}5$ hydrolysed with no selectivity to the diacids with *Rhodococcus rhodochrous* NCIB 11216 grown on propionitrile while Yamada⁵ observed selective monohydrolysis of glutaronitrile ($n = 3$) using *R. rhodochrous* K22 in quantitative yield. Schneider⁶ made similar observations to Yamada with another *R. rhodochrous* strain while Turner⁷ showed that succinonitrile gave solely 3-cyanopropionic acid while glutaronitrile gave both mono- and diacids, ratios depending upon time of reaction. Prochiral glutaronitriles were hydrolysed by Kakeya⁸ and by Turner⁹ to give enantio-efficient monohydrolysis in some cases, although not in others. A chiral malonitrile has been converted¹⁰ into its monoacid monoamide derivative utilising an *R. rhodochrous* strain. Other organisms have been used for the efficient monohydrolysis of 1,4-cyclohexanedicarbonitrile,¹¹ while the conversion of adiponitrile to adipic acid has been closely studied by Moreau¹² utilising a *Brevibacterium*.

We first examined the selectivity of hydrolysis in a series of α,ω -dinitriles, monitoring the reaction against time (Table 1). As we noted in earlier work,¹ the low molecular weight nitriles tended to be metabolised by other enzymes in the organism, limiting overall yields. However, a clear pattern emerged: dinitriles with more than four methylenes separating the functions gave solely diacids irrespective of reaction time. With less than four methylenes, regioselective monohydrolysis occurred.

We next examined a series of α,ω -dinitriles $\text{NC}[\text{CH}_2]_n\text{X}[\text{CH}_2]_n\text{CN}$ **4** containing a heteroatom X in the chain (Table 2). With the oxygen or sulfur series, reactions were rapid and generally efficient, whereas with NH or NMe the nitriles proved unstable. We therefore included the NAr series which, probably for steric reasons, reacted slowly and with $n > 2$, not at all, even with added co-solvents such as acetone or methanol. A remarkable feature emerges: regioselectivity is principally dependent upon the placement of the heteroatom, not the overall chain length. Thus a δ -oxygen is optimally efficient, with regiocontrol being effective with a β - or γ -oxygen also.

However a γ -sulfur allows optimal regiocontrol, the β -analogue showing selectivity but not the δ -analogue. In the nitrogen series we cannot yet define the limits of regiocontrol with chainlength but good selectivity is shown with a β -nitrogen. Interestingly, in this slowly hydrolysed series, a much greater sensitivity to p -substituents is evident than in the hydrolysis of mononitriles.

We explain the regioselectivity on the basis of chelation-deactivation of the enzyme. We believe that hydrolysis occurs by complexation of the nitrile nitrogen to a metal (iron or cobalt) followed by hydration of the nitrile function by the presumed

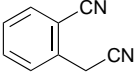
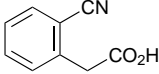
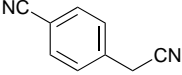
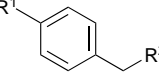
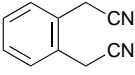
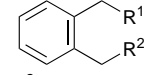
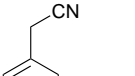
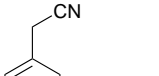
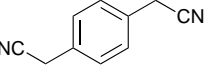
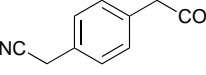
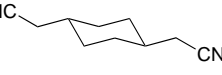
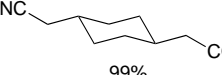
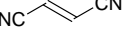
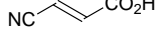
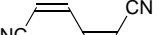
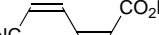
Table 1 Conversion of $\text{NC}[\text{CH}_2]_n\text{CN}$ **1** into $\text{NC}[\text{CH}_2]_n\text{CO}_2\text{H}$ **2** and/or $\text{HO}_2\text{C}[\text{CH}_2]_n\text{CO}_2\text{H}$ **3**

Entry	Substrate 1 <i>n</i>	Conditions		Product yields	
		Conc./ mmol	t/h	2 (%)	3 (%)
1	2	5	3	30	—
2	2	5	24	17	—
3	3	5	3	41	—
4	3	5	16	35	—
5	4	5	3	41	4
6	4	5	24	28	23
7	4	5	48	—	26
8	5	3	3	—	46
9	5	3	48	—	74
10	6	3	48	—	78
11	7	3	48	—	88
12	8	3	48	—	89

Table 2 Conversion of $\text{NC}[\text{CH}_2]_n\text{X}[\text{CH}_2]_n\text{CN}$ **4** into $\text{NC}[\text{CH}_2]_n\text{X}[\text{CH}_2]_n\text{CO}_2\text{H}$ **5** and/or $\text{HO}_2\text{C}[\text{CH}_2]_n\text{X}[\text{CH}_2]_n\text{CO}_2\text{H}$ **6**

Entry	Substrate 4		Conditions		Yield (%)		
	<i>n</i>	X	Conc./ mmol	t/h	5	6	4
1	2	O	3	48	61	—	—
2	3	O	5	1	35	tr	35
3	3	O	3	72	—	70	—
4	4	O	3	2	73	—	—
5	4	O	3	120	75	5	—
6	5	O	3	2	—	25	60
7	5	O	3	48	—	97.5	—
8	2	S	5	1	45	—	10
9	2	S	3	48	—	60	—
10	3	S	3	2	52	—	29
11	3	S	3	2	83	—	—
12	4	S	3	2	—	83	tr
13	4	S	3	96	—	86	—
14	2	NPh	2	64	93	—	—
15	2	$\text{NC}_6\text{H}_4\text{Cl-}p$	2	168	71	—	26
16	2	$\text{NC}_6\text{H}_4\text{OMe-}p$	2	72	91	—	—
17	3	NPh	1.5	168	—	—	95
18	3	$\text{NC}_6\text{H}_4\text{OMe-}p$	1.5	168	—	—	92
19	4	NPh	1.5	168	—	—	94
20	5	NPh	1.5	168	—	—	99

Table 3 Conversion of miscellaneous dinitriles into acids

Entry	Substrate	Conditions		Product(s) and yields
		Conc./mmol	t/h	
1		2	24	 80%
2		2	24	 R ¹ = CN, R ² = CO ₂ H 65% R ¹ = CO ₂ H, R ² = CN 16%
3		3	24	 R ¹ = R ² = CN 13% R ¹ = CN, R ² = CO ₂ H 11% R ¹ = R ² = CONH ₂ 65%
4		3	30	 67%
5		3	30	 69%
6		1.5	39	 99%
7		5	14	 67%
8		5	14	 86%

cofactor present in such nitrile hydratases, pyrroloquinoline quinone, as documented elsewhere.¹³ When a suitably placed ligand atom is also present in the nitrile a bidentate complexation to the metal occurs, which interferes with the hydration of the nitrile function. This ligand may be a suitably placed CO₂H function or a heteroatom. The glutaronitrile **1** ($n = 3$) bears a δ -oxygen ligand as does the ether **4** ($n = 4$, X = O). The C–S bond is considerably longer than the C–O bond (1.81 and 1.43 Å respectively) and not surprisingly therefore a γ placement lends optimal regioselectivity. If this mechanism is correct, the above ligands should behave as competitive inhibitors in the hydrolysis of other easily hydrolysed nitriles.† This is indeed found to be the case. Thus when the hydrolysis of benzonitrile is followed (by HPLC) in the presence or absence of NCCH₂CH₂CH₂SCH₂CH₂CH₂CO₂H, we find that the rate of benzonitrile disappearance is dramatically slower in the former case. Thus after 5 min reaction, about 20% of unreacted benzonitrile remained with no added cyano acid. In the presence of the inhibitor almost 40% remained. After 10 min the figures were *ca.* 2 and 25%. Furthermore the rate of decrease of amide

and rate of formation of acid are both significantly slower in the presence of the cyano acid.

When the aliphatic α,ω -dinitrile chain is interrupted by vinyl or aryl substituents, regiocontrol is efficiently observed in almost all cases that we have examined except for *o*-phenylenediacetonitrile (Table 2, entry 3). Although the regiocontrol here (and in the case of aromatic dinitriles¹⁴) may derive from a different basis, it is tempting to suggest that the π -systems can also act as effective ligands for the iron when chelation allows (Table 3). Also included in Table 3 is *trans*-cyclohexane-1,4-diacetonitrile (entry 6) which shows total regioselective hydrolysis, presumably by way of chelation of its axial or twist-boat conformer.

In conclusion, we have defined the extent to which regiocontrol can be expected in the hydrolysis of dinitriles bearing O, S and to a limited extent N substituents, and considered the control with π -functionalised dinitriles. The application of these ideas to prochiral and chiral systems is now being actively examined.

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Footnotes

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