Pergamon

Tetrahedron Letters, Vol. 36, No. 52, pp. 9561-9564, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

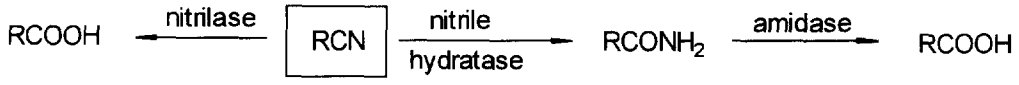
0040-4039(95)02048-9

A Powerful New Nitrile Hydratase For Organic Synthesis - Aromatic And Heteroaromatic Nitrile Hydrolyses - A Rationalisation

Otto Meth-Cohn* and Mei-Xiang Wang
Chemistry Department, University of Sunderland, Sunderland SR1 S3D, UK.
(*Email*: otto.meth-cohn@sunderland.ac.uk)

Abstract: A powerful new nitrile hydratase organism, Rhodococcus rhodocrous AJ270 has been isolated that efficiently hydrolyses all kinds of nitriles to amides and/or acids. This paper shows that aromatic and heterocyclic nitriles are readily hydrolysed to acids but, that those bearing an adjacent-substituent (which may be an ortho substituent or an adjacent heteroatom in the ring) give amides in good yield but only slowly proceed to acids.

Lipase mediated hydrolyses of esters are well established whereas nitrile hydrolysing organisms and enzymes have only come to the fore within the past 20 years, most notably the use of a *Rhodococcus rhodocrous J1* by Japanese workers to produce acrylamide in tens of thousands of tonnes per annum[1]. Unfortunately this organism is not generally available for synthetic application. Other applications have utilised a Novo immobilised *Rhodococcus rhodocrous* which also is currently not available. Although other organisms are occasionally used such as *Pseudomonas chloraphis* and *Corynebacterium*, *Rhodococcus rhodocrous* systems appear to be the most robust, useful and versatile. They operate either by direct hydrolysis to the acid (utilising a nitrilase enzyme) or by sequential action of an enzyme that hydrates the nitrile to the amide and another that hydrolyses the amide to the acid (Scheme 1)[2].



Scheme 1

The hydration stage is believed to involve complexation of the nitrile nitrogen to a transition metal (cobalt or iron) followed by hydration mediated by a pyrroloquinoline quinone. The nitrilase enzyme is totally different in action, *not* being mediated by a metal. A long used nitrilase system is exemplified by *R.r* N.C.I.B. 11216. It is mistakenly assumed that aliphatic hydrolyses are mediated by the hydratase enzyme, while aromatic and heterocyclic systems utilise a nitrilase enzyme[2].

At Sunderland over a number of years biologists have screened a large number of soil-derived nitrile hydrolysing organisms and focused on the most robust and versatile, *R.r. AJ270*, which is a nitrile hydratase system, and which is under active development[3]. We herein report the first applications of this organism in synthesis, with aromatic and heterocyclic nitriles as the target and show for the first time that most of the literature data and ours can be sensibly rationalised. Later publications will report our work on aliphatic and in particular chiral nitriles.

The literature data on aromatic nitrile hydrolysis is rather confused in terms of predicting effective hydrolysis, and the associated regioselectivities. This is partly due to the variety of systems used and their mode of generation. Since nitrilases are totally different in structure and thus reaction style, their use will not be featured here though interesting results from Thimann & Mahadevan[4a], Harper[4b] and Gutman[4c] and others have been reported.

Yamada[1,5] has reported that a variety of aromatic and heteroaromatic nitriles give amides when reacted with *R.r. J1,* the commercial organism used for acrylamide production which is almost devoid of amidase action toward aromatic amides so only amide formation is observed. Three different groups[6,2] showed that the immobilised Novo system (a hydratase system) slowly hydrolysed various *para*- and and *meta*-substituted -benzonitriles to acids though with poor conversions. Turner[2,6b] also demonstrated that various aromatic dinitriles gave nitrilo-acids (though phthalonitrile gave the diacid) and showed that unsymmetrical dinitriles showed poor regioselectivity in their mono-hydrolysis. Also a variety of perfluorodinitriles tend to give mono-amides rather than acids, also with poor regioselectivity. Griengl[6c] examined several heterocyclic nitriles which slowly gave amides or acids.

We now wish to show that *all the past work and our present observations indicate that the presence of an adjacent substituent* (an *ortho*-substituent or an adjacent ring heteroatom) *allows conversion of aromatic and heteroaromatic nitriles into amides; in many cases this is efficient and rapid; however the conversion of the amides to acids is slow.* On the contrary, non-adjacently substituted nitriles proceed rapidly in high yield to give acids (Tables 1-4; Yields in parentheses are from the literature for comparison). This generalisation applies to most of the published examples including the perfluoronitriles. In other words, while the first step, hydration of the linear nitrile substituent, is not significantly hindered by steric or electronic factors, the second amidase-mediated step is very sensitive to steric factors. This electronic and steric insensitvity in the nitrile hydration step accounts for the poor regioselectivity in mono-hydrolysis of dinitriles. In the case of *non-adjacently*-substituted nitriles the amide is occasionally visible by TLC after a few hours action (see examples with an asterisk in Table 1) but is hardly evident on work-up in most cases. In other words, with 'non-adjacent' compounds the amidase reaction is significantly faster than the hydratase step while with 'adjacent' compounds the reverse is true. In the 33 examples

Table 1
Hydrolysis of *para*-substituted benzonitriles *p*-RC_6H_4CN

R	Time (d)	Amide (%)	Acid (%)	Nitrile (%)
H	1	-	97.5	-
Me	1 (20[6a])	-	82 (28)	-
Cl*	2 (18[6a])	-	89 (43)	-
F	1	-	93	-
NO_2*	2	-	71.5	-
Ac	1	-	99	-
MeOOC	1	-	96.5	-
MeO	1 (5[6a])	-	87 (52)	-
HO	1	-	29	-
H_2N#	2[9h]	30[64]	38[10]	

* trace $ArCONH_2$ observed after 2-4h
# [ ] data after 9h

Table 2
Hydrolysis of *meta*-substituted benzonitriles *m*-RC_6H_4CN

R	Time (d)	Amide (%)	Acid (%)	Nitrile (%)
H	1	-	97.5	-
NO_2	1	-	92	-
MeO	4 (18[6a])	-	91 (18)	-
HO	0.4	-	56	-
H_2N	0.4	-	79	-

Table 5
Hydrolysis of other aromatic nitriles

Substrate	Time	Amide (%)	Acid (%)	Nitrile (%)
3,4-$(OCH_2O)C_6H_3CN$	1d (1d[8])	-	89 (83)	-
2,6-$F_2C_6H_3CN$	1d	80	15	-
2,6-$(MeO)_2C_6H_3CN$	9d	-	-	80

Table 3
Hydrolysis of *ortho*-substituted benzonitriles *o*-RC_6H_4CN

R	Time	Amide (%)	Acid (%)	Nitrile (%)
H	1d	-	97.5	-
Me	5.5h	92	7	-
"	5d	-	99	-
MeO	4h	93	5	-
"	4d	-	95	-
Cl	6.5h	94	4	-
"	7d	-	80	-
NO_2	4h	90	10	-
"	4d	-	86	-
HO	3h	80	5	-
"	1d	-	69	-
H_2N	1h	98	-	-
"	1d	-	62	-

Table 4
Hydrolysis of heterocyclic nitriles

Heteroaryl	Time	Amide (%)	Acid (%)	Nitrile (%)
2-furyl	1h	82	12.5	-
"	3d	-	83	-
2-thienyl	2h	73	28	-
"	2d	-	86	-
1,5-diMepyrrolyl	2h	81	-	16
"	2d	87	-	11
3-indolyl	6d(3[6b])	-	50(38)	29
2-pyridyl	6d	-	30	46
3-pyridyl	1d	-	60	-
4-pyridyl	2h	59	-	-
"	3d	-	50	-
3-quinolyl	2d	-	58	-
1-isoquinolyl	5d	12	-	62.5

already published either amide is isolated or the reaction to the acid is significantly long. Our results particularly in Tables 3 and 5 show convincingly that this generalisation is held.

All the reactions were conducted in a similar manner using an orbital shaker whereby the nitrile (2-5mmol) and *AJ270* (2g wet weight) were treated at 30°C in 50mL aqueous phosphate buffer, pH 7.0. Yields refer to isolated, purified material.

Some key features of our results need underlining:

- *Para-* and *meta-* substituted benzonitriles (Tables 1 and 2) hydrolyse rapidly in high yield to the corresponding acids irrespective of the electronic nature of the substituent. However hydroxy- and amino- substituted nitriles give lower yields and appear to be alternatively metabolised competitively with hydrolysis.
- In the benzonitrile series only *adjacently*-substituted nitriles yield amides, the yields being high on short reaction (Table 3). 2,6-Difluorobenzonitrile hydrolyses efficiently to the amide stage but very slowly to the acid; however 2,6-dimethoxybenzonitrile is unchanged after 9 days action. Clearly the amide hydrolysis is critically dependent upon adjacent steric facors while even the nitrile hydration has some steric limitation.
- Heterocycles bearing an adjacent C=O/C=N group are inert to the hydratase as noted by Griengl[6c]. We concur with this finding with several examples.

We thank BBSRC for a post-doctoral grant (M-X.W.) and generous funding that made this work possible.

REFERENCES

1. Nagasawa,T. and Yamada, H., *Pure & Appl. Chem.*, **1988**, 62, 1441-1444.
2. for a useful overview see: Crosby, J. , Moilliet, J., Parratt, J. S. and Turner, N. J., *J. Chem. Soc., Perk. 1*, **1994**, 1679-1687.
3. Blakey, A. J., Colby, J. , Williams, E. and O'Reilly, C., *FEMS Microbiol. Lett.*, **1995**, 129, 57-62.
4. (a) Thimann, K. V. and Mahadevan, S. *Arch. Biochem. Biophys.*, **1964**, 105, 133-141; (b) Harper, D. B. *Biochem. J.*, **1977**, 165, 309-319; (c)Bengis-Garber, C. and Gutman, A. L. , *Tetrahedron Lett.*, **1988**, 29, 2598-2590; *ibid., Appl. Microbiol. Biotechnol.*, **1989**, 32, 11-16.
5. Nagasawa, T., Mathew, C. D., Mauger, J. and Yamada, H., *Appl. Environm. Microbiol.*, **1988**, 1766-1769. Mauger, J. , Nagasawa, T. and Yamada, H. ,*J. Biotechnol.*, **1988**, 8, 87-96; *ibid.*, *Tetrahedron*, **1989**, 45, 1347-1354.
6. (a) Hönicke-Schmidt , P. and Schneider, M. P. , *J. Chem. Soc. Chem. Commun.*, **1990**, 648; (b) Cohen, M. A., Sawden, J. and Turner, N. J. , *Tetrahedron Lett.*, **1990**, 31, 7223-7226; (c) Klempier, N., de Raadt, A., Faber , K. and Griengl, H., *Tetrahedron Lett.*, **1991**, 32, 341-344; *ibid., J. Heterocycl. Chem.*, **1992**, 29, 93-95.

(*Received in UK* 5 *October* 1995; *revised* 23 *October* 1995; *accepted* 27 *October* 1995)