Enzymatic Hydrolysis of Heterocyclic Nitriles

Norbert Klempier, Anna de Raadt and Herfried Griengl*

Christian-Doppler-Laboratory for the Chemistry of Chiral Compounds
at the Institute of Organic Chemistry, Graz University of Technology,
Stremayrgasse 16, A-8010 Graz, Austria

Gottfried Heinisch

Institute of Organic and Pharmaceutical
Chemistry, University of Innsbruck,
Innrain 52a, A-6020 Innsbruck, Austria

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Chemoselective hydrolysis of heterocyclic nitriles can be achieved by an easy to use immobilized biocatalyst prepared from *Rhodococcus sp.* Pyrimidine-2-carbonitrile (**2a**) and 3-chloropyridazine-4-carbonitrile (**4a**) were converted into the corresponding amides while from 2-ethoxycarbonyl-4-pyridinecarbonitrile (**1a**), 6-methylpyridazine-3-carbonitrile (**3a**), 3-chloropyridazine-4-carbonitrile (**4a**), 3-ethoxycarbonyl-4,5-dihydroisoxazole-5-carbonitrile (**8a**), indole-3-carbonitrile (**9a**) and indole-3-ylacetonitrile (**10a**) the acids were formed.

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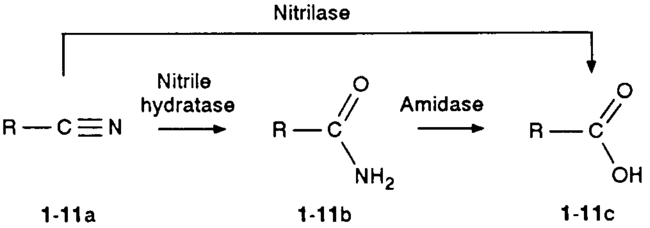
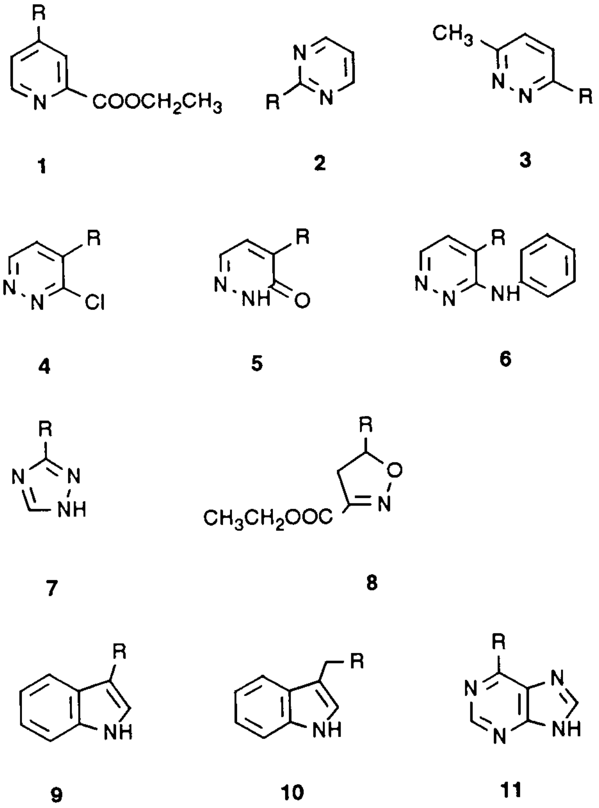
Hydrolysis of nitriles provides an important route to carboxylic acids and amides. This transformation usually requires rather strong acidic or basic conditions. As a consequence, when applied to sensitive compounds, side reactions are encountered. This is, for example, observed in acid hydrolysis of heterocyclic nitriles. It is well known that pyridinecarboxylic acids are prone to decarboxylation even at moderately elevated temperatures [1]. Decarboxylation in acidic medium is also reported for pyrimidine- [2,3] and indolecarboxylic acids [4]. Another disadvantage of chemical hydrolysis results from the impossibility to apply this methodology to molecules bearing other hydrolysable groups. To overcome these difficulties alternative chemical methods have been developed [5-8].

Nitriles can also be hydrolysed using microorganisms. The application of enzymes in organic synthesis [9-12] is playing an increasingly important role due to the mild reaction conditions employed and the high selectivities normally observed. For microbial nitrile hydrolysis two distinct pathways involving different enzymes have been recognized [13,14,15]: conversion to the carboxylic acid involving an intermediate amide, catalyzed by a two enzyme system, a *nitrile hydratase* and an *amidase*, or direct conversion to the acid, catalyzed by a single enzyme, a *nitrilase*. The use of various microorganisms for the hydrolysis of pyridine- [16-18]. thiophene- [17,19], furan- [17,19], pyrazine- [20] and quinolinenitriles [37] as well as thienyl- [37]

Scheme 1



Scheme 2



	a	b	c
R	CN	CONH$_2$	COOH

and indoleacetonitriles [19,21] has been reported. The compounds investigated did not contain other hydrolysable groups in the molecule.

For preparative purposes, however, handling of a microorganism is not usually the organic chemist's method of choice. Recently an enzyme complex obtained from *Rhodococcus sp.* became available in immobilized form [22]. This ready to use preparation has both nitrile hydratase and amidase activities. It has already been used for the hydrolysis of aliphatic and aromatic mono- and dinitriles [23,24]. The applicability of this immobilized enzyme to hydrolytic transformations of nitriles bearing additional groups, sensitive to acid or base hydrolysis, such as esters or acetals, is of special importance [25]. Herein the application of this enzyme preparation of the transformation of heterocyclic nitriles is reported.

Generally the hydrolyses are performed by shaking the substrate with the 5-fold excess of immobilised enzyme (w/w) in aqueous buffer (*p*H 7). Since a large proportion of the catalyst consists of the carrier the actual amount of enzyme is rather low. Depending of the substrate one to several days are required until all starting material has reacted. The results are given in Table 1.

Table 1

Compound	Product	Reaction time (hours)	Yield (%)
1a	**1c**	24	58
2a	**2b**	144	72
3a	**3c**	288	70
4a	**4b**	72	35
5a	–	120	–
6a	–	120	–
7a	–	288	–
8a	**8b**	60	9
8a	**8c**	60	54
9a	**9c**	72	40
10a	**10c**	24	90
11a	–	240	–

Cyanoesters **1a** [26] and **8a** [27] as well as chloronitrile **4a** [28] have been investigated as models for heterocyclic substrates with two hydrolysable groups in the molecule. In case of the cyanoesters, only the nitrile group was hydrolysed and thus 2-ethoxycarbonylisonicotinic acid (**1c**) (24 hours, 58%) and 3-ethoxycarbonyl-4,5-dihydroisoxazole-5-carboxylic acid (**8c**) (60 hours, 54%) could be obtained. A small amount of the intermediate amide **8b** was also isolated in the latter reaction. 3-Chloropyridazine-4-carbonitrile reacted more slowly and after three days 35% of the corresponding amide **4b** [28] was formed. Thus, for all three entries the course of the hydrolysis was chemospecific. In all cases starting material had reacted completely. The moderate yields obtained in these and the following examples can be attributed to loss of material during workup and purification by chromatography. No attempt has been undertaken to optimize this procedure. Among the other pyridazines investigated only 6-methylpyridazine-3-carbonitrile (**3a**) [29] gave - although rather slowly - the corresponding acid **3c** [30] (12 days, 70%).

3-Anilinopyridazine-4-carbonitrile (**6a**) [31] was transformed after 5 days only to a slight extent, probably due to either insolubility or steric effects. Therefore, this reaction was not investigated further. Pyridazinenitrile **5a** [32] was unreactive, possibly caused again by the low solubility of this compound. Also inhibition phenomena could be responsible. This reason might also be responsible for the exceedingly slow transformation of 6-cyanopurine (**11a**) [33] as well as 3-cyano-1,2,4-triazole (**7a**) [34] where again no attempts for product isolation have been undertaken. Addition of organic cosolvents such as methanol or dimethyl sulfoxide diminished the activity of the enzyme. The indole derivative **9a** and **10a** were transformed into the corresponding acids **9c** [35] (72 hours, 40%) and **10c** [36] (24 hours, 90%). The latter reaction had already been performed using the microorganisms *Rhodococcus rhodochrous* J 1 [37]. As an example for the pyrimidine series, pyrimidine-2-carbonitrile (**2a**) [38] was transformed into pyrimidine-2-carboxamide (**2b**) [39] (144 hours, 72%). Interestingly in this case as well as for compound **4a** the reaction did not exceed the formation of the amide, even after prolonged reaction time.

In summarizing the immobilized enzyme system from *Rhodococcus sp.* proved to be an efficient and convenient catalyst for chemoselective and mild hydrolysis of heterocyclic nitriles as has been shown on these selected examples. Limitations are set by bulky substituents, unsufficient solubility and possibly by inhibition phenomena.

EXPERIMENTAL

The nmr spectra were recorded on a Bruker MSL 300 at 300.13 MHz (^{1}H) and 75.47 MHz (^{13}C). Chemical shifts are in ppm relative to tetramethylsilane. The ir spectra were taken on Beckman IR-33 in nujol. Reactions were monitored by tlc using silica gel plates (Merck 60F$_{254}$). Flash chromatography was performed on silica gel (Merck 60, 70-230 mesh).

The following compounds were synthesized according to literature procedures: **1** [26], **2** [37], **3** [29], **4** [28], **5** [32], **6** [31], **7** [34], **8** [27] and **11** [33]. Compounds **9** and **10** were commercially obtained.

General Procedure for the Biotransformation.

The substrate (0.5 g) and the immobilized biocatalyst (2.5 g) [40] were suspended in 0.1 *M* phosphate buffer (50 ml, *p*H 7) and the suspension was shaken at room temperature for the period of time given in Table 1. The biocatalyst was removed by centrifugation and the remaining aqueous solution was evaporated to dryness. The residue was extracted with anhydrous methanol (50 ml), insoluble material was filtered off and the filtrate was concentrated *in vacuo*. The residue was adsorbed onto silica gel (1 g) and loaded on top of a silica gel column (2.5 x 30 cm, 5-10 g silica gel). Purification by flash chromatography was achieved using dichloromethane:methanol:acetic acid (9:1:0.1) as the eluent. The appropriate fractions were pooled and evaporated. Toluene was added and evaporated again, to remove residual acetic acid.

The structures of all products have been proved by analytical and spectral data.

3-Ethoxycarbonyl-4,5-dihydroisoxazole-5-carboxylic Acid (**8c**).

Purification by silica gel chromatography gave 600 mg (54%) of **8c**, a colorless glass; ir: (nujol): 3200-3420 (OH), 1715 (C=O) cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.31 (t, J = 7 Hz, 3H, $\mathit{C}H_3CH_2$), 3.42 (m, 2H, 4-H), 4.28 (q, J = 7 Hz, 2H, $CH_3\mathit{C}H_2$), 5.17 (m, 1H, 5-H), 7.0 (s, br, 1H, exchangeable with deuterium oxide, COOH); ^{13}C nmr (deuteriochloroform): δ 175.23 (COOH), 160.28 (COOEt), 152.20 (C-3), 81.24 (C-5), 62.49 ($CH_3\mathit{C}H_2$), 37.90 (C-4), 14.12 ($\mathit{C}H_3CH_2$).

Anal. Calcd. for $C_7H_9NO_5$: C, 44.92; H, 4.85; N, 7.48. Found: C, 44.27; H, 4.90; N, 7.55.

Ethyl 5-Carbamoyl-4,5-dihydroisoxazole-3-carboxylate (**8b**).

Chromatography afforded 95 mg (9%) of **8b**, a pale yellow oil; ir: (nujol): 3430, 3205 (NH_2), 1735 (C=O, ester), 1660, 1605 (C=O, amide) cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.37 (t, J = 7 Hz, 3H, $\mathit{C}H_3CH_2$), 3.55 (m, 2H, 4-H), 4.36 (q, J = 7 Hz, 2H, $CH_3\mathit{C}H_2$), 5.18 (m, 1H, 5-H), 6.05 and 6.67 (s, br, 2H, exchangeable with deuterium oxide, $CONH_2$); ^{13}C nmr (deuteriochloroform): δ 172.40 ($CONH_2$), 159.60 (COOEt), 152.26 (C-3), 80.45 (C-5), 62.60 ($CH_3\mathit{C}H_2$).

Anal. Calcd. for $C_7H_{10}N_2O_4$: C, 45.16; H, 5.41; N, 15.04. Found: C, 45.58; H, 5.66; N, 14.95.

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