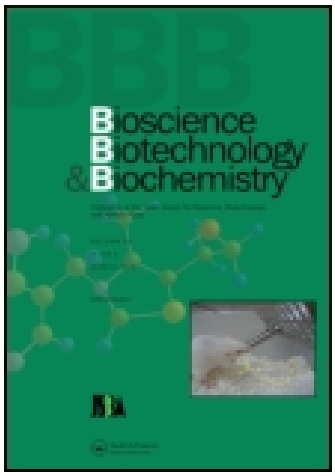


Bioscience, Biotechnology, and Biochemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbbb20

Action of Nitrile Hydratase from Rhodococcus rhodochrous IFO 15564 on Derivatives of 2,5-Anhydro-d-allononitrile

Masahiro Yokoyama[a], Yuko Nakatsuka[a], Takeshi Sugai[a] & Hiromichi Ohta[a]
[a] Department of Chemistry, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223, Japan
Published online: 12 Jun 2014.

To cite this article: Masahiro Yokoyama, Yuko Nakatsuka, Takeshi Sugai & Hiromichi Ohta (1996) Action of Nitrile Hydratase from Rhodococcus rhodochrous IFO 15564 on Derivatives of 2,5-Anhydro-d-allononitrile, Bioscience, Biotechnology, and Biochemistry, 60:9, 1540-1542, DOI: 10.1271/bbb.60.1540

To link to this article: http://dx.doi.org/10.1271/bbb.60.1540

Biosci. Biotech. Biochem., **60** (9), 1540-1542, 1996

Note

Action of Nitrile Hydratase from *Rhodococcus rhodochrous* IFO 15564 on Derivatives of 2,5-Anhydro-D-allononitrile

Masahiro YOKOYAMA, Yuko NAKATSUKA, Takeshi SUGAI, and Hiromichi OHTA†

Department of Chemistry, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223, Japan

Received April 24, 1996

The conversion of 2,5-anhydro-D-allononitrile derivatives by a nitrile hydratase from *Rhodococcus rhodochrous* IFO 15564 was studied. The activity of the enzyme was strongly effected by the steric bulkiness of the substituents at the 3-position of the substrates, and the corresponding amides were obtained in high yields from the nitriles with free hydroxyl groups at the 3- and 4-positions.

Key words: *Rhodococcus rhodochrous* IFO 15564; nitrile hydratase; 2,5-anhydro-D-allononitrile; 2,5-anhydro-D-allonamide; hydantocidin

Selective and mild hydrolyzing systems for nitriles by means of microorganisms recently became available for the production of fine chemicals.[1] Few examples, however, have been reported on the application to nitriles with a carbohydrate framework.[2] We were interested in the hydration of 2,5-anhydro-D-allononitriles **1** to amides **2**, since **2a** has been reported as the synthetic intermediate of nucleosides[3] and hydantocidin.[4]

Incubation of **1a**[5] with *Rhodococcus rhodochrous* IFO 15564 afforded a rather complex mixture of amides. It was observed that concomitant hydrolysis of the acetate protective groups (3-, 4-, and 6-positions) partially occurred during the incubation by an esterase in the microorganism.[1] Subsequently, the crude mixture was benzoylated to elucidate which acetates had been hydrolyzed by the esterase. This workup also facilitated the separation of products to give known amide **2a**[3,4] (35%), 3-acetate **2b**, and 4-acetate **2c** (**2b** + **2c**: 9%). This result indicated that hydrolysis of the 6-acetate had taken place faster than that of the 3-acetate and 4-acetate.

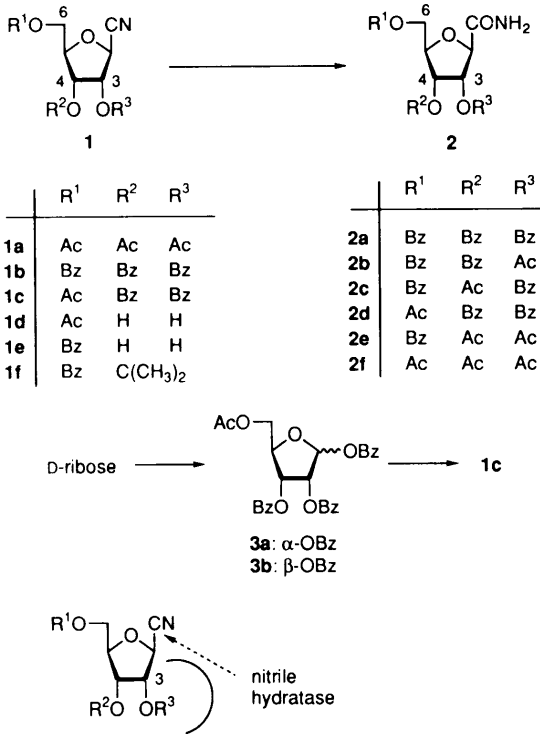
To simplify the situation, the 3-acetate and 4-acetate were substituted by benzoate, which was expected to be resistant to the esterase-mediated hydrolysis. Nitrile **1c** was prepared from D-ribose through the intermediates, a mixture of **3a** and **3b**, *via* regioselective lipase-catalyzed acetylation at the 6-position of ribose.[6,7] To our disappointment, hydration of the nitrile became very slow in the case of benzoates **1b**[5] and **1c**. The major product from **1c** was the corresponding hydroxy nitrile, only the 6-acetate being hydrolyzed.

These results indicated the importance of steric hindrance by the ester group at the 3-position. Indeed, the hydration of **1d**[8] and **1e**, which had free hydroxyl groups at the 3- and 4-positions, proceeded smoothly; especially in the case of 6-acetate **1d**, this hydration was extremely fast. Because of the shorter reaction time, the 6-acetate was scarcely affected, and thus amide **2d** was obtained as the major product (87%) after benzoylation. Although the 6-benzoate in **1e** retarded the hydration of the nitrile, desired product **2e** was obtained in as high a yield as 80% after an acetylative workup. All the results are listed in the Table.

The effect of steric hindrance at the 3-position on the nitrile hydrating activity was further studied. The activity of nitrile hydratase was almost lost by introducing a 3,4-acetonide group (**1f**).[8]

† Address correspondence to this author.

In conclusion, it was revealed that the rate of hydration of the nitrile group in 2,5-anhydro-D-allononitriles by the nitrile hydratase from *R. rhodochrous* IFO 15564 was strongly effected by the steric bulkiness at the 3-position. From substrates with free hydroxyl groups at the 3- and 4-positions, the corresponding amides were obtained in high yields (80–87%).



	R^1	R^2	R^3
1a	Ac	Ac	Ac
1b	Bz	Bz	Bz
1c	Ac	Bz	Bz
1d	Ac	H	H
1e	Bz	H	H
1f	Bz	$C(CH_3)_2$	

	R^1	R^2	R^3
2a	Bz	Bz	Bz
2b	Bz	Bz	Ac
2c	Bz	Ac	Bz
2d	Ac	Bz	Bz
2e	Bz	Ac	Ac
2f	Ac	Ac	Ac

Table Hydration of 2,5-Anhydro-D-allononitriles by *Rhodococcus rhodochrous* IFO 15564[a]

Substrate	Reaction time (h)	Products (Yield %)	
		Nitrile	Amide
1a	19	—	**2a** (35), **2b** + **2c** (9)[b]
1b	24	**1b** (91)	—
1c	22	**1b** (59)	**2a** (3)[b]
1d	2	—	**2a** (7), **2d** (87)[b]
1e	12	—	**2e** (80), **2f** (5)[c]
1f	72	**1f** (60)	—

[a] All reactions were carried out at 30°C.
[b] Products were isolated after benzoylation.
[c] Products were isolated after acetylation.

Experimental

IR spectra were recorded as thin films for oils or in KBr discs for solids. ^{1}H-NMR spectra were measured in chloroform-*d* with tetramethylsilane

as the internal standard at 400 MHz, unless otherwise stated.

Hydration of **1a**. A suspension of harvested cells of *Rhodococcus rhodochrous* IFO 15564[1j] (0.1 g) in a phosphate buffer solution (0.1 M, pH 6.0, 10 ml) was added to nitrile **1a** (17.7 mg, 0.062 mmol), and the mixture was stirred for 19 h at 30°C. The cells were removed by centrifugation, and the resulting supernatant was lyophilized. The residue was benzoylated in the conventional manner. The product was purified by silica gel preparative TLC (hexane–ethyl acetate, 1:1, developed three times) to afford **2a** (10.5 mg, 35%) and a mixture of **2b** and **2c** (2.5 mg, 9%). Analytical sample of **2a**: oil, ^{1}H-NMR δ: 4.67–4.77 (3H, m), 4.78 (1H, d, J=3.9 Hz), 5.72 (1H, dd, J=5.2, 6.0 Hz), 5.82 (1H, br. s), 5.96 (1H, dd, J=3.9, 5.2 Hz), 7.03 (1H, br. s), 7.30–7.63 (9H, m), 7.86–8.10 (6H, m). Its NMR spectrum was in good accordance with that reported previously.[3]

The minor product was revealed to be a mixture of 3-acetate **2b** and 4-acetate **2c** by its NMR spectrum. ^{1}H-NMR δ: 2.09 and 2.15 (each s, total 3H), 4.30–4.70 (4H, m), 5.49–5.84 (3H, m), 6.80 (br. s), 6.90 (br. s), 7.34–7.55 (6H, m), 7.73–8.02 (4H, m).

*5-O-Acetyl-1,2,3-tri-O-benzoyl-*D*-ribofuranose* (**3a** and **3b**). A mixture of D-ribose (1.509 g, 10.1 mmol), 2,6-di-*t*-butylphenol (BHT, a catalytic amount), and immobilized *Candida antarctica* lipase (2.02 g) was heated in vinyl acetate (10 ml) and pyridine (15 ml) at 45°C for 2 days under a slow, continuous flow of nitrogen. After removing the insoluble material by filtration through a pad of Celite, the filtrate and washings were combined and concentrated *in vacuo*. The residue was benzoylated in the conventional manner, and after the workup, the product was purified by silica gel column chromatography (250 g). Elution with toluene–ethyl acetate (30:1) afforded **3** (1.971 g, 39%) as an anomeric mixture. This mixture was employed in the next step without further purification.

The two anomers could be separated by silica gel preparative TLC. The fast-moving isomer was assigned to a β-anomer, and the slow-moving isomer to an α-anomer by comparing the coupling constants between H-1 and H-2 with the reported values for tetra-*O*-acetyl-D-ribofuranose[9]: δ 6.41, J=4.3 Hz, for H-1 of the α-anomer, and δ 6.18, J=0.8 Hz, for H-1 of the β-anomer. Fast-moving isomer (**3a**): R_f 0.58 (toluene–ethyl acetate, 9:1, developed twice), ^{1}H-NMR δ: 1.85 (3H, s), 4.21 (1H, dd, J=4.9, 12.2 Hz), 4.43 (1H, dd, J=3.9, 12.2 Hz), 4.66 (1H, ddd, J=3.9, 4.9, 6.8 Hz), 5.82 (1H, dd, J=4.9, 6.8 Hz), 5.85 (1H, d, J=4.9 Hz), 6.57 (1H, s), 7.27–7.58 (9H, m), 7.83–8.05 (6H, m).

Slow-moving isomer (**3b**): R_f 0.44 (the same solvent system), ^{1}H-NMR δ: 2.12 (3H, s), 4.35 (1H, dd, J=3.9, 12.2 Hz), 4.42 (1H, dd, J=2.9, 12.2 Hz), 4.71–4.73 (1H, m), 5.55 (1H, dd, J=4.4, 6.8 Hz), 5.70 (1H, dd, J=2.2, 6.8 Hz), 6.85 (1H, d, J=4.4 Hz), 7.20–7.56 (9H, m), 7.76–8.01 (6H, m).

*6-O-Acetyl-3,4-di-O-benzoyl-2,5-anhydro-*D*-allononitrile* (**1c**). A mixture of **3a** and **3b** (322 mg, 0.638 mmol) was treated with trimethylsilyl cyanide and boron trifluoride etherate.[5] After the workup, the product was purified by silica gel column chromatography (10 g). Elution with toluene–ethyl acetate (16:1) afforded **1c** (165 mg, 63%) as an oil, $[\alpha]_D^{21}$ −1.3 (c 1.02, chloroform). IR ν_{max} cm^{-1}: 1740, 1605, 1590, 1500, 1460, 1375, 1320, 1280, 1185, 1130, 1100, 1075, 1030, 965, 885, 815, 720, 670; ^{1}H-NMR δ: 2.20 (3H, s), 4.27 (1H, dd, J=2.9, 12.2 Hz), 4.55 (1H, dd, J=2.9, 12.2 Hz), 4.59–4.62 (1H, m), 4.95 (1H, d, J=4.4 Hz), 5.79 (1H, dd, J=4.9, 5.4 Hz), 5.95 (1H, dd, J=4.4, 4.9 Hz), 7.37–7.60 (6H, m), 7.93–7.96 (4H, m); ^{13}C-NMR δ: 20.89, 62.68, 69.71, 72.01, 74.73, 81.32, 115.92, 128.21, 128.48, 128.59, 128.63, 128.79, 128.83, 129.77, 129.86, 129.96, 133.86, 134.00, 164.90, 165.13, 170.44. *Anal.* Found: C, 64.27; H, 4.57; N, 3.53%. Calcd. for $C_{22}H_{19}NO_7$: C, 64.54; H, 4.68; N, 3.42%.

Hydration of **1c**. Nitrile **1c** (100.6 mg, 0.246 mmol) was incubated with *Rhodococcus rhodochrous* for 22 h at 30°C. After benzoylation and workup, the product was purified by silica gel column chromatography (20 g). Elution with hexane–ethyl acetate (6:1–1:1) and subsequent preparative TLC (hexane–ethyl acetate, 3:1) afforded **1b** (67.8 mg, 59%) and **2a** (3.8 mg, 3%).

*6-O-Acetyl-2,5-anhydro-*D*-allononitrile* (**1d**). A solution of **1a** (713 mg, 2.50 mmol) in chloroform (10 ml) was added to a saturated methanolic ammonia solution (20 ml) at 0°C in an argon atmosphere. The mixture was stirred for 2 h at 0°C, and then concentrated *in vacuo* while being kept at 0°C. The residue was purified by silica gel column chromatography (37 g). Elution with hexane–ethyl acetate–ethanol (25:25:1) afforded **1d** (456 mg, 91%) as an oil, $[\alpha]_D^{24}$ +18.9 (c 1.07, methanol). IR ν_{max} cm^{-1}: 3450, 2250, 1730, 1380, 1250, 1085, 1045, 980, 940, 720; ^{1}H-NMR (CD_3OD) δ: 2.13 (3H, s), 4.11–4.15 (1H, m), 4.14 (1H, dd, J=3.9, 13.2 Hz), 4.23 (1H, dd, J=4.9, 4.9 Hz), 4.41 (1H, dd, J=4.4, 4.9 Hz), 4.42 (1H, dd, J=4.4, 13.2 Hz), 4.60 (1H, d, J=4.4 Hz); ^{13}C-NMR δ: 20.77, 64.31, 72.16, 72.87, 76.56, 83.62, 118.96, 172.45; MS m/z (%): 142 (23), 141 (100). *Anal.* Found: C, 47.51; H, 5.41; N, 6.98%. Calcd. for $C_8H_{11}NO_5$: C, 47.76; H, 5.51; N, 6.96%.

*6-O-Acetyl-3,4-di-O-benzoyl-2,5-anhydro-*D*-allonamide* (**2d**). Nitrile **1d** (102.7 mg, 0.510 mmol) was incubated with *Rhodococcus rhodochrous* (2 g) in a phosphate buffer solution (0.01 M, pH 6.0, 20 ml) for 2 h at 30°C. During the incubation, the pH of the mixture was kept at 6.0 by using a pH controller. After benzoylation and workup, the product was purified by silica gel column chromatography (11 g). Elution with hexane–ethyl acetate (5:4–1:2) afforded **2a** (16.4 mg, 7%) and **2d** (190.8 mg, 87%).

Analytical sample of **2d**: oil, $[\alpha]_D^{22}$ +8.3 (c 1.10, chloroform); IR ν_{max} cm^{-1}: 3470, 3360, 1735, 1695, 1600, 1585, 1500, 1450, 1370, 1320, 1280, 1180, 1125, 1075, 1025, 810, 760, 715; ^{1}H-NMR δ: 2.15 (3H, s), 4.43 (1H, dd, J=3.4, 12.7 Hz), 4.47 (1H, dd, J=4.4, 12.7 Hz), 4.55–4.60 (1H, m), 4.74 (1H, d, J=3.4 Hz), 5.58 (1H, dd, J=5.4, 6.4 Hz), 5.91 (1H, dd, J=3.4, 5.4 Hz), 6.29 (1H, br. s), 6.98 (1H, br. s), 7.31–7.59 (6H, m), 7.86–8.06 (4H, m); ^{13}C-NMR δ: 20.90, 63.08, 71.36, 74.60, 79.99, 81.03, 128.42, 128.47, 128.71, 129.09, 129.76, 129.84, 133.50, 133.58, 165.08, 165.18, 170.85, 171.42. *Anal.* Found: C, 61.80; H, 5.07; N, 3.13%. Calcd. for $C_{22}H_{21}NO_8$: C, 61.82; H, 4.95; N, 3.28%.

*6-O-Benzoyl-3,4-di-O-acetyl-2,5-anhydro-*D*-allonamide* (**2e**) *and 3,4,6-tri-O-acetyl-2,5-anhydro-*D*-allonamide* (**2f**). Nitrile **1e** (254.8 mg, 0.968 mmol) was incubated with *Rhodococcus rhodochrous* (5 g) in a phosphate buffer solution (0.1 M, pH 6.0, 50 ml) for 12 h at 30°C. After acetylation and workup, the product was purified by silica gel column chromatography (30 g). Elution with hexane–ethyl acetate (1:2–1:5) afforded **2e** (283.6 mg, 80%) and **2f** (15.3 mg, 5%).

Analytical sample of **2e**: colorless prism, mp 127.6–128.0°C, $[\alpha]_D^{18}$ +34.9 (c 1.00, chloroform); IR ν_{max} cm^{-1}: 3450, 3330, 1770, 1755, 1720, 1700, 1665, 1600, 1455, 1435, 1380, 1370, 1320, 1240, 1105, 1090, 1070, 1055, 1025, 980, 965, 920, 900, 870, 815, 795, 720, 660, 600, 570; ^{1}H-NMR δ: 2.08 (3H, s), 2.13 (3H, s), 4.44 (1H, ddd, J=2.4, 4.4, 6.8 Hz), 4.50 (1H, d, J=3.9 Hz), 4.53 (1H, dd, J=4.4, 12.7 Hz), 4.64 (1H, dd, J=2.4, 12.7 Hz), 5.36 (1H, dd, J=4.9, 6.8 Hz), 5.57 (1H, dd, J=3.9, 4.9 Hz), 5.95 (1H, br. s), 6.87 (1H, br. s), 7.45–7.62 (3H, m), 8.03–8.05 (2H, m); ^{13}C-NMR δ: 20.50, 20.59, 63.31, 70.66, 73.79, 79.57, 80.54, 128.68, 129.26, 129.69, 133.61, 166.49, 169.42, 169.53, 171.33. *Anal.* Found: C, 55.87; H, 5.33; N, 3.84%. Calcd. for $C_{17}H_{19}NO_8$: C, 55.89; H, 5.24; N, 3.83%.

Analytical sample of **2f**: oil, $[\alpha]_D^{21}$ −0.2 (c 1.47, chloroform); IR ν_{max} cm^{-1}: 3480, 3370, 1750, 1700, 1595, 1440, 1380, 1240, 1100, 1060, 965, 910, 765, 725, 610; ^{1}H-NMR δ: 2.07 (3H, s), 2.12 (3H, s), 2.13 (3H, s), 4.30–4.36 (3H, m), 4.48 (1H, d, J=3.9 Hz), 5.21 (1H, dd, J=4.9, 5.4 Hz), 5.51 (1H, dd, J=3.9, 4.9 Hz), 5.98 (1H, br. s), 6.85 (1H, br. s); ^{13}C-NMR δ: 20.48, 20.59, 20.84, 62.76, 70.50, 73.76, 79.44, 80.64, 169.40, 169.52, 170.77, 171.46. HR-MS: Found, 261.0851, Calcd. for $C_{10}H_{15}NO_7$, 261.0849 ($M^+ + 1 - CH_3CO$).

Acknowledgments. The authors thank Novo Nordisk Co. for generously presenting *Candida antarctica* lipase. We are grateful to Dr. Takashi Matsumoto of this department for helpful discussions.

References

1) a) S. Geresh, Y. Giron, Y. Gilboa, and R. Glaser, *Tetrahedron*, **49**, 10099–10102 (1993); b) T. Beard, M. A. Cohen, J. S. Parratt, N. J. Turner, J. Crosby, and J. Moilliet, *Tetrahedron: Asymmetry*, **4**, 1085–1104 (1993); c) A. Kerridge, J. S. Parratt, S. M. Roberts, F. Theil, N. J. Turner, and A. J. Willetts, *BioMed. Chem.*, **2**, 447–455 (1994); d) M. Yokoyama, T. Sugai, and H. Ohta, *Tetrahedron: Asymmetry*, **4**, 1081–1084 (1993); e) F. Effenberger and J. Böhme, *BioMed. Chem.*, **2**, 715–721 (1994); f) Y. Hashimoto, M. Nishiyama, S. Horinouchi, and T. Beppu, *Biosci. Biotech. Biochem.*, **58**, 1859–1865 (1994); g) S. Masutomo, A. Inoue, K. Kumagai, R. Murai, and S. Mitsuda, *Biosci. Biotech. Biochem.*, **59**, 720–722 (1995); h) T. Sugai, O. Katoh, and H. Ohta, *Tetrahedron*, **51**, 11987–11998 (1995); i) O. Meth-Cohn and M.-X. Wang, *Tetrahedron Lett.*, **36**, 9561–9564 (1995); j) M. Yokoyama, N. Imai, T. Sugai, and H. Ohta, *J. Mol. Catal. B: Enzymatic*, **1**, 135–141 (1996) and references cited therein.

2) N. Klempier, A. de Raadt, K. Faber, and H. Griengl, *Tetrahedron Lett.*, **32**, 341–344 (1991); A. de Raadt, H. Griengl, N. Klempier, and A. E. Stütz, *J. Org. Chem.*, **58**, 3179–3184 (1993).
3) D. K. Buffel, B. P. Simons, J. A. Deceuninck, and G. J. Hoornaert, *J. Org. Chem.*, **49**, 2165–2168 (1984).
4) P. M. Harrington and M. E. Jung, *Tetrahedron Lett.*, **35**, 5145–5148 (1994).
5) K. Utimoto, Y. Wakabayashi, T. Horiie, M. Inoue, Y. Shishiyama, M. Obayashi, and H. Nozaki, *Tetrahedron*, **39**, 967–973 (1983).
6) R. Pulido, F. L. Ortiz, and V. Gotor, *J. Chem. Soc., Perkin Trans. 1*, **1992**, 2891–2897.
7) A. K. Prasad, M. D. Sørensen, V. S. Parmar, and J. Wengel, *Tetrahedron Lett.*, **36**, 6163–6166 (1995).
8) J. A. Montgomery and K. Hewson, *J. Heterocycl. Chem.*, **7**, 443–445 (1970).
9) B. L. Kam, J.-L. Barascut, and J.-L. Imbach, *Carbohydr. Res.*, **69**, 135–142 (1979).