Tetrahedron Letters,Vol.28,No.35,pp 4057-4060,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

BACTERIA IN ORGANIC SYNTHESIS : γ-ALKOXY-α-AMINOACIDS FROM RELATED α-AMINONITRILES.

Yen Vo-Quang*[1], Dominique Marais[1], Liliane Vo-Quang[1] and François Le Goffic.
Alain Thiéry[2], Marc Maestracci[2], Alain Arnaud[2] and Pierre Galzy[2].

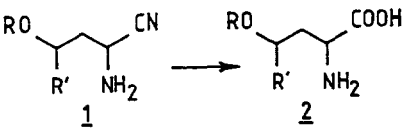
[1] : Laboratoire de Bioorganique et Biotechnologies, Ecole Nationale Supérieure de Chimie de Paris, 11 rue P. et M. Curie 75231 PARIS Cédex 05 (France).

[2] : Chaire de Génétique et Microbiologie, Ecole Nationale Supérieure Agronomique, place P. Viala 34060 MONTPELLIER Cédex (France).

Summary : The wild type strain Brevibacterium sp. R312 is able to hydrolyse water-soluble γ-alkoxyaminonitriles, under mild conditions, at pH value close to neutrality and at moderate temperature. The results reported here show that this technique is valuable for synthetic purposes.

α-Aminonitriles are important synthetic intermediates in the preparation of α-amino-acids (1,2). They are available by a variety of methods (3) and their hydrolysis is usually performed either directly or via an aminoester step (4). These techniques cannot however be applied to acid sensitive (5) or epimerizable molecules (6), therefore catalytic processes have recently been developed (7,8). The well documented bioconversion of nitriles (9) offers an interesting alternative for such a reaction as it can be performed under smooth conditions of pH and temperature (10,11). It has been shown that the wild-type strain Brevibacterium sp. R312 (12) is able to hydrolyse all water soluble nitriles into the corresponding acid via a transient amide. Two enzymes are involved in this bioconversion : a nitrile-hydratase (13-15) which converts the nitrile into the corresponding amide, and an amidase (16-19) which hydrolyses the amide into the acid. These enzymes having a wide substrate spectrum (17,19,20), Brevibacterium sp. R312 can accept a broad range of structurally different substrates, and therefore appears to be an attractive reagent for the planed transformation. But very little is apparently known so far about the applicability of such a technique for practical synthetic purposes (10).

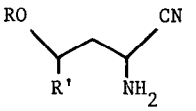
We now report the first results obtained in synthesizing γ-alkoxy-α-aminoacids 2 from the corresponding α-aminonitriles 1 by using Brevibacterium sp. R312 as a selective hydrolyzing reagent :



	a	b	c	d	e
R	Me	Et	iBu		iBu
R'	H	H	H	H	PhS

The γ-alkoxy aminonitriles 1 were readily prepared from γ-alkoxy aldehydes according to the Holland-Nayler procedure (21) (Table 1).

Table 1 : Aminonitriles 1



N°	R	R'	b.p.°C/mmHg	Yield %	^{1}H N M R (b)		
					H_2	H_3	H_4
1a	Me	H	64/0.01	82	3.88 t(6)	1.96 q(6 and 6)	3.82 t(6)
1b	Et	H	73/0.5	62	3.93 t(6)	1.98 q(6 and 6)	3.65 t(6)
1c	iBu	H	72/0.05	81	3.88 t(6)	1.95 q(6 and 6)	3.60 t(6)
1d		H	(a)	77	3.96 t(7)	2.11 q(6)	3.76 m(6)
1e	iBu	PhS	90/0.2	75	3.80 t(7)	2.20 q(6)	4.82 t(7)

(a) used crude without purification

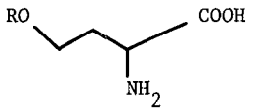
(b) T-60 ; $CDCl_3$ as solvent ; ppm/TMS, (JHz).

For the bioconversion of aminonitriles 1 to aminoacids 2, three different techniques are actually available : the use of free whole cells, of a crude bacterial extract, or whole cells entrapped in polyacrylamide gel (22). Preliminary kinetic assays revealed that the "free whole cells technique", easy to perform, could be used for the α-aminonitriles 1a,b,c but not for 1d, which uptake was probably not possible into the bacterial cells. In this case, the "disrupted bacteria process" was used.

The preliminary experiments were accomplished according to a previously described technique (13,16,17) on 50 mg of α-aminonitrile 1, in a phosphate buffer, at pH=7, and 28°C, with whole cells whose concentration in the culture medium was determined by measuring the optical density with a Klett-Summerson colorimeter (blue filter at 400-450 nm). The hydrolysis was monitored by thin-layer chromatography (on kieselgel 60 F 254) with an eluting system which allowed a good nitrile, amide and acid separation : propanol/ammonia 20 % (70/30) and 0.2 % ninhydrin in ethanol/acetic acid (80/20) as developer. The α-aminonitriles 1 were easily and quantitatively converted into α-aminoacids 2 after a few hours incubation, the rate of hydrolysis decreasing with increasing steric hindrance of substituent R. However, 1d was recovered practically unchanged in these conditions, even after a 8 hours incubation and the mixed thioacetal 1e, insoluble in water, was not structurally modified.

For synthetic purposes, 1.5 g of α-aminonitrile 1a, 1b, 1c were treated in the same way ; after a few hours of incubation, the suspension was centrifuged, new cells of Brevibacterium were added to the supernatant and incubated again in the same conditions. The enzymatic reaction products were isolated and purified by lyophilization and cationic exchange chromatography on a resin (Dowex 50WX2) (Table 2).

Table 2 : α-Aminoacids 2



R	m.p.°C	Yield % (a)	^{1}H-NMR (b)		
			H_2	H_3	H_4
Me	210	40	- 0.85 dd (6 and 6)	- 2.25 m	- 1.05 t (6)
Et	220	43	- 0.85 dd (6 and 6)	- 2.55 m	- 1.03 t (6)
iBu	235	35	- 0.86 dd (6 and 6)	- 2.83 m	- 1.06 t (6)

(a) Analytically pure product.

(b) T-60 spectrophotometer, D_2O as solvent, ppm/D_2O (JHz).

Disruption of bacteria by grinding and preparation of extracts as previously described (13,16,17) were performed to hydrolyse α-aminonitrile 1d. The same analytical assay showed that its conversion occurred ; however, longer reaction time was required to complete its hydrolysis.

These first not yet optimized results show that the enzymatic conversion of the γ-alkoxy-α-aminonitriles proceeds smoothly under mild conditions. The reaction occurs without any unwanted secondary product and the interesting γ-alkoxy-α-aminoacids may be cleanly purified. It could be of interest to extend this technique to water insoluble reactants, by adding for example a convenient cosolvent in the medium, to improve the value of this bioconversion for the synthesis of other functionalized α-aminoacids difficult to prepare by conventional ways.

References

(1) E.N. Safanova, V.M. Belikov, Russ. Chem. Rev. 43 (1974) 745.
(2) Y. Izumi, I. Chibata, T. Itoh, Angew. Chem. Int. Ed. 17 (1978) 176.
(3) K. Mai, G. Patil, Tetrahedron Lett. 25 (1984) 4583.
(4) R. Pascal, J. Taillades, A. Commeyras, Tetrahedron 34 (1978) 2275.
(5) K. Weinges, G. Graab, D. Nagel, B. Stemmle, Chem. Ber. 104 (1971) 3594.
(6) K. Nato, Tetrahedron Lett. 21 (1980) 4925.
(7) R. Sola, J. Brugidov, T. Taillades, A. Commeyras, A. Previéro, Tetrahedron Lett. 24 (1983) 1501.
(8) T. Mukaiyama, K. Kamio, S. Kobayashi, H. Takei, Chem. Letters (1978) 357.
(9) G. Whitesides, 5th International Conference on Organic Synthesis, FECS-IUPAC, Freiburg (F.R.G.) August 27-30, 1984.
(10) M.P. Schneider, Enzymes as catalysts in organic synthesis, Proceedings of the NATA advanced research Workshops (Reisenburg/Ulm, F.R.G., June 16-22, 1985), D. Reidl Publishing Company, Dordrecht, Holland, 1986.
(11) J.C. Jallageas, A. Arnaud, P. Galzy : Bioconversions of nitriles and their applications in Advances in Biochemical Engineering, Springer Verlag, Berlin 1980, p. 1-32.
(12) A. Arnaud, P. Galzy, J.C. Jallageas, C.R. Acad. Sci. Paris 287 (1976) 571.
(13) A. Arnaud, J.C. Jallageas, P. Galzy, Agric. Biol. Chem. 41 (1977) 2183.
(14) K. Bui, M. Maestracci, A. Thiéry, A. Arnaud, P. Galzy, J. Appl. Bacteriol. 57 (1984), 183.
(15) D. Tourneix, A. Thiéry, M. Maestracci, A. Arnaud, P. Galzy, Antonie Van Leeuwenhock 52 (1986), 173.
(16) J.C. Jallageas, A. Arnaud, P. Galzy, J. Gen. Appl. Microbiol. 24 (1978) 103.
(17) M. Maestracci, A. Thiéry, K. Bui, A. Arnaud, P. Galzy, Arch. Microbiol. 138 (1984), 315.
(18) M. Maestracci, K. Bui, A. Thiéry, A. Arnaud, P. Galzy, Biotechnol.Lett. 6 (1984), 14
(19) A. Thiéry, M. Maestracci, A. Arnaud, P. Galzy, M. Nicolas, J. Basic Microbiol. 26 (1986), 299.
(20) K. Bui, H. Fradet, A. Arnaud, P. Galzy, J. Gen. Microbiol. 130 (1984) 89.
(21) D.D. Holland, J.H.C. Nayler, J. Chem. Soc. (1952) 3402.
(22) K. Bui, A. Arnaud, P. Galzy, Enzyme Microb. Technol. 4 (1982) 195.

(Received in France 19 May 1987)