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STEREOSELECTIVE MICROBIAL HYDROLYSIS OF 2-ARYLOXYPROPIONITRILES

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2-Aryloxypropionic acids **3a-f**, compounds with herbicidal activity, have been prepared with high enantiomeric purity by microbial hydrolysis of the corresponding racemic nitriles and amides in presence of Brevibacterium imperiale cells.

INTRODUCTION

2-Aryloxypropionic acids are well known herbicides which are currently used as racemates although only the (R)-enantiomers are biologically active (Worthing, 1979). Therefore, the preparation of the (R)-isomer in a high optical purity is practically desirable.

Several procedures have been reported for the resolution of these compounds by chemical methods, such as crystallization of diastereomeric salts (Gottstein et al.,1965) or by biological methods involving lipase-catalysed enantioselective hydrolysis of racemic esters (Dernoncour et al., 1987). However, the process based on the enzymatic ester hydrolysis does not afford a stereoselectivity high enough to be competitive with the chemical approach.

In recent years the biological hydrolysis of nitriles has been proposed as an interesting alternative to the chemical methods to prepare carboxylic acids, since it can be performed at moderate temperature, pH values close to the neutrality and it does not lead to the production of large quantities of inorganic byproducts (Vo-Quang et al., 1897; Mauger et al., 1989; Hönicke-Schmidt et al., 1990).
In this work we report a new method for the preparation of optically active 2-phenoxypropionic acids 3a-f by stereoselective microbial hydrolysis of corresponding nitriles and amides.

MATERIALS AND METHODS

MICROORGANISMS

Brevibacterium imperiale B222 (CBS 498.78), Brevibacterium sp. C 211 (CBS 49974), Bacteridium sp. R 341 (CBS 49764) and Micrococcus sp. A 111 (CBS 49774) were purchased from Centraalbureau Voor Schimmelcultures (The Netherland).

CULTURE CONDITIONS

In typical experiments 500 ml of a sterilized culture medium (1% yeast extract, 2% peptone, 1% glucose, adjusted at pH 7) was inoculated with 5 ml of suspension of B. imperiale in the same medium. The media was cultured at 25°C for 48 hours, by shaking at 200 rpm.

ASYMMETRIC HYDROLYSIS

After culture, bacteria cells were harvested and suspended (6 g wet weight) in phosphate buffer 0.1 M, pH 7.5 (50 ml). The substrate **1a-f** or **2a-b** (7 mmol) was added and the suspension was shaken (200 rpm) at 25°C, the progress of the reaction was monitored by HPLC analysis. The reactions stopped spontaneusly at nearly 50% of conversion. After removal of the cells by centrifugation and adjusting the pH to 10, the amide **2a-f** was extracted with ethyl acetate. The acid **3a-f** was recovered from the remaining aqueous solution, after acidification to pH 2, by extraction with ethyl acetate. Both the products were purified by silica gel chromatography.

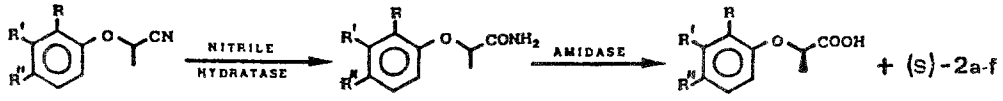
OPTICAL PURITY DETERMINATION

The optical purity of **2a,b,e,f** and **3a,b,e,f** was determined by HPLC analysis using a chiral column (Daicel Chiralcel OB, eluent hexane\2-propanol 95:5 v\v ; flow rate 0.8 ml\min), after transformation in the corresponding methyl ester. The optical purity of **2c,d** and **3c,d** was determined as described above after transformation in **2a** and **3a** by chemical reduction (H_2, Pd\C) (Noda et al., 1987).

RESULTS AND DISCUSSION

It is known that the bacterial strain Brevibacterium imperiale B 222 is able to hydrolyse a variety of nitriles into the corresponding acids via the amide intermediate (Commeyras et al., 1976). The enzymes involved in this bioconversion are a nitrile-hydratase, which converts the nitrile into the corresponding amide and an amidase, which hydrolyses the amide to the acid.

In order to investigate the stereospecificity of this enzymatic system, we studied the biological hydrolysis of a number of 2-aryloxypropionitrile derivatives 1a-f.



(R,S)-1a-f (R,S)-2a-f (R)-3a-f

	R	R'	R"
1a	H	H	H
b	H	H	Cl
c	H	Cl	H
d	Cl	H	H
e	Me	H	Cl
f	H	H	OPh

Using B. imperiale whole cells, all the tested substrates 1a-f were rapidly transformed into the corresponding racemic amides 2a-f by the non-stereoselective nitrile-hydratase and then slowly hydrolysed, by the stereoselective amidase, to give the acids 3a-f in the desired R form and the amides 2a-f in the S form (TABLE 1).

TABLE 1- Microbial hydrolysis of 1a-f and 2a-b

SUBSTRATE	TIME (hr)	CONV. (%)	(R)[a]-ACID 3 $[\alpha]_D^{25}$	e.e.%	(S)-AMIDE 2 $[\alpha]_D^{25}$ [b]	e.e.%
1a	4	47	+37.5[b]	≥95	+29.5	89
1b	3	47	+38.1[b]	95	+20.4	84
1c	48	51	+47.2[c]	88	+27.0	93
1d	48	52	+26.2[c]	86	+42.6	95
1e	24	44	+28.8[c]	≥95	+29.8	76
1f	3	51	+45.1[c]	92	+13.6	≥95
2a	6	49	+37.5	95	+30.8	93
2b	10	45	+39.6	≥95	+19.6	81

a) Determined by comparison with literature data (Gabard et al., 1986; Ohsuni et al., 1985).
b) C=1, EtOH
c) C=1, acetone

As shown in table 1, para-substituted compounds were hydrolysed 16 times faster than ortho- and meta- derivatives,

suggesting a strong dependence of the reaction rate on the steric hindrance of the substrate.
The enantioselectivity of the amidase was confirmed by the hydrolysis of racemic amides **2a,b**.
Other nitrile-hydrolysing miroorganisms such as Bacteridium sp. R 341, Micrococcus sp. A 111 and Brevibacterium sp. C 211 were tested in the resolution of **1a**, showing a comparable selectivity but a lower relative activity. For example, the reaction of **1a** with Micrococcus and Bacteridium cells yielded the acid **3a** with an enantiomeric excess ≥ 95% (47% conversion) in 10 and 7 hours, respectively.
The extension of this method for the resolution of different classes of chiral carboxylic acids is currently under investigation.

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