Bioorganic & Medicinal Chemistry Letters, Vol.3, No.4, pp. 681-684, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

ENANTIOSELECTIVE ACETYLATION OF PRIMARY AMINES BY *CYLINDROCARPON RADICICOLA*

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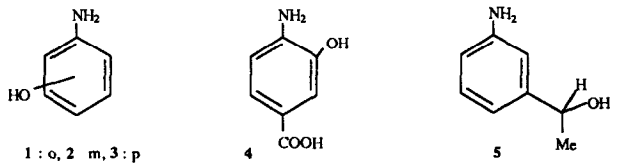
(*Received in USA* 10 *November* 1992)

Abstract: *Cylindrocarpon radicicola* ATCC 11011 resting cells catalyze kinetic resolution of primary amines via enantioselective N-acetylation in aqueous solution.

Esterifications catalyzed by hydrolytic enzymes have been successfully employed for kinetic resolution of racemic alcohols and acids[1]. This approach has been extended to chiral amines in the case of polyfonctionnal molecules with the aim of ensuring chemo- and stereoselectivity[1-5]. Klibanov and coworkers[2] have used hydrolases from several microbial strains to acylate racemic primary amines by carboxylic esters; subtilisin Carlsberg was shown to catalyze enantioselectively the N-acylation of 6-aminohexanol with N-Ac-(L)phe-OEtCl in tert-amyl alcohol at 45°C. This paper describes chemo- and enantioselective N-acetylation of primary amines in aqueous medium using *Cylindrocarpon radicicola* resting cells.

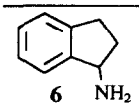
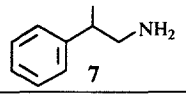
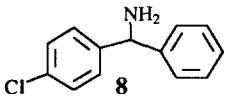
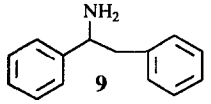
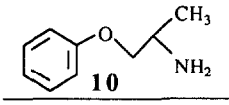
We have used resting cells of *C. radicicola* to N-acetylate substituted aromatic amines. Compounds **1** to **5** (figure 1) were transformed in gram scale to their amide derivatives, no trace of O-acetylated products was detected. In the case of (+/-) 3α-hydroxyethylaniline **5**, a stereorandom chemoselective N-acetylation was observed.



1 : o, 2 m, 3 : p 4 5

(figure 1) aminophenols 4-amino-2-hydroxybenzoic acid 3-hydroxyethyl aniline

Chiral primary aliphatic amines can also be N-acetylated by resting cells of *C. radicicola*[8]. Among the different substrates used racemic 1,2-diphenylethylamine **9** and 1-methyl-2-phenoxyethylamine **10** were resolved by enantioselective microbial N-acetylation (Table). The enantiomeric excess was determined by ^{1}H-NMR study after derivatization of the unreacted amine with (S)-O-acetyllactyl chloride[7] (Figure 2).

	Substrate concentration (mg/l)	Incubation time (h)	Amine/Amide[a] (%)	ee (%) Residual amine
 **6**	250	22	52/48	0
 **7**	500	10	65/35	0
 **8**	500	24	59/41	0
 **9**	300	18	48/52	80[b]
 **10**	500	24	53/47	95[b]

a : Determined by HPLC or GC

b: Determined by ^{1}H-NMR

The enantioselectivity coefficient calculated according to Sih[6] was higher than 30 for substrates **9** and **10** indicating that the acetylating enzyme involved in our reaction was highly enantioselective. With resting cells from *C. radicicola*, the reaction was carried out in distilled water, while in all enzymatic methods[1-5], anhydrous organic solvents are needed. The concentrations used in our case (0.2 to 0.5g/l) and the relatively short incubation time are interesting in terms of preparative approach. We verified that the racemic amides derived from compounds **9** and **10**, which have undergone incubation with *C. radicicola* were not hydrolyzed, thus the enzyme involved in our reaction is not reversible. Furthermore, this enzyme is not secreted from the cells because the incubation medium shows no activity. Since we used living microbial cells, the acetyl group is provided by the cell metabolism, while in the case of purified enzymes[1-5], acetyl donor molecules were added to the incubation medium.

We observed that the reaction does not take place in the case of chiral non aromatic amines. No acetylation is observed with 1-amino-1-cylohexane-carboxylic acid, trans-2-aminocyclohexanol or endo-2-aminonorbornane. Therefore, we postulate that aromatic substituent is necessary for the interaction of the substrate with the catalytic site of the enzyme. Selective acetylation of aliphatic polyfonctional molecules such as aminoalcohols or aminoacids bearing an aromatic substituent as protective group, are under current study.

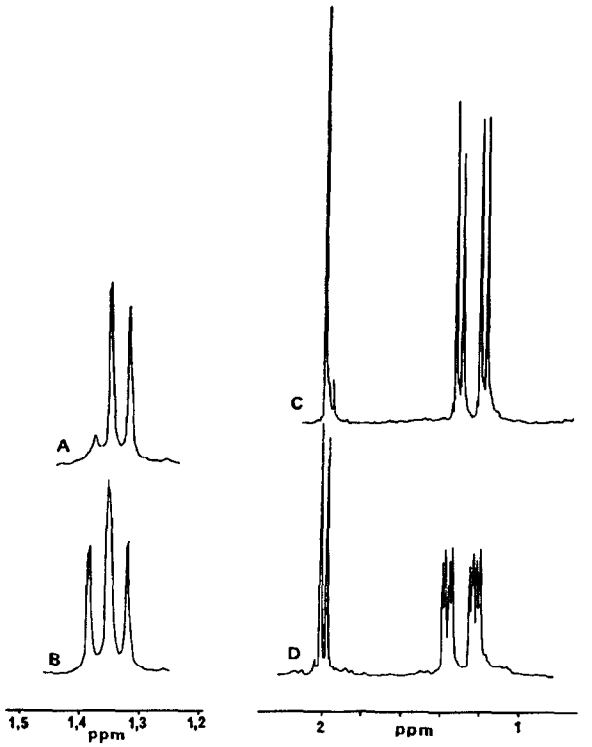


Figure 2: The chiral auxiliary (S)-O-acetyllactyl chloride and the derivatized amines are prepared according to Singer et al[7]. ^{1}HNMR (200MHz, $CDCl_3$ (±) **9**: 1.35 (3H,dd) ; 2.1 (3H,s) ; 3.1 (2H,m) ; 5.1 (1H,q) ; 5.3 (1H,m) ; 6.5 (1H,d) ; 7-7.4 (10H,m) (+) **10**: 1.2 (3H,dd) ; 1.4 (3H,dd) ; 2 (3H,d) ; 3.8 (2H,t) ; 4.3 (1H,m) ; 5.1 (1H,qt) ; 6.5 (1H,d) ; 6.8 (3H,m); 7.2 (2H,m). A: **9** C-Me corresponding to the microbial derivatized resting amine. B: **9** C-Me corresponding to the derivatized racemic amine. C: **10** -COO-Me and C-Me corresponding to the microbial derivatized resting amine. D: **10** -COO-Me and -C-Me corresponding to the derivatized racemic amine.

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8 - *Cylindrocarpon radicicola* ATCC 11011 was purchased from the American Type Culture Collection, Rockville, and maintained on agar slants (Diagnostic Pasteur, Paris). Spores of *C.radicicola* have been used to inoculate the liquid medium containing 1g KH_2PO_4, 0.5g K_2HPO_4, 10g corn steep liquor, 0.5g $MgSO_4$, 2g $NaNO_3$, 0.5g KCl, 0.02g $FeSO_4$ and 30g glucose per liter of distilled water. Cultures were grown in erlenmeyer flasks at 27°C on a rotary shaker (200 rpm). In a typical experiment, the two days grown mycelium of *C. radicicola* is recovered by filtration and incubated in distilled water containing the substrate at the desired concentration. The incubation was conducted at 27°C and 200rpm. Samples of the incubation medium are analyzed by HPLC or GC to determine the reaction rate. To stop the reaction, the medium was filtered and immediatly extracted with methylene chloride. The extract was then concentrated and the residual amine easily separated from the amide derivative by flash column chromatography (silica gel; ethyl acetate/heptane 8/2) . GC analysis was carried out on a ST1 capillary column operated from 60 to 290°C (10°C/mn) with FID detection and helium as carrier gas (60 Kpa). HPLC analysis was carried out on a C18 column (NOVA-PAK, 5µm, 100 x 45 mm); the eluent used is (30/67/3 , MeOH/H_2O/MeCOOH) and UV detection made at 250nm.