Biocatalysis in Organic Synthesis. 9. Highly Enantioselective Kinetic Resolution of Secondary Alcohols Catalyzed by Acylase.[1]

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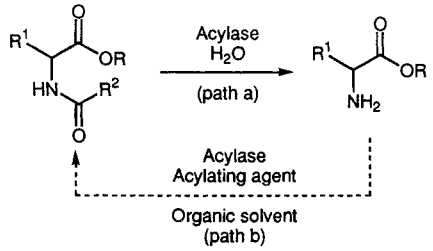
Abstract: A new catalytic activity of the enzyme acylase I (AA-I) from *Aspergillus* species has been found. Although this enzyme had previously been used only in the hydrolysis of N-acylamino acids, we have found that it is a highly efficient catalyst for transesterifications using vinyl esters as acyl donors. The method has been applied to the kinetic resolution of a variety of secondary alcohols.

Enzyme-mediated processes are becoming increasingly important technologies for the selective synthesis of organic compounds,[2] especially in enantiomerically pure form (EPC-synthesis[3]). An enzymatic method must satisfy several criteria to be useful in organic synthesis: a) the enzyme must be readily available and stable, b) the enzyme must accept a broad range of compounds as substrate, c) the transformations must be highly selective and prone to be modified by changes in the experimental conditions.[4] One of the future goals of this field is to find new synthetic applications of readily available enzymes.

In connection with our work on the synthetic applications of biocatalysts,[1,4] we have investigated the possibility of finding a novel reaction catalyzed by the enzyme acylase I (N-acylamino acid amide hydrolase, E. C. 3.5.1.14) from *Aspergillus species* (AA-I hereafter). It is an inexpensive enzyme which has been under-exploited in organic chemistry. The only previous use of this enzyme was the hydrolysis of the amide bond in N-acylamino acids (Scheme 1, path a).[5] At the outset of our work on the synthetic applications of acylases, we wondered if the reverse of this reaction (Scheme 1, path b) would be feasible, provided that a low water-content solvent and an acylating agent are present.



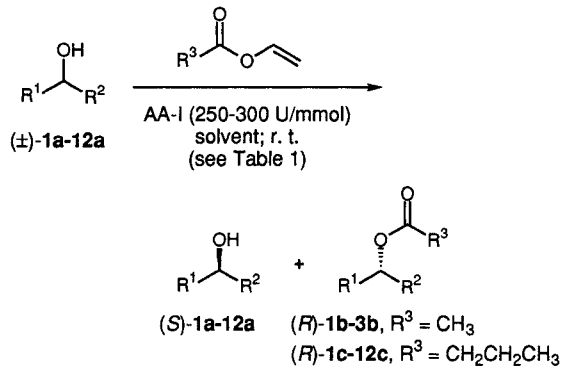
Scheme 1

Our first results on acylations catalyzed by AA-I were quite promising, demonstrating that AA-I was a catalyst for the acylation of alcohols and amines.[6] While the regioselectivity of the esterification of polyols and the chemoselectivity of the acylation of amino alcohols were from good to excellent,[6] the enantioselectivity of the kinetic resolution of racemic primary alcohols was from modest to good, and strongly dependent on remote substitution.[1a]

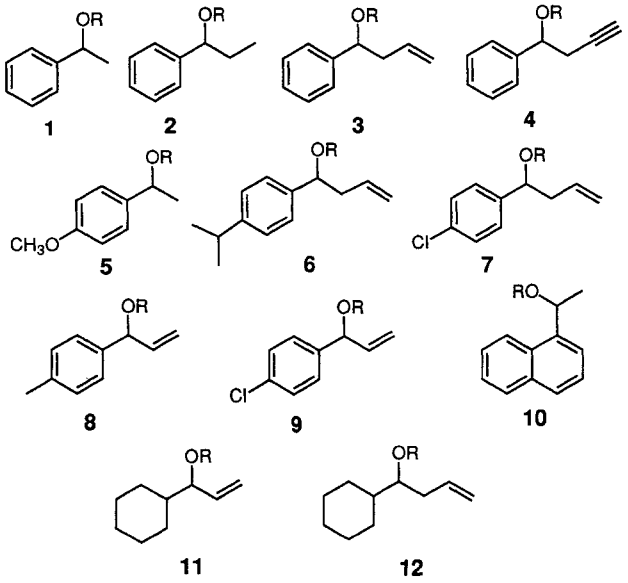
Continuing our investigation of this new reaction catalyzed by AA-I, we have carried out the kinetic resolution of a variety of racemic secondary alcohols using vinyl acetate and vinyl butyrate as acylating reagents (Scheme 2 and Table 1). The efficiency of the resolution has been calibrated by the values of the enantioselectivity E, as defined by Sih.[7] The substrates (±)-**1a-12a** presents a broad structural diversity (Chart 1). *The esterifications of all the substrates proceeds with good to excellent enantioselectivities*, giving the (*S*)-alcohols **1a-12a** and the (*R*)-esters either **1b-3b** or **1c-12c**, depending on the nature of the acylating agent.[9] This method compares favourably with other chemical or biocatalytic syntheses of the same or similar substrates.[10]

Several additional features deserve comment:

1) The acetylation of (±)-**1a** was carried out in a variety of solvents (Table 1, entries 1-8), ranging from hydrophobic and apolar, such as cyclohexane (entry 1) to polar and hydrophilic, such as acetone (entry 7) and acetonitrile (entry 8). It is worth mentioning that AA-I displays high



Scheme 2



stability and activity in polar solvents, a characteristic not shared for many enzymes.

2) It was found that both the velocity and the enantioselectivity of the esterifications of (±)-**1a**, (±)-**2a**, and (±)-**3a** increased considerably on using vinyl butyrate as acylating agent in dry toluene (compare entries 2 and 9, 10 and 11, and 12 and 13). In order to study the influence of the structure of the substrate on the selectivity, the rest of the reactions have been carried out using vinyl butyrate in dry toluene.

3) The influence of the nature of the side chain of the substrate on the enantioselectivity of the kinetic resolution was examined in the series α-methyl [(±)-**1a**, entry 9], α-ethyl [(±)-**2a**, entry 11], α-allyl [(±)-**3a**, entry 13], and α-propargyl [(±)-**4a**, entry 14] benzylalcohols. Although all the reactions were highly selective, the best substrates were those with shorter side chains (entries 9 and 11). The structure of the side chain had hardly any effect on the velocity of the reaction.

Table 1. Results of the kinetic resolution of **(±)-1a-12a** catalyzed by AA-I (Scheme 2 and Chart 1).[a)]

Entry	Starting material	Acylating agent (amount)[b)]	Solvent[c)]	Time (h)	%c[d)]	Ester %ee (%y) [e)]	Alcohol %ee (%y) [e)]	E[d)]
1	**(±)-1a**	Vinyl acetate (2.5)	Cyclohexane	71	53	(+)-(*R*)-**1b** 87 (30)	(-)-(*S*)-**1a** >99 (30)	75
2	**(±)-1a**	Vinyl acetate (2.5)	Toluene	41	50	(+)-(*R*)-**1b** 94 (48)	(-)-(*S*)-**1a** 94 (45)	>100 (115)
3	**(±)-1a**	Vinyl acetate (2.5)	CH_2Cl_2	42	50	(+)-(*R*)-**1b** 94 (48)	(-)-(*S*)-**1a** 93 (42)	>100 (115)
4	**(±)-1a**	Vinyl acetate (2.5)	$(^iPr)_2O$	71	54	(+)-(*R*)-**1b** 85 (33)	(-)-(*S*)-**1a** >99.5 (32)	>85
5	**(±)-1a**	Vinyl acetate (2.5)	^{t}BuOMe	73	55	(+)-(*R*)-**1b** 82 (48)	(-)-(*S*)-**1a** >99.5 (39)	>76
6	**(±)-1a**	Vinyl acetate (100)	Vinyl acetate	42	52	(+)-(*R*)-**1b** 90 (41)	(-)-(*S*)-**1a** >99.5 (38)	>100 (115)
7	**(±)-1a**	Vinyl acetate (2.5)	Acetone	72	51	(+)-(*R*)-**1b** 96 (42)	(-)-(*S*)-**1a** >99.5 (38)	>100 (336)
8	**(±)-1a**	Vinyl acetate (2.5)	Acetonitrile	42	50	(+)-(*R*)-**1b** 97 (40)	(-)-(*S*)-**1a** >98.5 (39)	>100 (374)
9	**(±)-1a**	Vinyl butyrate (1.0)	Toluene	25	50	(+)-(*R*)-**1c** 95 (45)	(-)-(*S*)-**1a** 95 (45)	>100 (177)
10	**(±)-2a**	Vinyl acetate (2.5)	Toluene	44	47	(+)-(*R*)-**2b** 97 (44)	(-)-(*S*)-**2a** 86 (44)	>100 (153)
11	**(±)-2a**	Vinyl butyrate (1.0)	Toluene	20	50	(+)-(*R*)-**2c** >97.5 (40)	(-)-(*S*)-**2a** 96 (38)	>100 (328)
12	**(±)-3a**	Vinyl acetate (1.5)	Toluene	177	34	(+)-(*R*)-**3b** 93 (25)	(-)-(*S*)-**3a** 48 (52)	42
13	**(±)-3a**	Vinyl butyrate (1.0)	Toluene	24	45	(+)-(*R*)-**3c** 94 (32)	(-)-(*S*)-**3a** 76 (42)	70
14	**(±)-4a**	Vinyl butyrate (1.0)	Toluene	23	51	(+)-(*R*)-**4c** 92 (46)	(-)-(*S*)-**4a** 95 (39)	89
15	**(±)-5a**	Vinyl butyrate (1.0)	Toluene	19	51	(+)-(*R*)-**5c** 95 (43)	(-)-(*S*)-**5a** 98 (42)	>100 (198)
16	**(±)-6a**	Vinyl butyrate (1.0)	Toluene	22	42	(+)-(*R*)-**6c** 97 (41)	(-)-(*S*)-**6a** 71 (46)	>100 (145)
17	**(±)-7a**	Vinyl butyrate (1.0)	Toluene	28	49	(+)-(*R*)-**7c** 85 (38)	(-)-(*S*)-**7a** 82 (38)	31
18	**(±)-8a**	Vinyl butyrate (1.0)	Toluene	8	51	(+)-(*R*)-**8c** 96 (38)	(-)-(*S*)-**8a** 99 (43)	>100 (224)
19	**(±)-9a**	Vinyl butyrate (1.0)	Toluene	6	46	(+)-(*R*)-**9c** 96 (35)	(-)-(*S*)-**9a** 82 (46)	>100 (117)
20	**(±)-10a**	Vinyl butyrate (1.0)	Toluene	139	30	(+)-(*R*)-**10c** 95 (30)	(-)-(*S*)-**10a** 41 (65)	55
21	**(±)-11a**	Vinyl butyrate (1.0)	Toluene	20	40	(+)-(*R*)-**11c** 94 (25)	(-)-(*S*)-**11a** 64 (42)	62
22	**(±)-12a**	Vinyl butyrate (1.0)	Toluene	183	21	(-)-(*R*)-**12c** 96 (19)	(-)-(*S*)-**12a** 26 (54)	69

a) AA-I is the enzyme Acylase I from *Aspergillus* species purchased from Aldrich or Sigma. Its specific activity is 0.5 U/mg (as defined in Sigma catalog: one unit hydrolyze 1.0 mmol of N-acetyl-L-methionine per hr at pH 7.0 at 25°C). The resolutions of substrates (±)-**1a**, (±)-**2a**, (±)-**5a**, and (±)-**10a** were carried out using 250 units of AA-I per mmol of racemic substrate. In the other resolutions, 300 units of AA-I per mmol of racemic alcohol were used. b) The amount refers to mmol of acylating agent per mmol of racemic alcohol. c) All the solvents were of the highest quality available (water content < 0.5%) kept over molecular sieves. d) The conversion degree c and the enantioselectivity E were calculated according to ref 7. e) All the yields refers to isolated yields after flash-chromatography. The enantiomeric excesses of all the alcohols (**1-12a**), the acetates **1b** and **2b** and the butyrates **1c**, **2c** and **5c** were determined directly by capillary gas-liquid chromatography. The enantiomeric excesses of the remainder esters were determined after methanolysis to the alcohol (K_2CO_3/MeOH). In all the cases a cyclodextrin-based chiral stationary phase was used (ref 8).

4) The influence of the substituent on the aromatic ring was briefly studied in the series of the α-methyl [(±)-**5a**], α-allyl [(±)-**6a** and (±)-**7a**], and α-vinyl [(±)-**8a** and (±)-**9a**] benzylalcohols. The enantioselectivity of the butyrylation of the *p*-methoxy substituted benzylic alcohol (±)-**5a** (entry 15) was slighty better than the unsubstituted analog (entry 9). A remarkable influence of the substituent on the selectivity of the reaction of the α-allyl substituted derivatives was observed; thus, while the *p-iso*-propyl derivative (±)-**6a** (entry 16) was acylated more enantioselectively than the unsubstituted analogue (±)-**3a** (entry 13), the *p*-chloro derivative (±)-**7a** (entry 17) reacted with lower selectivity than (±)-**3a**. The enantioselectivities of the acylations of the allylic alcohols (±)-**8a** and (±)-**9a** were very high (entries 18 and 19); and the influence of the substituent was also noted: the *p*-methyl substituted derivative (±)-**8a** reacted more enantioselectively than the *p*-chloro analog (±)-**9a**. Although more experiments are needed, these results seem to indicate an electronic effect on the selectivity of this kind of reaction, an effect rarely invoked in enzyme-mediated processes.[11]

5) The nature of the aromatic ring was also analyzed. The AA-I catalyzed butyrylation of the α-naphthyl derivative (±)-**10a** (entry 20) was slower[12] and less enantioselective than those of the phenyl derivative (±)-**1a** (entry 9).

6) Finally, to test the generality of the method, the transesterification catalyzed by AA-I was applied to functionalized non-aromatic alcohols [compounds (±)-**11a** and (±)-**12a**; entries 21 and 22]. These two aliphatic alcohols were kinetically resolved with good selectivity, and although the reaction of the homoallylic alcohol (±)-**12a** was slow, this inconvenience may be overcome by using a larger quantity of AA-I.

It is worth remarking that some of the chiral compounds reported in the present paper are useful as chiral auxiliaries for asymmetric synthesis[13] and chemical resolution,[14] as well as chiral building blocks for the synthesis of pharmacologically active compounds and natural products.[15] Especially important are the olefinic products; for instance, the homoallylic alcohols are precursors of chiral β-hydroxy carbonyl compounds[16] and γ- and δ-lactones,[17] which are ubiquitous structural features in natural products. It is also interesting that the kinetic resolution of the allylic alcohols is highly enantioselective and it might constitute an alternative to the Sharpless' kinetic resolution.[18]

Summarizing, the enzyme AA-I catalyzes the highly enantioselective acylation of a variety of secondary alcohols, providing compounds with high enantiomeric purities. This method satisfies all the requirements pointed out above for a useful biocatalytic method. Besides the synthetic usefulness of AA-I, the fact that AA-I is able to catalyze this "unnatural" reaction is interesting from a bioorganic point of view, posing questions on the mechanism of this novel biocatalytic reaction.[19]

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(7) (a) Sih, C. J.; Wu, S. H. *Topics Stereochem.* **1989**, *19*, 63-125. (b) The figures into brackets indicate the actual values of E calculated according to ref 7a. Values of E >100 should be judged with care because they are sensitive to small errors in measurements of enantiomeric excesses.

(8) These stationary phases have been prepared by Mrs. M[a] Isabel Jiménez (Instituto de Química Orgánica General, C. S. I. C.), to whom we thank for her assistance on the glc analysis.

(9) (a) The substrates not commercially available have been prepared from the corresponding aldehyde by reaction with either allyl bromide/Zn, or propargyl bromide/Zn, or vinylmagnesium bromide. (b) The absolute configurations of the products of the reactions have been assigned by comparing the sign of the optical rotation with commercial materials or compounds reported in the literature.[10] The absolute configurations of the non-reported chiral compounds (**6**, **8**, and **9**) have been assumed as indicated in Table 1 by analogy with the results of the other transformations. (c) All the new compounds gave satisfactory analytical (C, H ^{1}H- and ^{13}C-NMR, MS, and IR) data. The optical rotations of all the compounds are as follows: (-)-(*S*)-**1a** (of >99.5% ee): $[\alpha]_D$= -45.0 (MeOH, c = 1.0); (+)-(*R*)-**1b** (of 97% ee): $[\alpha]_D$= +105.1 ($CHCl_3$, c = 1.3); (+)-(*R*)-**1c** (of 95% ee): $[\alpha]_D$= +81.7 ($CHCl_3$, c = 1.2); (-)-(*S*)-**2a** (of 96% ee): $[\alpha]_D$= -27.9 (MeOH, c = 1.2); (+)-(*R*)-**2b** (of 97% ee): $[\alpha]_D$= +100.3 ($CHCl_3$, c = 1.6); (+)-(*R*)-**2c** (of >97.5% ee): $[\alpha]_D$= +82.8 ($CHCl_3$, c = 1.0); (-)-(*S*)-**3a** (of 83% ee): $[\alpha]_D$= -28.5 ($CHCl_3$, c = 1.2); (+)-(*R*)-**3b** (of 93% ee): $[\alpha]_D$= +30.4 ($CHCl_3$, c = 1.2); (+)-(*R*)-**3c** (of 94% ee): $[\alpha]_D$= +61.8 ($CHCl_3$, c = 0.7); (-)-(*S*)-**4a** (of 95% ee): $[\alpha]_D$= -50.8 ($CHCl_3$, c = 0.8); (+)-(*R*)-**4c** (of 92% ee): $[\alpha]_D$= +26.3 ($CHCl_3$, c = 0.8); (-)-(*S*)-**5a** (of 98% ee): $[\alpha]_D$= -34.2 ($CHCl_3$, c = 2.4); (+)-(*R*)-**5c** (of 95% ee): $[\alpha]_D$= +98.1 ($CHCl_3$, c = 1.0); (-)-(*S*)-**6a** (of 71% ee): $[\alpha]_D$= -25.6 ($CHCl_3$, c = 1.6); (+)-(*R*)-**6c** (of 97% ee): $[\alpha]_D$= +67.2 ($CHCl_3$, c = 0.9); (-)-(*S*)-**7a** (of 82% ee): $[\alpha]_D$= -29.4 ($CHCl_3$, c = 1.4); (+)-(*R*)-**7c** (of 85% ee): $[\alpha]_D$= +52.2 ($CHCl_3$, c = 0.9); (-)-(*S*)-**8a** (of 99% ee): $[\alpha]_D$= -3.8 ($CHCl_3$, c = 0.8); (+)-(*R*)-**8c** (of 96% ee): $[\alpha]_D$= +58.2 ($CHCl_3$, c = 0.9); (-)-(*S*)-**9a** (of 82% ee): $[\alpha]_D$= -13.2 ($CHCl_3$, c = 0.6); (+)-(*R*)-**9c** (of 96% ee): $[\alpha]_D$= +48.6 ($CHCl_3$, c = 1.0); (-)-(*S*)-**10a** (of 41% ee): $[\alpha]_D$= -33.7 ($CHCl_3$, c = 1.3); (+)-(*R*)-**10c** (of 95% ee): $[\alpha]_D$= +38.0 ($CHCl_3$, c = 0.9); (-)-(*S*)-**11a** (of 64% ee): $[\alpha]_D$= -4.8 ($CHCl_3$, c = 0.4); (+)-(*R*)-**11c** (of 94% ee): $[\alpha]_D$= +2.3 ($CHCl_3$, c = 1.5); (-)-(*S*)-**12a** (of 26% ee): $[\alpha]_D$= -0.9 ($CHCl_3$, c = 0.9); (-)-(*R*)-**12c** (of 96% ee): $[\alpha]_D$= -17.6 ($CHCl_3$, c = 0.5).

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(19) Although the mechanism of the acylations catalyzed by AA-I might be the reverse to the hydrolysis of N-acylamino acids (i. e., promoted by a metallic ion),[5] we hypothesize that the enzyme possesses a second active centre with esterase-like activity, which is responsible of the acylation reaction (i. e., similar to a serine protease). Work is in progress in order to understand the mechanism of this new reaction.