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Study of the resolution of amino acids and aminoalcohols in organic solvents

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Summary. The enzymatic resolution of racemic phenylglycine, phenylglycinol and phenylalaninol has been studied in organic solvents under a variety of experimental conditions. Subtilisin in 3-methyl-3-pentanol was effective for the resolution of phenylglycine esters, via N-acylation with trifluoroethyl butyrate. Porcine pancreatic lipase in ethyl acetate gave satisfactory results in the resolution of phenylglycinol and phenylalaninol; the α or β position of the phenyl group was found to influence both the rate and the chemioselectivity of the reaction.

Keywords: Amino acids – Aryl amino acids – Lipases – Subtilisin – Asymmetric resolution of amino acids and aminoalcohols in organic solvents

Introduction

As a part of our research on chiral auxiliares for asymmetric syntheses, we became interested in the resolution of racemic phenyl-substituted amino acids and aminoalcohols. The most common way to obtain optically pure amino acids is the separation of synthetically prepared racemic mixtures by chemical methods [e.g. fractional crystallization of diastereoisomeric mixtures (Barrett, 1985; Blaser, 1991) or chromatography on chiral stationary phases (Pirkle and Pochapsky, 1989)]. Recently, the preparation of optically pure amino acids on an intermediate or large scale has made use of enzymes. Examples are the hydrolysis of esters of N-protected amino acids by lipases/esterases and the cleavage of the amidic bond of N-acyl amino acids by proteases (Svedas and Galaev, 1983; Boland et al., 1991; Williams and Hendrix, 1992). Lipases (Miyazawa et al., 1989), aminopeptidases (Meijer et al., 1985), and hydanthoinases (Meijer et al., 1985) have also proven effective for the resolution of arylglycines. Enzymatic reactions were initially carried out in aqueous or aqueous-organic biphasic systems; however, during the last decade purely organic solvents have been explored and were found to be both successful and more convenient for many biotransformations (Klibanov, 1990).

Herein we report on the enzymatic resolution in organic solvents of racemic phenylglycine, the D-enantiomer of which is an important precursor for semisynthetic penicillins and cefalosporins. The resolution of racemic phenylglycinol and phenylalaninol was also investigated for comparison.

Materials and methods

Optical rotation was measured on a Perkin-Elmer 241 Polarimeter. ^1H and ^{13}C NMR spectra were obtained on a Varian FT 200 spectrometer (200 MHz) using $CDCl_3$ solutions with TMS as internal standard. Thin-layer chromatographic analysis (TLC) was carried out using silica gel 60 F_{254} plates (Merck).

Enzymes

Porcine pancreatic lipase and subtilisin Carlsberg (protease from Bacillus subtilis – subtilopeptidase A) were obtained from Sigma; the lipases from Pseudomonas fluorescens and from Candida cylindracea were obtained from Fluka. Subtilisin was liophilized from a buffered aqueous solution (pH = 7.5) to enhance its activity (Zacks and Klibanov, 1986) prior to use in the organic solvent.

Chemicals

All organic solvents were of analytical grade and were dried prior to use. Pyridine was refluxed over potassium hydroxide pellets (12 hrs), distilled, refluxed over calcium hydride, and distilled again before use. Ethyl acetate was distilled from phosphorus pentoxide. Tert-butyl alcohol and 3-methyl-3-pentanol were distilled from sodium wire. Phenylglycine (Aldrich), phenylglycinol and phenylalaninol (Fluka) were used without further purification. Phenylglicine methyl ester was prepared as described by Brook and Chan (1983); the trichloro- and trifluoroethyl butyrates were prepared according to Marinier et al. (1973).

The optically pure reference compounds N-acetyl-(S)-phenylglycinol, N,O-diacetyl-(S)-phenylglycinol, N-acetyl-(R)-phenylalaninol and N,O-di-acetyl-(R)-phenylalaninol, were prepared from (S)-phenylglicinol and (R)-phenylalaninol, respectively, by chemical acetylation with acetic anhydride in dry pyridine.

N-acetyl-(S)-phenylglycinol: $[\alpha]_D = +105°$, c = 41 mg/mL in methanol; ^1H NMR: 7.4-7.2 (5H), 6.5 (1H, d, J = 4, disappears upon exchange with D_2O), 5.0 (1H, dt, J = 5.3, 5.3, 4), 3.8 (2H, d, J = 5.3), 2.65 (1H, s, disappears upon exchange with D_2O)}, 2.0 (3H, s).

N,O-diacetyl-(S)-phenylglycinol: $[\alpha]_D = +71.5°$, c = 10.35 mg/mL in methanol; ^1H NMR: 7.4-7.2 (5H), 6.2 (1H, d, J = 7.5, disappears upon exchange with D_2O), 5.2 (1H, ddd, J = 7.5, 7.1, 5.5), 4.4 and 4.2 (2H, ABX system, $J_{AB} = 11.5$, $J_{AX} = 7.1$, $J_{BX} = 5.5$), 2.08 (3H, s), 2.0 (3H, s).

N-acetyl-(R)-phenylalaninol: $[\alpha]_D = +16.4°$, c = 41 mg/mL in methanol; ^1H NMR: 7.4-7.2 (5H), 6.4 (1H, d, J = 5.5, disappears upon exchange with D_2O), 4.1 (1H, m), 3.8 (1H, s, disappears upon exchange with D_2O), 3.5 (2H, m), 2.8 (2H, d, J = 4.4), 2.0 (3H, s).

N,O-di-acetyl-(R)-phenylalaninol: $[\alpha]_D = +10.7°$, c = 9.3 mg/mL in methanol; ^1H NMR: 7.4-7.2 (5H), 5.7 (1H, d, J = 5.5, disappears upon exchange with D_2O), 4.4 (1H, m), 4.0 (2H, d, J = 4.4), 2.8 (2H, d, J = 6.6), 2.1 (3H, s), 1.9 (3H, s).

N-Butyryl-(R)-phenylglycine methyl ester was prepared by reaction of (R)-phenylglycine methyl ester ($[\alpha]_D = -146$, c = 49 mg/mL in chloroform) (0.456 g, 2.76 mmol) with butyryl chloride (0.34 ml, 3.3 mmol) in dry methylene chloride (3 ml) at 0°C in the presence of dry triethylamine: $[\alpha]_D = -136°$, c = 90 mg/mL in chloroform; ^1H NMR: 7.4-7.0 (5H), 6.45 (1H, d, J = 5.2, disappears upon exchange with D_2O), 5.6 (1H, d, J = 5.2), 3.7 (3H, s), 2.2 (2H, t, J = 7.5), 1.6 (2H, tq, J = 7.5), 0.9, 3H, t, J = 7.5).

The optical purity of reference compounds was verified by ^{1}H NMR spectra taken in the presence of a chiral europium(III) shift reagent (tris [3(heptafluoropropylhydroxymethylene)-d-camphorato]-europium(III), Fluka).

The identity of the enzymatic reaction products was confirmed by TLC and ^{1}H NMR, and by comparison with authentic samples. The optical purity and the enantiomeric excesses were determined by comparison of $[\alpha]_D$ with that of authentic samples.

Typical procedures for the enzymatic reactions

a) *With lipases.* Porcine pancreatic lipase (0.5 g) and celite (0.5 g) were added with vigorous stirring to a solution of racemic phenylglycinol (0.274 g, 2 mmol) in ethyl acetate (4 ml) at room temperature. The reaction was monitored by thin-layer chromatography (silica gel, ethyl acetate/ethanol 9/1). Stirring was continued for 24 hrs ($\approx$ 50% conversion). Flash chromatography of the residue (silica gel, ethyl acetate/ethanol 9/1) afforded (R)-phenyl glycinol (97 mg, 0.71 mmol, 36% yield), 13% ee and of N-acetyl-(S)-phenylglycinol (68 mg, 0.38 mmol, 19% yield), 38% ee.

b) *With freshly activated subtilisin.* A solution of racemic phenylglycine methyl ester (0.74 g, 4.48 mmol) in dry 3-methyl-3-pentanol (4 ml) was warmed at 30°C and then trifluoroethylbutyrate (1.46 ml), freshly activated subtilisin (0.3 g) and celite (0.3 g) were added with vigorous stirring. The reaction was monitored by thin-layer chromatography (silica gel, ethylacetate/n-hexane 4/6). When conversion had reached 50% (22 hrs), the mixture was filtered and the solvent was evaporated under vacuum. Flash-chromatography of the residue (Still, 1978) on silica gel, eluting with ethyl acetate/n-hexane 4/6, afforded (R)-phenylglycine methyl ester (0.237 mg, 1.44 mmol, 32% yield), 16% ee and N-butyryl-(S)-phenylglycine methyl ester (0.347 mg, 1.57 mmol, 35% yield), 65% ee. No appreciable reaction was detected in the absence of the enzyme.

Results and discussion

Lipases were the first enzymes investigated in this work because of their low cost, convenience and reported versatility (Kirchner et al., 1985). These enzymes are known to successfully catalyze, via N- and O-acylation, the kinetic resolution of racemic alcohols and racemic aminoalcohols (Francalanci et al., 1987; Gotor et al., 1988; Fernandez et al., 1992), and indeed we found that lipases in organic solvents promoted the enantioselective acylation of phenylglycinol and phenylalaninol. The best results were obtained with porcine pancreatic lipase and are summarized in Table 1. The rate of the reaction was higher for phenylalaninol, which gave (S)-mono- and (R)-diacylated products. With phenylglycinol, in contrast, the diacylated product was observed only in traces by thin-layer chromatographic analysis. Thus, the position (α or β) of the phenyl group relative to the -NH_2 function influences both the rate and the chemioselectivity of the reaction. It should be noted that a reverse enantioselectivity was reported (Gotor et al., 1988) for the lipase-catalyzed acylation of aliphatic-only aminoalcohols (e.g. 2-amino-1-butanol gave (R)-mono-acylated and (S)-di-acylated products). No enantioselectivity was observed in our work using lipases for the enzymatic acylation of racemic phenylglycine. The following sets of conditions were explored. a) The acylating reagents were varied from simple ethyl acetate to the highly activated trichloroethyl acetate and trifluoroethyl butyrate. b) The reaction me-

Table 1. N-Acylation of (R,S)-phenylglycinol and (R,S)-phenylalaninol[a]

Substrate	Reaction time, h	Isolated yields (%)		ee (%)	
		Monoacylated	Diacylated	Monoac.	Diac.
(R,S)-Phenyl glycinol	48	5 (S)		14	
(R,S)-Phenyl glycinol[b]	25	19 (S)		38	
(R,S)-Phenyl alaninol	27	36 (S)	13 (R)	39	45

[a] The reactions were performed at 30°C in ethyl acetate, in the presence of porcine pancreatic lipase (enzyme/substrate ratio 1/1 by weight).
[b] The enzyme was supported on celite.

Table 2. N-Acylation of R,S-phenylglycine methyl ester

Acylating reagent	Enzyme	Solvent	Reactn. time, h	N-acylated Isol. yields (S)	ee %	Enzyme/ substrate w/w
EtOAc[a]	PPL	EtOAc	17	31	0	1
EtOAc	PPL	t-BuOH	72	6	0	1
EtOAc	PPL	3-Me-3-pentOH	72	8	0	1
TFEB[b]	PPL	Py	40	50	0	0.3
Vinyl acetate[c]	PPL	Toluene	1	31	0	1
TCEB[d]	PPL	t-BuOH	91	57	0	1.9
TCEB	PFL	3-Me-3-pentOH	43	40	0	0.5
TCEB	subtilisin	3-Me-3-pentOH	72[e]	36	0	0.2
TFEB	subtilisin	3-Me-3-pentOH	72	37	30	0.2
TFEB	subtilisin	3-Me-3-pentOH	71	35	56[f]	0.4
TFEB	subtilisin	3-Me-3-pentOH	22	35	65[g]	0.4

[a] The ethyl, n-butyl and t-butyl esters of R,S-phenyl glycine similarly showed no enantioselectivity. [b] Trifluoroethyl butyrate. [c] Vinyl acetate can not be used with subtilisin as the chemical acylation becomes competitive. [d] Trichloroethyl butyrate. [e] For reaction times >70 h, chemical acylation was observed. [f] The enzyme was dissolved in water containing 0.1 M phosphate, the solution was adjusted to pH = 7.8 and freeze-dried. [g] Prior to use the enzyme was activated (see the experimental) and supported on celite (enzyme/celite: 1/1 by weight).

dium was varied from a hydrocarbon (toluene) to a polar solvent (ethyl acetate, tetrahydrofuran and pyridine). c) The catalyst was obtained from different sources (Candida cylindracea, Porcine pancreas and Pseudomonas fluorescens). d) Alkoxy residues with increasing steric requirements (R = Et, n-But, t-But) were employed. The results of these experiments are summarized in Table 2 for the N-acylation only*.

* All attempts to enantioselectively transform the COOH group by either direct esterification or transesterification in organic solvents failed both with lipases and with subtilisin, and are not included in the Table.

Proteases are known to catalyze the synthesis of peptides (Schutt, 1985); accordingly, subtilisin was investigated as a possible catalyst for the resolution of phenylglycine methyl ester. The conditions of choice were trifluoroethyl butyrate as the acylating reagent and 3-methyl-3-pentanol as the solvent. Activation of the enzyme by "pH adjustment" (Zacks and Klibanov, 1986) and the use of celite as an enzyme support increased both the rate of reaction and the enantioselectivity (from 30% to 65%).

To determine whether the enantiomeric excesses obtained ($\approx$ 65%) could result from partial racemization during reaction, the (R)- and (S)-phenyl glycine methyl esters were treated separately with trifluoroethylbutyrate and activated subtilisin in 3-methyl-3-pentanol, as described above for the racemic phenylglycine methyl ester. No racemization was observed for the (S)-enantiomer; the acylated product, recovered in 62% yields, was enantiomerically pure. The acylated product from the (R)-isomer, recovered in 51% yield, showed instead partial racemization (ee 60%).

In order to determine at which step in the overall reaction the racemization of the (R)-enantiomer had occurred, the methyl esters of both (R)-phenylglycine and of N-butyryl (R)-phenylglycine were treated with the enzyme in absence of the acylating reagent. In this experiment the recovered methyl ester of N-butyryl-(R)-phenylglycine showed no racemization, whereas that of (R)-phenylglycine had a low optical activity.

Thus, it can be concluded that in the presence of subtilisin the acylation of both the (R)- and (S)-enantiomers of phenylglycine methyl ester can occur, with the latter being kinetically favoured. The racemization of the (R)-phenyl glycine methyl ester to the (S)-enantiomer, which is then preferentially acylated, could be an advantageous reaction were it not for the comparable rate of conversion of the enantiomers, which does not allow accumulation of products with higher enantiomeric excesses.

Finally, it should be mentioned that no appreciable acylation has been observed for both phenylglycinol and phenylalaninol in the presence of subtilisin. The enzymes investigated in this work therefore appear to be specific for each substrate – the lipases for aminoalcohols and subtilisin for amino acids.

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