Enzyme-Catalyzed Hydrolyses of *E*/*Z*-Diastereotopic and *E*/*Z*-Diastereomeric Esters. Affect on Selectivity by Reaction Media

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Received February 17, 1993 (Revised Manuscript Received May 26, 1993)

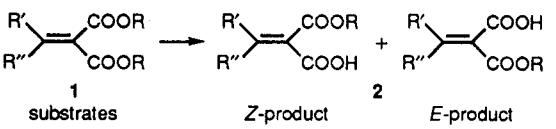
PLE-catalyzed hydrolyses of different types of *E*/*Z*-diastereotopic diesters and *E*/*Z*-diastereomeric monoesters have been studied. Arylidenepropanedioic diesters are specifically hydrolyzed to the *Z*-half esters, whereas the de values for dialkylated methylene propanedioic diesters range between 33 and 79% (*Z*). *D* values for the hydrolyses of the 3-methyleneazetidin-2-ones in detergent–buffer systems depend on the size of the substituent in the α-position. Diastereoselectivity of these substrates is affected by addition of the cosolvents acetonitrile and methanol.

The recent years have shown the increasing importance of enzymes as catalysts for asymmetric syntheses.[1] Stereoselective hydrolyses of prochiral diesters and racemic monoesters with PLE (PLE, EC 3.1.1.1) are well known,[2] but relatively few examples of diastereoselective transformations,[3] especially *E*/*Z*-selective reactions,[4] with this enzyme have been reported. Because of their different physical properties diastereomers can be separated more easily than enantiomers, but enzymatic hydrolyses may be the method of choice for transformations of labile molecules,[5] such as the esters of 3-methyleneazetidin-2-ones, which cannot be hydrolyzed by chemical methods. Since the hydrolyses of the β-lactams are referred to as a part of the development of new antibiotics, we investigated to what extent hydrolytic enzymes can differentiate between *E*/*Z*-diastereomeric ester groups.

Results

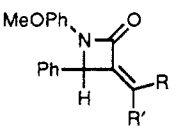
The diester and ester substrates were prepared by known methods. **1a–1g** (Figure 1) were synthesized by Knoevenagel reaction of the corresponding benzaldehydes[6d] or ketones,[6c] respectively, with diethyl or dimethyl malonate. Diesters **1h–1i** are commercially available (Aldrich). Peterson olefination of the C-3-silylated 1,4-diarylazetidin-2-one with α-keto esters led to the β-lactams **3a–3l** (Figure 2).[6a,b]

In our search for a hydrolase which can discriminate between diastereotopic ester groups, an enzyme screening with **1a** as a model substrate was carried out using different commercially available enzymes. The results are shown in Table I. Regarding selectivity and activity towards this substrate the best results were obtained with PLE.



a, R = Et, R′ = phenyl, R″ = H
b, R = Et, R′ = *p*-(dimethylamino)phenyl, R″ = H
c, R = Et, R′ = *p*-nitrophenyl, R″ = H
d, R = Et, R′ = Et, R″ = Me
e, R = Me, R′ = Et, R″ = Me
f, R = Me, R′ = propyl, R″ = Me
g, R = Et, R′ = pentyl, R″ = Me
h, R = Et, R′ = Me, R″ = H
i, R = Me, R′ = Me, R″ = H

Figure 1.



substrates 3	products 4
a, R = COOEt, R′ = COOEt	**a**, R = COOH, R′ = COOEt
b, R = COOEt, R′ = CH_3	**b**, R = COOH, R′ = CH_3
c, R = CH_3, R′ = COOEt	
d, R = COOMe, R′ = CH_3	**4b**
e, R = CH_3, R′ = COOMe	
f, R = COOEt, R′ = H	**f**, R = COOH, R′ = H
g, R = H, R′ = COOEt	
h, R = COOMe, R′ = H	**4f**
i, R = H, R′ = COOMe	**i**, R = H, R′ = COOH
k, R = COOMe, R′ = phenyl	**k**, R = COOH, R′ = phenyl
l, R = phenyl, R′ = COOMe	

Figure 2.

PLE-catalyzed hydrolyses of **1a–1c** were performed in 0.1 M phosphate buffer with or without addition of 10% acetone at pH 8 with the pH being maintained at this level by periodic addition of aqueous sodium hydroxide. Each reaction was terminated after the addition of 1 equiv of base, or the reactions stopped themselves and the acid ester products were isolated. PLE-catalyzed saponification specifically led to the *Z*-half esters independent from substitution in the para-position of the aryl ring and

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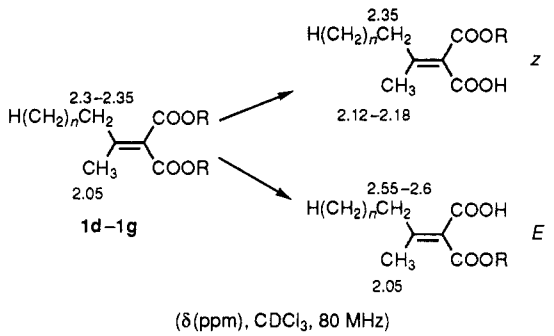

Figure 3.

Table I. Enzyme Screening of 1a

enzyme[a]	% c[b]	% de (Z)
without	no hydrolysis	
PLE	100	100
lipase from *Pseudomonas* sp. PsL	8	100
cholesterolesterase from *Pseudomonas* ChE	9	100
lipase from *Candida* cyl. CcL	58	62
lipase from *Aspergillus* nig. AnL	traces[c]	
lipase from *Rhizopus* arrh. RaL	36	60
α-chymotrypsin CTR	33	36
trypsin TR	13	100
subtilisin carlsb. STC	16	100
proteinase K	20	100
PPL	15	100
acetylcholinesterase AChE	traces[c]	

[a] Abbreviations taken from ref 1a. [b] After incubation of 3 d, at pH 7, 34 °C. [c] Traces of Z-product in TLC detectable. No hydrolyses took place with following enzymes: *Saccharomyces* cerev., lipase from *Pseudomonas* fl. Pf, Papain PP, Bromelain BL, Chymopapain CHP, Ficin FC, lipase from *Penicillium* roq. PrL.

Table II. PLE-Catalyzed Hydrolyses of 1a–1c in Different Media (Specific Activities (μmol min^{-1} mg^{-1})[a]

substrate	phosphate buffer	10% acetone
1a	3.4	5.2
1b	0.18	0.015
1c	0.07	0.16

[a] pH 8, 12.5 mM, 100 mg/L of PLE, 32 °C.

reaction conditions. Table II shows the initial rate velocities. In contrast to selectivity, we observed great differences in reaction velocities in different media.

In order to investigate if the selectivity depends on the size of the substituent in the β-position, we attempted to hydrolyze substrates **1h** and **1i** with a small substituent in this position. However, these molecules are not stable in water at pH 8,[7] so we chose substrates **1d–1g** as alternatives. Hydrolysis of these substrates no longer takes place specifically; the Z-half esters, however, are still the main products (Table III).[8]

Due to the very poor water solubility of the β-lactam esters **3a–3l**, reactions under the conditions described above did not work. These transformations could be carried out in detergent–buffer systems[9] or by lowering the buffer concentrations (0.05 M) and adding 10% acetonitrile or methanol. For determination of the initial rate velocities (Table IV), the diastereomeric monoesters of **3b–3l** were employed as single isomers after separation by column chromatography (silica gel 60, $CHCl_3$). The *D* values (Table V) were calculated in analogy to the *E* values for the enzymatic resolution of racemic monoesters[10] by $D = \ln([Z]/[Z_0])/\ln([E]/[E_0])$. Therefore, the hydrolyses of the mixture of the diastereomers of **3b–3l** were terminated before total conversion of the much better Z-substrates was reached. The Z-diastereomers **3b** and **3d** are hydrolyzed in detergent–buffer more than 100 times faster than the corresponding E-isomers **3c** and **3e**. The Z/E-ratio of reaction velocities for **3f/g** and **3h/i** is about 20–30. The E-isomer **3l** is not hydrolyzed at all. The diastereoselectivity is remarkably affected by addition of acetonitrile or methanol to the phosphate buffer. In these media, initial rate velocities of the mixtures are decreased, whereas those of the single E-isomers are increased. It should be noted that the hydrolyses of the E-isomers in all media stopped after 5–20% conversion. Only the product of **3i** could be isolated. Z_0/E_0-ratios, Z/E-ratios after hydrolyses, de values, and configurations were determined by ^{1}H-NMR. The conversions *c*, needed for

Table III. PLE-Catalyzed Hydrolyses of 1d–1g

substrate	% de (Z)[a]	substrate	% de (Z)[a]
1d	33–35	1f	79–80
1e	75–76	1g	36–37

[a] pH 8, 32 °C, 25 mM, 50 mg/L of PLE.

Table IV. PLE-Catalyzed Hydrolyses of 3a–3l in Different Media (Specific Activities (μmol min^{-1} mg^{-1}))

substrate	detergent–buffer[a]	10% ACN[b]	10% MeOH[b]
3a	0.07	0.017	0.016
3b	0.22		
3c	0.0022	0.062	
3b,c	0.18[c]	0.13	0.09
3d	0.2		
3e	0.0019	0.03	
3d,e	0.17[c]	0.08	0.04
3f	0.4		
3g	0.014	0.027	
3f,g	0.15[c]	0.038	0.048
3h	0.42		
3i	0.02		
3h,i	0.31[c]	0.12	0.05
3k	0.005		
3l	no hydrolysis		
3k,l	0.007[c]	0.004	0.01
1a	4	3	
1b	0.24	0.06	
1c	0.5	0.82	

[a] 12.5 mM, 100 mg/L of PLE, 0.13 M Nonidet P 40. [b] 10–20 mM, 50 mg/L of PLE. [c] 20–50 mM, 100 mg/L of PLE.

Table V. Hydrolyses of 3b–3l (*D* Values in Different Media)

substrate	detergent–buffer[a]	10% ACN[b]	10% MeOH[b]
3b,c	102	1.7	2
3d,e	186	27	4
3h,i	*c*	9	3.3
3k,l	*d*	77	60

[a] 20–50 mM, 100 mg/L of PLE, 0.05 M Triton X 100. [b] 10–20 mM, 50 mg/L of PLE. [c] Could not be determined because of too low initial concentration of Z-isomer. [d] E-isomer is not hydrolyzed.

(7) ^{1}H-NMR spectra show peaks for Z- and E-acid ester products of **2h** and **2i** in a ratio of $Z/E = 1/1$[7a] and as main product malonic acid half ester from retro-aldol reaction. The assignment of **2i** Z and E in ref 7a could be proven by 2D INEPT long-range experiments.[15] (a) Wentrup, C.; Lorencak, P. *J. Am. Chem. Soc.* **1988**, *110*, 1880–1883.

(8) The peaks in ^{1}H-NMR spectra for the methyl and methylene protons in the β-position were assigned to the Z- and E-isomers of **2d–2g** according to the principle that substituents cis-standing to the *acid* show a downfield shift and substituents cis-standing to the *ester* have nearly the same chemical shift as in the diesters (see Figure 3). This assignment could exemplarily be proved by NOE difference spectra of **2d** E/Z.

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the calculation[10] of the concentrations of Z and E ($[Z] + [E] = 1 - c$), were determined by the amount of base added.

Discussion

All enzymes that hydrolyze substrate **1a** prefer the ester group in the trans-position to the phenyl ring. It is interesting that only serine-proteases and not cysteine-proteases are able to transform this substrate. Diesters **1b** and **1c** are much poorer substrates for PLE, probably also due to their lower water solubility. In contrast to **1d–1g**, there is no difference in selectivity between methyl and ethyl esters, but differences occur in reaction velocity, depending on the medium.

In hydrolyses of **1d–1g**, selectivity could depend on chain length and on the alcohol part of the ester.[11]

The diester **3a** is specifically hydrolyzed to the E-half ester. This is in agreement with hydrolyses of **3d–3l** where the Z-isomers are the much better substrates for PLE. The D values in the system with Triton X 100 as detergent correspond to the ratio of Z/E-reaction velocities in the comparable Nonidet P 40-buffer. These reaction velocities seem to depend on the size of the substituent in the α-position and decrease with increasing size (H > CH_3 > COOEt > phenyl). The much lower D values in acetonitrile and methanol may be due to better activity toward the E-esters. But in these media, total conversion of the E-isomers could also not be reached. Hydrolyses of the mixture of the diastereomers under these conditions occur less rapidly than in the media with detergents used for improving the water solubility. The reason may be a poorer activity toward the Z-esters. No significant differences between ethyl and methyl esters were observed.

Experimental Section

Pig liver esterase (PLE EC 3.1.1.1, suspension in 3.2 M $(NH_4)_2SO_4$, activity 120 U/mg protein, ethyl butyrate), AnL, RaL, PrL were obtained from Fluka; all other enzymes were products from Sigma.

Enzyme Screening of 1a. **1a** (0.4 mmol) in 20 mL of phosphate buffer (pH 8) were incubated in a shaker at 34 °C with the following amounts of enzymes: PLE 1 mg, PsL 3.6 mg, ChE 30 mg, CcL 3.6 mg, AnL 50 mg, RaL 85 mg, CTR 90 mg, TR 100 mg, STC 25 mg, Proteinase K 10 mg, PPL 200 mg, AChE 5 mg, Yeast 10 g in 150 ml, PFL 10 mg, PP 150 mg, BL 170 mg, CHP 20 mg, FC 60 mg, PrL 50 mg. After 3 d the reactions were stopped by acidifying at pH 2 with 2 N HCl, and the suspensions were extracted with EtOEt (5 × 20 mL). The residues obtained after drying and evaporating were measured by ^{1}H-NMR spectroscopy to determine the diastereomeric excess (de) and the conversion.

PLE-Catalyzed Hydrolyses of the Esters and Diesters in Systems without Detergents. The following procedure is representative. PLE was added to a rapidly stirred suspension of the ester in 0.1 M phosphate buffer of pH 8 with or without addition of 10% acetone or 0.05 M phosphate buffer of pH 8 containing 10% ACN or MeOH at 32 °C. The pH was maintained at 8 by addition of 0.1 N aqueous NaOH. The reactions were allowed to proceed until the desired extent of hydrolyses, as determined by the volume of base added, had been achieved. The reaction mixture was washed with EtOEt (**1a**, **1c–1g**) or CH_2Cl_2 (**1b**) (5 × 20 mL) to remove unreacted diester and then acidified to pH 2 with 2 N HCl. Extraction with ether or methylene chloride (3–5 × 20 mL) followed by drying ($MgSO_4$) and evaporation yielded the acid esters **2a–2g**.

PLE-Catalyzed Hydrolyses in Detergent–Buffer Systems. The following procedure is representative. Phosphate buffer (0.1 M, pH 8) was added dropwise to a rapidly stirred suspension of ester in detergent. Then PLE was added, and the reaction was allowed to take place in the above-described manner. After termination of the reaction the mixture was acidified at pH 2 and extracted with methylene chloride (3 × 50 mL). The separation of the layers can be improved by centrifugation (7000 U/min, 5 min). The organic layers were extracted with saturated $NaHCO_3$ solution (8 × 30 mL) and the aqueous layers acidified with concentrated HCl and again extracted (3 × 30 mL) to yield the acid ester **4a** and the acids **4b**, **4f**, **4i**, and **4k** after washing the organic layer with water at pH 2 (5 × 30 mL), drying, and evaporation.

Determination of Specific Activities. The values result from the initial rate velocities.

Determination of D Values. The following procedure is representative. The mixtures of diastereomers are hydrolyzed in the above-described manner. The reactions are terminated after conversion of 10–80% depending on the Z_0/E_0-ratio before total hydrolysis of the Z-isomer has occurred (control by TLC, silica gel 60, cyclohexane/ethyl acetate/CHOOH = 49/49/2) by acidifying at pH 2 with 2 N HCl. The residue obtained after extraction with methylene chloride, drying, and evaporation is measured by ^{1}H-NMR spectroscopy to determine the resting Z/E-ratio. $[Z]$- and $[E]$-concentrations were calculated from $1 - c = [Z] + [E]$, conversion c deriving from the amount of base added. The D values were calculated by $D = \ln([Z]/[Z_0])/\ln([E]/[E_0])$.

(Z)-2-(Ethoxycarbonyl)-3-phenylprop-2-enoic acid (2a):[12] mp 87–89 °C; ^{1}H-NMR, $CDCl_3$, δ 1.26 (3 H, t, J = 7.1 Hz), 4.35 (2 H, q, J = 7.1 Hz), 7.4 (5 H, s), 7.93 (1 H, s), 11.6 (1 H, br s).

(Z)-2-(Ethoxycarbonyl)-3-(4-(dimethylamino)phenyl)-prop-2-enoic acid (2b):[13] mp 125–127 °C; ^{1}H-NMR, $CDCl_3$, δ 1.33 (3 H, t, J = 6.8 Hz), 3.05 (6 H, s), 4.36 (2 H, q, J = 6.8 Hz), 6.62 (2 H, d, J = 9 Hz), 7.43 (2 H, d, J = 9 Hz), 7.90 (1 H, s), 9.8 (1 H, br s); ^{13}C-NMR,[14] gated dec, $CDCl_3$, 100 MHz, δ 14.0 (q, J = 127 Hz), 40.0 (q, J = 126 Hz), 62.0 (t, J = 148 Hz), 111.5 (d, J = 159 Hz), 117.5 (s), 120 (s), 133 (d, J = 158 Hz), 147 (d, J = 154 Hz), 152.2 (s), 168 (dt, ^3J (CO, β-H) = 12.1 Hz, ^3J (CO, ester-H) = 3 Hz), 170 (d, ^3J = 7.6 Hz).

(Z)-2-(Ethoxycarbonyl)-3-(4-nitrophenyl)prop-2-enoic acid (2c):[13] mp 134–137 °C; ^{1}H-NMR $CDCl_3$, δ 1.25 (3 H, t, J = 6.8 Hz), 4.32 (2 H, q, J = 6.8 Hz), 7.61 (2 H, d, J = 8.3 Hz), 7.95 (1 H, s), 8.3 (2 H, d, J = 8.3 Hz), 8.8 (1 H, br s).

(Z/E)-2-(Ethoxycarbonyl)-3-methylpent-2-enoic acid (2d): ^{1}H-NMR, $CDCl_3$, δ 1.0–1.45 (6 H, m), 2.05 (s, E) and 2.18 (s, Z) (3 H), 2.35 (q, Z) and 2.55 (q, E) (2 H), 4.2 (2 H, q), 11.4 (1 H, br s); IR (film) 3320, 2981, 2941, 2880, 1732, 1700, 1629, 1404, 1368 cm^{-1}.

(Z/E)-2-(Methoxycarbonyl)-3-methylpent-2-enoic acid (2e): ^{1}H-NMR, $CDCl_3$, δ 1.1 (3 H, t), 2.05 (s, E) and 2.12 (s, Z) (3 H), 2.35 (q, Z) and 2.6 (q, E) (2 H), 3.7 (3 H, s), 11.0 (1 H, br s); IR (film) 3321, 2980–2880, 1739, 1702, 1630, 1434, 1406, 1377 cm^{-1}.

(Z/E)-2-(Methoxycarbonyl)-3-methylhex-2-enoic acid (2f): ^{1}H-NMR, $CDCl_3$, δ 0.95 (3 H, t), 1.3–1.75 (s, H, m), 2.05 (s, E) and 2.15 (s, Z) (3 H), 2.2–2.5 (2 H, m), 3.8 (3 H, s), 9.8 (1 H, br s); IR (film) 2960, 2880, 1730, 1700, 1630, 1435 cm^{-1}.

(Z/E)-2-(Ethoxycarbonyl)-3-methyloct-2-enoic acid (2g): ^{1}H-NMR, $CDCl_3$, δ 0.7–1.0 (3 H, m), 1.2–1.5 (9 H, m), 2.05 (s, E) and 2.17 (s, Z) (3 H), 2.22–2.5 (2 H, m), 4.2 (2 H, q), 10.9 (1 H, br s); IR (film) 3320, 2960, 2933, 2864, 1737, 1702, 1625, 1465, 1404, 1375, 1260 cm^{-1}.

(E)-3-(α-Carboxy-α-(ethoxycarbonyl)methylene)-1-(4-methoxyphenyl)-4-phenylazetidin-2-one (4a): mp 160–162 °C dec; ^{1}H-NMR, $CDCl_3$, δ 1.0 (3 H, t, J = 7 Hz), 3.75 (3 H, s),

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4.0 (2 H, q, J = 7 Hz), 5.8 (1 H, s), 6.7–7.45 (9 H, m), 8.0 (1 H, br s); IR (KBr) 3420, 2980, 2620, 1742, 1727, 1610, 1580, 1512, 1455, 1367, 1300, 1250, 1228 cm^{-1}. Anal. Calcd for $C_{21}H_{19}N\,O_6$: C, 66.14; H, 5.02; N, 3.67. Found: C, 65.29; H, 5.20; N, 3.55.

(*Z*)-3-(α-Carboxyethylidene)-1-(4-methoxyphenyl)-4-phenylazetidin-2-one (4b): mp 196–198 °C dec; ^{1}H-NMR, $CDCl_3$, δ 1.75 (3 H, s), 3.75 (3 H, s), 5.7 (1 H, s), 6.7–7.5 (9 H, m), 12.5 (1 H, br s); IR (KBr) 3420, 3110, 3070, 3010, 2930, 2850, 2700, 2640, 1740–1710, 1686, 1605, 1584, 1514 cm^{-1}. Anal. Calcd for $C_{19}H_{17}N\,O_4$: C, 70.58; H, 5.29; N, 4.33. Found: C, 70.42; H, 5.30; N, 4.25.

(*Z*)-3-(α-Carboxymethylene)-1-(4-methoxyphenyl)-4-phenylazetidin-2-one (4f): mp 155 °C dec; ^{1}H-NMR, $CDCl_3$/DMSO-*d*-6, δ 3.75 (3 H, s), 5.55 (1 H, d, J = 1.1 Hz), 5.8 (1 H, d, J = 1.1 Hz), 6.75–7.5 (9 H, m); IR (KBr) 3420, 3064, 1745–1720, 1680, 1610, 1513, 1454, 1373, 1302, 1254 cm^{-1}. Anal. Calcd for $C_{18}H_{15}N\,O_4$: C, 69.89; H, 4.89; N, 4.53. Found: C, 69.89; H, 5.22; N, 4.37.

(*E*)-3-(α-Carboxymethylene)-1-(4-methoxyphenyl)-4-phenylazetidin-2-one (4i): mp 187 °C dec; ^{1}H-NMR, $CDCl_3$/DMSO-*d*-6, δ 3.72 (3 H, s), 5.75 (1 H, d, J = 1.5 Hz), 6.3 (1 H, d, J = 1.5 Hz), 6.7–7.6 (9 H, m); IR (KBr) 3430, 2930, 1740, 1715, 1670, 1580, 1513, 1455, 1384, 1300, 1253 cm^{-1}; HREIMS found 309.1000, $C_{18}H_{15}N\,O_4$ requires 309.3226.

(*Z*)-3-(α-Carboxyphenylidene)-1-(4-methoxyphenyl)-4-phenylazetidin-2-one (4k): mp 186–187 °C dec; ^{1}H-NMR, $CDCl_3$, δ 3.7 (3 H, s), 5.6 (1 H, s), 6.75–7.4 (14 H, m); IR (KBr) 3430, 3057, 2644, 1730, 1711, 1610, 1585, 1512, 1452, 1442, 1410, 1367, 1302, 1252 cm^{-1}. Anal. Calcd for $C_{24}H_{19}N\,O_4$: C, 74.79; H, 4.97; N, 3.63. Found: C, 74.09; H, 5.01; N, 3.42.

Acknowledgment. Support of this research by Fonds der Chemischen Industrie, Frankfurt/Main, is gratefully acknowledged.

Supplementary Material Available: NMR spectra of **2h** *E*/*Z* (2D INEPT long range) and NOE difference spectra of **2d** *E*/*Z* (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.