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Selective and Convenient Enzymatic Separation of Geometric Isomers of N-Protected-α-dehydrophenylalanine Esters

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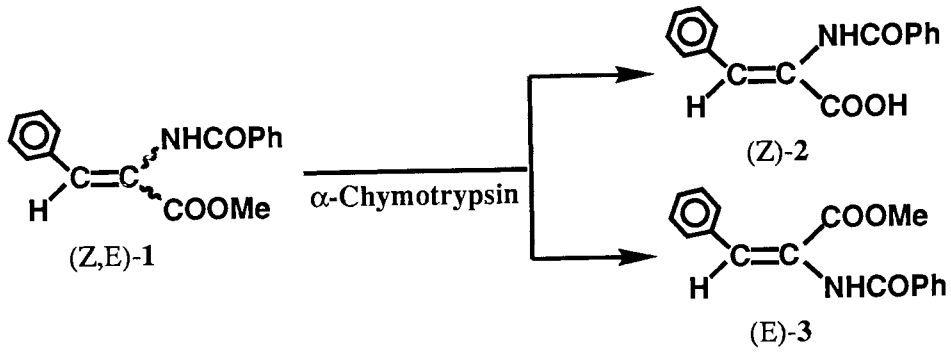
Selective enzymatic hydrolysis of a mixture of (Z)- and (E)-N-protected-ΔPhe-OMe with α-chymotrypsin A took place to give (Z)-ΔPhe-OH and (E)-ΔPhe-OMe.

In the previous papers,[1,2] we have reported a highly selective enzymatic hydrolysis of α- and γ-esters of methyl (Z)-N-benzyloxycarbonyl-γ-methyl-α-dehydroglutamate with papain and α-chymotrypsin A (CT), respectively. Here, we wish to report a convenient hydrolytic separation of geometric isomers of N-benzoyl-α-dehydrophenylalanine methyl ester [(Z,E)-Bz-ΔPhe-OMe; 1] by using CT (EC 3.4.22.2).

As the substrate of the enzymatic reaction, a 1 : 1 mixture of geometric isomers of 1[3] was prepared according to the method of Schmidt et al.[4] A solution of (Z,E)-1 [10 mM (1 M = 1 mol dm^{-3}), 14 mg] and CT (39 units/mg, 0.04 mM) in buffer (McIlvaine buffer : DMSO = 95 : 5 v/v) was incubated in the presence of $CaCl_2$ (15 ml), with shaking at pH 8 at 35 °C for 24 h. Then 10% citric acid (20 ml) was added, and the resulting solution was extracted with AcOEt. The organic layer was washed with brine and dried over anhydrous Na_2SO_4 and then concentrated in vacuo. The obtained products were identified and determined by comparing their HPLC (eluent MeOH : H_2O = 60 : 40 v/v) peaks with those of the compound prepared independently.[4,5] Thus, it was found that two products, (Z)-Bz-ΔPhe-OH (2) as the hydrolyzate and unchanged (E)-Bz-ΔPhe-OMe (3), were isolated separately (Scheme 1). The optimal pH value of the ester hydrolysis of (Z)-1 was about 9.

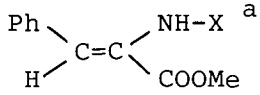
In order to purify the respective products, the crude mixture of (Z)-2 and (E)-3 obtained above was dissolved in AcOEt (10 ml) and the resulting solution was treated with a saturated $NaHCO_3$ aqueous solution (20 ml). The aqueous layer was acidified to pH 2 with 6 M-HCl and extracted with AcOEt. The extract was washed with brine and dried over anhydrous Na_2SO_4. Concentration in vacuo and further purification gave (Z)-2 as crystals.[6] On the other hand, a similar work-up of the organic layer, followed by



Scheme 1.

purification of the residue gave (E)-3.[7]

To examine the effect of the N-protecting groups, the hydrolysis of various (Z)-X-ΔPhe-OMe derivatives was carried out under same conditions. As the results summarized in Table 1 show, the benzoyl group was found to be superior to the urethane type groups [X=COOMe, COOEt, $COOBu^n$, and COOBzl].[5]

Table 1. Effect of N-protecting groups in  [a]

X	Yield/%	X	Yield/%
-COMe	0	-COOMe	52
-COPh	76	-COOEt	58
	90[b]	$-COOBu^n$	64
		-COOBzl	49

a) The reaction mixture (5 ml), containing 10 mM substrate and 1.6 g/l CT in buffer (McIlvaine buffer : DMSO = 95 : 5 v/v, pH 8.0), was shaken at 35 °C for 24 h. b) At pH 9.0.

References

1) C. Shin, N. Takahashi, and Y. Yonezawa, Chem. Lett., 1988, 2001.
2) C. Shin, M. Seki, and N. Takahashi, Chem. Lett., 1990, 2089.
3) (Z,E)-1: Yield 81%, colorless crystals. (Z)-1; NMR ($CDCl_3$): δ 3.88 (s, 3H, OCH_3), 7.79 (bs, 1H, NH). (E)-1: δ 3.68 (s, 3H, OCH_3), 8.55 (bs, 1H, NH).
4) U. Schmidt, A. Lieberknecht, and J. Wild, Synthesis, 1984, 53.
5) C. Shin, N. Takahashi, and Y. Yonezawa, Chem. Pharm. Bull., 38, 2020 (1990).
6) (Z)-2: Yield 90%, mp 215-216 °C (dec.), colorless needles from hexane-AcOEt. IR (KBr): 3310 (NH), 1690 (C=O) cm^{-1}. NMR (DMSO-d_6): δ 9.95 (s, 1H, COOH), 7.67-7.36 (m, 12H, $2C_6H_5$ + =CH + NH).
7) (E)-3: Yield 95%, mp 131-132 °C, colorless prisms from hexane-AcOEt. IR (KBr): 3310 (NH), 1725 (C=O) cm^{-1}. NMR (DMSO-d_6): δ 8.55 (bs, 1H, NH), 7.94-7.28 (m, 11H, $2C_6H_5$ + =CH), 3.66 (s, 3H, $COOCH_3$).

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