Enzymatic Hydrolysis of β-Lactam Esters by PLE

Young Key Shim*, Joong-Youn Shim, Young Soon Cho, and Wan Joo Kim

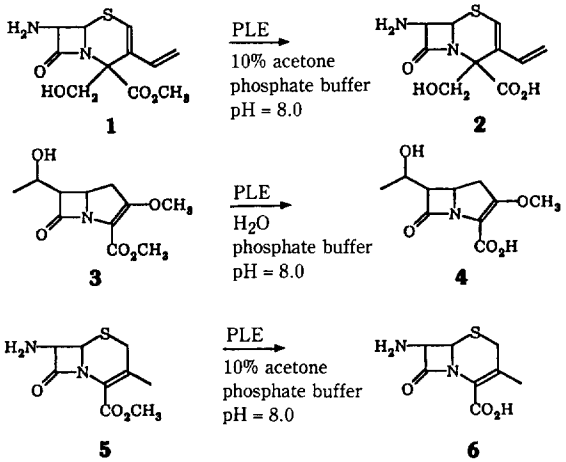
Korea Research Institute of Chemical Technology, Dae-Jeon 302-323. *Received July 23, 1988*

Pig Liver Esterase (PLE) which has been known to cleave a broad range of diesters to prepare stereoselective enantiomers is applied to hydrolysis of β-lactam esters imparting the corresponding acids in various yields.

Introduction

Pig Liver Esterase (PLE) was widely used to prepare enantiomeric excess chiral compounds. Although natural substrates for PLE have not been identified in biological systems, it is now known that PLE cleaves a broad range of esters. M. Ohno[1] reported the specificity on the hydrolysis of dialkyl esters that methyl esters gave much better enantioselectivity and higher yield of the acids than the corresponding diethyl esters.

Hydrolyzing ester groups in the presence of other sensitive functional groups were reported also. A. Hazato *et al.*[2] reported that PLE cleaves the methyl ester group of prostaglandin E1 without destroying the rest of the molecule to afford prostaglandin E1 in a good yield. Several attempts of simple hydrolysis on the labile molecules have been reported, however there are no reports on the β-lactam esters. So far the protecting groups for the esters of the β-lactam are very limited because of the uniqueness of the ring systems. *t*-Butyl, trichloroethyl, *p*-nitrobenzyl, allyl and diphenylmethyl groups have been used in laboratories and in industries. However the usage of t-butyl and trichloroethyl esters has problems of the low yields of preparation and their deblocking. In other hands *p*-nitrobenzyl, allyl and diphenylmethyl esters are obtained easily and in high yields, but toxic hazardous reagents, e.g., *p*-nitrobenzyl bromide for *p*-nitrobenzyl ester and mercury oxide in preparation of diphenyldiazomethane, and the costy palladium catalyst for the deblocking of the allyl ester are the other problems. Because of those real problems many researchers have been searched for the better blocking groups in the β-lactam antibiotics. Now we report the results of PLE hydrolysis on the β-lactam esters, e.g., cephalosporines and carbapenems (Scheme 1), to give the hydrolyzed products in varying yield. This new method can give the another possibility of the use of labile protecting group of β-lactam esters in laboratories and industries.



Scheme 1

Results and Discussion

Methyl esters of 3-vinyl-2-cephem **1** and carbapenem **3** were prepared easily by using diazomethane to the acids and methyl ester of 7-aminodeacetylcephalosporanic acid **5** (7-ADCA) was prepared by standing in 5% H_2SO_4-methanolic solution overnight in quantitative yield.

For the hydrolysis the methyl esters of cephalosporanic acid were dissolved in 10% acetone/water solution with 0.1M phosphate buffer (pH = 8.0) and stirred overnight at 25 °C with PLE(Sigma Type I, EC 3.1.1.1) to give the acids. 2-Cephem ester **1** gave 2-cephem acid **2** in 50% yield, 3-methoxy carbapenem **3** gave 15% yield of the acid compound **4**, and 7-ADCA **6** was obtained almost quantitatively. Unfortunately the reaction of 3-cephem derivatives instead of 2-cephems, e.g., 3-vinyl-3-cephem and 3-acetoxymethyl-3-cephem with PLE gave a mixture of several decomposed products, which can be explained by promoting the ring opening of the β-lactams by vinyl function or leaving ability of acetoxymethyl group.

Experimentals

Melting points were determined on a Thomas Hoover melting point apparatus and were uncorrected. ^{1}H-NMR spectra were obtained on Varian EM-360A and Bruker AM-300 nmr spectrometers. Chemical shift values from TMS were reported on the δ scale. Mass spectra were recorded on Zeol JMS-DX 303 spectrometer.

3-Vinyl-4-hydroxymethyl-7-amino-2-cepham-4-carboxylic Acid 2. The methyl ester **1** (slightly modified from the known procedure[3] by using diazomethane) (2.7g, 11 mmol) was suspended in the mixture of acetone (75 m*l*), water (613 m*l*) and 0.1M phosphate buffer (pH = 8.0) solution (75 m*l*). To this 3 m*l* (8580 units) of PLE (Sigma Type I, EC 3.1.1.1, suspension of ammonium sulfate buffer) was added and stirred at 25 °C overnight with continuously adjustment of the pH = 7.5-8.0 with 0.1N of NaOH. The reaction mixture was extracted with ethyl acetate twice, the solvent evaporated, and 500 m*l* of HCl solution (pH = 2.6) was added into the resulting liquid to obtain the pure acid (1.3g, 50% yield) as a white solid.

Mass *m/e* (relative intensity); 256(M^+, 18), 44(100)

NMR(CF_3CO_2D + $CDCl_3$) δ = 4.40(q,2H,-CH_2O-), 5.13-5.30 (m, 3H, C_6-H, =CH_2), 5.55(t, 1H,C_7-H), 6.10-6.45(m,1H, -CH=), 6.60(s, 1H,-SCH-)

6α-[1-(R)-Hydroxyethyl]-3-methoxy-7-oxo-1-azabicyclo(3,2,0)-hept-2-ene-2-carboxylic Acid 4. Into the solution of the methyl ester **3**(1g, 4.12 mmol), which was obtained from the hydrogenation of *p*-nitrobenzyl 6a-[1-(R)-hydroxyethyl]-3-hydroxy-7-oxo-1-azabicyclo(3,2,0)-hept-2-ene-2-carboxylate[4] followed by treatment with excess of etherial diazomethane [mass of methyl ester; *m/e*(relative intensity); 241(M^+,13), 259($M+H_2O^+$,45), 112(100), NMR($CDCl_3$) δ; 1.33(d,3H,-CH_3), 2.78(dd,2H,-CH_2-), 2.94(dd,1H,-C_6H), 3.66(s,3H,-OCH_3), 3.70(s,3H,-OCH_3), 3.99-4.25(m,3H,-CHO-,-C_5H,-OH)], in 0.05M-phosphate buffer (50 m*l*) and acetone (3 m*l*) was added PLE (1.5 m*l*, suspension of ammonium sulfate buffer) at 25 °C and stirred 2 days in constant adjustment of pH 8.0. Excess of acetone was poured and stirred for 10 min and filtered off any insoluble material. The filterate was washed with methylene chloride and the water solution was adjusted with HCl to pH = 2.0 and extracted with ethyl acetate. The organic solvent was washed with brine, dried over $MgSO_4$ and evaporated to give the desired product (147 mg, 15% yield).

Mass *m/e*(relative intensity); 227(M^+,7), 112(100)

NMR($CDCl_3$)δ = 1.10(d,3H,-CH_3), 2.40-2.90(m,3H,-CH_2-,C_6-H), 3.58(s,3H,-OCH_3), 3.70-4.10(m,3H,C_5-H,-CHO-,-OH)

7-Aminodeacetylcephalosporanic Acid (7-ADCA) 6. 7-ADCA (1.88g, 0.88mmol) was stirred at room temperature overnight in 5%-H_2SO_4 in anhydrous methanolic solution (100 m*l*). Water (100 m*l*) was added and extracted with methylene chloride, dried over $MgSO_4$, solvent evaporated to give the desired methyl ester which was pure enough to use for the next hydrolysis reaction. Into the solution of the methyl ester **5** (0.1g, 0.44 mmol) in water (14 m*l*), 0.1 M-phosphate buffer (4 m*l*) and acetone (2 m*l*) was added PLE (0.2 m*l*, suspension of ammonium sulfate buffer) at 25 °C and stirred overnight in constant adjustment of pH = 8.0. Excess of acetone was poured and stirred for 10 min and filtered off any insoluble material. The filterate was washed with methylene chloride and the water solution was adjusted with HCl to pH = 2.0 and extracted with ethylacetate. The organic solvent was washed with brine, dried over $MgSO_4$ and evaporation of the solvent gave the product which was identical to the commecially available 7-ADCA (90 mg, 95% yield).

References

1. M. Ohno, S.Kobayashi, and Adachi, in Enzymes as Catalysts in Organic Synthesis, M. P. Schneider, Ed., D. Reidel Publishing, Dordrecht, 1986, pp.123-142.
2. A. Hazato, T. Tanaka, T. Toru, N. Okamura, K. Bannai, S. Sugiura, K. Manabe, and S. Kurozumi, *Nippon Kagaku Kaishi*, **9**, 1390 (1983).
3. A. H. Shingler and N. G. Weir, in Recent Advances in the Chemistry of β-Lactam Antibiotics, J. Elks, Ed., The Chemical Society Burlington House, London, Special Publication No. 28, 1977, pp.155-157.
4. D. G. Melillo, I. Shinkai, T. Liu, K. Ryan, and M. Sletzinger, *Tetrahedron Lett.*, **21**, 2783 (1980).

A Study of the Parameters of the Retention of Monosubstituted Benzenes in Reversed-Phase Liquid Chromatography

Dai Woon Lee*, Keun Sung Yook, Ho Seob Kim†, and Won Lee‡

Department of Chemistry, Yonsei University, Seoul 120-749

†Lucky Central Research Institute, 84 Changdong, Chungku, Daejeon 302-343

‡Department of Chemistry, Kyunghee University, Seoul 130-701. *Recerived July 26, 1988*

The relationship between the solute retention and physical parameters describing the interaction between the solute and mobile phase was investigated to predict the solute retention easily in RPLC. The retention data of monosubstituted benzenes were measured on the μ-Bondapak C_{18} and phenyl columns with methanol-water systems. The linear relationship between dielectric increment(ε′) and retention data was observed. When the solute form hydrogen bonding with solvent molecules, the slope of the ln k′ vs. ε′ plot is changed as the compositions is varied. The quadric relationship between mixed solvent solubility parameter (δ_M) and retention data was observed.

Introduction

In recent years, many theories have been reported about the solute retention in reversed-phase liquid chromatography (RPLC)[1-3]. However, most of them are too complicated to apply for practical purposes and are not easy to predict the retention in several different conditions. Therefore, in this paper we report the results of our study on the relationship between the solute retention and physical parameters describing the interaction between the solute and mobile phase. This will allow the parameter to be used to predict the solute retention easily in RPLC.

Experimental

Instruments. Waters Associates liquid chromatographic system used consisted of M-440 Absorbance Detector, M-45 Solvent Delivery System, and M-U6K Universal Injec-