84-58-2; 9-methylanthracene, 779-02-2; 1,2-dihydroaceanthrylene, 641-48-5; 5,6-dihydrobenz[e]aceanthrylene, 3697-25-4; 11-methyl-6,7,16,17-tetrahydro-15H-cyclopenta[a]phenanthrene, 115338-38-0; 1,2,3,4,5,6-hexahydrochrysene, 2091-91-0; 6-methoxy-2-methylnaphthalene, 26386-94-7; 7-methylbenz[a]anthracene, 2541-69-7; 8,9,10,11-tetrahydrobenz[a]anthracene, 67064-62-4; 3-methylcholanthrene, 56-49-5; 7,8-dihydro-9H-cyclopenta[a]-pyrene, 82979-72-4; 3-methoxy-6,7,16,17-tetrahydro-15H-cyclopenta[a]phenanthrene, 115338-42-6; 6-methoxytetralin, 1730-48-9; 9-anthracenecarboxaldehyde, 642-31-9; 1,2-dihydroaceanthrylen-1-one, 51752-51-3; 5,6-dihydrobenz[e]aceanthrylen-5-one, 115482-66-1; benz[e]aceanthrylene, 199-54-2; 7,8,9,10-tetrahydrobenzo[a]pyren-10-one, 57652-65-0; 11-methyl-6,7,16,17-tetrahydro-15H-cyclopenta[a]phenanthren-17-one, 115338-39-1; 1,2,3,4,5,6-hexahydrochrysen-1-one, 115482-67-2; 6-methoxy-2-naphthalenecarboxaldehyde, 3453-33-6; 7-benz[a]anthracenecarboxaldehyde, 7505-62-6; 8,9,10,11-tetrahydrobenz[a]anthracen-11-one, 60968-15-2; 3-methylcholanthren-1-one, 3343-07-5; 3-methylbenz[j]aceanthrylene, 3343-10-0; 7,8-dihydro-9H-cyclopenta[a]pyren-9-one, 82979-73-5; 3-methoxy-6,7,15,16-tetrahydro-17H-cyclopenta[a]phenanthren-17-one, 17521-83-4; 6-methoxytetralin-1-one, 1078-19-9.

Papain-Catalyzed Synthesis of Aspartame Precursor in Biphasic System

S. T. Chen and K. T. Wang*

Institute of Biological Chemistry, Academia Sinica and Institute of Biochemical Sciences, National Taiwan University, P.O. Box 23-106 Taipei, Taiwan, Republic of China

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The synthesis of aspartame and its precursors has attracted the attention of many investigators.[1] The most interesting synthetic method is a thermolysin-catalyzed reaction that gives the products stereospecifically[2,3] However, this method has two drawbacks: (1) thermolysin is expensive and not readily available and (2) one extra equivalent of D- or L-Phe-OMe* is required to precipitate the product. In an attempt to use papain as a catalyst for the synthesis of P-L-Asp-L-Phe-OMe (P = Boc, Cbz, or Moz, abbreviations: Boc = *tert*-butyloxycarbonyl; Cbz = benzyloxycarbonyl; Moz = [(*p*-methoxybenzyl)oxy]-carbonyl; Asp = aspartic acid; Phe = phenylalanine) from P-Asp-OH and H-Phe-OMe, we found that P-L-Asp-L-Phe-OH instead of P-L-Asp-L-Phe-OMe was the only product when the reaction was carried out in McIlvaine buffer (McIlvine buffer consists of citric acid and sodium phosphate, see ref 10) because the initially formed methyl ester of the desired product was hydrolyzed by papain after the peptide bond was formed.[4] In order to avoid the hydrolysis, we used a McIlvaine/ethyl acetate biphasic system instead of a single phase and obtained P-L-Asp-L-Phe-OMe as the only product.

The procedure[5] for this biphasic reaction is to dissolve the two substrates in a minimum amount of buffer and to extract the product into a large volume of the immiscible organic solvent. In preliminary work, we found the solubility of the 1:1 mixture of P-Asp and Phe-OMe in McIlvaine buffer (pH 6.2) was higher than 0.5 M and that the pH of this solution had decreased to pH 5.5. The partition coefficient of P-Asp-Phe-OMe in 0.2 M solution (pH 5.5) and ethyl acetate is about 1 to 30 (calculated by HPLC quantitation).[6] Change of protecting groups (P = Boc, Cbz, and Moz) did not affect the partition coefficient significantly. In a typical study, 2.5 mmol each of the substrate in 6 mL of McIlvaine buffer (pH 6.2) and 95 mL of ethyl acetate was incubated at 37 °C for 72 h, and a yield of 70% was obtained for Moz-Asp-Phe-OMe. In a larger scale reaction, 20 mmol of the substrates were dissolved in 50 mL of McIlvaine buffer and 750 mL of ethyl acetate. Yields of and physical properties of the products are shown in Table I. Salt formation similar to that occurring in the thermolysin-catalyzed reaction was not observed for two reasons: (1) the weak acidity of reaction solution may not be suitable for the salt formation and (2) the product formed in the aqueous layer is extracted into the ethyl acetate layer without precipitation of the salt. Although the yield with use of Boc-L-Asp as substrate was acceptable, the best yield was obtained by using Moz-L-Asp. After separation of the organic layer, the enzyme solution was incubated again with the substrates as in the first run. The enzyme was still active but gave a lower yield (35%). Enzyme specificity was tested by reaction of the D,L substrate under the same conditions, and it was found that the P-L-Asp-L-Phe-OMe was the only product. The results are shown in Table II. The immobilized enzyme reaction was studied by adsorbing papain on Amberlite XAD-7.[7,8] After both substrates in ethyl acetate presaturated with McIlvaine buffer were passed through the column, the yield of Moz-L-Asp-L-Phe-OMe in the eluent was 45% as determined by HPLC.

Table I. Reaction of Various N-Protected L-Asp with L-Phe-OMe

substrates[a]	yield, %	product[b]	$[\alpha]^{25}_{D}$,[c] deg	mp, °C
Boc-L-Asp + L-Phe-OMe	54	Boc-L-Asp-L-Phe-OMe	−17.0	164–166
Cbz-L-Asp + L-Phe-OMe	70	Cbz-L-Asp-L-Phe-OMe	−16.0	121–123
Moz-L-Asp + L-Phe-OMe	75	Moz-L-Asp-L-Phe-OMe	−13.0	107–108

[a] Abbreviations: Boc = *tert*-butyloxycarbonyl; Cbz = benzyloxycarbonyl; Moz = [(*p*-methoxybenzyl)oxy]carbonyl. [b] The products were characterized by comparison with authentic samples (melting point and NMR and IR spectra). [c] The optical rotations were measured in methanol (*c* = 1).

Experimental Section

L-Amino acids were purchased from Kyowa Fermentation Co. Tokyo, Japan. D,L-Amino acids were obtained from Sigma. Solvents for synthesis and HPLC were from ALPS Chemicals Inc. Taipei, Taiwan. Amino acid derivatives were prepared according to the established method.[9] Papain (carica papaya 3.5 mAnson-E/mg) was from E. Merck. The McIlvaine buffer containing 0.5% mercaptoethanol was prepared according to Elving's procedure.[10] TLC was performed on silica gel G. 60 (E. Merck) precoated on a glass plate. The HPLC system consists of two Waters Model 6000 pumps, a Waters U6K valve–loop injector, a Waters Model 450 variable-wavelength UV detector, Waters Model 660 solvent programmer, and Shimadzu C-R2AX chro-

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Table II. Reaction of Various D,L-Substrates

substrates	yield,[a] %	product	$[\alpha]^{25}_D$,[b] deg	mp, °C
Cbz-L-Asp + D,L-Phe-OMe[c]	46	Cbz-L-Asp-L-Phe-OMe	-16.0	118-120
Cbz-D,L-Asp + L-Phe-OMe	54	Cbz-L-Asp-L-Phe-OMe	-15.0	119-121
Cbz-D,L-Asp + D,L-Phe-OMe	52	Cbz-L-Asp-L-Phe-OMe	-15.7	118-120
Moz-L-Asp + D,L-Phe-OMe	59	Moz-L-Asp-L-Phe-OMe	-11.9	105-108
Moz-D,L-Asp + L-Phe-OMe	50	Moz-L-Asp-L-Phe-OMe	-12.0	104-107
Moz-D,L-Asp + D,L-Phe-OMe	51	Moz-L-Asp-L-Phe-OMe	-11.9	105-107

[a] Cbz(or Moz)-L-Asp was used as basis to calculate the yield. [b] The optical rotations were measured in methanol (c = 1). [c] Two equivalents of the D,L derivative were used in every reaction.

matopac. Melting points were taken on a Büchi 510 melting point apparatus and are uncorrected. Optical rotation was measured on Polartronic Universal polarimeter (Schmidt & Haensch).

1. Synthesis of P-Asp-Phe-OMe. (Moz-Asp-Phe-OMe as Example). Into a 1-L flask were added Moz-Asp (5.94 g, 20 mmol), L-Phe-OMe·HCl (4.30 g, 20 mmol), McIlvaine buffer (pH 6.2, 50 mL), triethylamine (2.8 mL, 20 mmol), and papain (2 g), and the mixture was stirred to reach clear solution. Ethyl acetate (750 mL) was added, and the final mixture was incubated on a orbital shaker at 37 °C for 72 h. At the end of the reaction, the organic layer was separated and washed with 2% citric acid (3 × 50 mL) and water (2 × 50 mL) and dried over anhydrous sodium sulfate. Evaporation of solvent under reduced pressure gave the crude product, which was crystallized from water/acetone (2:1, 100 mL) to give 6.86 g (75%) of pure Moz-Asp-Phe-OMe: mp 107-108 °C; $[\alpha]^{25}_D$ -13.0° (c 1, MeOH). It was identical with the authentic sample of Moz-Asp-Phe-OMe prepared by thermolysin-catalyzed reaction. In addition, deprotection gave Asp-Phe-OMe.

2. Continuous-Flow Reaction by Immobilized Papain Column. Immobilization was prepared according to the procedure of Oyama.[7] Into a 1000-mL Wheaton Bio-reaction flask were added papain (25 g), Amberlite XAD-7 (150 mL), and McIlvaine buffer (500 mL). The mixture was stirred at room temperature for 3 h and packed into a column (20 × 800 mm). After draining out the buffer solution, a solution of 0.2 M each of the substrates dissolved in ethyl acetate presaturated with McIlvaine buffer was passed through the wet column (flow rate of 0.5 mL/min). After 1.5 bed volumes were eluted, a small aliquot was taken from eluent and quantitated with HPLC by the method of Durrant.[6] It was found to have 0.09 M of the product in ethyl acetate solution. The yield is calculated to be 45% by taking into account the volume of ethyl acetate.

Registry No. BOC-Asp-OH, 13726-67-5; Cbz-Asp-OH, 1152-61-0; Moz-Asp-OH, 20890-95-3; Cbz-DL-Asp-OH, 4515-21-3; Moz-DL-Asp-OH, 115705-34-5; H-Phe-OMe·HCl, 7524-50-7; H-DL-Phe-OMe, 15028-44-1; BOC-Asp-Phe-OMe, 40944-73-8; Cbz-Asp-Phe-OMe, 33605-72-0; Moz-Asp-Phe-Me, 68802-03-9; papain, 9001-73-4.

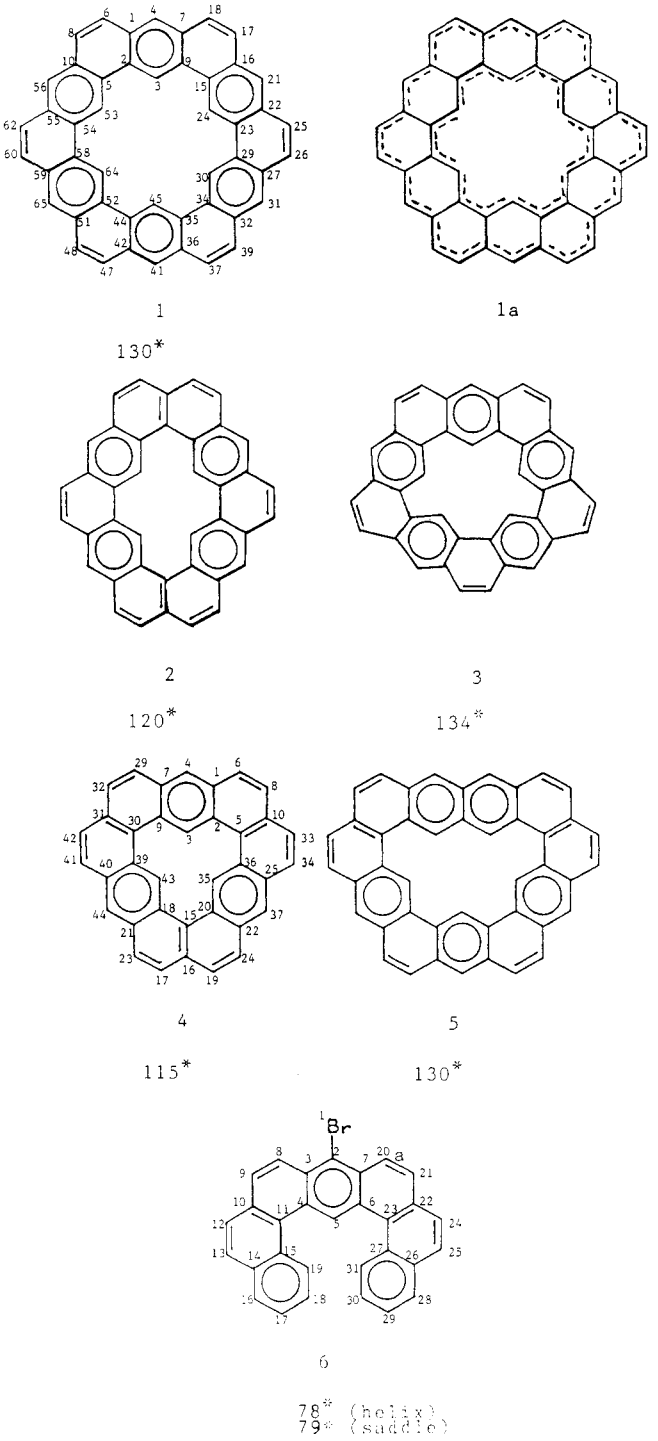

Figure 1. Cycloarenes and computed MMPMI strain energies. The asterisks (*) deonte MMPMI computed strain energies (kcal/mol). Final geometries are described in the supplementary material.

Localization of Aromaticity in Fused-Ring Cycloarene Systems: Prediction by an Effective Molecular Mechanics Model

Paul M. Lahti

Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003

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Condensed polycyclic aromatic molecules of the circulene and cycloarene families[1-3] have been the subject of synthetic and theoretical scrutiny for some time. Cycloarenes (exemplified by molecules **1-5**) have been of considerable theoretical interest concerning their possible superaromatic nature and their highly strained internal hydrogens, but to date kekulene[3] (1) and the related molecule **2**[4] appear to be the sole nontrivial representatives

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