Anal. Found: C, 41.0; H, 7.0; N, 7.9.

4-Thioquinolizone (XVIII). Method A.—A solution of 60 g. of XVI in 300 ml. of water was treated with decolorizing charcoal and filtered. A solution of 60 g. of sodium sulfide nonahydrate in 300 ml. of water was added slowly, with constant stirring. After 3 hours' stirring, the bright yellow solid which formed was collected, washed with water, and dried to give 30.8 g. of XVIII, m.p. 104°.[5] Recrystallization from a mixture of toluene and ligroin did not change the melting point.

Method B.—A mixture of 6 g. of XVI, 1.5 g. of thiourea and 100 ml. of ethanol was refluxed for 1 hr. and then evaporated to dryness under vacuum. The residue was heated with 20 ml. of 10% sodium hydroxide solution for 15 min. on the steam bath, the solution saturated with sodium chloride, and the organic material extracted with chloroform. The chloroform was removed from the extract and the residue recrystallized from ligroin (b.p. 90–120°) to yield 2.8 g. of XVIII, m.p. 97–99°.

4-Methylmercaptoquinolizinium Perchlorate (XIX).—Thioquinolizone (29.3 g.) and 30 ml. of dimethyl sulfate were mixed. A vigorous exothermic reaction ensued and the reaction mixture solidified. The mixture was heated for 1 hr. at 90°, 200 ml. of water was added, and the heating was continued until solution was complete. To the solution was added 15 ml. of 70% perchloric acid. On cooling, 24 g. of XIX separated, m.p. 135° (from water).

Anal. Calcd. for $C_{10}H_{10}NSClO_4$: C, 43.6; H, 3.6; N, 5.1. Found: C, 42.9; H, 3.6; N, 5.0.

Attempt to Make the Perchlorate of Quinolizone.—A solution of 2.0 g. of XV in 15 ml. of water was treated with 1 ml. of 70% perchloric acid in 3 ml. of water. On cooling, a product separated which was crystallized from ethanol; yield, 1.2 g.; m.p., 155°.

Anal. Calcd. for $C_{18}H_{14}O_5N_2Cl$: C, 55.5; H, 3.6; N, 7.2. Found: C, 55.2; H, 3.9; N, 7.0.

A Novel Peptide Cleavage Reaction

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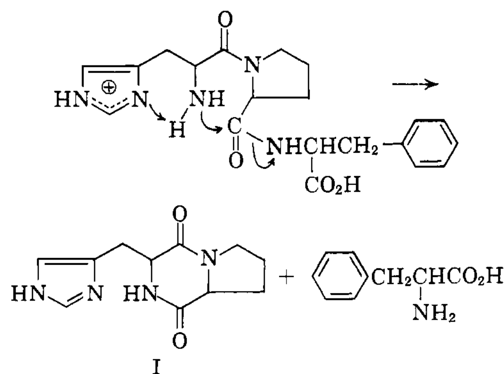
It has been observed that under mild acid treatment, the tripeptide His·Pro·Phe·OR is cleaved to yield histidylproline diketopiperazine (I) and Phe·OR· The reaction appears to be unusually rapid for the N-terminal sequence His·Pro but not completely specific.

We wish to report a reaction in which a peptide bond is cleaved under unexpectedly mild conditions. During some preliminary work on the synthesis of angiotensin II[1] the tripeptide ester His·Pro·Phe·OCH_3[2,3] and the unprotected tripeptide His·Pro·Phe were desired. Attempted preparation of the latter by detritylation with hot 50% acetic acid of Tr·Tr·His·Pro·Phe gave, surprisingly, phenylalanine and a second product which was ninhydrin negative and Pauly positive. The only other material obtained was triphenylcarbinol. We were able to identify the unexpected product as histidylproline diketopiperazine (I) (His·Pro). A plausible schematic mechanism for this reaction is shown.



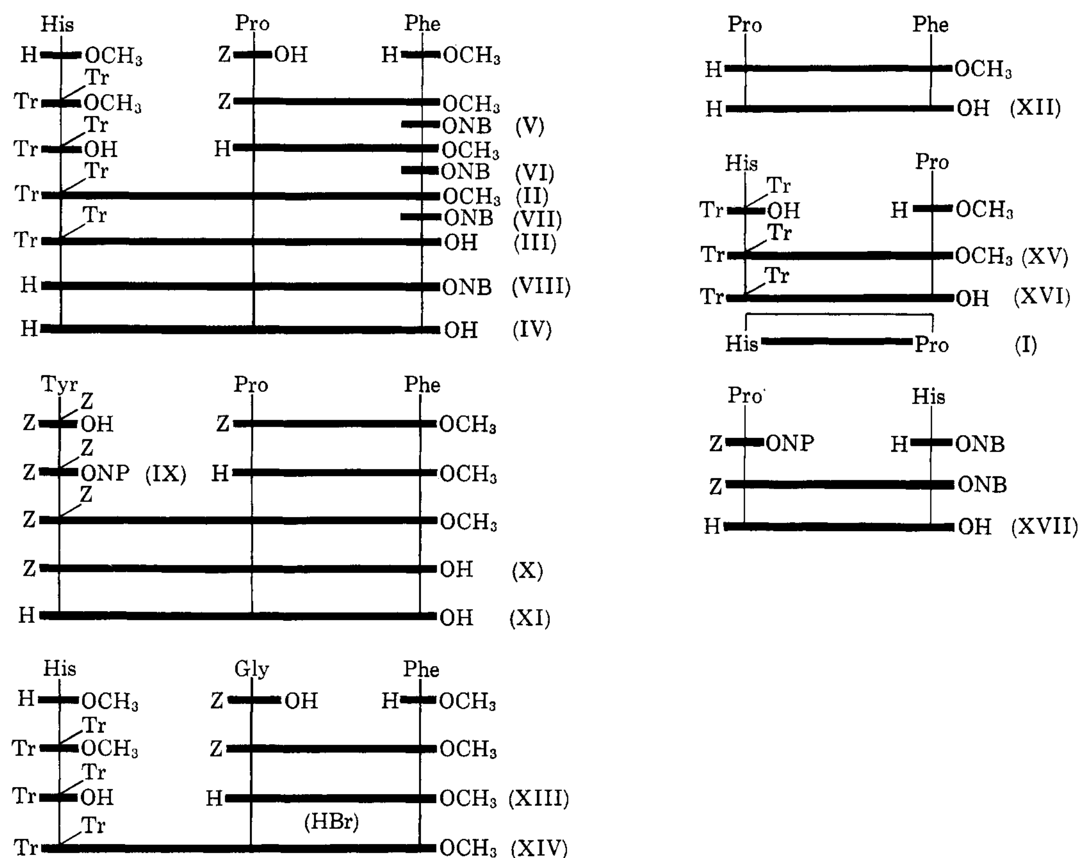
In view of the potential utility of this reaction for protein degradation, we have attempted to determine what structural features are required. Our first observation was that there was a great difference in rate of cleavage between Tr·Tr·His·Pro·Phe· and Tr·Tr·His·Pro·Phe·OCH_3. The acid was completely hydrolyzed and cleaved in 50% acetic acid after 24 hours at room temperature while the ester was detritylated but otherwise unchanged under the same conditions. Heating the ester in 50% acetic acid on the steam bath caused the formation of His·Pro and Phe·OCH_3. The unexpected difference in reactivity between the acid and ester where the structural change is rather remote from the reaction center can be rationalized by hydrogen bonding between the carboxyl of phenylalanine and the amide carbonyl of proline. The effect is to produce a partial positive charge on the proline carbonyl facilitating nucleophilic attack by the histidine amino group. This rate enhancing mechanism, not available to the ester, follows.

Attempts to synthesize His·Pro·Phe were partially successful. Brief hydrolysis of Tr·Tr·His·Pro·Phe at 60° in 50% acetic acid gave predominantly His·Pro·Phe as indicated by paper chromatography. However, an absolutely pure material could not be isolated, and attempted crystallization from methanol resulted in complete cleavage.

An alternate approach was made through Tr·Tr·His·Pro·Phe·ONB, which behaved analogously to the methyl ester. Hydrolysis with 50% acetic acid at 90° yielded His·Pro and Phe·ONB while at 60° for ten minutes the principal product was His·Pro·Phe·ONB. Hydrogenolysis of Tr·Tr·His·Pro·Phe·ONB gave mixtures of His·Pro·Phe and cleavage products. Ad-

(1) R. H. Mazur, *Can. J. Chem.*, **40**, 1098 (1962).

(2) All amino acids (except glycine) have the L-configuration. The abbreviations are adapted from the proposal of Brand and Edsall.[3] Additional abbreviations are Tr = triphenylmethyl, Z = carbobenzoxy, ONB = p-nitrobenzyl ester, and ONP = p-nitrophenyl ester.



dition of one equivalent of hydrochloric acid before hydrogenolysis resulted in virtually complete cleavage of the tripeptide.

The type of reaction we have described is reminiscent of imidazole catalysts of other hydrolytic processes,[4] but the presence of histidine alone is not enough to produce the observed cleavage. Furthermore, the role of proline is probably to hold the peptide molecule in a preferred orientation for participation of the imidazole ring but, again, proline alone does not suffice. These facts were brought out by the following experiments. Tyr·Pro·Phe, Pro·Phe, and Tr·Tr·His·Gly·Phe·O-CH_3 were heated at 100° with 50% acetic acid and the reaction followed by paper chromatography. Under these conditions, Tr·Tr·His·Pro·Phe·OCH_3 was completely cleaved in a few minutes. The tripeptide Tyr·Pro·Phe showed a slow hydrolysis with liberation of phenylalanine and a ninhydrin negative, Pauly positive material, presumably $\overline{\text{Tyr·Pro}}$. At sixty minutes, an appreciable quantity (<50%) of diketopiperazine seemed to be present. On the contrary, Pro·Phe under the same conditions showed no evidence of hydrolysis at all. The protected tripeptide Tr·Tr·His·Gly·Phe·-OCH_3 was immediately hydrolyzed to His·Gly·Phe·O-CH_3 as expected. After sixty minutes, a very small amount of Phe·OCH_3 and a substance assumed to be $\overline{\text{His·Gly}}$ had also appeared.

We have also investigated in some detail reactions of His·Pro, Pro·His, and their derivatives by paper chromatography. Tr·Tr·His·Pro·OCH_3 in 50% acetic acid at room temperature gave about equal quantities of His·Pro·OCH_3 and $\overline{\text{His·Pro}}$ after four hours. At 90°, almost complete conversion to $\overline{\text{His·Pro}}$ had occurred after one quarter of an hour. Tr·Tr·His·Pro in 50% acetic acid gave His·Pro at room temperature, the trityl groups being removed after one hour. The free dipeptide was stable for several hours at room temperature but was completely cyclized after one hour at 90°. On the other hand, Pro·His was readily prepared and proved to be quite stable in acetic acid solution. We may conclude from these qualitative experiments that the sequence His·Pro represents a special case in which resonance and steric factors combine to facilitate cyclization to $\overline{\text{His·Pro}}$ with the liberation of whatever group is attached to the C-terminal end of the proline, *e.g.*, OH, OR, NHCH $(CO_2H)CH_2$-C_6H_5.

During the course of this work we have developed a method for the preparation of *p*-nitrobenzyl esters which seems to be an improvement over that of Carpenter.[5] The amino acid, three to five equivalents of *p*-toluenesulfonic acid monohydrate and five equivalents of *p*-nitrobenzyl alcohol in dry chloroform are heated under vigorous reflux and the condensate returned to the

(3) E. Brand and J. T. Edsall, *Ann. Rev. Biochem.*, **16**, 223 (1947).

(4) T. C. Bruice and J. J Bruno, *J. Am. Chem. Soc.*, **84**, 2128 (1962), and references cited therein.

(5) J. E. Shields, W. H. McGregor, and F. H. Carpenter, *J. Org. Chem.*, **26**, 1491 (1961).

pot through a bed of calcium sulfate. Using chloroform gives a homogeneous reaction and the large excesses of acid and alcohol permit the reaction to go to completion in a few hours. The unchanged p-nitrobenzyl alcohol may be recovered. Examples using phenylalanine, histidine, alanine, and serine are described in Experimental.

Experimental

We wish to thank R. T. Dillon and associates for analyses, rotations, and spectra. Melting points are uncorrected. The analytical samples were dried at room temperature under water pump pressure. This procedure tended to give a stable analytical preparation although not one necessarily solvent-free.

Paper chromatography was carried out on Whatman no. 1 filter paper using n-butyl alcohol–acetic acid–water 7:1:2 in ascending system. Spots were detected with ninhydrin in isopropyl alcohol[6] (nin), diazotized sulfanilamide in n-butyl alcohol[7] (Pauly), vapor phase t-butyl hypochlorite followed by starch-iodide[8] (t-BuOCl), and ultraviolet scanning (UV).

Data for plotting countercurrent distribution curves were obtained by an improved method. An aliquot from each tube was taken to dryness and a complete ultraviolet spectrum run on the residue. Partition coefficients were calculated from optical density vs. tube number in the usual way. The shape of the ultraviolet curve and the absorption maximum from tube to tube gave additional information as to homogeneity and allowed detection of impurities that were not always readily apparent from the distribution curve itself, especially when obtained by scanning at a fixed wave length.

Nuclear magnetic resonance spectroscopy was very helpful in confirming the presence of cyclohexane of crystallization and in calculating the number of moles present. This was done by comparing the observed ratio of aromatic protons to aliphatic protons with calculated values. N.m.r. spectra were determined on a Varian A-60 spectrometer at 10% concentration in deuteriochloroform using tetramethylsilane as an internal standard.

Ditritylhistidylprolylphenylalanine Methyl Ester (II).—A general procedure was used. Ditritylhistidine[9] 16.72 g. (0.0105 mole) was dissolved in 200 ml. of methylene chloride, the solution concentrated to approx. 40 ml. and a solution of 3.57 g. 0.01 mole) of prolylphenylalanine methyl ester hydrobromide[1] in 40 ml. of methylene chloride containing 1.01 g. (0.01 mole) of triethylamine added. To the combined solutions was added 2.27 g. (0.011 mole) of dicyclohexylcarbodiimide and the resulting mixture allowed to stand overnight at 5°. The dicyclohexylurea was removed by filtration, washed with methylene chloride, the combined filtrates washed with 1 N hydrochloric acid, 1 N potassium bicarbonate, 10% sodium sulfate, and dried over sodium sulfate. Distillation of the methylene chloride gave 9.2 g. of a brittle foam. Crystallization from cyclohexane solution gave the desired tripeptide II as a powder, 7.98 g. (89%), m.p. 112–115°; R_f 0.91 (t-BuOCl +, one spot only); $[\alpha]^{25}D$ −6° (c 1, chloroform).

Anal. Calcd. for $C_{59}H_{55}N_5O_4$: C, 78.90; H, 6.17; N, 7.80. Found: C, 78.73; H, 6.64; N, 7.87.

Ditritylhistidylprolylphenylalanine (III).—Tripeptide ester II (2.0 g. 0.0022 mole) in 20 ml. of methanol was treated with 2 ml. of 4 N lithium hydroxide. The starting material oiled out but redissolved on stirring. The solution was allowed to stand 1 hr. at room temperature and was neutralized with 2 ml. of 4 N hydrochloric acid. The product was taken up in chloroform and the organic phase was washed with water, dried over sodium sulfate, and distilled. The crude acid was crystallized from cyclohexane to yield ditritylhistidylprolylphenylalanine as a microcrystalline powder, 1.7 g. (86%), m.p. 135–155° (no meniscus), R_f 0.90 (t-BuOCl +, one spot only); $[\alpha]^{26}D$ −1° (c 1, methanol).

Anal. Calcd. for $C_{58}H_{53}N_5O_4 \cdot H_2O$: C, 77.31; H, 6.15; N, 7.77. Found: C, 77.58; H, 6.65; N, 7.92.

Histidylprolylphenylalanine (IV).—Tripeptide acid III (4.50 g., 0.005 mole) in 100 ml. 50% acetic acid was heated 10 min. at 60° and concentrated nearly to dryness at room temperature. The residue was slurried with water, triphenylcarbinol removed by filtration, and the filtrated passed over a weakly basic anion exchange resin (IR 45). The eluate was lyophilized and the residue rubbed with acetone to give tripeptide IV, 1.76 g. (88%) as a powder, m.p. 130–145° dec., principal spot R_f 0.41 (nin +, Pauly +), very weak spots R_f 0.52 (nin +, Pauly-, phenylalanine) and R_f 0.28 (nin − Pauly +, diketopiperazine I); $[\alpha]^{27}D$ −25° (c 1, methanol).

Anal. Calcd. for $C_{20}H_{25}N_5O_4 \cdot 2H_2O$: C, 55.16; H, 6.71; 16.08. Found: C, 55.22; H, 6.68; N, 16.23.

At steam bath temperature, the protected tripeptide acid III was completely cleaved after a few minutes to diketopiperazine I and phenylalanine as shown by paper chromatography.

When the crude tripeptide IV was boiled in methanol, phenylalanine (identified by paper chromatography) crystallized from the solution. Essentially complete liberation of phenylalanine was observed after a few hours. This decomposition may have been due to traces of acid still present in the tripeptide.

Synthesis of p-Nitrobenzyl Esters. Alanine p-Nitrobenzyl Ester p-Toluenesulfonate.—A typical preparation follows. Alanine (8.91 g., 0.1 mole), p-toluenesulfonic acid monohydrate (57.0 g., 0.3 mole) and p-nitrobenzyl alcohol (76.5 g., 0.5 mole) were suspended in 300 ml. of dry chloroform (the chloroform was dried by shaking with concentrated sulfuric acid and filtering through Hyflo-Supercel to remove traces of sulfuric acid). A clear solution was obtained after a few minutes at reflux. The distillate was returned to the pot through a bed of calcium sulfate (Drierite). After 6 hr. under rapid reflux, paper chromatography showed no more alanine to be present. The dark solution was concentrated to a small volume and dry ether added to give crude alanine p-nitrobenzyl ester p-toluenesulfonate as a yellow-orange powder, 42.20 g., m.p. 140–145°. Crystallization from 250 ml. of isopropyl alcohol yielded pale yellow needles, 36.38 g. (92%), m.p. 155–157.5°. A second crystallization from isopropyl alcohol raised the melting point to 156–158°; R_f 0.64 (nin +, UV +, one spot only); λ_{max}^{MeOH} 263 mμ, ε 10,500; $[\alpha]^{29}D$ −4° (c 1, methanol).

Anal. Calcd. for $C_{17}H_{20}N_2O_7S$: C, 51.51; H, 5.09; N, 7.07. Found: C, 51.56; H, 4.82; N, 6.84.

The original ether filtrate was washed twice with water, once with 1 N potassium bicarbonate and the ether distilled. The residue was slurried with benzene and the p-nitrobenzyl alcohol filtered and dried. Recovery 41.7 g. (68%), m.p. 96.5–98° (lit.,[10] m.p. 93.4–93.6°).

Phenylalanine p-Nitrobenzyl Ester p-Toluenesulfonate.—Phenylalanine (16.5 g., 0.1 mole), p-toluenesulfonic acid monohydrate (95.0 g., 0.5 mole) and 76.5 g. (0.5 mole) p-nitrobenzyl alcohol yielded 45.0 g. (95%) desired ester, yellow needles from isopropyl alcohol, m.p. 180–182°; R_f 0.88 (nin +, UV +, one spot only); λ_{max}^{MeOH} 263 mμ, ε 10,600; $[\alpha]^{27}D$ +0.5° (c 1, methanol).

Anal. Calcd. for $C_{23}H_{24}N_2O_7S$: C, 58.46; H, 5.12; N, 5.93. Found: C, 58.55; H, 5.12; N, 5.58.

The above ester (1.0 g.) in 10 ml. of methanol was treated with 4.0 ml. of 4 N lithium hydroxide and the solution allowed to stand 1 hr. at room temperature. The solution was concentrated to a small volume, diluted with water, the pH adjusted to 6 with 2 N hydrochloric acid, and p-nitrobenzyl alcohol removed by chloroform extraction. The aqueous layer was concentrated to about 8 ml. and cooled to give phenylalanine, 0.25 g. (71%), identical with authentic phenylalanine on paper chromatography, $[\alpha]^{27}D$ −34° (c 1, water). Authentic phenylalanine had $[\alpha]^{26}D$ −34° (c 1, water).

Histidine p-Nitrobenzyl Ester Di-p-toluenesulfonate.—From 21.0 g. (0.1 mole) of histidine hydrochloride monohydrate, 95.0 g. (0.5 mole) of p-toluenesulfonic acid monohydrate and 76.5 g. of (0.5 mole) of p-nitrobenzyl alcohol was obtained, after crystallization from methanol–isopropyl alcohol, 61.0 g. (96%) of p-nitrobenzyl ester as tiny needles, m.p. 217–219°; R_f 0.54 (nin +, UV +, one spot only); $[\alpha]^{24}D$ +6° (c 1, methanol).

Anal. Calcd. for $C_{27}H_{30}N_4O_{10}S_2$: C, 51.09; H, 4.76; N, 8.33. Found: C, 50.77; H, 4.64; N, 8.64.

Serine p-Nitrobenzyl Ester p-Toluenesulfonate.—Serine (10.51 g., 0.1 mole), p-toluenesulfonic acid monohydrate (95.0 g., 0.5 mole) and p-nitrobenzyl alcohol (76.5 g., 0.5 mole) gave the p-

(6) R. J. Block, *Anal. Chem.*, **22**, 1327 (1950).

(7) D. Bolling, H. A. Sober, and R. J. Block, *Federation Proc.*, **8**, 185 (1949).

(8) R. H. Mazur, B. W. Ellis, and P. S. Cammarata, *J. Biol. Chem.*, **237**, 1619 (1962).

(9) G. Amiard, R. Heymes, and L. Velluz, *Bull. Soc. chim.*, 191 (1955).

(10) R. Paul and N. Joseph, *ibid.*, 550 (1952).

nitrobenzyl ester, needles from isopropyl alcohol, m.p. 158–163°, 38.76 g. (94%); R_f 0.58 (nin +, UV +, one spot only); $[\alpha]^{26}D$ −18° (*c* 1, methanol.).

Anal. Calcd. for $C_{17}H_{20}N_2O_8S$: C, 49.51; H, 4.89; N, 6.80. Found: C, 49.31; H, 4.99; N, 6.58.

A sample of the above ester (0.412 g.) in 6 ml. of 1 *N* hydrochloric acid was hydrogenated over 0.40 g. 10% palladium on carbon. The catalyst was rinsed with 4 ml. of 1 *N* hydrochloric acid. The combined filtrates, now 1% in serine, had $[\alpha]^{24}D$ +16° and paper chromatography showed no unchanged serine *p*-nitrobenzyl ester present. Authentic serine had $[\alpha]^{25}D$ +14° (*c* 1, 1 *N* hydrochloric acid).

Carbobenzoxyprolylphenylalanine *p*-Nitrobenzyl Ester (V).—Phenylalanine *p*-nitrobenzyl ester toluenesulfonate (42.0 g., 0.089 mole), 13.6 ml. (0.098 mole) of triethylamine, 24.3 g. (0.0975 mole) of carbobenzoxyproline[11] and 20.2 g. (0.098 mole) of dicyclohexylcarbodiimide following the procedure for compound II yielded crude V as an oil which solidified on standing. Crystallization from ethyl acetate–cyclohexane gave 33.0 g. (70%) of carbobenzoxyprolylphenylalanine *p*-nitrobenzyl ester as needles, m.p. 91–92°; $[\alpha]^{23}D$ −53° (*c* 1, chloroform).

Anal. Calcd. for $C_{29}H_{29}N_3O_7$: C, 65.52; H, 5.50; N, 7.91. Found: C, 65.14; H, 5.63; N, 8.07.

Prolylphenylalanine *p*-Nitrobenzyl Ester (VI).—The above dipeptide (10.6 g., 0.02 mole) in 50 ml. of 2 *N* hydrobromic acid in acetic acid was allowed to stand 2 hr. at room temperature. The solution was diluted with a large volume of ether and the residual oil, after decantation of the ether, taken up in water. The solution was washed twice with ether, the pH adjusted to 7 with sodium bicarbonate, the desired ester extracted with ethyl acetate, the ethyl acetate dried over magnesium sulfate, and distilled to yield compound VI as an oil which solidified on standing. Yield 6.20 g. (78%), m.p. 64–72°. Crystallization from isopropyl acetate–hexane raised the melting point to 74–75°; R_f 0.88 (nin +, UV +, one spot only); $[\alpha]^{26}D$ −33° (*c* 1, methanol).

Anal. Calcd. for $C_{21}H_{23}N_3O_5$: C, 63.46; H, 5.83; N, 10.58. Found: C, 63.48; H, 5.91; N, 10.89.

Ditritylhistidylprolylphenylalanine *p*-Nitrobenzyl Ester (VII).—Ditritylhistidine[9] (41.2 g., 0.063 mole), prolylphenylalanine *p*-nitrobenzyl ester (25.0 g., 0.063 mole) and 14.5 g. (0.069 mole) dicyclohexylcarbodiimide in methylene chloride gave 67.8 g. of compound VII as a brittle foam. Crystallization from ethyl acetate–cyclohexane gave ditritylhistidylprolylphenylalanine *p*-nitrobenzyl ester as irregular prisms, 51.0 g. (68%), m.p. 127–135° (effervescence). Recrystallization from ethyl acetate–cyclohexane raised the melting point to 131–140° (effervescence); $[\alpha]^{26}D$ −20° (*c* 0.7, methanol). The tripeptide would not crystallize in the absence of cyclohexane; analysis and n.m.r. showed the presence of two moles of cyclohexane of crystallization (yield and rotation calculated on this basis).

Anal. Calcd. for $C_{65}H_{58}N_6O_6 \cdot 2C_6H_{12}$: C, 77.88; H, 6.95; N, 7.08. Found: C, 77.77; H, 6.91; N, 7.15.

Histidylprolylphenylalanine *p*-Nitrobenzyl Ester (VIII).—Protected tripeptide VII (5.0 g., 0.0042 mole) was dissolved in 75 ml. of acetic acid and 75 ml. of hot water added. The mixture was heated 10 min. at 60° and the solvents distilled at 50°. The residue was partitioned between water and methylene chloride, neutralized to pH 7 with sodium bicarbonate and the product extracted with methylene chloride. The methylene chloride solution was washed with water, dried over sodium sulfate, and distilled to give histidylprolylphenylalanine *p*-nitrobenzyl ester as a glass-like material, 1.67 g. (74%); R_f 0.59 (nin +, Pauly +, very strong), 0.42 (nin +, Pauly +, trace); $[\alpha]^{26}D$ −36° (*c* 1, methanol).

Anal. Calcd. for $C_{27}H_{30}N_6O_6$: C, 60.66; H, 5.66; N, 15.72. Found: C, 60.51; H, 5.79; N, 15.38.

O,N-Dicarbobenzoxytyrosine *p*-Nitrophenyl Ester (IX).—Dicarbobenzoxytyrosine[12] (8.98 g., 0.02 mole) and 3.06 g. (0.022 mole) of *p*-nitrophenol were dissolved in 50 ml. of methylene chloride. Dicyclohexylcarbodiimide (4.32 g., 0.021 mole) in 15 ml. of methylene chloride was added and the solution allowed to stand overnight at 5°. The filtrate after removal of dicyclohexylurea was washed with 1 *N* hydrochloric acid, 10% sodium sulfate, 1 *M* potassium carbonate (twice), 10% sodium sulfate, dried over sodium sulfate, and the methylene chloride distilled. The residual solid was slurried with cyclohexane to yield O,N-dicarbobenzoxytyrosine *p*-nitrophenyl ester, 10.95 g. (96%), m.p. 132–135°. Crystallization from ethyl acetate–cyclohexane gave colorless needles, m.p. 137–138°; λ_{max}^{MeOH} 266 mμ, ε 9,300; $[\alpha]^{24}D$ −9° (*c* 1, methanol).

Anal. Calcd. for $C_{31}H_{26}N_2O_9$: C, 65.26; H, 4.59; N, 4.91. Found: C, 65.30; H, 4.78; N, 5.28.

Carbobenzoxytyrosylprolylphenylalanine (X).—Finely powdered prolylphenylalanine methyl ester hydrobromide[1] (3.57 g., 0.01 mole) and 4.80 g. (0.0084 mole) of dicarbobenzoxytyrosine *p*-nitrophenyl ester were dissolved in 20 ml. of chloroform and 1.54 ml. (0.011 mole) of triethylamine added. The solution was allowed to stand 72 hr. at room temperature, diluted with chloroform, washed with 1 *N* hydrochloric acid, thrice with 1 *M* potassium carbonate, once with 10% sodium sulfate, the chloroform dried over sodium sulfate, and distilled.

The residual glass-like substance (5.95 g.) was dissolved in 30 ml. of methanol, 10 ml. of 4 *N* lithium hydroxide added and the solution allowed to stand 1 hr. at room temperature. The solution was diluted with 50 ml. of water, acidified with 4 *N* hydrochloric acid and the product extracted with ethyl acetate. The ethyl acetate was washed with 10% sodium sulfate, dried over sodium sulfate and distilled to yield carbobenzoxytyrosylprolylphenylalanine as a glass-like material 5.56 g. Countercurrent distribution, thirty transfers, 5-ml. phases, methanol–water–chloroform–carbon tetrachloride 37:10:26:27, indicated the product to be homogeneous, $K = 1.14$.

The crude product slowly solidified under cyclohexane and the resulting powder had m.p. 78–80° with softening from 50°. Analysis and n.m.r. indicated the presence of cyclohexane of solvation. $[\alpha]^{26}D$ −25° (*c* 1, methanol); λ_{max}^{MeOH} 278 mμ, ε 1,400.

Anal. Calcd. for $C_{31}H_{33}N_3O_7 \cdot C_6H_{12}$: C, 69.03; H, 7.05; N, 6.53. Found: C, 69.27; H, 7.14; N, 6.30.

Tyrosylprolylphenylalanine (XI).—Compound X (2.80 g., 0.005 mole) in 20 ml. of 2 *N* hydrobromic acid in acetic acid was allowed to stand 2 hr. at room temperature. The solvent was distilled under vacuum and the residue rubbed with ether to give the tripeptide hydrobromide as a pale yellow powder. The crude salt was dissolved in water and the tripeptide liberated by passage through a column of weakly basic anion exchange resin (IR-45, hydroxide form). Paper chromatography showed one principal product, R_f 0.70, with small amounts of impurities at R_f 0.28 and R_f 0.44. The crude tripeptide, 2.00 g., was purified by countercurrent distribution, thirty transfers, 5-ml. phases, *n*-butyl alcohol–water 1:1, $K = 1.95$. From tubes 15–25 was isolated 1.29 g. (61%) of tyrosylprolylphenylalanine, powder under acetone, gas evolution above 210° (no m.p.), R_f 0.70 (nin +, Pauly +, one spot only); $\lambda_{max}^{H_2O}$ 274 mμ, ε 1,070; $[\alpha]^{26}D$ −9° (*c* 1, water).

Anal. Calcd. for $C_{23}H_{27}N_3O_5$: C, 64.92; H, 6.40; N, 9.88. Found: C, 64.44; H, 6.96; N, 9.45.

Prolylphenylalanine (XII).—Prolylphenylalanine methyl ester hydrobromide[1] (10.71 g., 0.03 mole) in 20 ml. of water was treated with cold 50% potassium carbonate and the dipeptide ester extracted with ethyl acetate. The ethyl acetate was dried over sodium sulfate, distilled, and the residue let stand 1 hr. at room temperature with 50 ml. of 2 *N* lithium hydroxide in 50% methanol. Neutralization of the solution with exactly one equivalent of 4 *N* hydrochloric acid caused the desired dipeptide to crystallize, 6.85 g. (87%), m.p. 230–233° dec. Recrystallization from aqueous acetic acid raised the m.p. to 234–236° dec. Paper chromatography showed one spot only, R_f 0.64 (nin +); $[\alpha]^{26}D$ −37° (*c* 1, 1 *N* hydrochloric acid). [Lit.,[13] m. p. 247°; $[\alpha]^{20}D$ −41° (*c* 5, 20% hydrochloric acid).]

Anal. Calcd. for $C_{14}H_{18}N_2O_3$: C, 64.10; H, 6.92; N, 10.68. Found: C, 63.79; H, 6.67; N, 10.64.

Glycylphenylalanine Methyl Ester Hydrobromide (XIII).—Phenylalanine methyl ester hydrochloride[14] (4.30 g., 0.02 mole), 2.94 ml. (0.021 mole) of triethylamine, 4.18 g. (0.02 mole) of carbobenzoxyglycine[15] and 4.53 g. (0.022 mole) of dicyclohexylcarbodiimide in methylene chloride gave carbobenzoxyglycylphenylalanine methyl ester as an oil. The latter in 40 ml. of 2 *N* hydrobromic acid in acetic acid was allowed to stand 2 hr. at room temperature, the solution concentrated to a small volume and diluted with ether to give a white powder, 5.87 g. (93%), m.p. 167–170° (gas evolution). Crystallization was effected by dissolving in five volumes methanol, adding fifty volumes of ethyl acetate and concentrating until crystallization began.

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The dipeptide ester hydrobromide XIII was obtained as long needles, m.p. 176–177° (gas evolution); R_f 0.61 (nin +, one spot only); $[\alpha]^{26}D$ +14° (c 1, methanol).

Anal. Calcd. for $C_{12}H_{16}N_2O_3 \cdot HBr$: C, 45.44; H, 5.40; N, 8.83; Br, 25.20. Found: C, 45.33; H, 5.63; N, 8.87; Br, 25.48.

Ditritylhistidylglycylphenylalanine Methyl Ester (XIV).—Ditritylhistidine[9] (6.39 g., 0.01 mole), glycylphenylalanine methyl ester hydrobromide (3.17 g., 0.01 mole), 1.50 ml. (0.011 mole) of triethylamine and 2.27 g. (0.011 mole) of dicyclohexylcarbodiimide in methylene chloride yielded the protected tripeptide XIV, 7.88 g. (92%), as a powder, m.p. 110–115°. Recrystallization from acetone–cyclohexane by concentrating to remove acetone yielded a microcrystalline powder, m.p. 110–115°, R_f 0.93 (nin −, Pauly −, *t*-BuOCl +, one spot only); $[\alpha]^{26}D$ −8° (c 1, methanol). Analysis and n.m.r. indicated that the product contained cyclohexane of crystallization.

Anal. Calcd. for $C_{56}H_{51}N_5O_4 \cdot C_6H_{12}$: C, 79.03; H, 6.74; N, 7.43. Found: C, 79.38; H, 6.82; N, 7.37.

Ditritylhistidylproline Methyl Ester (XV).—Ditritylhistidine[6] (7.73 g., 0.012 mole), proline methyl ester hydrochloride[16] (2.16 g., 0.013 mole), 2.0 ml. (0.014 mole) of triethylamine, 3.74 g. (0.013 mole) of dicyclohexylcarbodiimide in methylene chloride gave the protected dipeptide as a powder, 8.44 g. Chromatography on silica gel and elution with 40% ethyl acetate-benzene yielded 7.38 g. (82%) XV, m.p. 207–214°. Crystallization from acetone–cyclohexane gave needles, m.p. 212–216°, $[\alpha]^{25}D$ +29° (c 1, methanol); R_f 0.92 (*t*-BuOCl +, one spot only).

Anal. Calcd. for $C_{50}H_{46}N_4O_3$: C, 79.97; H, 6.18; N, 7.46. Found: C, 79.86; H, 6.28; N, 7.18.

Ditritylhistidylproline (XVI).—Protected dipeptide XV (3.75 g., 0.005 mole) was hydrolyzed as described for compound III. The crude product was crystallized from acetone–cyclohexane to yield ditritylhistidylproline, 2.66 g. (72%), gas evolution from 115°. On drying 2 hr. under vacuum at 100°, the product apparently lost solvent of crystallization and melted from 157° (no meniscus); $[\alpha]^{25}D$ −26° (c 1, methanol); R_f 0.92 (*t*-BuOCl +, one spot only).

Anal. Calcd. for $C_{49}H_{44}N_4O_3$: C, 79.86; H, 6.02; N, 7.60. Found: C, 79.83; H, 6.58; N, 7.32.

Compounds XV and XVI behaved quite differently on standing in 50% acetic acid at room temperature. Tr·Tr·His·Pro·OCH_3 (XV) had completely reacted after 1 hr. and yielded about equal amounts of His·Pro·OCH_3, R_f 0.24 (nin +, Pauly +, *t*-BuOCl +) and His·Pro (cyclic), R_f 0.30 (nin −, Pauly +, *t*-BuOCl +). Tr·Tr·His·Pro (XVI) also had completely reacted after 1 hr. but the only product was His·Pro, R_f 0.08 (nin +, Pauly +, *t*-BuOCl +). At 90°, the initially formed dipeptide was rapidly converted to His·Pro (cyclic).

Histidylproline Diketopiperazine (I).—Ditritylhistidylproline methyl ester (15.02 g., 0.02 mole) in 150 ml. of 50% acetic acid was heated 2 hr. on the steam bath and concentrated to dryness. The residue was concentrated twice more to dryness with water, slurried with water and the triphenylcarbinol (9.98 g., 96%) removed by filtration. Acetic acid was removed by passing the solution through an IR-45 ion exchange column (hydroxide form) and lyophilization of the eluate yielded the diketopiperazine (3.82 g., 82%) as a brittle foam, R_f 0.29 (nin −, Pauly +, *t*-BuOCl +, one spot only). This material proved to be extremely difficult to crystallize. On standing for 6 weeks, part of the product had solidified. From a boiling ethyl acetate extract of the partially crystallized material was obtained, on cooling, 0.12 g. of thick prisms, m.p. 167–170°. Recrystallization from ethyl acetate changed the m.p. to 168–170°. The crystalline material and the crude product behaved identically on paper chromatography; $[\alpha]^{25}D$ −66° (c 1, water).

Anal. Calcd. for $C_{11}H_{14}N_4O_2$: C, 56.40; H, 6.02; N, 23.92. Found: C 56.39; H, 5.84; N, 24.05.

Prolylhistidine (XVII).—Carbobenzoxyproline *p*-nitrophenyl ester[17] (9.26 g., 0.025 mole), 15.86 g. (0.025 mole) of histidine *p*-nitrobenzyl ester di-*p*-toluenesulfonate and 7.6 ml. (0.055 mole) of triethylamine were dissolved in 200 ml. of methylene chloride and allowed to stand 1 week at room temperature. The reaction mixture was washed thrice with water, thrice with 0.2 *N* potassium hydroxide and with water until neutral. The organic layer was dried over magnesium sulfate and the solvent distilled to give carbobenzoxyprolylhistidine *p*-nitrobenzyl ester as a thick oil, 9.85 g. (76%), R_f 0.73 (nin −, Pauly +, *t*-BuOCl +, one spot only).

Protected dipeptide (5.43 g., 0.0104 mole) in 250 ml. of methanol plus 2.65 ml. (0.032 mole) of 12 *N* hydrochloric acid was hydrogenated over 1.2 g. of palladium black. The solution after removal of the catalyst was taken to dryness, the residue dissolved in 50 ml. of water and passed through an IR-45 anion exchange column (hydroxide form) to liberate the dipeptide from its hydrochloride salt. The eluate was distilled to dryness and the residue crystallized by dissolving in 3 ml. of water and adding 30ml. of methanol. Prolylhistidine was obtained as fine prisms, 1.90 g. (72%), m.p. 191–192° (effervescence), R_f 0.12 (nin + (yellow), *t*-BuOCl +, one spot only); $[\alpha]^{25}D$ −22° (c 1, water).

Anal. Calcd. for $C_{11}H_{16}N_4O_3 \cdot 1/2H_2O$: C, 50.56; H, 6.56; N, 21.45. Found: C, 50.41; H, 6.57; N, 21.41.

A sample was dried 2 hr. at 100° under high vacuum.

Anal. Calcd. for $C_{11}H_{16}N_4O_3$: C, 52.37; H, 6.39; N, 22.21. Found: C, 52.28; H, 6.42; N, 22.16.

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The Effect of Initiators on the "Homogeneous" Friedel–Crafts Isomerization of Hexane

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The effect of hexene-1 and of ethylene and hydrogen bromide as co-catalysts with aluminum bromide for the isomerization of an *n*-paraffin has been studied. A kinetic analysis of the data with ethylene and hydrogen bromide suggests a dual role for the co-catalyst: that of facilitating carbonium ion formation and increasing rates at low initiator levels, and of facilitating inhibition at high initiator levels due to allylic hydride transfer between ionic and olefinic intermediates. The postulate of allylic hydride transfer leading to inhibition is supported by the effect of hexene-1 upon the reaction.

The structural rearrangement of a paraffin is customarily facilitated by the use of strong acid catalysts. Usually the reaction is conducted in a two-phase system since highly polar acids are not particularly compatible with nonpolar paraffins. Such systems are neither easily adaptable to kinetic analysis, nor to mechanistic interpretation of reaction paths.

This paper is primarily concerned with the problem of understanding the effect of a co-catalyst upon the rate of isomerization of an *n*-paraffin. The studies were