Failure Sequence in Solid-Phase Peptide Synthesis Due to the Presence of an *N*-Alkylamino Acid[1,2]

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Abstract: In an attempted synthesis of the octapeptide, Asp-Arg-Val-Tyr-Ile-His-Pro-MePhe ([MePhe^8]-angiotensin II), by the solid-phase procedure, the N-terminal hexapeptide was obtained as the main product. Further investigation revealed that because of the presence of the C-terminal *N*-methylphenylalanine residue, prolyl-*N*-methylphenylalanine was cleaved from the polymer, as *cyclo*-(Pro-MePhe-), during neutralization of the α-amine hydrochloride salt of the dipeptide polymer while Boc-N^{im}-benzylhistidine was acylated on the hydroxymethyl polymer so formed. The cleavage of the C-terminal dipeptide is not peculiar to the sequence with proline, but to a secondary amino group on the C-terminal amino acid attached to the polymer. However, the extent of cleavage depends upon the nature of the *N*-alkylamino acid. Whereas 80–85% of Pro-MePhe and Ala-MePhe were cleaved from the polymer, cleavage of Pro-Pro and Ala-Pro occurred to the extent of 40–60%. The hexapeptide, Asp-Arg-Val-Tyr-Ile-His ([Des-(Pro^7,Phe^8)]-angiotension II), has 0.04% pressor activity of Hypertensin Ciba and very slight myotropic activity, but no antagonistic activity to angiotensin II. These results indicate the importance of the C-terminal part of angiotension II for pressor or antagonistic activities.

A number of studies have been undertaken to determine the conformation of angiotensin II and the results obtained appear contradictory.[3] Recently it has been suggested[4] that angiotensin II does possess a definite conformation or several forms in equilibrium in solution and that the protons in the peptide backbone participate in hydrogen bonding. If this is so, substitution of natural amino acids in the peptide by *N*-methylamino acids should markedly affect peptide conformation and biological activity by disrupting hydrogen bonds and decreasing the rigidity of peptide bonds involved. To test this view we thought it of interest to synthesize two series of angiotensin II analogs. In the first series, each amino acid in the angiotensin molecule was to be replaced with the corresponding *N*-methylamino acid consecutively, while in the second series these were to be replaced progressively. As a first step we decided to synthesize the octapeptide, Asp-Arg-Val-Tyr-Ile-His-Pro-MePhe ([8-(*N*-methylphenylalanine)]-angiotensin II) by the solid-phase procedure of Merrifield[5] incorporating various recently suggested modifications.[6] In the course of this synthesis a failure resulted which may have been observed by others[7] but has gone unrecognized.

Results and Discussion

Boc-*N*-methylphenylalanine was esterified to the chloromethyl polymer and the Boc group was removed with 50% CF_3COOH in CH_2Cl_2. Further coupling with Boc-proline to the *N*-methylphenylalanine polymer ester was difficult. Three consecutive couplings were required to obtain an equal ratio of proline and *N*-methylphenylalanine. Similar difficulty was also experienced by Andreatta and Scheraga[7] when they attempted to couple Boc-N^{im}-benzylhistidine to the *N*-methylalanylphenylalanine polymer ester. The next six amino acids were added under the usual conditions. However, at the end of the synthesis the purified compound contained aspartic acid, arginine, valine, tyrosine, and histidine while only a trace of proline was present and isoleucine was high. Since the chromatographic peak for *N*-methylphenylalanine merged into that of isoleucine and since ninhydrin color intensity of this amino acid is low, it was difficult to judge the presence or absence of *N*-methylphenylalanine. Similarly, elemental analysis of the compound obtained did not check for either the desired octapeptide or the desproline analog.

Recently Dorman and Markley[8] have suggested that 50% CF_3COOH in CH_2Cl_2 used for removing the Boc group partially cleaved peptides at every stage. Since this same reagent was used in this synthesis we thought that perhaps proline or C-terminal dipeptide was being cleaved in a similar manner. Another synthesis of this octapeptide was attempted substituting 1 *N* HCl in acetic acid as the deprotecting reagent. Hydrolysis of the Boc-prolyl-*N*-methylphenylalanyl polymer before and after removal of the Boc group gave an equal ratio of proline and *N*-methylphenylalanine. However, the addition of Boc-N^{im}-benzylhistidine did not yield the expected tripeptide. The possibility of formation of *cyclo*-(His-Pro-) was therefore studied. This speculation was based on a report by Mazur and Schlatter[9] that under mild acid treatment the tripeptide histidyl-

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(2) All amino acids are of the L variety. Abbreviations used are: Boc, *tert*-butyloxycarbonyl; MePhe, *N*-methylphenylalanine; DCI, *N*,*N*′-dicyclohexylcarbodiimide; DMF, *N*,*N*-dimethylformamide; Et_3N, triethylamine.

(3) F. M. Bumpus and R. R. Smeby in "Renal Hypertension," I. H. Page and J. W. McCubbin, Ed., Year Book Medical Publishers, Inc., Chicago, Ill., 1968, p 87.

(4) (a) P. Fromageot, private communication; (b) E. C. Jorgensen, S. R. Rapaka, G. C. Windridge, and T. C. Lee, *J. Med. Chem.*, **14**, 899 (1971).

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(6) (a) M. Manning, E. Coy, and W. H. Sawyer, *Biochemistry*, **9**, 3925 (1970); (b) F. C. Westall and A. B. Robinson, *J. Org. Chem.*, **35**, 2842 (1970); (c) F. C. H. Chou, R. K. Chawla, R. F. Kibler, and R. Shapira, *J. Amer. Chem. Soc.*, **93**, 267 (1971); (d) M. C. Khosla, R. R. Smeby, and F. M. Bumpus, unpublished work.

(7) R. H. Andreatta and H. A. Scheraga, *J. Med. Chem.*, **14**, 489 (1971).

(8) L. C. Dorman and L. D. Markley, *ibid.*, **14**, 5 (1971).

(9) R. H. Mazur and J. M. Schlatter, *J. Org. Chem.*, **28**, 1025 (1963).

prolylphenylalanine ester was cleaved to yield *cyclo*-(His-Pro-) and phenylalanine ester. A similar observation was also reported by Sivanandaiah, *et al.*,[10] when they tried to synthesize histidylhydroxyprolylphenylalanine methyl ester by the solution procedure. However, neither *cyclo*-(His-Pro-) nor proline could be observed in the filtrates following coupling reaction with Boc-N^{im}-benzylhistidine.

Since the problem may have occurred prior to coupling Boc-N^{im}-benzylhistidine, synthesis was conducted again and polymer and washings were analyzed at each stage to detect the lost components. The data of Table Ia indicate that the hydrolysate of C-terminal dipeptide

Table I. Amino Acid Content in the Hydrolysates of Peptide Polymer Esters after Each Step

	Expected peptide polymer ester	mmol of amino acid/g of polymer		
a.	Boc-His(Bzl)-Pro-MePhe-*P*[a]	His(Bzl)	Pro	MePhe
	Boc-MePhe-*P*			0.620
	Boc-Pro-MePhe-*P*		0.592	0.600
	HCl·H-Pro-MePhe-*P*		0.592	0.562
	H-Pro-MePhe-*P*		0.087	0.110
	Boc-His(Bzl)-Pro-MePhe-*P*	0.580	0.054	0.108
b.	Boc-His(Bzl)-Ala-MePhe-*P*	His(Bzl)	Ala	MePhe
	Boc-MePhe-*P*			0.700
	Boc-Ala-MePhe-*P*		0.634	0.704
	HCl·H-Ala-MePhe-*P*		0.572	0.764
	H-Ala-MePhe-*P*		0.056	0.083
	Boc-His(Bzl)-Ala-MePhe-*P*	0.552	0.020	0.060
c.	Boc-Ala-Pro-*P*		Ala	Pro
	HCl·H-Ala-Pro-*P*		0.708	0.708
	H-Ala-Pro-*P*		0.406	0.412
d.	Boc-Pro-Pro-*P*			Pro
	HCl·H-Pro-Pro-*P*			1.12
	H-Pro-Pro-*P*			0.678

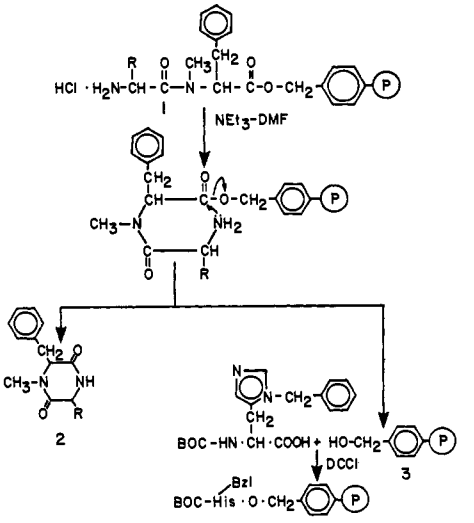
[a] *P* = -OCH_2–polystyrene–2% DVB copolymer.

polymer, before or after removal of the Boc group, gave an almost equal ratio of proline and *N*-methylphenylalanine and that the quantities of both these amino acids were present in the expected range. However, when the hydrochloride salt of this dipeptide was neutralized (10% Et_3N in DMF), almost 80–85% of proline and *N*-methylphenylalanine was removed from the polymer. Subsequent coupling with Boc-N^{im}-benzylhistidine followed by cleavage of the product from the polymer with HBr revealed that almost the entire quantity of Boc-N^{im}-benzylhistidine was directly esterified to the polymer. However, it was difficult to judge from these results whether the cleavage of C-terminal dipeptide was peculiar to the sequence histidylprolyl-*N*-methylphenylalanine, or whether it was entirely due to the presence of the *N*-methyl group. For the clarification of this point, synthesis of Boc-N^{im}-benzylhistidylalanyl-*N*-methylphenylalanyl polymer was undertaken. Analysis at each step revealed (Table Ib) exactly the same results as obtained with the former tripeptide, thereby suggesting that the cleavage of C-terminal dipeptide is not peculiar to the sequence with proline but to a secondary amino group on the C-terminal amino acid attached to the polymer. To confirm this, Boc-alanylproline polymer ester (Table Ic) and Boc-prolylproline polymer ester (Table Id) were also synthesized. In both cases, cleavage of the C-terminal dipeptide occurred at the neutralization step to the extent of 40–60%.

Investigation of the Et_3N–DMF wash in each case at the dipeptide stage revealed that none of the cleaved amino acids was present in free form. However, hydrolysis of the residue gave an equal ratio of both amino acids, and the quantities obtained accounted for the loss from the polymer. The combined Et_3N–DMF filtrates and washings, obtained during the neutralization step of the hydrochloride salt of the prolyl-*N*-methylphenylalanine polymer ester, were evaporated to dryness *in vacuo;* the product formed was isolated by repeated extraction of the cold aqueous solution of the residue (pH 3.5) with ethyl acetate. The crystalline compound obtained was found to be homogeneous on tlc. It gave a negative ninhydrin test but was easily detected with iodine vapor. On treatment with 6 *N* HCl at room temperature it gave a positive ninhydrin reaction, with a progressive increase in intensity, indicative of ring opening. Following acid hydrolysis, an equal ratio of proline and *N*-methylphenylalanine was obtained. The infrared spectrum lacked absorption due to amide N–H or carboxyl O–H in the region 3600–3200 cm^{-1} and a doublet at 1660 and 1640 cm^{-1} is assignable to the –CO–NR function in a six-membered cyclic system. The above results, along with the elemental analysis, suggest the formation of *cyclo*-(Pro-MePhe-) during the cleavage of Pro-MePhe from the polymer. Similar patterns of hydrolysis and infrared spectra were also obtained with other cyclic dipeptides thus obtained.

A plausible mechanism for the cleavage of C-terminal dipeptide is shown in Scheme I. When the hydro-

Scheme I



chloride (or trifluoroacetate) salt of a dipeptide polymer ester, which contains *N*-alkylamino acid as the C-terminal residue, is neutralized, the free dipeptide has a marked tendency toward intramolecular cyclization with the formation of the corresponding cyclic dipeptide **2**. The extent of cyclization in each case depends upon the nature of the C-terminal *N*-alkylamino acid. After the cleavage of cyclic dipeptide, the incoming Boc-amino acid, such as Boc-N^{im}-benzylhistidine in this case, is esterified on the hydroxymethyl polymer **3** in the presence of DCI. This esterification in the pres-

(10) K. M. Sivanandaiah, R. R. Smeby, and F. M. Bumpus, *Biochemistry*, 5, 1224 (1966).

ence of DCI was rather surprising since Beyerman and in't Veld[11] reported very poor yields when DCI was used as a coupling agent for the esterification of Boc-amino acid to the hydroxymethyl polymer. However, our experimental conditions were different than those reported by Beyerman and in't Veld in that we used DMF as the solvent instead of methylene chloride and our procedure utilized stirring under nitrogen. To confirm the results of esterification, an authentic sample of hydroxymethyl polymer[12] was prepared and treated under exactly the same conditions with Boc-N^{im}-benzylhistidine using DCI as the coupling agent. Weight increase[13] and amino acid analysis of the hydrolysate showed the expected amount of esterification.

On the basis of the above mechanism, the compound formed in the original synthesis of [MePhe^8]-angiotensin II should be the N-terminal hexapeptide. When the elemental analysis for this hexapeptide was calculated, it checked with the values found for C, H, and N.

A similar difficulty may have been observed by Andreatta and Scheraga[7] when attempting to couple Boc-N^{im}-benzylhistidine to *N*-methylalanylphenylalanine polymer ester. These authors suggested the coupling problems at this stage may be caused by the amino group of *N*-methylalanine being less susceptible to acylation than that of proline. In conclusion, it must be emphasized that extreme care is necessary during the synthesis of peptides by the solid-phase procedure, particularly if an *N*-alkylamino acid occurs in the sequence.

Biological Activity. The pressor activity of the N-terminal hexapeptide, Asp-Arg-Val-Tyr-Ile-His ([Des-(Pro^7,Phe^8)]-angiotensin II) was determined in the vagotomized, ganglion-blocked rat[14] to be 0.036% of the pressor activity of [Asn^1,Val^5]-angiotensin II (Hypertensin, Ciba). It should be pointed out that the N-terminal heptapeptide Asn-Arg-Val-Tyr-Ile-His-Pro ([Des-Phe^8]-angiotensin II) has been reported[15] to have 0.05% of the pressor activity of [Asn^1,Val^5]-angiotensin II. The myotropic activity was determined on rabbit aortic strip and the compound was found to have very slight myotropic activity and was inactive as an antagonist of angiotensin II. These results indicate the importance of the C-terminal part of angiotensin for pressor as well as antagonistic activities.

Experimental Section

All solvents used were spectral grade. DMF was dried over P_2O_5, distilled, and kept over Linde Molecular sieves 4A (4–8 mesh). CF_3COOH used for the cleavage of peptides from the polymer was freshly distilled. Solvents used for ascending paper chromatography (PC) on No. 1 Whatman filter paper and tlc were: (a) *n*-BuOH–AcOH–H_2O (BAW) (4:1:5); (b) *n*-BuOH–AcOH–H_2O–Pyr (BAWP) (30:6:24:20); (c) *n*-PrOH–AcOH–H_2O (PAW) (7:1:2); (d) *n*-BuOH–Pyr–H_2O (BPW) (65:35:65); tlc was carried out on Brinkmann silica gel or cellulose chromatogram plates.

Ionophoresis was carried out on filter paper strips (S & S 2043A gl) in a Beckman electrophoresis cell (Durrum type), Model R, series D at 400 V, using formic acid (60 ml)–acetic acid (240 ml) buffer diluted to 2 l. with water (pH 1.9). Glutamic acid was used as a reference compound, and *E*(Glu) indicates the electrophoretic mobility relative to glutamic acid. Detection of the compounds on the chromatograms was carried out with ninhydrin and/or with diazotized sulfanilic acid. Cyclic dipeptides were detected on silica gel tlc plates with iodine vapor.

For amino acid analysis, peptide–polymer was hydrolyzed overnight in a 1:1 mixture of 12 *N* HCl and propionic acid in a sealed tube under N_2 by the procedure reported by Scotchler, *et al.*[16] The free peptides were hydrolyzed in sealed tubes with 6 *N* HCl at 110° for 24 hr. Amino acid analyses were performed on a Jeolco-5 AH amino acid analyzer. The elemental analyses were performed by Micro-Tech Laboratories, Skokie, Ill.

Boc-*N*-methylphenylalanine. Methylation of Boc-*N*-methylphenylalanine was carried out by a modified procedure of Olsen.[17] A solution of Boc-Phe (11 g) in DMF (200 ml) was magnetically stirred at 45° with Ag_2O (25 g) in a 500-ml flask fitted with a reflux condenser. After 30 min, the mixture was cooled in ice–water (0–5°) and treated with a solution of CH_3I (17 ml) in DMF (50 ml). The mixture was stirred for 2 hr at 0–5° and 16 hr at room temperature. Solvent was removed *in vacuo* on a rotary evaporator, keeping the temperature below 30°, and the residue extracted with chloroform. The extract was washed with water, 5% citric acid solution, water, and saturated NaCl solution. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The residual oil was extracted with anhydrous ether (25 ml), the ethereal layer filtered, and the clear filtrate evaporated under N_2 to give Boc-Me-Phe-OMe as a pale yellow oil. The ir spectrum lacked absorption due to amide NH or carboxyl OH in the region of 3200–3600 cm^{-1}; absorption peaks were noted at 1730 and 1690 cm^{-1} (C=O). If the ir spectrum showed the presence of bands in the region of 3200–3600 cm^{-1}, the oil was treated again with MeI and Ag_2O as described above.

Anal. Calcd for $C_{16}H_{23}NO_4$: C, 65.45; H, 7.90; N, 4.77. Found: C, 65.35; H, 7.92; N, 4.86.

The above methyl ester was saponified by the procedure described by Olsen to give Boc-MePhe-OH as an oil (overall yield 75%). Silica gel tlc (MeOH) showed a homogeneous compound having R_f 0.61 as compared to R_f 0.45 obtained for Boc-Phe; ir (film) 3400–2500 cm^{-1} (carboxyl OH).

Anal. Calcd for $C_{15}H_{21}NO_4$: C, 64.49; H, 7.75; N, 5.01. Found: C, 64.10; H, 7.74; N, 5.01.

A solution of Boc-MePhe (5 mg) in 1:1 trifluoroacetic acid–methylene chloride was kept at room temperature for 1 hr, the solvent removed *in vacuo*, and the residue washed with ether. The crystalline trifluoroacetate salt so obtained showed one peak corresponding to *N*-methylphenylalanine on amino acid analyzer, while the peak for phenylalanine was totally absent.

Boc-*N*-methylphenylalanine Polymer Ester. A mixture of 10 g of bromomethyl polymer,[18] Boc-MePhe (3.35 g; 12 mmol) and Et_3N (1.78 ml; 12 mmol), and DMF (60 ml) was stirred magnetically for 36 hr at room temperature. The polymer was filtered and washed with DMF, CH_2Cl_2, EtOH, 0.5 *N* AcOH, H_2O, and MeOH. The polymer was dried over P_2O_5 in a vacuum desiccator for 36 hr. Amino acid analysis of the hydrolysate of an aliquot and weight increase (1.869 g) indicated that 0.565 mmol of Boc-MePhe was esterified per gram of the polymer.

General Procedure for Solid-Phase Peptide Synthesis. Synthesis was carried out basically according to the method developed by Merrifield[5] with some modifications.[6] The manual apparatus used consisted of a cylindrical vessel fitted at the bottom with a fritted coarse glass disk, in which percolation and filtration were carried out under N_2 pressure.[6d] The following cycle of reactions was used to introduce each new residue: (1) washed with glacial acetic acid (three times for 3 min); (2) washed with methylene chloride (three times for 3 min); (3) Boc group removed by treatment with 50% (v/v) CF_3COOH in CH_2Cl_2 for 25 min; a prewash with this reagent for 2 min was necessary to avoid dilution of CF_3COOH by the previous CH_2Cl_2 wash (when 1 *N* HCl in acetic acid (30 min) was used as the deblocking agent, step 2 was deleted and the polymer was washed three times with glacial acetic acid after the cleavage of the Boc group); (4) washed with CH_2Cl_2 (five times for 2 min); (5) washed with DMF (three times for 2 min); (6) free amino group liberated by treatment with 50 ml of 10% of Et_3N in DMF (two times for 6 min); (7) washed with DMF until free of

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chloride; (8) washed with CH_2Cl_2 (three times for 2 min); (9) Boc-amino acid (two-fold excess) in CH_2Cl_2 was added and mixed for 10 min; Boc-Arg(NO_2) and Boc-N^{im}-benzylhistidine were dissolved in DMF and both these amino acids were used in threefold excess; (10) added DCI (twofold excess) in CH_2Cl_2 and mixed for 2.5 hr; (11) washed with DMF (three times for 3 min); (12) steps 9 and 10 repeated using 1:1 DMF–CH_2Cl_2 as the solvent; (13) washed with DMF (three times for 3 min); (14) washed with a 1:2 mixture of methanol–chloroform (three times for 2 min).

Aspartylarginylvalyltyrosylisoleucylhistidine. Boc-*N*-methylphenylalanine polymer ester (5.75 g, 3.25 mmol of *N*-methylphenylalanine) was placed in the reaction vessel and the Boc group removed at each cycle with 50% trifluoroacetic acid in CH_2Cl_2. Boc-amino acids coupled successively were: proline, N^{im}-benzylhistidine, isoleucine, *O*-benzyltyrosine, valine, nitroarginine, and β-benzylaspartic acid. At the end, the polymer, after washing once with CF_3COOH, was suspended in freshly distilled CF_3COOH and a slow stream of HBr (prewashed through a gas washer containing 10% resorcinol in acetic acid) was passed through the suspension for 35 min. The mixture was filtered and washed twice with CF_3COOH and the combined filtrates were evaporated to dryness. The oily residue was triturated with dry ether and the powdery mass filtered, washed twice with dry ether, and dried *in vacuo* over P_2O_5 and KOH pellets. The crude product (3.00 g) was hydrogenated over palladium black (2 g) in a mixture of methanol–acetic acid–water (5:1:1) under 2 atm of H_2 for 36 hr to give 2.62 g of the crude, unblocked peptide. The product was dissolved in deionized water, a small amount of insoluble material removed by filtration, and the solution passed through a column of anion exchange resin IRA-410 (acetate form) (column dimensions: 2 × 75 cm). The product was eluted with additional water and the aqueous eluant indicating absorption at 280 mμ was pooled and lyophilized. Silica gel tlc (BAW) of the product showed one major component and two minor components.

A portion of the lyophilized powder (600 mg) was dissolved in a minimum volume of 0.01 *N* acetic acid and the solution subjected to purification on a column of CM cellulose (4 × 75 cm) by the procedure reported by Khairallah, *et al.*[19] The major component yielded 0.417 g of amorphous powder: loss of water at 100°, 4.84%; $[\alpha]^{25}D$ −4.5° (*c* 1, AcOH); tlc (cellulose) R_f 0.21 (BAW), R_f 0.26 (BAWP), R_f 0.50 (PAW); PC R_f 0.10 (BAW), R_f 0.43 (BAWP), *E*(Glu) 1.07. Amino acid ratios found were: Asp 0.92; Arg 1.00; Val 1.10; Tyr 0.80; Ile 1.11; His 1.03; Pro 0.00.

(19) P. A. Khairallah, I. H. Page, F. M. Bumpus, and R. R. Smeby, *Science*, **138**, 523 (1962).

Anal. Calcd for $C_{36}H_{53}N_{11}O_9 \cdot H_2O \cdot 2CH_3COOH$: C, 52.09; H, 6.80; N, 16.72. Found: C, 51.90; H, 6.67; N, 16.79.

Formation of *cyclo*-(Pro-MePhe-) during the Synthesis of Prolyl-*N*-methylphenylalanine Polymer Ester. From Boc-*N*-methylphenylalanyl polymer ester (3 g, containing 1.86 mmol of Boc-MePhe) the corresponding hydrochloride salt of prolyl-*N*-methylphenylalanine polymer ester was synthesized by the general procedure. The free amino group was liberated by treatment with 30 ml of 10% Et_3N in DMF (two times for 6 min). The polymer was washed with DMF and the effluent combined with the Et_3N wash. The polymer on hydrolysis at this stage showed very little proline or *N*-methylphenylalanine (Table Ia). The combined filtrates were evaporated to dryness on a rotary evaporator, the residue dissolved in water (15 ml), the pH adjusted to 3.5 with 1 *N* HCl under ice-cold conditions, and the aqueous solution extracted with AcOEt (six 10-ml portions). The organic phase was washed with H_2O (three 10-ml portions) and saturated NaCl solution (two 10-ml portions), dried (Na_2SO_4), and evaporated to give 250 mg of crude product. Crystallization from a small volume of AcOEt yielded *cyclo*-(Pro-MePhe-): mp 137° (softens at 132°); tlc (silica gel) R_f 0.68 (BAW), R_f 0.77 (BAWP), R_f 0.70 (BPW); amino acid ratios found were Pro 1.01, MePhe 1.00; ir (KBr) 2960, 2907, 1660, 1640 (d), 1453, 1395, 1294 cm^{-1}.

Anal. Calcd for $C_{15}H_{18}N_2O_2$: C, 69.72; H, 7.02; N, 10.85. Found: C, 69.96; H, 6.96; N, 10.86.

Similarly, filtrates obtained during the neutralization of the hydrochloride salts of alanyl-*N*-methylphenylalanyl polymer, alanylprolyl polymer and prolylprolyl polymer were evaporated *in vacuo*. Hydrolysis of the residue in each case gave an equal ratio of the corresponding acids in the expected range.

Boc-N^{im}-benzylhistidine Polymer Ester. Hydroxymethyl polymer (2 g) prepared according to the procedure reported by Stewart and Young[12] was washed with DMF and treated with a solution of Boc-N^{im}-benzylhistidine (1.726 g, 5 mmol) in DMF (30 ml). The mixture was stirred for 10 min followed by treatment with a solution of DCI (1.03 g; 5 mmol) in CH_2Cl_2 (5 ml). The mixture was percolated with N_2 for 4 hr, filtered, and washed with DMF. The polymer was again treated with Boc-N^{im}-benzylhistidine (0.863 g) and DCI (0.5 g) as above, and the mixture stirred overnight under N_2. The polymer was filtered, washed with DMF, MeOH, AcOH, $CHCl_3$, and EtOH, and dried over P_2O_5 and KOH pellets. Weight increase (0.444 g) and amino acid analysis of the hydrolysate indicated that 0.5 mmol of N^{im}-benzylhistidine was esterified per gram of the polymer.

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Communications to the Editor

A Complete Thermodynamic Analysis of the "Anomalous Order" of Amine Basicities in Solution[1,2]

Sir:

It has been recognized for many years[3–5] that the basicity order of simple aliphatic amines in aqueous solution at 25° (NH_3 < primary < secondary > tertiary) cannot be explained by any single cumulative substituent factor such as the inductive effect. The source of the peculiar inverted order has merited the attention of a number of able chemists.[6–9] It is not idiosyncratic to any alkyl series (such as the methylamines) nor is it limited to aqueous solution or 25°.

The availability of accurate gas-phase basicities,[10] when combined with appropriate thermodynamic properties for solution of the amines from the gas phase to water,[11] now makes it possible to separate all thermodynamic properties for the ionization into internal (gas phase) terms and those for solvation. Previous estimates of relative gas-phase basicities

(1) Taken in part from the thesis of F. M. J., III, University of Pittsburgh, 1971.

(2) Supported by grants from the National Science Foundation (GP-6550-X) and the Office of Saline Water.

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