Ammonolysis of *N*-Acylpyroglutamic Acid. Permanganate-oxidation of Peptides. II.

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Synopsis. The reaction of *N*-acylpyroglutamic acid with ammonia proceeds in two pathways. The one results in *N*-acylglutamine as a product, and the other in pyroglutamic acid and an amide formed from the *N*-acyl group. The ratio of the yields of the two reactions changes with the *N*-acyl group, being correlated with the acidity of the carboxylic acid from which the *N*-acyl group is derived, and also with the steric hindrance of the group.

In a previous paper,[1] it was reported that Z–Pro–OH[2] was oxidized to Z–pGlu–OH with $KMnO_4$ in the presence of strong acid. In further study, it has been found that Bz–Pro–OH is also oxidized to give Bz–pGlu–OH, and that the ammonolysis of Bz–pGlu–OH gives H–pGlu–OH as a main product. The latter result is in contrast to the well-known fact that Z–pGlu–OH is ammonolyzed to give Z–Gln–OH.[3] That is to say, two pathways seem possible in the ammonolysis of *N*-acyl–pGlu–OH.

In order to elucidate the factors determining the pathway, we synthesized several *N*-acyl–pGlu–OH's and their amides, and investigated the relationship between the *N*-acyl group and the reaction pathway.

Results and Discussion

The pathways in the ammonolysis of *N*-acyl–pGlu–OH are shown in Fig. 1. The reaction proceeds to produce *N*-acyl-Gln–OH with accompanying ring-opening in path I, and H–pGlu–OH and the amide in path II.

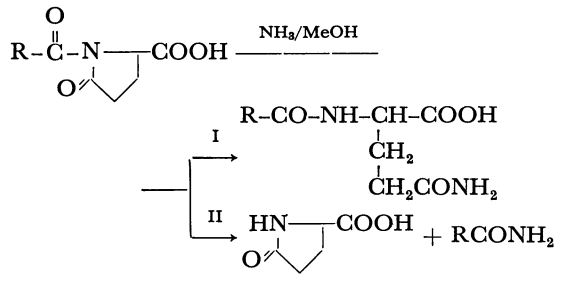

Fig. 1.

The results of ammonolysis are summarized in Table 1. Boc–pGlu–OH gave Boc–Gln–OH quantitatively, while pNBz–pGlu–OH gave H–pGlu–OH. The products vary with the acidity of the carboxylic acid from which the *N*-acyl group was derived. Namely, path II becomes predominant over I with increasing acidity of the carboxylic acid.

When the tosyl group was used as an *N*-protector, the product was Tos–Gln–OH only, although TosOH is a strong acid, showing deviation from the above correlation.

Ammonolysis of (A) Z–Ala–pGlu–NH_2 and (B) Z–Val–pGlu–NH_2 was carried out. In the case of (A), the peptide bond was cleaved almost quantitatively to give Z–Ala–NH_2 and H–pGlu–NH_2 (path II). In the case of (B), the reaction proceeded in two pathways, giving Z–Val–NH_2 and H–pGlu–NH_2 (path II), and Z–Val–Gln–NH_2 (path I) in the ratio 7 : 3.

Table 1. Ratio of the products in ammonolysis of *N*-acyl–pGlu–OH[a]

Acyl group	*N*-acyl–Gln–OH(Path I)	H–pGlu–OH (Path II)		pK_a[4]
Boc	1.00	0		
Z	0.83	0.17		
Ac	0.60	0.40	AcOH	4.67(5.84)[b]
Bz	0.15	0.85	BzOH	4.21(5.42)[b]
pNBz	0.04	0.96	pNBzOH	3.44(4.55)[b]
Tos	1.00	0	TosOH	1.70

a) Optically active (L-) in case of Boc, Z, and Tos; optically inactive in case of Ac, Bz, and pNBz. b) Measured in 50% MeOH at 27 °C.

The pK_a of Z–Ala–OH in 50% MeOH at 27 °C was determined to be 4.96, smaller than that of BzOH. Consequently the result of the ammonolysis of Z–Ala–pGlu–NH_2 is compatible with that of *N*-acyl–pGlu–OH. The pK_a of Z–Val–OH was 5.00 under the same conditions. It is conceivable that the ammonolysis of Z–Val–pGlu–NH_2 would give a similar result to that of Z–Ala–pGlu–NH_2. However, path I in the reaction of the valine peptide was not negligible. This shows that not only acidity but also steric hindrance of the *N*-acyl group affects the reaction pathway.

Z–Ala–pGlu–NH_2 and Z–Val–pGlu–NH_2 used in this study were obtained by the oxidation of Z–Ala–Pro–NH_2 and Z–Val–Pro–NH_2 by the method reported.[1]

Experimental

All melting points were uncorrected. NMR spectra were obtained on a Hitachi-Perkin Elmer R-20A Spectrometer, and IR spectra on a Hitachi Grating Infrared Spectrophotometer, Model 215. All *N*-acyl–pGlu–OH compounds were synthesized by the method reported.[3,5] All amino acids in peptide amides were of the L-series.

Ac–pGlu–OH·DCHA: Recrystallized from EtOH–EtOAc; yield[6] 32%; mp 174—175 °C; NMR (measured as free acid in $CDCl_3$) δ 2.54 (3H, s, $C\underline{H}_3$–CO), 2.00—2.90 (4H, m, CH–$C\underline{H}_2$–$C\underline{H}_2$–CO), 4.73 (1H, m, >$C\underline{H}$–COOH); IR (KBr) 1630, 1695, 1735 cm^{-1} (>C=O). Found: C, 64.83; H, 9.54; N, 8.11%. Calcd for $C_{19}H_{32}O_4N_2$: C, 64.74; H, 9.15; N, 7.95%.

Bz–pGlu–OH: Purified with a column of Sephadex LH-20 (solvent, benzene–EtOH–H_2O, 50 : 15 : 1), and recrystallized from EtOH–EtOAc; yield[6] 38%; mp 142—144 °C; NMR ($CDCl_3$–CD_3OD) δ 2.00—2.80 (4H, m, CH–$C\underline{H}_2$–$C\underline{H}_2$–CO), 4.88 (1H, m, >$C\underline{H}$–COOH), 7.20—7.80 (5H, m, $C_6\underline{H}_5$–CO); IR (KBr) 1655, 1710, 1730, 1770 cm^{-1} (>C=O). Found: C, 61.43; H, 4.63; N, 5.95%. Calcd for $C_{12}H_{11}O_4N$: C, 61.80;

H, 4.75; N, 6.01%.

pNBz–pGlu–OH: Recrystallized from EtOAc; yield[6] 41%; mp 180—181 °C; NMR ($CDCl_3$–CD_3OD) δ 1.80—2.80 (4H, m, CH–C$\underline{H}_2$–C$\underline{H}_2$–CO), 4.92 (1H, m, >C$\underline{H}$–COOH), 7.50—8.40 (4H, m, NO_2–$C_6\underline{H}_4$–CO); IR (KBr) 1695, 1705, 1750 cm^{-1} (>C=O). Found: C, 51.60; H, 3.34; N, 10.23%. Calcd for $C_{12}H_{10}O_6N_2$: C, 51.81; H, 3.62; N, 10.07%.

Z–Ala–pGlu–NH$_2$: Recrystallized from EtOAc–ether; yield[7] 38%; mp 156—158 °C; $[\alpha]_D^{26}$ −67.6° (*c* 1.0, EtOH); NMR ($CDCl_3$–CD_3OD) δ 1.37 (3H, d, >CH–C$\underline{H}_3$), 1.90—2.80 (4H, m, CH–C$\underline{H}_2$–C$\underline{H}_2$–CO), 4.56 (1H, m, >C$\underline{H}$–$CONH_2$), 5.06 (2H, s, C_6H_5–C$\underline{H}_2$O), 5.43 (1H, q, NH–C$\underline{H}$–CO), 7.31 (5H, s, $C_6\underline{H}_5$–CH_2); IR (KBr) 1670, 1690, 1745 cm^{-1} (>C=O). Found: C, 57.79; H, 6.05; N, 12.44%. Calcd for $C_{16}H_{19}O_5N_3$: C, 57.65; H, 5.75; N, 12.61%.

Z–Val–pGlu–NH$_2$: Purified with a column of silica gel (solvent, $CHCl_3$–EtOAc, 1 : 4), and recrystallized from EtOAc–hexane; yield[7] 17%; mp 145—147 °C; $[\alpha]_D^{26}$ −33.1° (*c* 1.2, MeOH); NMR ($CDCl_3$–CD_3OD) δ 0.80 (3H, d, CH–C$\underline{H}_3$), 1.05 (3H, d, CH–C$\underline{H}_3$), 1.90–2.80 (5H, m, CH–C$\underline{H}_2$–C$\underline{H}_2$–CO and CH_3–C$\underline{H}$–CH_3), 4.65 (1H, m, >C$\underline{H}$–$CONH_2$), 5.09 (2H, s, C_6H_5–C$\underline{H}_2$–O), 5.50 (1H, m, NH–C$\underline{H}$–CO), 7.34 (5H, s, $C_6\underline{H}_5$–CH_2); IR (KBr) 1670, 1685, 1745 cm^{-1} (>C=O). Found: C, 60.08; H, 6.46; N, 11.89%. Calcd for $C_{18}H_{23}O_5N_3$: C, 59.82; H, 6.42; N, 11.63%.

Method for Ammonolysis and Analysis of Products. To 1—3 mg of samples was added NH_3-saturated MeOH at 0 °C. The resulting solutions were allowed to stand at 0 °C overnight, and concentrated *in vacuo*. The products were identified with authentic samples[8] by TLC[9] on Kieselgel 60F-254 plate. The products were separated also by preparative TLC.[10] Silica gel layers at the position containing the products were raked up and treated with 6 M HCl for 10 h at 110 °C for hydrolysis. The ratios of amino acids in the hydrolyzates were determined by an amino acid analyzer (JEOL JLC-6AS).

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References

1) I. Muramatsu, Y. Motoki, Y. Yabuuchi, and H. Komachi, *Chem. Lett.*, **1977**, 1253.

2) Z=benzyloxycarbonyl; Boc=*t*-butoxycarbonyl; Ac=acetyl; Bz=benzoyl; pNBz=*p*-nitrobenzoyl; Tos=*p*-toluenesulfonyl; H–pGlu–OH=pyroglutamic acid. The abbreviation for amino acids and peptides is in accordance with the rules of the IUPAC-IBU Commission of Biochemical Nomenclature.

3) H. Gibian and E. Klieger, *Ann. Chem.*, **640**, 145 (1961).

4) J. A. Dean, "Handbook of Chemistry," 11th ed McGraw Hill Company (1973).

5) E. Schröder and E. Klieger, *Ann. Chem.*, **673**, 196 (1964).

6) From *N*-acyl–Glu–OH.

7) From *N*-acyl–Pro–NH_2.

8) Z–Val–Gln–NH_2 was prepared from Z–Val–Glu–$(OMe)_2$ by treatment with NH_3; mp 274—276 °C; $[\alpha]_D^{26}$ −18.3° (*c* 0.6, AcOH). Found: C, 56.85; H, 7.08; N, 14.51%. Calcd for $C_{18}H_{26}O_5N_4$: C, 57.13; H, 6.93; N, 14.81%.

9) Solvents for *N*-acyl–pGlu–OH; *n*-BuOH–HCOOH–H_2O, 4 : 1 : 1; *n*-BuOH–AcOH–H_2O, 4 : 1 : 1; *n*-BuOH–pyridine–AcOH–H_2O, 4 : 1 : 1 : 2; for *N*-acyl–pGlu–NH_2; $CHCl_3$–MeOH–AcOH, 18 : 2 : 1; $CHCl_3$–EtOH–HCOOH, 18 : 2 : 1; $CHCl_3$–MeOH–AcOH–pyridine, 18 : 2 : 1 : 1.

10) Solvent for *N*-acyl–pGlu–OH; *n*-BuOH–HCOOH–H_2O, 4 : 1 : 1; for *N*-acyl–pGlu–NH_2; $CHCl_3$–MeOH–AcOH, 18 : 2 : 1.