peptide, *Rf* (A) 0.77, *Rf* (B) 0.93, single Ehrlich positive spot; *Anal.* Calcd. for $C_{73}H_{90}O_{21}N_{20}$: C, 55.36; H, 5.73; N, 17.69. Found: C, 55.27; H, 5.63; N, 17.88.

H-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Asn-Arg-OH (IV)—III (74 mg) was treated with anhydrous HF (5 ml) in the presence of anisole (0.7 ml) under ice-cooling for 50 min. After evaporation of the excess HF in vacuum, the residue was extracted with 1% AcOH (10 ml $\times$ 2) and the combined extracts were washed with peroxide free ether. The clear solution thus obtained was passed through a column of Dowex 1 $\times$ 2 (acetate form, 1.8 $\times$ 7 cm) and the column was washed with 2% AcOH. Sakaguchi reaction positive eluates were collected and lyophilized. The crude decapeptide (59 mg) was dissolved in H_2O (2 ml). A small amount of insoluble material was removed by centrifugation and the supernatant was applied on a column of CM-cellulose (1.8 $\times$ 15 cm), which was eluted with a linear gradient elution from H_2O (300 ml) in mixing chamber to 0.1M NH_4OAc (pH 6.5, 300 ml) in reservoir at a flow rate of 5 ml/4 min with an automated fraction collector. Fractions of 5 ml each were collected and the absorbancy of each fraction was determined at 280 nm. The fraction of tubes No. 40 to 51 were pooled and lyophilized to constant weight (41 mg). The product was dissolved in H_2O (0.6 ml) and submitted to preparative TLC (Wakogel B-5, 20 $\times$ 20 cm $\times$ 5) using the system of BuOH-pyridine-AcOH- H_2O (30:20:6:24) as a developing solvent. Zone corresponding to *Rf* 0.35 was separated and extracted with 1% AcOH. The extract was evaporated to small volume. The solution was applied on a column of CM-cellulose (1.8 $\times$ 12 cm), which was eluted with H_2O (200 ml) and then with 2% AcOH (200 ml). The Sakaguchi reaction positive fractions were pooled and lyophilized; amorphous powder, yield 21 mg (38%); mp 193–199° (decomp.); $[\alpha]_D^{25} + 40.2^\circ$ ($c=0.5$, H_2O); *Rf* (A) 0.13, *Rf* (B) 0.34, single ninhydrin, Ehrlich and Sakaguchi positive spot; amino acid ratios in the acid hydrolysate: Arg 1.92, Asp 1.02, Ser 0.96, Glu 1.03, Gly 2.14, Ala 1.00, Phe 1.02; amino acid ratios in the AP-M digest: Trp 1.05, Arg 1.95, (Asn+Ser) 1.72, Glu 0.93, Gly 2.10, Ala 1.00, Phe 0.96.

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Syntheses of [Phe(4NO₂)⁵, Tyr(Me)⁸]- and [Tyr(Me)⁵, Phe(4NO₂)⁸]- Bradykinin¹⁾

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Synthesis of [Tyr(Me)⁵, Tyr(Me)⁸]-bradykinin showing relatively high bradykinin-like activity and antibradykinin activity as well has been reported by Stewart, *et al.*³⁾ Such a peptide exhibiting antibradykinin activity is a quite few among many synthetic bradykinin analogs.⁴⁾ Seeking for antibradykinin peptides exhibiting no bradykinin-like activity is of interest. In this context, it is of interest to examine further the biological activity of bradykinin analogs in which two phenylalanine residues of bradykinin are substituted with another

- 1) The amino acid residues except glycine are of the L-configuration. The abbreviations used to denote amino acid derivatives and peptides are those recommended by IUPAC-IUB Commission on Biochemical Nomenclature; *Biochim. Biophys. Acta*, **263**, 205 (1972). Other abbreviations: DMF=dimethylformamide, Et₃N=triethylamine, TFA=trifluoroacetic acid, DCC=dicyclohexylcarbodiimide.
- 2) Location: Komatsushima, Sendai, 983, Japan.
- 3) J.M. Stewart, and D.H. Woolly, "Hypotensive peptides," ed. by E.G. Erdös, N. Back, F. Sicuteri, and A.F. Wilde, Springer-Verlag, New York Inc., 1966, p. 23.
- 4) E. Schröder, "Handbook of Experimental Pharmacology," Vol. 25, ed., E.G. Erdös, Springer-Verlag, Berlin-New York, 1970, p. 324.

amino acid residues having side chains of electrophilic on aromatic rings of H-Phe-OH such as H-Phe(4NO₂)-OH.⁵⁾ In addition, dipole moments created by substituent group and the stereochemical structure of the side chain of the substituted amino acid residues are expected to influence for biological activity. In the present paper, the syntheses of [Phe(4NO₂)⁵, Tyr(Me)⁸]- (IX) and [Tyr(Me)⁵, Phe(4NO₂)⁸]-bradykinin (XV) and the results of biological assay of the two synthetic bradykinin analogs are described. The synthetic routes for the nonapeptides IX and XV are illustrated in Fig. 1 and 2 respectively.

Fig. 1. Synthesis of [Phe(4NO₂)⁵, Tyr(Me)⁸]-bradykinin Fig. 2. Synthesis of [Tyr(Me)⁵, Phe(4NO₂)⁸]-bradykinin5) B. Bergel and J.A. Stock, *J. Chem. Soc.*, 1954, 2489.

H-Arg(NO₂)-OBzl was condensed with Z-Tyr(Me)-ONp (I), which was prepared from Z-Tyr(Me)-OH⁶⁾ in the usual manner, to yield Z-Tyr(Me)-Arg(NO₂)-OBzl (II). II was treated with hydrogen bromide-acetic acid solution in the presence of anisole and the resulting dipeptide ester was condensed with Z-Pro-ONp⁷⁾ to yield Z-Pro-Tyr(Me)-Arg(NO₂)-OBzl (III). After removal of the Z group of III, the resulting tripeptide ester was condensed with Boc-Ser-N₃ which was prepared from Boc-Ser-NHNH₂⁸⁾ to yield Boc-Ser-Pro-Tyr(Me)-Arg(NO₂)-OBzl (IV). After removal of the Boc group of IV with TFA, the resulting tetrapeptide ester was condensed with Boc-Phe(4NO₂)-ONSu (V) which was prepared from Boc-Phe(4NO₂)-OH⁹⁾ to yield Boc-Phe(4NO₂)-Ser-Pro-Tyr(Me)-Arg(NO₂)-OBzl (VI). After removal of the Boc group of VI with TFA, the resulting pentapeptide ester was condensed with Z-Pro-Gly-ONp¹⁰⁾ to yield Z-Pro-Gly-Phe(4NO₂)-Ser-Pro-Tyr(Me)-Arg(NO₂)-OBzl (VII). After removal of the Z group of VII, the resulting heptapeptide ester was condensed with Z-Arg(NO₂)-Pro-ONp⁶⁾ to yield Z-Arg(NO₂)-Pro-Pro-Gly-Phe(4NO₂)-Ser(OAc)-Pro-Tyr(Me)-Arg(NO₂)-OBzl (VIII). The fully protected nonapeptide (VIII) was treated with anhyd. hydrofluoride (HF) in the presence of anisole.¹¹⁾ The deblocked [Ser(OAc)⁶]-nonapeptide was purified through a carboxymethyl (CM-) cellulose column to obtained [Phe(4NO₂)⁵, Tyr(Me)⁸]-bradykinin (IX). The nonapeptide (IX) thus obtained was found to be homogeneous from the result of paper chromatography using two different solvent systems. [Tyr(Me)⁵, Phe(4NO₂)⁸]-bradykinin (XV) was similarly prepared as shown in Fig. 2. The nonapeptide (XV) was found to be homogeneous from the result of paper chromatography using two different solvent systems. Ratios of amino acids in the acid hydrolysates of peptides IX and XV were agreed well those of theory except for H-Phe(4NO₂)-OH were in which was variably contaminated with traces of *p*-aminophenylalanine as described by A.V. Schally, *et al.*¹²⁾ The two synthetic peptides IX and XV were tested quantitatively for bradykinin-like activity on the isolated guinea pig ileum by Magnus method. As shown in Table I, the two peptides, IX and XV, exhibited 10 and 5 percent of the activity of synthetic bradykinin respectively. Antibradykinin activity of the two peptides was not tested further, because of relatively high bradykinin-like activity of the peptides.

TABLE I. Biological Activities of Bradykinin Analogs^{a)}

	Bradykinin-like activity ^{b)}
Bradykinin	1.00
[Phe(4NO ₂) ⁵ , Tyr(Me) ⁸]-bradykinin (IX)	0.10
[Tyr(Me) ⁵ , Phe(4NO ₂) ⁸]-bradykinin (XV)	0.05

a) Assayed by Magnus method on an isolated guinea pig ileum (male).

b) Activity of analogs was compared with that of synthetic bradykinin, based on cause a half-maximal isotonic contraction.

Experimental

Melting points are uncorrected. For paper chromatography, the protected peptides were deblocked with HBr in AcOH unless otherwise mentioned and resulting hydrobromides were chromatographed on a

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- 10) M.A. Ondetti, *J. Med. Chem.*, **6**, 10 (1963).
- 11) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Japan*, **40**, 2164 (1967).
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filter paper Toyo Roshi No. 51, at room temperature. Rf^1 value refer to the Partridge system,¹³⁾ and Rf^2 value to the system of BuOH-pyridine-AcOH-H₂O (30:20:6:24).¹⁴⁾ The amino acid composition of the acid hydrolysates determined with Hitachi Model KLA-3B amino acid analyzer according to the directions given by Moore, *et al.*¹⁵⁾

Z-Tyr(Me)-ONp (I)—Z-Tyr(Me)-OH⁶⁾ (2.00 g) and *p*-nitrophenol (0.94 g) were dissolved in EtOAc (25 ml) and the solution was kept at 0°, when dicyclohexylcarbodiimide (DCC) (1.40 g) was added. The reaction mixture was stirred for 1 hr at 0° and 2 hr at room temperature. To the reaction mixture, a few drops of AcOH was added and the solution was stirred for 30 min. The precipitated dicyclohexylurea was filtered off and washed with EtOAc. The combined filtrates were evaporated to dryness in vacuum and the residue was recrystallized from EtOH (40 ml) twice; yield 2.30 g (84%), mp 114–116°, $[\alpha]_D^{25}$ –37.1° ($c=0.7$, DMF), *Anal.* Calcd. for C₂₄H₂₂O₇N₂: C, 63.99; H, 4.92; N, 6.22. Found: C, 64.27; H, 5.24; N, 6.90.

Z-Tyr(Me)-Arg(NO₂)-OBzl (II)—To a solution of H-Arg(NO₂)-OBzl benzenesulfonate¹⁶⁾ (2.2 g) in DMF (20 ml) was added Z-Tyr(Me)-ONp (2.5 g), followed by Et₃N to keep the solution slightly alkaline. After 24 hr at room temperature, the reaction mixture was diluted with 1N NH₄OH (22 ml), stirred for 1 hr, and then poured into 1N NH₄OH (120 ml) with stirring. The precipitate formed was collected on a filter and washed successively with 1N NH₄OH, H₂O, 1N HCl, and H₂O. The dried precipitate was refluxed with EtOAc (25 ml) for 30 min and overnight cooling caused the protected dipeptide ester to crystallise; yield 2.3 g (74%), mp 180–182°, $[\alpha]_D^{25}$ +15.0° ($c=1.0$, DMF), *Anal.* Calcd. for C₃₁H₃₆O₈N₂: C, 59.99; H, 5.85; N, 13.54. Found: C, 59.77; H, 5.78; N, 13.40. Deblocked peptide ester: Rf^1 0.71, Rf^2 0.94; single ninhydrin positive spot.

Z-Pro-Tyr(Me)-Arg(NO₂)-OBzl (III)—The protected dipeptide ester (II) (2.1 g) was dissolved in AcOH (6.5 ml), anisole (0.3 ml) and 5.2N HBr in AcOH (6.5 ml). After 40 min at room temperature, the reaction mixture was shaken vigorously with dry (C₂H₅)₂O. The precipitate was washed with dry (C₂H₅)₂O and dried over KOH pellets in vacuum. To a solution of the resulting peptide ester HBr salt in DMF (20.0 ml) was added Z-Pro-ONp (1.3 g), followed by addition of Et₃N to keep the solution slightly alkaline. After 24 hr at room temperature, the reaction mixture was diluted with 1N NH₄OH (10.0 ml), stirred for 1 hr, and mixed with EtOAc (100.0 ml). The EtOAc solution was washed successively with 1N NH₄OH, H₂O, 1N HCl, and H₂O, and dried over MgSO₄. The solution was concentrated to a small volume and petroleum ether was added to the residue. The precipitate was reprecipitated from EtOAc and petroleum ether; yield 1.8 g (75%), mp 64–74°, $[\alpha]_D^{25}$ +12.0° ($c=1.0$, DMF), *Anal.* Calcd. for C₃₆H₄₃O₉N₇: C, 60.24; H, 6.04; N, 13.66. Found: C, 59.90; H, 6.34; N, 13.22. The deblocked peptide ester: Rf^1 0.77, Rf^2 0.94; single ninhydrin positive spot.

Boc-Ser-Pro-Tyr(Me)-Arg(NO₂)-OBzl Monohydrate (IV)—The protected tripeptide ester (III) (1.50 g) was treated with HBr in AcOH as described above. To a solution of the tripeptide ester hydrobromide in DMF (15 ml) containing Et₃N was added EtOAc solution (10 ml) of Boc-Ser-N₃ (prepared from 0.71 g of the hydrazide and 0.32 g of NaNO₂ in 18 ml of 1N HCl in 10% NaCl at 0°). The reaction mixture was stirred at 5° for 12 hr. The reaction mixture was diluted with EtOAc and washed successively with H₂O, 1N citric acid, H₂O, 1N NaHCO₃, and H₂O. The EtOAc solution was dried over MgSO₄ and the solvent was evaporated in vacuum. The residue was reprecipitated from EtOAc and (C₂H₅)₂O; yield 1.30 g (78%), mp 88–98°, $[\alpha]_D^{25}$ –22.0° ($c=1.1$, DMF), *Anal.* Calcd. for C₃₆H₅₀O₁₁N₈·H₂O: C, 54.81; H, 6.64; N, 14.21. Found: C, 55.61; H, 6.35; N, 14.45. For paper chromatography Boc group of this peptide derivative was removed with TFA, Rf^1 0.74, Rf^2 0.88, single ninhydrin positive spot.

Boc-Phe(4NO₂)-ONSu (V)—To a cold solution of Boc-Phe(4NO₂)-OH¹⁷⁾ (1.00 g) and N-hydroxysuccinimide (0.44 g) in EtOAc (15 ml) was added DCC (0.77 g). The reaction mixture was stirred for 24 hr at 5°. To the reaction mixture a few drops of AcOH was added and the solution was stirred for 20 min at room temperature. The precipitated dicyclohexylurea was filtered off and washed with EtOAc. The combined filtrates was evaporated to dryness in vacuum and the residue was recrystallized from isopropanol (80 ml) twice; yield 1.00 g (77%), mp 178–179°, $[\alpha]_D^{25}$ +23.0° ($c=1.1$, DMF), *Anal.* Calcd. for C₁₈H₂₁O₈N₃: C, 53.07; H, 5.20; N, 10.32. Found: C, 53.20; H, 5.70; N, 10.18.

Boc-Phe(4NO₂)-Ser-Pro-Tyr(Me)-Arg(NO₂)-OBzl Sesquihydrate (VI)—The protected tetrapeptide ester (IV) (642 mg) was dissolved in TFA (2 ml) and the solution was kept at room temperature for 30 min,

13) S.M. Partridge, *Biochem. J.*, **42**, 238 (1948).

14) S.G. Waley and G. Watson, *Biochem. J.*, **55**, 328 (1953).

15) S. Moore, D.H. Spackmann, and W.H. Stein, *Anal. Chem.*, **30**, 1185 (1958).

16) H. Otsuka, K. Inoue, M. Kanayama, and F. Shinozaki, *Bull. Chem. Soc. Japan*, **39**, 882 (1966).

17) K. Suzuki, personal communication, H-Phe(4NO₂)-OH monohydrate as a starting material of the Boc derivative was prepared as follows: H-Phe-OH (5.0 g) was dissolved in conc. H₂SO₄ and added NH₄NO₃ (2.5 g) portionwise in 40 min at room temperature with stirring was continued for another 1 hr. The reaction mixture was poured into ice and adjusted to pH 4.6 with conc. NH₄OH, yield 3.2 g (46%). This compound was identical to the compound prepared according to the direction given by B. Bergel, *et al.*⁵⁾

then dry $(C_2H_5)_2O$ was added. The precipitate formed was dried over KOH in vacuum. To a solution of this product in DMF (6 ml) was added Boc-Phe(4NO₂)-ONSu (373 mg), followed by Et₃N to keep the solution slightly alkaline. After 24 hr at room temperature, the reaction mixture was poured into 1 N NaHCO₃ with stirring. The precipitate formed was washed successively with 1 N NaHCO₃, H₂O, 1 N citric acid, and H₂O; yield 590 mg (72%), mp 104–110°, $[\alpha]_D^{25}$ –9.0° ($c=1.1$, DMF), *Anal.* Calcd. for C₄₅H₅₈O₁₄N₁₀·1½H₂O: C, 54.59; H, 6.21; N, 14.14. Found: C, 54.60; H, 5.90; N, 14.11. For paper chromatography Boc group of the protected peptide was removed with TFA: R_f^1 0.78, R_f^2 0.94, single ninhydrin positive spot.

Z-Pro-Gly-Phe(4NO₂)-Ser-Pro-Tyr(Me)-Arg(NO₂)-OBzl (VII)—This protected peptide (VI) was prepared essentially in the same manner as described above from V (385 mg) and Z-Pro-Gly-ONp (205 mg). The product was reprecipitated from MeOH and $(C_2H_5)_2O$; yield 336 mg (73%), mp 116–121°, $[\alpha]_D^{25}$ –12.0° ($c=0.9$, DMF), *Anal.* Calcd. for C₅₆H₆₆O₁₆N₁₂·2H₂O: C, 56.08; H, 5.88; N, 14.02. Found: C, 55.90; H, 5.63; N, 14.16. The deblocked peptide ester: R_f^1 0.81, R_f^2 0.92; single ninhydrin positive spot.

Z-Arg(NO₂)-Pro-Pro-Gly-Phe(4NO₂)-Ser(OAc)-Pro-Tyr(Me)-Arg(NO₂)-OBzl (VIII)—This protected peptide (VII) was prepared essentially in the same manner as described above from VI (145 mg) and Z-Arg(NO₂)-Pro-ONp (94 mg). The product was reprecipitated from MeOH with $(C_2H_5)_2O$; yield 90 mg (60%), mp 125–132°, $[\alpha]_D^{25}$ –11.0° ($c=0.8$, DMF), *Anal.* Calcd. for C₆₈H₈₆O₂₁N₁₈: C, 54.76; H, 5.81; N, 16.91. Found: C, 54.73; H, 5.87; N, 16.79. The deblocked peptide ester: R_f^1 0.63, R_f^2 0.89; single ninhydrin positive spot.

Arg-Pro-Gly-Phe(4NO₂)-Ser-Pro-Tyr(Me)-Arg Triacetate (IX)—The fully protected nonapeptide (VII) (100 mg) was deblocked by treatment with anhyd. HF (5 ml) in the presence of anisole (0.2 ml) at 0° for 30 min. After evaporation of the HF in vacuum, the residue was dried over NaOH pellets in vacuum. The solution of the product in 1% AcOH (10 ml) was washed with EtOAc 3 times and the H₂O layer was added to a (2.0 × 6.0 cm) Dowex 1-X2 acetate type column which was eluted with 1% AcOH. Fractions of 13 ml each were collected with an automatic fraction collector. Arginine-containing peptide was located in the eluate by sakaguchi reaction. The eluates in tubes No. 2 to 9 containing the nonapeptide were pooled and evaporated to dryness in vacuum. The O-acetyl-nonapeptide in H₂O (0.6 ml) was saponified with 1 N NaOH (0.6 ml) for 1 hr. The solution neutralized with 1 N AcOH, was added to a (2.0 × 6.0 cm) CM-cellulose column which was eluted with a linear gradient elution method from H₂O (300 ml) to 0.15 M pyridinium acetate buffer (PH 5.1) (300 ml). Fractions of 13 ml each were collected with an automatic fraction collector at a flow rate of 3 to 4 ml/min. The arginine-containing peptide was located in the eluate by Sakaguchi reaction. The eluates in tubes No. 36 to 51 containing the nonapeptide were pooled, evaporated to dryness in vacuum, lyophilized; yield 49 mg (55%), mp 180–185°, $[\alpha]_D^{25}$ –79.2° ($c=0.4$, H₂O), R_f^1 0.35, R_f^2 0.50, single ninhydrin and Sakaguchi positive spot; amino acid ratio in the acid hydrolysate: Arg 2.03, Gly 1.10, Tyr 0.89, Pro 2.97, Phe(4NO₂) 1.10, Ser 1.08.

Boc-Phe(4NO₂)-Arg(NO₂)-OBzl Hemihydrate (IX)—This compound was prepared from H-Arg(NO₂)-OBzl benzenesulfonate (2.2 g) and Boc-Phe(4NO₂)-ONSu (2.2 g) essentially in the same manner as described in the preparation of II; yield 2.0 g (65%), mp 80–88°, $[\alpha]_D^{25}$ –18.0° ($c=1.2$, DMF), *Anal.* Calcd. for C₂₇H₃₅O₉N₇·½H₂O: C, 53.11; H, 5.94; N, 16.06. Found: C, 53.10; H, 5.93; N, 16.17. For paper chromatography the Boc group was removed with TFA: R_f^1 0.71, R_f^2 0.92, single ninhydrin positive spot.

Z-Pro-Phe(4NO₂)-Arg(NO₂)-OBzl (X)—This compound was prepared from IX (1.5 g) and Z-Pro-ONp (1.0 g) essentially in the same manner as described in the preparation of III; yield 1.8 g (98%), mp 75–85°, $[\alpha]_D^{25}$ +9.0° ($c=0.9$, DMF), *Anal.* Calcd. for C₃₅H₄₀O₁₀N₈: C, 57.37; H, 5.50; N, 15.29. Found: C, 57.43; H, 5.67; N, 15.17. Deblocked peptide ester, R_f^1 0.70, R_f^2 0.91, single ninhydrin positive spot.

Boc-Ser-Pro-Phe(4NO₂)-Arg(NO₂)-OBzl (XI)—This compound was prepared from X (733 mg) and Boc-Ser-N₃ prepared from the hydrazide (353 mg), essentially in the same manner as described in the preparation of IV. The product was reprecipitated from EtOAc with $(C_2H_5)_2O$; yield 500 mg (64%), mp 104–112°, $[\alpha]_D^{25}$ –22.0° ($c=1.0$, DMF), *Anal.* Calcd. for C₃₅H₄₇O₁₂N₉: C, 53.49; H, 6.03; N, 16.04. Found: C, 53.06; H, 5.99; N, 15.94. For paper chromatography the Boc group was removed with TFA: R_f^1 0.71, R_f^2 0.86, single ninhydrin positive spot.

Z-Tyr(Me)-Ser-Pro-Phe(4NO₂)-Arg(NO₂)-OBzl Monohydrate (XII)—This compound was prepared from XI (778 mg) and Z-Tyr(Me)-ONp (500 mg), essentially in the same manner as described in the preparation of VI. The product was reprecipitated from EtOAc and $(C_2H_5)_2O$; yield 750 mg (74%), mp 100–106°, $[\alpha]_D^{25}$ –16.0° ($c=1.1$, DMF), *Anal.* Calcd. for C₄₈H₅₆O₁₄N₁₀·H₂O: C, 56.79; H, 5.76; N, 13.80. Found: C, 56.73; H, 5.55; N, 14.26. Deblocked peptide ester: R_f^1 0.91, R_f^2 0.94, single ninhydrin positive spot.

Z-Pro-Gly-Tyr(Me)-Ser(OAc)-Pro-Phe(4NO₂)-Arg(NO₂)-OBzl Sesquihydrate (XIII)—This compound was prepared from XII (332 mg) and Z-Pro-Gly-ONp (156 mg) essentially in the same manner as described in the preparation of III. The product was reprecipitated from EtOAc and $(C_2H_5)_2O$; yield 241 mg (59%), mp 106–116°, $[\alpha]_D^{25}$ –29.0° ($c=1.1$, DMF), *Anal.* Calcd. for C₅₇H₆₃O₁₇N₁₂·1½H₂O: C, 56.10; H, 5.87; N, 13.78. Found: C, 56.37; H, 5.65; N, 14.18. Deblocked peptide ester: R_f^1 0.62, R_f^2 0.83, single ninhydrin positive spot.

Z-Arg(NO₂)-Pro-Pro-Gly-Tyr(Me)-Ser(OAc)-Pro-Phe(4NO₂)-Arg(NO₂)-OBzl Dihydrate (XIV)—This compound was prepared from XIII (200 mg) and Z-Arg(NO₂)-Pro-ONp (143 mg) essentially in the same manner as described in the preparation of VII. The product was recrystallized from EtOAc; yield 141 mg (55%), mp 131–137°, $[\alpha]_D^{25}$ –13.0° ($c=0.8$, DMF), *Anal.* Calcd. for C₆₈H₈₆O₂₁N₁₈·2H₂O: C, 53.46; H, 5.94;

N, 16.51. Found: C, 53.84; H, 5.61; N, 16.31. Deblocked peptide ester.: Rf^1 0.62, Rf^2 0.83, single ninhydrin positive spot.

Arg-Pro-Pro-Gly-Tyr(Me)-Ser-Pro-Phe(4NO₂)-Arg Triacetate (XV)—The fully protected nonapeptide (XIV) (100 mg) was deblocked in the same manner as described in the preparation of VIII. The deblocked O-acetylnonapeptide was saponified as described in the preparation of VIII; yield 41 mg (47%), mp 175—185°, $[\alpha]_D^{25}$ —69.3° ($c=0.3$, H₂O), Rf^1 0.22, Rf^2 0.50, single ninhydrin and sakaguchi positive spot; amino acid ratios in the acid hydrolysate: Arg 1.96, Pro 3.06, Gly 1.01, Tyr 0.84, Ser 0.99, Phe(4NO₂) 1.06.

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C-13 NMR Spectra of Some Aminosugars and Sugar-Antibiotics, Neomycin and Kanamycin

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Aminosugars occur widely in the world of animals and micro-organisms as mucopolysaccharides and sugar-proteins. Also, almost all sugar-antibiotics contain aminosugars. C-13 nuclear magnetic resonance (NMR) spectroscopy is a useful tool for the structural determination of biologically important substances because of the C-13 large chemical shift difference and susceptibility to conformational and configurational changes.²⁾ In this paper its application to aminosugars and sugar-antibiotics is showed.

Several groups have studied the C-13 NMR spectra of sugars,³⁻⁶⁾ N-acetyl aminosugars,⁷⁾ and sugar-antibiotics, hygromycin⁸⁾ and gentamicin.⁹⁾ The present assignment of aminosugars was easily accomplished by a comparison with those studied. The signals of carbons directly attached to amino or acetamido groups appear almost 20 ppm higher field than those of the same carbons of parent sugars like as norbornane derivatives.¹⁰⁾ Instead of little lower

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