Chem. Pharm. Bull.
25(10)2731—2734(1977)

UDC 547.757.04 : 547.466.1.04

Synthesis and Biological Activity of Tetragastrin Analogues modifying the Tryptophan Residue[1]

YUICHIRO YABE, AKIRA MORITA, CHIEKO MIURA,
SHINSAKU KOBAYASHI, and YOSHIHIKO BABA

Central Research Laboratories, Sankyo Co., Ltd.[2]

(Received March 3, 1977)

Three analogues of tetragastrin, in which the tryptophan residue was substituted by 3-(1-naphthyl)-L-alanine, 3-(2-naphthyl)-L-alanine or 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, were synthesized and evaluated for their gastric juice stimulating activity. The results suggest that the indolyl NH function in the tryptophan residue plays an important role and that the role of the tryptophan residue in an interaction between tetragastrin and its receptor is different from that in the case of luteinizing hormone-releasing hormone.

Keywords——peptide; tetragastrin analogues modifying the Trp residue; gastric juice stimulating activity; male rats of the Donryu strain; structure-activity relationship; tetragastrin Nal(1) analogue; tetragastrin Nal(2) analogue

In naturally occurring biologically active oligopeptides so far investigated, Trp residue was found not so often compared with other kinds of amino acid residues. However, in most of Trp containing peptides, Trp residue has been noted to play an important role to elicit full biological activity. Replacement by other amino acid residues including nonprotein amino acids caused usually a significant loss of activities. Recently, in somatostatin almost eight times increase in the hormonal potency was reported by the change in configuration of the Trp residue at position 8.[3] On the other hand, in our previous paper,[4] we reported the syntheses of luteinizing hormone-releasing hormone (LH-RH) analogues modifying the Trp residue at position 3 by the nonprotein amino acids. The potency of analogue replaced by Nal(1) was about twice that of original LH-RH. This result prompted us to synthesize this kind of analogues of other Trp containing peptides. First we chose the C-terminal tetrapeptide amide of gastrin, Z-Trp-Met-Asp-Phe-NH_2 (tetragastrin). In extensive studies on structure-function relationship, Morley, *et al.* suggested that the Trp residue in tetragastrin is one of the binding sites along with the Met and Phe residues.[5] On the other hand, Lin, *et al.* have recently described that the removal of Trp in tetragastrin showed a marked decrease (about 1/4000 that of tetragastrin on a molar basis) in gastric juice stimulating activity.[6]

In the present study, we synthesized tetragastrin analogues substituted by Nal(2)[4] or Tca[7] as well as Nal(1).[4] Tca-tetragastrin was already reported as inactive by Morley.[5b]

---

[1] Amino acids, peptides and their derivatives mentioned in this paper are of the L-configuration. Abbreviations used are those recommended by IUPAC-IUB Commission of Biochemical Nomenclature in May 1971: *J. Biol. Chem.*, **247**, 977 (1972). Abbreviations of the less-common amino acids are as follows: Nal(1), 3-(1-naphthyl)-L-alanine; Nal(2), 3-(2-naphthyl)-L-alanine; Tca, 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid. The other abbreviations are as follows: Ac, acetyl; Boc, *tert*-butoxycarbonyl; Bzl, benzyl; Z, benzyloxycarbonyl.

[2] Location: *1-2-58, Hiromachi, Shinagawa-ku, Tokyo, 140, Japan.*

[3] J. Rivier, M. Brown, and W. Vale, *Biochem. Biophys. Res. Commun.*, **65**, 746 (1975).

[4] Y. Yabe, C. Miura, H. Horikoshi, and Y. Baba, *Chem. Pharm. Bull.* (Tokyo), **24**, 3149 (1976).

[5] *a*) J.S. Morley, H.L. Tracy, and R.A. Gregory, *Nature*, **207**, 1356 (1965); *b*) J.S. Morley, *Proc. R. Soc. London, Ser. B*, **170**, 97 (1968).

[6] T.-M. Lin, *Gastroenterology*, **63**, 922 (1972); T.-M. Lin, G.L. Southard, and G.F. Spray, *ibid.*, **70**, 733 (1976).

[7] D.G. Harvey, E.J. Miller, and W. Robson, *J. Chem. Soc.*, **1941**, 153.

However, we wanted to reevaluate this analogue because we found that Tca^3-LH–RH was a comparatively potent LH–RH antagonist.[8]

DL-Nal(1),[9] DL-Nal(2)[10] and Tca[7] were synthesized following the already described methods. The optical resolution was perfomed by acylase digestion of the N$^\alpha$-Ac-DL-Nal(1)-OH or N$^\alpha$-Ac-DL-Nal(2)-OH according to the method described in our previous paper.[4]

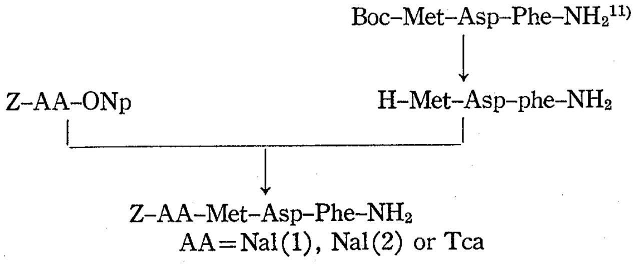

Chart 1

The analogues including Nal(1), Nal(2) or Tca were synthesized by the conventional classical methods as depicted in Chart 1. The α-amino groups of AA [Nal(1), Nal(2) or Tca], Asp and Phe, the α-amino group of Met and the β-carboxyl group of Asp were protected by Z, Boc and Bzl groups, respectively.

Benzyloxycarbonylation of Nal(1), Nal(2) or Tca by the usual Schotten–Baumann procedure gave the corresponding Z-amino acids. The individual *p*-nitrophenyl esters used in these syntheses were prepared from the above Z-amino acids, *p*-nitrophenyl and N,N'-dicyclohexylcarbodiimide (DCCD), following the general method of Bodanszky.[12]

Condensation of the triethylammonium salt of H-Met-Asp-Phe-NH$_2$[11] with Z-Nal(1)-ONp, Z-Nal(2)-ONp or Z-Tca-ONp afforded the Z-AA-Met-Asp-Phe-NH$_2$ [AA=Nal(1), Nal(2) or Tca] in 55—68% yield. These analogues obtained thus exhibited a single spot on thin-layer chromatography by the three different solvent systems, their hydrolysate showed the

TABLE I. Gastric Juice Stimulating Activity of Tetragastrin Analogues Modifying the Trp Residue

Compounds	μg/kg,[a] *i.v.*	% gastric juice stimulating activity *in vivo*
Tetragastrin	0.77	Assumed 100%
Nal(1)-tetragastrin	39	1.97
Nal(2)-tetragastrin	58	1.33
Tca-tetragastrin	67	1.15

a) Drug dosages required to lower the pH of the perfusate by 0.8.

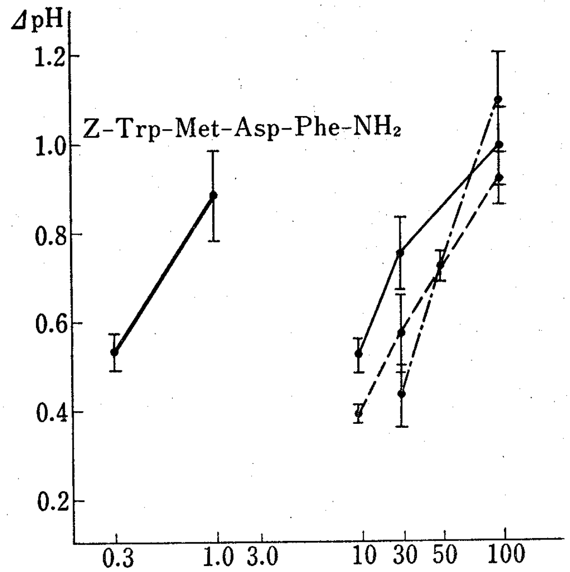

Fig. 1. ΔpH induced by Various Doses of Tetragastrin and Its Analogues modifying the Trp Residue

———, Z-Nal(1)-Met-Asp-Phe-NH$_2$.
------, Z-Nal(2)-Met-Asp-Phe-NH$_2$.
-·-·-, Z-Tca-Met-Asp-Phe-NH$_2$.

---

8) Y. Yabe, H. Horikoshi, and Y. Baba, *Chem. Pharm. Bull.* (Tokyo), in preparation.
9) H. Erlenmeyer and W. Grubenmann, *Helv. Chim. Acta*, 30, 297 (1947).
10) K. Dittmer, W. Herz, and S.J. Cristol, *J. Biol. Chem.*, **173**, 323 (1948).
11) J.S. Davey, A.H. Laird, and J.S. Morley, *J. Chem. Soc.* (*C*), **1966**, 555.
12) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).

correct amino acid ratios by an automatic amino acid analyzer, and their elemental analyses agreed with their respective theoretical values.

Gastric juice stimulating activities of these analogues were estimated by the procedure based on the rat stomach perfusion technique of Ghosh and Schild.13) The results are summarized in Table I and Fig. 1. Contrary to the expectation, replacement of the indole ring in the Trp residue by the naphthalene ring resulted in marked decrease of the activity. The activity of Nal(1)-tetragastrin was somewhat higher compared to that of Nal(2)-tetragastrin as in the case of Nal(1)3- and Nal(2)3-LH-RH. The present results strongly suggest that the indolyl NH function in the Trp residue of tetragastrin plays an important role for generating the full hormonal activity.

On the other hand, Tca-tetragastrin exhibited a weak but clear agonistic action unlike the above-mentioned literature.5b) These results apparently demonstrate the function of the Trp residue in an interaction between tetragastrin and its receptor is different from that in the case of LH-RH.

Experimental

Melting points are uncorrected. Optical rotations were measured with a Perkin-Elmer polarimeter 141. Thin-layer chromatography was performed on Merck Silica gel $60F_{254}$. *Rf* values refer to the following solvent systems: Rf^{I} $CHCl_3$–EtOH–AcOH (95: 5: 3, v/v/v), Rf^{II} $CHCl_3$–EtOH–AcOH (20: 5: 1), Rf^{III} $CHCl_3$–MeOH–32% aq. AcOH (6: 3: 1), Rf^{IV} $CHCl_3$–MeOH–conc. NH_4OH (6: 3: 1) and Rf^{V} *n*-BuOH–AcOH–H_2O–pyridine (30: 6: 24: 20). Amino acid analyses of the Z-tetrapeptide amides were carried out on samples that had been hydrolyzed with constant boiling HCl containing 4% thioglycolic acid for 24 hr in evacuated, sealed tubes at 110°, and were recorded on a Hitachi amino acid analyzer KLA-2 and Technicon's amino acid analysis assembly.

Z-Nal(1)-OH (1)——Nal(1)4) (1.09 g, 5 mmol) was dissolved in 1 N NaOH (5 ml) and the solution was cooled to 0°. Z–Cl (1 g, 6 mmol) and 1 N NaOH (7.5 ml) were added alternately in about five equal portions over a period of 30 min with vigorous stirring. After the addition was completed, stirring was continued at room temperature for 3 hr. The reaction mixture was washed with ether (about 20 ml) to remove excess Z–Cl, and the aqueous layer was acidified slowly with conc. HCl, with cooling in an ice-bath. The separated colourless crystals were collected by filtration, washed with water, and dried: Yield 1.48 g (84%). mp 143—147°. Rf^{II} 0.85. $[\alpha]^{22}_{D}$ −56.5° (*c*=1.15, MeOH). *Anal.* Calcd. for $C_{21}H_{19}NO_4$: C, 72.19; H, 5.48; N, 4.01. Found: C, 72.55; H, 5.24; N, 3.92.

Z-Nal(2)-OH (2)——The benzyloxycarbonylation of Nal(2)4) (1.09 g, 5 mmol) was achieved in the same manner as described above for Nal(1). The reaction mixture was washed with ether (about 20 ml) and the aqueous layer was acidified with conc. HCl. The precipitate was extracted with AcOEt and the organic layer was washed with the saturated brine. After drying over anhyd. Na_2SO_4, the solvent was evaporated *in vacuo* to an oily residue. The product was allowed to stand for a while to crystallize. Recrystallization from AcOEt–*n*-hexane gave colourless needles: Yield 1.55 g (88%). mp 105—107°. $[\alpha]^{22}_{D}$ 6.5° (*c*=1.1, MeOH). Rf^{II} 0.86. *Anal.* Calcd. for $C_{21}H_{19}NO_4$: C, 72.19; H, 5.48; N, 4.01. Found: C, 72.62; H, 5.29; N, 3.94.

Z-Tca-OH (3)——Tca7) (2.89 g, 13.4 mmol) was dissolved in 1 N NaOH (13.4 ml) and water (120 ml), and the solution was cooled to 0°. To this solution, Z–Cl (3 g, 18 mmol) and 1 N NaOH (22.5 ml) were added alternately in about five equal portions over a period of 30 min, with vigorous stirring. After the addition was completed, stirring was continued at room temperature for 3 hr. The reaction mixture was washed with ether (about 30 ml) and the aqueous layer was acidified with conc. HCl. The separated colourless precipitate was collected by filtration and redissolved in AcOEt. The solution was washed with the saturated brine, dried over anhyd. Na_2SO_4, and concentrated *in vacuo* to an oily residue. The product was dissolved in ether (30 ml) and the solution was permitted to stand overnight to separate out colourless needles: Yield 2.22 g (48%). mp 186—188° (lit.14) 183—184°). $[\alpha]^{22}_{D}$ 56.3° (*c*=0.96, MeOH). Rf^{II} 0.85. *Anal.* Calcd. for $C_{20}H_{18}N_2O_4$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.45; H, 5.48; N, 7.88.

Z-Nal(1)-ONp (4)——A solution of **1** (1.05 g, 3 mmol) and *p*-nitrophenol (0.5 g, 3.3 mmol) in a mixture of N,N-dimethylformamide (DMF; 1 ml) and AcOEt (5 ml) was treated with DCCD (0.68 g, 3.3 mmol) at 0°, and the mixture was stirred at 0° for 1 hr. Stirring was continued at room temperature for 3 hr and the precipitated dicyclohexyl (DC) urea was filtered off. The filtrate was concentrated *in vacuo* to dryness

---

13) M.M. Ghosh and H.O. Schild, *Brit. J. Pharmacol. Chemother.*, **13**, 54 (1958).

14) H. Gregory and J.S. Morley, *J. Chem. Soc.* (*C*), **1968**, 910.

and the oily residue crystallized by the addition of EtOH. Recrystallization from EtOH gave colourless needles: Yield 1.04 g (73%). mp 132—133°. $[\alpha]^{22}_{D}$ 1.8° (c=1.06, AcOEt). Rf^{I} 0.87. *Anal.* Calcd. for $C_{27}H_{22}N_2O_6$: C, 68.93; H, 4.71; N, 5.95. Found: C, 69.50; H, 4.54; N, 5.82.

Z-Nal(2)-ONp (5)——Prepared from **2** (1.05 g, 3 mmol), *p*-nitrophenol (0.5 g, 3.3 mmol) and DCCD (0.68 g, 3.3 mmol) by the method described for **4**. Recrystallization from EtOH gave colourless needles: Yield 1.0 g (70%). mp 112—113°. $[\alpha]^{22}_{D}$ −4.2° (c=1.13, AcOEt). Rf^{I} 0.89. *Anal.* Calcd. for $C_{27}H_{22}N_2O_6$: C, 68.93; H, 4.71; N, 5.95. Found: C, 69.54; H, 4.52; N, 5.89.

Z-Tca-ONp (6)——A solution of **3** (1.05 g, 3 mmol) and *p*-nitrophenol (0.5 g, 3.3 mmol) in AcOEt (7 ml) was treated with DCCD (0.68 g, 3.3 mmol) at 0°. The mixture was stirred at 0° for 1 hr and at room temperature for 4 hr. The precipitated DC urea was filtered off and the filtrate was concentrated *in vacuo* to dryness. The residual oil was crystallized by the addition of EtOH. Recrystallization from EtOH gave pale-yellowish needles: Yield 0.77 g (55%). mp 69—70°. $[\alpha]^{22}_{D}$ 100.2° (c=1.09, AcOEt). Rf^{I} 0.81. *Anal.* Calcd. for $C_{26}H_{21}N_3O_6$: C, 66.24; H, 4.49; N, 8.91. Found: C, 66.76; H, 4.57; N, 8.71.

Z-Nal(1)-Met-Asp-Phe-NH$_2$ (7)——Water (1 ml), followed by Et_3N (101 mg, 1 mmol), were added to a solution of H–Met–Asp–Phe–NH_2 trifluoroacetate[11] (**8**; 262 mg, 0.5 mmol) and **4** (260 mg, 0.55 mmol) in DMF (5 ml) at 0°, and the mixture was stirred at 5° for 22 hr. Water (20 ml) and AcOH (0.1 ml) were then added. The resulting precipitate was collected, washed with water and AcOEt, and stirred for 5 min with boiling MeOH. After cooling, the colourless precipitate was collected: Yield 206 mg (55%). mp 241—242.5°. $[\alpha]^{22}_{D}$ −44.4° (c=0.93, DMF). Rf^{III} 0.86, Rf^{IV} 0.34, Rf^{V} 0.66. *Anal.* Calcd. for $C_{39}H_{43}N_5O_8S$: C, 63.14; H, 5.84; N, 9.44; S, 4.32. Found: C, 62.96; H, 5.79; N, 9.40; S, 4.68. Amino acid ratios: Nal(1) 1.19, Met 0.98, Asp 1.03, Phe 1.00.

Z-Nal(2)-Met-Asp-Phe-NH$_2$ (9)——Prepared as described for **7** using **5**. Colourless powder: Yield 254 mg (68%). mp 238—239°. $[\alpha]^{22}_{D}$ −14.9° (c=1.2, DMF). Rf^{III} 0.86, Rf^{IV} 0.33, Rf^{V} 0.67. *Anal.* Calcd. for $C_{39}H_{43}N_5O_8S$: C, 63.14; H, 5.84; N, 9.44; S, 4.32. Found: C, 63.34; H, 5.97; N, 9.60; S, 4.21. Amino acid ratios: Nal(2) 1.15, Met 0.96, Asp 1.02, Phe 1.00.

Z-Tca-Met-Asp-Phe-NH$_2$ (10)——Water (1 ml), followed by Et_3N (101 mg, 1 mmol), were added at 0° to a solution of **8** (262 mg, 0.5 mmol) and **6** (259 mg, 0.55 mmol) in DMF (5 ml), and the mixture was stirred at 5° for 45.5 hr. Water (20 ml) and AcOH (0.1 ml) were then added. The resulting gelatinous precipitate was collected, washed with water and ether, and dried. pale-yellowish powder: Yield 226 mg (61%). mp 151—153° (lit.[14] 146—148°). $[\alpha]^{22}_{D}$ 3.0° (c=1.18, DMF) (lit.[14] 5.8° in aq. EtOH). Rf^{III} 0.86, Rf^{IV} 0.33, Rf^{V} 0.67. *Anal.* Calcd. for $C_{38}H_{42}N_6O_8S$: C, 61.44; H, 5.70; N, 11.30; S, 4.32. Found: C, 61.34; H, 5.89; N, 10.92; S, 4.42. Amino acid ratios: Tca 0.95, Met 0.88, Asp 0.96, Phe 1.00. Tca was identified by Technicon's amino acid analysis assembly using a nine chamber gradient systems. The elution volume based on His was 1.207. CHROMO-BEADS (type C-2, Technicon, 0.6×66 cm) column at 70°. Buffer system (Na-citrate buffer) was as follows: (Chamber I) 0.2 N, pH 2.75 (33 ml) +MeOH (2 ml), (II) 0.2 N, pH 2.75 (11 ml)+0.2 N, pH 2.875 (24 ml), (III) 0.2 N, pH 2.875 (35 ml), (IV and V) 0.2 N, pH 3.80 (35 ml), (VI—IX) 1.2 N, pH 6.10 (35 ml).

Z-Trp-Met-Asp-Phe-NH$_2$ (Tetragastrin, 11)——Z–Trp–ONp[15] (253 mg, 0.55 mmol) and **8** (262 mg, 0.5 mmol) were dissolved in DMF (5 ml) and the solution was cooled to 0°. Water (1 ml) and Et_3N (101 mg, 1 mmol) were added to this solution and the mixture was stirred for 17 hr at 5°. Water (20 ml) and AcOH (0.1 ml) were added and the resulting colourless precipitate was collected. The product was washed with water and AcOEt, and stirred for 5 min with boiling MeOH. After cooling, the colourless precipitate was collected: Yield 215 mg (59%). mp 237—239° (lit.[11] 237—238°). $[\alpha]^{22}_{D}$ −42.0° (c=1.25, DMF) [lit.[11] −41.0° (c=1.0, DMF)]. Rf^{III} 0.85, Rf^{IV} 0.32, Rf^{I} 0.66. *Anal.* Calcd. for $C_{37}H_{42}N_6O_8S$: C, 60.81; H, 5.79; N, 11.50; S, 4.39. Found: C, 60.59; H, 5.89; N, 11.57; S, 4.74. Amino acid ratios: Trp 0.80, Met 0.97, Asp 1.02, Phe 1.00.

Biological Activity——Solution were prepared immediately prior to the biological test by dissolving the sample (5 mg) in a mixture of polyethylene glycol 400 and saline (1: 1) containing 2 equivalents of 0.1 N aq. ammonia (5 ml) with the aid of the ultrasonic homogenizer. Biological activity was estimated according to the rat stomach perfusion technique of Ghosh and Schild[13] with slight modification. Male rats of the Donryu strain, weighing 200—250 g, were used. The stomach of the starved, urethane anesthetized (1.25 g/kg, *s.c.*) rat was perfused *via* cannulae placed in the esophagus and in the pyloric antrum. Perfusion was carried out with 0.067 mM NaOH–saline solution warmed at 37° and pH of the perfusate recorded continuously with glass electrode. The body temperature of the rat was maintained at 37° throughout the experiment. All samples were administered intravenously.

---

15) M. Wilchek and A. Patchornik, *J. Org. Chem.*, **28**, 1874 (1963).