[Chem. Pharm. Bull. 32(10)4157—4160(1984)]

Amino Acids and Peptides. IV. Synthesis and Analgesic Effects of Tyr-Containing Dipeptide Phenethylamides[1)]

MITSUKO MAEDA, SATOSHI OKUSADA, and KOICHI KAWASAKI*

Faculty of Pharmaceutical Sciences, Kobe-Gakuin University, Ikawadani-cho, Nishi-ku, Kobe 673, Japan

(Received February 16, 1984)

Several tyrosyl-5-aminovaleramide analogs were synthesized and their analgesic effects were examined. *N*-(Tyrosyl-5-aminovaleryl)-*N*-methylphenethylamine showed analgesic action in mice on peripheral administration.

Keywords——analgesic peptide; synthetic dipeptide amide; tyrosine-containing dipeptide amide; tyrosylaminovaleramide

In the preceding paper,[2)] we reported that H–Tyr–Ava–Phe–Met–OH (I, in which the Gly–Gly amide bond of Met–enkephalin is replaced by an ethylene bond) exhibited analgesic action in mice on intracisternal administration, but not after peripheral administration. We have now synthesized several dipeptide phenethylamides (II, III, IV, V, VI, VII) to study the structure–activity relationships, and in particular to identify the minimum structure for analgesic effect.

(Me)Ava was prepared as shown in Fig. 2. Acid hydrolysis of *N*-methylpiperidone gave hygroscopic crystals of (Me)Ava·HCl, which was converted to the free amino acid by treating it with ion-exchange resin. This synthetic procedure gave a much better result than the Eschweiler–Clarke reaction[3)] of Ava, which gave a mixture of (Me)Ava and $(Me)_2$Ava. Synthetic schemes for II and V are shown in Fig. 3. Compounds III, IV, VI, and VII were synthesized in the same manner as II. For the synthesis of V, Z–(Me)Tyr(Bzl)–OH (prepared by methylation of Z–Tyr(Bzl)–OH[4)]) was coupled with (Me)Ava–(Me)PHA by the mixed anhydride method[5)] followed by hydrogenation.

Analgesic effedts of the synthetic peptides on mice were examined by the tail-pinch method[6)] and the results are shown in Table I. The peptides were administrated by

TABLE I. Analgesic Effects of Synthetic Peptides in Mice

			5 min	15 min	30 min
I	*i.c.*	40 μg/mouse	4	1	0 (10)
	i.v.	100 mg/kg	0	0	0 (5)
II	*s.c.*	83 mg/kg	0	0	0 (8)
III	*s.c.*	40 mg/kg	5	1	0 (10)
	i.v.	40 mg/kg	4	2	0 (8)
IV	*s.c.*	40 mg/kg	0	0	0 (8)
V	*s.c.*	40 mg/kg	0	0	0 (8)
VI	*i.v.*	40 mg/kg	2	0	0 (10)
VII	*i.v.*	40 mg/kg	1	0	0 (10)

Numbers indicate total mice showing analgesia.
Numbers in parentheses indicate total mice examined.

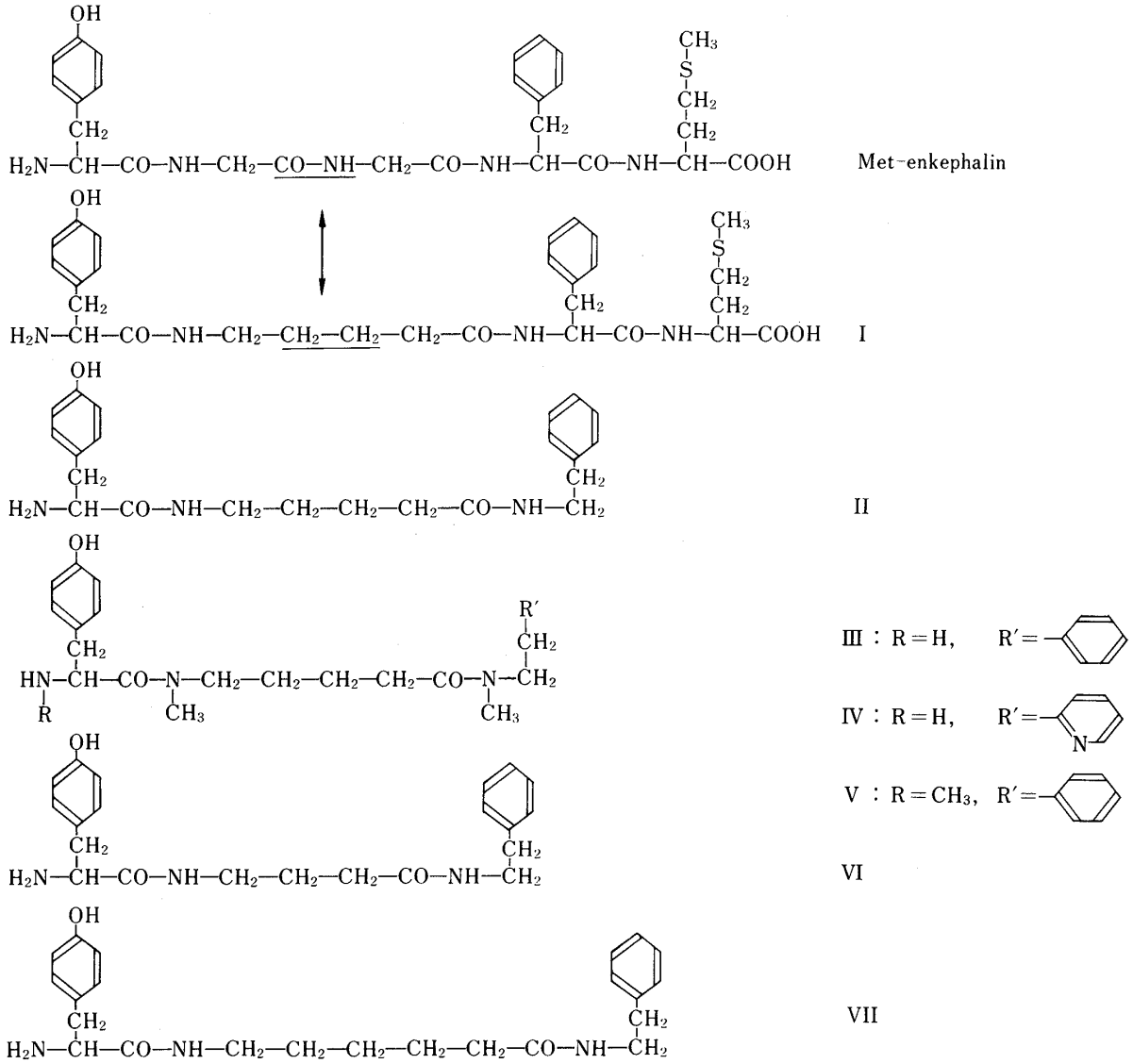

Fig. 1. Synthetic Dipeptide Phenethylamides

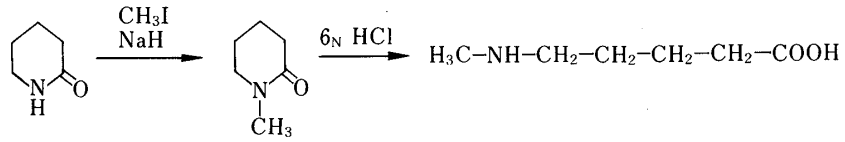

Fig. 2. Synthetic Scheme for (Me)Ava

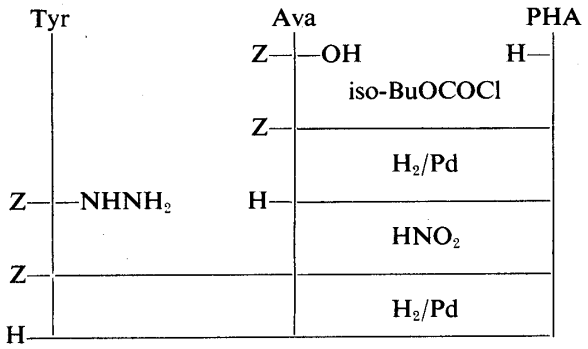

Fig. 3. Synthetic Scheme for II

subcutaneous or intravenous injection. Compound II was not effective at a dose of 40 mg/kg but its N-methylated derivative, III, showed analgesic action at the same dose. N-Methylation of the amide bonds might assist the peptide to pass the blood-brain barrier and might prevent hydrolysis of the peptide by enzymes. Kiso *et al.*[7] reported that a dipeptide amide, Tyr–D-Met(O)–PHA, exhibited an analgesic effect. Thus, kyotorphin, Tyr–D-Met(O)–PHA and III are all analgesic dipeptides; a comparison of their analgesic mechanisms should be of interest.

A comparison of the analgesic effects of III and IV suggests that a benzene ring may be more effective than a pyridine ring in the amine component of the valeramide portion.

Compound V did not exhibited analgesic effect at a dose of 40 mg/kg, contrary to our expectation. The reason for this is not clear.

Compounds VI and VII exhibited weak analgesic effects at a dose of 40 mg/kg. This result suggests that the distance between Tyr and PHA in the primary structure may not be crucial for analgesic effect.

Experimental

Melting points are uncorrected. Solvent systems for ascending thin-layer chromatography on Silica gel G (type 60, E. Merck) are indicated as follows: Rf^1 = *n*-BuOH–AcOH–H_2O (4 : 1 : 5, upper phase), Rf^2 = *n*-BuOH–pyridine–AcOH–H_2O (4 : 1 : 1 : 2), Rf^3 = $CHCl_3$–MeOH–H_2O (8 : 3 : 1, lower phase), Rf^4 = AcOEt–benzene (1 : 1). Acid hydrolyses were performed in constant-boiling HCl at 110 °C for 24 h in evacuated tubes.

(Me)Ava——2-Piperidone (2.88 g) was treated with NaH (1.08 g) and CH_3I (2.9 ml) in THF (30 ml) to give 1-methyl-2-piperidone [bp_{30} 150 °C, nuclear magnetic resonance (NMR) ($CDCl_3$) δ: 2.92 (3H, s, NCH_3)], which was hydrolyzed with 6 N HCl (20 ml) at 110 °C for 6 h. The solvent was evaporated off and the resulting hydrochloride was converted to the free amino acid by Dowex 50 (H^+) column chromatography using pyridine–acetate buffer (0.1 M, pH 3.8—5.5) as the eluent. Yield 1.89 g (70%), mp 117—119 °C, Rf^1 0.26, Rf^2 0.45. *Anal.* Calcd for $C_6H_{13}NO_2$: C, 54.9; H, 10.0; N, 10.5. Found: C, 54.7; H, 10.2; N, 10.5.

Z–Ava–PHA——Z–Ava–OH[9] (2 g) and PHA (0.96 ml) were coupled in THF by the mixed anhydride method[5] in the usual manner. Recrystallized from AcOEt/petro. ether. Yield 2.49 g (88%), mp 124—128 °C, Rf^4 0.43. *Anal.* Calcd for $C_{21}H_{26}N_2O_3$: C, 71.2; H, 7.4; N, 7.9. Found: C, 71.3; H, 7.3; N, 7.9.

Z–Tyr–Ava–PHA——Z–Tyr–$NHNH_2$[10] (988 mg) was coupled with H–Ava–PHA (prepared from 1 g of Z–Ava–PHA by hydrogenation) in DMF by the azide method[11] in the usual manner. Recrystallized from MeOH/ether. Yield 822 mg (61%), mp 132—135 °C, $[\alpha]_D^{35}$ −1.6° (c = 1.0, MeOH), Rf^3 0.69. *Anal.* Calcd for $C_{30}H_{35}N_3O_5$: C, 69.6; H, 6.8; N, 8.1. Found: C, 69.6; H, 6.8; N, 8.2.

II——Z–Tyr–Ava–PHA (460 mg) was hydrogenated over Pd in MeOH in the usual manner. Yield 350 mg (94%), hygroscopic material, $[\alpha]_D^{35}$ +11.0° (c = 1.0, MeOH), Rf^1 0.63, Rf^2 0.83, Rf^3 0.51. *Anal.* Calcd for $C_{22}H_{29}N_3O_3 \cdot H_2O$: C, 65.8; H, 7.8; N, 10.5. Found: C, 65.7; H, 7.6; N, 10.1.

Z–(Me)Ava–OH——(Me)Ava (9.18 g) was benzyloxycarbonylated[12] in the usual manner. Yield 15.4 g (81%), syrupy material, Rf^4 0.81. Characterized as its dicyclohexylamine salt: mp 85—89 °C. *Anal.* Calcd for $C_{14}H_{19}NO_4 \cdot C_{12}H_{23}N$: C, 69.9; H, 9.5; N, 6.3. Found: C, 69.7; H, 9.5; N, 6.1.

Z–(Me)Ava–(Me)PHA——Prepared from Z–(Me)Ava–OH (4 g) and (Me)PHA[13] (2 g) by the mixed anhydride method in the usual manner. The product was purified by silica gel column chromatography using $CHCl_3$ as the eluent. Yield 4.6 g (80%), syrupy material, Rf^4 0.44. *Anal.* Calcd for $C_{23}H_{30}N_2O_3$: C, 72.2; H, 7.9; N, 7.3. Found: C, 72.0; H, 8.1; N,7.0.

Z–Tyr–(Me)Ava–(Me)PHA——Z–Tyr–$NHNH_2$ (1.98 g) and H–(Me)Ava–(Me)PHA (prepared from 2 g of Z–(Me)Ava–(Me)PHA by hydrogenation) were coupled by the azide method. The product was purified by silica gel column chromatography using 1% MeOH/$CHCl_3$ as the eluent. Yield 1.51 g (53%), amorphous material, $[\alpha]_D^{20}$ +9.8° (c = 1.0, MeOH), Rf^4 0.38. *Anal.* Calcd for $C_{32}H_{39}N_3O_5$: C, 70.4; H, 7.2; N, 7.7. Found: C, 70.7; H, 7.2; N, 7.7.

III——Z–Tyr–(Me)Ava–(Me)PHA (200 mg) was hydrogenated in the usual manner. Yield 137 mg (91%), hygroscopic material, $[\alpha]_D^{21}$ +48.5° (c = 1.0, MeOH), Rf^3 0.89. *Anal.* Calcd for $C_{24}H_{33}N_3O_3$: C, 67.1; H, 8.2; N, 9.9. Found: C, 67.4; H, 8.1; N, 9.7.

Z–(Me)Ava–(Me)AEP——Prepared from Z–(Me)Ava–OH (5.3 g) and (Me)AEP[14] (2.7 g) by the mixed anhydride method. Yield 5.08 g (66%), syrupy material, Rf^3 0.96. *Anal.* Calcd for $C_{22}H_{29}N_3O_3$: C, 68.9; H, 7.6; N, 11.0. Found: C, 69.2; H, 7.7; N, 10.7.

Z–Tyr–(Me)Ava–(Me)AEP——Z–Tyr–$NHNH_2$ (1.98 g) and H–(Me)Ava–(Me)AEP (prepared from 2 g of Z—(Me)Ava–(Me)AEP by hydrogenation) were coupled by the azide method. The product was purified by silica gel column chromatography using 2% MeOH/$CHCl_3$ as the eluent. Yield 1.28 g (64%), amorphous material, $[\alpha]_D^{19}$ +8.7° (c = 1.0, MeOH), Rf^3 0.92. *Anal.* Calcd for $C_{31}H_{38}N_4O_5$: C, 68.1; H, 7.0; N, 10.2. Found: C, 67.9; H,

7.0; N, 10.0.

IV——Prepared from Z–Tyr–(Me)Ava–(Me)AEP (200 mg) by hydrogenation in the usual manner. Yield 121 mg (80%), hygroscopic material, $[\alpha]_D^{21}$ +50.8° (c=1.0, MeOH), Rf^3 0.78. *Anal.* Calcd for $C_{23}H_{32}N_4O_3 \cdot 2H_2O$: C, 61.6; H, 8.1; N, 12.5. Found: C, 62.0; H, 8.0; N, 12.8.

Z–(Me)Tyr(Bzl)–OH——Z–Tyr(Bzl)–OH[4] (1 g) was methylated with CH_3I (0.94 ml) and NaH (0.34 g) in THF in the usual manner. Yield 904 mg (67%), mp 103—108°, $[\alpha]_D^{23}$ −53.9° (c=1.0, MeOH), Rf^1 0.91, Rf^3 0.78. *Anal.* Calcd for $C_{25}H_{25}NO_5$: C, 71.6; H, 6.0; N, 3.4. Found: C, 71.3; H, 5.8; N, 3.4.

Z–(Me)Tyr(Bzl)–(Me)Ava–(Me)PHA——Prepared from Z–(Me)Tyr(Bzl)–OH (1.26 g) and H–(Me)Ava–(Me)PHA (0.74 g) by the mixed anhydride method. The product was purified by silica gel column chromatography using 1% MeOH/$CHCl_3$ as the eluent. Yield 1.07 g (55%), syrupy material, $[\alpha]_D^{24}$ +2.6° (c=1.1, MeOH), Rf^4 0.44. *Anal.* Calcd for $C_{40}H_{47}N_3O_5 \cdot 1/2H_2O$: C, 72.9; H, 7.5; N, 6.4. Found: C, 72.9; H, 7.5; N, 6.8.

V——Z–(Me)Tyr(Bzl)–(Me)Ava–(Me)PHA (300 mg) was hydrogenated in a mixture of 1 N HCl and MeOH. Yield 181 mg (85%), hygroscopic material, $[\alpha]_D^{25}$ +14.0° (c=1.0, MeOH), Rf^3 0.50. *Anal.* Calcd for $C_{25}H_{35}N_3O_3 \cdot HCl \cdot 3/4H_2O$: C, 63.1; H, 8.0; N, 8.8. Found: C, 63.1; H, 7.9; N, 9.0.

Z–Abu–PHA——Prepared from Z–Abu–OH[9] (5 g) and PHA (2.7 ml) by the mixed anhydride method, and recrystallized from AcOEt/petro. ether. Yield 6.92 g (96%), mp 113—115 °C, Rf^3 0.81, Rf^4 0.24. *Anal.* Calcd for $C_{20}H_{24}N_2O_3$: C, 70.6; H, 7.1; N, 8.2. Found: C, 70.8; H, 7.1; N, 8.0.

Z–Tyr–Abu–PHA——Z–Tyr–$NHNH_2$ (4.31 g) and H–Abu–PHA (2 g, prepared from Z–Abu–PHA by hydrogenation) was coupled by the azide method. Recrystallized from MeOH. Yield 4.61 g (70%), mp 192—196 °C, $[\alpha]_D^{35}$ −1.5° (c=1.0, MeOH), Rf^3 0.82. *Anal.* Calcd for $C_{29}H_{33}N_3O_5$: C, 69.2; H, 6.6; N, 8.4. Found: C, 68.9; H, 6.5; N, 8.4.

VI——Prepared from Z–Tyr–Abu–PHA (2 g) by hydrogenation. Yield 1.38 g (93%), mp 115—120 °C, $[\alpha]_D^{35}$ +12.2° (c=1.0, MeOH), Rf^1 0.60, Rf^2 0.78, Rf^3 0.53. *Anal.* Calcd for $C_{21}H_{27}N_3O_3$: C, 68.3; H, 7.4; N, 11.4. Found: C, 68.0; H, 7.4; N, 11.2.

Z–Aca–PHA——Prepared from Z–Aca–OH[9] (5 g) and PHA (2.4 ml) by the mixed anhydride method. Recrystallized from AcOEt/petro. ether. Yield 6.36 g (92%), Rf^1 0.83, Rf^4 0.32. *Anal.* Calcd for $C_{22}H_{28}N_2O_3$: C, 71.7; H, 7.7; N, 7.6. Found: C, 72.0; H, 7.7; N, 7.9.

Z–Tyr–Aca–PHA——Z–Tyr–$NHNH_2$ (1.78 g) and H–Aca–PHA (1.27 g, prepared from Z–Aca–PHA by hydrogenation) were coupled by the azide method. Recrystallized from AcOEt. Yield 2.0 g (70%), mp 155 °C, $[\alpha]_D^{34}$ −6.4° (c=1.0, DMF), Rf^3 0.82. *Anal.* Calcd for $C_{31}H_{37}N_3O_5$: C, 70.0; H, 7.0; N, 7.9. Found: C, 70.1; H, 6.8; N, 7.8.

VII——Prepared from Z–Tyr–Aca–PHA (1.67 g) by hydrogenation. Yield 0.87 g (71%), mp 117—118 °C, $[\alpha]_D^{35}$ +7.9° (c=1.0, MeOH), Rf^1 0.32, Rf^2 0.70. *Anal.* Calcd for $C_{23}H_{31}N_3O_3$: C, 69.5; H, 7.9; N, 10.6. Found: C, 69.5; H, 7.9; N, 10.8.

Bioassay——Analgesic effects of the synthetic peptides in mice were examined by the tail-pinch method.[6]

Acknowledgements The authors are grateful to the staff of the Department of Pharmacology, Research Institute, Daiichi Seiyaku Co., Ltd. for carrying out the analgesic activity assays.

References and Notes

1) Standard abbreviations for amino acids, protecting groups, and peptides are used [*J. Biol. Chem.*, **247**, 977 (1972)]. Other abbreviations include: Ava, 5-aminovaleric acid; (Me)Ava, 5-methylaminovaleric acid; PHA, phenethylamine; (Me)PHA, *N*-methylphenethylamine; (Me)AEP, 2-(β-methylaminoethyl)pyridine; Abu, 4-aminobutyric acid; Aca, 6-aminocaproic acid; (Me)Tyr(Bzl), *N*-methyl-*O*-benzyltyrosine; DMF, dimethylformamide; THF, tetrahydrofuran.
2) K. Kawasaki and M. Maeda, *Biochem. Biophys. Res. Commun.*, **106**, 113 (1982).
3) W. Eschweiler, *Chem. Ber.*, **38**, 880 (1905); H. T. Clarke, H. B. Gillespie, and S. Z. Weisshaus, *J. Am. Chem. Soc.*, **55**, 4571 (1933).
4) J. S. Moley, *J. Chem. Soc., C*, **1967**, 2410.
5) J. R. Vaughan, Jr. and R. L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).
6) H. Takagi, T. Inukai, and M. Nakama, *Jpn. J. Pharmacol.*, **16**, 287 (1966).
7) Y. Kiso, T. Miyazaki, M. Inai, K. Kitagawa, T. Akita, H. Moritoki, and H. Nakamura, "Peptide Chemistry 1982," ed. by S. Sakakibara, Protein Res. Found., Osaka, 1983, pp. 245—250.
8) H. Takagi, H. Shiomi, H. Ueda, and H. Amano, *Europ. J. Pharmacol.*, **55**, 109 (1979).
9) J. A. Davies, C. H. Hassal, and I. H. Rogers, *J. Chem. Soc., C*, **1969**, 1358.
10) M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **118**, 405 (1937).
11) J. Honzel and J. Rudinger, *Collect. Czech. Chem. Commun.*, **26**, 2333 (1961).
12) M. Bergmann and L. Zervas, *Chem. Ber.*, **65**, 1192 (1932).
13) Purchased from Aldrich Chem. Co.
14) L. A. Walter, W. H. Hunt, and R. J. Fosbinder, *J. Am. Chem. Soc.*, **63**, 2771 (1941).