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AMINO ACIDS AND PEPTIDES.II. MODIFICATION OF GLYCYLGLYCINE BOND IN METHIONINE ENKEPHALIN

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SUMMARY : An enkephalin analog, tyrosyl-5-aminovalerylphenylalanyl-methionine, has been prepared and shown to be 2 times as active as methionine enkephalin in the analgesic activity.

Smith et al.(1) reported that the conformation of leucine enkephalin was produced by Gly-Gly β-bend stabilized by antiparallel hydrogen bonding between tyrosine and phenylalanine. Nakauma et al.(2) also reported that Gly-Gly β-bend structure of methionine enkephalin was one of active conformations. We are interested in a role of Gly-Gly amide bond which might participate in holding a conformation and synthesized H-Tyr-Ava-Phe-Met-OH which corresponded to an analog of methionine enkephalin replaced its Gly-Gly amide bond with ethylene bond as shown in Fig.1.

MATERIALS AND METHODS

Synthetic scheme for H-Tyr-Ava-Phe-Met-OH is shown in Fig.2. Boc-Ava-OSu was coupled with H-Phe-Met-OH(3) to give the tripeptide followed by TFA treatment to remove the Boc group. The deblocked material was reacted with Boc-Tyr-$NHNH_2$(4) by the azide method followed by TFA treatment to give H-Tyr-Ava-Phe-Met-OH which was purified by ion-exchange column chromatography.

Boc-Ava-OH --- H-Ava-OH(5g) was tert-butoxycarbonylated with 2-tert-butoxycarbonyloxyimino-2-phenylacetonitril(5) in the usual manner. Recrystalized from ethyl acetate/petro.ether;yield 8.4g(91%), mp 53-55°, Rf^1 0.78. Anal. Calcd. for $C_{10}H_{19}NO_4$: C, 55.3; H, 8.8; N, 6.5. Found: C, 55.0; H, 9.0; N, 6.2.
Boc-Ava-OSu --- Boc-Ava-OH(6.5g) was converted to its N-hydoxysuccimide ester by the dicyclohexylcarbodiimide method in the usual ma-

Standard abbreviations for amino acids, protecting groups, and peptides are used[J.Biol.Chem.,247,977-983]. Other abbreviations include:Ava=5-amino valeric acid;-OSu=N-hydroxysuccimide ester. Sovent systems for ascending thin-layer chromatography on silica gel G(type60,E.Merck) are indicated as follows:Rf^1n-BuOH,AcOH,H_2O(4:1:5,upper phase);Rf^2n-BuOH, AcOH,pyridine,H_2O(4:1:1:2);$Rf^3$$CHCl_3$,MeOH,$H_2O$(8:3:1,lower phase).

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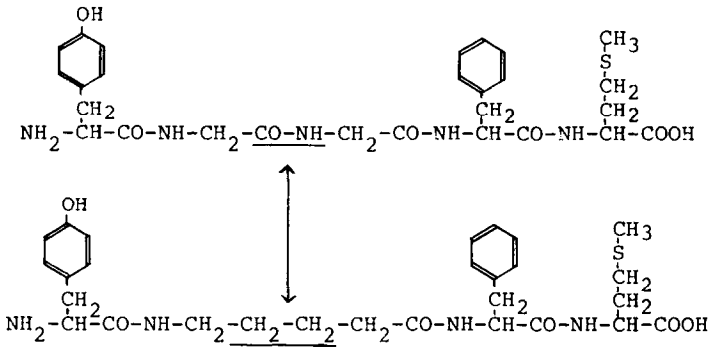

Fig. 1. Comparison of Met-enkephalin and H-Tyr-Ava-Phe-Met-OH

nner. Recrystallized from EtOH; yield 6.6g(70%), mp 98-101°, Rf^1 0.85. Anal. Calcd. for $C_{14}H_{22}N_2O_6$: C, 53.5; H, 7.1; N, 8.9. Found: C, 53.7; H, 6.9; N, 8.6.

Boc-Ava-Phe-Met-OH --- Boc-Ava-OSu(1.89g) was added to a solution of H-Phe-Met-OH(prepared from 2g of the corresponding Boc derivative by TFA treatment) in a mixture of 80% dioxane(10ml) and triethyamine(0.8 ml), and the mixture was stirred over night in a cold room. The solvent was evaporated off and the residue was dissolved in a mixture of ethyl acetate and 1% aqueous triethylamine. The aqueous layer was washed 3 times with ethyl acetate and acidified with citric acid. The resulting precipitate was extracted with ethyl acetate and the organic layer was washed with water, dried over Na_2SO_4 and evaporated down. The residue was purified by silica gel column chromatography. The desired material came out from the column in 1% MeOH/$CHCl_3$ eluate. The desired fractions were washed with water, dried over Na_2SO_4 and evaporated down. The residue was precipitated from petro.-ether/ethyl acetate; yield 1.2g(61%), mp 141-142°, $[\alpha]_D^{23}$ +10.6°(c=1.0, MeOH), Rf^1 0.72. Anal. Calcd. for $C_{24}H_{37}N_3O_6S$: C, 58.2; H, 7.5; N, 8.5. Found: C, 57.9; H, 7.7; N, 8.2. Amino acid ratios in an acid hydrolysate: Ava, 1.09; Phe, 1.00; Met, 0.89(average recovery 91%).

Boc-Tyr-Ava-Phe-Met-OH --- tert-butyl nitrite(0.47ml) was added to a solution of Boc-Tyr-$NHNH_2$(927mg) in a mixture of dimethylformamide

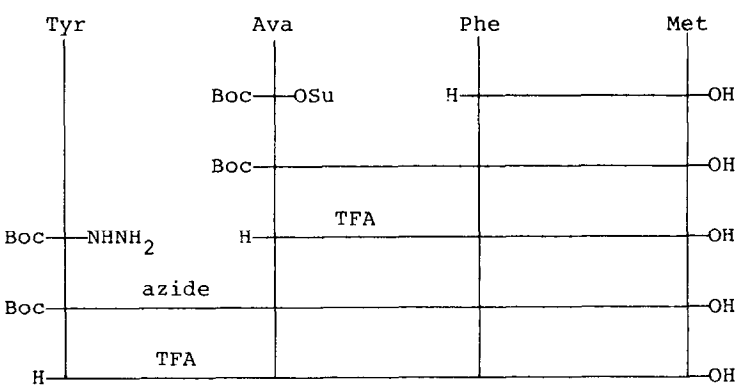

Fig. 2. Synthetic Scheme for H-Tyr-Ava-Phe-Met-OH

(8ml) and 7.5N HCl/dioxane(1.6ml) at -40° and the mixture was stirred for 10 min. The mixture was neutralized with triethylamine(1.7ml) and combined with a solution of H-Ava-Phe-Met-OH(prepared from 1g of the corresponding Boc derivative by TFA treatment) in a mixture of dimethylformamide(8ml) and triethylamine(0.33ml). The reaction mixture was stirred overnight at 4° and the solvent was evaporated off. The residue was extracted with water and the water layer was washed with ethyl acetate. The aqueous layer was then acidified with citric acid and the resulting precipitate was extracted with ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4 and evaporated down. The residue was recrystallized from ethyl acetate; yield 888mg(67%), mp 129-135°, $[\alpha]_D^{20}$-11.5°(c=0.5, MeOH), Rf^1 0.88, Rf^3 0.28. Anal. Calcd. for $C_{33}H_{46}N_4O_8S$: C, 60.2; H, 7.0; N, 8.5. Found: C, 59.9; H, 7.3; N, 8.1. Amino acid ratios in an acid hydrolysate: Tyr, 0.92; Ava, 1.06; Phe, 1.00; Met, 0.91(average recovery 89%).

H-Tyr-Ava-Phe-Met-OH --- Boc-Tyr-Ava-Phe-Met-OH(100mg) was treated with 90% TFA(0.3ml) at room temperature for 50 min. Ether was added and the resulting precipitate was collected by centrifugation, washed with ether and dried. The deblocked material was purified by Dowex 50 column chromatography(H^+ form, 1.7x8cm) using 0.05M pyridine acetate buffer for pH gradient elution(pH 3.8 - 5.8). Fractions showed Rf^2 0.49 were pooled and evaporated down. The residue was lyophilized from water to give fluffy powder; yield 63mg(75%), $[\alpha]_D^{20}$+16.8° (c=1.3, 20%CH_3COOH), Rf^1 0.31, Rf^2 0.49. Anal. Calcd for $C_{28}H_{38}N_4O_6S$: C, 60.2; H, 6.9; N, 10.0. Found: C, 59.9; H, 7.0; N, 9.7. Amino acid ratios in an acid hydrolysate: Tyr, 0.92; Ava, 1.07; Phe, 1.00; Met, 0.91(average recovery 83%).

The analgesic effect of the synthetic peptide was tested according to the method reported by Ueda et al.(6). The peptide was intracisternally administered to mice with a J-shaped needle and the analgesic effect was evaluated by the tail-pinch test.

RESULTS AND DISCUSSION

On the synthesis of Boc-Ava-Phe-Met-OH, ß-alanine was found in an acid hydrolysate of the crude product. Since ß-alanine was not found in an acid hydrolysate of Boc-Ava-OSu, it did not come from an impurity produced by Lossen rearrangement(7) in the active ester. The impurity might be produced during the coupling reaction. The impurity could be removed by silica gel column chromatography.

The synthetic tetrapeptide, H-Tyr-Ava-Phe-Met-OH, was homogeneous on thin layer chromatography and its amino acid ratios in an acid hydrolysate agreed with the expected values. Elemental analysis also showed good agreement with the theoretical values.

The analgesic effect of the synthetic material is shown in table 1. The analgesic effect of the synthetic tetrapeptide was found 2 times as active as methionine enkephalin and was antagonized by naloxone. No

Table 1. The Analgesic Effect of H-Tyr-Ava-Phe-Met-OH intracisternally administered to Mice

	ED 50 μg/mouse (95% confidence limits)
H-Tyr-Ava-Phe-Met-OH	40
Met-Enkephalin	84
Morphine	0.5

analgesic effect was observed when the material was administered by intra-venous injection.

The result suggests that replacement of Gly-Gly amide bond in methionine enkephalin with ethylene bond does not affect an active conformation. A little stronger analgesic effect of the synthetic tetrapeptide comparing with methionine enkephalin might be caused by a more flexble active conformation or by slower hydrolysis of Tyr-Ava bond by an enzyme. Conformational analysis of the synthetic tetrapeptide will be done in future.

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