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SYNTHESIS OF C-TERMINAL HEPTAPEPTIDE ANALOGS OF SUBSTANCE P: THE SUBSTITUTION OF GLUTAMINE BY GLYCINE OR 5-AMINOVALERIC ACID

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C-Terminal heptapeptide analogs of substance P, in which the glutaminyl residues were substituted by Gly or δAva, were synthesized by the liquid phase method. These analogs showed substance P activity. The results suggest that glutaminyl residues are not essential for the intrinsic activity on a guinea-pig ileum.

Substance P(SP), a smooth-muscle stimulating peptide, was first detected in extracts of brain and intestine by von Euler and Gaddum[1] in 1931. It was purified in 1970[2] and was found to be an undecapeptide, H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2, by Chang et al.[3] Since the first synthesis of SP by Tregear et al.,[4] numerous analogs have been synthesized and their structure-activity relationships have been discussed. In studies[5,6] of the relationships between chain length and activity, it was revealed that the C-terminal hexa- and heptapeptides of SP had higher potency than SP, although the pentapeptide fragment had much lower activity. This suggested that glutaminyl residues of SP were very important for inducing activity. Recently, Chipkin et al.[7] proposed a hypothetical SP receptor model, postulating that position 4-6(Pro-Gln-Gln) of SP was not critical for the intrinsic activity but had the ability to enhance the potency related to the affinity of the peptide for the receptor. In the present work, in order to investigate the biological effect of the substitution of glutaminyl residues, we synthesized four analogs (shown below), and tested their contractile activity on isolated guinea-pig ileum.

H-Gln-Gly-Phe-Phe-Gly-Leu-Met-NH_2 1
H-Gly-Gln-Phe-Phe-Gly-Leu-Met-NH_2 2
H-Gly-Gly-Phe-Phe-Gly-Leu-Met-NH_2 3
H-δAva-Phe-Phe-Gly-Leu-Met-NH_2 4

The synthetic route of 1 is shown in Fig. 1.[8] The protected N-terminal pentapeptide was obtained by stepwise coupling, and the C-terminal dipeptide amide was derived from Boc-Leu-Met-OMe by treatment with NH_3. The protected heptapeptide was prepared by condensation of the penta- and dipeptide with WSCD·HCl and HOBt. The peptide was deblocked by TFA, and converted to the corresponding acetate by ion exchange chromatography on Dowex 1-X2 which was purified by partition chromatography on Sephadex G-25. Other peptides 2, 3 and 4 were also obtained in a similar way. Their homogeneities were confirmed by tlc. Elemental analyses of

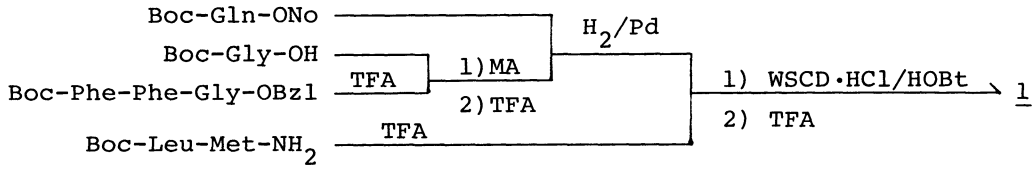

Fig. 1. The synthetic route of 1

the peptides gave satisfactory results. Some of their properties and relative potencies compared with authentic SP are summarized in Table 1.[9)]

Table 1. The characterization of the analogs(1-4), and their biological activities

	Mp(dec.),°C	$[\alpha]_D^{23}$(c,DMF)	Amino Acid Ratios Glu	Phe	Gly	Leu	Met	δAva	Relative Potency (SP: 100)
1	248-52	-47.0°(0.3)	0.96	2.15	2.06	0.96	0.87	—	22
2	223-7	-50.2°(0.3)	1.06	2.12	2.12	0.91	0.81	—	43
3	214-6	-49.6°(0.3)	—	2.07	3.17	0.92	0.83	—	18
4	204-6	-40.8°(0.4)	—	2.19	0,94	0.84	0.96	1.06	76

Analog 2 had the highest activity among the three analogs(1-3) substituted by Gly. Apparently Gln^6 of SP contributes much more than Gln^5 to high potency. Analog 4 showed about 80% of the potency of SP, a higher potency than that of 3. Thus, the peptide bond between positions 5 and 6 (Gln-Gln) of SP is not essential. That is to say, these results suggest that glutaminyl residues are not essential for the intrinsic activity.

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8) Amino acids used are of L-configuration. The abbreviations for amino acids and peptides are in accordance with the rules of the IUPAC-IBU Commission on Biochemical Nomenclature. Other abbreviations are as follows: Boc=t-butoxycarbonyl, TFA=trifluoroacetic acid, OBzl=benzyl ester, ONo=o-nitrophenyl ester, WSCD·HCl=1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, HOBt=1-hydroxybenzotriazole, δAva=5-aminovaleric acid, MA=mixed anhydride method.
9) Amino acid ratios were determined in the hydrolyzates with 6M HCl at 110°C for 20h.

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