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Synthesis and pharmacological properties of [1-N^4-dimethyl-asparagine]-angiotensin II

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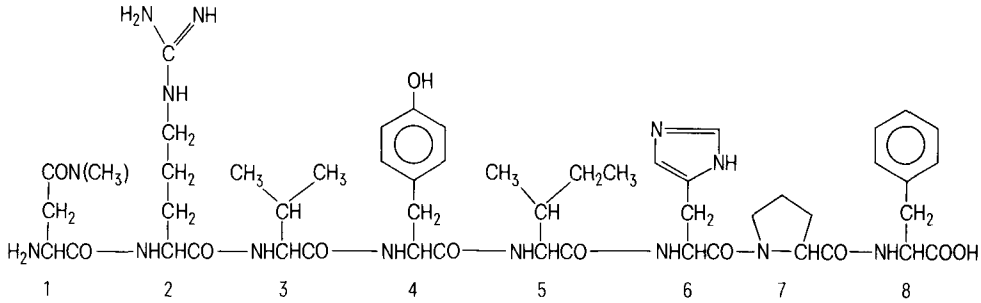
Summary. [1-N^4-Dimethyl-asparagine]-angiotensin II was synthesized by Merrifield's solid-phase procedure. The analogue gave rat blood pressure about 70% relative potency to Hypertensin (Ciba). Rabbit aorta strips gave intrinsic activity $\alpha_E = 1$, a PD_2 of 6.92 ± 0.09 and an affinity relative to [Asn^1]-angiotensin II of 6.5%.

It has been reported that substitution in the 1 position of angiotensin II, e.g. [Sar^1, Ile^8]-angiotensin II, enhanced the agonistic potency of this hormone by an increased binding affinity for the receptor and by a decreased rate of breakdown due to the enzyme angiotensinase A; moreover, the basicity of an α-nitrogen at the N-terminus is important for maximum agonistic properties of angiotensin II. Thus, [1-dimethylglycine]-angiotensin II showed higher pressor activity than [Sar^1]- and natural angiotensin II, while in the antagonistic series it has been proved that [Sar^1, Ile^8]- and [Me_2Gly^1, Ile^8]-angiotensin II enhanced the in vitro antagonistic potency of these analogues[3–5].

Since the N-terminus part in angiotensin II is hydrophilic in nature, we thought it of interest to investigate whether a change to lipophilic character of the carboxamide group of the asparagine residue may yield an analogue of different conformation and hence of different biological profile. Therefore, [N^4-dimethyl-Asn^1]-angiotensin II (figure) was synthesized according to the general solid-phase procedure of Merrifield[6] as follows. Chloromethylated polystyrene resin cross-linked with 1% divinyl benzene containing 0.75 mEq Cl/g was obtained from Bio-Rad Lab. Inc. (Bio-Beads S-Xl, 200–400 mesh). Boc-phenylalanine-resin was prepared by the Loffet[7] esterification procedure. A degree of substitution of 0.56 mmoles of Boc-Phe per g of resin was obtained. The cycles of deprotection (25% trifluoroacetic acid in methylene chloride, twice for 15 min), neutralization (10% triethylamine in methylene chloride) and coupling (120–240 min) were carried out on a Beckman 990 peptide synthesizer. Between individual operations, extensive washing was performed with CH_2Cl_2 or DMF, followed by absolute methanol. All amino acid derivatives[8] were added in 2.5-fold excess. DCCI was used as coupling agent (0.25 M in CH_2Cl_2). After each cycle, completeness of the reaction (> 99% yield) was confirmed with the Kaiser ninhydrin test[9].

Cleavage of the octapeptide from the resin[10], with concomitant removal of the protecting groups, was accomplished by treatment with HF containing 10% by volume of anisole for 1 h at 0 °C. After removal of HF and drying under vacuum, the resin was washed several times with ether and then was extracted with acetic acid (2 M). Lyophilization of the latter extract yielded the crude peptide (1.2 g, 100%) in solid form. A 220-mg portion of the lyophilizate was partially purified by gel filtration on a 2.6 × 100 cm column of Sephadex G-15 in acetic acid (5% v/v). Fractions of 7 ml were collected at a flow rate of 25 ml/h. Peptide material was detected by monitoring the absorbancy at 280 nm. On the basis of the elution profile and amino acids analysis, fractions 25–38 were collected and lyophilized to give 85 mg (39%). Thin-layer chromatography of this product showed one major spot R_f 0.16 and 2 contaminants with R_f 0.12 and 0.19 in the upper phase of the solvent system (BAW) n-BuOH-AcOH-H_2O (4:1:5).

A 80-mg portion of the above material was further purified by partition chromatography on a 2.6 × 100 cm column of



Aminoacid sequence of [N^4-dimethyl-Asn^1, Ile^5]-angiotensin II; numbers indicate sequence positions of individual residues.

Sephadex G-15 previously equilibrated with both phases of the solvent system n-BuOH-AcOH-H_2O (4:1:5). The column was eluted with upper phase at 10 ml/h and collected in fractions of 8 ml. The product was detected by monitoring the absorbancy at 280 nm. The fractions 68–73 were pooled with H_2O, the organic phase was removed in vacuum, and the aqueous phase was lyophilized. Yield 26 mg (12% overall yield based on initial phenylalanine attached to the polymer). The final product gave single spots on TLC (precoated plates of silica gel G) when loads of 10–15 µg were used, with R_f 0.16 (BAW), R_f 0.40 in the solvent system (upper phase) n-BuOH-AcOH-H_2O-pyridine (30:6:24:20) and R_f 0.53 in ethyl acetate-pyridine-AcOH-H_2O (5:5:1:3). Compound was detected on the chromatogram with ninhydrin, chlorine peptide spray and with diazotized sulfanilic acid. M.p. 217–219 °C (dec); $[\alpha]_D^{24}$ −55.3° (c 0.5, 1 N AcOH). Amino acid analysis gave the following molar ratios: Asp, 1.07; Arg, 0.96; Val, 1.03; Tyr, 0.90; Ile, 0.90; His, 0.90; Pro, 1.02; Phe, 1.05. Elemental analysis gave the following values: $C_{52}H_{76}N_{14}O_{11} \cdot C_2H_4O_2 \cdot 3H_2O$ (1187.36) calculated: C, 54.62; H, 7.31, N, 16.51; found: C, 54.40; H, 7.29; N, 16.45.

NMR-spectra run with a Varian HR-220 spectrometer at pHs 1.5, 7.5 and 9.0 indicated the analogue to be towards a trans-configuration around the His-Pro peptide bond.

Rat blood pressure test was performed according Regoli[11] and was about 70% relative potency to Hypertensin (Ciba) ([Asn^1]-angiotensin II). Rabbit aorta strips[12] gave intrinsic activity $a_E = 1$, a PD_2 of 6.92±0.09 and an affinity relative to [Asn^1]-angiotensin II of 6.5%. In both systems the action of the analogue was specific for the angiotensin II-receptor. Its action was competitively inhibited by [Leu^8]- and [Sar^1, Leu^8]-angiotensin II, while addition of a maximal dose of angiotensin II after maximal contraction caused by the analogue did not give any further contraction. As the relaxation time of the tissues after removing of the analogue was about as quick as for natural angiotensin II, it seems likely that it is degraded similarly to angiotensin II by aminopeptidases.

In conclusion it seems likely that the reduced affinity of the analogue tested should be explained by some steric hindrance of its N^4-dimethyl group in position 1, although modification of other type of interactions with the receptor (e.g. hydrogen bonding) cannot be completely excluded.

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A new synthesis of β-fluoroaspartic acid[1]

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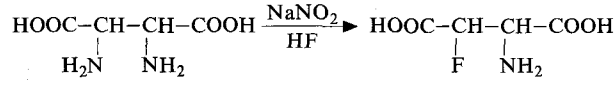
Summary. β-Fluoroaspartic acid, a new amino acid, was synthesized by a diazotization of diaminosuccinic acid in liquid hydrogen fluoride.

Halogenoamino acids, halogen-containing α-amino acids, have frequently been isolated from natural sources and have become attractive as a class of biologically interesting α-amino acids[3]. In particular, fluoro-α-amino acids are noteworthy because they act as metabolic antagonists to the naturally occurring α-amino acids[4], and show antibacterial activities against various microorganisms[5]. From this point, a considerable number of the fluoroamino acids have been synthesized; as monofluorinated α-amino acids, fluoroalanine, fluorobutyrine, fluorovaline, fluoroleucine, fluoroisolecine, fluorophenylalanine, fluorothreonine, fluoroproline, fluorohistidine, fluoroglutamic acid, etc., being reported[6–13].

Among these fluoroamino acids, however, fluoroaspartic acid[14], which is of great interest from the biological viewpoint, has never appeared as yet, though some attempts to synthesize have been made by several authors[8,9,15]. In this work, we have attempted the synthesis of fluoroaspartic acid[16] and found a convenient preparation of the desired β-fluoroaspartic acid from diaminosuccinic acid by the following scheme.



The process involves a simple diazotization in liquid hydrogen fluoride; the method and results are as follows. meso-Diaminosuccinic acid (14.8 g, 0.1 moles) was dissolved in liquid hydrogen fluoride (25 ml) kept at −30– −10 °C in a polyethylene bottle. To the mixture was added well dried sodium nitrite (6.2 g, 0.09 moles) in small portions for a period of 10 min under vigorous stirring. After the stirring