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Notes

Synthesis of a Proposed Pepsitensin

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Pepsitensin is a vasopressor peptide obtained by peptic proteolysis of the α_2-globulin fraction of blood plasma.[1] Its biological activities are qualitatively identical with those of angiotensin II[2] but the two peptides were shown to be different.[3] Franze, *et al.*,[4] isolated from plasma, after incubation with pepsin at pH 6, a pressor substance that was identified as the decapeptide angiotensin I. More recently, a pepsitensin was isolated from bovine plasma after hydrolysis with pepsin at pH 3, for which the following sequence was proposed: Asp·Arg·Val·Tyr·Val·His·Pro·Phe·His·Leu·Leu (**1**).[5] These results might indicate that peptic proteolysis of the plasma protein substrate would differ according to whether pH 6 or 3 is employed in the incubation, leading to the production of either angiotensin I or the undecapeptide,[5] respectively.

In order to verify this hypothesis, and to study the pharmacological properties of the proposed pepsitensin, we have synthesized both **1** and its Ile[5] analog **2**, which would be the homologous peptide obtained from horse plasma.

The peptides were tested for their biological activity on the rat's blood pressure, the isolated guinea pig ileum, and the isolated rat uterus using a four-point assay design,[6] with the results shown on Table I. The activities of the two peptides on the rat's blood pressure and the guinea pig ileum were surprisingly low compared to angiotensin I,[7] from which they differ only by an additional leucine residue at the C terminus. The observed activities appear to be intrinsic to **1** and **2**, and not due to their conversion to angiotensin II, because of the great differences in "converting enzyme" activity in the rat's blood plasma, uterus, and the guinea pig ileum.[8]

The conversion of both **1** and **2** to the respective angiotensin II octapeptides was easily obtained by the action of carboxypeptidase. An illustration of this conversion is presented in Figure 1 which shows that the incubation of **1** with porcine carboxypeptidase A resulted in a very large

Table I. Biological Activity[a] of Proposed Pepsintensins

Assay	Compd 1[b]	Compd 2[b]
Rat's blood pressure	1.28 (0.08)	1.42 (0.09)
Rat uterus	0.82 (0.10)	1.40 (0.06)
Guinea pig ileum	0.88 (0.09)	1.68 (0.06)

[a] Relative to Ile[5]-angiotensin II = 100, on a molar basis. [b] Each value was obtained from a four-point assay made with at least eight groups of four doses. Standard deviations are shown inside parentheses.

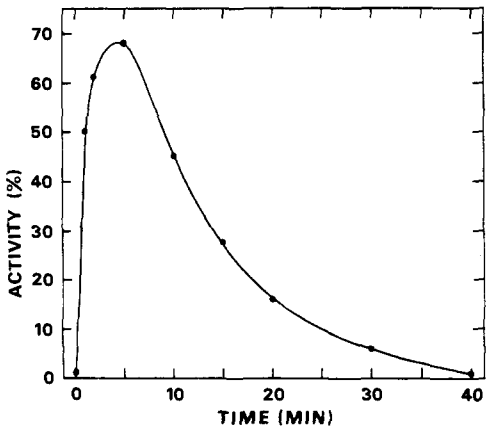

Figure 1. The effect of carboxypeptidase A on the biological activity of **2**. The peptide (7×10^{-4} *M*) was incubated with the enzyme (0.34 g/l.) at pH 8.4, 0.1 *M* phosphate buffer, and aliquots were removed, boiled for 5 min, and diluted in Tyrode's solution for the assay on the isolated guinea pig ileum.

increase of the biological activity, reaching a maximum of about 70% the activity of angiotensin II. This was followed by inactivation, as would be expected, since carboxypeptidase removes the C-terminal phenylalanine of angiotensin II with complete inactivation.[9]

Experimental Section

The same procedure was followed in the synthesis of both compounds 1 and 2. Merrifield's solid-phase method,[10] with an automatic peptide synthesizer,[11] was performed as described by Stewart and Young,[12] with some modifications. The synthesis started with 0.4 mmol of *N*-α-Boc-L-leucine-resin. The coupling reaction was performed with a 2.5 *M* excess of Boc-amino acid and DCI. The side-chain protecting groups were the usual ones employed in solid-phase peptide synthesis,[12] with the exception of histidine where the imidazole was protected with the *p*-Ts group[13] recently introduced for this purpose in solid-phase synthesis.[14,15] Because this group was not stable to 4 *N* HCl in dioxane, usually employed in the deprotection step of the solid-phase method, a solution of 25% (v/v) trifluoroacetic acid in $CHCl_3$ was used in this step.

The coupling of the last residue (Boc-β-benzylaspartic acid) was followed by a deprotection step, after which the peptide-resin was washed with EtOH. After washing with CH_2Cl_2 to allow the peptide-resin to swell, the peptides were cleaved from the resin by stirring for 60 min at 0° in anhydrous HF containing 5% (v/v) anisole. The HF was then removed by distillation *in vacuo* and the residue was washed free of anisole with EtOAc. The peptides were then extracted with glacial AcOH and freeze-dried. Purification of the two peptides was accomplished by countercurrent distribution in the system *n*-BuOH:AcOH:H_2O (4:1:5), on a Post Model 3 automatic instrument. In both cases, 200 transfers were sufficient to obtain the pure peptide acetates that were concentrated in a rotary evaporator and freeze-dried.

Asp·Arg·Val·Tyr·Val·His·Pro·Phe·His·Leu·Leu (1) behaved on countercurrent distribution as a homogeneous ninhydrin- and Pauly-positive peptide with a distribution coefficient $K = 0.29$. Only one component was seen by tlc on silica gel with *n*-BuOH:AcOH:H_2O (4:1:1) ($R_f = 0.24$) and with *n*-BuOH:pyridine:AcOH:H_2O (30:20:6:24) ($R_f = 0.55$). High-voltage paper electrophoresis with pyridine acetate buffer, pH 4.9, and with 2 *M* AcOH, pH 2.4, also showed only one ninhydrin- and Pauly-positive spot with the expected mobility. The amino acid analysis, performed on a Beckman Model 120 C amino acid analyzer, gave the following composition: Asp, 1.03; Arg, 1.00; Val, 1.95; Tyr, 0.94, His, 2.10; Pro, 0.98; Phe, 0.97; Leu, 1.99. The yield of the pure peptide was 34% of the theoretical, based on the starting amount of Boc-leucyl-resin.

Asp·Arg·Val·Tyr·Ile·His·Pro·Phe·His·Leu·Leu (2) also was homogeneous on countercurrent distribution ($K = 0.31$), tlc with *n*-BuOH:AcOH:H_2O (4:1:1) ($R_f = 0.27$), and high-voltage paper electrophoresis at pH 4.9 (pyridine acetate) and pH 2.4 (2 *M* AcOH), where it showed the expected mobility. The amino acid composition was found to be: Asp, 1.05; Arg, 0.98; Val, 1.04; Tyr, 0.94; Ile, 0.93; His, 1.96; Pro, 1.01; Phe, 0.97; Leu, 2.07. The yield of the pure peptide was 25% of the theoretical, based on the starting amount of Boc-leucyl-resin.

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Antimicrobials. New Nitrofuran Derivatives

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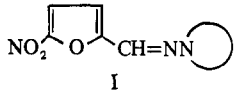
Since the introduction of nitrofurans in human therapeutics, as a result of the investigations of Dodd and Stillman,[1] a tremendous number of compounds of this type have been synthetized. Most of them are derivatives of 5-nitro-2-furfural with an amino compound and possess the general structure I. The linkage -CH=NN< seems to be as essential for the antibacterial activity as the 5-nitro-2-furan residue. Antibacterial compounds derived from the



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vinylogous 3-(5-nitro-2-furyl)acrolein have not been so extensively studied. We hoped that the introduction in the side chain of a vinyl linkage in addition to the imino bond will procure more active compounds. Furthermore, the amine to be condensed with the nitro aldehyde was chosen among the amino acids.

Modified amino acids have already been used as antagonists of natural ones in bacterial cells. For instance,