THE STRUCTURE OF ELASTATINAL, AN ELASTASE INHIBITOR OF MICROBIAL ORIGIN

Sir:

Elastatinal[1] is an elastase inhibitor which was found in culture filtrates of various species of actinomycetes. In this communication, its structural elucidation is reported.

The properties of elastatinal have already been described in a previous paper[1]. It has the molecular formula $C_{21}H_{36}N_8O_7$. The IR [1725, 1655 (broad) and 1550 (broad) cm^{-1}] and UV [$\lambda_{max}^{0.1N\ HCl}$: 275 nm ($E_{1cm}^{1\%}$, 1.5)] spectra, color reactions (RYDON-SMITH, FOLIN, triphenyltetrazolium chloride, silver nitrate and 2, 4-dinitrophenylhydrazine tests are positive, but ninhydrin is negative) and preliminary degradation studies suggested that elastatinal is an N-masked oligopeptide containing an aldehyde function. Potentiometric titration showed the presence of one carboxyl (pKá 3.7) and one strongly-basic (pKá >11) function. The latter was suggested to be a disubstituted guanidino group from the color reactions (nitroprusside-ferricyanide reaction is positive, but SAKAGUCHI is negative)[2]. Oxidation of the aldehyde group with potassium permanganate in aqueous solution gave elastatinic acid and its structure was determined.

Elastatinic acid (pKá 3.2, 4.3 and >11) was hydrolyzed with 6 N HCl at 105°C for 60 hours in a sealed tube. The amino acid analysis of the hydrolyzate showed the presence of three common amino acids (glutamic acid, alanine and leucine; molar ratio 1.0: 1.0:0.1) and one unusual basic amino acid designated compound **I**. A large scale hydrolyzate was separated into three fractions by carbon column chromatography developed with water followed by aqueous acetone. The effluent contained a mixture of glutamic acid, alanine and leucine, and compound **I** was isolated from the water-eluate. The aqueous acetone eluate contained a mixture of hydantoins from leucine and compound **I**.

L-Glutamic acid ($[\alpha]_D^{20}$ +31.0°, *c* 1.0, 6 N HCl), L-alanine ($[\alpha]_D^{20}$ +17.3°, *c* 0.36, AcOH) and L-leucine ($[\alpha]_D^{20}$ −8.0°, *c* 1.0, H_2O) were separated by silica gel column chromatography developed with chloroform, methanol and 15% ammonia (2:2:1 in volume). Optical rotation of the isolated alanine and leucine suggested that partial racemization had occurred.

Compound I was obtained as colorless crystals. It gave positive ninhydrin and nitroprusside-ferricyanide, and negative SAKAGUCHI reactions. It had the molecular formula $C_6H_{12}N_4O_2$ and three pKá values (<2, 7.3 and >12). The PMR spectrum showed that it is a mixture of diastereomers containing in common the moiety of $-CH_2-CH_2-\underset{|}{CH}-\underset{|}{CH}-$. These properties suggested that **I** is a diastereoisomeric mixture of α-(2-iminohexahydro-4-pyrimidyl)-glycines, such as was recently isolated from chymostatin[3]. The diastereomers of **I** were separated by fractional crystallization of their diflavianates following the procedure used in the studies of chymostatin[3]. The major component of **I** was identified as α-[2-iminohexahydro-4(S)-pyrimidyl]-(S)-glycine (**I-a**) and the minor component as the R-glycine derivative (**I-b**) by comparison of their IR spectra and optical rotations with those of the authentic samples.

The mixture of hydantoins composed of leucine and **I** was found to contain at least four isomers. Details of the separation, characterization, racemization, and structural relations of these isomers will be reported elsewhere. When elastatinic acid was hydrolysed under mild conditions the content of the major component (**II**) of the hydantoin mixture was larger. This suggests that **II** retains the stereochemistry of the leucine and **I** moieties of elastatinal. Deuterium exchange examinations by PMR spectrometry showed that in 20% ND_4OD solution at room temperature the α-methine proton of the leucine moiety of **II** was easily racemized, which suggested that the hydantoin ring of **II** includes the carboxyl group of leucine[4].

Compound **II** was synthesized from L-leucine and **I-a** (Fig. 1). To a toluene suspension of L-leucine benzyl ester hydrochloride was added an excess of trichloromethoxycarbonylchloride. After reflux for 4.5 hours, the isocyanate of L-leucine benzyl ester was obtained in almost quantitative yield. The isocyanate was reacted with **I-a** in DMSO solution for 20 hours at room temperature, affording the linear ureide in about 90%

Fig. 1. Preparation of compound II

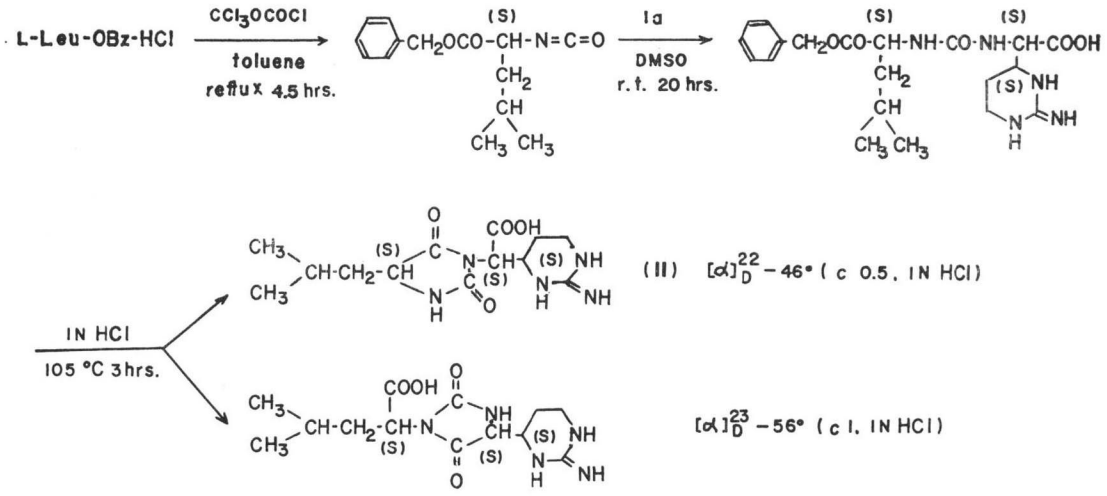
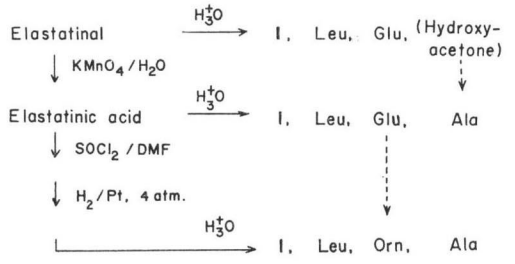


Fig. 2. Amino acids obtained by acid hydrolysis of elastatinal and its derivative

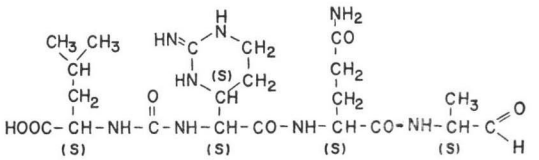


yield. The hydantoin cyclization was achieved by heating the ureide at 105°C for 3 hours in 1 N HCl. Under these conditions racemization of the hydantoin was not observed. Compound **II** and another hydantoin which contained the carboxyl group of **I-a** in the hydantoin ring, were separated by silica gel column chromatography. Synthetic and natural **II** had identical IR spectra, chromatographic behavior and optical rotation ($[\alpha]_D^{22}$ −46°; *c* 0.5, 1 N HCl).

Carboxypeptidase A digestion of elastatinic acid liberated alanine. This product was not obtained by acid hydrolysis of elastatinal. Instead, hydroxyacetone was isolated (Fig. 2) and identified as its 2, 4-dinitrophenylhydrazone. These results indicated that the C-terminal unit of elastatinal was L-alaninal. Hydrazinolysis of elastatinic acid gave alanine and leucine, which indicated that the N-terminal unit of elastatinal was **I-a**, in which

Fig. 3. The structure of elastatinal

N-[(S)-1-Carboxy-isopentyl] carbamoyl-α-[2-iminohexahydro-4(S)-pyrimidyl]-(S)-glycyl-(S)-glutaminyl-(S)-alaninal



the amino group is masked by ureide formation with L-leucine.

Glutamic acid, which was liberated by acid hydrolysis of elastatinal and elastatinic acid, was considered to be derived from glutaminyl moiety from the following evidence. When elastatinic acid was reacted with thionyl chloride at room temperature for 3 hours in DMF solution, it formed a product with IR absorption at 2450 cm^{-1}. This suggested that a nitrile function had been generated by dehydration of a carboxamide group. Catalytic hydrogenation at 4 atmospheres pressure with platinum catalyst in acetic acid at room temperature, followed by acid hydrolysis gave ornithine (Fig. 2).

From all of these results, the structure of elastatinal was deduced to be N-[(S)-1-carboxy-isopentyl]carbamoyl-α-[2-iminohexahydro-4(S)-pyrimidyl] - (S) - glycyl - (S) - glutaminyl - (S) - alaninal (Fig. 3). The proposed structure was confirmed by a total synthesis, which will be published elsewhere.

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