Communication to the editor

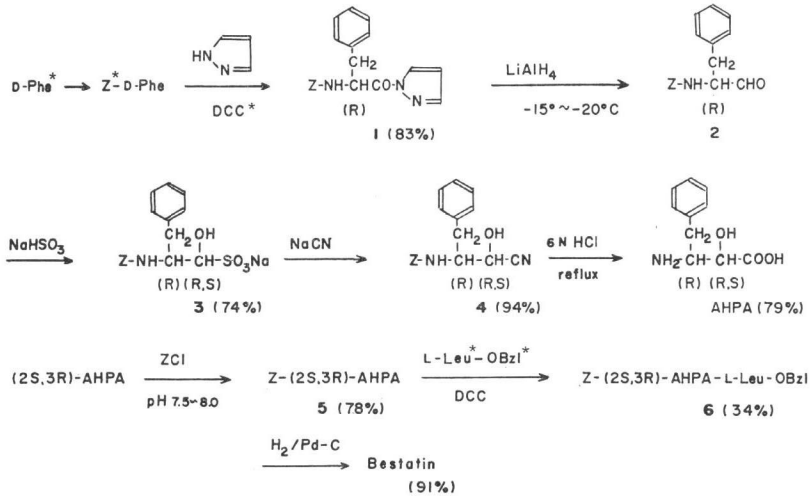
THE CHEMICAL SYNTHESIS OF BESTATIN

Sir:

Bestatin is a specific inhibitor of aminopeptidase B and leucine aminopeptidase.[1] It was isolated from a culture filtrate of *Streptomyces olivoreticuli*. Its structure was elucidated as [(2S,3R)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine.[2,3] (2S,3R)-3-Amino-2-hydroxy-4-phenylbutanoic acid [abbreviated as (2S,3R)-AHPA], an acid hydrolysis product of bestatin, is a new naturally-occuring amino acid. In this communication, the chemical synthesis of bestatin by the scheme shown in Fig. 1, is reported.

To an ethyl acetate solution of benzyloxycarbonyl-D-phenylalanine kept at 0°C was added one equivalent of dicyclohexylcarbodiimide (DCC). Thirty minutes after addition, one equivalent of pyrazole was added. The reaction mixture was stirred for 16 hours at 0°C. After removal of resulting dicyclohexylurea by filtration, the filtrate was evaporated under reduced pressure to yield crude pyrazolide of benzyloxycarbonyl-D-phenylalanine (**1**). It was crystallized from ethyl acetate, mp 108~109°C (83% yield). To a tetrahydrofuran (THF) solution of **1** kept at −15~−20°C was added 2 eq. of $LiAlH_4$ in THF intermittently over a period of 30 minutes. The reaction mixture was stirred for 30 minutes at the same temperature. After decomposition of the excess reagent by addition of 2 N HCl, the solvent was evaporated and the residue was extracted with ethyl acetate. The extract was washed with water and then dried. The dried material, benzyloxycarbonyl-D-phenylalaninal (**2**), was used in the following reaction without further purification. An aqueous suspension of **2** was treated with 2 eq. of $NaHSO_3$ at 60°C for 2 hours to form the adduct (**3**). It was extracted from the clear reaction mixture with ethyl acetate and crystalline powder of **3** was obtained after evaporation of the solvent in 74% yield starting from the pyrazolide. To an ice-cold aqueous solution of **3** was added 1 eq. of NaCN over a period of 1 hour to form the cyanohydrin (**4**). It was extracted with ethyl acetate in 94% yield. Reflux of **4** in 6 N HCl gave AHPA in 79% yield. As expected, AHPA thus obtained was a mixture of (2S,3R)- and (2R,3R)-isomers. (2S,3R)-AHPA was separated from its diastereoisomer by Dowex 50 chromatography using linear gradient elution between 0.36 M pyridine-acetate (pH 3.52) and 0.48 M pyridine-acetate (pH 3.80) buffer. (2S,3R)-AHPA was eluted later than the diastereoisomer. The synthetic (2S,3R)-AHPA was identical with natural material, $[\alpha]_D^{22}$ +27.7° (*c* 1.0, 1 N HCl) [Lit.[2] $[\alpha]_D^{22}$ +27.9° (*c* 0.717, 1 N HCl)]. On a cellulose thin-layer chromatogram using butanol saturated with

Fig. 1. Synthesis of bestatin



* Phe, Phenylalanine; Z, Benzyloxycarbonyl; DDC, Dicyclohexylcarbodiimide; Leu, Leucine; Bzl, Benzyl.

water, (2S,3R)-AHPA can be distinguished from (2R,3R)-AHPA. The Rf value of the former was 0.45 and that of the latter was 0.37.

The benzyloxycarbonyl derivative of (2S,3R)-AHPA (**5**) was obtained by treatment with benzyloxycarbonyl chloride under pH control between 7.5~8.0 by automatic pH titrator (Metrohm Herisau) to avoid oxazolidone formation. It was recrystallized from ethyl acetate, mp 158.5~159.5°C (78% yield). Compound **5** and L-leucine benzylester tosylate were coupled by the DCC method to yield **6** in 34% yield, mp 126~128°C. Catalytic hydrogenolysis of **6** with Pd-C yielded bestatin in 91% yield.

Anal. Calcd. for $C_{16}H_{24}N_2O_4$: C, 62.32; H, 7.82; N, 9.08; O, 20.75.
Found: C, 61.62; H, 7.58; N, 8.65; O, 20.56.

Synthetic bestatin showed the same optical rotation {$[\alpha]_D^{22}-15.6°$ (*c* 1.0, 1 N HCl), Lit.[1], $[\alpha]_D^{22}-15.5°$}, IR spectrum, and biological activities as the natural material.

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References

1) UMEZAWA, H.; T. AOYAGI, H. SUDA, M. HAMADA & T. TAKEUCHI: Bestatin, an inhibitor of aminopeptidase B, produced by actinomycetes. J. Antibiotics 29: 97~99, 1976
2) SUDA, H.; T. TAKITA, T. AOYAGI & H. UMEZAWA: The structure of bestatin. J. Antibiotics 29: 100~101, 1976
3) NAKAMURA, H.; H. SUDA, T. TAKITA, T. AOYAGI, H. UMEZAWA & Y. IITAKA: X-Ray structure determination of (2S, 3R)-3-amino-2-hydroxy-4-phenylbutanoic acid, a new amino acid component of bestatin. J. Antibiotics 29: 102~103, 1976