

ISOLATION AND CHARACTERIZATION OF A NEW AMINO ACID,  
5-CHLORO-D-TRYPTOPHAN FROM ANTIBIOTIC LONGICATENAMYCIN<sup>1)</sup>

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A new antibiotic with the peculiar amino acid components was isolated from a streptomyces strain S-520 by J. Shoji et al,<sup>2a,b)</sup> and named tentatively the antibiotic S-520 which is now renewed to longicatenamycin.<sup>3)</sup> This is a mixture of congeners of cyclic peptides with three exchangeable amino acids. Consistent amino acids were reported to be each one mole of glycine, threo- $\beta$ -hydroxy-L-glutamic acid, L- $\alpha$ -aminoisooctanoic acid (L- $\alpha$ -aminoisooctanoic acid, L- $\alpha$ -aminoisooheptanoic acid), D-ornithine (D-lysine) and D-valine (D-isoleucine).<sup>2,4)</sup>

However, a consideration on the molecular weight suggested a presence of an unknown amino acid which escaped from the amino acid analysis. Furthermore, the UV absorption at 298, 289, 282, 226 nm, and the positive Ehrlich reaction of the original antibiotic, may indicate an existence of a chromophore such as tryptophan-like amino acid which was degraded on the acid hydrolysis.

Therefore, in our study, longicatenamycin (1 g) was first hydrolyzed with 10% Ba(OH)<sub>2</sub> and a new amino acid (50 mg) of molecular formula C<sub>11</sub>H<sub>11</sub>O<sub>2</sub>N<sub>2</sub>Cl was isolated by extraction with isobutyl alcohol from the hydrolyzate, Mp. 250-255°C (dec.), UV:  $\lambda_{\max}^{\text{MeOH}}$  298 nm ( $\epsilon$  3800), 289 (5300), 282 (5200), 226 (36700), NMR (100 MHz, C<sub>5</sub>D<sub>5</sub>N-D<sub>2</sub>O, 1 : 1): H-4:  $\delta$ 8.03 (1H, d, J=2.0 Hz), H-6: 7.11 (1H, dd, J=2.0, 9.0), H-7: 7.49 (1H, d, J=9.0), H-2: 7.78 (1H, s), H- $\beta$ : 3.66 (1H, dd, J=4.0, 16.0), H- $\beta'$ : 4.00 (1H, dd, J=10.0, 16.0), H- $\alpha$ : 4.55 (1H, dd, J=4.0, 10.0), MS: m/e, 238, 193, 164, 127. From all the above data, the structure of 5-chloro-tryptophan was deduced.

However, this amino acid was completely racemized during the basic

hydrolysis. In order to clarify a configuration of the  $\alpha$ -carbon atom, longicatenamycin (250 mg) was next hydrolyzed with 6N HCl at 110°C for 1 hr in a degassed and sealed tube. From the hydrolyzate, an optically active 5-chlorotryptophan (10 mg) was thus obtained. Mp. 238-245°C (dec.),  $[\phi]_{320} + 3410^\circ$  (peak),  $[\alpha]_D^{25} + 40^\circ$  (c, 0.58, MeOH).

Although L- $\alpha$ -amino acids show positive Cotton effects in general, L-tryptophan is known to give a negative effect exceptionally. Therefore, (+)-5-chlorotryptophan was supposed to have D-configuration. To certify this supposition, (+)-5-chlorotryptophan was ozonized and then treated with hydrogen peroxide to give aspartic acid<sup>5)</sup> which was purified by Dowex 50 column and then 2,4-dinitrophenylated. DNP-aspartic acid thus obtained showed negative specific optical rotation,  $[\alpha]_D^{18} - 8.0^\circ$  (c 0.03, MeOH);  $[\alpha]_D^{20} - 45^\circ$  (c 0.11, 1% NaHCO<sub>3</sub>), indicating that this compound possesses D-configuration. Accordingly, 5-chlorotryptophan in longicatenamycin must be also of D-configuration.

For a confirmation of this supposed structure, this amino acid was synthesized starting from  $\beta$ -cyanoacrylonitrile through Bücherer's reaction to hydantoin nitrile, reduction of nitrile to aldehyde with poisoned nickel catalyst, Fischer's indole reaction with p-chlorophenylhydrazine and then alkaline hydrolysis successively.<sup>6)</sup> The synthetic DL-5-chlorotryptophan, mp. 239-242°C (dec.), was completely identical with the natural amino acid in respects of TLC, IR, NMR, amino acid analysis, and mass spectrum. This amino acid is a new naturally occurring amino acid which has never been reported so far.

#### REFERENCES AND FOOTNOTES

- 1) This work was presented at the 30th Annual Meeting of the Chemical Society of Japan, Osaka, Japan, April, 1974.
- 2) a) J. Shoji, S. Kozuki, M. Mayama, and N. Shimaoka, *J. Antibiotics*, 23, 429 (1970); b) J. Shoji, and R. Sakazaki, *ibid.*, 23, 432 (1970); c) J. Shoji, and R. Sakazaki, *ibid.*, 23, 519 (1970).
- 3) This term is based on the presence of special long chain  $\alpha$ -amino acids with seven, eight and nine carbon atoms as constituents in the antibiotic.<sup>1c)</sup>
- 4) Amino acids in parenthesis show the replaceable ones at each position.
- 5) Y. Maeno, and S. Seno, *Nippon Kagaku Zasshi*, 87, 1240 (1966).
- 6) Y. Komachiya, S. Suzuki, T. Yamada, H. Miyashiki, and S. Sakurai, *ibid.*, 86, 104 (1965).