Notes

β-ETHYNYLSERINE, AN ANTIMETABOLITE OF L-THREONINE, FROM *STREPTOMYCES CATTLEYA*

MINORU SANADA*, TETSUJI MIYANO and SHUICHI IWADARE

Okazaki Research Laboratories,
Banyu Pharmaceutical Co., Ltd.,
3-9-1 Kamimutsuna, Okazaki, Aichi 444, Japan

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As reported in our previous paper[1], 4-fluorothreonine, an antimetabolite of L-threonine, was found in the culture broth of the thienamycin producer *Streptomyces cattleya*[2]. Thereafter, we isolated another antimetabolite in the course of purification of 4-fluorothreonine. This antibiotic was also an antimetabolite of L-threonine and was identified as β-ethynylserine from its physico-chemical properties. Here we report on the isolation, identification and biological properties of this antibiotic.

Seed cultures of *S. cattleya* NRRL 8057 were prepared in a medium containing sucrose 3%, distiller's solubles 1.5%, yeast autolysate 0.5%, and corn gluten meal 0.5% per liter of tap water (initial pH 7.5). Seed developed in this medium was inoculated into a 200-liter fermentor containing 130 liters of production medium consisting of glycerol 3%, distiller's solubles 1%, corn steep liquor 2.3%, Pharmamedia 0.75%, dry yeast 0.75%, potassium fluoride 0.015%, Na-succinate 0.1%, glycine 0.15%, $CaHPO_4 \cdot 5H_2O$ 0.05%, $CoCl_2 \cdot 6H_2O$ 0.0002%, $MgSO_4 \cdot 7H_2O$ 0.001%, and KM-75 (Shinetsu Chem.) 0.05% per liter of tap water (initial pH 7.5). The fermentation was carried out at 28°C for 5 days with aeration at 100 liters/minute and agitation at 100 rpm.

The mycelia were filtered off and the filtrate was passed through a column of Amberlite IR-120 (H^+, 15 liters bed volume). The column was washed with water and the absorbed activity was eluted with 2 M NH_4OH. The active fractions from the column were combined and concentrated *in vacuo* to a volume of about 1 liter. The concentrated fraction was applied to a column of activated charcoal and developed with water. The active fractions were concentrated to 200 ml *in vacuo* and yielded a precipitate (42 g) by adding methanol (1 liter). Further purification was carried out by chromatography on a column of silica gel (Wako gel W-200) equilibrated and eluted with a mixture of acetonitrile - acetic acid - water (9 : 1 : 1). The antimetabolite-rich fractions were combined and concentrated *in vacuo*, and passed through a column of Amberlite IR-120 (H^+, 140 ml bed volume). The column was washed with water and the activity was eluted with 2 M NH_4OH. The active fractions were concentrated *in vacuo* to dryness to yield a pale yellow powder (6 g). The active substance was purified by crystallization from water. It was apparent from the IR spectrum that the crystals thus obtained contained two components, 4-fluorothreonine and another compound.

The crystals were dissolved in a small amount of hot water and the solution was gradually cooled to 5°C to yield colorless prisms free of 4-fluorothreonine. Recrystallization from water resulted in colorless prisms (0.32 g): mp 205 ~ 207°C; $[\alpha]_D^{20}$ −71.5° (*c* 1, H_2O). The purified compound gave a positive color reaction (yellow to brown) with ninhydrin reagent. *pKa* Values of 2.6 and 8.15 suggested an α-amino acid structure; titration equivalent was 129.

Anal Calcd for $C_5H_7NO_3$: C 46.51, H 5.47, N 10.85.
Found: C 46.35, H 5.56, N 10.87.

Field desorption mass spectrometry gave a peak at *m/z* 130 (M^+ +1).

The IR spectrum showed absorptions at 3260 and 2110 cm^{-1} characteristic of a terminal acetylenic group. In the UV spectrum no peaks of absorption were observed except end absorption. From these properties, it was concluded that the active substance was an amino acid which contained a terminal acetylenic group.

The ^1H NMR spectrum showed signals at δ 3.15 (1H, doublet, *J*=2 Hz), 4.35 (1H, doublet, *J*=3 Hz), and 5.16 (1H, apparent triplet; actually doublet of doublets). The signals at δ 3.15 and 4.35 are ascribable to CH≡C– and –CHC*H*C–

respectively, and a carbon skeleton of CH≡CCHCHC– was deduced from proton-proton decoupling analysis.

The ^{13}C NMR spectrum (recorded in D_2O with dioxane as an internal reference) is composed of five ^{13}C signals which support the above molecular formula. There are signals at δ 59.7 (d, CHN), 60.8 (d, CHO), 77.7 (s, C≡CH), 80.7 (d, C≡CH), and 171.4 (s, COOH). On the basis of the additional evidence provided by these NMR studies, the active substance is 2-amino-3-hydroxypent-4-ynoic acid.

The optical rotation $[\alpha]_D^{20}$ $-71.5°$ (*c* 1, H_2O) was very close to literature values[3); $[\alpha]_D$ $-76.9°$ (*c* 2.3, H_2O). On the basis of these considerations, the active substance was confirmed to be 2(*S*),3(*R*)-2-amino-3-hydroxypent-4-ynoic acid.

This compound has antibacterial activity against *Pseudomonas aeruginosa* MB-2835 on Davis minimal medium; an inhibitory zone of 17.5 mm was obtained with 15 μg of β-ethynylserine on a paper disk. In studies with this bacterial strain, the reversal of the antibiotic activity by amino acids was examined. The growth inhibition was readily reversed by L-threonine while L-serine and L-alanine were partially effective.

The LD_{50} of β-ethynylserine in mice was 320 mg/kg when administered intravenously.

POTGIETER *et al.* isolated β-ethynylserine, 2(*S*),3(*R*)-2-amino-3-hydroxypent-4-ynoic acid, from dried sclerotia of the fungus *Sclerotium rolfsii* (Sacc.)[3]. They reported that it was a toxic amino acid lethal to New Hampshire chickens (LD_{50} 150 mg/kg), but did not comment on any other physiological properties of this amino acid.

We present the first report on production of β-ethynylserine by *S. cattleya* and on its antimetabolite activity against microorganisms.

References

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