NOTE

L-3-(2,5-DIHYDROPHENYL)ALANINE, AN ANTIMETABOLITE OF L-PHENYLALANINE PRODUCED BY A STREPTOMYCETE*

JAMES P. SCANNELL, DAVID L. PRUESS, THOMAS C. DEMNY, THOMAS WILLIAMS and ARTHUR STEMPEL

Chemical Research Department,
Hoffmann-La Roche Inc.,
Nutley, New Jersey 07110, U.S.A.

(Received for publication October 17, 1970)

An unclassified species of a Streptomycete, X-13,185, when grown in a medium containing glucose, 10 g/liter; asparagine, 0.5 g/liter; yeast extract (Difco), 2 g/liter and K_2HPO_4, 0.5 g/liter produced a substance (100 mg/liter at 48 hours) inhibitory to the growth of *Bacillus subtilis* and *Escherichia coli* on DAVIS and MINGIOLI minimal agar[1]. The growth inhibition was relieved by addition of L-phenyalanine to the agar.

The antimetabolite was isolated from the filtered broth by adsorption onto Dowex 50WX4 resin (50~100 mesh) in the hydrogen ion form. The activity was then eluted with a 5% aqueous solution of pyridine. After removal of the pyridine by evaporation at reduced pressure the antimetabolite was adsorbed onto Norite A at pH 6.5 and eluted from the charcoal with 50% aqueous ethanol. The antimetabolite was then separated from aromatic amino acids by cation-exchange chromatography. A solution containing 5 g of crude material at pH 2.3 was adsorbed onto a 57 cm×5 cm column containing AG 50WX4 resin (200~400 mesh) in the sodium ion form and then eluted with 0.4 M sodium citrate buffer at pH 4.2. The active fractions free of phenylalanine were desalted by adsorption onto AG50W×4 resin (50~100 mesh) in the hydrogen ion form followed by elution with 5% aqueous pyridine solution. After evaporation at reduced pressure, the pH was adjusted to 0.8 with 5 N hydrochloric acid and the hydrochloride salt was crystallized from ethanol. Recrystallization from ethanol resulted in colorless needles of L-3-(2,5-dihydrophenyl) alanine hydrochloride m. p. 206°~208°C; $[\alpha]_D^{25}$ −33.7 (*c* 1, 5 N HCl) −47.9 (*c* 1, 0.2 N sodium phosphate pH 6.5). Anal. calcd. for $C_9H_{13}NO_2 \cdot HCl$: C 53.08, H 6.93, N 6.88, Cl 17.60. Found: C 52.99, H 7.03, N 6.85, Cl 17.52. When crystallized as the zwitterion a rapid partial conversion of the anhydrous crystals to phenylalanine occurred on exposure to air.

The oxidative conversion to phenylalanine and the elemental analysis suggested a dihydrophenylalanine. The mass spectrum of the zwitterion with peaks at m/e 167, 166, 165, 122, 120, 93, 91, 74 contained the molecular ion, m/e 167, and the M-45 (loss of COOH), and M-74 (loss of $H_2NCHCOOH$) peaks. In addition a series of peaks two mass units lower at m/e 165, 120 and 91 corresponds to the mass spectrum of phenylalanine. The lack of ultraviolet absorption at wave lengths higher than 230 nm in samples free of phenylalanine excluded conjugated double bonds and the nmr spectrum of the zwitterion (D_2O, ext. TMS) pointed to a 2,5 rather than a 1,4 dihydro system since there were three olefinic protons, δ 6.19(s, 2), 6.12(s, 1) and four allylic protons, δ=3.12. Confirmation was obtained by comparison of the properties with authentic[2,3] material synthesized by reduction of L-phenylalanine with lithium in ammonia.

Since L-3-(2,5-dihydrophenyl)alanine is new in nature we carried out a preliminary investigation into its biosynthesis. No incorporation of label from L-phenylalanine-C^{14} (U) at a detection limit of 0.02% was observed. Label from shikimic acid-C^{14} (U) was incorporated into L-3-(2, 5-dihydrophenyl)alanine to the extent of 2.5%. Furthermore, over 90% of the label in the extracellular amino acid pool was present in L-3-(2,5-dihydrophenyl)alanine. Presumably, biosynthesis is accomplished by way of a branch in the aromatic amino acid pathway.

A report[4] on the microbiological activity of L-3-(2,5-dihydrophenyl)alanine has appeared. In addition, we have observed

* Presented at the 160 th National Meeting of the American Chemical Society, Sept. 15, 1970 (Chicago).

activity against sarcoma-180 in the mouse when administered intraperitoneally and against *Trichomonas vaginalis* when administered locally.

Acknowledgements

We are indebted to members of the Physical Chemistry Department under Dr. P. BOMMER, the Fermentation Pilot Plant under Mr. B. TABENKIN and the Chemotherapy Department under Dr. E. GRUNBERG for their contributions to this work.

References

1) DAVIS, B. D. & C. S. MINGIOLI: Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. 60 : 17～28, 1950
2) SNOW, M. L.; C. LAUINGER & C. RESSLER: 1,4-Cyclohexadiene-1-alanine (2,5-dihydrophenylalanine), a new inhibitor of phenylalanine for the rat and *Leuconostoc dextranicum* 8086. J. Org. Chem. 33 : 1774～1780, 1968
3) SHOULDERS, B. A.; R. M. GIPSON, R. J. JANDACEK, S. H. SIMONSEN & W. SHIVE: Conformation and biological activity of 1,4-cyclohexadiene derivatives. J. Am. Chem. Soc. 90 : 2992～2993, 1968
4) GENGHOF, D. S.: 2,5-Dihydrophenylalanine as an inhibitor of microbial growth. Canad. J. Microbiol. 16 : 545～547, 1970.