OCCURRENCE OF 1,2,4-TRIAZOLE RING IN ACTINOMYCETES

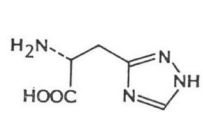
Sir:

In the course of our screening for new antimetabolites from actinomycetes, it was found that strain KM-10329 produced L-1,2,4-triazole-3-alanine. Many synthetic compounds containing the 1*H*-1,2,4-triazole ring are known, however, their natural occurrence has never previously been reported. In this present communication, the isolation and identification of the compound are described.

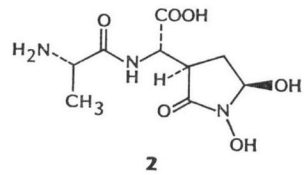
Strain KM-10329 was isolated from a soil sample collected at Joetsu-city, Niigata Prefecture, Japan. On the basis of taxonomic studies, it was classified as *Streptomyces* sp.

This organism was cultured at 27°C for 48 hours in 500-ml Erlenmeyer flasks containing 100 ml of a seed medium, composed of glucose 0.1%, starch 2.4%, peptone 0.3%, meat extract 0.3%, yeast extract 0.5% and $CaCO_3$ 0.4% (pH 7.0). Two hundred milliliters of the seed culture were transferred into 20 liters of a medium containing wheat bran 4.0%, soybean meal 2.0% and NaCl 0.3% (pH 7.0) in a 30-liter jar fermentor, and the fermentation was carried out at 27°C for 140 hours under aerobic conditions.

The culture filtrate (15 liters) was applied to a column of Amberlite IR-120B (H^+). The column was washed with H_2O, and then the active material was eluted with 1.0 N NH_4OH. The eluate was concentrated *in vacuo*, and applied to a Diaion PA-416 (OH^-) column. After washing the column with H_2O, the active principle was eluted with 0.1 N AcOH. The active eluate was concentrated to a small volume *in vacuo*, and poured into MeOH. After the flocculated material was removed by filtration, the filtrate was evaporated to dryness, and chromatographed on an Avicel column with EtOH-0.15 M aq NH_4OH. The active fractions were collected, concentrated *in vacuo*, and lyophilized to yield a brown powder. The powder was dissolved in a small amount of H_2O and purified on an activated carbon column with 10% MeOH in H_2O. Acetone was added to the concentrated active eluate to yield crystalline **1**: 150 mg; mp 258~261°C (dec); λ_{max}^{MeOH} nm 255 (sh); IR (KBr) 2850, 1605, 1525, 1430, 1410, 1020 cm^{-1}; EIMS *m/z* 157 $(M+H)^+$, HIMS Calcd for $C_5H_9N_4O_2$: 157.073; Found: 157.073;



1



2

Anal Calcd for $C_5H_8N_4O_2$: C 38.46, H 5.16, N 35.88; Found C 38.15, H 5.05, N 34.86; $[\alpha]_D^{18}$ −18.0° (*c* 1.0, H_2O).

The ^1H NMR spectrum of **1** in D_2O showed one methylene [3.28 (d, J=7.6 Hz) and 3.31 (d, J=5.4 Hz) ppm] coupled with a methine [4.08 (1H, dd, J=7.6 and 5.4 Hz) ppm] which corresponded to the α-proton of α-amino acid, and an isolated olefinic proton [8.28 (1H, s) ppm]. These data suggested the presence of a β-substituted alanine moiety. The ^{13}C NMR spectrum of **1** in D_2O showed 5 carbon signals, *i.e.*, one methylene (28.9 ppm, $^1J_{CH}$=130.4 Hz), a methine (54.1 ppm, $^1J_{CH}$=145.3 Hz), a protonated and a non-protonated sp^2 carbon (146.9 ppm, $^1J_{CH}$=212.7 Hz and 157.3 ppm), and a carboxylic acid (173.6 ppm). The large one bond ^{13}C-^1H coupling constant of the protonated sp^2 carbon indicated that the carbon was between two nitrogens.[1] Since the remaining carbon had to attach to nitrogen through a double bond and the ^{13}C NMR chemical shift of methylene was similar with that of the β-carbon of histidine, the structure of **1** was concluded to be 1,2,4-triazole-3-alanine. In order to confirm this, the ^1H NMR spectrum of **1** was compared with that of authentic D,L-1,2,4-triazole-3-alanine, and these spectra were superimposable.

The specific optical rotation of **1** for D line was −18.0° and that of L-histidine was known to be −39.7°, consequently the stereochemistry of **1** is the L form. Furthermore, positive Cotton effect in CD curve was observed both in **1** and L-histidine, confirming that they are L form. Therefore, the structure of **1** is L-1,2,4-triazole-3-alanine. The synthesized D,L-1,2,4-triazole-3-alanine has been known as a histidine antagonist,[2] and the mechanism of action has been investigated.[3]

The strain was also found to co-produce a proline antagonist,[4] nourseimycin (**2**), having collagen proline hydroxylase inhibiting activity.[5] The compound also contains an unusual amino acid.[6] Thus, the strain KM-10329 produces two unusual amino acids.

This is the first report of the natural occurrence of a compound containing 1,2,4-triazole ring. There are no structural differences except that in **1** nitrogen is substituted for the δ-carbon in L-histidine. Biosynthesis of L-histidine has been well investigated, *i.e.*, the first step is the condensation of ATP with phosphoribosylpyrophosphate, and the δ-carbon of L-histidine is derived from the anomeric carbon of ribose. Though the structure of **1** is closely related to L-histidine, the biosynthesis of **1** seems not to relate with that of L-histidine. Thus, it is an interesting target for biosynthetic investigation.

NOBUTAKA IMAMURA
MASATSUNE MURATA
TIANJUE YAO†
RUIKO ŌIWA
HARUO TANAKA
SATOSHI ŌMURA*

The Kitasato Institute
and Kitasato University
Minato-ku, Tokyo 108, Japan

(Received April 1, 1985)

† Present address: Institute of Antibiotics, Chinese Academy of Medical Sciences, Tiantan, Beijing, China

References

1) STOTHERS, J. B.: Carbon-13 NMR Spectroscopy. p. 343, Academic Press, New York, 1972
2) JONES, R. G. & C. AINSWORTH: 1,2,4-Triazole-3-alanine. J. Am. Chem. Soc. 77: 1538~1540, 1955
3) LEVIN, A. P. & P. E. HARTMAN: Action of a histidine analogue, 1,2,4-triazole-3-alanine, in *Salmonella typhimurium*. J. Bacteriol. 86: 820~828, 1963
4) NISHIDA, H.; M. TAGAWA, A. HIROTA, A. ISOGAI & H. SAKAI: Isolation and biological activities of nourseimycin, a new antimetabolite of L-proline. Agric. Biol. Chem. 47: 1599~1604, 1983
5) HIGASHIDE, E.; S. HORII, H. ONO, N. MIZOKAMI, T. YAMAZAKI, M. SHIBATA & M. YONEDA: Alahopcin, a new dipeptide antibiotic produced by *Streptomyces albulus* subsp. *ochragerus* subsp. nov. J. Antibiotics 38: 285~295, 1985
6) HORII, S.; H. FUKASE, E. HIGASHIDE, M. YONEDA, H. NISHIDA, H. SAKAI, A. HIROTA & A. ISOGAI: Structure of alahopcin (nourseimycin), a new dipeptide antibiotic. J. Antibiotics 38: 302~311, 1985
7) BENDER, D. A.: Amino Acid Metabolism. pp. 143~150, John Wiley & Sons, London, 1975