PRODUCTION OF FOSFOMYCIN (PHOSPHONOMYCIN) BY *PSEUDOMONAS SYRINGAE*

JUN'ICHI SHOJI*, TOSHIYUKI KATO, HIROSHI HINOO, TERUO HATTORI, KEIICHIRO HIROOKA, KOICHI MATSUMOTO, TATSUO TANIMOTO and EIJI KONDO

Shionogi Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan

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In recent years, it has become to be recognized that bacteria can produce antibiotics of wide structural diversity, some of which are known also as the products of Actinomycetales[1~5]. Here we report the isolation of fosfomycin (phosphonomycin)[6~8] from a bacterial culture, which was noticed in our screening work by causing spheroplast formation of *Escherichia coli* LS-1 (a supersensitive mutant to β-lactam antibiotics) in a hypertonic medium.

The producing organism, PB-5,123, was isolated from pond water in Kyoto city, and identified as *Pseudomonas syringae*[9,10] by the following characteristics. The organism was aerobic, Gram-negative, non-sporulating rods ($1.0\times 1.8\sim 2.0\ \mu$m) with rounded ends. Motility was observed with polar multi-trichous flagellation. Colonies on a nutrient agar were circular, convex, smooth and shining with orange color. Glucose was metabolized oxidatively. The following tests gave positive results; catalase, gelatin liquefaction, citrate utilization, arginine dihydrolase, and formation of fluorescent pigments. But, the following were negative; oxidase, starch hydrolysis, nitrate reduction, and accumulation of poly-β-hydroxybutyrate. Growth was observed at 28°C and 37°C, but not at 5°C or 42°C.

The producing organism was cultivated by jar fermentation using a medium consisting of glycerol 3.0%, glucose 0.1%, Polypeptone 0.5%, beef extract 0.5%, NaCl 0.5%, under aerobic conditions for 24 hours. The antibiotic in the culture filtrate (*ca.* 160 liters) was adsorbed on a Dowex-1X2 (Cl^-) column and eluted with 5% NaCl. The active eluate was freeze-dried to give a powder (120 g). Extraction with methanol from the powder was repeated several times. The extract was concentrated and passed through a carbon column with water. The effluent was concentrated and applied to a Biogel P-2 column with water. The active eluate from the column was freeze-dried to give a glassy residue (3 g). It was chromatographed on a DEAE-Sephadex column in linear gradient manner with water and 0.4 M NH_4HCO_3. The active eluate was once freeze-dried and then passed through an Amberlite CG-50 (Na^+) column with water. Freeze-dry of the effluent gave a crude powder (230 mg) of the antibiotic as a sodium salt. It was then purified by chromatographies on an Avicel cellulose column with 75% propanol followed by 75% acetonitrile. Concentration and freeze-dry of the active eluate gave a colorless powder (21 mg) of the antibiotic as a sodium salt.

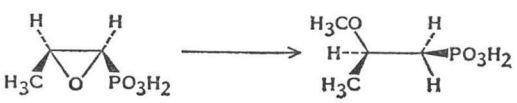
The antibiotic obtained as above is a water-soluble acidic substance. No UV absorption was observed with an aqueous solution of the sodium salt. The IR spectrum (KBr) and the ^{1}H NMR spectrum (D_2O) were substantially identical with those of a commercially available fosfomycin sodium salt (Meiji Seika Kaisha, Ltd.). The identity with fosfomycin was also shown by color reaction and TLC experiments. The same color tones were observed by reaction with molybdate reagent. Identical mobilities on TLC were shown as follows: Silica gel plate (Merck); $CHCl_3$ - MeOH - 28% ammoniacal water (1: 3: 2), Rf 0.52; 70% propanol, Rf 0.30; cellulose plate (Avicel): butanol - acetic acid - H_2O (3: 1: 1), Rf 0.30; 70% propanol, Rf 0.40; butanol - pyridine - acetic acid - H_2O (10: 6: 1: 4), Rf 0.27; 75% acetonitrile, Rf 0.20.

Methanolysis products (**2**) were prepared according to the method of CHRISTENSEN *et al.*[7] from both our specimen and authentic fosfomycin (**1**) and their optical activities were compared.

Product from our specimen: $[\alpha]_D$ $-5.1\pm0.5°$, $[\alpha]_{365}$ $-17.3\pm0.6°$ (*c* 0.00909, H_2O).

Product from fosfomycin: $[\alpha]_D$ $-7.9\pm0.3°$, $[\alpha]_{365}$ $-25.3\pm0.4°$ (*c* 0.0157, H_2O).

Fig. 1.



1 **2**

From the above, the antibiotic produced by *Pseudomonas syringae* was concluded to be identical with fosfomycin including its stereochemistry.

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