Communications to the editor

S-2,3-DICARBOXY-AZIRIDINE, A NEW METABOLITE FROM A *STREPTOMYCES*

Sir:

In the course of our screening of new antibiotics, a new metabolite (**I**), which shows antibacterial activity against *Aeromonas salmonecida* by cup-assay method, was isolated from a cultured broth of a streptomyces. The producing strain (MD398-A1) was isolated from a soil collected in Nara City, Japan. The structure of **I** was determined to be S-2,3-dicarboxy-aziridine. In this communication, the production, isolation, physicochemical properties and structural determination of this new metabolite are reported.

A medium containing 1 % glucose, 2.0 % starch, 0.5 % yeast extract, 0.5 % casamino acids (Difco), 0.4 % $CaCO_3$ was used for the production of **I**. The maximum production was obtained at the third or fourth day in shaken culture at 24°C. The filtrate showed activity against *Aeromonas salmonecida* by cup-assay method. Four liters of the filtrate was treated with 60 g of active charcoal and the colorless filtrate was passed through a column (800 ml) of Amberlite IR-120 (H-form). The effluent and washing was concentrated under reduced pressure to yield a syrup which was triturated with 100 ml of methanol. The crude solid material (5.5 g) thus obtained was dissolved in 500 ml of water and charged on a column (100 ml) of Dowex 50W-X8 (H-form). The column was developed with water. Antibacterial activity was not shown in the effluent. The active fraction of the eluate developed with water was dried to yield 1.57 g of colorless crystalline material. Recrystallization with water gave 1.10 g of pure crystalline **I**.

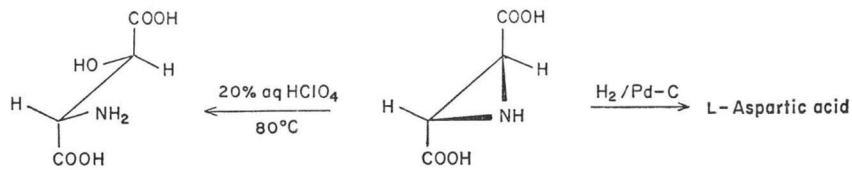
Compound **I** crystallized as colorless plates and decomposed at 178°C, $[\alpha]_D^{24}+54°$ (*c* 0.5, H_2O).

Anal. Calcd. for $C_4H_5NO_4$: C, 36.65; H, 3.84; N, 10.69; O, 48.82.
Found: C, 36.83; H, 3.88; N, 10.41; O, 48.98.

There is no UV absorption except end absorption. Ninhydrin and RYDON-SMITH reactions were positive. The presence of two dissociable groups at pKá 2.1, and 5.2 was observed by potentiometric titration and the titration equivalent was 131 (Calcd. for $C_4H_5NO_4$: 131.09). On high-voltage paper electrophoresis using formic acid - acetic acid - water (25 : 75 : 900 in volume), compound **I** moved slightly toward the anode like an acidic substance, which suggested the existence of another strongly acidic function which could not be detected by titration. The PMR spectrum in hexadeuterodimethylsulfoxide (DMSO-d_6) showed the presence of a singlet peak at δ 2.59 and a broad peak centered at δ 7.8. The broad peak disappeared by addition of deuterium oxide. The CMR spectrum in DMSO-d_6 showed only two signals: one is a methine signal at δ 35.9, which was confirmed by off-resonance method, and the other is a carbonyl signal at δ 174.6. These NMR data indicate that **I** should have symmetrical structure.

Catalytic hydrogenation of **I** with palladium-charcoal (5 %) in water under atmospheric pressure gave L-aspartic acid ($[\alpha]_D^{25}+25°$ in 6 N HCl) in quantitative yield after consuming one mole of hydrogen. These results suggested that **I** should be S-2,3-dicarboxy-azilidine.

Compound **I** was hydrated in 20 % aqueous perchloric acid solution after keeping at 80°C for 30 hours. Most of the perchloric acid



L-Erythro-β-hydroxy aspartic acid

S-2,3-Dicarboxy-aziridine

was removed from the reaction mixture as the sparingly soluble potassium salt by neutralization with potassium hydroxide. The hydrated product was isolated after adsorption on sulfonic acid resin (Dowex 50W-X4) followed by elution with ammonia and neutralization with carboxylic acid resin (Amberlite CG-50). It was crystallized from water (Yield 62 %) as plate-like crystals, m.p. >250°C (gradually darkening), $[\alpha]_D^{25}+55.5°C$ (*c* 0.6, 1 N HCl). The hydrated product was identical with L-*erythro*-β-hydroxyaspartic acid as expected. It was confirmed by direct comparison of IR spectrum and optical rotation with the authentic material which was kindly supplied by Prof. T. SHIBA, Osaka University, Japan. Not even a trace of the *threo*-isomer could be found in the reaction product, which suggests that the hydration is strictly stereospecific. Thus, the structure of **I** is determined to be S-2,3-dicarboxy-aziridine.

S-2,3-dicarboxy-aziridine did not show any antibacterial and antifungal activity at 100 mcg/ml including *Aeromonas salmonecida* by agar dilution test. But by ordinary cup-assay method using *Aeromonas salmonecida* at the test organism, it showed growth inhibition of 30 mm diameter at 25 mcg/ml.

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