A New Metabolite, 6-Oxo-7-aza-tridecanedioic Acid, a Microbial Product from Cyclic Dimer of ϵ -Caprolactam

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As yet few investigations have been made of the utilization of oligomers in nylon 6 extractables as a microbial substrate. Only a few group of workers^{1~4)} have reported the isolation of microbes which can utilize ϵ -caprolactam and its oligomers as a sole source of carbon and nitrogen, and also they have given some informations on hydrolytic enzymes which act on them.

The authors have isolated several different bacteria which can grow on a medium containing the oligomers as a sole source of carbon and nitrogen. One of these was capable of converting the cyclic dimer of ϵ -caprolactam to a new compound, 6-oxo-7-aza-tridecanedioic acid, as the major metabolite.

The oligomers-utilizing bacteria were isolated from a sample of soil in the nylon 6 manufacturing plant (Toyobo Co. Ltd., Tsu-ruga) by the usual enrichment and plate culture methods. The basal medium for isolation was composed of 10 g of the oligomers (mainly cyclic dimer and trimer), 0.25 g of yeast extract and 1000 ml of the basal mineral solution (KH_2PO_4 0.95 g, $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ 4.65 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g, $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ 2.5 mg, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 5.0 mg, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 15 mg, and deionized water 1000 ml, pH 7.0). The isolated bacteria were cultured and stocked on the slant having the same composition as this

medium.

Two of the bacteria thus isolated were selected for further studies because of their high abilities to utilize the cyclic dimer of ϵ -caprolactam as a sole carbon and nitrogen source. These two bacteria, based on their morphological and physiological observations, were tentatively identified as belonging to the genus *Achromobacter* and the genus *Alcaligenes*, respectively. The details of the taxonomical study on these bacteria will be published elsewhere.

In the present study the bacterium identified as *Achromobacter* was cultured at 30°C for 8 days with shaking in 300 ml of the basal mineral medium supplemented with 75 mg of yeast extract and, instead of the oligomers, 3 g of the cyclic dimer, which was purified by the method of Heikens *et al.*⁵⁾ After removal of the cells by centrifugation, the pH of the supernatant culture broth was 6.28.

Paper chromatographic analysis of the culture broth (*sec*-BuOH: 28% ammonia water: water=8:2:3 as a developer) showed the presence of ϵ -aminocaproic acid and its linear dimer. Colorimetric analysis⁶⁾ based on a reaction with ninhydrin reagent revealed it to contain amino compounds amounting to 450 mg per 100 ml (calculated as ϵ -aminocaproic acid).

The culture broth (300 ml) was neutralized and concentrated to one-tenth of its initial volume under reduced pressure. After removal of water-insoluble substances resulted, the culture broth was passed through cation exchanger resin (Amberlite IR-120 H^+ form) to remove amino compounds. The eluate was continuously extracted with ether for 4 days and the ether was removed by distillation to give 1.63 g of crude crystals which, after three-times repeated recrystallizations from hot water, melted at 108~109°C (Compound I). Anal. Found: C, 55.25; H, 8.16; N, 5.38. Calcd. for $\text{C}_{12}\text{H}_{21}\text{O}_5\text{N}$: C, 55.58; H, 8.16; N, 5.40%.

Compound I is acidic and its neutralization equivalent was 128.6, suggesting it to be a dibasic acid (molecular weight of $\text{C}_{12}\text{H}_{21}\text{O}_5\text{N}$

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259.3).

The IR spectrum of I showed characteristic bands as follows. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3320 (NH of CONH), 2945 and 2872 (methylene), 1700 (CO of CO_2H), 1637 (CO of CONH), and 1535 (NH of secondary amide). The spectrum was identical to that of an authentic sample of 6-oxo-7-aza-tridecanedioic acid (mp $108 \sim 109^\circ\text{C}$, neutralization equivalent 128.3. *Anal.* Found: C, 55.38; H, 8.20; N, 5.30%), which was prepared by the reaction of ϵ -aminocaproic acid with adipic acid anhydride. A mixed melting point of I with the authentic sample showed no depression.

The NMR spectrum of I, determined at 80°C and at 60 MHz, was in good agreement with the structure of 6-oxo-7-aza-tridecanedioic acid. NMR $\delta_{\text{Me}_4\text{Si}}^{\text{CD}_3\text{SO}_2}$: 1.44 (10H, multiplet, CH_2 at C-3, C-4, C-9, C-10 and C-11), 2.17 (6H, multiplet, CH_2 at C-2, C-5 and C-12), 3.05 (2H, multiplet, CH_2 at C-8) and 7.84 (1H, broad line, NHCO). The signal due to the proton of the CO_2H group was not identified because of its chemical exchange with the proton of water contained in the solvent as an impurity.

On the basis of the above-mentioned experimental data, it seems reasonable to conclude that compound I, obtained from the culture broth of *Achromobacter* sp., is 6-oxo-7-aza-tridecanedioic acid.

In the earlier stage of the culture of the

Achromobacter, a considerable amount of a compound which could react with 2,4-dinitrophenylhydrazine was observed, suggesting that a precursor of 6-oxo-7-aza-tridecanedioic acid might be an aldehyde. A study on the metabolic pathway is now being undertaken.

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