Note

Formation of Pimelic and Azelaic Acids, Biotin Intermediates, Derived from Oleic Acid by *Micrococcus* sp.

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The participation of oleic acid in biotin biosynthesis has not yet been elucidated. Our earlier investigation of the utilization of oleic acid by a strain of *Brevibacterium* sp. focused on the possibility that oleic acid could serve as a biotin intermediate to yield desthiobiotin.¹⁾ After that we presented evidence of a role of oleic acid in biotin biosynthesis; the radioactivity of oleic acid-1-¹⁴C was incorporated into biotin-vitimers such as 7-keto-8-aminopelargonic acid, desthiobiotin and biotin by *Cryptococcus neoformans*.²⁾

In the course of our studies on microbial biotin biosynthesis, we have investigated a biotin intermediate with oleic acid by a strain of *Micrococcus* sp., which has already been reported by us as a bacterium forming pimelic acid from azelaic acid.³⁾ The evidence for the formation of pimelic acid from oleic acid is the subject of our report in this communication.

The organism was grown at 28°C for 4 days with shaking in a 500-ml of a medium consisted of oleic acid (99% purity, Nakarai Chemicals) 0.5%, (NH₄)₂SO₄ 0.3%, K₂HPO₄ 0.2%, KH₂PO₄ 0.2% and MgSO₄·7H₂O 0.05%. After growth, the cultures (1,000 ml) were centrifuged to remove the cells, and concentrated to 110 ml under reduced pressure at 40°C. The small amount of a biotin-vitamer in the concentrate was found by assay with *Saccharomyces cerevisiae*, in a concentration of 1.5 µg per 1 ml. Desthiobiotin was identified as a product derived from oleic acid by paper chromatography used Toyo Roshi # 53 paper and a butanol-acetic acid-H₂O (12:3:5 v/v) solvent, and bioassay. In order to remove the biotin-vitamer, the concentrate was adjusted to pH 2 with conc. HCl and the vitamer was filtered off with adsorption on 3 g of active carbon. The resulting filtrate was extracted three times with a 100 ml volume of ether. The extract was again evaporated to dryness under vacuum. The residue was dissolved in 10 ml of H₂O and adjusted to pH 7.0 with 4N KOH (Sample I). A 0.5-ml aliquot of Sample I was subjected to methylation with BF₃-methanol reagent,⁴⁾

FIG. 1. Gas-liquid Chromatogram of Sample I.

The conditions of GLC were as follows: instrument, Shimadzu GC-4BP (FID); column, a glass column (3 mm i.d. × 2.0 m) packed with 10% DEGS on Shimalite W (60~80 mesh); column temp., initial 100°C, final 200°C; programming rate, 5°C/min; injection and detector temp., 240°C; carrier gas, nitrogen at 60 ml/min; hydrogen, 50 ml/min; air, 800 ml/min; sensitivity × range, 10² × 64 (1.6 × 10⁻⁹ ampere in full scale). Each peak was identified as shown in the figure: A, pimelic acid; B, azelaic acid; C, oleic acid.

and analyzed by gas-liquid chromatography (GLC). The result is shown in Fig. 1. The two peak (compounds A and B in figure) were separated on DEGS 10% column (Shimadzu), where the retention times of 9.4 min and 11.5 min, were marked respectively. The peaks eluting early (compound A), and another (compound B), were shown to be identical with authentic pimelic and azelaic acids, respectively. The conversion of oleic acid to azelaic and pimelic acids was quantitated by GLC and found in about 2.8 and 1.1 per cent yields, respectively. These low values for the conversion can probably be ascribed to the subsequent utilization of the intermediates during cultivation. Furthermore, the separation and identification of compounds A and B by GLC were achieved under the same conditions on two types of columns such as Shinchrome E-71 and Fon (both Shimadzu), where the retention times of 9.2 min and 11.2 min on the former column, 11.5 min and 16.2 min on the latter, were marked respectively.

Compound A was characterized with the resting cell system of *Bacillus sphaericus* to form a biotin-vitamer, desthiobiotin.⁵⁾ Various amounts of Sample I were incubated with 48 mg resting cells in 200 mM phosphate buffer (pH 7.0) for 2 hours at 28°C under shaking. The volume of Sample I was varied from 0.01 to 0.3 ml per 1 ml of the reaction solution. Desthiobiotin synthesized was determined by assay with *Saccharomyces cerevisiae*.⁶⁾ The desthiobiotin yields at 0, 0.01, 0.03, 0.1 and 0.3 ml Sample I were 0, 0.015, 0.03, 0.11 and 0.28 µg per ml, respectively. This result shows that the formation of desthiobiotin is directly proportional to the amount of Sample I introduced into the assay solution. Therefore, the above result does provide evidence for the presence of pimelic acid in Sample I. The organism, as described in the previous report,³⁾ possesses the ability to act on azelaic acid and form pimelic acid, so that the last step is undoubtedly attributable to a β-oxidation reaction which results in the formation of pimelic acid from azelaic acid.

The mechanism of the formation of azelaic acid is under investigation.

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