Isolation of Fumaryl-pyruvic Acid as an intermediate of the Gentisic acid Oxidation by *Pseudomonas ovalis* var. S-5

Sir:

In a previous paper\(^1\), we presumed that the initial intermediate after the oxidative ring cleavage of gentisic acid by *Ps. ovalis* S-5 might be maleyl-pyruvic acid or fumaryl-pyruvic acid.

Recently, we have succeeded in isolating one of the suggested intermediates in a crystalline form. (Fig. 1)

The crude extracts of *m*-hydroxybenzoate induced cells of *Ps. ovalis* S-5, prepared as described elsewhere\(^2\), were dialyzed against distilled water.

When the dialyzed crude extracts were incubated with sodium gentisate, an o-phenylenediamine positive β-diketo substance was accumulated. Such accumulation of the β-diketo substance was not detected when the non-dialyzed crude extracts were employed.

After sodium gentisate was incubated at 30°C. for 40 min. with the dialyzed extracts, the reaction mixture was deproteinized with meta-phosphoric acid and continuously extracted with peroxide free ether. Ether was removed *in vacuo* and a yellow substance was obtained. From this yellow substance the β-diketo intermediate was obtained in a crystalline form by several recrystallizations from ethylacetate-petroleum ether and ethanol-benzene.

The crystalline substance was readily hydrolyzed by the non-dialyzed crude extracts of our *Pseudomonas* cells and also by the acylpyruvase from rat liver\(^3\), forming two acids, i.e., fumaric and pyruvic acids. These two acids were detected and identified by paper chromatography. However, maleic acid was not detected. The formation of a dicarboxylic acid as the intermediate during gentisic acid oxidation was also observed manometrically.

Analytical data of the crystalline intermediate were as follows:


This substance readily reacted with carbonyl reagents such as sodium niroprusside,

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\(^1\) Arima, K., et al. This Bulletin 19, 51 (1955).


\(^3\) Meister, A. & Greenstein, J.P., *J. Biol. Chem.* 175, 575 (1948)
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FIG. 2. Infra-red Spectrum of Fumaryl-pyruvic acid in Nujol Mull. Frequency (cm\(^{-1}\))

The authors wish to express their appreciation to Professor Kin-ichiro Sakaguchi of Univ. of Tokyo for his guidance and encouragement, to Assist. Prof. Takehiko Shimanouchi for his instruction on the infra-red analysis and to the members of Professor Sumiki's laboratory for their helpful advice. They are also deeply indebted to the Central Institute of the Japan Monopoly Corpora-

2,4-dinitrophenyl-hydrazine, and semicarbazide. The reaction with \(\sigma\)-phenylenediamine was also strongly positive.

The UV-absorption spectra of the crystalline intermediate showed the maximum at 335 m\(\mu\) (\(\varepsilon=8300\)) in 0.1 N HCl; at 340 m\(\mu\) (\(\varepsilon=9400\)) in phosphate buffer pH 7.3; and at 350 m\(\mu\) (\(\varepsilon=10300\)) in 0.1 N NaOH.

The infra-red spectra in Nujol mull (Fig. 2) showed the presence of monoenol-\(\beta\)-diketone (1560-1570 cm\(^{-1}\)); \(\alpha\), \(\beta\)-unsaturated acid HOOC-C=C:1710 cm\(^{-1}\)); and -C=C- H H trans (980 cm\(^{-1}\)).

All the facts observed for the crystalline intermediate are compatible with the structure (Fig. 3, III) of fumalyl-pyruvic acid. Therefore, the gentisic acid oxidation by Pseudomonas ovalis S-5 is demonstrated in the following scheme:

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