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Reduction of 5-Keto-D-gluconic Acid to L-Idonic Acid by *Fusarium* species

Sir:

5-Keto-D-gluconic acid (5KGA) and L-idonic acid are the two main intermediates for the synthesis of L-ascorbic acid in the method described by B. E. Gray¹⁾ in 1947. In his method, L-idonic acid is prepared from 5KGA through chemical reduction. Formation of L-idonic acid by microorganisms has never been reported. During our studies on the metabolism of 5KGA by various microorganisms, it was found that certain strains of *Fusarium* formed L-idonic acid from 5KGA. This is the first report on L-idonic acid formation in nature. Out of a number of microorganisms examined, including various molds, yeasts and bacteria, only nine strains of *Fusarium**, i.e. *Fusarium lini* IAM 1-7, *Fusarium oxysporum* IAM 1-8 and seven *Fusarium* species, formed L-idonic acid from 5KGA.

Fusarium sp. No. 125 was grown for 40~48 hours in a potato extract medium containing 1% glucose and 0.2% monosodium glutamate or in a synthetic medium containing 5KGA as a sole carbon source, at 30°C on a shaker. Potassium salt of 5KGA was then added to each culture to a concentration of 1%, cotton plugs were replaced with rubber stoppers and flasks were incubated further for 20~30 hours at 30°C. Concentration of L-idonic acid in the incubation mixture reached around 1.0~1.5 mg per ml (i.e. about 10% conversion of the

5KGA added).

The identification of L-idonic acid was carried out by isolation of dibenzal derivative and paperchromatography²⁾ as follows. Cells were removed from the incubation mixture by centrifugation. The supernatant was decolorized with charcoal, passed through a column of Amberlite IR-120 (H⁺) and concentrated under vacuum at 15~20°C to a syrup. Residual 5KGA was removed as Ca-salt. The syrup was used for the formation of dibenzal derivative. Crystals of the dibenzal-L-idonate obtained were recrystallized from 10% KOH solution by the addition of HCl and then from hot alcohol, and washed with ethyl-ether. The chemical properties of these needle crystals were as follows:

Melting point: 214~215.5°C.

Anal. Found: C, 64.26%; H, 5.48%.

Calcd. for C₂₀H₂₀O₇: C, 64.52%; H, 5.38%.

The infrared spectrum of these crystals and that of the synthetic dibenzal-L-idonate prepared by chemical reduction of 5KGA are shown in Fig. 1. Upon paperchromatography of the culture filtrate and the syrup, L-idonic acid was detected as the lactone as well as the free acid form, but D-gluconic acid, the D-isomer of L-idonic acid was not detected. L-Idonic acid was not formed from glucose, D-gluconic acid, 2-keto-L-gulonic acid or 2-keto-D-gluconic acid.

It is most interesting that L-idonic acid which has not been found in nature, is formed

1) B.E. Gray: U.S. Patent, 2,421,611 and 2,421,612 (1947).

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2) M. Abdel-Aker and F. Smith, *J. Am. Chem. Soc.*, **73**, 5859 (1951).

FIG. 1. Infrared Absorption Spectra of the Crystal and Synthesized Dibenzal-L-idonate (Nujol Mull).

A: The Crystal.
B: Synthesized Dibenzal-L-idonate.

from 5KGA by microorganisms. It is believed that an enzyme system of this reductive reaction will be different from 5-ketogluconoreductase system described by De Ley and others³⁻⁶.

These studies will be published in details

elsewhere.

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- 6) H. Murooka, The Annual Meeting of the Agricultural Chemical Society of Japan, Fukuoka, on April 1, 1961.

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