Accumulation of Isocitric Acid by a *Penicillium* Strain

Sir:

The accumulation of isocitric acid from glucose by *Penicillium purporogenum* Stoll var. *rubri-sclerotium* Thom (non-sclerotigenic type) no. 1148¹⁾ in medium was proved by the isolation and purification of the above acid. This acid, known as one of the members of the TCA cycle, was found to be contained in some higher plants, in considerable amounts^{2,3)}. Although the occurrence of the acid in the TCA cycle of bacteria has been proved, the accumulation of this acid by microorganisms has, so far, never been reported.

This fungus was grown aerobically in a medium containing 10% glucose, 0.3% peptone and 1% CaCO₃ by shaking culture. From the culture filtrate, the acid was extracted with ether and the extract evaporated to a syrup which was then heated at 100° in vacuum so as to dehydrate the acid to its γ -lactone. The lactone was purified by recrystallization from ethylacetate and chloroform.

The γ -lactone, γ -lactone-dimethylester, and

tri-acid-amide were obtained in the crystalline form.

The lactone was titrated as a dibasic acid in the cold, but after treatment with excess alkali, one more acidic group appeared; it melted at 140–141° to yellow oil.

Anal. Calculated for C₆H₆O₆: C, 41.33; H, 3.47. Found: C, 41.24; H, 3.54.

$[\alpha]_D^{20}$; +42.3° (c=4.83, H₂O)

Dimethyl ester of lactone: m.p. 49–50°

Anal. Calculated for C₈H₁₀O₆: C, 47.53; H, 4.99. Found: C, 47.80; H, 5.34.

$[\alpha]_D^{20}$; +56.2° (c=0.85, MeOH)

Tri-acidamide, m.p. 189° with decomposition.

Anal. Calculated for C₆H₁₁O₄N₃: C, 38.09; H, 5.88; N, 22.20. Found: C, 38.27; H, 5.58; N, 21.70.

The infrared spectrum of tri-sodium salt of the isolated acid was almost completely identical with that of an authentic specimen. (Fig 1, 2)

By dry distillation of the lactone, citraconic and itaconic anhydrides were obtained. The former was identified by paper chromatography. The latter acid was purified from

FIG. 1. Infrared Spectrum of the Tri-sodium Salt of the Isolated Acid in Nujol mull.

1) S. Abe, *J. General and Applied Microbiol.* **2** Nos. 1–3 (1956).

2) E.K. Nelson, *J. Am. Chem. Soc.*, **47**, 571 (1925).

3) G.W. Pucher, *J. Biol. Chem.*, **145**, 511 (1942).

this distillate by silica gel chromatography. Admixture with authentic itaconic acid showed no melting point depression.

FIG. 2. Infrared Spectrum of the authentic Tri-sodium Isocitrate in Nujol mull.

However, as shown in the melting points and $[\alpha]_D$ values, the isolated acid was far different from the ordinary optically active isocitric acid which was found to occur in the TCA cycle. Therefore, it is supposed that the isolated acid might be the diastereoisomer of isocitric acid, i.e., allo-isocitric acid. This was supported by the result of paper chromatography with the synthetic product containing a considerable amount of the allo form which was synthesized by the method originally described by Fittig-Miller^{4,5)}. The paper chromatographic behavior of the isolated acid and the synthetic allo-acid were completely identical. (Table I)

From the above results, it was concluded that the isolated acid should be allo-isocitric acid. The presence of allo-isocitric acid in

TABLE I
PAPERCHROMATOGRAPHIC IDENTIFICATION
OF ALLO-ISOCITRIC LACTONE

solv: <i>n</i> -BuOH-formic acid-water (4:1.5:1 v/v)	
R_F value of the isolated lactone	0.69
" the synthetic product	0.69
containing allo-acid	0.60
(three spots)	0.91*
" <i>dl</i> -isocitric lactone	0.60

* Small amount, probably due to polymerization.

nature, has yet never been reported.

The yield of isocitric acid in weight, in some cases, exceeded 20% of consumed glucose. There were no other acids detectable in the culture medium.

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4) R. Fittig and H.E. Miller, *Ann. Chem. Justus Liebigs*, **255**, 43 (1889).

5) G.W. Pucher and H.B. Vickery, *J. Biol. Chem.*, **163**, 169 (1946).