STEREOCHEMISTRY OF THE ISOCITRATE LYASE REACTION*

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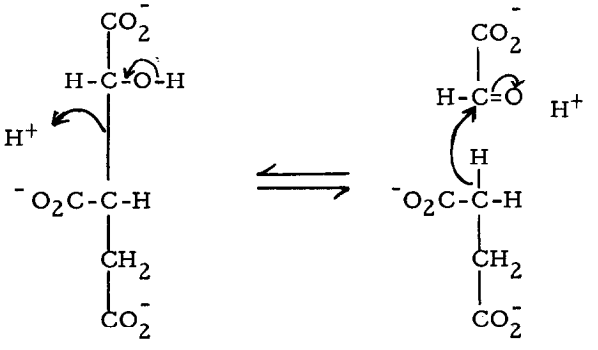
The cleavage of $\underline{D}_s$-isocitrate by isocitric lyase (1-4) may be regarded as an electrophilic substitution on C-3, a proton from the medium replacing the carbonyl carbon of glyoxylate (Scheme 1). Enzymic



Scheme 1

electrophilic displacements at saturated carbon having received only limited study, it was considered of interest to determine the stereochemistry of this reaction.

Since the absolute configuration of natural isocitrate is known (5-7), the steric course of the protonation in the isocitrate lyase

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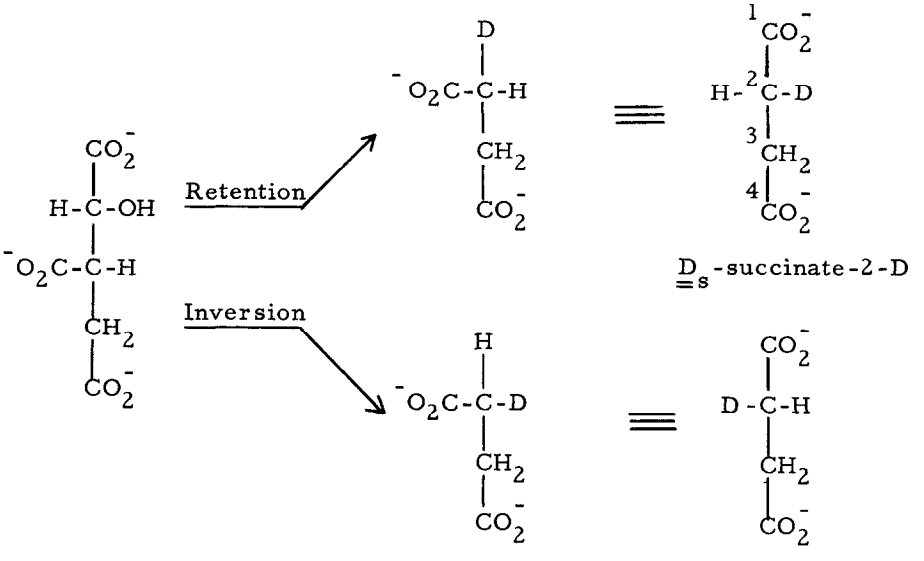
reaction can be readily studied in D_2O. If a deuterium atom is introduced with retention of the original configuration $\underline{\underline{D}}_s$-succinate-2-D[1] would result. However, if a deuterium atom is introduced with inversion of C-3 of isocitrate, $\underline{\underline{L}}_s$-succinate-2-D[1] would be formed (Scheme 2).



Scheme 2

These two possibilities can be distinguished as a result of the elegant work of Cornforth et al. (9), who found that $\underline{\underline{D}}_s$-succinate-2-D had a plain negative optical rotatory dispersion curve in the 350 to 250 mμ region (cf. 10).

In the present investigation a partially purified preparation of isocitrate lyase from baker's yeast (3) was incubated with $\underline{\underline{DL}}$-isocitrate in D_2O. The isolated deuteriosuccinate had a positive plain optical rotatory dispersion curve, and must therefore be $\underline{\underline{L}}_s$-succinate-2-D. Hence

1. According to the configurational nomenclature of Cahn, Ingold and Prelog (8) $\underline{\underline{D}}_s$-succinate-2-D would be R-deuteriosuccinate, and $\underline{\underline{L}}_s$-succinate-2-D would be S-deuteriosuccinate.

the isocitrate lyase reaction proceeds by inversion of configuration of the β-carbon (see Scheme 2).

It had been reported that isocitric lyase did not catalyze the incorporation of tritium from the medium into succinate in the absence of glyoxylate (11), nor did exchange take place when pyruvate or acetaldehyde was added (12). It appeared desirable to repeat similar experiments in a medium of D_2O. On the assumption that compounds which resemble glyoxylic acid might be able to stimulate exchange in D_2O although they cannot react with succinate, incubations were carried out with the addition of pyruvate and glyoxal. The previous observations were confirmed: succinate did not become labeled in the absence or in the presence of the carbonyl compounds.

The rate of isocitrate cleavage was approximately 20% slower in D_2O than in H_2O. Since the assay procedure was based on the continuous measurement of glyoxylate semicarbazone formation, a still larger difference in rates may have been obscured by the slow rate of the latter reaction.[2]

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2. In control experiments it was observed that semicarbazone formation from glyoxylate under assay conditions was relatively slow, requiring 5 to 6 minutes for completion.

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