THE STEREOCHEMISTRY OF THE ISOCITRIC ACID DEHYDROGENASE REACTION*

Sasha Englard and Irving Listowsky

(Department of Biochemistry, Albert Einstein College of Medicine, Yeshiva University New York 61, New York)

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The TPN^+ specific isocitric acid dehydrogenase of hog heart catalyzes the oxidative decarboxylation of isocitric acid to α-ketoglutaric acid. The reaction proceeds by direct hydrogen transfer from substrate to the nucleotide (1) and is characterized by stereospecific addition of this hydrogen atom to the A side of the pyridine ring (2). In the conversion of isocitric acid to α-ketoglutaric acid, the β-carboxyl group must be replaced by an hydrogen atom from the medium; this reaction proceeds stereospecifically. Thus, equilibration of β-ditritiated α-ketoglutaric acid with the TPN^+ specific isocitric acid dehydrogenase in the presence of TPNH and Mg ions results in the labilization of only a single tritium atom (3). On the basis of this exchange phenomenon and by analogy with nonenzymatic metal-catalyzed decarboxylation of β-keto acids.(4), it has been suggest that the immediate product of the oxidative decarboxylation of isocitric acid is an enzyme-bound enc anion of α-ketoglutaric acid (3). It must be assumed that the bound enolate intermediate cannot be liberated from the enzyme, or eventual loss of isotope from both β-hydrogen positions of the β-ditritic α-ketogluatric acid would occur instead of the observed stereospecific exchange of only one β-hydro (3).

The absolute configuration of naturally occurring dextrorotatory isocitric acid has recently been determined and found to be α-OH-D_s, β-COOH-L_s, (5-7). In addition, the two isomers of monodeuterosuccinic acid can be distinguished by application of optical rotatory dispersion data (8). Sinc monodeuterosuccinic acid of known configuration can be prepared by reduction of 3-deutero-L-malic

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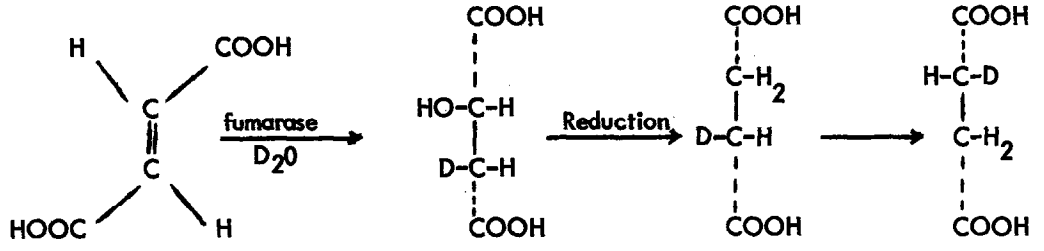
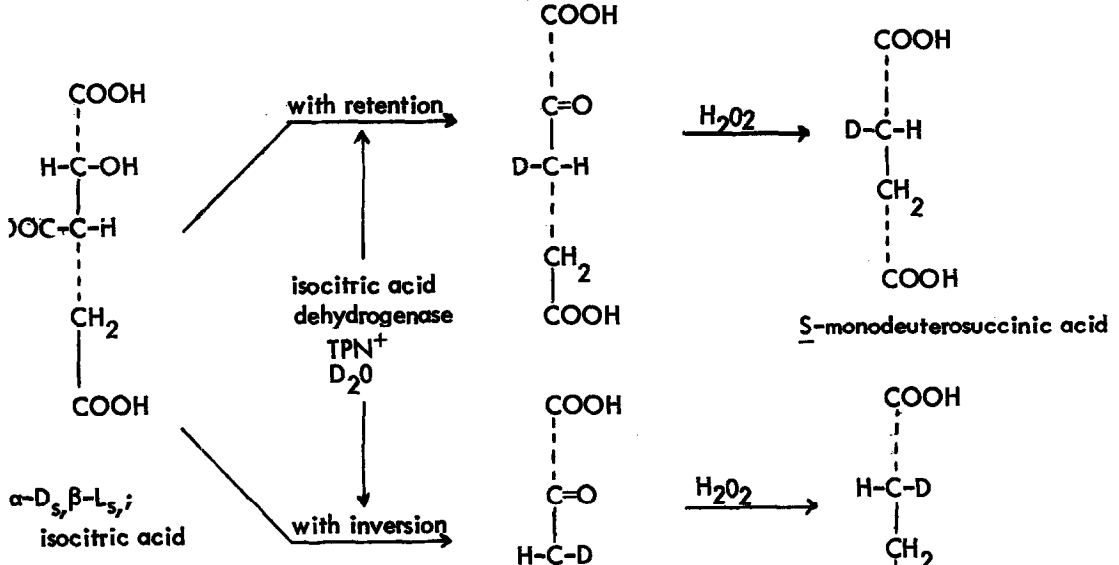
acid obtained by the fumarase catalyzed trans-hydration of fumaric acid in D_2O (9-11), a reference compound is therefore available permitting one to relate optical rotation to absolute configuration. By converting α-ketoglutaric acid obtained by enzymatic oxidative decarboxylation of isocitric acid in D_2O to the corresponding monodeuterosuccinic acid one could therefore deduce the stereochemistry of the reaction. One would thus be able to determine the position of the hydrogen (or deuterium) addition to the enzyme-enolate complex, relative to the displaced carboxyl group. The sequence of reactions is shown below:



fumaric acid — 3-monodeutero-L-malic acid — R*-monodeuterosuccinic acid



α-D_s,β-L_s; isocitric acid — β-monodeutero-α-ketoglutaric acid — S-monodeuterosuccinic acid — R-monodeuterosuccinic acid

*R and S according to the Sequence Rule of Cahn, Ingold and Prelog (12)

α-D_s,β-L_s isocitric acid was incubated with aconitase-free isocitric acid dehydrogenase in the presence of $MnCl_2$, excess TPN^+, 0.05 M glycylglycine at pH 7.35 in a medium of 99.8% D_2O. The reaction was followed spectrophotometrically by the increase in optical density of 340 mμ due to the appearance of TPNH. When the reaction was complete, the β-monodeutero-α-ketoglut acid was decarboxylated by addition of a slight excess of hydrogen peroxide. After disappearance of the α-ketoglutaric acid excess peroxide was destroyed by addition of a trace of catalase. The monodeuterosuccinic acid was then obtained by continuous ether extraction, purified by sublimation and recrystallized from water-acetone. (Yield, 70%).

Optical rotatory dispersion studies were performed using solutions of the monodeutero-succinic acid in water or methanol; the data are summarized in Table I.:

TABLE I

Optical Rotatory Dispersion Data for Monodeuterosuccinic Acid

Wavelength mμ	$[\alpha]_\lambda$ in Methanol (a)	$[\alpha]_\lambda$ in Water (b)
400	+2.27	
384.7	+2.48	
370.4	+2.71	
357.1	+3.13	
344.8	+3.37	
333.3	+3.68	+1.55
322.6	+4.07	+1.67
312.5	+4.54	+1.82
303.0	+5.44	+1.95
294.1	+6.45	+2.10
285.7	+7.89	+3.00
277.8	+9.16	+4.05
270.3	+10.19	+5.21
263.2	+12.10	+6.99
256.4	+15.92	+10.00
250.0	+20.63	+13.39
243.9	+28.84	+18.37
238.1	+39.11	+27.65
232.6	+48.49	+38.15
227.3		+49.31

a) conc. = 0.70%, cell length = 1 cm
b) conc. = 0.585%, cell length = 1 cm

As expected, specific rotations were very small at wavelengths above 400 mμ, but assumed

significant positive values at approximately 300 mμ and increased even more at wavelengths in the range of chromophore absorption.

Negative specific rotation values have been observed (8, 13) with R-monodeuterosuccinic acid obtained by reduction, on the one hand, of 3-monodeutero-L-malic acid prepared by enzymatic stereospecific trans-hydration of fumaric acid in D_2O (9-11) and, on the other hand, of 3-monodeutero-L-aspartic acid prepared by enzymatic stereospecific trans-amination of fumaric acid in D_2O (14, 15). The product obtained by the oxidative decarboxylation of isocitric acid in D_2O must therefore be of the opposite configuration. Values for rotation obtained for this monodeuterosuccinic acid were approximately equal but opposite in sign to values observed recently by Sprecher (personal communication) for R-monodeuterosuccinic acid prepared by chloramine T degradation of 4-deutero-L-glutamic acid obtained by the enzymatically catalyzed rearrangement of 3-deutero-L-threo-3-methyl aspartic acid (13).

From the assigned absolute configuration for naturally occurring dextrorotatory isocitric acid, it is possible to relate the absolute configuration of the resulting S-monodeuterosuccinic acid to the starting substrate. As shown by the indicated sequence of reactions the data are compatible with the conclusion that the overall reaction occurs with retention of configuration. In the isocitric acid dehydrogenase reaction one may conclude therefore, that precisely that position vacated by displacement of the carboxyl group to form the enzyme-bound enolate intermediate is taken by a replacing hydrogen or deuterium atom.

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