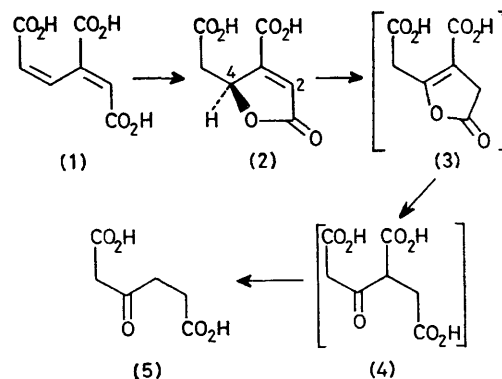


An Intramolecular, 1,3-Suprafacial Hydrogen Shift in the Enzymic Conversion of 3-Carboxymuconolactone into 3-Oxoadipic Acid

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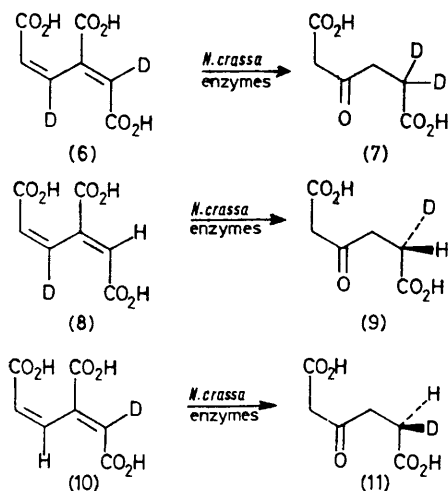
Summary The conversion of 3-carboxymuconolactone (2) into 3-oxoadipic acid (5), catalysed by a cell-free preparation from *Neurospora crassa*^{1,2} has been shown, by deuterium labelling, to involve an intramolecular, 1,3-suprafacial hydrogen shift consistent with an initial, allylic isomerisation of the substrate.

CELL-FREE extracts and purified protein fractions of *Neurospora crassa*^{1,2} and other fungi² catalyse the conversion of *cis-cis*-3-carboxymuconic acid (1) into 3-oxoadipic acid (5) *via* the lactone (2) (Scheme 1). No intermediates have been detected in the transformation of (2) into (5) but the enol-lactone (3) and tricarboxylic acid (4) are mechanistically plausible possibilities. We report here experiments with deuterio-derivatives of (1) which throw light on the stereochemistry and mechanism of this process.



SCHEME 1

The dideuterio-acid (6)[†] was incubated with a cell-free preparation¹ of *N. crassa* SY4a to afford 3-oxoadipic acid (7) with, surprisingly, retention of both deuterium atoms. Incubation of an equal mixture of (1) and (6) gave 3-oxoadipic acid containing equal amounts of dideuterio- and diprotio-species (mass spectral analysis); the small amount (*ca.* 10%) of monodeuteriated material in the product corresponded to that present in the starting mixture. Thus, intramolecular migration of deuterium had occurred during enzyme-catalysed conversion of (6) into (7). Deuterium was located in (7), as shown, by n.m.r. spectroscopy with the help of a routine set of chemical transformations.[‡]



SCHEME 2

The enzyme preparation from *N. crassa* converted (8) (50% monodeuteriated) into the monodeuteriated acid (9) (Scheme 2). This was purified by crystallisation, then cleaved with aqueous alkali, to yield 2-deuteriosuccinic acid,

[†] The substrates (6), (8), and (10) were prepared from appropriately deuteriated vanillins.³ Deuterium was located unambiguously by ¹H n.m.r. spectroscopy. Full details will be reported elsewhere.

[‡] 4-Oxopentanoic (laevulinic) acid with NaOD-D₂O gave, by exchange α to the ketonic group, a pentadeuterio-derivative showing (n.m.r.) a broad singlet, δ [(CD₃)₂CO] 2.55, for the remaining, C-2, methylene group. Reduction (NaBH₄) of this material confirmed (n.m.r. spectrum of the derived alcohol) this assignment. Acid-catalysed decarboxylation of (7) gave 2,2-dideuterio-4-oxopentanoic acid, δ [(CD₃)₂CO] 2.76 (br. s, CH₂) and 2.15 (br. s, Me).

¹ S. R. Gross, R. D. Gafford, and E. L. Tatum, *J. Biol. Chem.*, 1956, **219**, 781; G. W. Kirby, G. J. O'Loughlin, and D. J. Robins, *J.C.S. Chem. Comm.*, 1975, 402.

² R. B. Cain, R. F. Bilton, and J. A. Darrah, *Biochem. J.*, 1968, **108**, 797; D. R. Thatcher and R. B. Cain, *European J. Biochem.*, 1975, **56**, 193.

³ Cf. A. T. Ainsworth and G. W. Kirby, *J. Chem. Soc. (C)*, 1968, 1483.

⁴ J. W. Cornforth, R. H. Cornforth, C. Donninger, G. Popjak, G. Ryback, and G. J. Schroepfer, *Proc. Roy. Soc.*, 1966, **B**, **163**, 436.

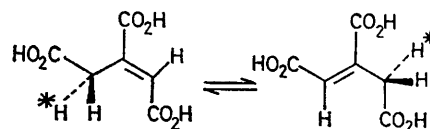
⁵ See D. J. Cram, 'Fundamentals of Carbanion Chemistry,' Academic Press, New York, 1965.

⁶ K. R. Hanson and I. A. Rose, *Accounts Chem. Res.*, 1975, **8**, 1.

⁷ J. P. Klinman and I. A. Rose, *Biochemistry*, 1971, **10**, 2259.

which was recrystallised several times. The optical rotation of this product after comparison with standard data⁴ (263—333 nm), showed it to be (*S*)-(+) -2-deuteriosuccinic acid, thus establishing the absolute configuration of (9). In a complementary experiment, (10) (92% monodeuteriated) was converted *via* (11) into (*R*)-(–)-2-deuteriosuccinic acid.

These findings establish that enzymic conversion of (2) into (5) involves a 1,3-suprafacial, intramolecular shift of hydrogen and provide persuasive evidence for the pathway in Scheme 1. However, direct proof of the involvement of (3) and (4) is still lacking. We propose that a basic group of an enzyme removes the hydrogen from C-4 of (2) to give a conjugated anion or, with concurrent protonation on oxygen, the related 2-hydroxyfuran. Reprotonation at C-2 from the same face of the molecule would generate the enol-lactone (3). Non-enzymic, base-catalysed, allylic isomerism is a familiar process.⁵ A few examples of the corresponding, enzymic transformation, presumed to involve



SCHEME 3

base-catalysis, have been recorded.⁶ The one⁷ most closely resembling ours in structural and stereochemical features is the interconversion of *cis*- and *trans*-aconitic acid (Scheme 3). The enzyme causes stereospecific exchange of one of the methylene hydrogens (asterisks in formulae) in each substrate with hydrogen in the aqueous medium. However, only a small (*ca.* 4%) intramolecular shift of hydrogen was observed, hydrogen exchange of the protonated enzyme with the medium being, presumably, faster than isomerisation.

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