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

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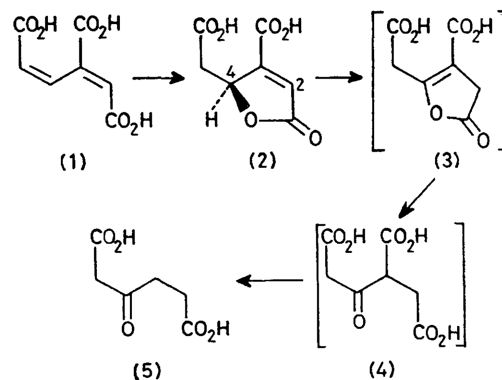
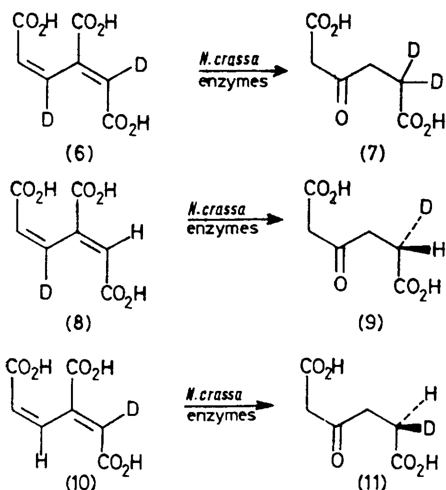
Summary The conversion of 3-carboxymuconolactone (**2**) into 3-oxoadipic acid (**5**), catalysed by a cell-free preparation from *Neurospora crassa*, has been shown, by deuterium labelling, to involve an intramolecular, 1,3-suprafacial hydrogen shift consistent with an initial, allylic isomerisation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

Cell-free extracts and purified protein fractions of *Neurospora crassa*[1,2] and other fungi[2] 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

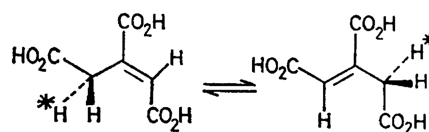
The dideuterio-acid (**6**)† was incubated with a cell-free preparation[1] of *N-crassa* SY4a to afford 3-oxoadipic acid (**7**) with, surprisingly, retention of both deuterium atoms. Incubation of an equal mixture of (**1**) and (**6**) gave 3-oxoadipic acid containing equal amounts of dideuterio- and 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, which was recrystallised several times. The optical rotation of this product after comparison with standard data[4] (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 one[7] most closely resembling ours in structural and stereochemical features is the interconversion of *cis*- and *trans*-aconitic aid (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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† The substrates (**6**), (**8**), and (**10**) were prepared from appropriately deuteriated vanillins.[3] Deuterium was located unambiguously by ^{1}H n.m.r. spectroscopy. Full details will be reported elsewhere.

‡ 4-Oxopentanoic (laevulinic) acid with NaOD–D_2O gave, by exchange α to the ketonic group, a pentadeuterio-derivative showing (n.m.r.) a broad singlet, δ [$(CD_3)_2CO$] 2·55, for the remaining, C-2, methylene group. Reduction ($NaBH_4$) 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 [$(CD_3)_2CO$] 2·76 (br. s, CH_2) and 2·15 (br. s, Me).

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