The Stereochemistry of the Enzymic Cyclisation of 3-Carboxymuconic Acid to 3-Carboxymuconolactone

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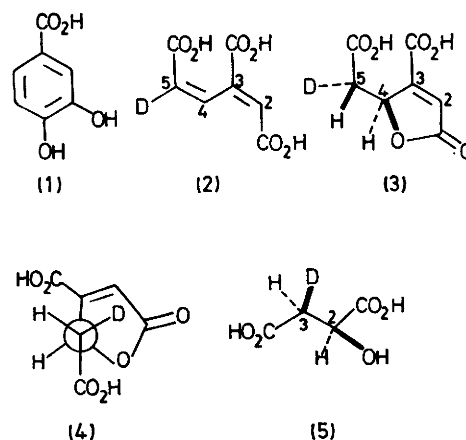
Summary Cyclisation of *cis-cis*-3-carboxy-[5-^{2}H]muconic acid catalysed by the lactonising enzyme of *Neurospora crassa* proceeds with *syn*-addition to the 4,5-double bond to give (4*S*,5*S*)-C-carboxy-[5-^{2}H]muconolactone.

BIOCHEMICAL degradation[1] of protocatechuic acid (**1**) by the fungus, *Neurospora crassa,* involves oxidative cleavage to *cis-cis*-3-carboxymuconic acid (**2**; D = H). This acid is cyclised to 3-carboxymuconolactone (**3**; D = H) which is further transformed into 3-oxoadipic acid. We report here the relative absolute stereochemistry of the enzyme-catalysed cyclisation, (**2**) → (**3**).

The deuterio-acid (**2**), prepared[2] from [5-^{2}H]vanillin, was incubated with a crude preparation[1] of the lactonising enzyme from *N. crassa* SY4a. The resulting (−)-lactone (**3**) was isolated and purified in the usual way[1] then examined by ^{1}H n.m.r. spectroscopy. The non-deuteriated lactone (**3**; D = H) showed a well-separated ABX system for the methylene and methine protons with allylic coupling between H(4) and H(2), τ [$(CD_3)_2CO$] 7·21 (J 8·1 and 16·8), 6·76 (J 3·3 and 16·8), and 4·41 (J 8·0, 3·3, and 2·2 Hz). The deuteriated lactone (**3**) gave signals for only one diastereoisomer, τ 6·76 (J_{HH} *ca.* 3 and J_{HD} *ca.* 3 Hz) and 4·41 (multiplet). In contrast, the racemic lactone obtained from (**2**) by cyclisation in trifluoroacetic acid gave H(5) signals, τ 7·22 and 6·76, for two diastereoisomers. Thus enzymic cyclisation had occurred, as expected, stereospecifically.

The n.m.r. spectrum of (**3**) defines the relative stereochemistry shown if it is assumed that the predominant conformation in solution is (**4**) with the large carboxyl group and lactone residue antiperiplanar. The single methylene proton should then show, as observed, the smaller, averaged



vicinal coupling. The relative stereochemistry was confirmed, and the absolute stereochemistry established, by degradation. Successive treatment of (**3**) with ozone, manganese dioxide, and formic acid–hydrogen peroxide

gave (2*S*, 3*S*)-[3-^{2}H]malic acid (**5**). The relative configuration of (**5**) followed from the small vicinal coupling constant (*J* 3·0 Hz) observed in alkaline D_2O since (2*S*, 3*R*)-[3-^{2}H]-malic acid, prepared enzymically from fumaric acid in D_2O, shows[3] the alternative, larger vicinal coupling (*J* 9·7 Hz). Similar degradation of the unlabelled (−)-lactone (**3**; D = H) gave partially racemic† (2*S*)-malic (L-malic) acid, $[\alpha]_D^{15}$ −23·6° (*c* 1·5 in pyridine) (L-malic acid, −28·6°). The absolute configuration of (**3**) is therefore as shown and enzymic cyclisation of (**2**) takes place *syn* from the *re-si*-face of the olefinic double bond.

Avigad and Englard[4] have studied a related cyclisation. Incubation of *cis-cis*-muconic acid with the lactonising enzyme from *Pseudomonas putida* in tritiated water gave the correspondingly tritiated (+)-muconolactone which was degraded to (2*S*,3*R*)-[3-^{3}H]malic acid. Thus, in both fungi and bacteria, lactonisation of structurally similar acids occurs by the rarely observed[5] *syn* addition to a double bond.

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† Partial racemisation was not unexpected since oxidative degradation of (**3**) places a carbonyl group next to the chiral centre at C(2) in (**5**).

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