Stereochemistry of Enzymic Cyclisation of 3-Methyl-*cis,cis*-muconic Acid to form 3- and 4-Methylmuconolactone

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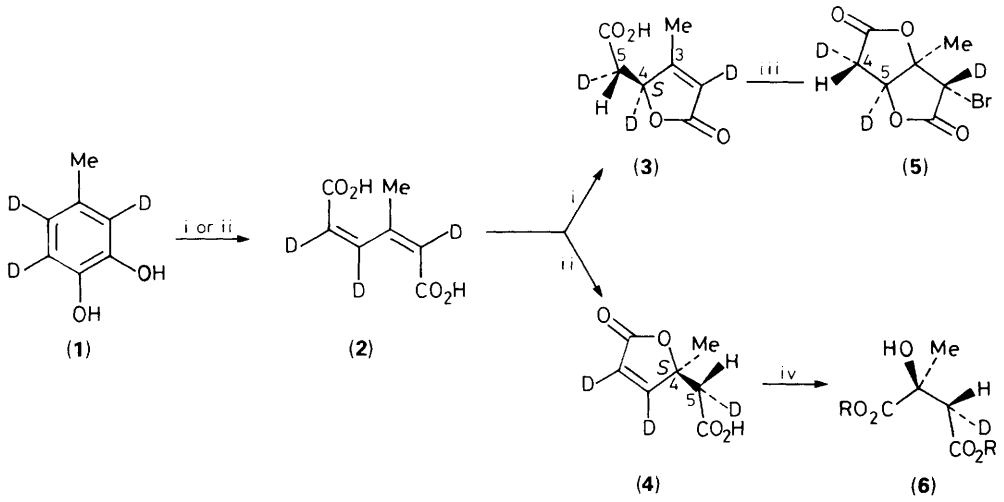
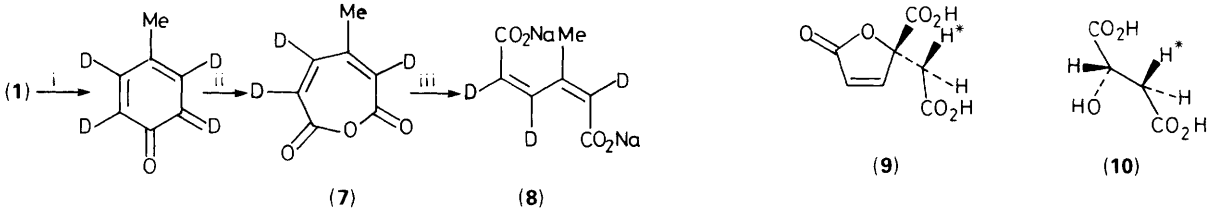
Enzyme-catalysed cyclisation of 3-methyl-*cis,cis*-muconic acids proceeds by *syn* addition of carboxyl groups to double bonds to form (4*S*)-3-methylmuconolactone in *Aspergillus niger* and (4*S*)-4-methylmuconolactone in *Pseudomonas putida*.

The muconic acid pathways[1] provide important routes for the microbial degradation of benzene derivatives present in soil or industrial wastes. In particular (Scheme 1), toluene, *p*-cresol, and *p*-toluic acid are degraded *via* 4-methylpyrocatechol [(**1**); H in place of D] and 3-methyl-*cis,cis*-muconic acid [(**2**); D = H].[2] In the yeast *Trichosporon cutaneum*,[2] this 3-methylmuconic acid is converted into (*S*)-3-methylmuconolactone [(**3**); D = H] and thence *via* 4-methyl-3-oxoadipic acid into acetic and pyruvic acids. However, in the bacterial genus pseudomonas cyclisation of 3-methylmuconic acid characteristically occurs in the alternative manner to give (*S*)-4-methylmuconolactone [(**4**); D = H], a metabolically 'dead-end' product.[3] Unexpectedly, strains of *Alcaligenes eutrophus* and several nocardioform actinomycetes (bacteria) have recently been shown[4] to effect the enzymic transformation of (*S*)-4-methylmuconolactone into (*S*)-3-methylmuconolactone, thereby overcoming this bacterial 'block' whereas in the fungus *Aspergillus niger* there is no comparable enzymic activity. We report here the stereochemistry of enzymic cyclisation of the 3-methylmuconic acid (**2**) to form the 3-methylmuconolactone (**3**) in *Aspergillus niger* and the 4-methylmuconolactone (**4**) in *Pseudomonas putida*.

The deuteriated pyrocatechol (**1**), prepared from 4-methylpyrocatechol by exchange[5] in 4 M DCl at 90 °C, was fed to a mutant strain of *A. niger* known[6] to accumulate (*S*)-3-methylmuconolactone. The ^{1}H n.m.r. spectrum [200 MHz; $(CD_3)_2CO$] of the resulting lactone (**3**) showed, as expected, that highly stereoselective cyclisation had occurred; δ 2.98 (t, $J_{H,D}$ 2.3 Hz, 5-H) [the undeuteriated lactone[4] gave signals at δ 2.55 and 3.00 (5-CH_2)]. In confirmation, the ^{2}H



Scheme 1. *Reagents and conditions*: i, *Aspergillus niger* culture; ii, *Pseudomonas putida* culture; iii, Br_2–$NaHCO_3$ in CH_2Cl_2–H_2O; iv, O_3 in CH_2Cl_2, −70 °C then HNO_3–H_2O, 90 °C.



Scheme 2. *Reagents*: i, Ag_2O–Na_2SO_4 in Et_2O; ii, monoperphthalic acid in Et_2O; iii, NaOH (2 mol equiv.) in H_2O.

n.m.r. spectrum (30.7 MHz; Me_2CO) showed a strong signal at δ 2.51 (d, $J_{H,D}$ 2.3 Hz, 5-D) and a much weaker (*ca.* 3%) signal, δ 2.96, which might have arisen from the lactone formed non-enzymically from the muconic acid (**2**) (see below). The lactone (**3**) was then converted[7] into the rigid bromo dilactone (**5**). The ^{1}H n.m.r. spectrum [200 MHz; $(CD_3)_2CO$] of the undeuteriated dilactone [(**5**); D = H] shows signals, δ 2.92 (ddd, *J* 18.7, 1.0, and 0.7 Hz, 4-H_R) and 3.36 (dd, *J* 18.7 and 4.9 Hz, 4-H_S), for the 4-methylene protons. Unambiguous assignment of these signals follows from the near-zero, vicinal coupling between the *trans* protons 4-H_R and 5-H [dihedral angle[7] H(5)–C(5)–C(4)–H_R(4) 99°]. The ^{1}H n.m.r. spectrum of the deuteriated dilactone (**5**) showed a signal, δ 2.89 (t, $J_{H,D}$ 2.8 Hz), corresponding to 4-H_R, and the complementary ^{2}H spectrum showed a signal, δ 3.34 (d, $J_{H,D}$ 2.9 Hz), corresponding to 4-D_S.

Similarly, the pyrocatechol (**1**) was fed to *P. putida* (ATCC 12633). The n.m.r. spectra of the resulting 4-methylmuconolactone[2,3] (**4**) again indicated highly stereoselective lactonisation; δ_H [200 MHz; $(CD_3)_2CO$] 2.76 (t, $J_{H,D}$ 2.3 Hz, 5-H) and δ_D (30.7 MHz; $CHCl_3$; ^{1}H decoupled) 2.94 (s, 5-D). The lactone (**4**) was degraded by successive treatment with ozone and nitric acid[8] to give the (*S*)-citramalic acid [(**6**); R = H], which was then esterified with diazomethane. The ^{1}H n.m.r. spectrum of the dimethyl ester [(**6**); R = Me] corresponded closely with that reported[8] for synthetic material of unambiguously determined relative configuration; δ (200 MHz; $CDCl_3$) 2.64 (t, $J_{H,D}$ 2.2 Hz, 3-H). Unexpectedly, the biosynthetic 4-methylmuconolactone (**4**) was accompanied by a substantial amount (*ca.* 8% of the mixture) of racemic, deuteriated 3-methylmuconolactone consisting of a mixture of 5*R* and 5*S* (**3**) diastereoisomers (*ca.* 3:1). Presumably, this must have arisen by non-enzymic cyclisation of the muconic acid (**2**) formed *in vivo* from the methylpyrocatechol (**1**). To test this interpretation, the disodium salt (**8**) was prepared (Scheme 2) from the anhydride[9] (**7**) by cleavage with sodium hydroxide, and fed to cultures, at pH 7.2, of *P. putida*. The derived, optically pure 4-methyl-lactone (**4**) was accompanied by a greatly increased amount (77% of the mixture) of racemic 3-methyl-lactone. In a separate experiment, non-deuteriated 3-methyl-*cis*,*cis*-muconate [(**8**); D = H] was found to isomerise rapidly even at pH 7.2 to give the corresponding, enzymically-inactive 2-*cis*,4-*trans*-muconate. The latter cyclised at lower pH to afford racemic 3-methylmuconolactone.†

In conclusion, lactonisation of 3-methyl-*cis*,*cis*-muconic acid occurs in both *A. niger* and *P. putida* by *syn* addition of carboxyl groups to *cis* double bonds. The same relative and absolute stereochemistry of lactonisation obtains for the parent (*S*)-muconolactone[11,12] in *P. putida* and for (*S*)-3-carboxymuconolactone[13] in *Neurospora crassa* (a fungus). Curiously, the same strain of *P. putida* converts 3-carboxy-*cis*,*cis*-muconic acid into (*R*)-4-carboxymuconolactone [(**9**); H* represents a proton from the medium] by *anti* addition.[12] However, a feature common to all five enzymic lactonisations is the absolute stereochemistry of the newly created methylene groups [see (**9**)]; this is the same as that in (*S*)-malic acid

† In our hands, 3-methyl-*cis*,*cis*-muconic acid did not cyclise at pH 6.5 to form (±)-4-methylmuconolactone;[10] instead we observed (^{1}H n.m.r. monitoring in D_2O) rapid formation of 3-methyl-2-*cis*,4-*trans*-muconic acid and 3-methylmuconolactone followed by slower conversion of the former into the latter. Dr. D. H. Pieper (Universität Stuttgart) has kindly repeated our experiment and confirmed this result.

(**10**) formed by fumarase-catalysed, *anti* hydration of fumaric acid.[14] The lactones (**3**) and (**4**) will serve as key reference compounds for studies on methylmuconolactone isomerisation[4] in nocardioform actinomycetes.

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