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(+)-Muconolactone from Arene Biotransformation in *Pseudomonas putida*: Production, Absolute Configuration and Enantiomeric Purity.

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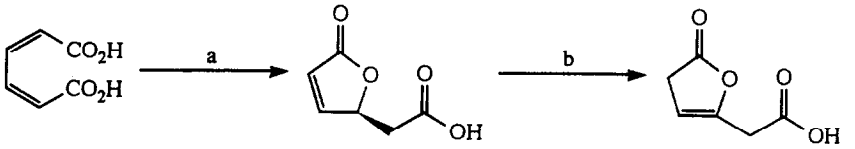
Abstract: The biotransformation of racemic mandelate by a mutant of *Pseudomonas putida* to give the title compound (1) in high yield and enantiomeric purity is reported. An apparent inconsistency in previous assignments of the absolute configuration of 1 and the corresponding methyl ester is resolved to show that (+)-1 has (5S) stereochemistry.

Chiral , non-racemic γ-lactones are key moieties in a wide range of natural products[2,3] and are also of considerable value as synthetic intermediates.[3-5] A variety of synthetic approaches to these compounds continue to be reported: thus the use of biological[2,6,7] and transition metal[8,9] asymmetric catalysts, chiral auxiliaries[10,11] and the "chiral pool"[12,13] have been used. Although powerful, none of these routes have been demonstrated to have general applicability and hence there remains considerable scope for the development of new, complementary procedures. To this end we have been investigating biotransformation mediated routes to the highly functionalized muconolactone [5-carboxymethyl-2,5-dihydrofuran-2-one (1), Scheme 1] as a prototype to a group of butenolides.



(2) (1)

a) Muconate cycloisomerase

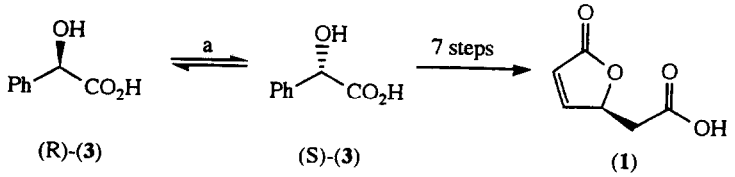
b) Muconate isomerase, defective in *Pseudomonas putida* mutant PRS 2912

Scheme 1

(+)-Muconolactone has been identified as a key intermediate in the catechol branch of the β-ketoadipate pathway for the degradation of many arenes by a variety of organisms including *Pseudomonas putida*.[14] (+)-**1** is formed by an enzyme catalyzed cycloisomerization of (Z,Z)-muconic acid (**2**, Scheme 1).[15] (-)-Muconolactone has also been reported to be formed by the action of the same enzyme on (E,Z)-muconic acid, but at a very much slower rate (0.02%).[14] The absolute configuration of the (+)-lactone was held to be (5S) on the basis of a combination of chemical and enzymatic degradations,[16] but no assessment of the enantiomeric purity has been reported.

To evaluate the utility of muconolactone as a starting material in asymmetric synthesis it was therefore necessary that we develop methodology to accurately assess the enantiomeric purity of the lactone formed by *Pseudomonas putida* - particularly given that the possible lability of the 5-proton of the furanone system and the, albeit slow, Z,Z- to E,Z-isomerization of muconic acid at room temperature[17] could have a deleterious effect on the enantiomeric purity of the product during the biotransformation and isolation procedures. A further requirement was that we develop suitable biotransformation conditions to accumulate suitable amounts of the product (+)-**1**.

We have studied the synthesis of (+)-**1** by the degradation of a range of arenes using a mutant strain *Pseudomonas putida* PRS 2912[18] which is defective in muconolactone isomerase - the enzyme that converts (+)-muconolactone to the corresponding achiral enol lactone (Scheme 1).[14] Using this strain (+)-**1** accumulates in the growth medium in near equimolar yields (≥97%) from various simple arenes, e.g. catechol, benzoic acid, **R-**, **S-**, and (±)-mandelic acids (**3**) when these substrates were supplied concomitantly with glucose, arginine or succinate as substrates for growth and respiration. Mandelate was the least toxic arene and could be supplied at high concentrations (up to 20mM in racemate). An easy protocol uses media containing 20mM glucose and 20mM (±)-mandelate in a conventional mineral salts medium at pH 6.7 - 7.3. The low toxicity of (±)-**3** allows the enantioconvergent eight step biotransformation to (+)-**1** in 97% yield (19.5 mM; 2.7 g l^{-1}). Higher concentrations of (+)-**1** are obtainable by continuous or batch additions of glucose and (±)-**3**.[19] Although the oxidation sequence of **3** is solely initiated by a membrane bound (S)-mandelate oxidase, both enantiomers of **3** are converted to (+)-**1** due to the coordinate induction of a mandelate racemase (Scheme 2).[20,21]

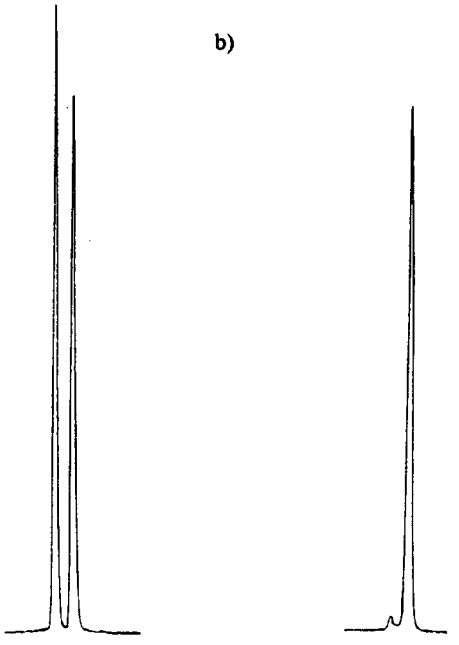


(R)-(**3**) (S)-(**3**) (**1**)

a) Mandelate racemase

Scheme 2

The measurement of the enantiomeric excess of (+)-1 so formed was achieved readily by gas chromatographic analysis, on a Lipodex D® column, of the methyl ester (4) (prepared by the treatment of 1 with diazomethane, Scheme 3) by comparison with the racemic compound.[22] Enantiomeric excesses in the range 97 - 99% were determined consistently over a series of biotransformations (Figure 1).



Gas chromatograph of a) (±)-4 and b) (+)-4 of 97.4% ee.

Figure 1

Both enantiomers of methyl ester (4) have been isolated from sponge extracts {$[\alpha]_D$ = +80.3 (c = 0.27, $CHCl_3$), $[\alpha]_D$ = -96.1 (c = 1.05, $CHCl_3$)},[23] and the (+)-enantiomer was assigned as (5R) by CD correlation. The methyl ester prepared by us from (+)-1, reported to be of (5S) configuration,[16] had a very similar optical rotation {namely $[\alpha]_D$ = +87.0 (c = 0.28, $CHCl_3$), $[\alpha]_D$ = +96.0 (c = 1.05, $CHCl_3$)} to that reported for (+)-4. Given that an inversion of configuration in the transformation of (+)-1 to (+)-4 is unlikely, the two assignments appear to be inconsistent.

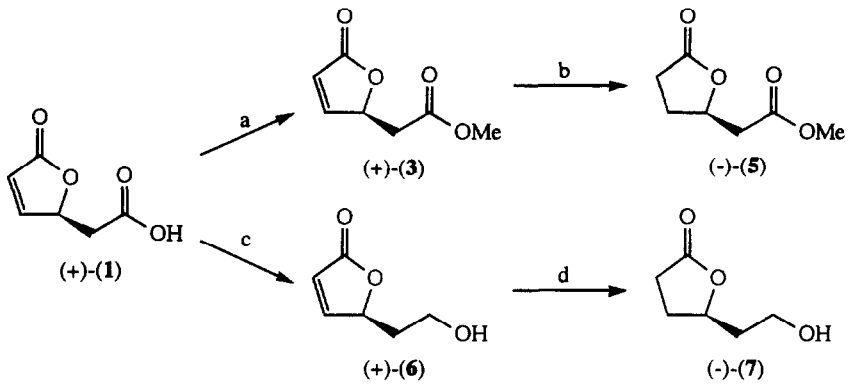
We therefore determined unambiguously the absolute configuration of (+)-1 by conversion to three derivatives of known absolute configuration (Scheme 3). Thus catalytic hydrogenation of the methyl ester (+)-4 gave the fully saturated lactone (-)-5. This enantiomer has been prepared previously via a classical

resolution procedure and shown to be of (5R) configuration by conversion to a carbohydrate degradation product of known absolute configuration.[24]

Furthermore, reduction of (+)-1 with borane.dimethylsulfide complex gave the primary alcohol (+)-6. The antipode (-)-6 has been prepared recently in several steps from (R)-malic acid.[13]

Finally, catalytic hydrogenation of (+)-6 gave the butanolide (-)-7 of similar optical rotation to material prepared via a yeast-mediated reduction and shown to be of (5R) configuration by conversion to the antipode of a beetle pheromone of known stereochemistry.[6]

Our syntheses of (R)-5, (S)-6 and (R)-7 are all consistent with the (+)-enantiomer of muconolactone (1) having (5S) configuration and hence suggest that (+)-4 isolated from sponges has (5S) and not (5R) configuration.



a) CH_2N_2, THF, Et_2O (86%); b) H_2, Pd/C, EtOAc (98%);
c) $BH_3.SMe_2$, THF (58%); d) H_2, Pd/C, EtOAc (99%).

Scheme 3

With a high yielding route to essentially optically pure (+)-muconolactone now in hand, future work will concentrate on biotransformation based approaches to (-)-1 and the use of both enantiomers as starting materials in asymmetric synthesis, for example in approaches to therapeutically important analogues of compactin and mevinolin.

EXPERIMENTAL

Melting points are uncorrected. All ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Brucker AM250 spectrometer at 250MHz in deuterochloroform, unless stated otherwise. IR spectra were recorded on a Perkin-Elmer 297 spectrophotometer. Optical rotations were measured on an Optical Activity AA-1000 polarimeter in a 5 cm path length cell and are given in 10^{-1} deg. cm^2 g^{-1}. All products were shown to be homogenous by ^{1}H-NMR and ^{13}C-NMR spectroscopic analysis, and all spectroscopic data was consistent with that reported in the cited literature.

(+)-5-Carboxymethyl-2,5-dihydrofuranone **(1)**: A minimal growth medium supplemented with glucose (3.60 g, 0.020 mol) and (±)-mandelic acid (3.04 g, 0.020 mol) (1 l in a 2 l Erhlenmeyer flask) was inoculated with 20 ml of an overnight (14 - 18 h) suspension of *P. putida* strain PRS 2912,[18] cultivated on Lab-Lemco (Oxoid Ltd.) broth, and shaken for 24 - 48 h at 30°C. Progress of the biotransformation was monitored by analysis of clarified and diluted samples by HPLC on C-18 reverse phase eluting with 10% H_3PO_4-isopropanol (1:9) which also revealed the transient accumulation of some intermediates (benzoate, catechol, (Z,Z)-muconate) between (±)-**3** and (+)-**1**. When the concentration of (±)-**3** was less than 0.1 mM the culture was clarified by centrifugation, concentrated to 100 ml, acidified and extracted with ether continuously for 18-24 h. Crystalline (+)-**1** (2.64 g, 93%) was obtained by evaporation of the ether extract, m.p. 65-70°C (lit.[14b] m.p. 76-77.5°C); $[\alpha]_D$ = +57.0 (c = 0.51, EtOH); δ_H (d_6-DMSO)= 12.4 (br. s, 1H), 7.80 (dd, J=5.7, 1.4 Hz, 1H), 6.25 (dd, J=5.7, 1.9 Hz, 1H), 5.42 - 5.37 (m, 1H), 2.85 (dd, J=16.5, 4.8 Hz, 1H), 2.51 (dd, J=16.5, 8.2 Hz, 1H); δ_C ($CDCl_3$ / d_6-DMSO) 172.4 (s), 170.8 (s), 155.8 (d), 121.6 (d), 79.1 (d), 37.6 (t); ν_{max} (nujol) 3500 - 2500, 1735, 1700 cm^{-1}.

(+)-5-Methoxycarbonylmethyl-2,5-dihydrofuran-2-one **(4)**: (+)-5-Carboxymethyl-2,5-dihydrofuran-2-one (0.28 g, 2.0 mmol) was stirred in THF (10 ml) at room temperature and ethereal diazomethane **(CAUTION!)**[25] added slowly dropwise until thin layer chromatography on silica gel, eluting with ethyl acetate-acetic acid (9:1), indicated the disappearance of starting material. The mixture was evaporated and the residue chromatographed over silica eluting with ethyl acetate-light petroleum (b.p. 40-60°C) (1:3 → 1:1) to give the title compound (0.27 g, 86%) as a white solid, m.p. 44.5-46.5°C (lit.,[23] oil); $[\alpha]_D$ = +87.0 (c = 0.28, $CHCl_3$), $[\alpha]_D$ = +96.0 (c = 1.05, $CHCl_3$); δ_H = 7.58 (dd, J=5.5, 1.5 Hz, 1H), 6.16 (dd, J=5.5, 1.5 Hz, 1H), 5.39 (dddd, J=7, 7, 1.5, 1.5 Hz, 1H), 3.72 (s, 3H), 2.85 (dd, J=16, 7 Hz, 1H), 2.64 (dd, J=16, 7 Hz, 1H); δ_C 172.3 (s), 169.5 (s), 155.5 (d), 122.3 (d), 79.0 (d), 52.3 (q), 37.7 (t); ν_{max} ($CHCl_3$) 1757, 1735, 1159 cm^{-1}.

(-)-5-Methoxycarbonylmethyl-2,3,4,5-tetrahydrofuran-2-one (5): (+)-5-Methoxycarbonylmethyl-2,5-dihydrofuran-2-one (3) (0.156 g, 1.0 mmol) was stirred with 10% palladium on carbon (0.013 g) in ethyl acetate (3 ml) at room temperature under an atmosphere of hydrogen for 90 min. The mixture was filtered and concentrated to yield the alkane (0.155 g, 98%) as a colourless oil (lit.,[24] oil); $[\alpha]_D$ = -44.2 (c = 1.0, EtOH); δ_H = 4.91 - 4.78 (m, 1H), 3.66 (s, 3H), 2.76 (dd, J=16, 6.5 Hz, 1H), 2.61 (dd, J=16, 6 Hz, 1H), 2.56 - 2.35 (m, 3H), 2.02 - 1.85 (m, 1H); δ_C 176.7 (s), 170.1 (s), 76.3 (d), 52.0 (q), 39.7 (t), 28.5 (t), 27.5 (t); ν_{max} (film) 1776, 1734, 1167 cm^{-1}.

(+)-5-(2'-Hydroxyethyl)-2,5-dihydrofuran-2-one (6): (+)-5-Carboxymethyl-2,5-dihydrofuran-2-one (1) (0.71 g, 5.0 mmol) was stirred in dry THF (25 ml) under nitrogen in an ice bath and borane.dimethylsulfide complex (5.0 ml of a 2.0 M solution in THF, 10.0 mmol) added slowly dropwise.[26] The mixture was stirred at room temperature for a further 5 h, poured into methanol (25 ml) in an ice bath, then concentrated under reduced pressure. Column chromatography on silica gel using dichloromethane-ethyl acetate (100:0 → 0:100) gave the alcohol (0.38 g, 58%) as a colourless oil ((-)-form lit.,[13] oil); $[\alpha]_D$ = +40.0 (c = 2.2, $CHCl_3$); δ_H = 7.57 (dd, J=5.5, 1.5 Hz, 1H), 6.04 (dd, J=5.5, 2.0 Hz, 1H), 5.21 (dddd, J=8.5, 5.0, 2.0, 1.5 Hz, 1H), 3.78 - 3.71 (m, 2H), 3.03 (br. s, 1H), 2.06 - 1.91 (m, 1H), 1.86 - 1.71 (m, 1H); δ_C 173.9 (s), 157.9 (d), 120.7 (d), 81.5 (d), 58.2 (t), 35.9 (t); ν_{max} (film) 3442 (br), 1756 cm^{-1}.

(-)-5-(2'-Hydroxyethyl)-2,3,4,5-tetrahydrofuran-2-one (7): (+)-5-(2'-Hydroxyethyl)-2,5-dihydrofuran-2-one (6) (0.192 g, 1.5 mmol) was stirred with 10% palladium on carbon (15 mg) in ethyl acetate (7.5 ml) under hydrogen at room temperature. After 18 h, the mixture was filtered and concentrated to give the title compound (0.194 g, 99%) as a colourless oil (lit.,[6] oil); $[\alpha]_D$ = -62.5 (c = 1.3, $CHCl_3$); δ_H = 4.74 - 4.61 (m, 1H), 3.77 (t, J=6 Hz, 2H), 2.56 - 2.47 (m, 2H), 2.35 (dddd, J=12.5, 6, 6, 6 Hz, 1H), 2.11 - 1.78 (m incorporating br. s at 2.03, 5H); δ_C 178.0 (s), 78.7 (d), 58.6 (t), 38.1 (t), 28.9 (t), 28.1 (t); ν_{max} (film) 3449 (br), 1765 cm^{-1}.

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