Synthesis and absolute configurations of the naturally occurring 3- and 4-methylmuconolactones: X-ray structures of (*S*)-1-phenylethylammonium salts and an 8-bromo-1-methylmuconodilactone

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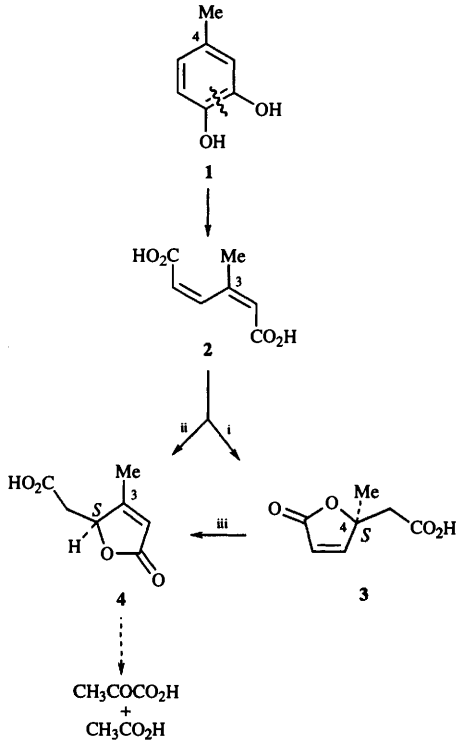
(±)-3-Methylmuconolactone (±)-4 is resolved by fractional crystallisation of the (*S*)-(−)-1-phenylethylammonium salts. The X-ray crystal structures of both salts are determined. Salt A (Fig. 1) gives (*S*)-(−)-3-methylmuconolactone 4, which is identical to the lactone from fungi. The lactone is converted with bromine into the bromo dilactone 5 and thence with tributyltin hydride into the (−)-1-methylmuconodilactone 6. This dilactone with aqueous sodium hydroxide gives (*S*)-(+)-4-methylmuconolactone 3, which is identical to the lactone from bacteria, together with (*S*)-(−)-3-methylmuconolactone 4. The X-ray structure of the bromo dilactone 5 (Fig. 3) confirms the absolute configurations of both the fungal and bacterial muconolactones. (*S*)-(+)-4-Methylmuconolactone 3 gives the corresponding (−)-bromo dilactone 9, which is also reduced with tributyltin hydride to yield the (−)-1-methylmuconodilactone 6. The isomeric bromo dilactones (±)-5 and 9 are similarly converted into the dibromo dilactones (±)-8 and 11 *via* the corresponding 2-bromomuconolactones (±)-7 and 10, respectively.

Disodium 3-methyl-*cis*,*cis*-muconate 17 is prepared non-enzymically by treatment of 3-methylmuconic anhydride 16 with 2 mol equiv. of aqueous sodium hydroxide. Unexpectedly, the salt 17 rapidly gives 3-methyl-2-*cis*,4-*trans*-muconate even in weakly alkaline solutions. Contrary to an earlier report, at pD 6.5 the salt 17 is converted at approximately equal rates into 3-methyl-2-*cis*,4-*trans*-muconic acid 18 and (±)-3-methylmuconolactone (±)-4.

The methylmuconic acid pathways (Scheme 1) provide important routes for the complete microbial degradation of toluene derivatives in soil or industrial waste.† Thus, substrates such as toluene, *p*-xylene, *p*-toluic acid and *p*-cresol may be converted enzymically into 4-methylpyrocatechol **1** and thence 3-methyl-*cis*,*cis*-muconic acid **2**.‡ In bacteria generally, cycloisomerisation (path i) of the muconic acid **2** gives (*S*)-4-methylmuconolactone **3**,‡ which in typical bacteria is a 'dead-end' metabolite since the 4-methyl group prevents further degradation. In contrast, fungi convert (path ii) the muconic acid **2** into (*S*)-3-methylmuconolactone **4**, which may be degraded further, ultimately to acetic and pyruvic acids. In this way, certain fungi are able to exploit toluene derivatives as sole sources of metabolic carbon and energy.

However, the discovery that the bacterium *Alcaligenes eutrophus* JMP 134[2] and certain nocardioform actinomycetes (bacteria), including *Rhodococcus rhodocrous* N75,[3] could metabolise 4-methylpyrocatechol **1** to the characteristically *fungal* lactone **4** led to the identification of a new type of enzymic transformation (step iii)[4] and prompted us to study in detail the enzymic transformations of the methylmuconic acid pathways.[1] In particular, we showed that the enzymic formation of (*S*)-4-methylmuconolactone **3** in the bacterium *Pseudomonas putida* and of (*S*)-3-methylmuconolactone **4** in the fungus *Aspergillus niger* proceeds by *syn* addition of alternative carboxy groups to the distal double bonds of the muconic acid



Scheme 1 *Organisms:* i, bacteria; ii, fungi; iii, 'specialised' bacteria, *e.g. Alcaligenes eutrophus* and *Rhodococcus rhodocrous*

† For further details of the muconic and 3-methyl- and 3-carboxymuconic acid pathways see ref. 1 and the literature citations therein.
‡ The IUPAC name for the muconic acid **2** is (2*E*,4*E*)-3-methylhexa-2,4-dienedioic acid and for compound **4** is 2-(2-methyl-5-oxo-2,5-dihydro-2-furyl)acetic acid.

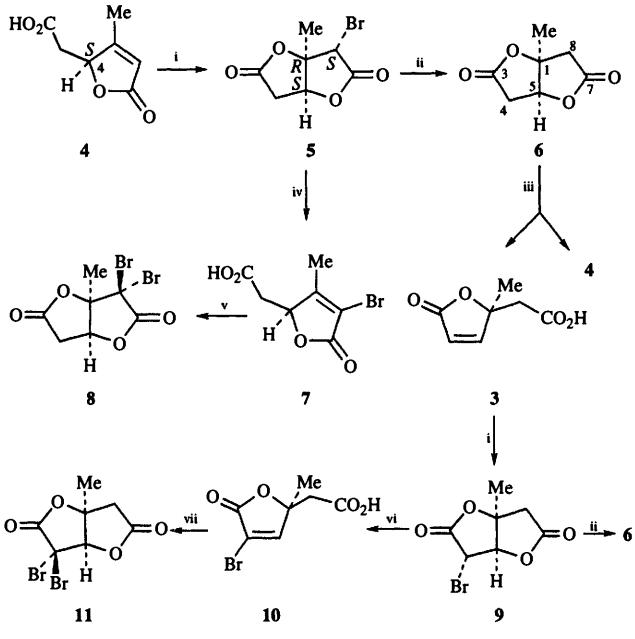
non-enzymic syntheses of 3-methyl-*cis,cis*-muconic acid **2** and the lactone enantiomers **3** and **4** were available. However, (±)-3-methylmuconolactone (±)-**4** is readily obtained by treatment of 4-methyl-2-nitrophenol with hot, concentrated sulfuric acid.[5] Resolution of this racemic lactone provided the starting point for the following studies.[6]

Resolution of (±)-3-methylmuconolactone (±)-4 and the absolute configuration of the natural (−)-enantiomer 4 (Scheme 1)

(±)-3-Methylmuconolactone was converted with (*S*)-(−)-1-phenylethylamine into a mixture of diastereoisomeric salts, which were separated by fractional crystallisation. X-Ray analyses were carried out on single crystals of both salts. Salt A (Fig. 1), mp 104–108 °C (decomp.), when treated briefly in methanol with an ion-exchange resin (H^+ form), gave (*S*)-(−)-3-methylmuconolactone **4**. Similarly, salt B (Fig. 2), mp 119–122 °C (decomp.) gave (*R*)-(+)-3-methylmuconolactone. The spectroscopic properties and optical rotation of the synthetic (−)-lactone **4** agreed well with those reported[2,3] for the natural, fungal product. To confirm the identity of the synthetic and natural lactones, 4-methylpyrocatechol **1** was fed to a mutant strain of *Aspergillus niger* known[7] to accumulate (−)-3-methylmuconolactone. Unexpectedly, when the crude, natural lactone was crystallised slowly from dilute solution, a small quantity (*ca.* 3%) of *racemic* 3-methylmuconolactone was obtained. Nevertheless, the mother liquors yielded (−)-3-methylmuconolactone **4** as the major product, as expected. We believe that the racemic lactone was most likely formed *in vivo* by non-enzymic cyclisation of the muconic acid **2**.[1] The natural (−)-3-methylmuconolactone was then treated with (*S*)-(−)-1-phenylethylamine and the resulting salt was shown by X-ray crystallography to be identical with the foregoing, synthetic salt A.

Synthesis and absolute configuration of the natural (+)-4-methylmuconolactone 3 (Scheme 2)

To our knowledge, no efficient, non-enzymic synthesis of racemic or optically active 4-methylmuconolactone has previously been reported. In particular, acid-catalysed cyclisation[5,8] of the *cis,cis*-acid **2** or *cis,trans*-acid **18** gives



Scheme 2 *Reagents (all at room temp.):* i, Br_2 in CH_2Cl_2 and aq. $NaHCO_3$; ii, Bu_3SnH in C_6H_6 with $[Me_2C(CN)N]_2$; iii, 1 mol equiv. aq. NaOH; iv, aq. $NaHCO_3$ or Et_3N in CH_2Cl_2; v, Br_2 in CH_2Cl_2 with **7** or **5** and aq. $NaHCO_3$; vi, aq. $NaHCO_3$; vii, Br_2 in CH_2Cl_2 with **10** or **9** and aq. $NaHCO_3$

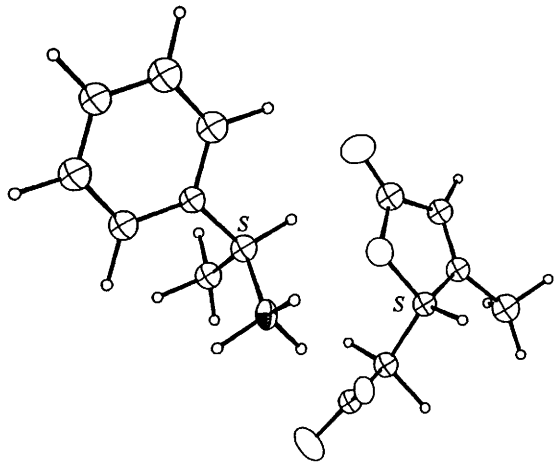


Fig. 1 X-Ray structure of the (*S*)-1-phenylethylammonium salt (salt A) of (*S*)-3-methylmuconolactone **4**

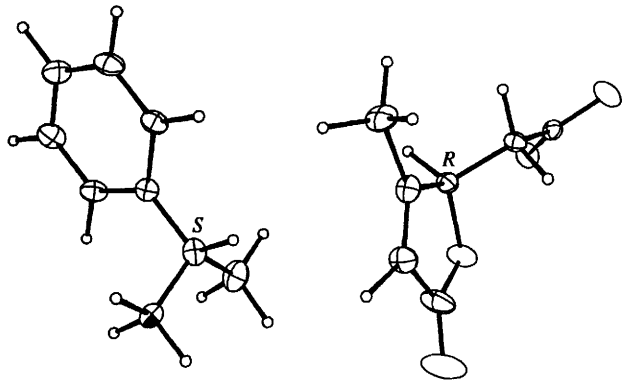


Fig. 2 X-Ray structure of the (*S*)-1-phenylethylammonium salt (salt B) of (*R*)-3-methylmuconolactone

predominantly or exclusively 3-methylmuconolactone (±)-**4**. We developed the synthetic route shown in Scheme 2 for the following reasons: (1) the dilactone **6** is possibly an intermediate in the enzyme-catalysed transformation **3** ⟶ **4** (step iii in Scheme 1),[4] (2) X-ray analysis of the bromo dilactone **5**, exploiting bromine as a heavy atom, would provide an independent check on the absolute configurations of both the lactones **3** and **4**, and (3) the rigid bromo dilactone **5** would allow the configuration of enzymically generated 5-deuterio-3-methylmuconolactone to be determined by ^1H NMR spectroscopy.[1] Therefore, the lactone **4** in aqueous sodium hydrogen carbonate, was treated with bromine in dichloromethane to afford the crystalline bromo dilactone **5**, in high yield. A two-phase system was essential since the product **5** was rapidly converted into the bromo lactone acid **7** under even weakly basic conditions. The bromo dilactone **5**, suspended in benzene, reacted readily with tributyltin hydride, in the presence of azoisobutyronitrile, to give the dilactone **6**, which crystallised out of the reaction mixture in high yield. The ^1H NMR spectrum, mp and optical rotation of this dilactone **6** agreed well with those reported[9,10] for material formed by acid-catalysed cyclisation of natural (+)-4-methylmuconolactone **3**, which must therefore have the 4*S* configuration. X-Ray analysis of the bromo dilactone **5** (Fig. 3) provided independent proof of the relative and absolute configurations of all the foregoing products **3** to **7**. The synthesis of (+)-4-methylmuconolactone **3** was then completed by treatment of the dilactone **6** with aqueous sodium hydroxide (1 mol equiv.). The lactones **3** and **4** were formed in the ratio **3**:**4** = *ca.* 3:2 and were separated by TLC. The physical constants for the synthetic lactone **3** were in excellent agreement with those reported[9,10] for the lactone from bacteria.

Remarkably, the typical bacterial lactone, (*S*)-(+)-methyl-

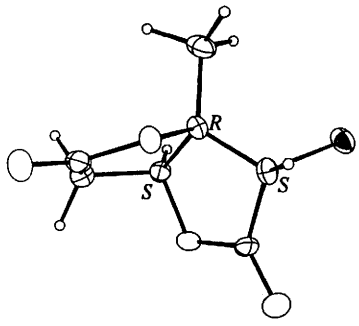
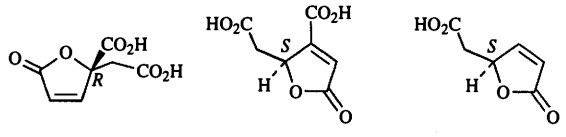


Fig. 3 X-Ray structure of the bromo dilactone **5** derived from (*S*)-3-methylmuconolactone **4**

muconolactone **3**, first described as a metabolite of *Pseudomonas desmolyticum*[9] and produced by *Pseudomonas putida*,[11] has therefore an absolute configuration opposite to that of the bacterial 4-carboxymuconolactone **12** produced by the same strain of *P. putida* and by *Acinetobacter calcoaceticus*.[12] In contrast, (−)-3-methylmuconolactone **4** and (−)-3-carboxymuconolactone[13] **13**, both produced by fungi, have the same absolute configuration (4*S*), identical with that of the unsubstituted (+)-muconolactone[12,14] **14** from fungi and bacteria. These observations, and the relative stereochemistries (*syn* or *anti* addition) of enzymic lactonisation, have been discussed elsewhere.[1]

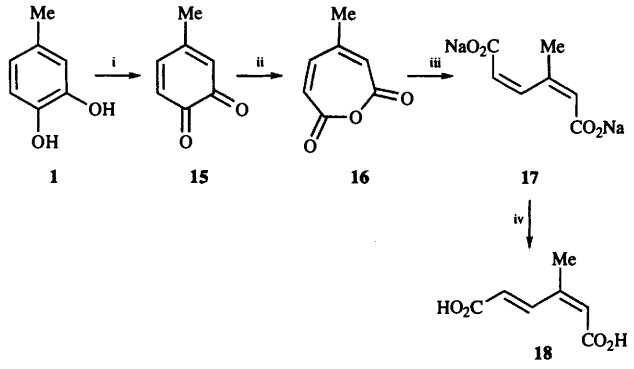


12 **13** **14**

Conditions for the reactions shown in Scheme 2 were first developed with (±)-3-methylmuconolactone (±)-**4**, which was more readily available than the natural enantiomer **4**. The racemic form of the dilactone **6** was employed in attempts to achieve selective ring-opening in either of the two possible modes. Indeed, with triethylamine or 1,5-diazabicyclo[4.3.0]-non-5-ene in dichloromethane at room temperature, (±)-3-methylmuconolactone (±)-**4** was formed exclusively. However, no conditions were found that gave better yields of (±)-4-methylmuconolactone (±)-**3** than those obtained (see above) with aqueous sodium hydroxide. Other transformations shown in Scheme 2 produced potential substrates or inhibitors of the enzymes of the methylmuconic acid pathways. Thus, the racemic bromo dilactone (±)-**5** was treated with aqueous sodium hydrogen carbonate to give the sodium salt of the bromo lactone acid **7**. This was brominated directly to yield the dibromo dilactone (±)-**8**, which has been found[15] to be an irreversible inhibitor of methylmuconolactone methylisomerase,[4] the enzyme that catalyses path iii in Scheme 1. Similarly, the isomeric (−)-dibromo dilactone **11** was prepared from the (+)-lactone **3**, and the intermediate (−)-bromo dilactone **9** was reduced with tributyltin hydride to give the dilactone **6**, which was, as expected, identical with that obtained from the isomeric bromo dilactone **5**.

Synthesis and *cis,trans* isomerism of disodium 3-methyl-*cis,cis*-muconate 17 (Scheme 3)

Elvidge *et al.*[8] studied the hydrolysis of 3-methylmuconic anhydride **16** under various conditions but were unable to obtain the *cis,cis*-muconic acid **2**; the corresponding *cis,trans*-acid **18** and the racemic lactone (±)-**4** were the only products isolated. It was clear that the *cis,cis*-acid **2** had undergone acid-catalysed isomerisation even more rapidly than the corresponding 3-carboxy derivative[16] **13** (*cis,cis*-muconic acid itself **14** is relatively stable under mildly acidic conditions). However, we

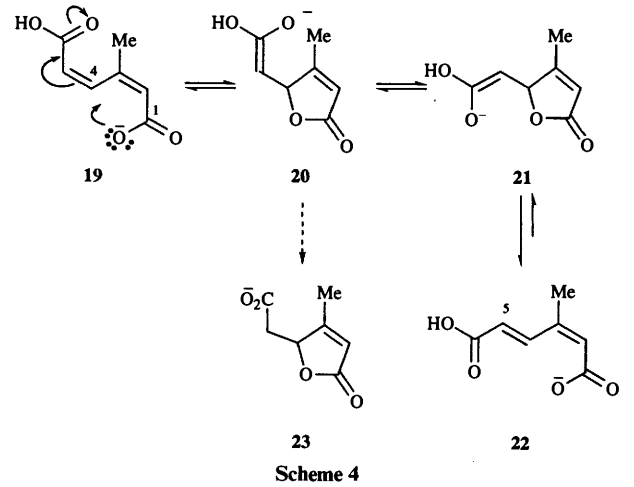


1 **15** **16** **17** **18**

Scheme 3 *Reagents* (*all at room temp.*): i, Ag_2O in dry Et_2O; ii, monoperphthalic acid in Et_2O; iii, aq. NaOH (2 mol equiv.); iv, H_3O^+

reasoned that the disodium salt **17**, required as a substrate for the cycloisomerase (lactonising) enzymes, would be configurationally stable. Indeed, when the anhydride **16** was treated with aqueous sodium hydroxide (2 mol equiv.), the disodium *cis,cis*-muconate **17** was obtained quantitatively. The ^1H NMR spectrum showed no significant signals for the corresponding *cis,trans* isomer. Unexpectedly, when the anhydride **16** was treated with aqueous sodium hydrogen carbonate (2 mol equiv.) or disodium carbonate (1 mol equiv.), the initially formed *cis,cis*-muconate rapidly isomerised to the *cis,trans* form (^1H NMR monitoring in D_2O solutions), even though the solutions remained distinctly alkaline throughout the experiments, as expected.

Our interpretation of these findings is outlined in Scheme 4.



19 **20** **21** **23** **22**

Scheme 4

Weakly alkaline solutions of 3-methyl-*cis,cis*-muconate will contain appreciable amounts of the monoanion **19**. Addition of the 1-carboxylate anion to the 4,5-double bond will be facilitated by the undissociated 6-carboxy group, as shown. The conformational change **20** ⟶ **21** followed by ring-opening would then produce the *cis,trans*-muconate **22**. Apparently, the proposed enolate intermediate **20**, which is the α-carbanion of a carboxylic acid, undergoes the transformations **20** ⟶ **21** ⟶ **22** faster than tautomerism to give the much more stable carboxylate anion **23**. Significantly, the isomerisation **19** ⟶ **22** cannot have proceeded through the lactone **23** because, when the reaction was monitored in deuterium oxide by ^1H NMR spectroscopy, the product **22** was seen to have incorporated no deuterium at C-5. However, under slightly acidic conditions, formation of the lactone **4** competes with *cis–trans* isomerisation of the muconic acid **2**. At pD *ca.* 6.5, 3-methyl-*cis,cis*-muconic acid **2** was observed (^1H NMR monitoring in D_2O) rapidly to form both the lactone **4** and the *cis,trans*-muconic acid **18**, in approximately equal amounts; the latter product in turn cyclised to form the lactone **4**, but at a

much slower rate. In contrast, Schmidt *et al.*[17] reported that 3-methyl-*cis,cis*-muconate, obtained enzymically, cyclised at pH 6.5 to give the isomeric, bacterial lactone **3**. Dr D. H. Pieper (Gesellschaft für Biotechnologische Forschung, Braunschweig) kindly repeated our experiment and confirmed our finding that the lactonic product is **4**, not **3**. Intermediates of the type **20** may be involved in the reversible, enzymic lactonisation of *cis,cis*-muconic acids.[18] Measurements of kinetic isotope effects[19] indicate that the enzyme-catalysed conversion of muconolactone **14** into *cis,cis*-muconic acid is a two-step process, base-catalysed deprotonation being followed by ring opening. The intermediate is unlikely for energetic reasons to be the α-carbanion of a carboxylate anion. More plausibly, the monoanion **20** (H replacing Me) might be formed, either by deprotonation of the carboxylic acid or by concerted protonation on oxygen and deprotonation from carbon of the carboxylate anion.[18]

X-Ray crystallographic analyses

Both the diastereoisomeric (*S*)-1-phenylethylammonium salts of 3-methylmuconolactone were subjected to single crystal X-ray analysis. It was recognised that accurate structural data (Figs. 1 and 2) for the anion of the lactone had potential value in later studies on the mode of action of the 3-methylmuconate cycloisomerase (step ii) and 4-methylmuconolactone methylisomerase (step iii) enzymes. The X-ray analysis of the (−)-bromo dilactone **5** (Fig. 3) confirmed the expected *cis* ring fusion and, with bromine serving as a heavy atom, established independently the absolute configuration of the dilactone itself and of both the muconolactones **3** and **4**. Importantly, the magnitude (99°) of the torsion angle H(5)–C(5)–C(4)–$H_{pro\text{-}R}$ (4) explains the small (*ca.* 0.7 Hz) coupling between the *trans* protons 4-$H_{pro\text{-}R}$ and 5-H observed in the ^{1}H NMR spectrum. In this way, signals at δ 2.92 and 3.36 could be unambiguously assigned to 4-$H_{pro\text{-}R}$ and -$H_{pro\text{-}S}$, respectively, enabling the stereochemistry (*syn* addition) of the enzymic cycloisomerisation **2** ⟶ **4** to be determined with an appropriately deuteriated precursor.[1]

Experimental

Mps were determined on a Kofler hot-stage microscope. IR spectra were recorded on either a Perkin-Elmer 580 or 953 spectrometer. ^{1}H NMR spectra at 90 and 200 MHz were obtained with Perkin-Elmer R34 and Bruker WP200 spectrometers, respectively. *J* Values are in Hz. Mass spectra were produced by EI at 70 eV with an AEI MS9 instrument. Optical rotations were measured with an Optical Activity Ltd. AA-100 Polarimeter. $[\alpha]_D$ Values are given in units of 10^{-1} deg cm^2 g^{-1}.

Resolution of (±)-3-methylmuconolactone (±)-4

(±)-3-Methylmuconolactone (±)-**4** was prepared[5] by heating 4-methyl-2-nitrophenol in conc. sulfuric acid. This racemic lactone (1.045 g, 6.7 mmol) in ethyl acetate (15 cm^3) was mixed with (*S*)-(−)-1-phenylethylamine (0.811 g, 6.7 mmol) in ethyl acetate (10 cm^3) and the combined solutions were kept at room temp. for 3 h. A mixture of diastereoisomeric salts (1.57 g) crystallised out as needles, mp 104–112 °C (decomp.) (Found: C, 64.9; H, 6.8; N, 5.0. $C_{15}H_{19}NO_4$ requires C, 65.0; H, 6.9; N, 5.1%). The crystals were collected and repeatedly recrystallised from ethyl acetate to yield salt B as fine needles (96 mg). The mother liquors from the early crystallisations were concentrated; repeated recrystallisation from ethyl acetate then afforded salt A as flakes (91 mg). The salts were identified by X-ray crystallography (see below). *Salt A*, the (S)-(−)-1-*phenylethylammonium salt of* (S)-(−)-3-*methylmuconolactone* had mp 104–108 °C (decomp.) (Found: C, 64.8; H, 7.0; N, 5.1. $C_{15}H_{19}NO_4$ requires C, 65.0; H, 6.9; N, 5.1%); $[\alpha]_D^{20}$ −5 (*c* 1.2 in MeOH); ν_{max}(KBr)/cm^{-1} 2400–3200 (br), 1740 and 1570. *Salt B*, the (S)-(−)-1-*phenylethylammonium salt of* (R)-(+)-3-*methylmuconolactone* had mp 119–122 °C (decomp.) (Found: C, 65.0; H, 7.1; N, 5.0. $C_{15}H_{19}NO_4$ requires C, 65.0; H, 6.9; N, 5.1%); $[\alpha]_D^{20}$ −2 (*c* 0.75 in MeOH); ν_{max}(KBr)/cm^{-1} 2400–3200 (br), 1740 and 1570.

Amberlite IR-120 ion-exchange resin (H^+ form, 525 mg, *ca.* 1 mmol equiv.) was added to a stirred solution of salt A (139 mg, 0.5 mmol) in methanol (2.5 cm^3) at room temp. After 15 min (longer reaction times gave significant amounts of the lactone methyl ester) the mixture was filtered and the resin washed with methanol. Evaporation of the combined filtrate and washings gave the lactone **4** (75 mg, 96%). (S)-(−)-3-*Methylmuconolactone* **4** had mp 81–83 °C (from ethyl acetate–hexane) (lit.,[3] 77–78 °C), $[\alpha]_D^{20}$ −37 (*c* 1.26 in H_2O) (lit.,[3] −35.8); δ_H[200 MHz; $(CD_3)_2CO$] 2.13 (br s, Me), 2.50 (dd, *J* 16.2 and 8.5, 5-H), 2.99 (dd, *J* 16.3 and 3.9, 5-H), 5.26 (m, 4-H) and 5.84 (q, *J* 1.5, 2-H). The ^{1}H NMR spectrum was indistinguishable from that of the racemic form. A sample of natural (*S*)-(−)-3-methylmuconolactone was obtained[1] by feeding 4-methylpyrocatechol to a mutant strain of *Aspergillus niger* known[7] to accumulate this lactone. Crystallisation from a dilute solution in ethyl acetate gave a small quantity (*ca.* 3% of the total) of (±)-3-methylmuconolactone, mp 127–130 °C. The mother liquors, after concentration, yielded the required (−)-lactone **4**. This was treated with (*S*)-(−)-1-phenylethylamine to form a salt, which was shown by X-ray crystallography to be identical with the foregoing synthetic salt A. Similarly, treatment of salt B with the ion-exchange resin afforded (R)-(+)-3-*methylmuconolactone*, mp 82–84 °C (from ethyl acetate–hexane), $[\alpha]_D^{20}$ +33 (*c* 1.0 in H_2O).

Conversion of 3-methylmuconolactone 4 and 4-methylmuconolactone 3 into the corresponding bromo dilactones 5 and 9

Bromine (160 mg, 1.0 mmol) in dichloromethane (3 cm^3) was added to a solution of (*S*)-(−)-3-methylmuconolactone **4** (156 mg, 1.0 mmol) in water (2 cm^3) containing sodium hydrogen carbonate (84 mg, 1.0 mmol), at room temp. The mixture was stirred for 4 h, during which time the colour of bromine was almost completely discharged. Residual bromine was decomposed by the addition of a small amount of aq. disodium thiosulfate. The dichloromethane and aq. layers were separated and the latter was extracted with dichloromethane (3 × 5 cm^3). The combined dichloromethane solutions were washed with water, dried ($MgSO_4$) and evaporated to yield crystals (217 mg, 92%) of essentially pure product **5**. (1R,5S,8S)-8-*Bromo*-1-*methyl*-2,6-*dioxabicyclo*[3.3.0]*octane*-3,7-*dione* **5** had mp 84.5–86 °C (from chloroform–hexane) (Found: C, 35.8; H, 2.8; Br, 34.2. $C_7H_7BrO_4$ requires C, 35.7; H, 3.0; Br, 34.0%); $[\alpha]_D^{20}$ −87 (*c* 1.0 in MeOH); ν_{max}(KBr)/cm^{-1} 1790; δ_H[200 MHz; $(CD_3)_2CO$] 1.77 (s, Me), 2.92 (ddd, *J* 18.7, 1.0 and 0.7, 4-$H_{pro\text{-}R}$), 3.36 (dd, *J* 18.7 and 4.9, 4-$H_{pro\text{-}S}$), 4.87 (d, *J* 1.0, 8-H) and 5.34 (dt, *J* 4.85 and 0.5, 5-H). Similarly, (±)-3-methylmuconolactone gave the *racemic bromo dilactone* (±)-**5** (92%), mp 88–90 °C (from chloroform–hexane) (Found: C, 35.8; H, 2.6; Br, 34.4%; M^+, 235.9506 and 233.9525. $C_7H_7BrO_4$ requires C, 35.7; H, 3.0; Br, 34.0%; *M*, 235.9507 and 233.9528); ν_{max}($CHCl_3$)/cm^{-1} 1800.

Bromolactonisation of (*S*)-(+)-4-methylmuconolactone **3**, obtained[1] from *Pseudomonas putida*, under the foregoing conditions gave (1S,4S,5R)-4-*bromo*-1-*methyl*-2,6-*dioxabicyclo*[3.3.0]*octane*-3,7-*dione* **9** (74%), mp 124–125 °C (from ethyl acetate–hexane) (Found: C, 35.3; H, 2.6; Br, 34.0. $C_7H_7BrO_4$ requires C, 35.7; H, 3.0; Br, 34.0%); $[\alpha]_D^{20}$ −70 (*c*, 1.4 in MeOH); δ_H[90 MHz; $(CD_3)_2CO$] 1.90 (s, Me), 3.08 and 3.09 (AB_q, *J* 19, 8-H_2), 4.84 (s, 4-H) and 5.27 (s, 5-H). Prepared similarly from the racemic lactone (±)-**3**, (±)-4-*bromo*-1-*methyl*-2,6-*dioxabicyclo*[3.3.0]*octane*-3,7-*dione* (±)-**9** (82%) had mp 143–145 °C (Found: M^+, 235.9515 and 233.9530. $C_7H_7BrO_4$ requires *M*, 235.9509 and 233.9528); ν_{max}(KBr)/cm^{-1} 1785.

Conversion of the bromo dilactones 5 and 9 into the dilactone 6

Tributyltin hydride (291 mg, 1.0 mmol) was injected under nitrogen into a stirred suspension of the bromo dilactone **5** (118 mg, 0.5 mmol) in benzene (1 cm^3) containing azoisobutyronitrile (7 mg). The mixture was warmed briefly to initiate reaction then set aside at room temp. until all the bromo dilactone had dissolved and crystals of the product **6** had formed. After 1 h, hexane was added to the mixture, and the crystals (77 mg, 98%) were collected and washed with hexane. (1S,5S)-1-*Methyl*-2,6-*dioxabicyclo*[3.3.0]*octane*-3,7-*dione* **6** had mp 107–108 °C (from diethyl ether) (lit.,[9] 108 °C) (Found: C, 53.9; H, 5.2. $C_7H_8O_4$ requires C, 53.8; H, 5.1%); $[\alpha]_D^{20}$ −134 (*c* 1.0 in H_2O) (lit.,[9] −131); ν_{max}(KBr)/cm^{-1} 1780. The ^1H NMR spectrum corresponded reasonably well with that reported earlier[9] and exactly with that of the following racemic dilactone. Similarly, the racemic bromo dilactone (±)-**5** gave (±)-1-*methyl*-2,6-*dioxabicyclo*[3.3.0]*octane*-3,7-*dione* (±)-**6**, mp 90–91 °C (from chloroform–hexane) (Found: C, 53.9; H, 5.1%; M^+, 156.0426. $C_7H_8O_4$ requires C, 53.8; H, 5.1%; *M*, 156.0422); ν_{max}(KBr)/cm^{-1} 1783; ν_{max}($CHCl_3$)/cm^{-1} 1798; δ_H[200 MHz; $(CD_3)_2CO$] 1.63 (s, Me), 2.77 (ddd, *J* 18.7, 0.9 and 0.5, 4-H), 2.91 (ddd, *J* 18.4, 0.9 and 0.4, 8-H), 3.00 (d, *J* 18.4, 8-H), 3.23 (dd, *J* 18.7 and 5.4, 4-H) and 5.09 (d with fine splitting, *J* 5.4, 5-H). Again, the isomeric bromo dilactone **9** was converted with tributyltin hydride into the (−)-dilactone **5**, which had physical properties identical with those of the foregoing compound formed from the bromo dilactone **5**.

Synthesis of (*S*)-(+)-4-methylmuconolactone 3 and (*S*)-(−)-3-methylmuconolactone 4 from the (−)-dilactone 6

An ice-cooled suspension of the (−)-dilactone **6** (78 mg, 0.5 mmol) in water (0.5 cm^3) was treated with aq. sodium hydroxide (2 mol dm^{-3}, 0.25 cm^3, 0.5 mmol). The mixture was shaken until a homogeneous solution had formed (*ca.* 5 min), which was allowed to warm up to room temp. An excess of Amberlite ion exchange resin (1R-120, H^+ form) was added and the acidified mixture was then filtered. The resin was washed with water and the combined aq. filtrate and washings were evaporated at room temp. to give a mixture of the lactones **3** and **4** (74 mg; ratio **3**:**4** = 6:4 as determined by ^1H NMR spectroscopy). The mixture was separated on Merck GF_{254} silica gel plates developed with diisopropyl ether–formic acid–water (200:7:3). The lactones **3** and **4** formed semi-transparent bands, R_F 0.40 and 0.21, respectively, when the plates had dried. The bands were barely detectable under UV light. The upper band was eluted with acetone to give (S)-(+)-4-*methylmuconolactone* **3** (37 mg, 47%), mp 46–48 °C (from diisopropyl ether–hexane) (lit.,[10] 49–51 °C); $[\alpha]_D^{20}$ +36 (*c* 1.32 in H_2O) (lit.,[10] +38); δ_H[90 MHz; $(CD_3)_2CO$] 1.54 (s, Me), 2.78 (d, *J* 15.7, 5-H), 2.91 (d, *J* 15.7, 5-H), 6.06 (d, *J* 5.7, 2-H) and 7.80 (d, *J* 5.7, 3-H). The ^1H NMR data agreed well with those reported earlier.[10] Elution of the lower band with acetone gave (S)-(−)-3-*methylmuconolactone* **4** (23 mg, 29%), mp 80–83 °C, $[\alpha]_D^{20}$ −36 (*c* 1.07 in H_2O). These data and the ^1H NMR spectrum corresponded closely with those reported above for the starting material of this synthetic cycle (Scheme 2). Similarly, the racemic dilactone (±)-**6** gave (±)-4-*methylmuconolactone* (±)-**3** as an oil (Found: M^+, 156.0415. $C_7H_8O_4$ requires *M*, 156.0423) having a ^1H NMR spectrum identical with that of the foregoing (+)-lactone **3**.

Synthesis of the (±)-dibromo dilactone (±)-8 and the (−)-dibromo dilactone 11

The racemic bromo dilactone (±)-**5** (235 mg, 1.00 mmol) was added to a stirred solution of sodium hydrogen carbonate (84 mg, 1.00 mmol) in water (2 cm^3) at room temp. Stirring was continued until a homogeneous solution of the sodium salt of the bromo lactone acid (±)-**7** was obtained. Bromine (160 mg, 1.00 mmol) in dichloromethane (3 cm^3) was added and the two-phase mixture was stirred for 5 h. Traces of residual bromine were removed with aq. sodium thiosulfate. The aqueous layer (pH 7.5) was extracted with dichloromethane (3 × 10 cm^3) and the combined dichloromethane solutions were dried and evaporated to give (±)-(1R*5S*)-8,8-*dibromo*-1-*methyl*-2,6-*dioxabicyclo*[3.3.0]*octane*-3,7-*dione* (±)-**8** (165 mg, 53%), mp 154–156 °C (Found: C, 27.0; H, 1.5; Br, 51.3%; M^+, 315.8597, 313.8606 and 311.8630. $C_7H_6Br_2O_4$ requires C, 26.8; H, 1.9; Br, 51.0%; *M*, 315.8594, 313.8614 and 311.8634); ν_{max}(KBr)/cm^{-1} 1795 and 1810; ν_{max}($CHCl_3$)/cm^{-1} 1805 and 1815; δ_H[90 MHz; $(CD_3)_2CO$] 1.88 (s, Me), 3.05 (d, *J* 18.5, 4-H), 3.43 (dd, *J* 18.5 and 4.5, 4-H) and 5.43 (d, *J* 4.5, 5-H). Acidification of the aqueous layer and extraction with diethyl ether gave (±)-2-*bromo*-3-*methylmuconolactone* (±)-**7** (102 mg, 43%), mp 78–79 °C (from chloroform) (Found: M^+, 235.9511 and 233.9531. $C_7H_7BrO_4$ requires *M*, 235.9507 and 233.9527); δ_H(90 MHz; $CDCl_3$) 2.21 (s, Me), 2.69 (dd, *J* 16 and 8, 5-H), 2.98 (dd, *J* 16 and 4, 5-H), 5.30 (dd, *J* 8 and 4, 4-H) and 9.5 (br s, OH, exch. with D_2O). This bromo lactone (±)-**7** was also obtained, in good yield, by treatment of the racemic bromo dilactone (±)-**5** with triethylamine in dichloromethane at room temp. Similarly, bromination of the (−)-dibromo dilactone **9** in aq. sodium hydrogen carbonate gave (1S,5R)-4,4-*dibromo*-1-*methyl*-2,6-*dioxabicyclo*[3.3.0]*octane*-3,7-*dione* **11** (47%), mp 152–154 °C (from chloroform) (Found: C, 26.7; H, 1.2; Br, 51.0%; $[M - CO_2]^+$ 271.8685, 269.8699 and 267.8722. $C_7H_6Br_2O_4$ requires C, 26.8; H, 1.9; Br, 51.0%; *M* − 44, 271.8697, 269.8716 and 267.8736); $[\alpha]_D^{20}$ −77 (*c* 1.0 in MeOH); ν_{max}(KBr)/cm^{-1} 1785 and 1800; δ_H(90 MHz; $CDCl_3$) 1.92 (s, Me), 2.80 (d, *J* 18, 8-H), 3.13 (d, *J* 18, 8-H) and 5.15 (s, 5-H). Acidification of the aqueous layer then extraction, as before, gave the bromo lactone acid **10** (57%) as an oil; δ_H(90 MHz; $CDCl_3$) 1.64 (s, Me), 2.80 (d, *J* 16, 5-H), 2.98 (d, *J* 16, 5-H), 7.75 (s, 3-H) and 8.45 (br s, OH); *m*/*z* 234 and 236 (M^+). This intermediate was not purified further.

Disodium 3-methyl-*cis*,*cis*-muconate 17

Powdered 3-methylmuconic anhydride[8] **16** (345 mg, 2.5 mmol) was added to stirred, ice-cooled, aq. sodium hydroxide (2 mol dm^{-3}, 2.5 cm^3). After 5 min the resulting homogeneous solution was evaporated to dryness at room temp. to give the *disodium salt* **17** as a white powder (450 mg, 2.25 mmol), mp 300–350 °C (decomp.); ν_{max}(KBr)/cm^{-1} 1570; δ_H(90 MHz; D_2O; standard Bu^tOH, δ 1.22) 6.71 (d, *J* 12.5, 4-H), 6.01 (d, *J* 12.5, 5-H), 5.83 (m, 2-H) and 1.94 (s with fine splitting, Me) [no signals for the *cis*, *trans* isomer (see below) were apparent].

Hydrolysis of 3-methylmuconic anhydride 16 to give 3-methyl-2-*cis*,4-*trans*-muconate

The anhydride **16** (45 mg, 0.33 mmol) was shaken with a solution of sodium hydrogen carbonate (56 mg, 0.67 mmol) in D_2O (0.5 cm^3) at room temp. until almost all the anhydride had dissolved. The mixture was filtered and the filtrate was examined by NMR spectroscopy. The major product was 3-methyl-2-*cis*,4-*trans*-muconate; δ_H(90 MHz; D_2O; standard Bu^tOH, δ 1.22) 7.73 (d, *J* 16.5, 4-H), 6.08 (d, *J* 16.5; 5-H), 6.01 (m, 2-H) and 1.91 (br s, Me). A weak doublet at δ 6.70 (*J* 12.5) indicated the presence of the *cis*,*cis*-muconate **17** (*ca.* 15% of the total product). A similar result was obtained with disodium carbonate (0.33 mmol) in place of sodium hydrogen carbonate.

X-Ray crystal data

Salt A (Fig. 1) of *S*-(−)-1-phenylethylamine and (*S*)-(−)-3-methylmuconolactone 4. $C_8H_{12}N^+ \cdot C_7H_7O_4^-$, $M = 277.3$, orthorhombic, space group $P2_12_12_1$, $a = 5.988(3)$, $b = 12.472(3)$, $c = 20.857(3)$ Å, $U = 1.557.6$ Å^3, $F(000) = 592$, $D_C = 1.18$ g cm^{-3}, $Z = 4$, μ(Mo-Kα) = 0.80 cm^{-1}. Final $R = 0.067$ for 611 independent reflections ($I \geqslant 3.0\sigma_I$).

Salt B (Fig. 2) of (*S*)-(−)-1-phenylethylamine and (*R*)-(+)-3-methylmuconolactone (enantiomer of 4). $C_8H_{12}N^+ \cdot C_7H_7O_4^-$, $M = 277.3$, orthorhombic, space group $P2_12_12_1$, $a = 6.192(3)$, $b = 12.599(3)$, $c = 19.376(3)$ Å, $U = 1511.6$ Å^3, $F(000) = 592$,

D_C = 1.22 g cm^{-3}, Z = 4, μ(Mo-Kα) = 0.82 cm^{-1}. Final R = 0.058 for 879 independent reflections ($I \geqslant 3.0\sigma_I$).

(1*R*,5*S*,8*S*)-8-Bromo-1-methyl-2,6-dioxabicyclo[3.3.0]octane-3,7-dione 5 (Fig. 3). $C_7H_7BrO_4$, M = 235.0, orthorhombic, space group $P2_12_12_1$, a = 7.044(2), b = 9.934(1), c = 12.209(2) Å, U = 854.3 Å^3, F(000) = 464, D_C = 1.83 g cm^{-3}, Z = 4, μ(Mo-Kα) = 47.3 cm^{-1}. Final R = 0.039 for 584 independent reflections ($I \geqslant 3.0\sigma_I$). The absolute configuration was determined unambiguously using both conventional anomalous scattering calculations on R (the value increased to 0.051 for the inverted structure) and the value of ETA,[20] which was +1.25 for the enantiomer **5**.

The data sets were collected on an Enraf-Nonius CAD-4 automatic diffractometer. The structures were solved by direct phasing techniques using MITHRIL.[21]

Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited[6] at the Cambridge Crystallographic Data Centre.

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