Resolution of (±)-3-Methylmuconolactone and the Absolute Configurations of the Naturally Occurring 3- and 4-Methylmuconolactones: *X*-Ray Crystal Structures of (*S*)-1-Phenylethylammonium Salts and a Bromodilactone

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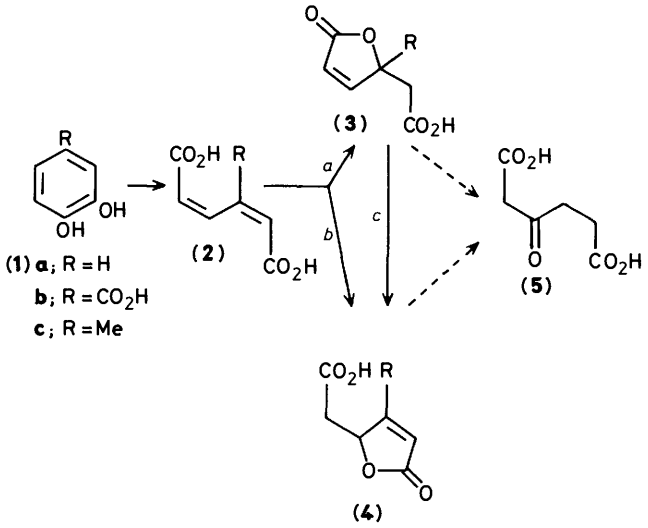
(%)-3-Methylmuconolactone (**4c**) has been resolved *via* the diastereoisomeric salts of (*S*)-(−)-1-phenylethylamine and the absolute configurations of fungal (4*S*)-(−)-3-methylmuconolactone (**6**) and bacterial (4*S*)-(+)-4-methylmuconolactone (**9**) have been determined by combined use of *X*-ray crystallographic analysis and chemical correlation.

The muconic acid pathways[1] (Scheme 1) constitute important routes for the microbial degradation of benzene derivatives present in soil or industrial wastes. In both bacteria and fungi, the pyrocatechol (**1a**) is converted enzymically *via* *cis,cis*-muconic acid (**2a**) into muconolactone (**3a**) [≡ (**4a**)] and thence

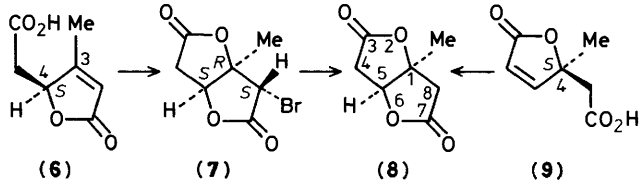


Scheme 1.

into 3-oxoadipic acid (**5**), a metabolic source of acetic and succinic acid. However, the pathways leading from protocatechuic acid (**1b**) diverge after 3-carboxy-*cis,cis*-muconic acid (**2b**); in bacteria (path *a*) 4-carboxymuconolactone (**3b**) serves as an intermediate for 3-oxoadipic acid (**5**), whereas in fungi (path *b*) the 3-carboxy isomer (**4b**) fulfils the same role.

Until recently, it was believed that degradation of 4-methylpyrocatechol (**1c**) to 4-methyl-3-oxoadipic acid could occur only in fungi,[2] *via* path *b* involving 3-methyl-*cis,cis*-muconic acid (**2c**) and 3-methylmuconolactone (**4c**). In bacteria, cyclisation of this muconic acid (**2c**) characteristically gives 4-methylmuconolactone (**3c**) as a 'dead-end' product.[3,4] The discovery[5] that certain nocardioform actinomycetes (bacteria) could metabolise 4-methylpyrocatechol to the typical *fungal* lactone (**4c**) led to the identification of a new type of enzymic transformation (path *c*). Thus, a laboratory-constructed strain of *Alcaligenes eutrophus*[6] and several naturally occurring nocardioform actinomycetes, including *Rhodococcus ruber*,[7] all produce an enzyme able to catalyse the transformation (**3c**) ⟶ (**4c**) and, thereby, overcome the bacterial 'block'.

To initiate a general investigation of the stereochemistry of the methylmuconate pathways (Scheme 1) we have resolved (±)-3-methylmuconolactone (**4c**) and determined the absolute configurations of 'fungal' (−)-3-methylmuconolactone (**6**) and 'bacterial' (+)-4-methylmuconolactone (**9**) (Scheme 2).



Scheme 2.

The racemic lactone (**4c**), prepared from 4-methyl-2-nitrophenol,[8] was treated in ethyl acetate with (*S*)-(−)-1-phenylethylamine and the resulting mixture of diastereoisomeric salts was separated by fractional crystallisation from the same solvent. *X*-Ray crystallographic analyses were carried out on both salts.† Salt A (Figure 1), m.p. 104—108 °C (decomp.), $[\alpha]_D$ −5° (*c* 1.2 in MeOH), was treated briefly in methanol with an ion-exchange resin (H^+ form) to give (4*S*)-(−)-3-methylmuconolactone (**6**). Similarly, salt B, m.p. 119—122 °C

† *Crystal data:* Salt A (Figure 1) of (*S*)-(−)-phenylethylamine and (4*S*)-(−)-3-methylmuconolactone (**6**), $C_8H_{12}N^+\cdot C_7H_7O_4^-$, 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-K_α) = 0.80 cm^{-1}. Final R = 0.067 for 611 independent reflections [$I \geqslant 3.0\sigma_I$].

Salt B of (*S*)-(−)-phenylethylamine and (4*R*)-(+)-3-methylmuconolactone [enantiomer of (**6**)], $C_8H_{12}N^+\cdot C_7H_7O_4^-$, M = 277.3, orthorhombic, space group $P2_12_12_1$, a = 6.192(3), b = 12.599(3), c = 19.376(3) Å, U = 1 511.6 Å^3, $F(000)$ = 592, D_c = 1.22 g cm^{-3}, Z = 4, μ(Mo-K_α) = 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 (**7**), $C_7H_7BrO_4$, 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-K_α) = 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,[16] which was +1.25 for the enantiomer (**7**).

The data sets were collected on an Enraf-Nonius CAD-4 automatic diffractometer. The structures were solved by direct phasing techniques using MITHRIL.[17]

Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. For details of this deposition scheme, see 'Instructions for Authors,' (1989), *J. Chem. Soc., Perkin Trans. 1*, 1989, Issue 1.

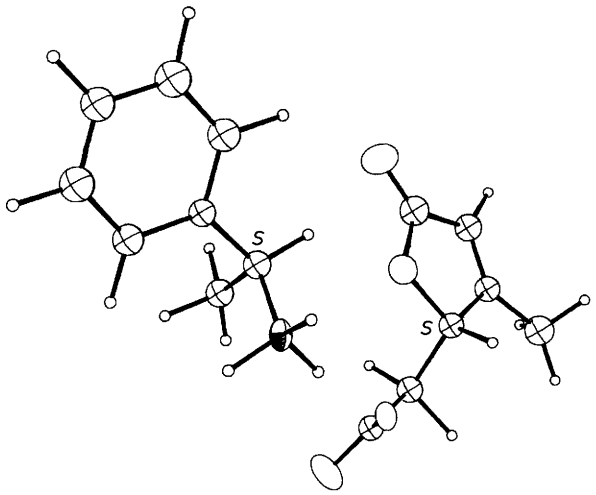


Figure 1. *X*-Ray structure of the (*S*)-1-phenylethylammonium salt (salt A) of (4*S*)-3-methylmuconolactone (**6**)

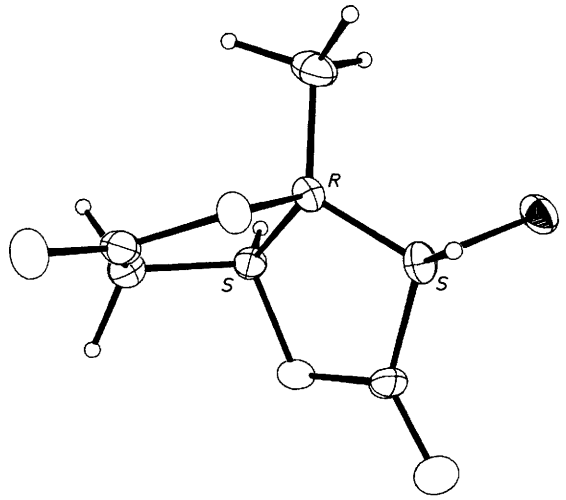
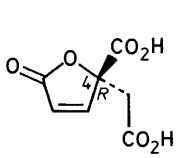


Figure 2. *X*-Ray structure of the bromo dilactone (**7**) derived from (4*S*)-3-methylmuconolactone (**6**)

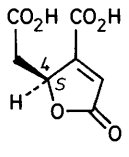
(decomp.), $[\alpha]_D$ −2° (*c* 0.75 in MeOH), gave (4*R*)-(+)-3-methylmuconolactone. The spectroscopic properties and optical rotation of the synthetic (−)-lactone (**6**) agree well with those reported[2,5–7] for the natural product. To confirm the identities of the natural and synthetic lactones, 4-methylpyrocatechol was fed to a mutant strain of *Aspergillus niger* known[9] to accumulate (−)-3-methylmuconolactone. The lactone was purified by recrystallisation and treated with (*S*)-1-phenylethylamine. The resulting salt was shown by *X*-ray analysis to be identical with the foregoing, synthetic salt A.

The absolute configuration of natural (+)-4-methylmuconolactone was determined by chemical correlation with the 3-methyl isomer (Scheme 2). Treatment of the (−)-lactone (**6**) in aqueous sodium hydrogen carbonate (1 mol equiv.) with bromine (1 mol equiv.) in dichloromethane at room temperature gave the (−)-bromodilactone (**7**) (92%), m.p. 84.5—86 °C (from $CHCl_3$–hexane), $[\alpha]_D$ −86.5° (*c* 1.0 in MeOH). *X*-Ray analysis* established the relative configuration, as shown (Figure 2), and confirmed the absolute configuration at C-5. The (−)-bromo dilactone (**7**) reacted with tributyltin hydride (1.2 mol equiv.), in benzene containing azoisobutyronitrile (0.1 mol equiv.), at room temperature under nitrogen to give the (−)-dilactone (**8**) (98%), a possible intermediate[10] in the enzymic transformation (**3**) ⟶ (**4**). The ^1H n.m.r. spectrum, m.p., and optical rotation of the synthetic (−)-dilactone (**8**) agreed well with those reported[3,4] for material formed by non-enzymic cyclisation of natural (+)-4-methylmuconolactone, which must now be assigned the 4*S* configuration (**9**).

Remarkably, the (+)-4-methylmuconolactone (**9**), first described as a metabolite of *Pseudomonas desmolyticum*,[3] has an absolute configuration opposite to that of the 4-carboxymunocolactone (**10**) produced by *Pseudomonas putida* and



(**10**)



(**11**)

Acinetobacter calcoaceticus.[11] In contrast, the (−)-3-methylmuconolactone (**6**) reported here and the (−)-3-carboxymuconolactone (**11**)[12] produced by fungi both have configurations (4*S*) identical with that of the unsubstituted (+)-muconolactone[11,13] produced by fungi and bacteria. Experiments in progress will relate the relative stereochemistries (*syn* or *anti* addition or elimination) of lactone closure and opening of the methylmuconate pathways with those reported for the carboxymuconate[11,12,14,15] and muconate[11,13,15] derivatives.

Acknowledgements

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References

1 R. Y. Stanier and L. N. Ornston, 'Advances in Microbial Physiology,' eds. A. H. Rose and D. W. Tempest, Academic Press, London, 1973, vol. 9, p. 89.
2 J. B. Powlowski and S. Dagley, *J. Bacteriol.*, 1985, **163**, 1126.
3 D. Catelani, A. Fiecchi, and E. Galli, *Biochem. J.*, 1971, **121**, 89.
4 H.-J. Knackmuss, M. Hellwig, H. Lackner, and W. Otting, *Eur. J. Appl. Microbiol.*, 1976, **2**, 267.
5 D. J. Miller, 'Actinomycetes,' *Zbl. Bakt. Suppl., 11*, eds. Shaal and Pulverer, Gustav Fischer Verlag, Stuttgart, 1981, p. 355.
6 D. H. Pieper, K.-H. Engesser, R. H. Don, K. N. Timmis, and H.-J. Knackmuss, *FEMS Microbiol. Lett.*, 1985, **29**, 63.
7 N. C. Bruce and R. B. Cain, *FEMS Microbiol. Lett.*, 1988, **50**, 233.
8 H. Pauly, R. Gilmour, and G. Will, *Annalen*, 1914, **403**, 119; J. A. Elvidge, R. P. Linstead, and P. Sims, *J. Chem. Soc.*, 1951, 3386.
9 E. F. Ahlquist and R. B. Cain, unpublished observations; E. F. Ahlquist, Ph.D. Thesis, University of Kent in Canterbury, 1977.
10 N. C. Bruce and R. B. Cain, submitted for publication in the *Biochem. J.*
11 R. V. J. Chari, C. P. Whitman, J. W. Kozarich, K.-L. Ngai, and L. N. Ornston, *J. Am. Chem. Soc.*, 1987, **109**, 5514.
12 G. W. Kirby, G. J. O'Loughlin, and D. J. Robins, *J. Chem. Soc., Chem. Commun.*, 1975, 402.
13 G. Avigad and S. Englard, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, 1969, **28**, 345.
14 R. A. Hill, G. W. Kirby, and D. J. Robins, *J. Chem. Soc., Chem. Commun.*, 1977, 459.
15 R. V. J. Chari, C. P. Whitman, and J. W. Kozarich, *J. Am. Chem. Soc.*, 1987, **109**, 5520.
16 D. Rodgers, *Acta Crystallogr., Sect. A*, 1981, **37**, 734.
17 C. J. Gilmore, *J. Appl. Crystallogr.*, 1984, **17**, 42.

* See footnote on p. 202.

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