Biosynthetic Studies on Pseudomonic Acid (Mupirocin), a Novel Antibiotic Metabolite of *Pseudomonas fluorescens*

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^{2}H and ^{18}O Isotope shifts observed in the ^{13}C n.m.r. spectra of pseudomonic acid (mupirocin) enriched from [1-^{13}C, $^{2}H_3$]- and [1-^{13}C, $^{18}O_2$]-acetates provide information on the mechanisms of formation of the tetrahydropyran and ester functions. The results of incorporation studies with ^{14}C- and $^{13}C_2$-labelled β-hydroxy-β-methylglutarates do not support previous proposals for its involvement in the biosynthesis of pseudomonic acid.

Pseudomonic acid A is a structurally unique antibiotic produced by *Pseudomonas fluorescens*[1] and which is now being used clinically under the generic name mupirocin for topical treatment of skin infections. It has a complex structure (**1**) consisting of a C_{17} unsaturated carboxylic acid entity containing epoxide, diol, and tetrahydropyran functions, esterified by a 9-hydroxynonanoic acid entity.[2] On the basis of preliminary biosynthetic studies[3] the pathway summarised in Scheme 1 was proposed. According to this, pseudomonic acid is essentially polyketide in origin and is elaborated *via* C_{12}, C_5, and C_9 units. Among the unusual features of the pathway are

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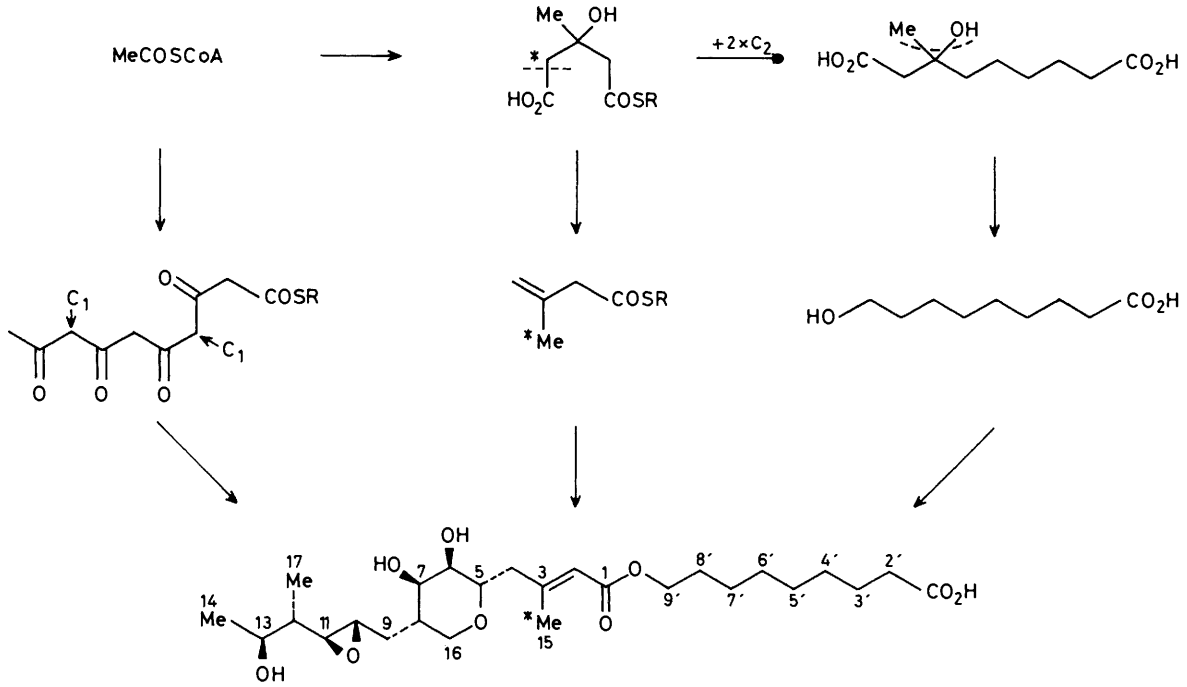
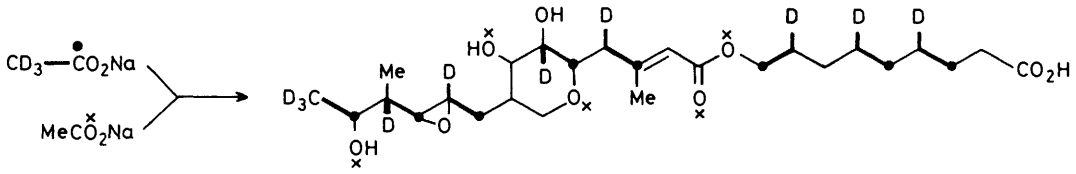
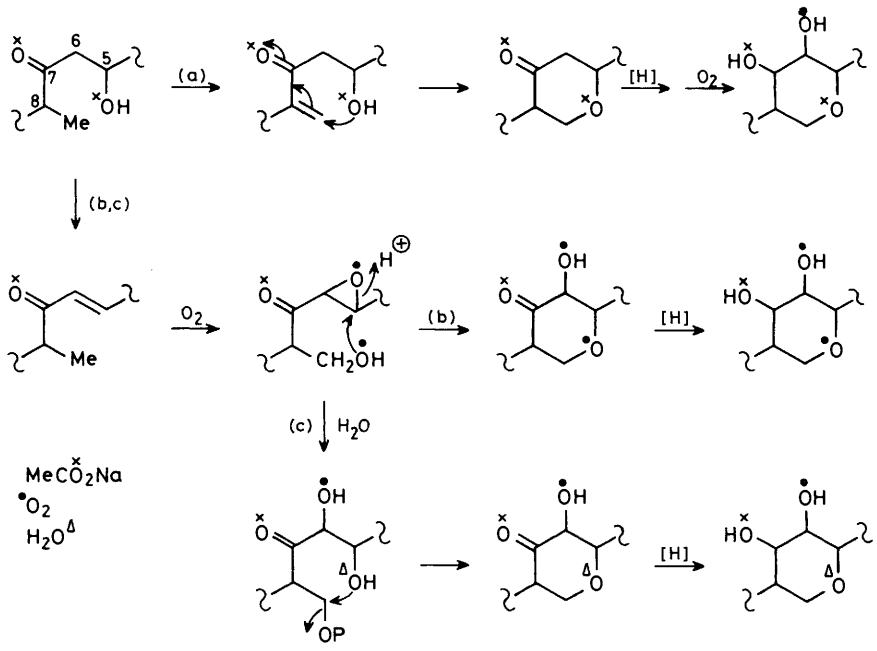


(1)

Scheme 1.



Scheme 2.



Scheme 3.

Table. ^{2}H and ^{18}O Isotope-induced shifts observed in the 90.56 MHz ^{13}C n.m.r. spectrum of pseudomonic acid A (1)

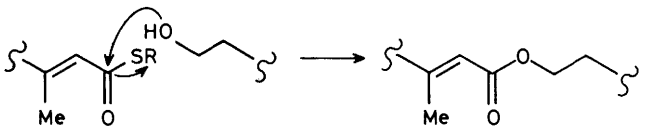
Carbon	δ_C (p.p.m.)	$\Delta\delta \times 100$ (p.p.m.)[a]	$\Delta\delta \times 100$ (p.p.m.)[b]
1	166.7	3.7	
3	156.5		5.1
5	74.7	1.7	5.5
13	71.3	2.3	4.9, 9.1, 13.7
7	70.3	2.3	
9′	63.7	2.9	2.9
11	61.2		7.2
9	31.5		13.7
5′	29.0		10.1
3′	24.8		9.8

[a] [1-^{13}C, $^{18}O_2$]Acetate-enriched. [b] [1-^{13}C, $^{2}H_3$]Acetate-enriched.

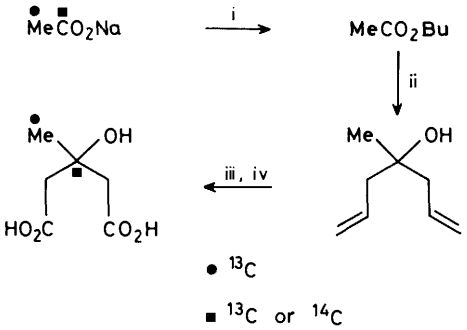
the proposed involvement of β-hydroxy-β-methylglutarate in the formation of both the C_5 and C_9 moieties and the origin of the C-15 methyl from the methyl carbon of a cleaved acetate unit. This feature has been observed more recently in the biosynthesis of virginiamycin,[4] myxovirescin,[5] and myxopyronin[6] but no satisfactory biosynthetic explanation has been established as yet.

A number of incorporation experiments with precursors labelled with stable isotopes which provide more information on the biosynthetic pathway to pseudomonic acid are now reported.

The ^{13}C n.m.r. spectrum of pseudomonic acid has been rigorously assigned.[7] Incorporations of [1-^{13}C, $^{18}O_2$]- and [1-^{13}C, $^{2}H_3$]-acetates and analysis of the enriched metabolites by highfield ^{13}C n.m.r. (Table) revealed the origins of the hydrogen, and more importantly, the oxygen atoms indicated in Scheme 2. The oxygens attached to C-1, C-5, C-7, C-13, and C-9′ are all derived from acetate. Of a number of biogenetically reasonable mechanisms which can be proposed for the formation of the tetrahydropyran moiety (Scheme 3) only path (a) is consistent with the oxygen-labelling results. These results also confirm that the ester linkage in pseudomonic acid is formed *via* separate C_{17} and C_9 moieties (Scheme 4) and not *via e.g.* a Baeyer-Villiger-type cleavage of a single long-chain ketone intermediate.



Scheme 4.



Scheme 5. *Reagents:* i, $(BuO)_3PO$, reflux; ii, CH_2=$CHCH_2Br$, Mg, tetrahydrofuran, Et_2O; iii, O_3, HOAc, CH_2Cl_2; iv, H_2O_2, HOAc

The proposed involvement of β-hydroxy-β-methylglutaric acid has been tested by the synthesis[8] of [3-^{14}C]- and [3,6-$^{13}C_2$]-β-hydroxy-β-methylglutarates (Scheme 5). The ^{14}C-labelled precursor was incorporated with high efficiency into pseudomonic acid (dilution value[9] 7.2). However, analysis of the ^{13}C n.m.r. spectrum of the metabolite derived from the $^{13}C_2$-labelled precursor, showed ^{13}C–^{13}C coupling satellites throughout the molecule, consistent with incorporation of label from the glutarate entirely *via* prior breakdown to acetyl coenzyme A and subsequent re-incorporation. There was no evidence whatsoever for the preferential enrichments of C-3 and C-4 or C-7′ required by the pathway shown in Scheme 1.

Acknowledgements

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