A Study of the Biosynthesis of the Polyketide Polivione in *Penicillium frequentans*, using ^{13}C-, ^{2}H-, and ^{18}O-Labelled Precursors

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Polivione, a polyketide metabolite of *Penicillium frequentans* is shown to incorporate ^{13}C-, ^{2}H-, and ^{18}O-labelled acetates, and ^{18}O-labelled oxygen gas, in a manner consistent with a biosynthesis *via* a naphthalene intermediate having the same carbon skeleton as fusarubin.

Polivione (**1**) (major tautomer) is the main metabolite of *Penicillium frequentans*.[1] It is readily converted chemically into citromycetin (**2**) which has hitherto been considered to be the major metabolite of this organism. There is, therefore, a strong possibility that citromycetin is not a true product, but that it is a chemical artefact produced from (**1**) in the culture medium and in the isolation procedure. Fulvic acid (**3**) is a third member of this family of metabolites, which is characterised by the carbon skeleton (**4**). Equivalent carbons of all three compounds are at the same oxidation level, and it is likely that they are built up using a common biosynthetic strategy.

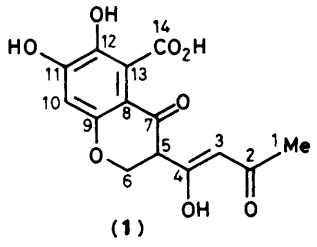
Both citromycetin (**2**)[2] and fulvic acid (**3**)[3] are known to incorporate acetate in a manner consistent with a polyketide biosynthesis, but the branched structure (**4**) is not consistent with a straightforward derivation from a single polyketide chain. Two fundamentally different biosynthetic strategies have been proposed.[4] According to the first, the skeleton would be produced by combination of two separately formed chains, as exemplified by pathways (a) and (b) of Scheme 1. The alternative strategy involves cleavage of a carbon–carbon bond in a polyketide skeleton produced in the conventional way by cyclisation of a single unbranched polyketide chain. Two reasonable candidates, both with skeletons corresponding to naphthalene derivatives, are shown in pathways (c) and (d). It has been established that the fungal metabolite rubrofusarin (**7**) which has the carbon skeleton (**5**), and is suitably functionalised, does incorporate acetate in a manner consistent with this hypothesis.[5] We now describe biosynthetic studies with polivione (**1**) which provide a definitive answer to this long-standing biosynthetic puzzle.

In the course of the structure determination,[1] the carbon

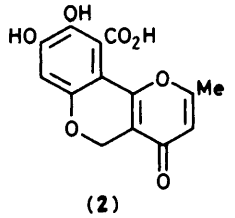
Table 1. ^{18}O-Shifts in the ^{13}C n.m.r. spectra[a] of ^{18}O-enriched samples of polivione (**1**).

Source of ^{18}O	Carbons showing ^{18}O-shifts (Hz)						
	C-2	C-4	C-7	C-9	C-11	C-12	C-14
[1-^{13}C,1-$^{18}O_2$]Acetate[b]			3.4	1.6	1.2		
$^{18}O_2$[b]		5.5				1.2	2.3, 4.6
$H_2{}^{18}O$[c]	2.6	5.6	3.4				

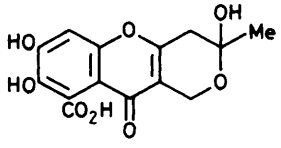
[a] At 100 MHz in CD_2Cl_2. [b] Biosynthetic experiment. [c] Exchange *in vitro*.



(1)



(2)



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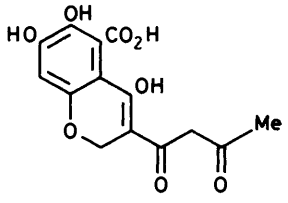

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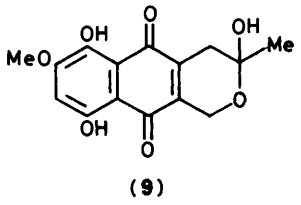
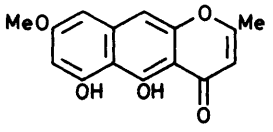
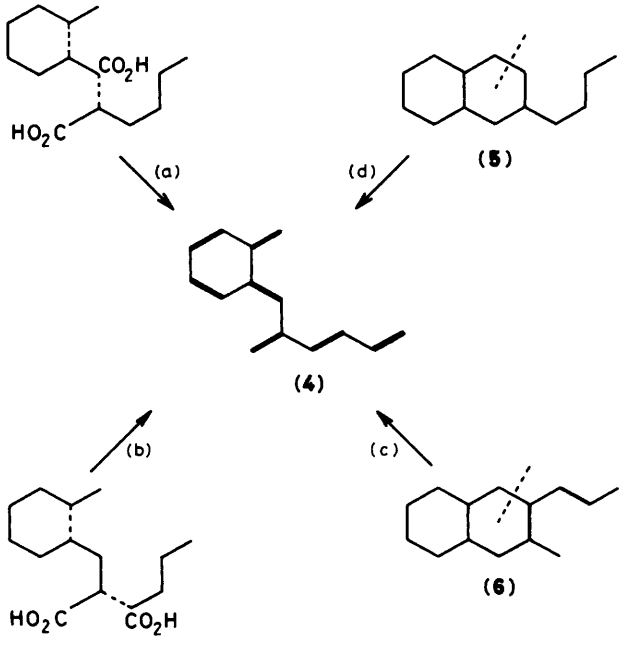


(9)



(7)



Scheme 1

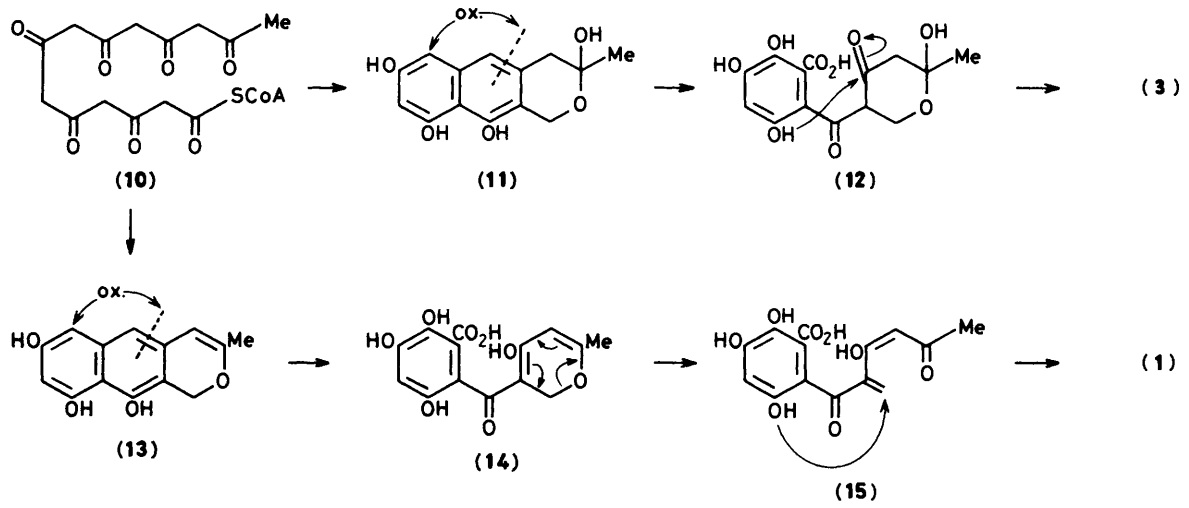
skeleton of polivione (**1**) was shown to incorporate [1,2-$^{13}C_2$]-acetate to produce the typical polyketide labelling pattern shown in (**4**) (where intact C_2-units are indicated by heavy lines). This pattern is consistent with the proposed biosynthetic relationship, since both citromycetin (**2**) and fulvic acid (**3**) have been shown earlier to incorporate acetate in equivalent ways.

The origins of the hydrogens in the C–H groups of polivione were investigated next, by administration of [2-$^2H_3$]acetate (150 mg) to 3 day old cultures of *P. frequentans*, before isolation of the metabolite as reported previously.[1] In the ^2H n.m.r. spectrum of the derived polivione there was a strong signal from the methyl group at C-1, the presumed starter unit of the polyketide chain, and a weaker signal for H-10, but, disappointingly, no deuterium could be detected at the key position C-3 in either of the two principal tautomers of the metabolite. Presumably any label incorporated at that site is subsequently lost to the medium through interconversion of (**1**) with its tautomer (**8**).

The decisive experiments were those aimed at establishing the origins of the oxygen atoms of polivione. Firstly, [1-^{13}C, 1-$^{18}O_2$]acetate (100 mg) was administered to the organism; the ^{13}C n.m.r. spectrum of the resulting metabolite (**1**) showed ^{18}O-shifted peaks at C-7, C-9, and C-11 (see Table 1). Secondly, a growing culture of the organism was exposed to $^{18}O_2$ gas (20% enriched) for 24 h, at the time polivione production reached its maximum. The resulting metabolite showed ^{18}O-shifted peaks in its ^{13}C n.m.r. spectrum for C-4, C-12, and for C-14 (two shifted peaks). The crucial finding was the presence of an isotopically shifted peak for C-4. Since this was a relatively weak peak (presumably due to loss of labelled oxygen by exchange with water), its assignment was checked by exposing unlabelled metabolite to ^{18}O-enriched H_2O (50% enriched) for 24 h. The resulting polivione showed isotopically shifted peaks for C-4 (at the expected position) and C-2, the shifted peaks being in each case approximately equal in intensity to the unshifted peak.

The finding that the oxygen at C-4 of polivione is derived from molecular oxygen points to an oxygenation reaction at that site at some stage of the biosynthesis. This would be expected for pathway (c), in which an intermediate with the carbon skeleton (**6**), possibly related to fusarubin (**9**), undergoes oxidative cleavage of a carbon–carbon bond to that carbon; in the other three pathways the oxygen at C-4 would be expected to derive from acetate (or from water by exchange).

A possible biosynthetic scheme to account for these results is presented in Scheme 2. A heptaketide chain (**10**) is cyclised and reduced at the chain terminus to produce (**11**) or (**13**) [quite likely *via* (**11**)], both of which are related to fusarubin



Scheme 2

(9). Oxidative cleavage at the sites indicated would give intermediates (**12**) or (**14**) respectively. In the former case cyclisation of phenolic hydroxy as indicated would lead ultimately to fulvic acid (**3**). In the case of (**14**) this potential cyclisation might be pre-empted by a facile electrocyclic rearrangement leading to (**15**). The favoured cyclisation would now be the one leading to polivione (**1**). The absence of a detectable isotopically shifted peak for C-2 in the ^{13}C n.m.r. spectrum of the polivione produced by incorporation of [1-^{13}C,1-$^{18}O_2$]acetate is in accord with this proposal, even though the attached oxygen is derived from acetate: the relevant oxygen is transferred to C-2 from a different acetate unit, and so, at the low level of isotopic enrichment in the metabolite, there is only a low probability that ^{18}O- and ^{13}C-labels will coincide at that site.

Recently a similar scheme was proposed by Hutchinson[3] to account for the biosynthesis of fulvic acid (**3**). However, none of the evidence previously obtained discriminates between pathways (c) and (d) for this metabolite; the $^{18}O_2$-incorporation experiment which provided decisive evidence in our studies of (**1**) would probably not work with (**3**) because the key oxygen at C-4 would almost certainly be lost in the cyclisation and dehydration of (**11**) to form the pyrone ring of (**3**). Nevertheless, the close structural relationship which exists between (**1**) and (**3**) strongly suggests a common biosynthesis. The reactions proposed in Scheme 2 therefore represent an attractive strategy for future work on the biomimetic synthesis of both (**1**) and (**3**).

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References

1 A. K. Demetriadou, E. D. Laue, F. J. Leeper, and J. Staunton, *J. Chem. Soc., Chem. Commun.*, 1985, preceding communication.
2 A. J. Birch, P. Fitton, E. Pride, A. J. Ryan, H. Smith, and W. B. Whalley, *J. Chem. Soc.*, 1958, 4576; S. Gatenbek and K. Mosbach, *Biochem. Biophys. Res. Commun.*, 1963, **11**, 166; A. J. Birch, S. F. Husain, and R. W. Rickards, *J. Chem. Soc.*, 1964, 3494; G. E. Evans and J. Staunton, *J. Chem. Soc., Chem. Commun.*, 1976, 760.
3 I. Kurobane, C. R. Hutchinson, and L. C. Vining, *Tetrahedron Lett.*, 1981, **22**, 493.
4 W. B. Whalley, in 'Recent Developments in the Chemistry of Natural Phenolic Compounds,' ed. W. D. Ollis, Pergamon Press, Oxford, 1961, p. 49; C. E. Stickings and A. Mahmoudian, *Chem. Ind.*, 1962, 1718; T. Money, *Nature*, 1963, **199**, 592; W. B. Turner, 'Fungal Metabolites,' Academic Press, London, 1971, 154.
5 F. J. Leeper and J. Staunton, *J. Chem. Soc., Chem. Commun.*, 1982, 911; F. J. Leeper and J. Staunton, *J. Chem. Soc., Perkin Trans. 1*, 1984, 2919.