The Biosynthesis of the Polyketide Metabolite Palitantin from Deuterium and Oxygen Labelled Acetates in *Penicillium brefeldianum*

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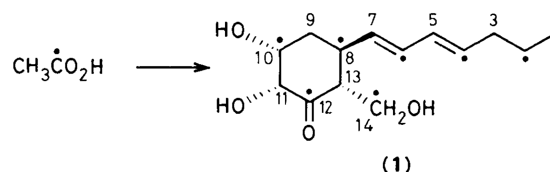
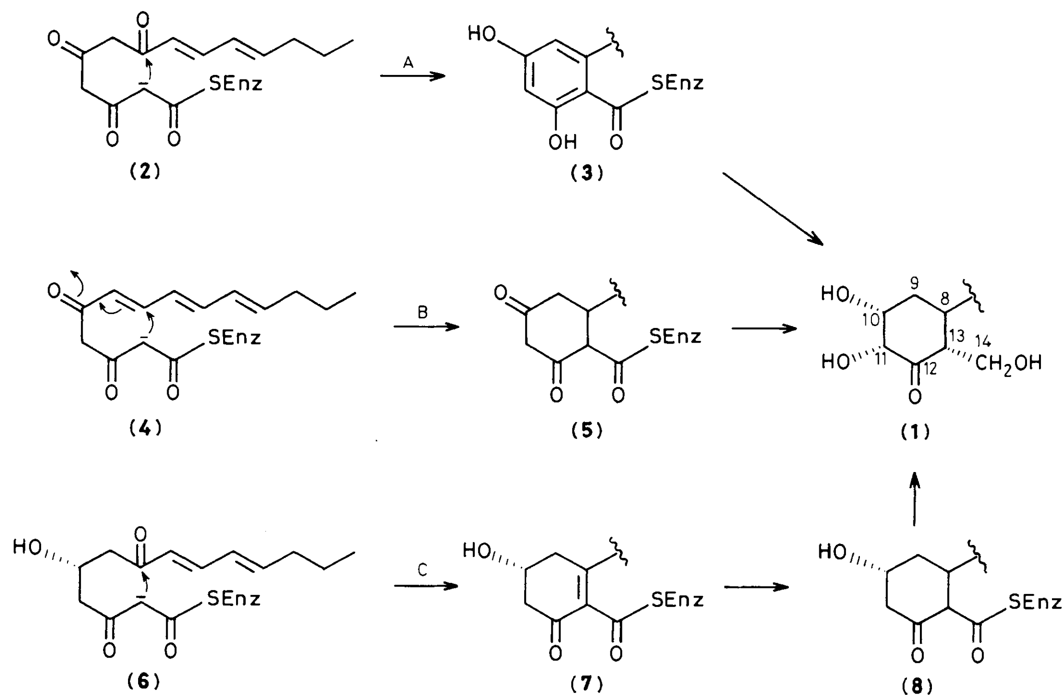
Isotopic shifts in the ^{13}C n.m.r. spectrum of the polyketide metabolite palitantin labelled by incorporation of ^2H and ^{18}O labelled acetates establish that no aromatic intermediates are involved in the generation of the six-membered carbocyclic ring.

Palitantin (**1**), a metabolite of *Penicillium brefeldianum* and other species, was first isolated and characterized in 1936 by Birkinshaw and Raistrick.[1] Further attempts[2] to elucidate the structure were unsuccessful, and it was not until 1959 that the problem was finally solved by Bowden, Lythgoe, and Marsden[3] who proposed the structure (**1**). Early biosynthetic work by Chaplen and Thomas[4] showed that radioactivity was incorporated from [1-^{14}C]acetate in a manner consistent with polyketide biosynthesis (Scheme 1).

Our interest in the biosynthesis of palitantin was aroused by the presence of an isolated non-aromatic carbocyclic ring. This unusual feature for a polyketide metabolite is also found in ICI 139603,[5] and in the avermectins.[6] Three possible pathways for the production of the ring in palitantin are shown in Scheme 2. According to pathway A, the carbocyclic ring is formed by a standard cyclisation of a linear polyketide intermediate (**2**) (aldol condensation followed by dehydration) to form an aromatic intermediate such as (**3**). The aromatic ring would then be reduced in subsequent steps. In the cyclisation step of pathway B a carbanion is added to an enone to produce the carbocyclic ring of (**5**) which is not prone to aromatise. Pathway C involves an aldol condensation



Scheme 1



Scheme 2

followed by dehydration to produce a non-aromatic intermediate (**7**). This could aromatise but instead the enone residue is reduced to give (**8**), in which aromatisation is prevented. All three uncyclised intermediates (**2**), (**4**), and (**6**) have an array of functional groups consistent with their being generated on a polyketide synthetase by standard biosynthetic reactions. The stereochemistry at C-10 of (**6**) is consistent with precedents set by the closely related fatty acid synthetases.

We have investigated the cyclisation mechanism by determining the origins of various key residues in the metabolite. First, the derivation of the carbon skeleton was confirmed by administration of [1,2-$^{13}C_2$]acetate to a six-day-old culture of *P. brefeldianum*. In the ^{13}C n.m.r. spectrum of the derived material each carbon gave rise to a ^{13}C–^{13}C doublet in addition to the natural abundance singlet; the doublets could be paired unambiguously by coupling constant (Table 1), thus confirming the incorporation of seven intact acetate residues. The assignments shown were confirmed by ^{1}H–^{13}C two-dimensional correlation experiments.[7]

In a preliminary experiment to determine the origin of the hydrogen atoms in palitantin, CD_3CO_2Na was administered to the organism. The ^{2}H n.m.r. spectrum of the derived metabolite showed enriched signals for H-1, H-3, H-5, H-7, H-9, and H-11; of the methyl derived sites, therefore, only H-13 failed to give evidence for deuterium incorporation. Sodium [2-$^{2}H_3$, 2-^{13}C]acetate was then used as a precursor so that the number of deuterium atoms retained at individual sites could be determined by observation of α-shifted peaks in the ^{13}C n.m.r. spectrum.[8] The significant results (Table 1) are (a) the retention of three deuterium atoms at C-1, which is thereby confirmed as the starter methyl group; (b) the retention of only one deuterium atom at C-3, which is consistent with the intermediacy of a compound with a double bond at that site; and (c) the retention of two deuterium atoms at C-9, which rules out two of the pathways (A and B) of Scheme 2.

Table 1. ^{13}C n.m.r. data[a] for palitantin (**1**).

Carbon	$\delta(^{13}C)$	$J(^{13}C$-$^{13}C)^b$ /Hz	Isotopic[c] shift/p.p.m.	^{18}O Shift[d] /Hz
1	13.9	34.9	0.28, 0.58, 0.86	
2	23.5	34.9		
3	35.7	43.3	0.4	
4	135.0	43.3		
5	131.3	42.0		
6	133.2	42.0		
7	133.8	45.1	0.35	
8	40.4	45.1		
9	37.9	36.0	0.37, 0.74	
10	73.8	36.3		2.0
11	78.8	37.8	0.38	
12	210.9	38.6		
13	56.3	40.4		
14	59.4	40.1		1.45

[a] At 100 MHz in CD_3OD–CD_3COCD_3. [b] After incorporation of [1,2-$^{13}C_2$]acetate. [c] After incorporation of [2-$^{2}H_3$, 2-^{13}C]acetate. [d] After incorporation of [1-$^{18}O_2$, 1-^{13}C]acetate.

The origins of the oxygen atoms of palitantin were investigated next using [1-$^{18}O_2$, 1-^{13}C]acetate as a precursor; the ^{13}C n.m.r. spectrum of the resultant labelled metabolite showed ^{18}O shifted peaks at C-10 and C-14 (see Table 1). We were initially surprised to find no isotopically shifted peak for C-12, since the attached oxygen is almost certainly derived from acetate, but the following control experiment shows that an oxygen label at that site could have been lost by exchange in the course of the biosynthetic experiment. Unlabelled palitantin was exposed to ^{18}O labelled H_2O (50% enriched) containing a trace of toluene-*p*-sulphonic acid. Mass spectrometric analysis of the crude recovered material showed in addition to the normal M^+ peak an $M^+ + 2$ peak of equal

intensity, consistent with exchange to equilibrium at one site, presumably C-12. Following purification, this material failed to show an isotopically shifted peak in its ^{13}C n.m.r. spectrum. Mass spectrometric analysis of the sample recovered from the n.m.r. experiment showed that the ^{18}O label had been lost.

The cyclisation mechanism shown for pathway C is consistent with all these results. It bears a striking resemblance to that proposed for the generation of the carbocyclic ring of the aromatic polyketide metabolite 6-methylsalicylic acid.[9] All the corresponding carboxy-derived carbon atoms are at the same oxidation level on both pathways; the key difference is that the hydroxy group at the site corresponding to C-10 of (**6**) is dehydrated prior to cyclisation in the proposed 6-methylsalicylic acid biosynthesis, with the consequence that the six-membered ring of the intermediate corresponding to (**7**) can aromatise simply by enolisation of a ketone group. The carbocyclic ring in (**7**) is much less prone to aromatise, and with the reduction of the enone residue the propensity to aromatise is removed. Subsequent steps on the proposed pathway are unexceptional, involving the two-stage reduction of the thioester group to an alcohol, and the hydroxylation of the C-11 methylene, presumably at the expense of molecular oxygen.

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