Biosynthesis of Oncorhyncolide, a Metabolite of the Seawater Bacterial Isolate MK157

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Stable isotope incorporation experiments show that all of the carbon atoms, including the branching methyls at C-15 and C-16, in the marine bacterial metabolite oncorhyncolide **1** are derived from acetate.

Marine bacteria are potentially a rich source of novel bioactive secondary metabolites that could act as leads for the development of new therapeutic agents.[1] However, it is only recently that a significant number of interesting new natural products have been isolated from cultures of marine bacteria.[2] We have initiated a programme designed to discover antimicrobial and cytotoxic metabolites produced by marine bacteria. As part of this programme, it was found that liquid shake cultures of the bacterial isolate MK157, obtained from a surface seawater sample taken near a chinook salmon farming operation, produced the known antibiotic aminopyrrolonitrin and the biologically inactive but chemically novel metabolite oncorhyncolide **1**.[3] Although oncorhyncolide **1** has a relatively simple structure, it was not possible to predict the exact nature of its biogenetic origin with any degree of certainty. One of the alternatives represented a rarely encountered variation on the well known polyketide biosynthetic pathway. Therefore, a series of stable isotope incorporation studies were undertaken to establish the origin of the carbon atoms in oncorhyncolide and the results are reported in this communication.

Three alternative biogenetic origins for oncorhyncolide (labelled **a**, **b** and **c**) that were viewed as reasonable possibilities at the outset of the study are represented schematically in Fig. 1.[4] The most straightforward possibility involved a linear 'heptaketide' initiated with a starter acetate unit at C-1/C-2 and it incorporated branching methyls derived from either propionate units or SAM (*S*-adenosylmethionine) alkylations as depicted in **a**. Although alternative **a** readily accounts for the formation of the carbon skeleton of **1**, a substantial number of oxidation state adjustments (oxygena-

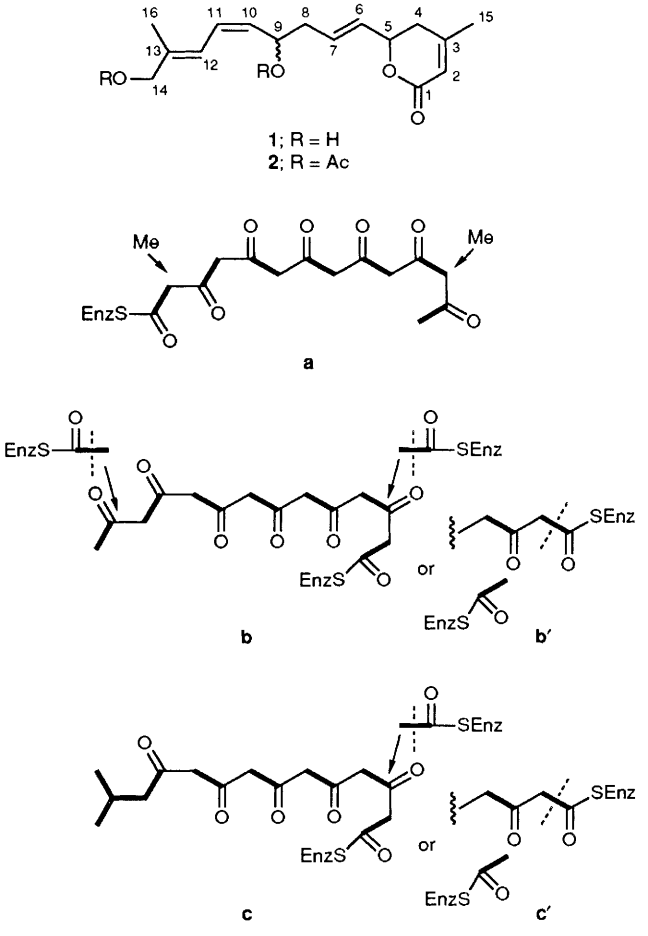

Fig. 1

tion of C-1, C-5 and C-9 and deoxygenation of C-4 and C-8) are required to reach the natural product. Alternative **b/b'** also incorporates a linear 'heptaketide' but with a starter acetate unit at either C-14/C-13 or C-16/C-13 and with methyl branches arising from C-2 of acetate. The pathway **b** proposes formation of both the C-15 and C-16 methyl groups *via* an aldol-like condensation between an acetyl–SEnz group and carbonyls at C-3 and C-13 followed by simultaneous decarboxylation and loss of hydroxy. A similar mechanism has been proposed previously to account for the formation of a methyl branch in the biosynthesis of virginiamycin.[5] A second pathway **b'** for formation of the C-15 methyl involves a standard decarboxylation of the linear 'heptaketide' and the incorporation of a separate intact acetate unit into C-1/C-2. One attractive feature of alternative **b/b'** was that only one carbon (C-14) requires an oxidation state adjustment apart from the standard PKS (polyketide synthetase) reduction–dehydration transformations. The final alternative **c/c'** envisages a five carbon starter unit derived from either mevalonate, valine or leucine and then proceeds as in **b/b'**.

In order to test the viability of the alternatives **a**, **b/b'** and **c/c'**, liquid shake cultures of MK157 (12 × 500 ml flasks) were fed [1-^{13}C: 1 g], [2-^{13}C: 0.5 g], [1,2-^{13}C: 0.25 g] and [2-^{13}C/^{2}H$_3$: 0.5 g] labelled acetate in separate experiments. The labelled acetates were added in two pulses 30 and 50 h after inoculation and the cultures were harvested after 4 days. Oncorhyncolide **1** was isolated from the cultures and converted into its diacetate **2** as previously described.[3] The extent of incorporation of the labelled substrates was determined by comparison of the ^{13}C NMR spectrum of the diacetate **2** isolated from each experiment with the spectrum of a natural abundance sample. Analysis of the results of the feeding studies (Table 1) revealed that seven intact acetate units had been incorporated

Table 1 Isotope incorporation data for oncorhyncolide diacetate **2**

Carbon	δ ^{13}C[a]	% Enrichment [1-^{13}C]	% Enrichment [2-^{13}C]	$^{1}J_{C,C}$/Hz	No. of ^{2}Hs incorporated [2-^{13}C/^{2}H$_3$]
1	163.5	7.8	0.2	68.3	—
2	117.3	0.6	2.9	68.3	1
3	154.9	9.9	0.1	37.7	—
4	34.4	0.0	3.5	36.9	2
5	76.3	7.9	0.0	49.8	—
6	131.5	0.4	4.0	50.6	1
7	128.3	9.6	0.4	43.1[b]	—
8	37.9	0.0	4.6	43.1[b]	2
9	69.0	11.4	0.3	50.2	—
10	128.9	0.4	4.5	49.8	1
11	127.3	10.0	0.3	56.2	—
12	121.7	0.0	3.6	56.2	1
13	136.0	10.3	0.3	47.4	—
14	68.8	0.6	4.5	47.4	2
15	22.1	0.3	2.6	s	2
16	14.0	0.4	4.1	s	2
Ac	20.4	0.4	0.2		
Ac	20.7	0.3	0.0		
Ac	169.8	0.7	0.0		
Ac	169.5	−0.3	0.1		

[a] Recorded in [^{2}H$_6$]benzene at 125 MHz.[b] Recorded in [^{2}H$_6$]acetone

into oncorhyncolide **1** and furthermore that both of the C-15 and C-16 branching methyl carbons were enriched when [2-^{13}C] acetate was fed. The results of the [2-^{13}C/^{2}H$_3$] acetate feeding experiment (Table 1) showed that the maximum possible number of ^{2}H atoms were incorporated at each site in the linear heptaketide chain derived from C-2 of acetate and it also showed that two ^{2}H atoms were incorporated at both the C-15 and C-16 methyls.

The observed labelling patterns found in the acetate feeding studies ruled out the biogenetic alternatives **a** and **c/c'** with valine or leucine starter units (Fig. 1) and they were consistent with alternative **b/b'** or alternative **c/c'** with a mevalonate derived starter unit.[6] In an attempt to distinguish between alternatives **b/b'** and **c/c'**, cultures of MK157 were fed [2-^{13}C]-mevalanolactone (0.1 g) following the same protocol used for the acetate feeding studies. There was no evidence for incorporation of mevalanolactone into oncorhyncolide in this experiment. The negative incorporation evidence did not by itself prove that mevalonic acid is not on the biosynthetic pathway to oncorhyncolide. However, if carbons 11, 12, 13, 14 and 16 are derived from acetate *via* mevalonate then it might be reasonable to expect lower levels of incorporation at these sites. The results listed in Table 1 show that carbons 11, 12, 13, 14 and 16 are labelled with acetate to virtually the same extent as the rest of oncorhyncolide. Taken together, the negative incorporation and almost uniform labelling results suggested that mevalonate is probably not an intermediate in oncorhyncolide biosynthesis.

In conclusion, stable isotope incorporation experiments have shown that all of the carbon atoms in oncorhyncolide are derived from acetate. Analysis of the labelling patterns revealed the incorporation of seven intact acetate units into the linear carbon chain extending from C-1 to C-14 and the incorporation of the C-2 carbon of acetate into the two branching methyls at C-15 and C-16. These results are consistent with the biogenetic alternative **b/b'** shown in Fig. 1. The observation of methyl branches derived from C-2 of acetate and attached to carbons in a linear chain that have been derived from C-1 of acetate as indicated in alternative **b** is rarely encountered in polyketide biosynthesis. This type of one carbon branching has only been previously reported in the biosyntheses of virginiamycin[5] and myxovirescin.[7] The authors of the virginiamycin work pointed out a potential mechanistic analogy with 'the formation of the methyl group

of mevalonic acid by a pathway involving an aldol condensation between acetoacetyl-CoA and acetyl-CoA',[5,7] Our results do not provide any insight into the mechanistic origin of the unusual methyl branching pattern observed in oncorhyncolide but they do provide another example of an interesting albeit infrequently encountered type of acetate incorporation that warrants further study.

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