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Biosynthesis of the Bicyclic Depsipeptide Salinamide A in *Streptomyces* sp. CNB-091: Origin of the Carbons[1]

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Abstract: The biosynthesis of the bicyclic depsipeptide salinamide A, a potent anti-inflammatory agent, in the marine bacterium *Streptomyces* sp. CNB-091 has been examined through feeding experiments with ^{13}C-labeled intermediates.

Salinamide A (**1**) is an anti-inflammatory agent produced by *Streptomyces* sp. CNB-091, a marine bacterium that was isolated from the surface of a jellyfish *Cassiopeia xamachana*.[2] Recently, five structurally related salinamides have been isolated from this marine bacterial strain, some of which appear to be biosynthetic shunt products.[3] Salinamide A has also been found in an edaphic *Streptomyces* sp. (NRRL 21611) and has been shown to exhibit strong inhibitory activity against bacterial RNA polymerases.[4] In this communication, we report initial findings on the biosynthesis of **1**, notably on the origin of the carbons in the unusual (*p*-alkoxyphenyl)glycine unit of this bicyclic depsipeptide (Figure 1).

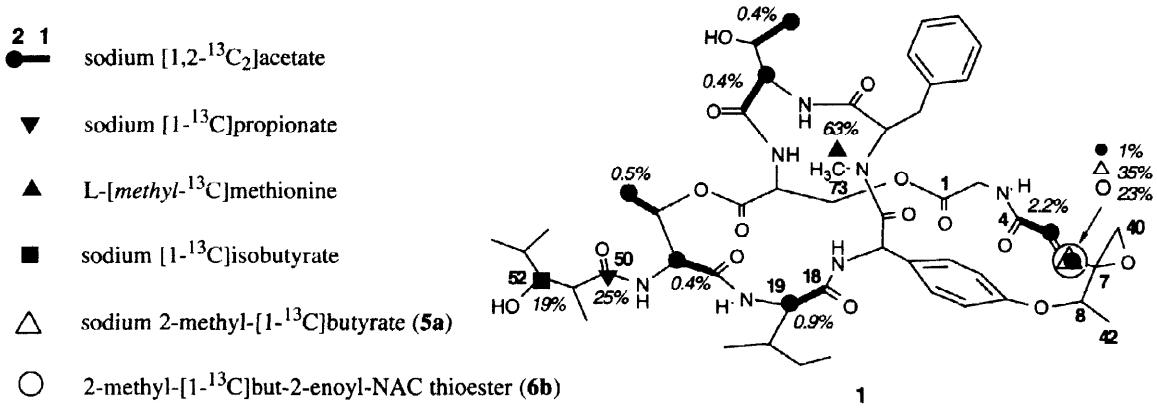

Figure 1. Structure and biosynthetic origin of carbons in salinamide A (**1**).

The peptide core of **1** is probably derived from the hexapeptide Thr-D-Ile-Hpg-MePhe-D-aThr-Ser (Scheme 1). Its biosynthesis most likely involves a nonribosomal peptide synthetase pathway,[5] as the hexapeptide core contains two D-amino acids, an *N*-methyl amino acid and a nonproteinogenic (*p*-hydroxyphenyl)glycine[6] (Hpg) unit. This hypothesis is supported by the fact that the D-isoleucine unit of **1**

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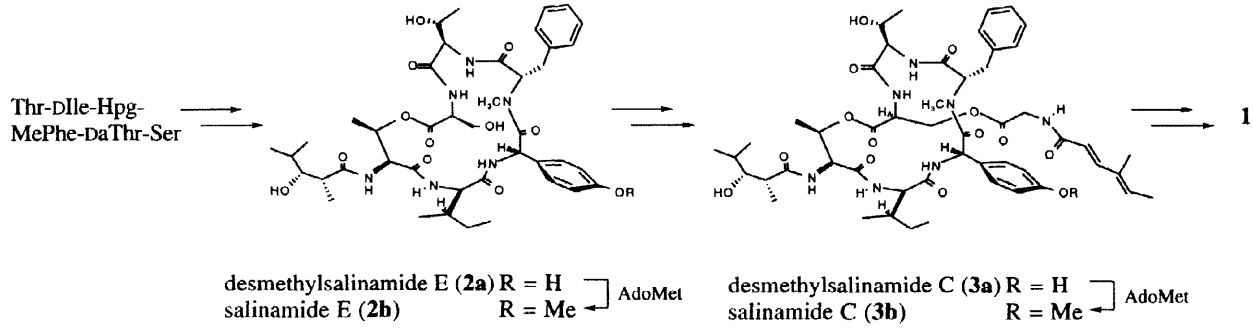
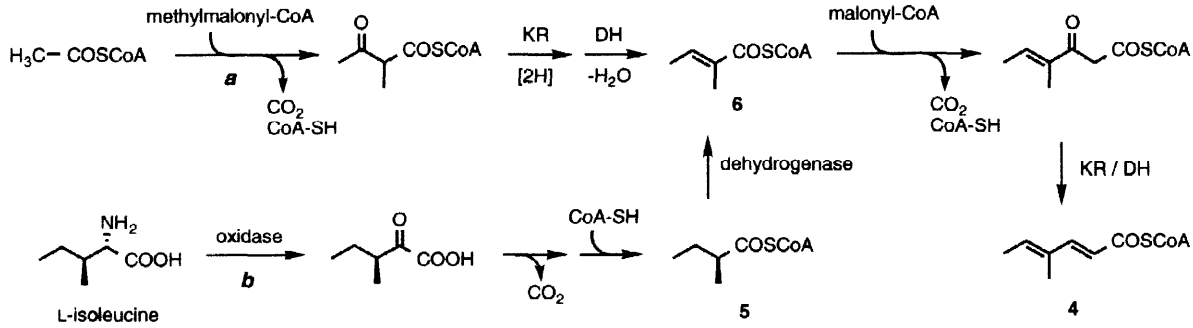
can be replaced by a D-valine residue to give salinamide D by supplementing the growth media with L-valine.[3] The proposed pathway proceeds through the hypothetical intermediates desmethylsalinamides E (**2a**) and C (**3a**). We suspect that the naturally occurring salinamides E (**2b**) and C (**3b**) are dead-end products of **2a** and **3a** whose Hpg phenolic groups have been methylated by *S*-adenosyl-L-methionine (AdoMet), thus preventing further conversion to **1**. The seven-carbon fragment connecting Hpg to glycine in **1** appears to be derived from (2*E*,4*E*)-4-methylhexa-2,4-dieneoyl coenzyme A (CoA) (**4**), as suggested by the structure of **3**.



Scheme 1. Proposed biosynthesis of salinamide A (**1**) via the hypothetical intermediates desmethylsalinamides E (**2a**) and C (**3a**).

Feeding experiments with stable isotope-labeled precursors were conducted to establish the building blocks of the proposed **4**-derived unit in **1** (Figure 1). Two hypothetical pathways are proposed in Scheme 2. If **4** is polyketide in origin, it should be derived from one propionate and two acetate units (path a). Alternatively, **4** may originate via a pathway involving successive isoleucine catabolism to 2-methylbutyryl-CoA (**5**) and dehydrogenation to (*E*)-2-methyl-2-butenoyl-CoA (**6**) (path b), a common intermediate of both pathways.



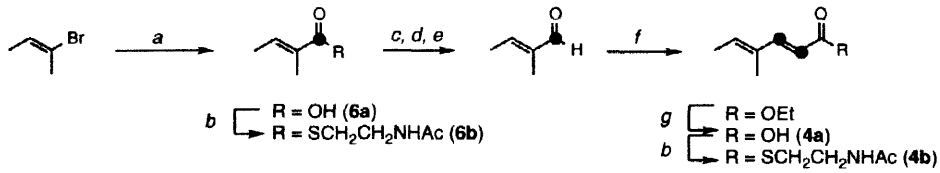
Scheme 2. Biosynthesis of (2*E*,4*E*)-4-methylhexa-2,4-dienoyl coenzyme A (**4**) via (a) polyketide biosynthetic and (b) isoleucine catabolic pathways. (KR = β-ketoreductase, DH = dehydratase)

[1,2-$^{13}C_2$]Acetate was incorporated into C4—C5 ($^1J_{4,5}$ = 65.9 Hz) but not into C8—C42 of the α,β-unsaturated amide residue of **1**, thus eliminating the polyketide pathway scenario (Scheme 2, path a). Moreover, the single incorporation (1%) of acetate at C6 of **1** further implied that the remaining 5-carbon fragment (C6-C8, C40, C42) was derived from **5**, as the internal isoleucine moiety was also enriched to the

same extent from [1,2-$^{13}C_2$]acetate at C18—C19 ($^1J_{18,19}$ = 52.1 Hz). The methyl carbon of acetate was determined to provide the olefinic C6 based on a subsequent feeding experiment with [2-^{13}C]acetate. The C73 methyl group on the *N*-methyl phenylalanine unit was the only carbon in **1** to be derived from the C_1 pool as shown by a feeding experiment with L-[*methyl*-^{13}C]methionine.

[1-^{13}C]Propionate labeled C50 of the (2*S*,3*S*)-3-hydroxy-2,4-dimethylpentanoic acid (HDMP) residue, but not C6, further supporting the proposed isoleucine catabolic pathway (Scheme 2, path b). This conclusion was corroborated by the high specific incorporation (35%) of 2-methyl-[1-^{13}C]butyrate (**5a**) at C6. The propionate-derived unit in **1** was also enriched at C50 (13%) in the latter feeding experiment, presumably via the degradation product [1-^{13}C]propionoyl-CoA formed by β-oxidation of **5a**. The remaining carbons in the HDMP side chain were shown to be isobutyrate-derived on the basis of a feeding experiment with sodium [1-^{13}C]isobutyrate[7] (19% ^{13}C-enrichment at C52).

The unsaturated 2-methyl-[1-^{13}C]but-2-enoate (**6a**) (Scheme 3) likewise enriched C6, but at a much lower specific incorporation (13%) than **5a**. As expected,[8] incorporation of the corresponding *N*-acetylcysteamine (NAC) thioester **6b**[9] was considerably higher (23%). In both cases, however, considerable degradation of the labeled precursors was once again observed.



Scheme 3. Reagents: *a.* (i) Mg,THF, (ii) $^{13}CO_2$; *b.* (i) $(EtO)_2P(O)Cl$, (ii) EtOTl, $HSCH_2CH_2NHAc$, THF; *c.* CH_2N_2; *d.* $LiAlH_4$; *e.* PCC; *f.* BuLi, $(EtO)_2P(O)^{13}CH_2CO_2Et$; *g.* K_2CO_3, $MeOH/H_2O$.

The advanced intermediate 4-methyl-[2,3-$^{13}C_2$]hexa-2,4-dienoate (**4a**), prepared by standard Horner-Emmons chemistry from 2-methyl-[1-^{13}C]but-2-enal and [2-^{13}C]triethylphosphonoacetate (Scheme 3), and the corresponding NAC thioester **4b** were next administered to the bacterium. In both cases, intact incorporation of **4a** and **4b** with enrichment at C5—C6 was not observed. Labeling was only measured at C6 and C50, which revealed that the substrate had undergone complete degradation before being taken up into **1**.

Interestingly, both of the 7-carbon, non-amino acid residues of **1** are biosynthesized by a single malonyl chain extension of a short-chain carboxylic acid derived from a branched amino acid. In the case of HDMP, the polyketide product is biosynthesized from isobutyrate, which is derived from valine, and methylmalonyl-CoA and undergoes a β-keto reduction. The diene **4**, on the other hand, is derived from the condensation of the isoleucine product **6** with malonyl-CoA followed by β-keto reduction and dehydration (Scheme 2, path b).

Further work is currently underway on the mode of cyclization of **3** to **1** (*e.g.* whether the oxidation/cyclization involves an epoxide intermediate which is opened by the Hpg phenol followed by dehydration and a second epoxidation or involves an Fe(II)-dependent oxygenase mediated [2+2] cycloaddition[10] followed by dehydrogenation[11]) and on the molecular genetics of salinamide biosynthesis (we have cloned and sequenced a peptide synthetase from *Streptomyces* sp. CNB-091).

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References and Notes

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