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Biosynthetic studies on the antibiotics PF1140: a novel pathway for a 2-pyridone framework

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Abstract—Incorporation of labeled acetate and L-serine into PF1140 in *Eupenicillium* sp. indicated that the skeleton of PF1140 is derived from five acetates and a L-serine. Upon administration of [1,3-$^{13}C_2$]glycerol, a precursor of biotransformation into L-[1,3-$^{13}C_2$]serine, the isotopic labels became contiguous in the resultant 2-pyridone of PF1140. Based on the feeding experiments, a novel and potentially general biosynthetic pathway for a 2-pyridone framework has been proposed, in which an acyl tetramic acid precursor could be converted via a ring expansion with loss of the hydroxyl group.

Numerous fungi produce a series of *N*-hydroxy-pyridone antibiotics exemplified by PF1140 (**1**),[1] tenellin (**2**),[2] ilicicolin H (**3**),[3] leporins (**4**),[4] fusaricides (**5**),[5] and pyridoxatins (**6**)[6] (Fig. 1). Certain members of this class are candidates for modulators of erythropoietin gene expression,[4b] free radical scavengers,[6a] inhibitors of ubiquinol-cytochrome *c* reductase,[7] and anti-malarial drugs.[8] In 1996, the structure of **1** with unspecified relative configuration was reported,[1a] and recently the stereochemistry of **1** including the absolute configuration has been elucidated by our group.[1b] It is likely that **1** could be biosynthesized by a polyketide synthase and non-ribosomal peptide synthetase hybrid,[9] and our biosynthetic studies focused on the formation of a 2-pyridone framework as well as the cyclization of a linear polyketide precursor leading to a fused carbocyclic skeleton.[10] Herein, we wish to propose a novel biosynthetic pathway for the 2-pyridone of **1** based on feeding experiments.

First of all, sodium [1-^{13}C]acetate was administered into the cultures of *Eupenicillium* sp. PF1140.[1a] Treatment of the resulting crude extract containing **1** with allyl bromide and K_2CO_3 in acetone provided a less-polar derivative **8**, which was purified by simple silica-gel chromatography (Fig. 2).[1b] Enhanced signals in the ^{13}C NMR of **8** were observed at the unambiguously assigned sites of C13, C9, C11, C7, and C2, compared with an unlabeled control sample (Table 1).[11] Next, administration of sodium [1,2-$^{13}C_2$]acetate and subsequent allylation yielded labeled **8** with characteristic satellite peaks flanking the natural abundance signal in the ^{13}C NMR spectrum because of the incorporation of intact acetate units. These results proved that a total of five intact acetate units are joined in a head-to-tail fashion to form a pentaketide unit. Furthermore, administration of L-[*Me*-^{13}C]methionine indicated that the branching three methyl groups on C8, C10, and C12 of the polyketide moieties are donated by the *S*-methyl group of L-methionine.

To verify the participation of amino acid precursors for the biosynthesis of the remaining part (N1 and C4-C6), L-[1-^{13}C]serine and L-[1-^{13}C]alanine were administered.[11] The isotopic label derived from L-[1-^{13}C]serine was detected at C4 in **8** (Table 1), whereas that of L-[1-^{13}C]alanine was almost negligible.[12] With these encouraging results, [1,3-$^{13}C_2$]glycerol, a precursor of the biotransformation into [1,3-$^{13}C_2$]serine **10**, was next introduced into *Eupenicillium* sp. (Fig. 3a).[11] The ^{13}C NMR showed that satellite peaks are apparent at C4 and C5 (^1J = 64 Hz) in the resultant **8** (Fig. 3b), which is indicative of an intramolecular carbon skeletal rearrangement.

Based on the feeding experiments, we have shown, for the first time, the incorporation of L-serine into the

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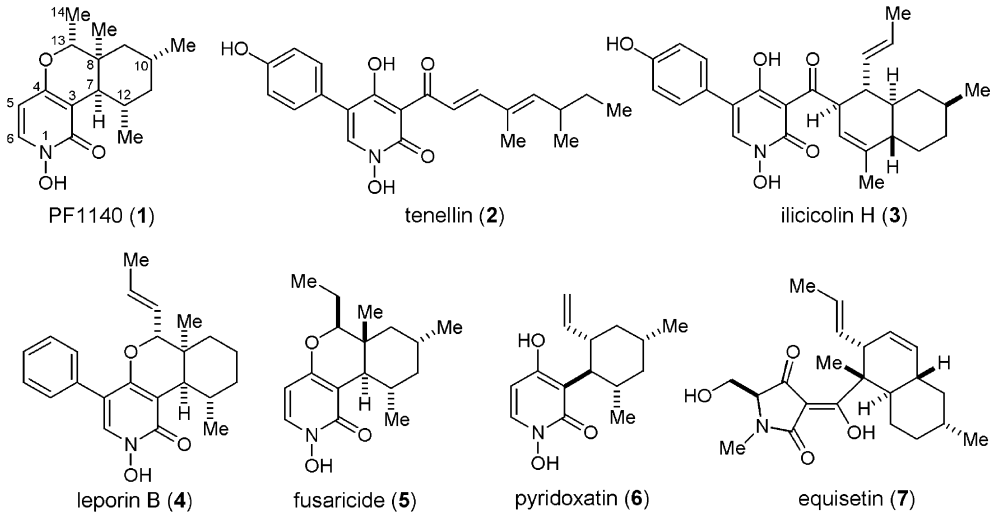


Figure 1. Naturally occurring 2-pyridone alkaloids (**1**–**6**) and an acyl tetramic acid **7**.

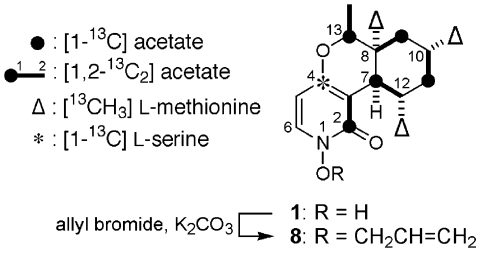


Figure 2. Biosynthetic origin of the carbon atoms of **1**.

Table 1. ^{13}C NMR data of **8** obtained from feeding experiments with ^{13}C-labeled precursors

	δ_C	Relative ^{13}C enrichments[a]			J_{C-C} (Hz)
		[1-^{13}C]-acetate	[*S*-^{13}C]-methionine	[1-^{13}C]-serine	[1,2-$^{13}C_2$]-acetate
C-14	14.6				41
C-13	74.2	2.5			41
C-8	37.8				33
C-9	44.5	2.7			33
C-10	27.0				33
C-11	44.2	2.5			33
C-12	33.7				33
C-7	44.8	2.6			33
C-3	111.7				73
C-2	160.1	2.7			73
C-6	133.6				
C-5	98.2				
C-4	160.0			1.4	
8-Me	20.6		24.2		
10-Me	21.4		17.9		
12-Me	22.8		18.0		

[a] Ratio of carbon signal intensities for enriched and natural abundance sample measured under identical conditions.

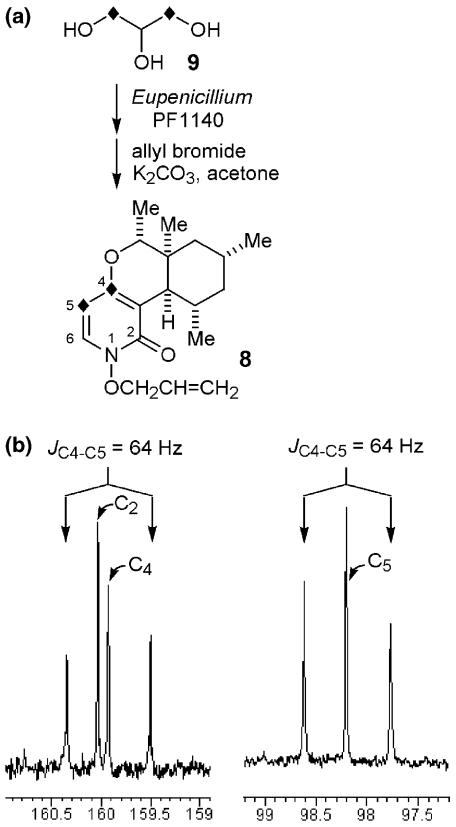
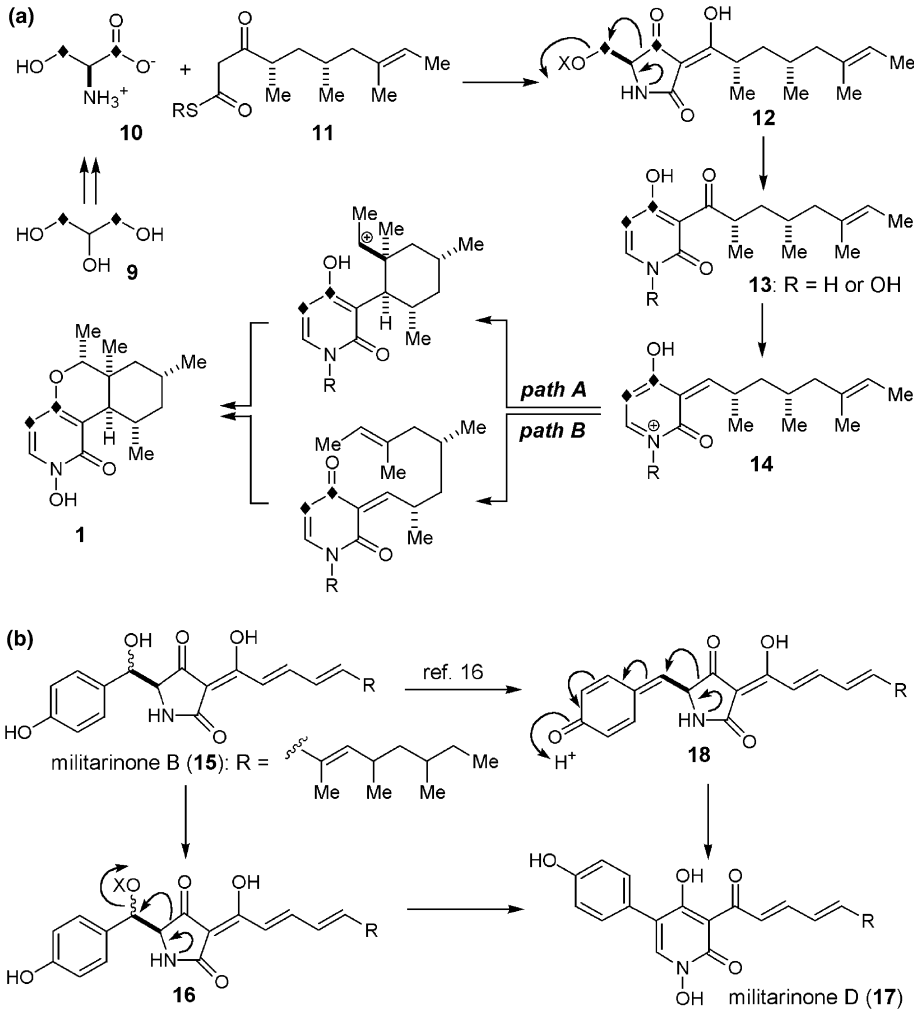


Figure 3. (a) Incorporation of [1,3-$^{13}C_2$]glycerol into 2-pyridone skeleton of **8**. (b) Sections of the ^{13}C NMR spectrum of **8**.

2-pyridone framework as well as the rearrangement of the original serine skeleton. In this context, a plausible biosynthetic pathway to **1** illustrated in Scheme 1a was derived. First, L-serine **10** is considered to combine with a polyketide moiety **11** to generate the acyltetramic acid intermediate **12**, which is analogous to the biosynthesis of acyl tetramic acids such as euisetins **7** and pramanicins.[9,13] Then, the critical ring expansion may occur by loss of the hydroxyl group and subsequent aromatization leading to **13**. Finally, the *cis*-fused carbon skeleton is considered to be constructed either via a stepwise cyclization (pathway A) or a hetero Diels–Alder reaction[14] (pathway B) to furnish **1**.



Scheme 1. (a) A proposed biosynthetic route to **1**. (b) A proposed biosynthetic mechanism for the 2-pyridone framework of **17**.

To date, a number of biosynthetic studies for **2** and **3** revealed that the origins of the 2-pyridone framework are an aromatic amino acid, phenylalanine (or tyrosine), and a polyketide.[15] Nonetheless, condensation of these precursors and the subsequent steps leading to the 2-pyridone remain uncertain. Recently, Hamburger and co-workers proposed a biosynthetic route to the 2-pyridone of militarinone D (**17**), which involves a rearrangement of the original tyrosine skeleton via a quinone methide intermediate **18** (Scheme 1b).[16] Whereas their biosynthetic proposal cannot be applied to those of **1** and **4**–**6**, our proposal for the rearrangement (**12**→**13**) featuring the loss of the hydroxyl group could be generally applicable for the biosynthesis of **5** and **6** via the possible serine-derived acyl tetramic acid intermediates. Similarly, we wish to propose an alternate mechanism for the conversion of the aromatic amino acid-derived acyl tetramic acid intermediates into **2**–**4** and **17** as exemplified in Scheme 1b (**16**→**17**).

In summary, a novel and potentially general biosynthetic pathway for a 2-pyridone framework has been proposed, in which an acyl tetramic acid precursor could be converted via a ring expansion with loss of the hydroxyl group. Currently, we are engaged in a genetic analysis clarifying the machinery of biosynthetic enzymes as well as a synthetic approach identifying the actual intermediate.

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