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Structures and Biosynthesis of the Pyridinopyrones, Polyenepyrones from a Marine-Derived *Streptomyces* Species

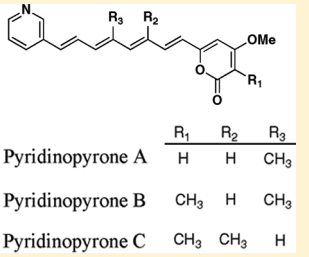
Takashi Fukuda,[†] Eric D. Miller,[†] Benjamin R. Clark,[‡] Ali Alnauman,[‡] Cormac D. Murphy,[‡] Paul R. Jensen,[†] and William Fenical*[,†]

[†]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093-0204, United States

[‡]School of Biomolecular and Biomedical Sciences, Centre for Synthesis and Chemical Biology, Ardmore House, University College Dublin, Belfield, Dublin 4, Republic of Ireland

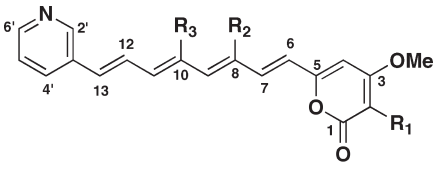
Supporting Information

ABSTRACT: Three polyenylpyrone metabolites, pyridinopyrones A to C (**1**−**3**), have been isolated from the culture broth of a marine-derived *Streptomyces* sp., strain CNQ-301. The structures of the pyridinopyrones were assigned on the basis of chemical modification and combined spectroscopic methods, focusing on interpretation of 1D and 2D NMR data. Pyridinopyrones B and C (**2**, **3**), examined as an inseparable mixture of methyl positional isomers, were ultimately defined by hydrogenation and NMR analysis of a saturated derivative. The biosynthesis of these metabolites was defined by the incorporation of stable isotope-labeled precursors, revealing that the biosynthetic starter unit is nicotinic acid, while the polyene chain and pendant methyl groups are acetate- and methionine-derived, respectively.



	R_1	R_2	R_3
1 Pyridinopyrone A	H	H	CH_3
2 Pyridinopyrone B	CH_3	H	CH_3
3 Pyridinopyrone C	CH_3	CH_3	H

As a continuation of our interest in the chemistry of marine sediment-derived actinomycete bacteria,[1] we examined populations of these organisms cultured from samples collected off shore in the La Jolla, California, coastal region.[2] One of the strains isolated was found to produce a unique series of polyenepyrone metabolites possessing pyridine end groups. These compounds, the pyridinopyrones (**1**−**3**), belong to a class of aromatic polyenepyrones, distant relatives of which are produced by fungi and in a rare instance by a marine protozoan.[3−6] Because this actinomycete is a unique source producing this class, we examined the structures and biosyntheses of these compounds in detail.



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3 Pyridinopyrone C	CH_3	CH_3	H

RESULT AND DISCUSSION

Streptomyces strain CNQ-301 was isolated from bottom sediments collected near La Jolla, California. The strain was cultured under saline conditions on a 36 L scale. The culture was resin-extracted (XAD-7), and the resin and cell mass were collected by filtration and extracted with acetone. The acetone was removed under vacuum, and the residue was partitioned between H_2O and EtOAc to yield the pyridinopyrone mixture (950 mg) after evaporation of the EtOAc fraction.

Pyridinopyrone A (**1**) was isolated by repeated chromatographic separation from the EtOAc fraction as an amorphous, yellow solid. The molecular formula for **1** was established as $C_{20}H_{19}NO_3$ ($[M + H]^+$ m/z 322.1439) on the basis of high-resolution ESI-TOFMS measurements, indicating that pyridinopyrone A contained 12 degrees of unsaturation. The high degree of unsaturation was further illustrated by an intense UV−vis absorption at 424 nm, which also suggested the presence of an extended conjugated system in the molecule. The IR spectrum of **1** showed a characteristic absorption at 1712 cm^{-1}, suggesting the presence of an ester carbonyl. The ^{13}C NMR spectrum (in acetone-d_6) showed 20 resolved signals, which were classified as derived from two methyl, 13 sp^2 methine, and five quaternary carbons including one ester carbonyl carbon (C-1) and two oxygenated sp^2 carbons (C-3 and C-5) (Table 1). The ^1H NMR spectrum of **1** in acetone-d_6 showed two methyl signals and 13 olefinic and aromatic methine signals. The connectivity of all protons and carbon atoms was established by interpretation of HSQC and HMBC NMR spectroscopic data (Table 1). Analysis of $^1H-^1H$ COSY NMR data allowed three partial structures,

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Table 1. NMR Spectroscopic Data for Pyridinopyrone A (1) (600 MHz for ^{1}H, 150 MHz for ^{13}C (acetone-d_6))[a]

C #	δ_C	δ_H mult (J in Hz)	HMBC[b]
1	162.4, C		
2	88.3, CH	5.51, s	1, 3, 4
3	170.9, C		
4	100.7, CH	6.08, s	2, 3, 5, 6
5	158.8, C		
6	122.5, CH	6.35, d (15.0)	4, 5, 7, 8
7	135.6, CH	7.15, dd (15.0, 11.0)	5, 6, 8, 9
8	127.8, CH	6.61, dd (15.0, 11.0)	6, 7, 10
9	142.6, CH	6.81, d (15.0)	7, 10, 11, 10-CH_3
10	137.0, C		
11	135.3, CH	6.55, d (10.9)	9, 12, 13, 10-CH_3
12	128.2, CH	7.47, dd (15.6, 10.9)	10, 11, 13, 3′
13	129.1, CH	6.78, d (15.6)	11, 2′, 4′
2′	145.3, CH	8.72, s	13, 4′, 6′
3′	134.1, C		
4′	132.6, CH	8.01, d (7.8)	13, 2′, 6′
5′	122.8, CH	7.35, dd (7.8, 4.8)	3′, 6′
6′	145.2, CH	8.44, d (4.8)	2′, 4′, 5′
3-OMe	55.7, CH_3	3.89, s	3
10-Me	12.0, CH_3	2.07, s	9, 10, 11

[a] Assignments made by interpretation of COSY, HSQC, and HMBC NMR data. [b] HMBC correlations are from the proton(s) stated to the indicated carbon.

C-6 to C-9, C-11 to C-13, and C-4′ to C-6′ to be assigned. Analysis of HMBC NMR spectroscopic data gave further structural information virtually defining the full structure of **1**. HMBC NMR cross-peaks from H-2 (δ 5.51) to C-1 (δ 162.4), C-3 (δ 170.9), and C-4 (δ 100.7), from H-4 (δ 6.08) to C-2 (δ 88.3), C-3, and C-5 (δ 158.8), and from the 3-OCH_3 protons (δ 3.89) to C-3 supported the connectivity of C-1 to C-5. Taking into consideration the number of oxygen atoms present in pyridinopyrone A, and the chemical shifts of C-1 and C-5, one oxygen atom must be inserted between these carbons. Thus, the existence of a 3-methoxy-α-pyrone unit in **1** was confirmed as shown in Figure 1. Additional HMBC NMR cross-peaks from H-9 (δ 6.81) to C-10 (δ 137.0), C-11 (δ 135.3), and 10-CH_3 (δ 12.0), from H-11 (δ 6.55) to C-9 (δ 142.6), C-10, and 10-CH_3, and from 10-CH_3 (δ 2.07) to C-9, C-10, and C-11 supported the existence of the C-10-methyl-substituted tetraene unit (C-6 to C13). Further HMBC correlations from H-2′ (δ 8.72) to C-4′ (δ 132.6) and C-6′ (δ 145.2), from H-4′ (δ 8.01) to C-2′ (δ 145.3) and C-6′, from H-5′ (δ 7.35) to C-3′ (δ 134.1) and C-6′, and from H-6′ (δ 8.44) to C-2′, C-4′, and C-5′ allowed the assembly of C-2′ through C-6′ of the pyridine ring.

Taking into consideration the molecular formula, chemical shifts, and vicinal coupling information, the existence of the polyene substituent at the 3′ position of the pyridine was established. HMBC NMR correlations from H-12 (δ 7.47) to C-3′, from H-13 (δ 6.78) to C-2′ and C-4′, from H-2′ to C-13, from H-4′ to C-13, and from H-5′ to C-3′ showed that the tetraene is linked at the 3′ position of the pyridine ring. Furthermore, HMBC correlations from H-4 to C-5 and C-6 (δ 122.5) and from H-6 (δ 6.35) to C-4 and C-5 allowed the tetraene and the α-pyrone functionalities to be joined at the C-5 pyrone position. Comprehensive analysis of HMBC data also clearly showed that the methyl group was positioned at C-10 (Figure 1). From the coupling

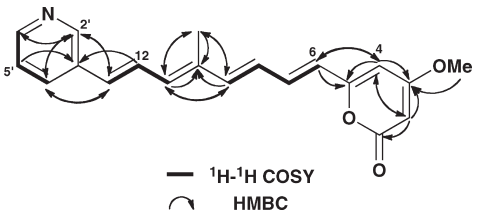


Figure 1. ^{1}H−^{1}H COSY and key HMBC correlations used to establish the structure of pyridinopyrone A (**1**).

constants ($J_{6,7}$ = 15.0 Hz, $J_{8,9}$ = 15.0 Hz, and $J_{12,13}$ = 15.6 Hz) and by comparison with similar compounds,[3,4] the tetraene unit was assigned all *E* geometrical configurations. On the basis of all of the data, the structure of pyridinopyrone A was confidently established as **1**.

Pyridinopyrones B and C (**2**, **3**) were isolated as an amorphous, yellow solid that contained pyridinopyrone B (**3**) as its major component. Although multiple HPLC attempts were made, this mixture could not be separated. Fortunately, the two-component mixture was realized to be composed of methyl group positional isomers sharing the same molecular formula. As a result, the molecular formula for both constituents was established by HR-ESI-TOFMS as $C_{21}H_{21}NO_3$ ($[M + H]^+$ m/z 336.1598). This molecular formula indicated the addition of 14 mass units to the formula for pyridinopyrone A, thus indicating an additional methyl group. The structures of pyridinopyrones B and C were established by analysis of 1D and 2D NMR spectroscopic data obtained from the mixture (Table 2). For pyridinopyrone B (**2**), the major isomer present, the presence of one methyl signal at δ 1.86 and the lack of the olefinic methine signal at δ 5.51, as observed in **1**, indicated that a new methyl group was attached in both compounds at C-2. Analysis of HMBC spectroscopic data fully supported this assignment as well as confirmed the presence of the 2-methyl-3-methoxy-α-pyrone unit. Thus, the structure of pyridinopyrone B was assigned as **2**.

The structure of pyridinopyrone C (**3**), a minor constituent of the mixture (ca. 10−20%), was established by comparison of all spectroscopic data with that from pyridinopyrone B (**2**) (see Supporting Information). The difference between these two metabolites was clearly the methyl group location in the tetraene unit. Analysis of the ^{1}H−^{1}H COSY NMR data showed connectivity between positions C-6 to C-7 and C-9 to C-13. Analysis of HMBC NMR data (Table 2), in particular the observed correlations from H-7 (δ 7.15) to C-9 (δ 135.8) and 8-CH_3 (δ 11.5), from H-9 (δ 6.63) to C-7 (δ 138.0) and 8-CH_3 and from 8-CH_3 to C-7, C-8 (δ 135.8), and C-9, illustrated that the methyl group was positioned at C-8. On the basis of these data, the structure of pyridinopyrone C was assigned as **3**.

For additional confirmation of these structure assignments, the pyridinopyrone B/C mixture was hydrogenated in the hopes of isolating the pure methyl positional isomers. The molecular formula of the hydrogenation products was established as $C_{21}H_{29}NO_3$ ($[M + H]^+$ m/z 344.2225) by interpretation of HR-ESI-TOFMS data. As with the natural product mixture, HPLC separation proved unsuccessful. As expected, the ^{1}H NMR spectrum, which was primarily defined by the major hydrogenation product of pyridinopyrone B (BH), showed a broad methylene band between δ 1.16 and 2.61 (Table 3). Fortuitously, the ^{1}H NMR spectral data for this mixture showed two well-resolved doublet methyl signals at δ 0.87 (major) and 0.90 (minor). Using these signals as a starting point for HMBC NMR analysis, cross-peaks

Table 2. NMR Spectroscopic Data for the Pyridinopyrone B/C Inseparable Mixture (2, 3) (600 MHz for ^{1}H, 150 MHz for ^{13}C (acetone-d_6))[a]

C #	(2) δ_C	(3) δ_C	(2) δ_H mult (J in Hz)	(3) δ_H mult (J in Hz)	(2) HMBC[b]	(3) HMBC[b]
1	163.3, C	163.3, C				
2	101.3, C	101.3, C				
3	165.5, C	165.5, C				
4	96.0, CH	96.2, CH	6.53, s	6.58, s	2, 3, 5, 6	2, 3, 5, 6
5	157.6, C	157.9, C				
6	122.7, CH	122.7, CH	6.38, d (15.1)	6.40, d (14.0)	4, 5, 7, 8	4, 5, 7, 8
7	134.9, CH	138.0, CH	7.17, dd (15.1, 11.2)	7.15, d (14.0)	5, 6, 8, 9	5, 6, 8, 8-Me
8	127.7, CH	135.8, C	6.61, dd (14.7, 11.2)		6, 7, 9, 10	
9	142.0, CH	135.8, CH	6.78, d (14.7)	6.63, d (11.4)	7, 8, 10, 11,10-Me	7, 8, 11, 8-Me
10	137.1, C	130.8, CH		6.94, dd (14.7, 11.4)	9, 10, 12, 13	8, 12
11	134.2, CH	135.5, CH	6.54, d (11.4)	6.71, dd (14.7, 10.6)	10-Me	9, 13
12	127.2, CH	131.4, CH	7.48, dd (15.5, 11.4)	7.24, dd (15.2, 10.6)	10, 11, 13, 3′	10, 3′
13	130.4, CH	130.0, CH	6.77, d (15.5)	6.76, d (15.2)	11, 12, 2′, 3′, 4′	11, 2′, 4′
2′	148.6, CH	148.6, CH	8.72, s	8.70, s	13, 3′, 4′, 6′	13, 4′, 6′
3′	133.5, C	133.1, C				
4′	132.4, CH	132.1, CH	7.99, d (8.0)	7.92, d (8.0)	13, 2′, 3′, 5′, 6′	13, 2′, 6′
5′	123.5, CH	123.5, CH	7.34, dd (8.0, 4.7)	7.34, dd (8.0, 4.7)	3′, 4′, 6′	3′, 6′
6′	148.5, CH	148.5, CH	8.43, d (4.7)	8.43, d (4.7)	2′, 4′, 5′	2′, 4′, 5′
2-Me	7.9, CH_3	7.9, CH_3	1.86, s	1.86, s	1, 2, 3	1, 2, 3
3-OMe	55.9, CH_3	55.9, CH_3	3.96, s	3.97, s	3	3
8-Me		11.5, CH_3		2.02, s		7, 8, 9
10-Me	11.6, CH_3		2.09, s			9, 10, 11

[a] Assignments made by interpretation of COSY, HSQC, and HMBC data. [b] HMBC correlations are from the proton(s) stated to the carbon indicated.

Table 3. NMR Spectroscopic Data for the Hydrogenation Product of Pyridinopyrone B (600 MHz for ^{1}H, 150 MHz for ^{13}C in acetone-d_6)[a]

C#	δ_C	δ_H mult (J in Hz)	COSY	HMBC[c]
1	164.5, C			
2	99.3, C			
3	166.0, C			
4	94.2, CH	6.39, s		2, 3, 5, 6
5	164.3, C			
6	33.3, CH_2	2.51, t (7.8)	7	4, 5, 7, 8
7	27.1, CH_2	1.64, m[b]	6, 8	5, 6, 8, 9
8	26.1, CH_2	1.34, m[b]	7, 9	6, 7, 9, 10
9	36.2, CH_2	1.16, 1.35, m[b]	8, 10	7, 8, 10, 11, 10-CH_3
10	32.1, CH	1.45, m	9, 11	8, 9, 11, 10-CH_3
11	36.2, CH_2	1.16, 1.35, m[b]	10, 12	9, 10, 12, 13 10-CH_3
12	28.3, CH_2	1.60, m[b]	11, 13	10, 11, 13, 3′
13	32.5, CH_2	2.61, m	12	11, 12, 2′, 3′, 4′
2′	150.2, CH	8.45, s		13, 3′, 4′, 6′
3′	137.8, C			
4′	135.5, CH	7.61, d (7.8)	5′	13, 2′, 6′
5′	123.3, CH	7.27, dd (7.8, 4.2)	4′, 6′	3′, 6′
6′	147.3, CH	8.40, d (4.2)	5′	2′, 4′, 5′
2-CH_3	7.6, CH_3	1.80, s		1, 2, 3
3-OCH_3	55.9, CH_3	3.94, s		3
10-CH_3	19.1, CH_3	0.87, d (7.2)		9, 10, 11

[a] Assignments made by interpretation of COSY, HSQC, and HMBC data. [b] Signals obscured by overlap. [c] HMBC correlations are from the proton(s) stated to the carbon indicated.

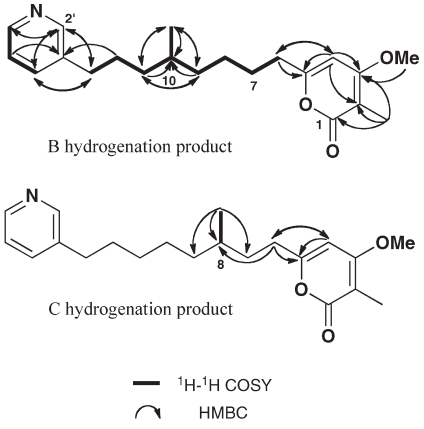
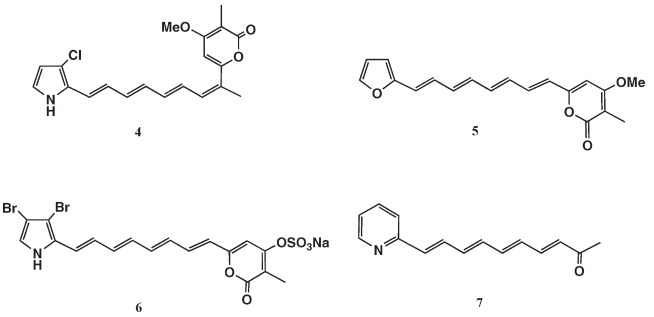


Figure 2. ^{1}H−^{1}H COSY correlations and key HMBC correlations used to establish the structures of the hydrogenation products from pyridinopyrones B (**2**) and C (**3**).

were observed that clearly confirmed the methyl group assignments at C-10 and C-8 for pyridinopyrones B and C, respectively (Figure 2 and Supporting Information).

The pyridinopyrones bear a strong similarity to the fungal metabolites rumbrin (**4**),[3] the auxarconjugatins,[4] and the gymnoconjugatins [gymnoconjugatin A (**5**)],[5] metabolites with identical tetraenepyrone functionalities produced by members of the terrestrial fungal family Onygenales. Very similar bromopyrrole polyene pyrones have also been isolated from the marine protist *Pseudokeronopsis rubra* [keronopsin A (**6**)].[6] In the case of these latter metabolites, however, the end group is chloro- or bromopyrrole,

pyrrole, or furan, rather than pyridine. The only other pyridine polyenes found in nature appear to be from the marine opisthobranchs *Navanax inermis* [navenone A (**7**)] and *Haminoea navicula*.[7,8]



In order to examine the biosynthetic origins of the pyridinopyrones, experiments were conducted using isotopically labeled precursors to determine whether these compounds are biosynthesized in an analogous fashion to the fungal polyenes. Labeled precursors were added to growing cultures after five days of incubation, and the cultures were filtered and extracted after another five days, ensuring production of the pyridinopyrones. The extracts were then analyzed by mass spectrometry to determine the degree of incorporation of the labeled substrates into the compounds of interest. For the biosynthetic studies, pyridinopyrones B and C (**2**, **3**) were produced in the highest quantities, and therefore the labeling studies described below focused on these as a model of production for all pyridinopyrones.

Initial labeling studies addressed the origins of the starter unit. The obvious candidate was nicotinic acid, which in most prokaryotes is biosynthesized from aspartic acid. When ^{15}N-aspartic acid (2.5 mM) was used to supplement the culture medium, good incorporation into pyridinopyrones B and C (10.2%) was observed. In contrast, a control experiment using ^{15}N-glycine (2.5 mM) gave only 4.3% incorporation, which can be attributed to nonspecific cycling of ^{15}N through the nitrogen pool. This strongly suggested aspartic acid is a precursor in pyridinopyrone biosynthesis. Nevertheless, in order to unambiguously confirm the identity of the starter unit, nicotinic acid-d_4 (2.5 mM) was used to supplement the growth medium. MS analysis of the polyenes produced in this experiment showed strong peaks at m/z 340 $[M + 5]^+$, corresponding to 88.6% incorporation of the labeled substrate into the pyridinopyrone B/C (**2**, **3**) mixture, confirming nicotinic acid as the starter unit for pyridinopyrone production. The very high incorporation of nicotinic acid into the pyridinopyrones suggests that precursor-directed biosynthesis studies to produce analogues should be readily achievable.

When [1-^{13}C]-acetate (20 mM) was added to the culture, an overall incorporation of 8.4% was observed, in concordance with the proposed polyketide origin of the metabolites. ^{13}C NMR analysis confirmed the locations of the enriched carbons at C-1, C-3, C-5, C-7, C-9, C-11, and C-13, as would be expected (Table S1, Supporting Information, Figure 3). Carbon-13 also showed significant enhancement, which can be attributed to the incorporation of labeled acetate into nicotinic acid, via aspartic acid, oxaloacetic acid, and the TCA cycle. On the other hand, [1-^{13}C]-propionate (2.5 mM) did not act as a precursor (0% enrichment), indicating that the pendant *C*-methyl groups of the pyridinopyrones are derived from another source. Indeed, [methyl-^{13}C]-L-methionine (2.5 mM) was found to be incorporated with high efficiency, with up to three labeled carbons (one label: 42.1%, two labels: 19.0%, three labels: 2.6%) incorporated into each molecule of the pyridinopyrone B/C mixture by MS analysis. This suggested that not only the *O*-methyl but also the *C*-methyl groups were methionine-derived. The ^{13}C NMR spectrum of pyridinopyrones B/C showed strongly enhanced resonances at 55.9 (OMe), 11.6 (10-Me), and 7.6 ppm (2-Me), indicating incorporations of 20%, 22%, and 23%, respectively.

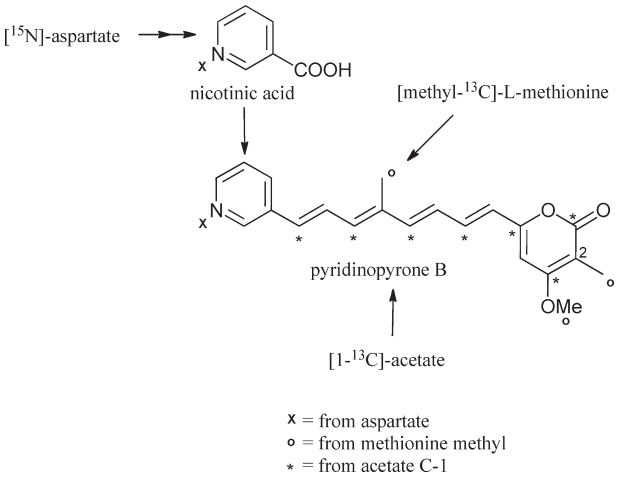


Figure 3. Overall results of ^{13}C incorporation studies illustrating the biosynthetic origins of the carbons in pyridinopyrone B (**2**).

The labeling patterns observed for the pyridinopyrones closely resemble those observed for rumbrin.[9] The use of methionine in the formation of the pendant *C*-methyl is characteristic of iterative fungal PKSs.[10] In contrast, in bacterial modular PKSs, pendant methyl groups are usually formed by incorporation of propionate (as methylmalonate) units during chain extension. The structures of the pyridinopyrones are unusual in that they are partially reduced linear structures, rather than the condensed aromatics typical of type II bacterial PKSs, or the larger, highly reduced compounds typical of modular type I systems. On the other hand, such linear polyene-pyrones are relatively common among fungal metabolites, such as alternapyrone[11] and the mycotoxin citreoviridin.[12] A small number of bacterial metabolites containing a similar motif are known, such as the salinipyrones,[13] luteoreticulin,[14] and aureothin.[15] Biosynthetic studies on the latter have shown that it is produced by a modular type I PKS in which one of the modules is used iteratively[16] and that the *C*-methyl groups are propionate-derived.[17] The biosynthesis of pyridine-containing natural products has also been investigated in marine mollusks. In a feeding study using labeled precursors, haminol-2, a 3-alkylpyridine pheromone, was found to be derived from acetate-extended nicotinic acid.[18]

The interesting structures of the pyridinopyrones, and the use of methionine in the production of the *C*-methyl groups, raises interesting questions about their biosynthesis. On the basis of the current evidence, it seems likely that they are produced by an iterative PKS in a fashion analogous to structurally related fungal polyenes. A study of their biosynthesis on a genetic level and comparison with those of structurally similar fungal and bacterial metabolites could yield interesting insights.

EXPERIMENTAL SECTION

General Experimental Procedures. The optical rotations were measured on a JASCO P-2000 polarimeter. UV spectra were measured

on a Beckman Coulter DU800 spectrophotometer with a 1 cm cell. IR spectra were obtained with a Thermo Nicolet IR100 FT-IR. ^{1}H and ^{13}C NMR spectra were obtained in acetone-d_6 on a Bruker DRX600 spectrometer, respectively. For biosynthetic experiments, ^{1}H and ^{13}C NMR spectra were obtained in acetone-d_6 using a Varian Inova 400 MHz spectrometer. HR-EIS-TOFMS data were obtained at The Scripps Research Institute using a Waters Micromass MALDI-R spectrometer. Reversed-phase HPLC separations were performed using a semipreparative C18 Phenomenex Luna column (250 × 10 mm) at a flow rate of 2.5 mL/min using a Waters 600E pump and a Waters Lambda-Max model 480 UV detector. Other HPLC separations were carried out using a Varian Prostar system consisting of two solvent delivery modules (210), DAD detector (335), autoinjector (410), and fraction collector (710), respectively. For the biosynthetic experiments, mass spectra were obtained using a Micromass Quattro mass spectrometer, coupled to a Waters Alliance 2695 solvent delivery system. Isotopically labeled compounds were purchased from CK Gas Products.

Collection, Identification, and Cultivation of Strain CNQ-301. *Streptomyces* strain CNQ-301 was isolated from a sediment sample collected at a depth of 133 ft near La Jolla, California (N 32°53.017′, W 117°16.166′) in 2002. Strain CNQ-301 was assigned as a member of the genus *Streptomyces*, most closely related to *S. sulphureus,* with 99.3% identity over 96% of the sequence. The GenBank accession number for the 16S sequence is EU214916 (1453 base pairs). Strain CNQ-301 was cultured in replicate 2.8 L Fernbach flasks, at 27 °C, with shaking (215 rpm) in the medium A1BFe+C (10 g of starch, 4 g of yeast extract, 2 g of peptone, 1 g of $CaCO_3$, 40 mg of $Fe_2(SO_4)_3 \cdot 4H_2O$, 100 mg of KBr, and 1 L of seawater). After 7 days, the 36 L culture was extracted with Amberlite XAD-7 resin (10 g/L). The culture broth and resin were shaken at 150 rpm for 2 h, and the resin and cell material were collected by filtration and extracted with acetone. After the acetone extract was concentrated, the resulting aqueous solution was extracted with EtOAc. The EtOAc layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to yield 950 mg of solid material.

Isolation of Pyridinopyrones. The EtOAc extract was dissolved in a small volume of isooctane, applied to a silica gel column (30 g, 3.2 × 15 cm, 200−450 mesh), and eluted stepwise with 100% isooctane, 50:1, 25:1, 10:1, 5:1, 3:1, 1:1 (v/v) of isooctane−EtOAc solvent, and 100% EtOAc (400 mL each). Pyridinopyrones were observed in the last fraction eluted with 100% EtOAc. This fraction was further purified by reversed-phase C-18 HPLC (250 × 10 mm, Phenomenex Luna), eluting with 50% CH_3CN at a flow rate 2.5 mL/min with UV detection at 210 nm. Under this condition, pyridinopyrone A and the mixture of B and C were eluted as peaks with retention times of 28 and 37 min. These peaks were collected and concentrated to yield 2.8 and 6.9 mg as yellow solids, respectively (for **1**, 0.078 mg/L; for **2**, 3: 0.192 mg/L).

*Pyridinopyrone A (**1**, 2.8 mg):* amorphous, yellow solid; UV ($CHCl_3$) λ_{max} (log ε) 424 (4.30), 404 (4.38), 380 (4.20), 308 (3.90); IR (film, NaCl) ν_{max} 3024, 1712, 1636, 1549 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESITOF $[M+H]^+$ *m*/*z* 322.1445 (calculated for $C_{20}H_{20}NO_3$, 322.1443).

*Pyridinopyrones B and C (**2**, **3**, 6.9 mg):* amorphous, yellow solid; UV ($CHCl_3$) λ_{max} (log ε) 433 (3.92), 412 (3.99), 395 (3.92), 318 (3.70); IR (film, NaCl) ν_{max} 3010, 1691, 1533, 1471, 1403 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; HRESITOF $[M+H]^+$ *m*/*z* 336.1598 (calculated for $C_{21}H_{22}NO_3$, 336.1600).

Hydrogenation of the Pyridinopyrone B and C Mixture. The mixture of pyridinopyrones B and C (3.8 mg) was dissolved in EtOAc (1 mL), and palladium on activated carbon (10%) (2.0 mg) was added with stirring. The gas phase above the reaction mixture was changed to H_2, and the reaction mixture was stirred at 25 °C for 15 min. The colorless liquid was filtered through Celite and concentrated to yield 3.1 mg of the hydrogenation products as a colorless oil. Pyridinopyrones BH and CH (**4**, **5**, 3.1 mg): colorless oil; UV ($CHCl_3$) λ_{max} (log ε) 300 (3.89), 271 (3.69); IR (NaCl) ν_{max} 2928, 1698, 1643, 1574, 1471 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; HRESITOF $[M+H]^+$ *m*/*z* 344.2225 (calculated for $C_{21}H_{30}NO_3$, 344.2226).

Feeding Studies. *Streptomyces* strain CNQ-301 was cultured using the same conditions as for the initial isolation studies, using 250 mL flasks containing 50 mL of medium A1BFe+C. Substrates for feeding studies were added after 5 days, and the cultures were then grown for 5 additional days. Diaion HP-20 resin (0.5 g) was added to each flask and shaken for a further 2 h to absorb any pyridinopyrones present in the culture medium. The resin was then extracted with acetone, reduced under vacuum, and extracted with EtOAc, as described for the initial isolation studies. Incorporation of the labeled precursors was determined by MS analysis of the crude extract by comparison of the heights of the MS peaks at M + 1 and M + 2 (and M + 3, M + 4, M + 5 as appropriate for the relevant metabolite(s). ^{13}C-Labeled pyridinopyrones were then produced on a larger scale by reculturing CNQ-301 using 10 culture flasks and combining the contents before extraction. The ^{13}C-labeled pyridinopyrones were partially purified before NMR analysis using reversed-phase HPLC: Zorbax C-18 StableBond column (4.6 × 250 mm, 5 μm particles) eluting with 65% MeOH−H_2O at a flow rate of 3 mL/min, with pyridinopyrone A (**1**) eluting at 57 min and pyridinopyrones B and C (**2**, **3**) as a single peak at 66 min. Percentage isotope incorporations were calculated by comparison of ^{13}C peak heights both internally and with a ^{13}C NMR spectrum of an unlabeled sample. While enhanced peaks were observed for both compounds, the signal-to-noise ratio for the minor compound, pyridinopyrone C, was too low to obtain reliable incorporation percentages.

■ ASSOCIATED CONTENT

Supporting Information. Proton and carbon NMR spectra (both 1D and 2D) for compounds **1** and **2**/**3** and the hydrogenation products of **2**/**3**, and a table of the % incorporation of ^{13}C into **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: wfenical@ucsd.edu. Tel: 1-858-534-2133. Fax: 1-858-534-1318.

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