Biosynthesis of Pyripyropene A

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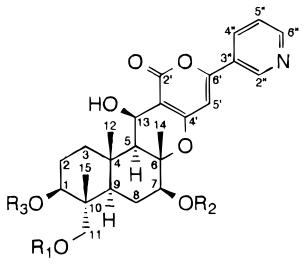
The biosynthetic origin of pyripyropene A (**1**) was studied by feeding sodium [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetates, D,L-[2-^{13}C]mevalonolactone, and [carboxy-^{14}C]nicotinic acid to the producing organism *Aspergillus fumigatus* FO-1289-2501. ^{13}C NMR and degradation experiments of ^{13}C- and ^{14}C-labeled **1** established that **1** is derived from three mevalonates, five acetates, and one nicotinic acid. The biosynthetic scheme for **1** proposes that (1) a pyridino-α-pyrone moiety is produced via condensation of a primer nicotinic acid with two acetates in a "head-to-tail" fashion, (2) an *all-trans* farnesyl pyrophosphate is produced via the mevalonate pathway, (3) the two parts are linked and cyclized to form the core skeleton, and (4) then three acetyl residues from acetates are introduced into the skeleton to yield **1**. This is the first demonstration that an intact nicotinic acid works as an acyl primer unit for oligoketide formation in fungal secondary metabolites.

Introduction

Pyripyropenes A−D (**1**−**4**, Figure 1) were isolated from the culture broth of *Aspergillus fumigatus* FO-1289 as inhibitors of acyl-CoA: cholesterol acyltransferase (ACAT).[1] The pyripyropenes represent the most potent natural product-derived ACAT inhibitors with nanomolar levels of IC_{50} values in rat liver microsomes. The relative and absolute stereochemistry of **1** has been elucidated by NOE experiments, X-ray crystallographic analysis, and Mosher's NMR method.[2] Pyripyropenes have a common skeleton consisting of polyoxygenated sesquiterpene, α-pyrone, and pyridine moieties, and three of the four hydroxyl residues at the sesquiterpene moiety are acylated by acetyl and propionyl groups to form different pyripyropenes. The pyridino-α-pyrone structure is relatively rare among natural products. Only anibine (**5**) and oxalicine A (**6**) have been reported to possess the pyridino-α-pyrone substructure. The former was isolated as an alkaloid of South American rosewood trees, *Aniba duekei* and *Aniba resaeodora*,[3] and the latter from *Penicillium oxalicum*.[4] The biosynthetic pathways of these compounds have not been established. In this paper, we describe the biosynthetic origin of pyripyropene A (**1**) as established by feeding experiments using various [^{13}C] and [^{14}C] precursors. A unique degradation method was employed to reisolate the intact labeled nicotinic acid precursor.

Results

[^{13}C]Precursor Feeding Experiments. To establish the biosynthetic origin of **1**, sodium [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetates and D,L-[2-^{13}C]mevalonolactone were fed to cultures of *Aspergillus fumigatus* FO-1289-2501. The ^{13}C-enriched samples of **1** were isolated, and the positions of the ^{13}C-enriched carbon atoms were determined by ^{13}C NMR spectroscopy. All the data from the feeding experiments are summarized in Table 1.



	R_1	R_2	R_3
Pyripyropene A (1)	-$COCH_3$	-$COCH_3$	-$COCH_3$
Pyripyropene B (2)	-$COCH_2CH_3$	-$COCH_3$	-$COCH_3$
Pyripyropene C (3)	-$COCH_3$	-$COCH_2CH_3$	-$COCH_3$
Pyripyropene D (4)	-$COCH_3$	-$COCH_3$	-$COCH_2CH_3$

Figure 1. Structures of pyripyropenes.

As illustrated in Figure 2, the 11 carbons (C-2, C-4, C-6, C-8, C-10, C-13, 1-O*C*OCH$_3$, 7-O*C*OCH$_3$, 11-O-*C*OCH$_3$, C-2′, and C-4′) and the 14 carbons (C-1, C-3, C-5, C-7, C-9, C-11, C-12, C-14, C-15, 1-OCO*C*H$_3$, 7-OCO*C*H$_3$, 11-OCO*C*H$_3$, C-3′, and C-5′) were enriched in the sodium [1-^{13}C]- and [2-^{13}C]acetate incorporation experiments, respectively. From the coupling constants of [1,2-$^{13}C_2$]-acetate-enriched **1** (Table 1), 11 pairs of doublet signals (C-1 to C-2, C-4 to C-12, C-5 to C-13, C-6 to C-14, C-8 to C-9, C-10 to C-15, 1-*C*OCOCH$_3$ to 1-COCO*C*H$_3$, 7-*C*OCOCH$_3$ to 7-COCO*C*H$_3$, 11-*C*OCOCH$_3$ to 11-COCO*C*H$_3$, C-2′ to C-3′, and C-4′ to C-5′) and three uncoupled signals (C-3, C-7, and C-11) were observed, confirming the intact 11 acetate incorporation into **1** (Figure 2). Clearly, the three acetyl residues at the 1-CO−, 7-CO−, and 11-CO− positions were derived from acetates, and the rings A and B were constructed with a sesquiterpene derived from three mevalonates. This was also corroborated by the enrichment of the three carbons, C-3, C-7, and C-11, in the D,L-[2-^{13}C]mevalonolactone feeding experiment (Table 1). However, the carbons of the pyridine moiety (C-2″,

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Table 1. ^{13}C NMR Chemical Shifts,[a] Enrichment Ratio of Pyripyropene A Derived from ^{13}C-Single-Labeled Precursors, and J_{C-C} of [1,2-$^{13}C_2$]AcONa-Labeled Pyripyropene A

			enrichment ratio[d] of **1** derived from			
carbon	^{13}C chemical shift,[a] ppm	mult[b]	[1-^{13}C]AcONa[c]	[2-^{13}C]AcONa[c]	D,L-[2-^{13}C]MVL[c]	[1,2-$^{13}C_2$]AcONa[c] J_{C-C}, Hz
C-1	73.5	d	0.68	2.04	0.43	37.4
C-2	22.6	t	2.77	0.97	0.42	37.4
C-3	36.1	t	0.65	3.21	6.59	s[e]
C-4	37.8	s	1.66	0.50	0.30	35.5
C-5	54.6	d	0.63	2.10	0.41	38.5
C-6	83.1	s	1.74	0.52	0.30	38.5
C-7	77.7	d	0.74	2.50	9.28	s[e]
C-8	25.1	t	2.92	1.00	0.48	34.3
C-9	45.3	d	0.63	2.10	0.41	34.3
C-10	40.2	s	1.98	0.60	0.33	35.5
C-11	64.8	t	0.80	2.47	6.29	s[e]
C-12	17.4	q	0.76	2.74	0.74	35.5
C-13	60.0	d	1.96	0.78	0.39	38.5
C-14	16.2	q	0.59	2.68	0.67	38.5
C-15	13.2	q	0.98	2.44	1.00	35.5
1-OCOCH_3	21.1	q	1.00	3.71	0.65	59.5
1-OCOCH$_3$	170.0	s	2.71	0.74	0.35	59.5
7-OCOCH_3	21.2	q	0.99	4.38	0.53	60.3
7-OCOCH$_3$	170.0	s	2.15	0.82	0.35	60.3
11-OCOCH_3	20.7	q	0.92	3.01	0.53	59.9
11-OCOCH$_3$	170.8	s	3.57	0.89	0.42	59.9
C-2′	163.8	s	1.94	0.67	0.48	78.6
C-3′	102.9	s	0.80	2.02	0.41	78.6
C-4′	162.1	s	1.48	0.54	0.35	64.5
C-5′	99.3	d	0.69	2.60	0.48	64.5
C-6′	157.2	s	0.65	0.22	0.42	s[e]
C-2″	146.7	d	0.41	0.31	0.27	s[e]
C-3″	127.1	s	0.30	0.10	0.10	s[e]
C-4″	132.9	d	0.56	0.48	0.41	s[e]
C-5″	123.6	d	0.19	0.13	0.15	s[e]
C-6″	151.4	d	0.49	0.42	0.38	s[e]

[a] Each sample was dissolved in $CDCl_3$. Chemical shifts are shown with reference to $CDCl_3$ as 77.0 ppm. [b] Multiplicities determined from DEPT spectra. [c] AcONa = sodium acetate; MVL = mevalonolactone. [d] Enrichment ratios were relative to the 1-OCOCH_3 signal ([1-^{13}C]AcONa), the C-8 signal ([2-^{13}C]AcONa), and the C-15 signal ([2-^{13}C]MVL) as 1.00. [e] Signal was observed as a singlet.

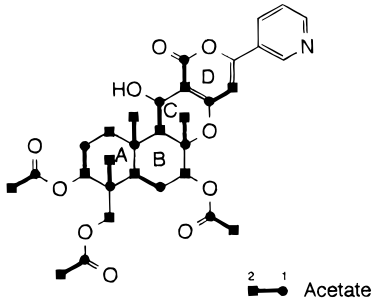
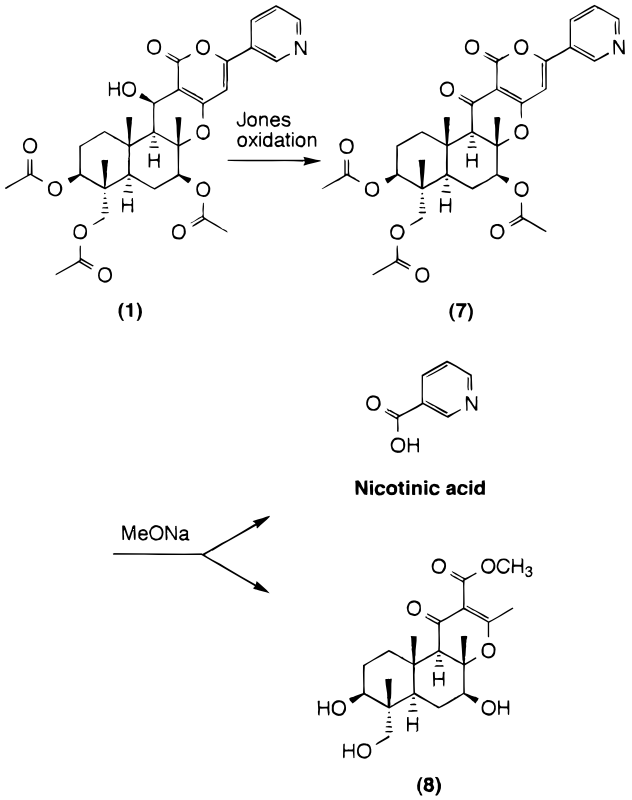


Figure 2. Summary of incorporation patterns of [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetate-enriched pyripyropene A.

C-3″, C-4″, C-5″, and C-6″) and C-6′ were not enriched in any of these [^{13}C] precursor feeding experiments (Table 1).

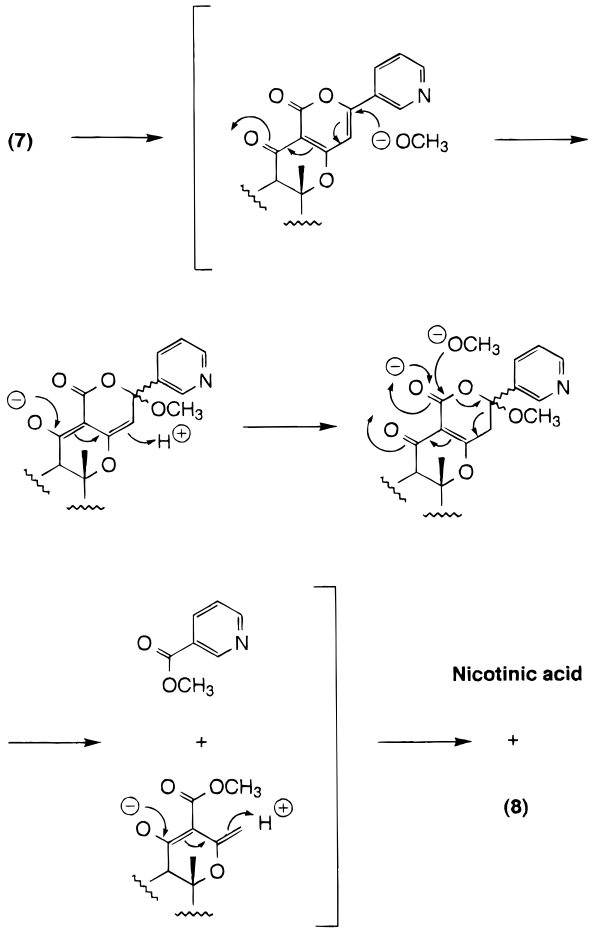
Intact [^{14}C]Nicotinic Acid Incorporation. When nicotinic acid was added to the fermentation medium, the production of **1** increased approximately 2-fold, suggesting the incorporation of nicotinic acid into the molecule. In fact, addition of [carboxy-^{14}C]nicotinic acid to the medium produced [^{14}C] labeled **1** in a yield of 12% (2.38 Ci/mol). This suggested that intact [^{14}C]nicotinic acid was incorporated into **1** without decarboxylation and that the C-6′ position was [^{14}C] labeled. To demonstrate this aspect, [carboxy-^{14}C]nicotinic acid-labeled **1** was chemically degraded to recover [^{14}C]nicotinic acid according to Scheme 1. This degradation scheme had been established earlier using unlabeled **1**. The ^{14}C-labeled **1** diluted with cold **1** (84 mCi/mol, 5.56 mg) was oxidized with Jones' reagent to yield 13-oxo **1** (**7**, 84 mCi/mol, 3.40 mg, yield 61.3%), and then **7** (2.16 mg) was treated with

Scheme 1. Degradation of Pyripyropene A



CH_3ONa to give **8** (no radioactivity, 1.21 mg, yield 85.2%) and nicotinic acid (84 mCi/mol, 0.25 mg, yield 54.7%). Thus, the same specific radioactivity between **1** and the

Scheme 2. Hypothetical Mechanism of Degradation from 7 to 8

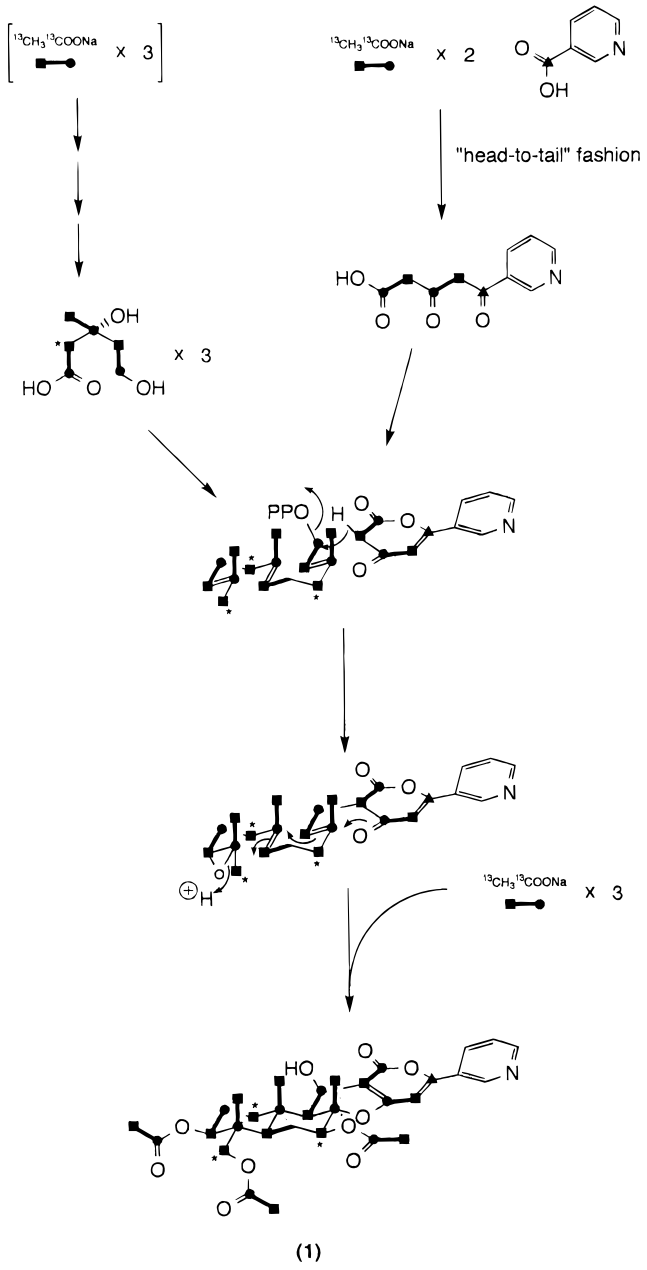


recovered nicotinic acid revealed that intact nicotinic acid was exclusively incorporated into the pyridine and C-6′ positions of **1**.

Discussion

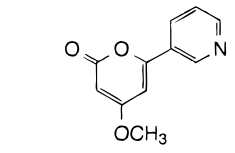
The biosynthetic origin of all the carbons in **1** was confirmed by the NMR analyses of [^{13}C]acetate- and [^{13}C]-mevalonate-enriched **1** (Figure 2 and Table 1) and the degradation experiment of [^{14}C]nicotinic acid-enriched **1** (Scheme 1). The degradation of **7** to **8** appears to be a very unique reaction. After treatment of **7** with sodium methoxide, nonradioactive **8** and radioactive methyl nicotinate would be produced. In fact, **8** was isolated by preparative HPLC, and the structure was confirmed by spectral analyses such as NMR and MS. A radioactive fraction was recovered with a retention time of 9.1 min through preparative HPLC, which did not correspond to those of methyl nicotinate or nicotinic acid. Since it was suggested to be converted to sodium nicotinate during post-treatment of the reaction, HPLC analysis was further carried out under the acidic conditions, resulting in identification of radioactive nicotinic acid. Eventually, the same specific radioactivity (84 mCi/mol) of **1**, **7**, and nicotinic acid was shown in this degradation experiment, revealing the exclusive incorporation of nicotinic acid into this specific portion of pyripyropene A. The hypothetical scheme for the degradation mechanism is shown in Scheme 2, where two molecules of sodium methoxide attack in turn the C-6′ and C-2′ carbons. The pyridino-α-pyrone moiety is derived from one nicotinic acid and

Scheme 3. Proposed Biosynthetic Pathway of Pyripyropene A

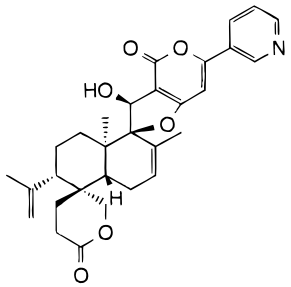


two acetates, the sesquiterpene moiety is from three mevalonates, and the three acetyl residues are from three acetates.

The proposed biosynthetic pathway of **1** is shown in Scheme 3. From the stereochemical point of view of ring junctures,[2] *all-trans*-farnesyl pyrophosphate is produced from three mevalonates. On the other hand, the acyl primer nicotinic acid is condensed sequentially with two acetates in a "head-to-tail" fashion to form 5-(3-pyridyl)-3,5-dioxopentanoic acid which is possibly converted to a pyridino-α-pyrone-like structure. Then, *all-trans*-farnesyl pyrophosphate and the putative 4-oxo-6-(3-pyridyl)-α-pyrone (pyridino-α-pyrononе) or 5-(3-pyridyl)-3,5-dioxopentanoic acid are condensed and cyclized to form the core skeleton, and finally three acetyl residues are introduced to yield **1**. Similar condensation and cyclization reactions between a polyketide and a sesquiterpene were reported for cochlinoquinones[5] production. The biosynthesis of pyripyropene A is the first demonstration that nicotinic acid is utilized as an acyl primer for

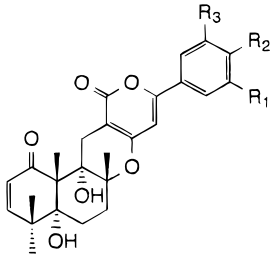


Anibine (5)



Oxalicine A (6)

Figure 3. Structures of anibine and oxalicine A.



	R_1	R_2	R_3
Territrem A (9)		$-OCH_2O-$	$-OCH_3$
Territrem B (10)	$-OCH_3$	$-OCH_3$	$-OCH_3$
Territrem C (11)	$-OCH_3$	-OH	$-OCH_3$

Figure 4. Structures of territrems.

polyketide biosynthesis. Thus, pyripyropenes are classified as a meroterpenoid, that is, a carbon skeleton derived from a mixed polyketide-terpene origin.[7] Anibine (**5**) and oxalicine A (**6**) (Figure 3) share the pyridino-α-pyrone structure, which is assumed to be biosynthesized in the same fashion. Interestingly, the structures of pyripyropenes including the stereochemistry resemble those of territrems (**9**−**11**), fungal tremorgens, and acetylcholine esterase inhibitors from *Aspergillus terreus*.[6a] The structures of territrems consist of common phenyl-α-pyrone and sesquiterpene moieties. Ling and Peng[6b] suggested that [U-^{14}C]shikimate was incorporated in the aromatic moiety and [2-^{14}C]mevalonate was incorporated into the nonaromatic moiety of territrem B (Figure 4). On the basis of our study on the biosynthesis of pyripyropene A, it is plausible that the phenyl-α-pyrone moiety in territrem B is formed by condensation of a primer benzoic acid derived from shikimate with two acetates.

Recently, eight other components of pyripyropenes have been further isolated from the culture broth of *A. fumigatus* FO-1289-2501.[8] They also contain the common skeleton comprising pyridino-α-pyrone and sesquiterpene. All of them are oxygenated at the R_3 position, but not at the R_1, R_2, and C-13 positions, suggesting that R_3 oxygenation occurs first presumably during cyclization of the farnesyl moiety initiated via an epoxidated intermediate (Scheme 3) to form the common skeleton, followed by sequential oxygenation at the R_1/R_2 and C-13 positions. Components with a free hydroxy group(s) at the R_1, R_2, and R_3 positions have not been isolated so far, suggesting that the acylation occurs immediately after the oxygenation at these positions.

Very recently, a total synthesis of (+)-pyripyropene A[9] and (±)-GERI-BP001,[10] the simplest member of the pyripyropene family, has been completed. In addition, Parker and Resnick[10] reported a "biomimetic" synthesis of the latter compound, indicating the full agreement between biosynthetic theory and practice.

Experimental Section

General Experimental Procedures. Materials. More than 99% enriched stable isotope precursors sodium [1-^{13}C]-acetate, sodium [2-^{13}C]acetate, sodium [1,2-$^{13}C_2$]acetate, and D,L-[2-^{13}C]mevalonolactone were purchased from ISOTEC. [Carboxy-^{14}C]nicotinic acid (250 μCi, 50−60 mCi/mmol) was purchased from ARC. The scintillation solution (ATOM-LIGHT) was purchased from New England Nuclear.

Pyripyropene A Production. To increase the fermentation titer of pyripyropene A, *Aspergillus fumigatus* FO-1289 (pyripyropene A production of about 1 mg/mL) was mutated with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine according to the established method. *A. fumigatus* FO-1289-2501 selected was found to produce pyripyropene A at a level 10 times greater than the parent strain. The mutant strain was grown on slants containing soluble starch 1.5%, yeast extract 0.4%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, and agar 2.0% in distilled water (pH 6.0). The slants were incubated at 27 °C and stored in tubes sealed with screw caps at room temperature. Prior to pyripyropene A production, the strain was precultivated in a 50-mL test tube (20 × 200 mm) containing 10 mL of a medium consisting of glucose 0.5%, yeast extract 0.5 %, and mannitol 0.5% in distilled water, adjusted to pH 6.0 before sterilization. The tube was shaken reciprocally at 300 rpm at 27 °C for 72 h. One milliliter of this culture broth was inoculated into a 500-mL Erlenmeyer flask containing 100 mL of a medium consisting of soluble starch 3.0%, glycerol 1.0%, soybean meal 2.0%, dry yeast 0.3%, KCl 0.3%, KH_2PO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, and $CaCO_3$ 0.2% in distilled water, adjusted to pH 6.5 before sterilization. The flask was incubated at 27 °C on a shaker (300 rpm) for 192 h. Maximum yield at 144 h of pyripyropene A under these conditions was estimated by HPLC to be 14.4 mg/L.

A nicotinic acid solution (Merck) (0.002% in H_2O adjusted to pH 6.5,) after filtration−sterilization was added at 24-h cultivation to a flask containing 100 mL of the same culture medium. Under these conditions, pyripyropene A production increased 1.8-fold with a maximum yield of 25.6 mg/L at 144 h.

[^{13}C]Acetate Incorporation. A solution of 100 mg of sodium [1-^{13}C]-, [2-^{13}C]-, or [1,2-$^{13}C_2$]acetate after filtration−sterilization, with pH adjusted to 6.5, was added at 72-h cultivation to each of three flasks containing 100 mL of culture medium (nicotinic acid 0.002%, soluble starch 2.0%, glycerol 0.67%, soybean meal 2.0%, dry yeast 0.3%, KCl 0.3%, KH_2PO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, and $CaCO_3$ 0.2%), and incubation was continued for 144 h. Peak intensity enhancements at the specific carbon signals were 1.48- to 3.57-fold ([1-^{13}C]acetate) and 2.02- to 4.38-fold ([2-^{13}C]acetate), respectively.

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D,L-[2-^{13}C]Mevalonolactone Incorporation. To a flask containing 100 mL of the same culture medium as that in the [^{13}C]acetate incorporation experiment was added an ethanol solution of 100 mg of D,L-[2-^{13}C]mevalonolactone in the same manner at 72-h cultivation, and incubation was continued for 144 h. Peak intensity enhancements at the specific carbon signals were 6.29- to 9.28-fold.

[Carboxy-^{14}C]nicotinic Acid Incorporation. A solution of 250 μCi of [carboxy-^{14}C]nicotinic acid (50−60 mCi/mmol) after filtration−sterilization, adjusted to pH 6.5, was added at 48-h cultivation to the flask containing 100 mL of culture medium (soluble starch 3.0%, glycerol 1.0%, soybean meal 2.0%, dry yeast 0.3%, KCl 0.3%, KH_2PO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, and $CaCO_3$ 0.2%). The fermentation was run for 144 h. ^{14}C-Labeled pyripyropene A of specific activity 2.38 Ci/mol was obtained after the usual workup.

Isolation Procedures. The whole fermentation broth (100 mL) was centrifuged at 3000 rpm for 5 min. The supernatant was extracted three times with an equal volume of ethyl acetate. To the mycelia was added 80% aqueous acetone (100 mL) to extract pyripyropenes. After centrifugation of this suspension at 3000 rpm for 5 min, the supernatant was concentrated to remove acetone. The aqueous solution was extracted three times with an equal volume of ethyl acetate. The ethyl acetate extracts of the broth and the mycelia were combined and concentrated. The residue was purified by preparative HPLC using an ODS column (YMC pack D-ODS-5 AM 343 (20 × 250 mm); UV at 232 nm; 45% acetonitrile at 6 mL/min). Under these conditions, pyripyropene A was eluted with a retention time of 49.5 min.

Degradation of ^{14}C-Labeled 1. [^{14}C]Nicotinic acid-labeled **1** (2.38 Ci/mol) was diluted with cold **1** to yield the specific radioactivity of 84 mCi/mol. The degradation scheme is shown in Scheme 1. To a solution of pyripyropene A (5.56 mg, 9.54 μmol, 84 mCi/mol) in acetone:H_2O (3.48 mL:170 μL) was added Jones reagent (3 M CrO_3, H_2SO_4) (50 μL). After the mixture was stirred for 2 h at room temperature, isopropyl alcohol (20 μL) was added. The reaction mixture was filtered and concentrated. The resulting solution was extracted with ethyl acetate (30 mL), washed with brine (20 mL), and dried over Na_2SO_4. Preparative HPLC of the residue (YMC pack D-ODS-5 AM 343 (20 × 250 mm); UV at 225 nm; 55% acetonitrile at 6 mL/min) afforded **7** (3.40 mg, 5.85 μmol, yield 61.3%, 84 mCi/mol). Under these conditions pyripyropene A and **7** were eluted with retention times of 26.0 and 24.0 min, respectively.

7: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (d, J = 3.0 Hz, H-1), 8.73 (dd, J = 5.0, 2.0 Hz, H-1), 8.16 (ddd, J = 8.0, 3.0, 2.0 Hz, H-1), 7.43 (dd, J = 8.0, 5.0 Hz, H-1), 6.49 (s, H-1), 5.22 (dd, J = 11.5, 5.3 Hz, H-1), 4.77 (dd, J = 11.5, 5.0 Hz, H-1), 3.75 (d, J = 12.0 Hz, H-1), 3.70 (d, J = 12.0 Hz, H-1), 2.77 (dq, J = 14.0, 4.0 Hz, H-1), 2.61 (s, H-1), 2.15 (s, H-3), 2.10 (s, H-3), 2.02 (s, H-3), 1.72−1.83 (m, H-4), 1.58 (m, H-1), 1.55 (s, H-3), 1.51 (m, H-1), 1.21 (s, H-3), and 0.85 (s, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.0, 172.1, 170.9, 170.4, 169.8, 162.5, 156.6, 152.6, 147.4, 133.7, 126.4, 123.7, 100.5, 97.7, 87.4, 76.6, 73.3, 64.8, 62.2, 44.6, 40.4, 37.2, 36.8, 24.8, 22.8, 21.12, 21.07, 20.7, 16.0, 15.5, and 13.2; mp 166−167 °C; HRMS calcd for $C_{31}H_{36}NO_{10}$ 582.2339, found 582.2347.

A solution of CH_3ONa (2.74 mg, 0.05 mmol) in 360 μL of H_2O was added to a solution of **7** (2.16 mg, 3.72 μmol, 84 mCi/mol) in methanol (360 μL), and the resultant mixture was stirred for 6 h at room temperature. This resulting mixture was separated directly by preparative HPLC under the following conditions: Shiseido Capcell Pak C18 AG120 (20 × 250 mm); UV detection at 225 nm; 20% CH_3CN at 6 mL/min. Under the conditions, a radioactive fraction and nonradioactive **8** were eluted with retention times of 9.1 and 39.0, respectively. The later fraction was concentrated to dryness to give **8** (1.21 mg, 3.17 μmol, yield 85.2 %).

8: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.97 (dd, J = 12.0, 5.0 Hz, H-1), 3.79 (s, H-3), 3.70 (d, J = 10.0 Hz, H-1), 3.68 (m, H-1), 3.49 (s, H-1), 3.39 (m, H-1), 2.85 (brs, H-1), 2.68 (dt, J = 14.0, 3.5 Hz, H-1), 2.35 (s, H-1), 2.16 (s, H-3), 1.79 (m, H-1), 1.78 (m, H-2), 1.61 (m, H-1), 1.58 (s, H-3), 1.47 (m, H-1), 1.40 (m, H-3), and 1.28 (s, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.5, 173.4, 166.2, 111.6, 87.6, 75.9, 74.4, 69.2, 60.6, 52.0, 50.9, 45.6, 42.1, 37.1, 26.9, 26.7, 20.6, 16.1, 14.2, and 11.6; HRMS calcd for $C_{20}H_{31}O_7$ 383.2070, found 383.2108.

The former radioactive fraction was concentrated under basic conditions, and the resulting material dissolved in 500 μL of water was analyzed by HPLC under acidic conditions: YMC pack-D-ODS-5 AM-343 (20 × 250 mm); 10% CH_3CN in 0.05% acetic acid at 6 mL/min; UV at 225 nm. After 5 μL injection, one peak appeared with a retention time of 17 min, which was identical with that of authentic nicotinic acid. The amount and radioactivity of nicotinic acid was calculated to be 20 nmol and 3750 dpm, respectively, showing the same specific radioactivity of 84 mCi/mol as those of the starting material (pyripyropene A) and **7**.

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra of **7** and **8** (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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