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Biosynthesis of the Fungal Polyketide Antibiotics TMC-151s: Origin of the Carbon Skeleton

JUN KOHNO*, MAKI NISHIO, NOBORU KISHI, TORU OKUDA and SABURO KOMATSUBARA

Discovery Research Laboratory, Tanabe Seiyaku Co., Ltd., 2-50, Kawagishi-2-chome, Toda-shi, Saitama 335-8505, Japan

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As a result of our continuing search for microbial metabolites, especially for the products of hypocreales, we have discovered novel cytotoxic antibiotics TMC-151A~F[1] and their 14,15-dedihydro analogs, TMC-154 and TMC-171A~C[2] from *Gliocladium* spp. TMC-151 family has an unusual, highly methylated, polyketide skeleton. Interestingly, the polyketide carbon skeleton (C-1 ~C-14 and C-21~C-27) of TMC-151 family are identical with that of 6-deoxyerythronolide, the first macrolide intermediate in erythromycins biosynthesis by actinomycetes.[3,4] This intermediate is known to be derived from seven propionate units. Fungal polyketide metabolites are, however, known to be biosynthesized from acetate and methionine.[5] In this regard, we took a great interest in the origin of the polyketide carbon skeleton of the TMC-151 family.

In this paper, we report the biosynthetic origin of polyketide carbon skeleton of TMC-151s, obtained by feeding experiments.

Materials and Methods

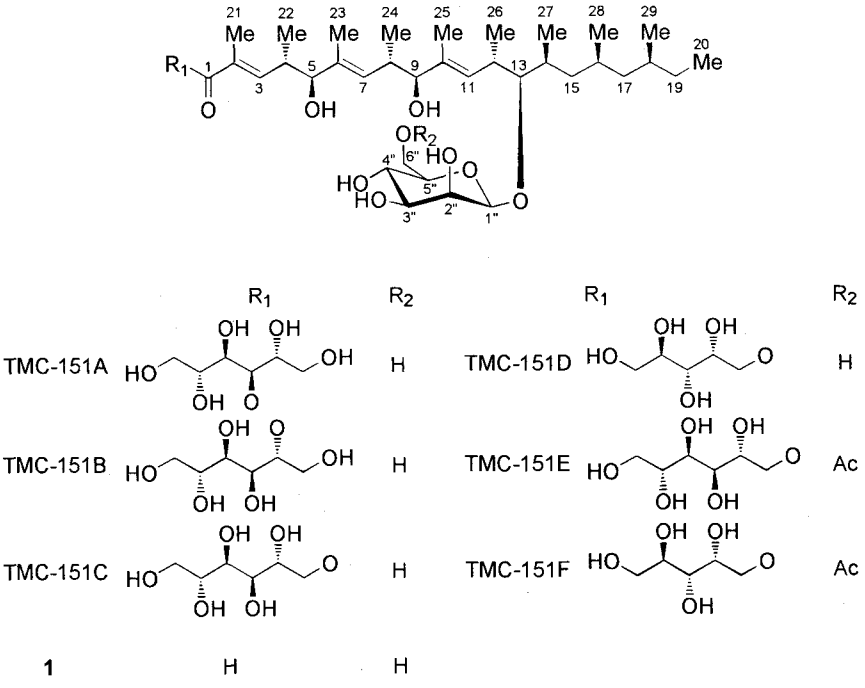
Labeled Compounds

^{13}C-labeled compounds were 99% ^{13}C atom purity. Sodium [1-^{13}C]acetate, sodium [2-^{13}C]acetate, sodium [1,2-$^{13}C_2$]acetate, sodium [1-^{13}C]propionate and L-[*Me*-^{13}C]-methionine were purchased from Aldrich Chemical Co.

Incorporation of Stable Isotope-labeled Precursors into **1**

Gliocladium catenulatum TC 1280 was inoculated into 250 ml Erlenmeyer flask containing pressed barley 10 g, yeast extract 0.02 g, Na tartrate 0.01 g, KH_2PO_4 0.01 g, and deionized water 10 ml. The static fermentation was conducted at 25°C. Each ^{13}C-enriched carbon source (132 mg [1-^{13}C]acetate, 132 mg [2-^{13}C]acetate, 132 mg [1,2-$^{13}C_2$]acetate, 230 mg [1-^{13}C]propionate, 164 mg L-[*Me*-^{13}C]methionine) was dissolved in 10 ml of sterile water and fed to the growing culture on the 7th day of cultivation. The

Fig. 1. Structures of TMC-151A~F and **1**.



incubation was continued for an additional 5 days.

Isolation of **1**

Each resultant solid obtained from two flasks was extracted with 1-butanol (80 ml) by shaking on a shaker at room temperature for 30 minutes. The solvent layer was separated and concentrated *in vacuo* to dryness. The residue was suspended in water (20 ml), washed twice with EtOAc (20 ml), and then extracted twice with 1-butanol (10 ml) to afford a crude solid containing of TMC-151 complex.

Hydrolysis was accomplished by the addition of 4 ml of 0.4 N KOH to the crude solid. After two hours at room temperature, the reaction mixture was neutralized with 1 N HCl, and then extracted twice with 1-butanol (2 ml) to give a crude ^{13}C-enriched **1**. The product was purified by column chromatography on a silica gel (EtOAc - MeOH - H_2O= 30 : 3 : 1 as an eluent). After Sehadex LH-20 column chromatography (CH_2Cl_2 - MeOH = 1 : 1), the pure ^{13}C-enriched **1** was obtained in the following amounts: [1-^{13}C]acetate, 41.5 mg; [2-^{13}C]acetate, 52.8 mg; [1,2-$^{13}C_2$]acetate, 34.1 mg; [1-^{13}C]propionate, 43.1 mg; L-[*Me*-^{13}C]methionine, 16.7 mg.

NMR

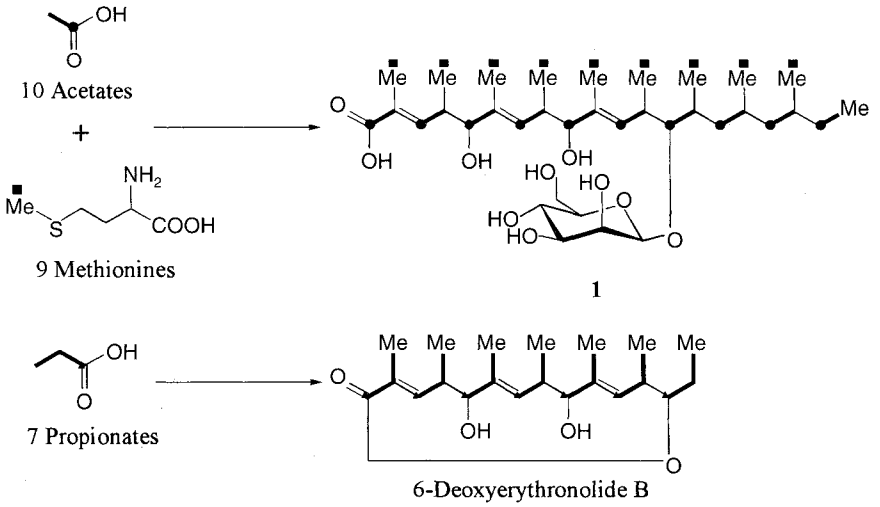
^{13}C-NMR spectra were recorded on a JEOL GSX-400

Table 1. Relative ^{13}C-enrichments in **1** obtained from feeding experiments with ^{13}C-labeled precursors.

Position	δ (ppm)	Enrichment ratios[a] of 1 derived from [1-^{13}C]-Acetate	[2-^{13}C]-Acetate	[1-^{13}C]-Propionate	L-[*Me*-^{13}C]-Methionine	[1,2-$^{13}C_2$]-Acetate $J_{C\text{-}C}$ (Hz)
1	169.0	**5.8**	1.9	1.4	1.1	68.6
2	126.8	1.3	**5.2**	1.4	0.8	68.6
3	145.8	**4.6**	1.6	1.4	0.9	43.0
4	36.9	1.0	**3.8**	1.0	1.0	43.0
5	80.6	**2.8**	1.1	0.9	0.9	47.1
6	136.1	0.9	**3.7**	1.0	1.0	47.1
7	131.1	**3.3**	1.1	1.0	1.0	43.8
8	36.2	1.0	**3.7**	1.0	1.0	43.8
9	81.1	**3.1**	1.1	1.0	1.0	47.1
10	134.6	0.9	**3.4**	0.8	0.9	47.1
11	130.0	**2.7**	1.0	0.9	0.9	43.0
12	34.5	0.9	**3.6**	1.0	1.0	43.0
13	85.3	**3.6**	1.1	1.1	1.0	38.9
14	33.0	1.0	**3.4**	1.0	1.0	38.0
15	42.1	**3.8**	1.0	1.0	1.0	34.7
16	27.2	1.0	**3.5**	1.0	0.9	34.7
17	43.8	**3.9**	1.0	1.0	1.1	34.7
18	30.9	1.0	**3.4**	1.1	1.0	34.7
19	28.0	**3.9**	1.1	1.0	1.0	34.7
20	10.8	1.0	**3.5**	1.0	1.0	34.7
21	12.4	1.0	1.1	0.9	**4.4**	
22	16.5	1.1	1.2	1.1	**4.9**	
23	11.3	1.0	1.3	1.1	**5.2**	
24	17.4	1.0	1.2	1.0	**4.4**	
25	11.2	0.9	1.2	1.0	**4.3**	
26	18.2	1.0	1.2	1.1	**5.1**	
27	15.8	1.0	1.1	1.0	**4.8**	
28	20.9	1.0	1.2	1.1	**4.9**	
29	20.0	1.0	1.2	1.1	**5.5**	
1"	101.3	1.0	1.0	1.0	1.0	
2"	70.7	1.0	1.1	1.1	1.0	
3"	73.9	1.0	1.0	1.0	1.0	
4"	67.0	1.1	1.0	1.1	1.0	
5"	77.3	0.9	1.1	1.0	0.9	
6"	61.4	1.0	1.1	1.0	1.0	

[a] Enrichment ratios were normalized to peak intensities for the C-1" signal.

Fig. 2. Biosynthesis of **1** and 6-deoxyerythronolide.



NMR spectrometer at 30°C. Samples of **1** (10 mg), natural abundance and ^{13}C-enriched, were dissolved in DMSO-d_6 (0.6 ml). Flip angle: 45°; memory size: 16k; relaxation delay: 1.0 s; scans: 18,000. The relative ^{13}C-enrichment of each carbon was determined by measuring the ratio of each signal intensity to the signal intensity of unenriched position (C-1″).

Results and Discussion

The different samples of **1** were obtained from alkaline hydrolysis of TMC-151 complex; the resulting ^{13}C NMR data are shown in Table 1.

In the experiment with [1-^{13}C]acetate, significant enrichment was observed in carbons C-1, 3, 5, 7, 9, 11, 13, 15, 17 and 19, and with [2-^{13}C]acetate, observed in carbons C-2, 4, 6, 8, 10, 12, 14, 16, 18 and 20. The feeding experiment using [1,2-$^{13}C_2$]acetate followed by analysis of ^{13}C-^{13}C couplings established the incorporation pattern of intact acetate units. Methyl carbons C-21~C-29 were labeled by L-[*Me*-^{13}C]methionine. On the other hand, none of the carbons in **1** were labeled by [1-^{13}C]propionate.

These results demonstrated that the polyketide carbon skeleton of TMC-151s was derived from ten acetates and nine methionines as illustrated in Fig. 2.

In this study, TMC-151s have been proved to be biosynthesized in the same manner as most fungal polyketides, *via* acetate and methionine; this is in contrast to the biogenetic origin of polyketide carbon skeletons in erythromycins. It is very interesting that the identical carbon skeleton is biosynthesized *via* the different pathways in fungi and actinomycetes. In addition, TMC-151s are unusual non-aromatic fungal polyketides possessing many *C*-methyl groups derived from methionine. The other fungal polyketide of this type is, to the best of our knowledge, only radiclonic acid.[6] Recently, we have found that production of TMC-151s, TMC-154 and TMC-171s was specific to *Gliocladium roseum* group.[7] Thus, studying the enzymology and molecular biology of polyketide biosynthesis in *Gliocladium* species would advance our overall understanding of natural product biosynthesis in filamentous fungi. In addition, such knowledge would provide the opportunity to apply combinatorial-biology to the production of novel polyketides in fungi.[8]

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