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Biosynthesis of the Monoterpenoid Moiety of Teleocidins *via* the Non-mevalonate Pathway in *Streptomyces*

Kazuhiro Irie*, Yu Nakagawa, Satoru Tomimatsu, and Hajime Ohigashi

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

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Abstract

The monoterpenoid moiety of teleocidin B-4 was shown to be biosynthesized from D-glucose *via* the non-mevalonate pathway proposed by Rohmer *et al.* using *Streptomyces blastmyceticum* NA34-17.

Keywords: biosynthesis; non-mevalonate pathway; *Streptomyces*; teleocidin; terpenoid

Tumor promoter teleocidins produced by *Streptomyces* are peculiar indole alkaloids with a variety of monoterpenoid moiety at positions 6 and 7 of the indole ring [1]. In 1990, we proposed a possible biosynthetic pathway of teleocidins based on feeding experiments with ^{2}H- or ^{13}C-labelled precursors and isolation of several key biosynthetic intermediates using *Streptomyces blastmyceticum* NA34-17 [2,3]. L-Tryptophan and L-valine proved to be important building blocks of the nine-membered lactam ring. The C-18 and C-29 methyl groups of teleocidin B-4 derived from L-methionine-d_3. However, as for the monoterpenoid moiety from C-19 to C-28 of teleocidin B-4, we could not identify suitable precursors. The most plausible candidates such as DL-[2-^{13}C]mevalonolactone, [1,2-$^{13}C_2$]sodium acetate, and L-leucine-d_3 gave only disappointing results [3].

Recently, Rohmer *et al.* [4,5] proposed the non-mevalonate pathway in the biosynthesis of some *Streptomyces* metabolites. Seto *et al.* [6,7] also added two examples showing that *Streptomyces* utilizes the non-mevalonate pathway for the formation of isopentenyl diphosphate (IPP). These results prompted us to examine the involvement of the non-mevalonate pathway in the biosynthesis of the monoterpenoid moiety of teleocidins.

S. blastmyceticum NA34-17 [8] maintained in Waksman's medium was transferred to a 500 mL shaking flask containing 100 mL of a medium consisting of 1% glucose, 1% polypeptone, 1% meat extract and 0.5% NaCl (pH 7.0), and the flask was shaken at 30 °C for three days. Two milliliters of the seed culture thus obtained was transferred to a 500 mL shaking flask containing 100 mL of a medium (1% polypeptone, 1% meat extract, 0.5% NaCl, and 1% D-[1-^{13}C]glucose or 2% [1-^{13}C]sodium acetate), and the flask was further shaken for 5 days. The mycelia were filtered and steeped in acetone. The acetone extracts were purified by the method reported previously [3] to give teleocidin B-4 at a yield of 3.1 mg/100 mL for cultivation with D-[1-^{13}C]glucose, and 0.2 mg/100 mL for [1-^{13}C]sodium acetate. The extremely low yield in the [1-^{13}C]sodium acetate-cultivation possibly dues to the slow growth rate of the microorganism.

The ^{13}C NMR spectrum of teleocidin B-4 derived from D-[1-^{13}C]glucose showed clear increment of the signals of C-20, C-22, C-24, and C-28 in the monoterpenoid moiety (Table 1). Significant incorporation of the label into these positions is explained by glycolysis of the labeled glucose to [3-^{13}C]pyruvic acid and [3-^{13}C]glyceraldehyde 3-phosphate to form [1,5-$^{13}C_2$]IPP by the non-mevalonate pathway [4,5]. The intensity of C-21, C-23, C-25, and C-27 signals is similar to that of background C-19 and C-26 signals, excluding the mevalonate pathway. Incorporation of the label from D-[1-^{13}C]glucose into C-2 of the indole ring is compatible with the shikimic acid pathway where tryptophan derives from phosphoenol pyruvate and erythrose 4-phosphate.

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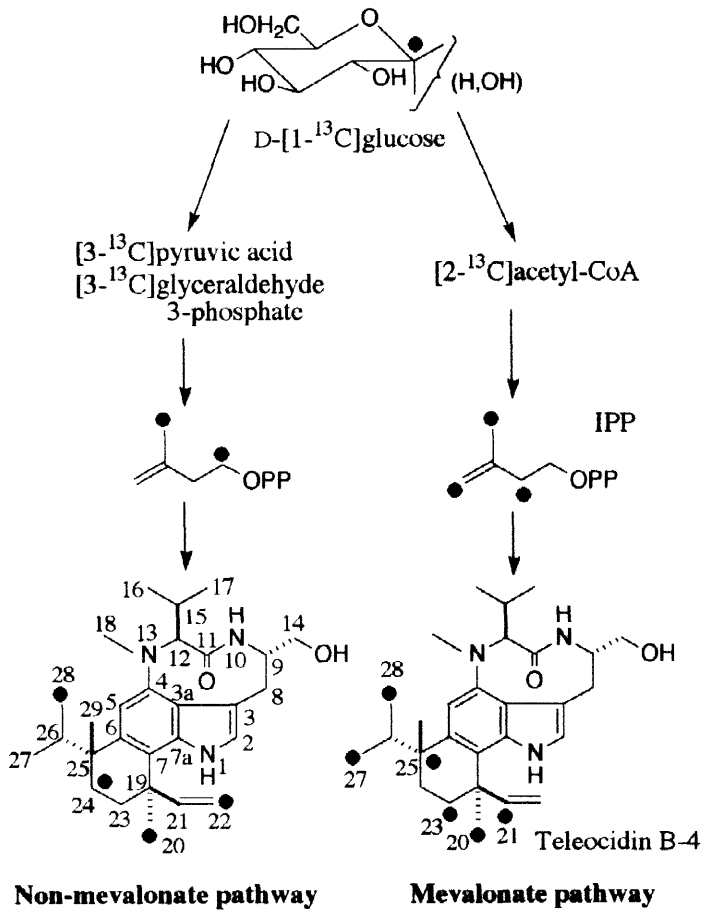

Figure 1. Expected labelling patterns of teleocidin B-4 *via* non-mevalonate and mevalonate pathway.

Table 1. ^{13}C-Chemical shifts and normalized peak hight of teleocidin B-4 derived from D-[1-^{13}C]glucose.

Carbon	Chemical shift[a] δ (ppm)	Normalized peak hight[b]
2	120.87	17.1
3	113.89	1.3
3a	116.73	1.1
4	146.03	6.1
5	106.17	1.0
6	137.85	8.4
7	118.04	1.4
7a	138.72	1.4
8	33.95	4.4
9	55.61	1.4
11	173.59	2.2
12	70.88	2.3
14	65.21	1.9
15	28.46	1.7
16	19.61	8.3
17	21.65	5.5
18	32.89	1.5
19	39.63	4.1
20	21.55	23.9
21	151.89	3.7
22	111.34	32.9
23	34.82	3.0
24	24.99	30.3
25	40.06	3.5
26	37.90	4.5
27	16.97	4.1
28	18.02	24.9
29	29.08	1.5

[a] Twist conformer (0.013 M, 125 MHz, $CDCl_3$, 300K).
[b] The signal intensities were corrected by those of unlabeled teleocidin B-4 and normalized to C-5.

In contrast to this observation, the ^{13}C NMR spectrum of teleocidin B-4 derived from [1-^{13}C]sodium acetate showed significant increment of only the C-23 and C-27 signals in the monoterpenoid moiety (data not shown). Although we currently can not explain how this labelling pattern arises, this pattern does not also fit the conventional mevalonate pathway shown in Fig. 1. Overall, the above results indicate that *S. blastmyceticum* NA34-17 exclusively uses the non-mevalonate pathway in the synthesis of teleocidin B-4. Recently, further additional example showing that *Streptomyces* utilizes the non-mevalonate pathway for the formation of IPP has been reported [9]. The next efforts are directed to isolate the enzymes involved in the biosynthesis of teleocidins.

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