Pergamon

Tetrahedron Letters 40 (1999) 4353–4354

TETRAHEDRON LETTERS

Biosynthesis of Diterpenoid Moiety of Brasilicardin A *via* Non-mevalonate Pathway in *Nocardia brasiliensis*

Hideyuki Shigemori, Hisayuki Komaki[a], Katsukiyo Yazawa[a], Yuzuru Mikami[a], Akira Nemoto[b], Yasushi Tanaka[b], and Jun'ichi Kobayashi*

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, [a]Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 260-8673, Japan, and [b]Research Laboratory, Higeta Shoyu Co., Ltd., Chiba 288-8680, Japan

Received 4 March 1999; revised 23 March 1999; accepted 26 March 1999

Abstract: The diterpenoid moiety of brasilicardin A (**1**), isolated from the actinomycete *Nocardia brasiliensis* IFM 0406, was shown to be biosynthesized from D-glucose *via* the non-mevalonate pathway.

Keywords: biosynthesis; non-mevalonate pathway; Nocardia; terpenoids

In our continuing search for new bioactive metabolites from pathogenic actinomycete *Nocardia* strains, we have isolated a novel tricyclic metabolite, brasilicardin A (**1**), with potent immunosuppressive activity from *N. brasiliensis* IFM 0406.[1,2] The perhydrophenanthrene skeleton of **1** seems to be derived from isopentenyl diphosphates. Recently, the non-mevalonate pathway in the biosynthesis of terpenoids from actinomycetes *Streptomyces* was proposed.[3,4] These results promoted us to examine the biosynthetic pathway of the aglycon of brasilicardin A (**1**).

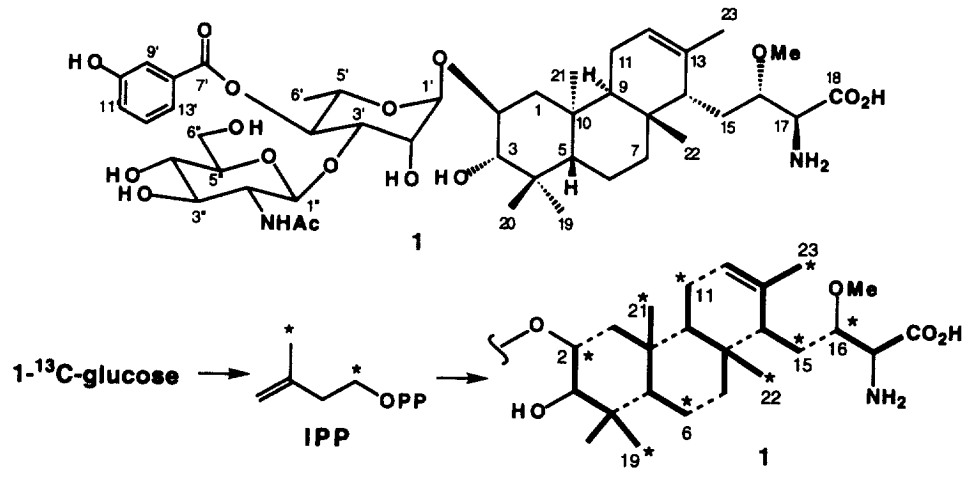

Figure 1. ^{13}C-Labelling pattern in brasilicardin A (**1**) derived from [1-^{13}C]glucose

0040-4039/99/$ - see front matter
PII: S0040-4039(99)00689-9

Table 1. ^{13}C-Chemical shifts and normalized peak height of brasilicardin A (**1**) derived from D-[1-^{13}C]glucose

carbon	chemical shift	peak height[a]	carbon	chemical shift	peak height[a]
1	44.47	2.0	13	138.96	2.7
2	80.26	13.1	14	52.75	1.0
3	83.78	1.3	15	32.45	22.2
4	41.67	1.6	16	80.86	21.3
5	46.73	1.0	OMe	59.01	2.8
6	19.18	14.9	17	55.07	1.9
7	31.76	1.8	18	170.35	4.0
8	39.02	4.1	19	17.79	10.1
9	47.88	1.0	20	29.71	2.1
10	38.06	1.5	21	29.39	10.7
11	27.55	33.0	22	23.00	21.1
12	124.11	1.9	23	23.02	13.9

a) The signal intensities were corrected by those of unlabeled **1** and normalized to C-9.

One loopful of the culture from slant culture of *N. brasiliensis* IFM 0406 was inoculated into 100 mL-Erlenmeyer flasks containing 20 mL of a seed culture medium (2% glycerol-enriched brain heart infusion medium) and the inoculated flasks were shaken at 32 °C for 4 days. Ten milliliters of the seed cultures were inoculated into 500-mL shake flasks containing 100 mL of the production medium (0.3 % glucose, 1 % polypeptone, and 0.6 % beef extract, pH 7.0), and incubated at 32 °C. After incubation for 9 h, D-[1-^{13}C]glucose (at a final concentration of 0.2 %) was added to the culture and cultivation was continued at 32 °C for 39 h. The supernatant of the fermentation broth was applied to a Diaion HP-20 column and further purified by the method described previously.[1,2] From 1 L of culture broth, 25 mg of brasilicardin A (**1**) was obtained.

The ^{13}C NMR spectrum of brasilicardin A (**1**) derived from D-[1-^{13}C]glucose showed clear increment of the signals of C-2, C-6, C-11, C-15, C-19, C-21, C-22, and C-23 in the perhydrophenanthrene skeleton (Fig. 1 and Table 1), indicating that the perhydrophenanthrene skeleton was a tricyclic diterpenoid derived from geranylgeranyl phosphate. The increment of a signal of C-16 suggested that the amino acid moiety (C-16 ~ C-18) might be derived from [3-^{13}C]pyruvate. Incorporation of the label into these positions is explained by glycolysis of the labeled glucose to [3-^{13}C]pyruvate and [3-^{13}C]glyceraldehyde 3-phosphate to form [1,5-$^{13}C_2$]isopentenyl diphosphate (IPP) by operation of the non-mevalonate pathway (Fig. 1).[5] Therefore, these results indicate that *N. brasiliensis* uses the non-mevalonate pathway in the synthesis of brasilicardin A (**1**). This is the first example showing that *Nocardia* utilizes the non-mevalonate pathway for the formation of IPP.

Acknowledgment: This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

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