COMMUNICATIONS TO THE EDITOR

Studies on the Biosynthesis of Terpenoidal Compounds Produced by Actinomycetes

2. Biosynthesis of Carquinostatin B *via* the Non-mevalonate Pathway in *Streptomyces exfoliatus*

Sir:

In contrast to vast number of terpenoid compounds produced by plants and fungi (more than 22,000), *Streptomyces* produce only small number of terpenoid compounds. Among them, naphterpin[1], furaquinocins[2], napyradiomycins[3] and terpentecin[4] were proved to be biosynthesized *via* the classical mevalonate pathway. Very curiously, however, the sesquiterpene pentalenolactone which is produced by several kinds of *Streptomyces* species, is synthesized *via* the non-mevalonate pathway[5] which has recently been proposed by ROHMER *et al.*[6,7] These results surprisingly imply that isopentenyl diphosphate (IPP), the building unit of terpenoids, is formed by the mevalonate pathway in some *Streptomyces* species, while it is prepared by a completely different pathway, *i.e.*, the non-mevalonate pathway in other *Streptomyces* species.

During studies for pursuing this intriguing problem, we reported that the producing organism of naphterpin, *Streptomyces aeriouvifer*, utilized the non-mevalonate pathway for the production of IPP at the earlier fermentation stage and that this pathway was gradually replaced by the mevalonate pathway as the fermentation proceeded[8].

As a further step to investigate this interesting phenomenon, we chose carquinostatin B[9] as a *Streptomyces* metabolite with the simplest terpenoid unit in the molecule (Fig. 1) and studied its biosynthesis by the use of ^{13}C-labeled precursors.

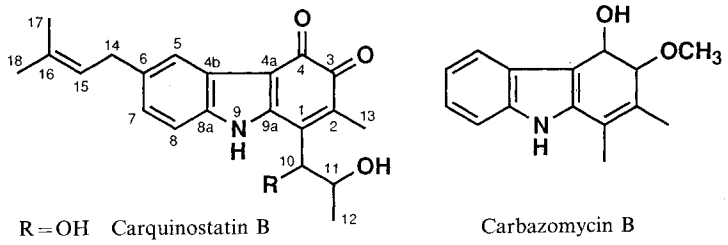
Carquinostatin B was isolated as a congener of carquinostatin A[10], a neuronal cell protecting substance from *Streptomyces exfoliatus* 2419-SVT2 in our laboratory†. The carquinostatins show structural similarity to carbazomycins[11,12] whose biosynthetic pathway had been clearly elucidated by labeling experiments using ^{13}C-labeled tryptophan[13] and alanine[14], a biological equivalent of pyruvic acid. Carquinostatins are assumed to be formed by introduction of a dimethylallyl side chain to the carbazole skeleton which is formed in a similar way as reported for carbazomycin[14].

A stock culture of *S. exfoliatus* 2419-SVT2 was inoculated to a 15 ml test tube containing a seed medium consisting of starch 1.0%, polypeptone 1.0%, molasses 1.0% and beef extract 1.0, pH 7.2. After incubation for 24 hours at 27°C on a rotary shaker, 2 ml of the culture was transferred to 500 ml Erlenmeyer flasks containing 100 ml of the production medium (dextrin 2.5%, soybean meal 2.2%, dried yeast 0.2% and $CaCO_3$ 0.4%, pH 6.2) and the fermentation was conducted on a rotary shaker for 24 hours at 27°C.

Sodium [1-^{13}C]acetate (1 mg/ml), sodium [2-^{13}C]-acetate (2 mg/ml) and [1-^{13}C]glucose (2 mg/ml) were separately added to the medium 9 hours after initiation of the fermentation. After cultivation for a total of 24 hours, the differently labeled samples of carquinostatin B were obtained according to the method previously reported[9].

The incorporation experiment using sodium [1-^{13}C]-acetate revealed that C-1 and C-11 of carquinostatin B were selectively labeled by the precursor with no in-

Fig. 1. Structures of carquinostatins B and A, and carbazomycin B.



For part 1 of this series, see ref 8.

† During storage of *S. exfoliatus* for several years, this strain has changed to produce carquinostatin B as the major component.

corporation into the dimethylallyl side chain (Table 1). A decreased production yield of the labeled carquinostatin caused by addition of sodium acetate precluded quantitative determination of the signal intensities. However, the signal intensities of C-1 and C-11 of carquinostatin B were apparently increased by about 6.5 and 5.5 times by addition of the ^{13}C-labeled precursor (Table 1). Use of sodium [2-^{13}C]acetate enriched the signal intensities of C-10 and C-12 by *ca.* 3.0 and 5.5, respectively. These experimental results proved clearly that only C-1, C-10, C-11 and C-12 of carquinostatin B are derived from acetic acid. The lack of incorporation of the labeled acetates into the isoprenoid side chain precludes the operation of the mevalonate pathway in the formation of IPP in *S. exfoliatus*.

In contrast, addition of [1-^{13}C]glucose to the fermentation broth increased *inter alia* the signal intensities of C-14 and C-17 in the dimethylally side chain (Table 1 and Fig. 2). Incorporation of the label into these two 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-$^{13}C_2$]IPP by operation of the non-mevalonate pathway[7]. Therefore, it is concluded that the carquinostatin producing organism *S. exfoliatus*

Table 1. ^{13}C-chemical shifts of carquinostatin B and incorporation of the ^{13}labeled precursors.

Carbon	Normalized peak height			
	Chemical shift	[1-^{13}C] AcOH	[2-^{13}C] AcOH	[1-^{13}C] glucose
1	148.05	6.5		1.6
2	138.64			1.8
3	190.07			1.0
4	178.51			2.2
4a	117.30			1.1
4b	131.33			1.0
5	125.09	1.4		2.5
6	143.49			1.4
7	131.06	1.4		1.6
8	119.99	1.0		1.3
8a	141.52			3.4
9a	150.59			2.3
10	79.96		3.5	4.4
11	75.30	5.5		1.1
12	25.58	1.4	5.0	5.6
13	18.19	1.3	1.3	5.4
14	39.91	1.3		5.4
15	129.76	1.2		1.3
16	137.62			1.9
17	23.75	1.0	1.0	4.7
18	31.58	1.0		1.2

The signal intensities were normalized to C-17, C-17 and C-4b for the samples labeled with [1-^{13}C]acetic acid, [2-^{13}C]acetic acid and [1-^{13}C]glucose, respectively.

Fig. 2. The ^{13}C-NMR spectrum of carquinostatin B labeled by [1-^{13}C]glucose. Closed circles show signals enriched by the precursor.

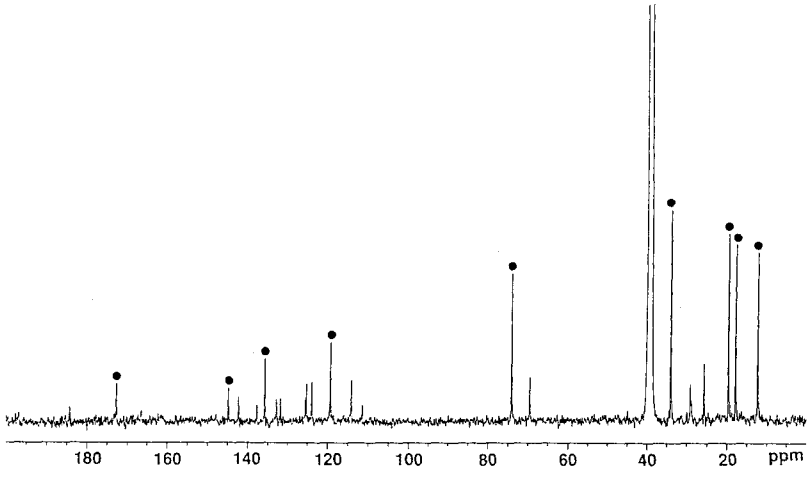
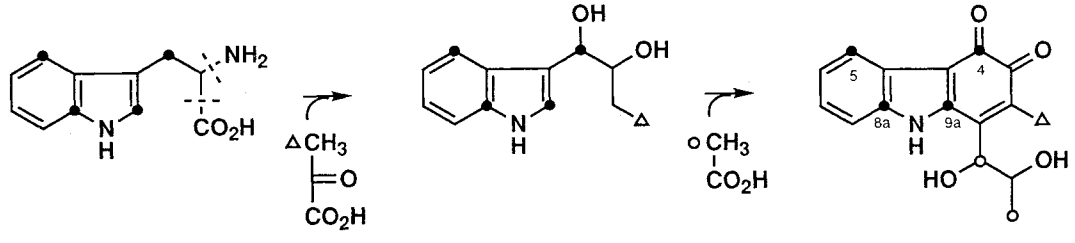


Fig. 3. Biosynthetic pathway for carquinostatins.



2419-SVT2 uses exclusively the non-mevalonate pathway[6,7] for the biosynthesis of IPP. This is the second example showing that *Streptomyces* utilizes the non-mevalonate pathway for the formation of IPP.

The incorporation of the label from [1-^{13}C]glucose into the carbazole nucleus at C-4, C-5, C-8a and C-9a is compatible with the operation of the shikimate pathway for the formation of an intermediate, tryptophan from phosphoenolpyruvate and erythrose 4-phosphate as in the case of the carbazomycin biosynthesis[11] (Fig. 3). Enrichment of C-13, C-10 and C-12 is due to the incorporation of labeled acetate originating from pyruvate.

Further studies on the biosynthesis of other terpenoid compounds produced by *Streptomyces* are now under way.

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