NOTES

Studies on the Biosynthesis of Terpenoid Compounds Produced by Actinomycetes

3. Biosynthesis of the Isoprenoid Side Chain of Novobiocin *via* the Non-mevalonate Pathway in *Streptomyces niveus*†

NOBUYO ORIHARA, TOMOHISA KUZUYAMA, SHUNJI TAKAHASHI, KAZUO FURIHATA†† and HARUO SETO*

Institute of Molecular and Cellular Biosciences, ††Division of Agriculture and Agricultural Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-0032, Japan

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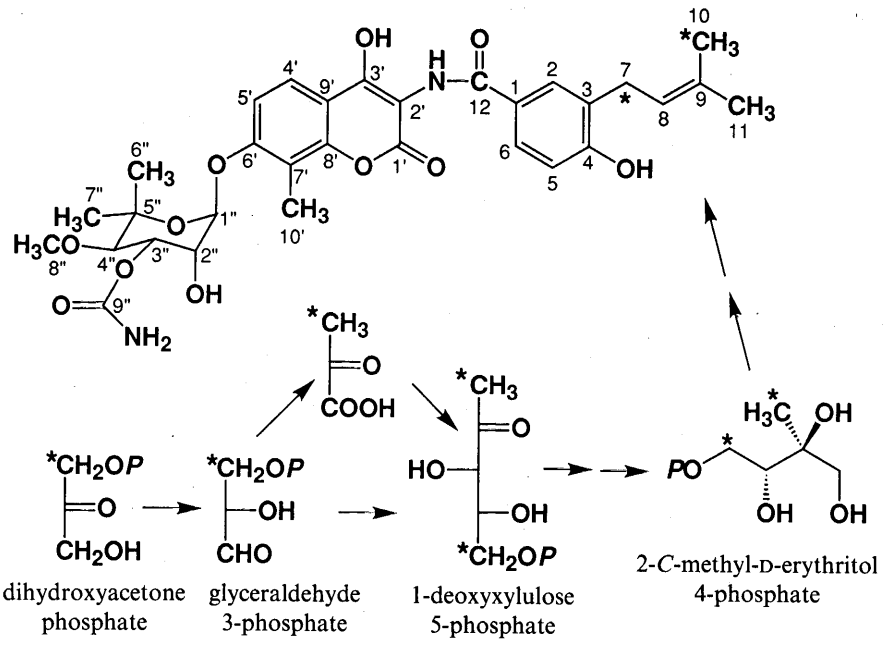
In contrast to vast number of terpenoid compounds produced by plants and fungi (more than 22,000), *Streptomyces* produce only small number of terpenoid compounds. Among them, naphterpin[1], furaquinocins[2], napyradiomycins[3] and terpentecin[4] were proved to be biosynthesized *via* the classical mevalonate pathway. Very curiously, however, pentalenolactone which is produced by several *Streptomyces* species, is synthesized *via* the non-mevalonate pathway[5] which has recently been proposed by ROHMER *et al.*[6,7]. In this pathway (see Fig. 1), glyceraldehyde 3-phosphate and pyruvic acid condense to form 1-deoxyxylulose 5-phosphate which is then converted to isopentenyl diphosphate (IPP) *via* 2-*C*-methyl-D-erythritol 4-phosphate. In addition to pentalenolactone, we have reported in a previous paper that the dimethylally side chain of carquinostatin, an antioxidative substance produced by *Streptomyces exfoliatus*, was biosynthesized *via* the same non-mevalonate pathway[8].

These results surprisingly imply that IPP, the building unit of terpenoids, is formed by the mevalonate pathway in some *Streptomyces* species, while it is formed by a completely different pathway, *i.e.*, the non-mevalonate pathway in other *Streptomyces* species. The question thus occurred to us was that "Are *Streptomyces* with the mevalonate pathway taxonomically different from those possessing the non-mevalonate pathway?" As a further step to pursue this interesting phenomenon, we chose

Fig. 1. Incorporation of [1-^{13}C]glucose to the dimethylally side chain of novobiocin *via* the non-mevalonate pathway.



† For Part 2, see ref. 8.

noboviocin as a metabolite of *Streptomyces niveus*[9] with a dimethylally side chain unit (Fig. 1), which was reasonably assumed to be originated from IPP, and studied its biosynthesis by the use of ^{13}C-labeled precursors. This antibiotic is produced by several other *Streptomyces*, such as *Streptomyces spheroides*, *Streptomyces griseus*, and *Streptomyces griseoflavus*.

Biosynthetic studies using ^{14}C-precursors revealed that the aminocoumarin and benzoic acid moieties of novobiocin were derived from tyrosine[10] and that the sugar unit was formed from glucose[11] with the introduction of a methyl group to either C-6″ or C-7″ from the methyl group of methionine[12]. The origin of the C_5 side chain at C-3, however, remained to be clarified.

S. niveus was used to inoculate to a 15 ml test tube containing a seed medium consisting of starch 1.0%, polypepton 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 seed culture was transferred to two 500 ml Erlenmeyer flasks containing 100 ml of the production medium (starch 2.5%, soybean meal 1.5%, dried yeast 0.2% and $CaCO_3$ 0.4%, pH 6.2) and the fermentation was conducted on a rotary shaker at 27°C.

Sodium [1-^{13}C]acetate (0.5 mg/ml) was added to the medium 18 hours after initiation of the fermentation, while [1-^{13}C]glucose (1 mg/ml) was added to separate cultures 6, 18 and 30 hours after initiation of the fermentation. After cultivation for a total of 96 hours, the differently labeled samples of novobiocin were isolated by AcOEt extraction followed by preparative TLC ($CHCl_3$: MeOH = 10 : 1) at a yield of *ca.* 17 mg.

Assignment of the ^{13}C-NMR spectrum of novobiocin was made mainly based on the reported values[13] and our own 2D-NMR data. Analysis of the ^{13}C-NMR spectrum of novobiocin labeled with sodium [1-^{13}C]-acetate showed no incorporation of the precursor into C-7 and C-9 excluding the operation of the mevalonate pathway for the formation of IPP in this organism (data not shown). On the other hand, addition of [1-^{13}C]-glucose into the fermentation broth increased the signal intensities of C-7 and C-10 in the dimethylally side chain by 1.4 to 2.2 times (Table 1 and Fig. 2). This labeling pattern is explained by incorporation of glucose *via* the non-mevalonate pathway to novobiocin after metabolism to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate by glycolysis[14] (Fig. 1).

The incorporation of [1-^{13}C]glucose to the side chain was apparently improved by addition of the precursor at a later stage showing sole operation of the non-mevalonate pathway throughout the fermentation

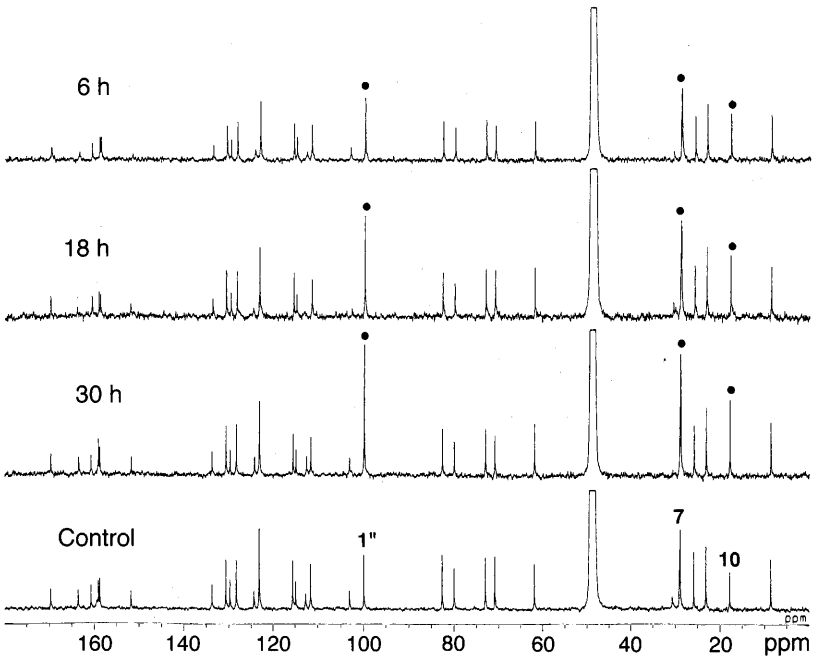
Table 1. ^{13}C-Chemical shifts of novobiocin and incorporation of [1-^{13}C]glucose added separately at three different times.

Carbon	Chemical shift	Normalized peak height: Added at 6 hours	Added at 18 hours	Added at 30 hours
1	124.4	0.8	0.6	1.1
2	130.8	1.0	1.1	1.1
3	129.8	0.9	1.0	0.9
4	160.8	0.9	1.0	0.9
5	115.6	1.0	1.0	0.9
6	128.4	1.1	1.1	1.1
7	29.2	1.4	1.7	2.0
8	123.2	1.0	1.0	1.0
9	133.8	0.8	0.9	1.1
10	17.9	1.7	1.9	2.2
11	26.0	1.0	1.0	1.0
12	169.7	0.9	1.2	1.1
1′	160.8	0.9	1.0	0.9
2′	103.1	0.9	0.6	1.0
3′	163.6	0.5	0.6	1.0
4′	111.6	1.0	1.0	1.0
5′	158.8	1.1	1.0	0.9
6′	114.9	1.0	0.8	1.0
7′	151.8	1.1	0.9	1.0
8′	112.7	0.5	0.8	1.0
9′	123.2	0.7	1.0	1.3
10′	8.7	1.2	1.2	1.2
1″	100.0	1.6	2.2	2.7
2″	70.9	0.9	1.0	0.8
3″	73.0	1.1	1.1	1.0
4″	82.7	1.0	1.0	0.9
5″	80.0	1.1	1.0	0.9
6″	29.0	1.3	1.3	1.5
7″	23.2	1.2	1.3	1.2
8″	61.9	1.2	1.3	1.3
9″	159.2	1.1	1.0	1.4

Signal intensity was normalized to C-4′. The spectra were taken at 125 MHz in CD_3OD.

period. This result is in sharp contrast to the case of naphterpin, where the non-mevalonate pathway was used for the production of IPP at the earlier fermentation stage with gradual replacement by the mevalonate pathway at the later fermentation stage[15]. Thus it is concluded that *S. niveus* possesses only the non-mevalonate pathway for the formation of IPP. This is the third example in which *Streptomyces* utilizes the non-mevalonate pathway for the formation of IPP. As far as our data are concerned, common *Streptomyces* seem to possess only the non-mevalonate pathway. Further studies on the biosynthesis of terpenoid compounds produced by *Streptomyces* are now under way to uncover the relationship between the ability to utilize

Fig. 2. ^{13}C-NMR spectra of novobiocin labeled with [1-^{13}C]glucose.



Enriched signals are indicated by closed circles.

the mevalonate pathway by *Streptomyces* and their taxonomical properties.

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