

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*[†]

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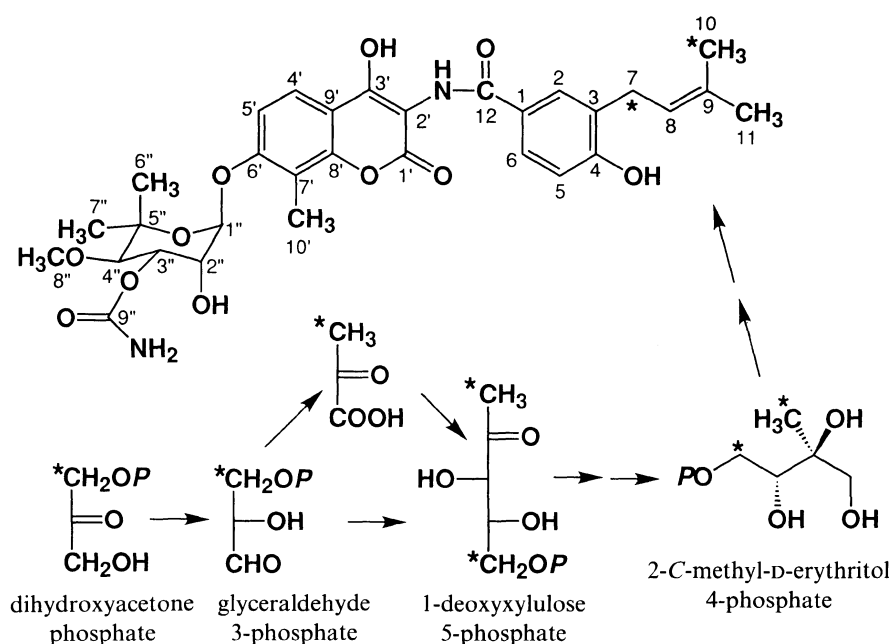
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In contrast to vast number of terpenoid compounds produced by plants and fungi (more than 22,000), *Streptomyces* produce only small number of terpenoid compounds. Among them, naphterpin¹⁾, furaquinocins²⁾, napyradiomycins³⁾ and terpentecin⁴⁾ were 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 pathway⁵⁾ 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 dimethylallyl side chain of carquinostatin, an antioxidative substance produced by *Streptomyces exfoliatus*, was biosynthesized *via* the same non-mevalonate pathway⁸⁾.

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 [1-¹³C]glucose to the dimethylallyl 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 dimethylallyl side chain unit (Fig. 1), which was reasonably assumed to be originated from IPP, and studied its biosynthesis by the use of ¹³C-labeled precursors. This antibiotic is produced by several other *Streptomyces*, such as *Streptomyces spheroides*, *Streptomyces griseus*, and *Streptomyces griseoflavus*.

Biosynthetic studies using ¹⁴C-precursors revealed that the aminocoumarin and benzoic acid moieties of novobiocin were derived from tyrosine¹⁰⁾ and that the sugar unit was formed from glucose¹¹⁾ with the introduction of a methyl group to either C-6'' or C-7'' from the methyl group of methionine¹²⁾. The origin of the C₅ 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 CaCO₃ 0.4%, pH 6.2) and the fermentation was conducted on a rotary shaker at 27°C.

Sodium [1-¹³C]acetate (0.5 mg/ml) was added to the medium 18 hours after initiation of the fermentation, while [1-¹³C]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 (CHCl₃: MeOH = 10:1) at a yield of ca. 17 mg.

Assignment of the ¹³C-NMR spectrum of novobiocin was made mainly based on the reported values¹³⁾ and our own 2D-NMR data. Analysis of the ¹³C-NMR spectrum of novobiocin labeled with sodium [1-¹³C]-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 [1-¹³C]-glucose into the fermentation broth increased the signal intensities of C-7 and C-10 in the dimethylallyl 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 glycolysis¹⁴⁾ (Fig. 1).

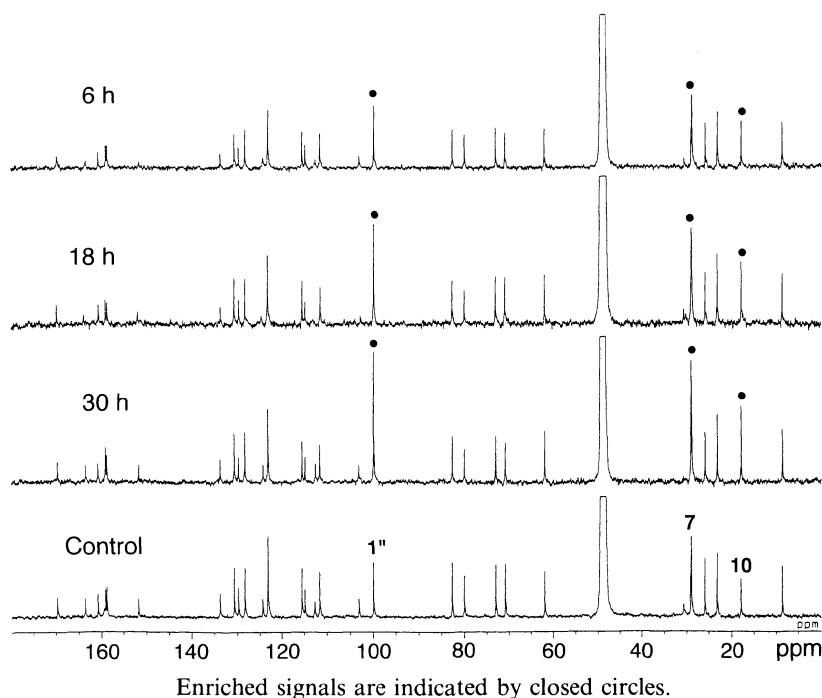
The incorporation of [1-¹³C]glucose to the side chain was apparently improved by addition of the precursor at a later stage showing sole operation of the non-mevalonate pathway throughout the fermentation

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

Carbon	Chemical shift	Normalized peak height		
		6 hours	18 hours	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 CD₃OD.

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 stage¹⁵⁾. 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 $[1-^{13}\text{C}]\text{glucose}$.

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

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