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Biosynthesis of the Dimethylallyl Moiety of Novobiocin *via* a Non-mevalonate Pathway

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Abstract: After feeding of [1-^{13}C]glucose to *Streptomyces spheroides* (NCIB 11891), the labelling pattern of the dimethylallyl moiety of novobiocin was consistent with a biosynthesis predominantly *via* the non-mevalonate (= pyruvate/glyceraldehyde 3-phosphate) pathway.

Isopentenyl diphosphate (IPP) is the common precusor of terpenoid compounds, occurring widespread in all living organisms, and of prenyl moieties of many hemiterpenoid compounds, e.g. prenylated flavonoids,[1] coumarins[2] etc. IPP was generally believed to be synthesized from the mevalonate pathway, i.e. from three molecules of acetyl coenzyme A *via* 3-hydroxy-3-methylglutaryl-coenzyme A and mevalonate.[3] Recent studies with ^{2}H- and ^{13}C-labelled precursors, however, revealed that many isoprenoids are formed by a mevalonate-independent pathway, i.e. from pyruvate and glyceraldehyde 3-phosphate *via* 1-deoxy-D-xylulose-5-phosphate.[4] Compounds formed by this pathway include plant secondary metabolites such as isoprene, mono- and diterpenes,[4b-e] but also primary metabolic prenyllipids in plant chloroplasts[4f-i] (e. g. carotenoids, plastoquinone and the phytyl side chain of chlorophyll) and in bacteria (e.g. the isoprenoid side chain of ubiquinone and menaquinone[4j-l]). First molecular genetic data of this pathway have been reported.[5]

Streptomyces species are an important source of secondary metabolites, and several of these metabolites contain isoprenoid moieties. Previous biosynthetic studies with these compounds indicate the involvement of the mevalonate pathway in their formation.[6] A recent study showed that in *Streptomyces aeriouvifer,* the biosynthesis of the secondary metabolite naphterpin proceeded predominantly *via* the mevalonate pathway, whereas the primary metabolite menaquinone was formed mostly *via* the non-mevalonate pathway. This finding was explained by the operation of the non-mevalonate pathway at the initial growth stage (formation of menaquinone), and its replacement by the mevalonate pathway at a later growth stage (formation of naphterpin).[7] In order to obtain more information on the origin of isoprenoid moieties in secondary metabolites in *Streptomyces*, we investigated the incorporation pattern of [1-^{13}C]glucose into novobiocin (**1**, Fig.1), a gyrase inhibitor produced by *Streptomyces niveus* and *Streptomyces spheroides.*[8]

Novobiocin was produced in a chemically defined medium,[9] containing as carbon sources citrate, proline and 10 g/l glucose (representing 17.1, 36.4, and 46.5 % of the total carbon, respectively). Citrate and a major part of proline are metabolized in the initial growth stage, and novobiocin production starts in a later growth stage when glucose is used as the main carbon source.[9] In our experiment, ordinary glucose was replaced in the medium with [1-^{13}C]glucose, containing 99 atom-% of ^{13}C at C-1. Novobiocin was isolated from the medium 10 days after inoculation and subjected to NMR analysis.[10] The assignments of the ^{13}C NMR signals given by Kuo *et al.*[11] was unequivocally confirmed by two-dimensional ^{1}H-^{13}C experiments for all but two carbons: for C-6‴ and C-7‴ (Fig. 1) we followed the assignment suggested by Kuo *et al.*,[11] although the reverse assignment could not be totally excluded. Relative enrichments of ^{13}C were obtained by comparison of ^{13}C integrals of the novobiocin isolated from the feeding experiment with those of a natural abundance

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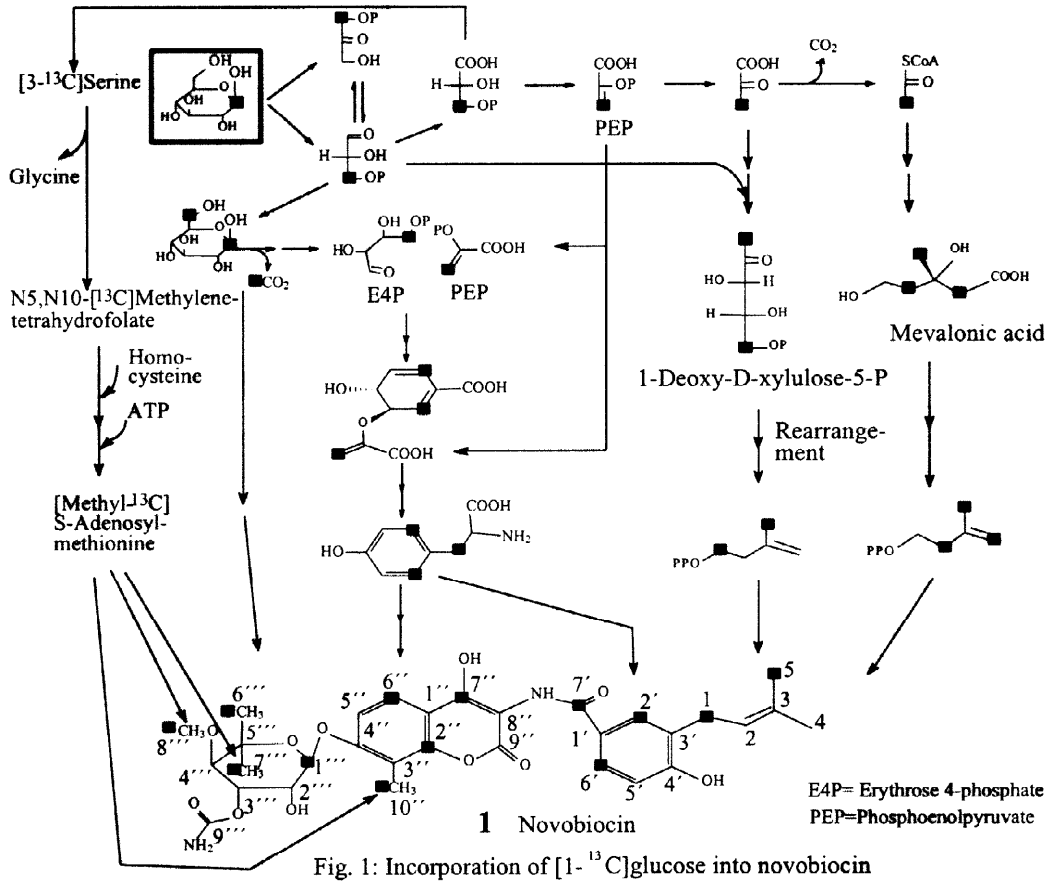

Fig. 1: Incorporation of [1-^{13}C]glucose into novobiocin

Table 1: ^{13}C Enrichment in novobiocin after feeding of [1-^{13}C]glucose (99 atom% ^{13}C)

C-Atom (see Fig. 1)	Chemical shift (δppm. CD_3OD)	Enrichment (%)	C-Atom (see Fig. 1)	Chemical shift (δppm. CD_3OD)	Enrichment (%)
1	29.17	25.5	4''	158.82	<1
2	123.18	-[a]	5''	111.65	<1
3	133.84	<1	6''	123.18	20.9
4	25.94	4.1	7''	159.11	20.2
5	17.90	21.0	8''	103.28	<1
			9''	163.48	<1
1'	124.23	<1	10''	8.62	16.6
2'	130.65	18.4			
3'	129.84	<1	1'''	100.05	44.0
4'	160.84	<1	2'''	70.91	<1
5'	115.64	<1	3'''	73.08	2.2
6'	128.35	19.2	4'''	82.72	<1
7'	169.48	22.9	5'''	80.01	<1
			6'''	23.24	16.6
1''	112.59	<1	7'''	29.03	9.5
2''	151.62	17.1	8'''	61.88	8.6
3''	114.91	<1	9'''	158.53	<1

[a] The ^{13}C signal of C-2 coincided with the signal of C-6'', and the ^{13}C satellites of H-2 overlapped with other proton signals. However, since the absolute enrichment of C-6'' was known from the ^{13}C satellites of H-6'', the enrichment of C-2 could be estimated to be between 0 and 5.3 %.

standard. Absolute ^{13}C enrichments of selected carbons were obtained from the ^{13}C satellites in the ^{1}H NMR spectrum, and the relative enrichments of the other carbons were then referenced to these carbons.[4i, 12]

As shown in Fig. 1 and Table 1, the labelling pattern of the isoprenoid side chain unequivocally proved that incorporation of label from [1-^{13}C]glucose proceeded predominantly *via* a non-mevalonate pathway. This is shown by the 25.5% and 21.0% enrichment in the position 1 and 5. A smaller contribution *via* the mevalonate pathway may be indicated by the observed 4.1% enrichment in position 4. However, a certain portion of this enrichment may also be attributed to the less-than-perfect stereocontrol in the DMAPP-IPP isomerase reaction as observed in similar feeding experiments.[4i] Enrichment in position 2 could only be estimated, suggesting an enrichment between 0 and 5.3% (see Table 1).

As shown in Fig. 1, [1-^{13}C]glucose is converted, *via* the Embden-Meyerhof-Parnas pathway, to two moles of interconvertible triose phosphates. Regeneration of glucose from the trioses diverts ^{13}C label to the position 6 of glucose; this explains the enrichment in C-6‴ of the noviose sugar moiety. This finding confirms earlier results that noviose is derived from intact glucose.[13] The deoxygenation at position 6 is expected to proceed with retention of the configuration at position 5 of the sugar.[14]

The 99 % ^{13}C abundance in the position 1 of labelled glucose is decreased to approximately 50% in position 3 of the triose phosphates. Loss of ^{13}C label occurs in the oxidative branch of the pentose phosphate pathway by oxidation of [1-^{13}C]glucose-6-phosphate and the removal of $^{13}CO_2$. The ^{13}C enrichment in the carbon atoms derived from C-3 of the triose phosphate intermediates was in the range of 20%, which is in very good agreement with the specific incorporation rates found in earlier studies.[12a] Enrichment in the carbons at position 10″, 7‴and 8‴, known to derive from S-adenosyl-methionine,[15] was somewhat lower, probably since degradation of serine is the major but not the only source of activated methyl groups.[16] The observed labelling pattern therefore confirms the earlier hypotheses about novobiocin biosynthesis, which were based on radioactive isotope feeding experiments during the 1960's and 1970's. [13, 17]

In *Escherichia coli*, 4-hydroxybenzoate is formed in a single step from chorismate by elimination of pyruvate[18] and is used for ubiquinone biosynthesis after prenylation of position 3 of the aromatic nucleus. Our experiments confirm that the 3-prenylated 4-hydroxybenzoate moiety of novobiocin, in contrast, is formed by side chain degradation of tyrosin, so that the carboxyl group at C-7′ (Fig. 1) is derived from C-3 of phosphoenolpyruvate rather than from the carboxyl group of shikimate.

Novobiocin, just as naphterpin in *Streptomyces aeriouvifer*, is a secondary metabolite produced exclusively at a later growth stage of the bacterial culture. In contrast to naphterpin,[7] however, incorporation of label from [1-^{13}C]glucose proceeds predominantly *via* the non-mevalonate pathway. *Streptomyces* species apparently can use both isoprenoid pathways for the formation of their secondary metabolites.

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