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# BIOSYNTHETIC STUDIES OF NAPHTERPIN, A TERPENOID METABOLITE OF *STREPTOMYCES*

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Summary. Naphterpin, a metabolite of *Streptomyces aeriouvifer*, was shown to be biosynthesized from two units, a polyketide derived naphthoquinone unit and a geranyl side chain. $^{13}C$-$^{13}C$ splitting patterns of naphterpin labeled with [1,2-$^{13}C_2$]acetate indicated the involvement of a symmetric naphthalene intermediate.

Although a variety of terpenoidal compounds are frequently produced by fungi and yeasts, it is quite rare that derivatives belonging to the terpene group or possessing a partial terpenoidal structure have been isolated as metabolites of *Streptomyces*. Such rare examples include pentalenolactone[1], terpentecin[2], napyradiomycins[3] and furaquinocins[4] whose biosynthetic origins have been unequivocally revealed by labeling experiments. In this regards, we have been very much interested in the biosynthetic mechanism of naphterpin, a metabolite of *Streptomyces aeriouvifer*, isolated as an antioxidative agent[5].

Naphterpin is a naphthoquinone metabolite with an additional $C_{10}$ ring unit of seemingly terpenoidal origin. Its structure has been established as shown in Fig. 1 by NMR and X-ray analyses[5]. In the present report, we describe biosynthetic studies of naphterpin by means of feeding experiments using $^{13}C$- and $^{2}H$-labeled precursors. *S. aeriouvifer* was cultured as described previously and 24 hrs after inoculation, [1-$^{13}C$] or [1,2-$^{13}C_2$]acetate and [$C^2H_3$]methionine were added at the level of 1 mg/ml and 0.2 mg/ml, respectively. The labeled naphterpins were isolated by solvent extraction followed by silica gel chromatography [5].

Table 1. Incorporation ratio of [1-$^{13}C$]acetate and $^{13}C$-$^{13}C$ coupling constants observed with [1,2-$^{13}C_2$]acetate-labeled naphterpin

| Carbon | $\delta_C$ | ratio* | $J_{C\text{-}C}$(Hz) | Carbon | $\delta_C$ | ratio* | $J_{C\text{-}C}$(Hz) |
|---|---|---|---|---|---|---|---|
| 1 | 183.1 | 2.7 | 59 | 10 | 120.0 | 1.1 | 43 |
| 2 | 153.5 | 1.0 | 59, 68 | 11 | 136.1 | 2.2 | 42 |
| 3 | 123.3 | 2.2 | 68, 57 | 12 | 29.6 | 1.1 | |
| 4 | 184.8 | 1.4 | 57, 54 | 13 | 20.4 | 2.2 | 34 |
| 4a | 131.4 | 2.2 | 54, 64 | 14 | 39.7 | 1.1 | 34 |
| 5 | 108.4 | 1.0 | 64, 64 | 15 | 80.8 | 2.2 | 41 |
| 6 | 161.5 | 2.2 | 64, 69 | 16 | 23.5 | 1.1 | 42 |
| 7 | 117.2 | 1.1 | 69, 69 | 17 | 25.6 | 1.2 | |
| 8 | 162.6 | 2.3 | 69, 64 | 18 | 25.1 | 1.2 | 41 |
| 8a | 107.9 | 1.2 | 64, 59 | 7-$CH_3$ | 7.8 | 1.2 | |
| 9 | 31.1 | 2.2 | 43 | | | | |

* normalized to C2.

The assignment of the $^{13}C$-NMR signals (125 MHz, $CDCl_3$, TMS) of naphterpin was fully established based on $^{13}C$-$^{1}H$ COSY along with HMBC experiments (Table 1)[5]. The $^{13}C$ NMR spectrum of naphterpin labeled with [1-$^{13}C$]acetate revealed the enrichment of 9 carbon signals (C1, C3, C4a, C6, C8, C9, C11, C13 and C15) with the incorporation ratio being 2.2 to 2.7 (Table 1). These data suggest that the acetate was incorporated into the naphthoquinone ring by way of a polyketide intermediate and into $C_{10}$ unit *via* a terpenoidal pathway.

In order to confirm this conclusion, naphterpin was labeled with [1,2-$^{13}C_2$]acetate and the obtained $^{13}C$-$^{13}C$ coupling constants ($J_{C\text{-}C}$) are summarized in Table 1. Unexpectedly and interestingly, all carbons in the naphthoquinone part (C1 to C8a) showed two direct $^{13}C$-$^{13}C$ couplings except for C1, C5 and C7, which

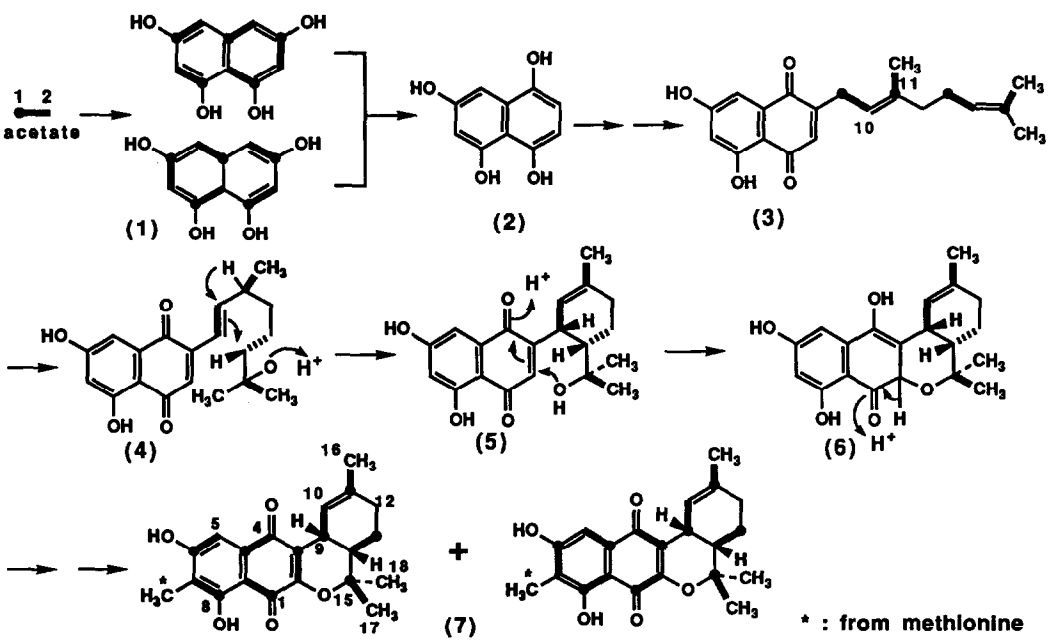
showed only one kind of coupling constants due to incidentally identical magnitude of the one bond coupling constants with their adjacent carbons.

These results suggest that the biosynthesis of the naphthoquinone ring should involve a symmetric intermediate such as 1,3,6,8-tetrahydroxynaphthalene (Fig. 1, (1)) which was preceded in the biosyntheses of scytalone[6] napyradiomycins[3] and furaquinocins[4]. As for the $C_{10}$ moiety, C9-C10, C11-C16, C13-C14 and C15-C17 were observed as $^{13}C$-$^{13}C$ coupled pairs, and C12 and C18 appeared as singlets revealing the terpenoidal origin of this unit. The origin of 7-$CH_3$ proved to be the methyl group of methionine by incorporation of [$C^2H_3$]methionine (12 %, detected by $^2H$-NMR spectroscopy) into this methyl group.

Based on these experimental results, we propose the biosynthetic pathway of naphterpin to be summarized as shown in Fig. 1. The symmetric intermediate (1) is transformed to a hydroquinone derivative (2) followed by alkylation with a geranyl residue. Isomerization of the C-10, C-11 double bond of (3) and cyclization initiated by ring opening of an epoxide intermediate (4) would result in the formation of naphterpin (7). At the moment, it remains unknown when the naphthoquinone nucleus is methylated.

Isolation of plausible intermediates of naphterpin is now under way.

**Fig. 1. Biosynthetic pathway of naphterpin**

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