Heliquinomycin, a New Inhibitor of DNA Helicase, Produced by *Streptomyces* sp. MJ929-SF2

III. Biosynthesis

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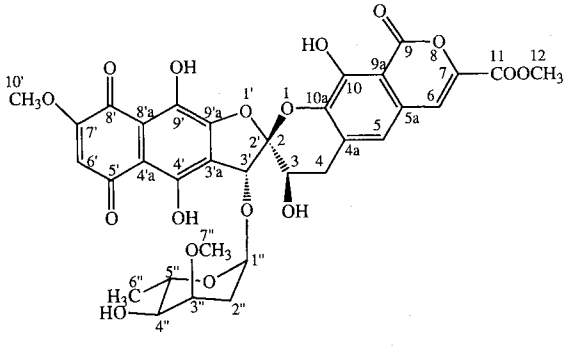
In the course of our screening program for a novel DNA helicase inhibitor, we have isolated heliquinomycin, which is produced by *Streptomyces* sp. MJ929-SF2[1]. The absolute structure of heliquinomycin (Fig. 1) was determined by X-ray analysis and the L-configuration of cymarose which was obtained from the acid hydrolysate of heliquinomycin[2]. Heliquinomycin is the first member of glycosylated rubromycins and griseorhodins group antibiotics. Although the structures of rubromycins and griseorhodins group were first reported by ECKARDT *et al.*[3], no their biosynthetic studies have been reported.

In this paper, we describe the studies on the incorporations of ^{13}C-labeled acetate, methionine and glucose that helped an elucidation of the biosynthetic origins for heliquinomycin, and discussed the biosynthetic pathway.

In the ^{13}C NMR spectrum from the labeling experiment with [1-^{13}C]acetate, enhancements of ^{13}C signal intensities were observed at C-3, C-4a, C-5a, C-7, C-9, C-10, C-3′, C-4′, C-5′, C-7′, C-8′a and C-9′a. Enrichment ratios are shown in Table 1. In the ^{13}C NMR spectrum from the labeling experiment with [2-^{13}C]acetate, enhancements of ^{13}C signals were observed at C-2.2′, C-4, C-5, C-6, C-9a, C-10a, C-11, C-3′a, C-4′a, C-6′, C-8′ and C-9′ as shown in Table 1.

Fig. 1. Structure of heliquinomycin.



The feeding experiment of [1,2-^{13}C]acetate revealed the twelve ^{13}C-^{13}C coupling pairs of C-3-C4, C-4a-C-5, C-5a-C-6, C-7-C-11, C-9-C-9a, C-10-C-10a, C-2.2′-C-3′, C-3′a-C-4′, C-4′a-C-5′, C-6′-C-7′, C-8′-C-8′a and C-9′-C-9′a, and nine singlet signals without ^{13}C-^{13}C coupling attributed to the sugar moiety and three methoxy groups in the ^{13}C NMR spectrum. The twelve ^{13}C-^{13}C coupling pairs are well consistent with the results of labeling patterns obtained by the singly labeled acetates. Table 2 shows the result of ^{13}C chemical shifts and ^{13}C-^{13}C coupling constants for heliquinomycin labeled with [1,2-^{13}C]acetate. The above described results indicate that the aglycon moiety of heliquinomycin are formed through a

Table 1. ^{13}C NMR data for heliquinomycins, by incorporation of ^{13}C-labeled precursors.

Position	δ (ppm)	[1-^{13}C]-Acetate	[2-^{13}C]-Acetate	L-[methyl-^{13}C]-Methionine
2, 2′	111.92	0.86	3.81*	1.00[c]
3	61.55	2.50*	0.88	1.06
4	30.17	0.68	4.21*	0.82
4a	130.24	1.88*	0.51	0.75
5	118.98	0.76	4.23*	0.88
5a	27.92	1.77*	0.60	0.69
6	113.58	0.69	3.92*	0.89
7	141.23	1.61*	0.50	0.57
9	164.96	1.45*	0.61	0.67
9a	106.72	0.47	2.64*	0.72
10	150.23	1.69*	0.61	0.74
10a	140.47	0.48	2.81*	0.54
11	160.53	0.60	3.20*	0.56
12	52.96	1.12	0.91	27.91*
3′	76.34	3.24*	1.19	1.08
3′a	122.78	0.66	2.38*	0.76
4′	159.98	1.68*	0.41	0.51
4′a	106.61	0.50	2.44*	0.66
5′	183.52	2.34*	0.26	0.42
6′	110.42	0.67	2.51*	0.76
7′	159.91	2.35*	0.41	0.51
8′	178.96	0.42	2.76*	0.29
8′a	114.06	2.28*	0.52	0.51
9′	150.56	0.47	2.83*	0.44
9′a	156.01	1.92*	0.42	0.65
10′	56.85	1.00[a]	0.31	34.67*
1″	94.12	0.85	0.78	0.89
2″	30.84	0.86	0.78	0.92
3″	76.33	1.13	0.97	1.01
4″	71.68	1.10	1.02	1.08
5″	65.91	1.06	0.81	1.01
6″	18.16	1.40	1.04	1.22
7″	57.35	1.46	1.00[b]	28.58*

[a] Relative enrichments were normalized to the peak intensity of C-10′-OCH_3 signal.
[b] Relative enrichments were normalized to the peak intensity of C-7″-OCH_3 signal.
[c] Relative enrichments were normalized to the peak intensity of C-2, 2′ signal.
* High level of enrichment was odserved.

polyketide precuesor derived from twelve intact acetic acid units.

The feeding of L-[methyl-^{13}C]methionine enriched the three methoxy carbon of the C-12, 10′ and 7″ position (Table 1). There are many facts that *O*-methyl groups are derived from the methyl group of methionine in various metabolites. KIHARA *et al.*[4] reported that 3-*O*-methyl group of cymarose moiety in cytovaricin was derived from methionine.

Table 2. ^{13}C Chemical shifts and J_{CC} coupling constants for heliquinomycin labeled with [1,2-^{13}C]acetate.

Position	δ (ppm)[a]	J_{CC} (Hz)
2, 2′	111.92	53.2
3	61.55	37.0
4	30.17	37.0
4a	130.24	60.5
5	118.98	60.5
5a	127.92	54.5
6	113.58	54.5
7	142.23	93.1
9	164.96	72.0
9a	106.72	72.0
10	150.23	81.0
10a	140.47	81.0
11	160.53	93.1
3′	76.84	53.2
3′a	122.78	72.0
4′	159.98	72.0
4′a	106.61	57.5
5′	183.52	57.5
6′	110.42	71.0
7′	159.91	71.0
8′	178.96	59.0
8′a	114.06	59.0
9′	150.56	73.5
9′a	156.01	73.5

[a] 125 MHz ^{13}C NMR spectrum in $CDCl_3$ (TMS as internal reference).

As for the sugar moiety in the heliquimnomycin, the feeding experiment with D-[U-^{13}C]glucose was carried out and resulted in low enrichments of ^{13}C incorporation. Although the sugar moiety was not enriched by the feeding experiments with ^{13}C-labeled acetates, the ^{13}C NMR spectrum of the sample labeled with D-[U-^{13}C]glucose showed all the signals having ^{13}C-^{13}C coupling except for the methoxy groups (C-12, 10′ and 7″). The ^{13}C signals attributed to cymarose were suggested that there were two types of carbon connectivities, type A: C_2 units such as C-1″-C-2″, C-3″-C-4″, C-5″-C-6″ and type B: C_3 units such as C-1″-C-2″-C-3″, C-4″-C-5″-C-6″ from the ^{13}C-^{13}C coupling constants and signal intensities as shown in Fig. 2. In the case of type A, the doublet of signals indicated that each was coupled with one vicinal ^{13}C carbon. In the case of type B, the splitting pattern at C-5″ indicated that the intensity of the triplet signal arising from the ^{13}C-^{13}C coupling with C-4″ and C-6″ was higher than that of the doublet arising from the coupling C-4″ or C-6″. It has been reported that the 6-deoxy sugar in acarbose was biosynthesized from C_3 unit and the deoxyhexose was biosynthesized from a glucose 6-phosphate[5~7]. Since there are variations for ^{13}C signal intensities derived from incorporation of C_2 and C_3 units, the cymarose moiety may be biosynthesized by various C_2 and C_3 units which are metabolized from the D-[U-^{13}C]glucose during its glycolysis pathway.

The results obtained from the above feeding experi-

Fig. 2. Coupling patterns of cymarose moiety in heliquinomycin derived biosynthically from [U-^{13}C]glucose.

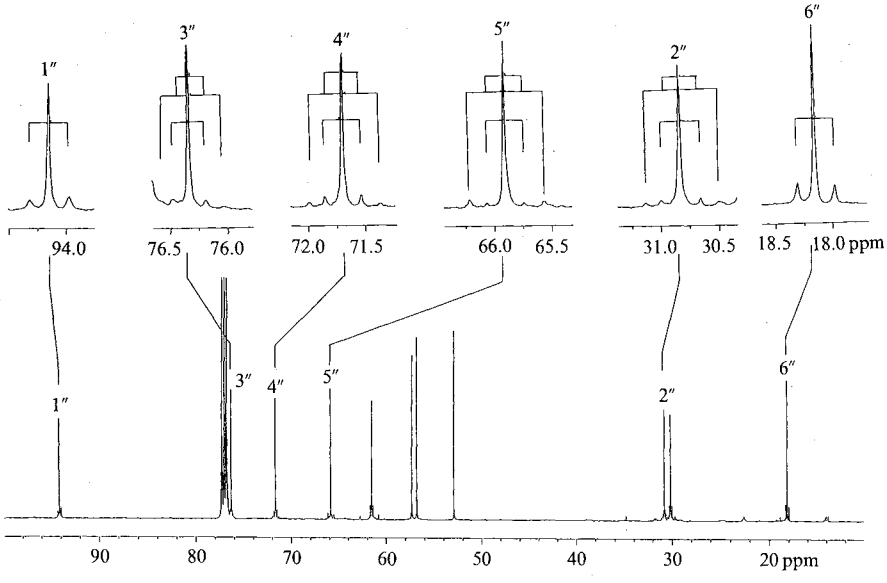
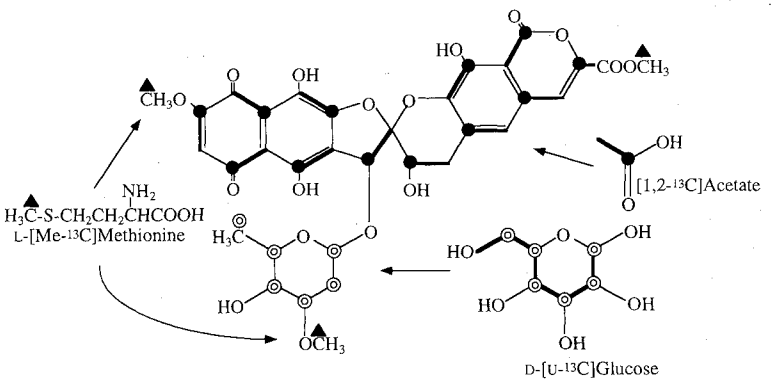


Fig. 3. Biosynthesis of heliquinomycin.



ments with ^{13}C-labeled precursors demonstrate that the aglycon of heliquinomycin is biosynthesized from twelve acetate units, the methoxy groups at C-12, 10', 7'' are derived from the methionine-*S*-methyl group, and the cymarose is biosythesized from metabolites of glucose. Thus, the origin of the all carbon atoms in heliquinomycin has been established and are summarized in Fig. 3.

Experimental

Incorporation of Isotope-labeled Compounds to Heliquinomycin

A slant culture of strain MJ929-SF2 was inoculated into 110 ml of culture medium consisting of galactose 2.0%; dextrin 2.0%; Bacto Soytone 1.0%; corn steep liquor 0.5%; $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2% (pH 7.4) in a 500 ml Erlenmeyer flask, ^{13}C-labeled compounds (30 mg per flask) were added to the culture twice at 48th and 72nd hour of cultivation and then the culture was incubated for an additional 24 hours. In a case of D-[U-^{13}C]glucose, the amount was 25 mg per a flask, and cultured at 27°C for 4 days on a rotary shaker (180 rpm)[1].

Labeled Compounds

^{13}C-labeled compounds were 99% ^{13}C atom purity. Sodium [1-^{13}C]acetate, sodium[2-^{13}C]acetate, sodium [1,2-^{13}C]acetate, L-[methyl-^{13}C]methionine and D-[U-^{13}C]glucose were purchased from Aldrich Chemical Co., U.S.A.

Isolation of Labeled Heliquinomycin

Each culture broth was separated to a mycelial cake and a culture supernatant by centrifugation. The mycelial cake was extracted with methanol and the extract was filtered and concentrated *in vacuo* to an aqueous solution. The solution was combined with the culture supernatant and extracted with an equal volume of butyl acetate at pH 2.0. The active extract was concentrated to dryness under reduced pressure. The residue was subjected to a centrifugal partition chromatography using CPC-L.L.N model NMF (Sanki Engineering Limited) with a solvent system of $CHCl_3$-MeOH-H_2O (5:6:4). The upper phase of the solvent system was introduced by ascending method. By changing of the flow direction (descending method), the active fractions were eluted with the lower phase of the solvent system to give a red fraction. The active fractions were collected and then evaporated to give a red powder, then the powder was applied onto a Sephadex LH-20 column (1 liter) and eluted with a solvent mixture of $CHCl_3$-MeOH (1:1). The active fractions were concentrated under reduced pressure to give red powder. The crude powder thus obtained was further purified by CPC using the solvent system of *n*-Hexane-MeOH-CH_3CN (5:1:4). The active fraction was evaporated to dryness to give pure heliquinomycin. The labeled heliquinomycins thus obtained were 28 mg ([1-^{13}C]acetate), 12 mg ([2-^{13}C]acetate), 25 mg ([1,2-^{13}C]acetate), 7.1 mg (L-[methyl-^{13}C]methionine) and 16.1 mg (D-[U-^{13}C]glucose), respectively.

NMR Spectroscopy

^{13}C NMR spectra were measured on a JEOL JNM-A500 at 125 MHz. Heliquinomycins which were obtained from the cultures feeding various ^{13}C labeled compounds, were dissolved in $CDCl_3$. TMS was used as an internal reference.

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