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

II. Structure Determination of Heliquinomycin

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The structure of heliquinomycin which was isolated from the culture broth of *Streptomyces* sp. MJ929-SF2 was studied by NMR spectroscopies, X-ray crystallographic analysis and degradation experiments. Heliquinomycin is the first member of glycosylated rubromycins and griseorhodins group antibiotics.

In the preceding paper[1], we have described the taxonomy, isolation, physico-chemical properties and biological activity of heliquinomycin (**1**), a novel inhibitor of DNA helicase. In this paper, we describe the structure determination of **1** (Fig. 1.).

Results and Discussion

Structure Determination

The IR spectrum of **1** indicated the presence of hydroxyl, ester and quinone groups at 3440, 1720 and 1620 cm^{-1}, respectively. The UV spectrum of **1** showed absorption maxima at 231, 321, 336, 507, 523 and 540 nm and this UV spectrum was similar to those of the rubromycins and griseorhodins[2,3].

The molecular weight and formula of **1** were elucidated as $C_{33}H_{30}O_{17}$ (MW 698) from the FAB-MS (negative) peak at *m/z* 698 (M^-) and HRFAB-MS [found *m/z* 698.1486 (M^-), calcd. *m/z* 698.1483 for $C_{33}H_{30}O_{17}$]. The ^{13}C NMR spectrum in $CDCl_3$ (Table 1) indicated 33 carbons and 15 of those carbons bound protons from DEPT spectrum, thus corroborating the molecular formula. Interpretation of the ^{13}C NNR signals showed as follows: $4\times CH_3$, $2\times CH_2$, $6\times CH$, $1\times C$, $3\times CH=$, $13\times C=$ and $4\times C=O$.

The comparison of NMR spectra including HMBC with those of 8-deoxygriseorhodin C[4] gave assignments of NMR signals in the aglycon moiety of **1** (Fig. 2).

^1H-^1H COSY spectrum of **1** suggested the presence of 2,6-dideoxy sugar, and the 1C-conformation of the sugar moiety was shown by the results of NOEs between 2″ax-H (δ 1.88) and 4″-H (δ 3.34) and between 3″-OCH_3 (δ 3.51) and 5″-H (δ 3.93), respectively. The observed coupling constants from 1″-H to 5″-H indicated that the sugar moiety was cymarose as shown in Fig. 3.

The connectivity between the aglycon and cymarose was demonstrated by the HMBC spectrum. The following cross peaks were observed between 3′-H (δ 5.81) and 1″-C (δ 94.1) and between 1″-H (δ 5.58) and 3′-C (δ 76.8), respectively.

Relative Stereochemistry

Heliquinomycin (**1**) was crystallized from a $CHCl_3$-EtOAc solution to give red prismatic crystals. A crystal was chosen for an X-ray analysis. As a result, the relative stereochemistry of **1** was determined as shown in Fig. 4. The ORTEP drawing of **1** was shown in Fig. 4. Crystal data were summarized in Table 2.

Fig. 1. Structure of heliquinomycin (**1**).

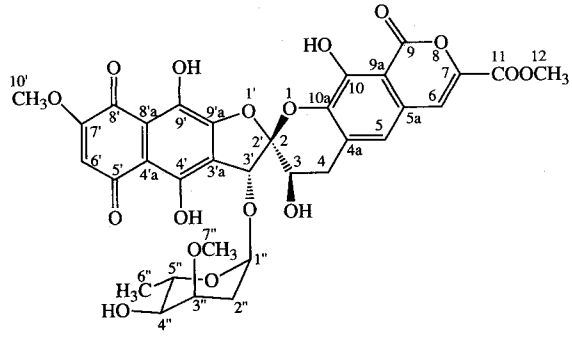


Table 1. ^{13}C and ^{1}H HNR data of **1**.

Position	δ_C (ppm)	δ_H (ppm)	J value (Hz)
2, 2′	111.92 s		
3	61.55 d	4.55 m	
		5.31 br d, (3-OH)	J=3.8
4	30.17 t	3.10 dd	J=2.1, 17.4
		3.56 dd	J=3.8, 17.4
4a	130.24 s		
5	118.98 d	6.95 s	
5a	127.92 s		
6	113.58 d	7.44 s	
7	142.23 s		
9	164.96 s		
9a	106.72 s		
10	150.23 s		
		11.01 s, (10-OH)	
10a	140.47 s		
11	160.53 s		
12	52.96 q	3.95 s	
3′	76.84 d	5.81 s	
3′a	122.78 s		
4′	159.98 s		
		13.55 s, (4′-OH)	
4′a	106.61 s		
5′	183.52 s		
6′	110.42 d	6.21 s	
7′	159.98 s		
8′	178.96 s		
8′a	114.06 s		
9′	150.56 s		
		12.18 s, (9′-OH)	
9′a	156.01 s		
10′	56.85 q	3.93 s	
1″	94.12 d	5.58 br d	J=3.4
2″	30.84 t	1.88 ddd	J=3.2, 3.4, 15.3
		2.32 ddd	J=3.2, 15.3
3″	76.33 d	3.71 ddd	J=3.2, 3.2, 3.2
4″	71.68 d	3.34 dd	J=3.2, 9.5
		2.18 br (4″-OH)	
5″	65.91 d	3.93 m	
6″	18.16 q	1.40 d	J=6.4
7″	57.35 q	3.51 s	

^{1}H NMR was measured at 500 MHz, ^{13}C NMR was measured at 125 MHz; in $CDCl_3$.

Fig. 2. Structure of **1** from ^{1}H-^{1}H COSY and HMBC spectra.

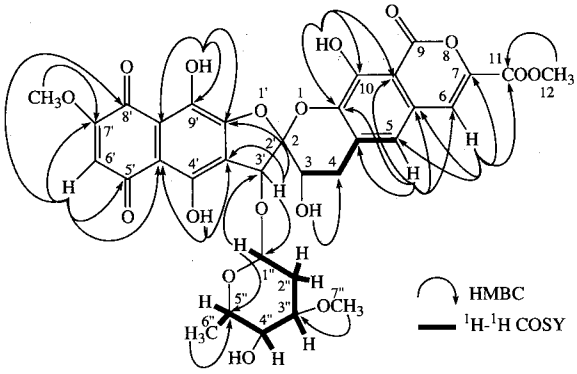


Fig. 3. Relative stereochemistry of the sugar moiety by NMR data.

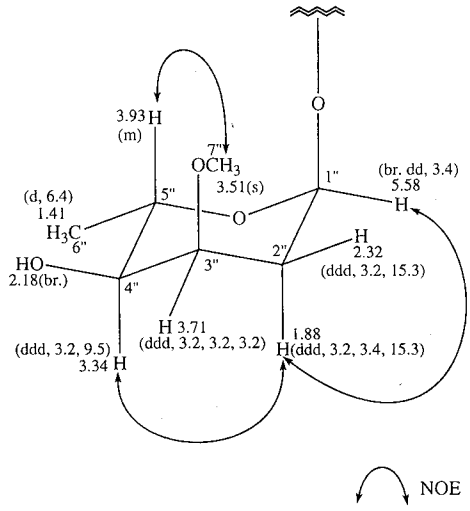


Fig. 4. Molecular structure of **1**.

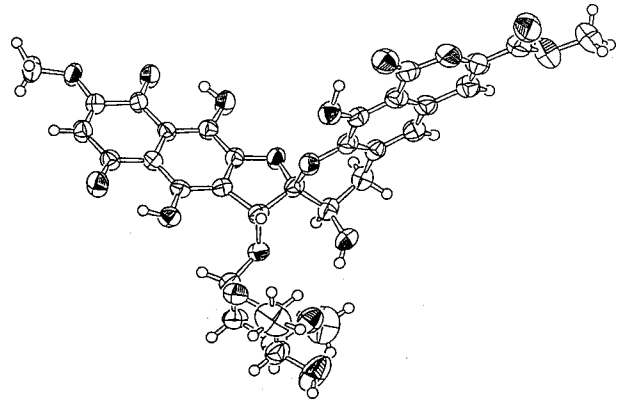
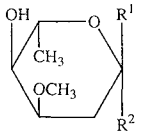


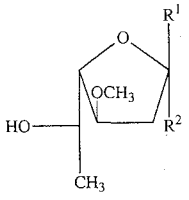
Table 2. Crystal data of **1**.

Empirical formula	$C_{33}H_{30}O_{17}\cdot 1/2C_4H_8O_2$
Formula weight	742.64
Crystal system	Tetragonal
Crystal color, habit	Red, prism
Space group	$P4_3$
Lattic Parameters	a=14.235(2) Å
	c=33.382(5) Å
	V=6764.1(9) Å^3
Z value	8
D_{calc}	1.458 g/cm^3
μ (CuKα)	10.25 cm^{-1}

Fig. 5. Structure of methyl cymaropyranosides and cymarofuranosides.



Methyl α-L-cymaropyraniside (**3**) : R^1=OMe, R^2=H
Methyl β-L-cymaropyranoside : R^1=H, R^2=OMe



Methyl α-L-cymarofuranoside (**2**) : R^1=OMe, R^2=H
Methyl β-L-cymrofuranoside : R^1=H, R^2=OMe

Absolute Stereochemistry

Cymarose was isolated by the acid hydrolysis of **1**. The observed optical rotation of sugar moiety was $[\alpha]_D^{22}$ −46°, which was in good agreement with L-cymarose ($[\alpha]_D^{13}$ −50.4°) reported by TSUKAMOTO *et al.*[5].

Methanolysis of **1** afforded methyl cymarosides. The mixture of methyl cymarosides was chromatographed on silica gel colum to give two anomeric mixtures. A more polar anomeric mixture contains methyl α-cymarofuranoside (**2**) (Fig. 5) and methyl α-cymaropyranoside (**3**) (3 : 1), a less polar anomeric mixture contains methyl β-cymarofuranoside and methyl β-cymaropyranoside (1 : 1). The observed specific rotation of the α-anomeric mixture was $[\alpha]_D^{26}$ −154.4°. All data of specific rotation and NMR data of **2** and **3** were well agreed with those described in reference 6 and 7.

The above data show that the sugar moiety of **1** is L-cymarose. Thus, the absolute structure of **1** was determined to be methyl (2*R*,3*R*,3′*R*)-3′-(2,6-dideoxy-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy-3,4-dihydro-3,4′,9′,10-tetrahydroxy-7′-methoxy-5′,8′,9-trioxo-spiro-[benzo[1,2-*b*: 5,4-*c*]dipyran-2(3*H*),2′(3′*H*)naphto[2,3-*b*]pyran]-7-carboxylate. It is the first report of an absolute structure in glycosylated rubromycins and griseorhodins group antibiotics.

Experimental

General

UV absorption spectra were measured with a Hitachi U-3210 spectrophotometer. IR absorption spectra were obtain with a Hitachi I-5020 FT-IR and Hitachi 260-10 spectrometer. FAB-MS and HRFAB-MS were obtained on a JEOL JMS-SX102 mass spectrometer. ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-A500 spectrometer. Chemical shifts are given in ppm from TMS as an internal standard. Optical rotations were taken by a Perkin-Elmer 241 polarimeter using a microcell (light path 10 cm).

X-Ray Crystallography

The crystal of **1** was recrystallized from a $CHCl_3$-EtOAc solution. A red prismatic crystal having approximate dimensions of 0.20 × 0.40 × 0.40 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα radiation. Cell constants and an orientation matrix for data collection were obtained from a least-squares refinement using the setting angles of 25 carefully centered reflections in the range $55.7 < 2\theta < 56.50°$. Crystal data are shown in Table 2. The reflection data were collected at a temperature of 21°C using the ω-2θ scan technique to a maximum 2θ value of 120.2°. Scans of $(1.57 + 0.30 \tan\theta)°$ were made at a speed of 8.0°/minute (in omega). A total of 5608 reflections was collected. The intensities of three standard reflections were measured after every 150 reflections, no significant intensity decay was observed. An absorption correction using the program DIFABS[8] was applied which resulted in transmission factors ranging from 0.89 to 1.21. The data were corrected for Lorentz and polarization effects. The structure was solved by a direct method (SIR88[9]) and expanded using Fourier techniques (DIRDIF[10]). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 5250 observed reflections ($I > 2.00\sigma(I)$) and 955 variable parameters and converged with unweighted and weighted agreement factors of R = 0.035 and Rw = 0.049. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.25 and $-0.14\,e^-/Å^3$, respectively. All calculation were performed using the teXsan crystallographic software package of Molecular Structure Corporation.

Isolation of Cymarose by Hydrolysis of 1

Compound **1** (65 mg) was hydrolyzed with 2 M HCl-THF (1 : 1, 10 ml) at 65°C for 1 hour. The reaction mixture was neutralized with Ag_2CO_3 (2 g). The precipitates were filtered off and the filtrate was concentrated under reduced pressere. The residue was purified by silica gel column chromatography (toluene-acetone (5 : 1)). The fractions containing cymarose were collected and evaporated in vacuum to give a residue (4.6 mg). The residue was dissolved in H_2O (1 ml) and was chromatographed ODS-silica gel column (i.d. 6 mm × 13 mmh) using H_2O to give colorless syrup (4.3 mg). $[\alpha]_D^{22}$ −46° (*c* 0.36, H_2O).

Isolation of Methyl Cymaroside by Methanolysis

A solution of **1** (124.8 mg) in MeOH (12 ml) was allowed to react with Amberlyst 15 (H^+, 270 mg) at room temperature for 24 hours then at 50°C for 3 hours. The precipitates were filtered off and the filtrate was evaporated to give a mixture. The products were chromatographed on silica gel (10 g) using *n*-hexane - ethyl acetate (1 : 2) to give a mixture of **2** and **3** (1.5 mg). The yield was quite low due to the volatility of the methyl cymaroside.

Mixture of **2** and **3** (3 : 1): A colorless syrup. $[\alpha]_D^{26}$ $-154.4°$ (*c* 0.136, $CHCl_3$).

2: ^1H NMR ($CDCl_3$) δ 1.23 (3H, d, $J=6.5$ Hz, 6-H), 1.95 (1H, d, $J=2.5$ Hz, 5-OH), 2.03 (1H, ddd, $J=1$, 2, 14 Hz, 2-H), 2.09 (1H, d, $J=5$, 7.3, 14 Hz, 2-H), 3.34, 3.39 (each 3H, s, 1,3-OCH_3), 3.90 (2H, m, 3,4-H), 4.00 (1H, dq, $J=2.5$, 6.5 Hz, 5-H), 5.07 (1H, dd, $J=1.5$, 4.8 Hz, 1-H).

Acknowledgements

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