4'-Deacetyl-(−)-griseusins A and B, New Naphthoquinone Antibiotics from an Actinomycete

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In the course of our screening for new antibiotics, new members of naphtoquinone antibiotics, 4'-deacetyl-(−)-griseusins A (**1**) and B (**2**) (Fig. 1), were isolated from a culture broth of an unidentified actinomycete strain designated as MJ932-SF3. The antibiotics are active against Gram-positive bacteria including methicillin-resistant strain of *Staphylococcus aureus* (MRSA). In this report, we wish to report the production isolation, physico-chemical properties, structure determination and biological properties of **1** and **2**.

A slant culture of the strain MJ932-SF3 on asparagine-glucose agar was inoculated into a 500-ml baffled Erlenmeyer flask containing 110 ml of a seed medium consisting of glycerol 2%, dextrin 2%, Bacto-Soytone (Difco) 1.0%, yeast extract 0.3%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2% in deionized water (pH 7.4 before sterilization). The culture was incubated on a rotary shaker (180 rpm) at 30°C for 2 days, and 1% of the seed culture was transferred into 500-ml baffled Erlenmeyer flask containing 110 ml of the same medium as described above. The fermentation was carried out at 27°C for 3 days on a rotary shaker.

The antibiotics were monitored by antibacterial activity against *Staphylococcus aureus* Smith during the purification process.

The fermentation broth (10 liters) was centrifuged at 2,500 rpm for 10 minutes to remove the mycelial cake. The supernatant was adjusted to pH 2.0 with 6N HCl, and was applied to a column of Diaion HP-20 (500 ml). The active substances were eluted with 50% aq acetone (2 liters) after washing with water (2 liters) and 50% aq MeOH (2 liters). The eluate was concentrated under reduced pressure and extracted with EtOAc (3 liters). The organic layer was concentrated to dryness under reduced pressure. The dried reside (2.5 g) was further purified by using silica gel column chromatography, sephadex LH 20 column chromatography, and centrifugal partition chromatography (CPC) succesively as shown in Scheme 1 (4'-deacetyl-(−)-griseusins A (**1**, 9.0 mg) and B (**2**, 4.5 mg)).

The physico-chemical properties of **1** and **2** are summarized in Table 1. The molecular formulas of **1** and **2** were established as $C_{20}H_{18}O_9$, and $C_{20}H_{20}O_9$ respectively, on the basis of HRFAB-MS and NMR spectral analyses.

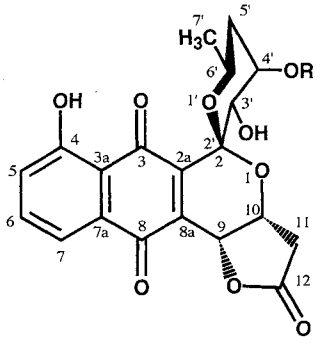
The UV spectra and color reaction with ethanolic $FeCl_3$ of **1** and **2** suggested the presence of perihydroxy-naphthoquinone chromophore in their structure. The IR spectrum of **2** indicated the presence of carboxyl carbonyl (1720 cm^{-1}, br), chelated quinone carbonyl (1648 cm^{-1}), and non-chelated quinone carbonyl (1670 cm^{-1}, sh). The ^1H and ^{13}C NMR spectral data of **1**, and **2** are shown in Table 2.

The ^1H-^1H COSY spectrum of **2** revealed the presence of a trisubstituted benzene and two partial structures as follows: CH_3–CH(O)–CH_2–CH(O)–CH(O)– and –CH_2–CH(O)–CH_2–CO–.

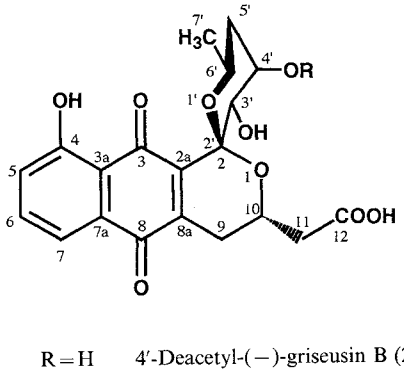
The connectivity among the partial structures of **2** was performed by HMBC experiment. In the HMBC spectrum of **2**, a methylene proton at δ_H 2.50 (9-H_a) was coupled to a quinone carbonyl at δ_C 183.1 (C-8) and two sp^2 carbons at δ_C 139.4 (C-8a), and 146.5 (C-2a). Another methylene protons at δ_H 2.70 and δ_H 2.82 (11-H_2) were coupled to a carboxyl carbon at δ_C 173.0 (C-12). An oxygen-bearing methine proton at δ_H 4.18 (4'-H) was coupled to a signal of two oxygens-bearing sp^3 carbon at δ_C 99.2 (C-2,2').

To clarify the signal assignment of C-2, 2', D-HMBC[1] spectrum of **2** was taken. This technique gives ^1H-^{13}C

Fig. 1. Structure of 4'-deacetyl-(−)-griseusins A (**1**), B (**2**), (−)-griseusin A, and B.

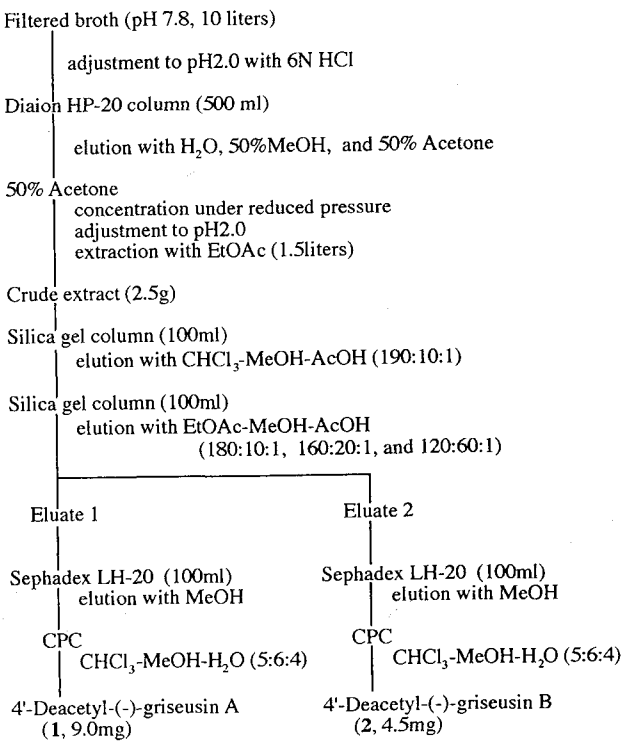


R=H 4'-Deacetyl-(−)-griseusin A (**1**)
R=Ac (−)-Griseusin A



R=H 4'-Deacetyl-(−)-griseusin B (**2**)
R=Ac (−)-Griseusin B

Scheme 1. Isolation of 4'-deacetyl-(−)-griseusins.



long range cross peaks with excellent sensitivities for the small ^{1}H-^{13}C coupling constants less than 2 Hz. The D-HMBC spectrum showed cross peaks between the quaternary carbon at δ_C 99.2 (C-2, 2') and the methylene (9-H_2) at δ_H 2.50, 2.88, and an oxygen bearing methine at δ_H 4.70 (3'-H), respectively. The results of HMBC

Table 1. Physico-chemical propaties of **1** and **2**.

	1	**2**
Appearance	Orange powder	Orange powder
MP (°C)	172~174(dec.)	195~200 (dec.)
Molecular formula	$C_{20}H_{18}O_9$	$C_{20}H_{20}O_9$
FAB-MS (*m/z*)	402 (M^-)	404 (M^-)
HRFAB-MS (*m/z*)		
Calcd :	402.0951	404.1107
Found :	402.0956	404.1108
$[\alpha]_D^{24}$	-198 (*c* 0.1, MeOH)	-162 (*c* 0.05, MeOH)
UVλ_{max} nm(ε)		
MeOH	212 (47,400) 252 (14,200) 431 (5,500)	212 (38,500) 250 (11,800) 427 (4,300)
MeOH-0.005N NaOH	216 (36,500) 266 (10,900) 348 (1,900) 530 (4,700)	214 (33,400) 274 (10,400) 345 (1,800, sh) 528 (4,000)
IRν_{max}(KBr)cm^{-1}	1795, 1673, 1659, 1630	1720 br, 1670, 1648, 1622

Table 2. ^{1}H and ^{13}C NMR data of **1** and **2**.

No	**1**		**2**	
	δ_C[a]	δ_H[b]	δ_C	δ_H
2	98.7	-	99.2	-
2a	142.9	-	146.5	-
3	187.4	-	187.6	-
3a	115.4	-	115.2	-
4	162.1		161.9	
4-OH		11.9(1H, s)		12.13(1H, s)
5	125.5	7.32(1H, dd, 1.8, 8.0)[c]	125.1	7.25(1H, dd, 2.0, 8.0)
6	137.0	7.69(1H, m)	136.2	7.58(1H, m)
7	119.6	7.66(1H, m)	119.0	7.60(1H, m)
7a	131.2	-	131.4	-
8	181.7	-	183.1	-
8a	138.4	-	139.4	-
9	68.4	5.30(1H,d, 2.8)	28.1	2.50(1H, dd, 11.4, 20.0)
				2.88(1H, dd, 18.8, 2.8)
10	66.4	4.82(1H, dd, 2.8, 4.6)	63.6	4.47(1H, m)
11	36.5	2.77(1H, d, 17.0)	39.5	2.70(1H, dd, 8.8, 15.0)
		3.06(1H, dd, 4.6, 17.0)		2.82(1H, dd, 3.0, 15.0)
12	173.1	-	173.0	-
3'	67.8	4.82(1H, m)	68.1	4.70(1H, d, 4.0)
3'-OH	-	2.64(1H, br d, 9)	-	-
4'	68.3	4.17(1H, m)	69.2	4.18(1H, m)
4'-OH	-	2.71(1H, br d, 6)	-	-
5'	39.1	1.93(1H, m)	39.2	1.89(1H, m)
		2.09(1H, m)		2.11(1H, m)
6'	62.7	4.20(1H, m)	61.5	4.33(1H, m)
7'	20.7	1.28(3H, d, 6.0)	20.8	1.25(3H, d, 6.0)

[a] 500 MHz ($CDCl_3$, ref TMS).
[b] 125 MHz ($CDCl_3$, ref TMS).
[c] Integration, multipulicity, coupling constant (Hz).

Fig. 2. Summary of ^{1}H-^{1}H COSY and HMBC experiments of **2**.

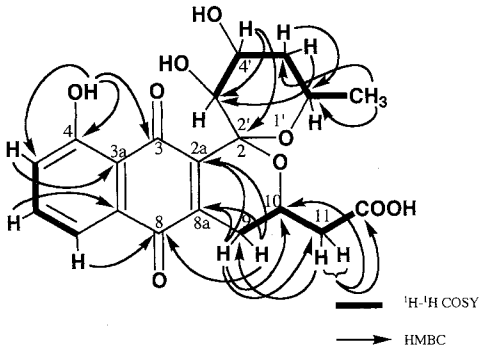


Fig. 3. D-HMBC experiment of **2**.

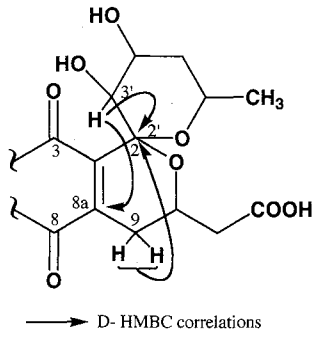
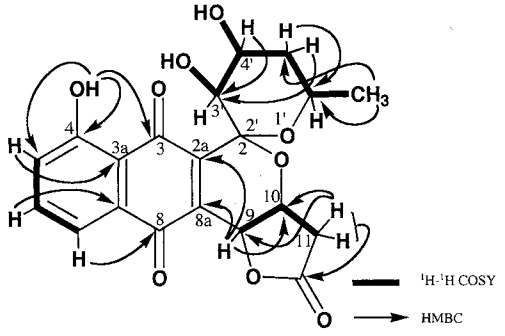


Fig. 4. Summary of ^{1}H-^{1}H COSY and HMBC experiments of **1**.



experiment and D-HMBC experiments of **2** are summarized in Figs. 2 and 3.

Comparisons of the ^{1}H, ^{13}C NMR, and HMBC (Fig. 4) spectra of **1** and **2** showed the compound **1** is very similar to the compound **2** except for the signals of methylene at C-9 in **2**. The IR spectrum of **1** indicated the presence of γ-lactone carbonyl (1785 cm^{-1}). This result was suggested that the carboxyl carbon cyclized the γ-lactone in **2**. In fact, **2** was easily converted to **1** by a treatment with pyridine[2]. The optical rotation values and CD spectrum of **1** and **2** were similar to those of griseusins A and B (**1**, $[\alpha]_D^{23}$ -198 (c 0.1, $CHCl_3$), CD (MeOH) θ_{251} -32000, θ_{289} $+5500$, θ_{351} 0, θ_{390} -950, θ_{460} -2200; griseusin A, $[\alpha]_D^{23}$ -147.8 (c 0.997, $CHCl_3$)[2], CD (MeOH) θ_{250} -33000, θ_{289} $+6400$, θ_{356} 0, θ_{390} -950, θ_{460} -2200; **2**, $[\alpha]_D^{23}$ -162 (c 0.08, $CHCl_3$), CD (MeOH) θ_{255} -32900, θ_{289} $+8200$, θ_{329} 0, θ_{350} -2100, θ_{445} -2100; griseusin B, $[\alpha]_D^{23}$ -190.2 (c 0.5, DMF)[2], CD (MeOH) θ_{255} -24700, θ_{289} $+8100$, θ_{325} 0, θ_{350} -1900, θ_{445} -1400). From the all results, the structure of **1** and **2** were determined to be 4′-deacetyl (−)-griseusin A and B[3], respectively. These antibiotics are new family of naphthoquinone antibiotics (−)-griseusin A and B, lactoquinomycin[4], and 3′-O-α-D-forosaminyl-(+)-griseusin A[5].

Table 3. Antimicrobial activities of 4′-deacetyl-(−)-griseusins A (**1**) and B (**2**).

Test organisms	MIC(μg/ml)	
	1	**2**
Staphylococcus aureus FDA209P	1.56	0.78
S. aureus Smith	0.78	1.56
S. aureus MS9610	0.78	1.56
S. aureus MS16526(MRSA)	0.78	1.56
S. aureus TY-04282(MRSA)	1.56	1.56
Micrococcus luteus IFO3333	0.39	0.78
M.luteus PCI1001	0.78	0.78
Bacillus subtilis NRRL B-558	1.56	1.56
B. cereus ATCC10702	1.56	1.56
Corynebacterium bovis 1810	1.56	1.56
Escherichia coli NIHJ	50	100
Shigella dysenteriae JS11910	12.5	25
Salmonella enteritidis	100	100
Proteus mirabilis IFM OM-9	50	50
Providencia rettgeri GN466	100	>100
Serratia marcescens	100	100
Pseudomonas aeruginosa A3	>50	>50
Klebsiella pneumonie PCI602	100	100
Mycobacterium smegmatis ATCC607*	>100	>100
Candida albicans 3147	100	>100

Mueller Hinton agar 37°C 18 hours.
* 37°C 42 hours.

The antimicrobial activities of 4′-deacetyl-(−)-griseusins A and B are shown in Table 3. They showed broad and strong antimicrobial activities against Gram-positive bacteria including MRSA and some Gram-negative bacteria.

It was found that another actinomycete strain, MJ934-SF1 of our institute also produced both antibiotics, **1** and **2**.

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