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STRUCTURES OF NOVEL ANTIBIOTICS, FURAQUINOCINS A AND B

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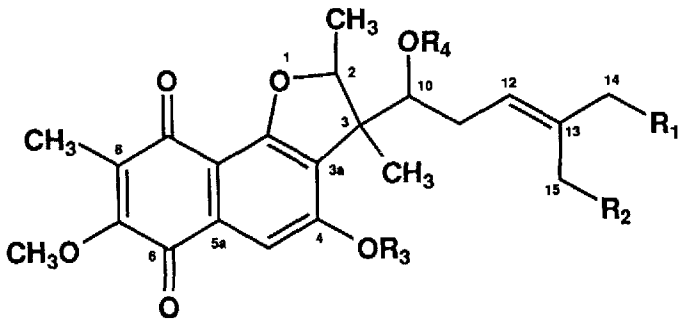
Summary: Structures of novel citocydal antibiotics furaquinocins A and B, isolated from the fermentation broth of *Streptomyces* sp. KO-3988, was elucidated on the basis of spectroscopic analyses.

In the course of a screening program for novel antibiotics possessing cytocidal activity, furaquinocins A (**1**) and B (**2**) were isolated from the fermentation broth of *Streptomyces* sp. KO-3988.[1] These antibiotics exhibited strong activity against HeLa S_3 cells *in vitro* at concentration of 3.1 and 1.6 μg/mL, respectively. This paper describes the structure elucidation of furaquinocins A (**1**) and B (**2**).



	R_1	R_2	R_3	R_4
1	H	OH	H	H
2	OH	H	H	H
3	H	OAc	Ac	Ac
4	H	OH	CH_3	H

Furaquinocin A (**1**),[2] yellow needles, mp 182-183 °C, $[\alpha]_D^{19}$ -46° (*c* 0.58, $CHCl_3$), and furaquinocin B (**2**),[3] yellow needles, mp 101-104 °C, $[\alpha]_D^{19}$ -132° (*c* 0.57, $CHCl_3$), showed similar UV and IR absorption spectra, suggesting the presence of the same chromophore. The EIMS of **1** and **2** showed mainly the molecular ion at *m/z* 402 in the beginning of measurement and then a gradual increase in an ion of the reduced form (*m/z* 404, M^++2) to predominate as described for quinones or related compounds.[4] A common molecular formula, $C_{22}H_{26}O_7$, for furaquinocins A and B was established by elemental analysis [**1**: Found, C 63.10, H: 6.77; Calcd, C 62.85, H 6.71 for $C_{22}H_{26}O_7 \cdot H_2O$] and high resolution EIMS [**2**: Found *m/z* 402.1681, Calcd for $C_{22}H_{26}O_7$: M, 402.1677].

^1H and ^{13}C NMR spectra of **1** and **2** were also analogous to each other and DEPT

experiments revealed that both compounds possessed the following groups: 4 x $-CH_3$, 1 x $-CH_2-$, 1 x -C-, 1 x CH_3O-, 1 x $-CH_2O-$, 2 x -CH-O-, 2 x =CH-, 8 x =C-, and 2 x -C=O. This accounts for 22 carbons and 23 protons. The three missing protons belong to 3 hydroxyl groups, which was confirmed by acetylation of **1** to form a triacetate [**3**, *m/z* 528 (M^+)]. One of three hydroxyl groups was on an aromatic ring, viz., a phenol, which was shown by treatment of **1** with trimethylsilyldiazomethane and diisopropylethylamine[5] to give an *O*-methyl ether [**4**, *m/z* 418 $(M+2)^+$].

The extensive 1D and 2D NMR experiments were carried out by using furaquinocin B (**2**). The ^1H-^1H COSY spectrum of **2** revealed cross peaks for Me-2/H-2, H-10/H_2-11, H_2-11/H-12, and H-12/H_3-15. The ^1H- ^{13}C COSY experiment provided definitive assignments for all protonated carbons as shown in Table 1. The presence of a

Table 1. ^1H and ^{13}C NMR Chemical Shifts (in $CDCl_3$) of Furaquinocin B (2) and Protons to Which a Long-Range Correlation Was Observed in the LSPD Experiment

	^{13}C	^1H	LSPD (^1H)
1	-		
2	88.9 d	4.69 q	Me-2, Me-3
Me-2	16.1 q	1.32 d (3H)	
3	52.4 s		Me-2, Me-3, H-10
Me-3	18.9 q	1.37 s (3H)	
3a	124.5 s		H-5, H-10
4	158.4 s		H-5
5	110.7 d	7.15 s	
5a	134.1 s		H-5
6	180.7 s		H-5, Me-8
7	156.9 s		MeO-7, Me-8
MeO-7	60.7q	4.00 s (3H)	
8	133.7 s		Me-8
Me-8	9.3 q	2.04 s (3H)	
9	183.7 s		Me-8
9a	109.2 s		H-5
9b	160.4 s		H-2
10	73.0 d	4.07 dd	H-11
11	31.9 t	2.57 dt	
		2.19 ddd	
12	120.1 d	5.52 m	
13	140.0 s		H_2-14, H_3-15
14	68.0 t	4.09 s (2H)	
15	14.3 q	1.74 brs (3H)	

J (H/H) in Hz: Me-2/2=6.5; 10/11=10, 10/11'=1; 11/11'=14.5; 11/12=10; 11'/12=4.5

naphthoquinone group as well as three partial structures **2a-2c** (Fig. 1) were thus deduced by these NMR data. The EIMS of **2** gave an intense peak at *m/z* 287 (M^+-

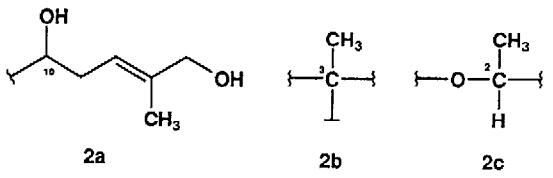


Fig. 1. Partial Structures of 2.

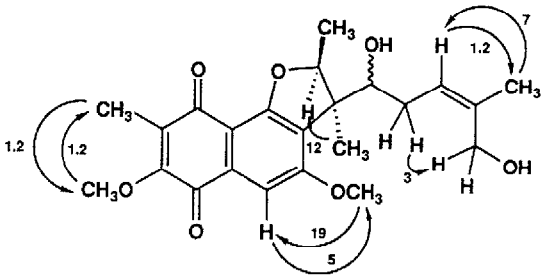


Fig. 2. NOE (%) of 4.

$C_6H_{11}O_2$) which was due to the fragment ion generated by loss of the segment **2a**. The high-field resonance for the olefinic methyl (C-15, δ_C 14.3) of **2** implied 12*E*-configuration.[6] Further information on the structural framework was obtained by analyzing the ^1H-^{13}C long range couplings observed through the LSPD experiments.[7] As summarized in Table 1, the location of the substituents on the naphthoquinone ring was clealy disclosed. The methyl protons at δ_H 2.04 were coupled obviously with C-7 (J=4.5 Hz), C-8 (J=6.5 Hz), and C-9 (J=3.5 Hz), and weakly with C-6 (J<1 Hz). The methoxy protons at δ_H 4.00 showed connectivity with C-7 (J=3.5 Hz). Thus the methyl and the methoxy groups were shown to be on C-8 and C-7, respectively. On irradiation of the aromatic proton at δ_H 7.15 signals for C-3a, C-4, C-5a, C-6, and C-9a were simplified. This observation along with consideration of the ^{13}C chemical shifts indicated that the aromatic proton was on C-5, an alkyl group on C-3a (δ_C 124.5), and oxygen atoms on C-4 (δ_C 158.4) and C-9b (δ_C 160.4). Three segments **2a-2c** and the naphthoquinone ring were connected with one another as follows. On irradiation of H-2 (δ_H 4.69), the doublet-like signal (J=5.5 Hz) for C-9b collapsed into a singlet, suggesting that the oxygen in the segment **2c** was attached to C-9b to form an ether linkage. The remaining oxygenated carbon (C-4), therefore, bore the phenol group. The methyl protons (δ_H 1.32) on C-2 showed connectivity with C-3 in addition to C-2, while the methyl protons (δ_H 1.37) on C-3 was coupled with C-2 as well as C-3. Thus C-2 in **2c** was connected with C-3 in **2c**. Irradiation of the hydroxymethine proton at δ_H 4.07 (H-10) simplified the signals for not only C-3 but also C-3a, which indicated that C-10 in **2a** was connected with C-3 in **2b**, and C-3 was in turn with C-3a, consequently to construct a dihydrofuran ring attached to the naphthoquinone. The structure of furaquinocin B was, therefore, concluded to be **2**.

The difference in the structure of furaquinocin A (**1**) from that of **2** was found only in the olefin-geometry in the side chain. The olefinic methyl of **1**, on comparison with that of **2**, resonated in the lower field (**1**: δ_C 23.2; **2**: δ_C 14.3), while the hydroxymethyl carbon of **1** was in the higher field (**1**: δ_C 61.4; **2**: δ_C 68.0). This observation revealed 12*Z* for **1**.[6] NOE results using the *O*-methyl ether (**4**) were coincident as shown in (Fig. 2) and provided additional proof for the adjacent location of the following groups: H-5/MeO on C-4, Me-8/MeO on C-7, and H-2/Me-3. Observation

of 12% NOE from Me-3 to H-2 suggested the relative configuration of Me-2 and Me-3 to be *trans*.

The biogenesis of furaquinocins A and B may raise an interesting subject since these compounds possess a unique structure consisting of the chromophore of naphtho[1,2b]furan-6,9-dione with an isoprenoid-like side chain. The biosynthetic pathway of **1** and **2** is currently under investigation. Studies on the absolute stereochemistry of **1** or **2** as well as other accompanying components in the *Streptomyces* sp. KO-3988 are in progress.

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References and Notes

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2. UV (MeOH) 222, 268, 292, and 408 nm; IR (KBr) 3450, 1672, 1643, 1381, and 1115 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 1.31 (3H, s; Me-3), 1.30 (3H, d, *J*=6 Hz; Me-2), 1.86 (3H, br s; H_3-14), 2.03 (3H, s; Me-8), 2.13 (1H, dd, *J*=14.5 and 6 Hz; H-11), 2.61 (1H, dt, *J*=14.5 and 10 Hz; H'-11), 3.95 (1H, d, *J*=10 Hz; H-10), 3.98 (3H, s; MeO), 4.00 (1H, d, *J*=11.5 Hz; H-15), 4.41 (1H, d, *J*=11.5 Hz; H'-15), 4.67 (1H, q, *J*=6 Hz; H-2), 5.50 (1H, dd, *J*=10 and 6 Hz; H-12), 7.12 (1H, s; H-5), and 11.32 (1H, br s; OH-4); ^{13}C NMR ($CDCl_3$) δ_C 9.3 (q, Me-8), 16.1 (q, Me-2), 18.9 (q, Me-3), 23.2 (q, C-14), 32.4 (t, C-11), 52.8 (s, C-3), 60.6 (q, MeO), 61.4 (t, C-15), 71.4 (d, C-10), 88.9 (d, C-2), 108.8 (s, C-9a), 111.0 (d, C-5), 124.6 (s, C-3a), 124.9 (d, C-12), 133.6 (s, C-8), 134.0 (s, C-5a), 138.3 (s, C-13), 156.9 (s, C-7), 158.9 (s, C-4), 160.6 (s, C-9b), 180.8 (s, C-6), and 183.8 (s, C-9); EIMS *m/z* 402 (M^+), 287, 273, and 259.
3. UV (MeOH) 223, 269, 294, and 410 nm; IR (KBr) 3450, 1659, 1635, 1368, and 1100 cm^{-1}; EIMS *m/z* 402 (M^+), 287, 273, and 259.
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