Oxidation of **5** with MCPBA gave the epoxide (**17**) in poor yield, but a better result was obtained via three conventional reactions. When **17** was reduced with $NaBH_4$, the expected 9*R* alcohol (**18**) was obtained in quantitative yield. This (**18**) was converted to maridonolide II (**4b**) via **19**–**21** as described for **3b**. Compound **4b** was also obtained from **16** via only two reactions: MCPBA oxidation and hydrolysis with K_2CO_3. Similarly, maridonolide I (**4c**) was synthesized from **5**.

The aglycons **2b,c**, **3a–c**, and **4b,c** thus synthesized were identical in all respects (NMR, MS, IR, $[\alpha]_D$) with those derived from natural antibiotics.[20]

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Supplementary Material Available: Physical data for compounds **2b,c**, **3a–c**, **4b,c**, **13**, **15**, **17**, and **18** and calculation data (MMP2-CONFLEX2) for **11** and **12** (26 pages). Ordering information is given on any current masthead page.

(20) These aglycons (isomeric mixtures with respect to the hemiacetal positions) except for **3b**[10,19] have never been reported.

Biosynthesis of Furaquinocins A and B

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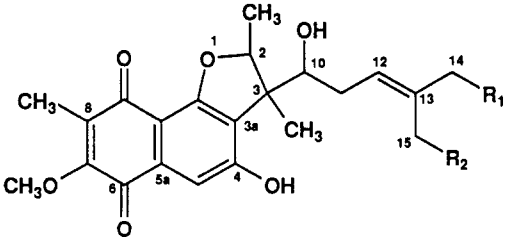
Summary: The biosynthetic pathway to furaquinocins was investigated by means of incorporation experiments with [1-^{13}C]acetate, [1,2-$^{13}C_2$]acetate, and [*methyl*-^{13}C]-L-methionine. Furaquinocins are derived from a pentaketide, two mevalonates, and two C_1 units from L-methionine.

The furaquinocins A (**1**) and B (**2**) comprise a novel polyketide antibiotic complex produced by *Streptomyces* sp. KO-3988, which exhibit cytotoxic activity against HeLa S_3 cells but no antimicrobial activity.[1] Extensive spectroscopic analyses have demonstrated that **1** and **2** possess a unique structure, which consists of a naphtho[1,2-*b*]-furan-6,9-dione chromophore with an isoprenoid-like side chain.[2]



1 R_1 = H, R_2 = OH
2 R_1 = OH, R_2 = H

In the present report, we describe studies on the biosynthetic pathway to furaquinocins A and B by means of feeding experiments using ^{13}C-labeled precursors.[3]

Assignment of the ^{13}C NMR signals of **1** and **2** was fully established on the basis of ^{1}H–^{13}C COSY along with LSPD experiments[2] and are presented in Table I. The ^{13}C NMR spectra of furaquinocins A (**1**) and B (**2**) labeled with [1-^{13}C]acetate revealed the enrichment of nine carbon signals (Me-2, C-3, C-4, C-5a, C-7, C-9, C-9b, C-11, and C-13). The intensity ratios of nonlabeled ones are also shown in Table I. In a feeding experiment with [1,2-$^{13}C_2$]acetate, 20 carbons were found to be derived from acetate, of which 11 carbons (C-2, Me-3, C-3a, C-5, C-6, C-8, C-9a, C-10, C-12, C-14, and C-15) arose from C-2 of acetate. The remaining two carbons (MeO-7 and Me-8) were not derived from acetate units. The ^{13}C–^{13}C coupling constants (J_{CC}) of **1** and **2** labeled with [1,2-$^{13}C_2$]acetate are given in Table I. The carbons in the naphthoquinone ring show two kinds of coupling with equal signal intensities. These coupling constants are coincident with those of the adjacent carbons.

Table I. ^{13}C NMR Chemical Shifts, Enrichment Ratio of [1-^{13}C]Acetate-Labeled Furaquinocins, and J_{CC} (in Hz) of [1,2-$^{13}C_2$]Acetate-Labeled Furaquinocins[a]

	1			2		
carbon	δ	enrichmt ratio[b]	*J*	δ	enrichmt ratio[b]	*J*
2	88.9	1.0	38	88.9	1.0	38
Me-2	16.1	5.1	38	16.1	5.2	38
3	52.8	3.3	35	52.4	3.2	34
Me-3	18.9	1.0	35	18.9	0.8	34
3a	124.6	0.4	61, 69	124.5	0.2	61, 70
4	158.9	6.9	69, 67	158.4	5.8	70, 67
5	111.0	0.8	67, 66	110.7	1.0	67, 66
5a	134.0	4.2	66, 54	134.1	4.3	66, 54
6	180.8	0.4	54, 57	180.7	0.3	54, 57
7	156.9	5.3	57, 74	156.9	3.8	57, 74
MeO-7	60.6	0.8	*c*	60.7	0.4	*c*
8	133.6	0.8	74, 52	133.7	0.6	74, 52
Me-8	9.3	1.0	*c*	9.3	0.8	*c*
9	183.8	4.5	52, 60	183.7	4.4	52, 60
9a	108.8	0.3	60, 73	109.2	0.2	60, 73
9b	160.6	4.0	73, 61	160.4	4.8	73, 61
10	71.4	0.8	s[d]	73.0	1.2	s[d]
11	32.4	6.0	43	31.9	7.7	44
12	124.9	0.7	43	120.1	0.9	44
13	138.3	4.0	46	140.0	5.0	43
14	23.2	1.0	s[d]	68.0	0.7	s[d]
15	61.4	0.8	46	14.3	0.8	43

[a] Each sample was dissolved in $CDCl_3$ and chemical shifts were shown with reference to $CDCl_3$ as 77.0 ppm. [b] Enrichment ratios were relative to the C-2 signal as 1.0. [c] Signal for the carbon not incorporated. [d] Signal was singlet, so the carbon had no coupling with others.

(1) Komiyama, K.; Funayama, S.; Anraku, Y.; Ishibashi, M.; Ōmura, S. *J. Antibiot.*, in press.
(2) Funayama, S.; Ishibashi, M.; Anraku, Y.; Komiyama, K.; Ōmura, S. *Tetrahedron Lett.*, in press.
(3) A seed culture of *Streptomyces* sp. KO-3988 was made by the method previously described.[1] The seed culture (1–2 mL) was inoculated into each 500-mL Sakaguchi flask containing 100 mL of medium composed of 2% starch, 1% soy bean meal, and 0.3% NaCl (pH 7.0 before sterilization). ^{13}C-labeled precursors in 2 mL of aqueous solution ([1-^{13}C]- and [1,2-$^{13}C_2$]acetate, 100 mg; [*methyl*-^{13}C]-L-methionine, 50 mg) was added to each flask after 6 h of cultivation and the cultures were then incubated for 66 h at 27 °C. ^{13}C-enriched furaquinocins (2–4 mg) were isolated from the cultured broth (0.5–1 L) by solvent extraction, silica gel chromatography, and Sephadex LH-20 gel filtration as previously reported.[1] ^{13}C NMR spectra were recorded on a Varian XL-400 spectrometer in deuteriochloroform.

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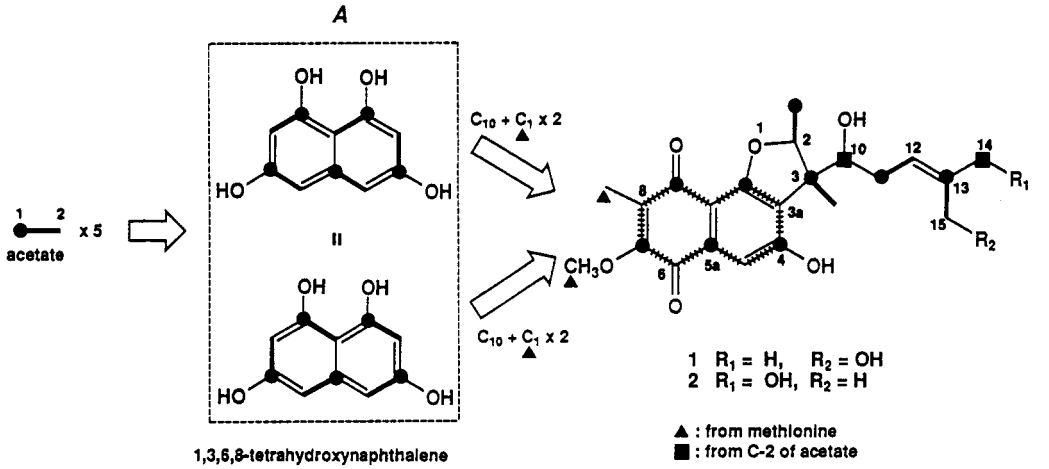
Scheme I. Biosynthetic Pathway of Furaquinocins A (1) and B (2)



This result suggests that the biosynthesis of the naphthoquinone ring may involve two different patterns of acetate arrangement, which is also revealed by the 2D INADEQUATE[4] spectrum of [1,2-$^{13}C_2$]acetate labeled **1**. The furaquinocins are therefore most likely produced through a symmetric intermediate such as 1,3,6,8-tetrahydroxynaphthalene (A; Scheme I), which was also proposed for the biosynthesis of scytalone[5-7] and napyradiomycins.[8] The labeling pattern of the C_{10} unit (a part of the dihydrofuran ring and the side chain: Me-2, C-2, C-3, Me-3, and C-10–C-15) is consistent with biosynthesis involving mevalonate. The ^{13}C NMR signals for C-10 and C-14 appear as singlets and eight additional satellite peaks are observed for the other carbons of the side chain, their J_{CC} values indicating four acetate units for Me-2/C-2, C-3/Me-3, C-11/C-12, and C-13/C-15. The only structural difference between **1** and **2** is the geometry of Δ^{12}-double bond. In each of the ^{13}C spectra of **1** and **2** labeled with [1,2-$^{13}C_2$]acetate, the signal for C-14 (CH_3 in **1** and CH_2OH in **2**) is observed as a singlet and that for C-15 (CH_2OH in **1** and CH_3 in **2**) is coupled with C-13 as shown in Table I. These results imply that both terminal methyl carbons (C-14 and C-15) could be oxygenated and that no *E–Z* isomerization took place during the biosynthetic process.

The ^{13}C NMR spectra of **1** and **2** labeled with [*methyl*-^{13}C]-L-methionine exhibit high incorporations (more than 90%) for two methyl carbons (MeO-7 and Me-8), thereby accounting for the origin of all 22 carbons of **1** and **2**.

It may be worth noting that the carbon for Me-8 does not arise from propionate, which had been suggested when the symmetrical intermediate (A) was postulated in the biosynthesis of the naphthoquinone ring. Concurrent attachment of an isoprenoid side chain and a C_1 unit from methionine onto the polyketide backbone is also interesting. Although a few isoprenoid antibiotics produced by actinomycetes have been reported,[8-11] furaquinocins may be the first example[12] involving a carbon–carbon bond between an inside position of the isoprenoid chain (C-3) and the polyketide nucleus (C-3a).

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(4) Bax, A.; Freeman, R.; Kempsell, S. P. *J. Am. Chem. Soc.* **1980**, *102*, 4849–4851.
(5) Sankawa, U.; Shimada, H.; Sato, T.; Kinoshita, T.; Yamasaki, K. *Tetrahedron Lett.* **1977**, 483–486.
(6) Seto, H.; Yonehara, H. *Tetrahedron Lett.* **1977**, 487–488.
(7) Bardshiri, E.; Simpson, T. J. *Tetrahedron* **1983**, *39*, 3539–3542.
(8) Shiomi,. K.; Iinuma, H.; Naganawa, H.; Isshiki, K.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1987**, *40*, 1740–1745.
(9) Cane, D. E.; Rossi, T.; Tillman, A. M.; Pachlatko, J. P. *J. Am. Chem. Soc.* **1981**, *103*, 1838–1843.
(10) Isshiki, K.; Tamamura, T.; Sawa, T.; Naganawa, H.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1986**, *39*, 1634–1635.
(11) Funayama, S.; Eda, S.; Komiyama, K.; Ōmura, S.; Tokunaga, T. *Tetrahedron Lett.* **1989**, *30*, 3151–3154.
(12) For those obtained from plants, see: Saitoh, T.; Shibata, S. *Tetrahedron Lett.* **1975**, 4461–4462.

Solution and Crystal Structures of (+)-Hitachimycin (Stubomycin)[1]

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Summary: The complete relative and absolute stereochemistry, as well as the solid-state and solution conformations of the antitumor–antibiotic (+)-hitachimycin (stubomycin) (**1**) have been defined via two-dimensional NMR experiments, single-crystal X-ray analysis, and computational methods.

[†]Deceased February 13, 1985.

(1) Dedicated to the memory of Professor Jerry Donohue.

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