# Structure and Biosynthesis of Xanthoquinodins, Anticoccidial Antibiotics

**Noriko Tabata, Hiroshi Tomoda, Keiichi Matsuzaki, and Satoshi Ōmura***

*Contribution from the Research Center for Biological Function, The Kitasato Institute, and School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo 108, Japan*

*Received March 22, 1993*®

**Abstract:** The structures of xanthoquinodins A1, A2, A3, B1, and B2, anticoccidial antibiotics, produced by *Humicola* sp. FO-888 were confirmed by analyzing $^{13}C$-labeled xanthoquinodins prepared biosynthetically in $^{13}C$-labeled precursor feeding experiments on the basis of extensive spectroscopic techniques including $^1H$–$^1H$ COSY, $^{13}C$–$^1H$ COSY, and HMBC. Xanthoquinodins are the first heterodimers of octaketide-derived xanthone and anthraquinone monomers connected in an "end-to-body" fashion. The stereochemistry of xanthoquinodin B2 was determined by NMR including NOE measurements. Accordingly, the stereochemistry of xanthoquinodins A1, A2, and B1 was also deduced by studying the mechanism of interconversion among xanthoquinodins.

## Introduction

Xanthoquinodins A1, A2, A3, B1, and B2 are a novel series of fungal metabolites produced by *Humicola* sp. FO-888.[1] These compounds were found to exhibit anticoccidial activity in an *in vitro* assay system using *Eimeria tenella* as a parasite and BHK-21 cells as a host. The physicochemical properties of xanthoquinodin A1 appear similar to those of M-4126 originally isolated as an antibacterial antibiotic.[2] However, its structure has not been reported. In this paper, we report the structures of the xanthoquinodins, which possess an interesting heterodimer consisting of xanthone and anthraquinone moieties. Beticolins[3] and cebetins,[4] both discovered as fungal toxins, are constructed with a heterodimer core analogous to that of the xanthoquinodins. However, thus far, the biosynthesis of this class of compounds has not been clarified. We also report the complete assignment of the $^{13}C$ NMR signals of the xanthoquinodins and the incorporation of various $^{13}C$-labeled precursors into the xanthoquinodins. Thus, the structures of the xanthoquinodins are secured, and a possible biosynthetic route to xanthoquinodin A1 is proposed. Furthermore, the relative configurations of the xanthoquinodins are determined by NMR including NOE measurements.

## Results

**Structure of Xanthoquinodin A1 and Incorporation of $^{13}C$-Labeled Precursors.** The molecular formula of xanthoquinodin A1 (**1**) was determined to be $C_{31}H_{24}O_{11}$ ($m/z$ found 572.1316, calcd 572.1317) on the basis of HREI-MS measurements. The fragment ion peak at $m/z$ 513 in the EI-MS spectrum suggested the presence of a methyl ester moiety in **1**. The $^{13}C$ NMR spectrum ($CDCl_3$) of **1** (Table I) showed 31 resolved peaks, which were classified into 1 $CH_3$, 1 $OCH_3$, 3 —$CH_2$—, 2 —CH—, 5 —CH═, and 19 quaternary carbons by analysis of the DEPT spectrum. The $^1H$ NMR spectrum of **1** (Table I) displayed 24 proton signals. The four downfield singlet signals (δ 14.80, 13.91, 11.93, and 11.69) were due to phenolic hydroxyl protons. The $^1H$ NMR signal at δ 2.54 disappeared by the addition of $D_2O$,

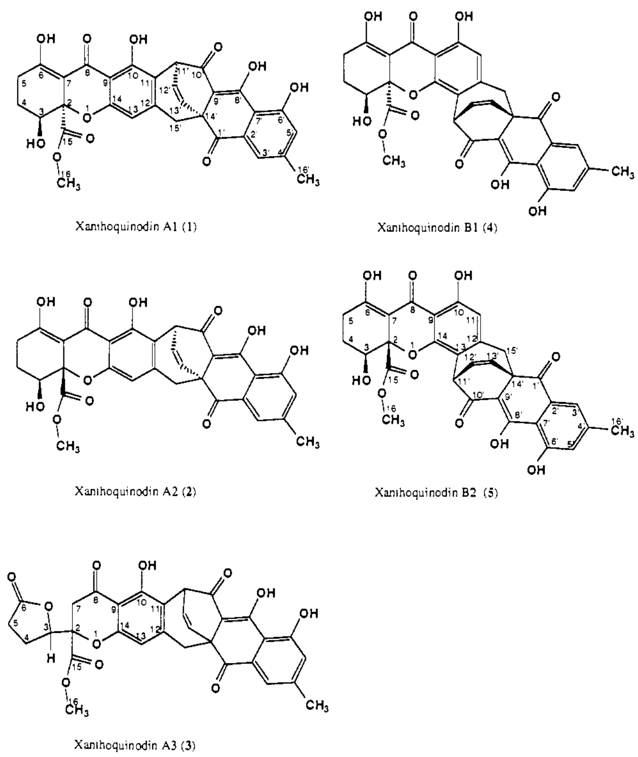



**Figure 1.** Structures of xanthoquinodins A1, A2, A3, B1, and B2.

indicating an exchangeable hydroxy proton. The singlet methyl signal (δ 3.69) was assigned to $OCH_3$ of a methyl ester group. The connectivity of proton and carbon atoms was confirmed by the $^{13}C$–$^1H$ COSY spectrum (Table I). Analyses of $^1H$–$^1H$ COSY and HMBC spectra revealed the three partial structures I, II, and III (Figure 2). Observation of a cross peak from H-3′ (δ 7.56) to C-1′ (δ 195.52) in the HMBC spectrum indicated the connection of C-1′ in II to C-3′ in III *via* a single carbon atom.

In order to complete the structure of **1**, [$^{13}C$]xanthoquinodins were prepared biosynthetically by sodium [1-$^{13}C$]-, [2-$^{13}C$]-, and [1,2-$^{13}C_2$]acetates and L-[*methyl*-$^{13}C$]methionine feeding experiments. All the $^{13}C$ NMR data of the $^{13}C$-enriched xanthoquinodin A1 are summarized in Table II. From the sodium [1-$^{13}C$]- and [2-$^{13}C$]acetates and L-[*methyl*-$^{13}C$]methionine incorporation experiments, the 14 carbons (C-2, C-4, C-6, C-8, C-10, C-12, C-14, C-2′, C-4′, C-6′, C-8′, C-10′, C-12′, and C-14′), the 16 carbons (C-3, C-5, C-7, C-9, C-11, C-13, C-15, C-1′, C-3′, C-5′,

* Abstract published in *Advance ACS Abstracts*, August 15, 1993.

(1) Tabata, N.; Suzumura, Y.; Tomoda, H.; Masuma, R.; Haneda, K.; Kishi, M.; Iwai, Y.; Ōmura, S. *J. Antibiot.* **1993**, *46*, 749.

(2) Mizuno, K.; Takada, M.; Furuhashi, H. Jpn. Kokai Patent JP 49 1794, Jan 9, 1974.

(3) Jalal, M. A. F.; Hossain, M. B.; Robeson, D. J.; Helm, D. *J. Am. Chem. Soc.* **1992**, *114*, 5967.

(4) Milat, M.-L.; Praange, T.; Ducrot, P.-H.; Tabet, J.-C.; Einhorn, J.; Blein, J.-P.; Lallelmand, J.-Y. *J. Am. Chem. Soc.* **1992**, *114*, 1478.

0002-7863/93/1515-8558$04.00/0 © 1993 American Chemical Society

**Table I.** $^1H$ and $^{13}C$ NMR Chemical Shifts of **1**, **2**, **3**, **4**, and **5**

| | **1** | | **2** | | **3** | | **4** | | **5** | |
|---|---|---|---|---|---|---|---|---|---|---|
| carbon no. | $^{13}C$ chemical shifts (ppm)[a] | $^1H$ chemical shifts (ppm)[b] | $^{13}C$ chemical shifts (ppm)[a] | $^1H$ chemical shifts (ppm)[b] | $^{13}C$ chemical shifts (ppm)[a] | $^1H$ chemical shifts (ppm)[b] | $^{13}C$ chemical shifts (ppm)[a] | $^1H$ chemical shifts (ppm)[b] | $^{13}C$ chemical shifts (ppm)[a] | $^1H$ chemical shifts (ppm)[b] |
| C-2 | 83.95 | | 84.41 | | 83.99 | | 84.63 | | 85.39 | |
| C-3 | 66.84 | 4.27 (1H, dd, $J$ = 3.9, 1.9 Hz) | 71.82 | 4.25 (1H, dd, $J$ = 12.5, 5.0 Hz) | 80.72 | 4.83 (1H, dd, $J$ = 7.0, 7.0 Hz) | 66.91 | 4.48 (1H, dd, $J$ = 4.0, 1.9 Hz) | 71.73 | 4.45 (1H, dd, $J$ = 12.3, 4.9 Hz) |
| C-3–OH | | 2.54 (1H, s) | | *c* | | *c* | | *c* | | *c* |
| C-4 | 23.00 | 1.91 (1H, dddd, $J$ = 14.0, 12.0, 6.8, 1.9 Hz), 2.12 (1H, ddd, $J$ = 14.0, 6.8, 3.9 Hz) | 23.85 | 2.05 (1H, m), 2.14 (1H, m) | 21.97 | 2.36 (2H, m) | 22.96 | 2.05 (1H, m), 2.18 (1H, ddd, $J$ = 14.5, 6.5, 4.0 Hz) | 23.87 | 2.13 (1H, m), 2.24 (1H, m) |
| C-5 | 24.41 | 2.38 (1H, dd, $J$ = 20.0, 6.8 Hz), 2.81 (1H, ddd, $J$ = 20.0, 12.0, 6.8 Hz) | 27.58 | 2.66 (2H, m) | 27.55 | 2.54 (1H, m), 2.60 (1H, m) | 24.45 | 2.38 (1H, dd, $J$ = 19.5, 7.0 Hz), 2.85 (1H, ddd, $J$ = 19.5, 12.0, 7.0 Hz) | 27.71 | 2.70 (2H, m) |
| C-6 | 179.79 | | 178.32 | | 175.30 | | 180.42 | | 178.73 | |
| C-6–OH | | 13.91 (1H, s) | | 13.83 (1H, s) | | | | 14.10 (1H, s) | | 13.95 (1H, s) |
| C-7 | 100.14 | | 101.18 | | 39.34 | 2.93 (1H, d, $J$ = 17.0 Hz), 3.12 (1H, d, $J$ = 17.0 Hz) | 100.18 | | 101.60 | |
| C-8 | 186.66 | | 186.42 | | 193.21 | | 186.83 | | 186.67 | |
| C-9 | 105.08 | | 105.09 | | 105.71 | | 105.60 | | 105.68 | |
| C-10 | 158.70 | | 158.55 | | 158.52 | | 160.39 | | 160.24 | |
| C-10–OH | | 11.93 (1H, s) | | 11.80 (1H, s) | | 12.00 (1H, s) | | 11.27 (1H, s) | | 11.09 (1H, s) |
| C-11 | 116.53 | | 116.00 | | 116.04 | | 113.96 | 6.17 (1H, s) | 113.72 | 6.14 (1H, s) |
| C-12 | 146.50 | | 147.02 | | 147.70 | | 146.54 | | 146.79 | |
| C-13 | 110.41 | 6.07 (1H, s) | 110.45 | 6.13 (1H, s) | 110.28 | 6.15 (1H, s) | 114.25 | | 114.80 | |
| C-14 | 156.13 | | 157.10 | | 157.52 | | 153.80 | | 154.98 | |
| C-15 | 170.95 | | 169.78 | | 168.65 | | 170.93 | | 169.81 | |
| C-16 | 53.49 | 3.69 (3H, s) | 53.35 | 3.69 (3H, s) | 53.75 | 3.75 (3H, s) | 53.59 | 3.76 (3H, s) | 53.29 | 3.74 (3H, s) |
| C-1′ | 195.52 | | 195.69 | | 195.34 | | 195.44 | | 195.46 | |
| C-2′ | 132.21 | | 132.24 | | 132.25 | | 132.27 | | 132.25 | |
| C-3′ | 120.97 | 7.56 (1H, d, $J$ = 1.0 Hz) | 120.97 | 7.56 (1H, s) | 121.11 | 7.58 (1H, d, $J$ = 1.0 Hz) | 121.08 | 7.57 (1H, d, $J$ = 1.5 Hz) | 121.09 | 7.57 (1H, s) |
| C-4′ | 147.52 | | 147.55 | | 148.31 | | 147.78 | | 147.65 | |
| C-5′ | 124.32 | 7.07 (1H, d, $J$ = 1.0 Hz) | 124.36 | 7.08 (1H, s) | 124.33 | 7.08 (1H, d, $J$ = 1.0 Hz) | 124.35 | 7.09 (1H, d, $J$ = 1.0 Hz) | 124.35 | 7.08 (1H, s) |
| C-6′ | 161.41 | | 161.41 | | 161.42 | | 164.45 | | 161.20 | |
| C-6′–OH | | 11.69 (1H, s) | | 11.72 (1H, s) | | 11.68 (1H, s) | | 11.70 (1H, s) | | 11.46 (1H, s) |
| C-7′ | 115.10 | | 115.12 | | 115.01 | | 115.05 | | 114.00 | |
| C-8′ | 182.76 | | 182.76 | | 182.89 | | 183.24 | | 181.74 | |
| C-8′–OH | | 14.80 (1H, s) | | 14.82 (1H, s) | | 14.80 (1H, s) | | 14.98 (1H, s) | | 14.93 (1H, s) |
| C-9′ | 106.49 | | 106.73 | | 106.50 | | 107.00 | | 106.88 | |
| C-10′ | 189.07 | | 188.97 | | 188.93 | | 188.46 | | 189.60 | |
| C-11′ | 37.85 | 4.79 (1H, dd, $J$ = 6.5, 1.0 Hz) | 37.90 | 4.77 (1H, d, $J$ = 6.5 Hz) | 37.84 | 4.77 (1H, dd, $J$ = 6.2, 0.1 Hz) | 38.51 | 4.79 (1H, dd, $J$ = 6.5, 1.0 Hz) | 38.94 | 4.86 (1H, d, $J$ = 6.2 Hz) |
| C-12′ | 131.44 | 6.47 (1H, dd, $J$ = 8.3, 6.5 Hz) | 131.60 | 6.48 (1H, dd, $J$ = 8.0, 6.5 Hz) | 131.31 | 6.48 (1H, dd, $J$ = 8.4, 6.2 Hz) | 131.29 | 6.49 (1H, dd, $J$ = 8.0, 6.2 Hz) | 131.45 | 6.54 (1H, dd, $J$ = 8.3, 6.2 Hz) |
| C-13′ | 132.73 | 6.66 (1H, dd, $J$ = 8.3, 1.0 Hz) | 132.64 | 6.66 (1H, d, $J$ = 8.0 Hz) | 132.87 | 6.69 (1H, dd, $J$ = 8.4, 0.1 Hz) | 132.90 | 6.69 (1H, dd, $J$ = 8.0, 1.0 Hz) | 132.39 | 6.69 (1H, d, $J$ = 8.3 Hz) |
| C-14′ | 50.01 | | 50.04 | | 49.95 | | 49.98 | | 50.00 | |
| C-15′ | 38.93 | 2.88 (1H, d, $J$ = 17.8 Hz), 3.04 (1H, d, $J$ = 17.8 Hz) | 38.98 | 2.94 (1H, d, $J$ = 18.0 Hz), 3.02 (1H, d, $J$ = 18.0 Hz) | 39.03 | 2.91 (1H, d, $J$ = 17.0 Hz), 3.08 (1H, d, $J$ = 17.0 Hz) | 38.86 | 2.95 (1H, d, $J$ = 17.8 Hz), 3.04 (1H, d, $J$ = 17.8 Hz) | 39.33 | 2.93 (1H, d, $J$ = 17.0 Hz), 3.04 (1H, d, $J$ = 17.0 Hz) |
| C-16′ | 22.06 | 2.45 (3H, s) | 22.08 | 2.45 (3H, s) | 22.11 | 2.45 (3H, s) | 22.11 | 2.45 (3H, s) | 22.06 | 2.45 (3H, s) |

[a] Each sample was dissolved in $CDCl_3$; chemical shifts are shown with reference to $CDCl_3$ at 77.0 ppm. [b] Chemical shifts are shown with reference to $CDCl_3$ at 7.26 ppm. [c] C-3–OH protons of **2**, **3**, **4**, and **5** were not detected under this condition.

C-7′, C-9′, C-11′, C-13′, C-15′, and C-16′), and the 1 carbon of C-16 were enriched, respectively. Thus, the origin of all 31 carbons was determined. Analysis of $^{13}C$–$^{13}C$ couplings for xanthoquinodin A1 prepared from the sodium [1,2-$^{13}C_2$]acetate incorporation experiment indicated 12 pairs of doublet signals (C-2 to C-15, C-3 to C-4, C-5 to C-6, C-7 to C-8, C-12 to C-15′, C-1′ to C-14′, C-2′ to C-3′, C-4′ to C-16′, C-6′, to C-7′, C-8′ to C-9′, C-10′ to C-11′, and C-12′ to C-13′), 2 uncoupled signals (C-16 and C-5′), and 3 pairs of signals comprised of two doublets (C-10 to C-9 and C-11, C-14 to C-9 and C-13, and C-9 to C-10 and C-14). The C-9 signal observed as a doublet happened to have the same coupling constant ($J$ = 65.3 Hz) to both C-10 and C-14. All the C–C bonds were also confirmed by cross peaks in the INADEQUATE spectrum (Figure 3).

Compilation of all the data from $^{13}C$-labeled precursor incorporation, HMBC, and NOE experiments for partial structures I, II, and III gave larger structures IV and V (Figure 4) which satisfy the molecular formula of **1**. Furthermore, it is reasonable to connect IV and V at C-8 to C-9 and at O-1 to C-14 because of (1) the higher field shift of C-9 ($\delta$ 105.08) suggesting the presence of an adjacent electrophilic moiety, (2) the lower field shift of C-14 ($\delta$ 156.13) suggesting the presence of an adjacent nucleophilic moiety, (3) the degree of unsaturation, and (4) the polyketide biosynthetic scheme (*vide infra*). The structure of

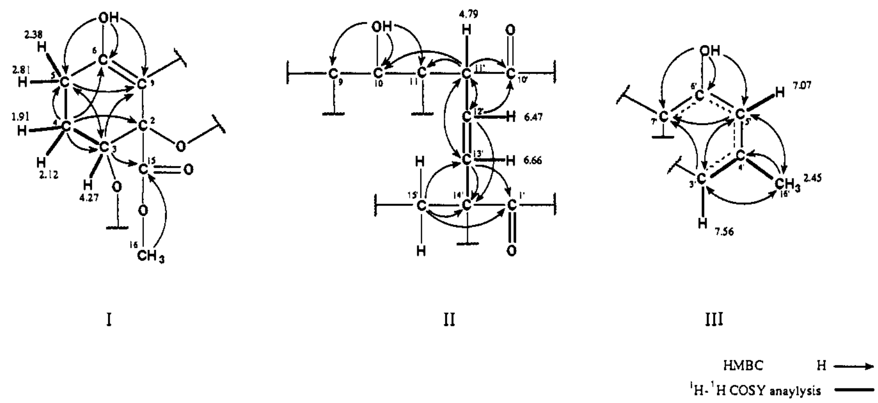



**Figure 2.** $^1$H–$^1$H COSY and HMBC experiments of xanthoquinodin A1.

**Table II.** Enrichment Ratio of **1**, **2**, **3**, and **5** Derived from $^{13}$C-Single-Labeled Precursors and $J_{C-C}$ of [1,2-$^{13}C_2$]AcONa-Labeled[a] **1**, **2**, **3**, and **5**

| | **1**[b] | | | | **2**[c] | | | **3**[d] | | | **5**[e] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | enrichment ratio | | | | enrichment ratio | | | enrichment ratio | | | enrichment ratio | | |
| carbon no. | [1-$^{13}$C]-AcONa[a] | [2-$^{13}$C]-AcONa[a] | [*Me*-$^{13}$C]-Met[a] | [1,2-$^{13}C_2$]-AcONa[a] $J_{CC}$ (Hz) | [1-$^{13}$C]-AcONa[a] | [2-$^{13}$C]-AcONa[a] | [1,2-$^{13}C_2$]-AcONa[a] $J_{CC}$ (Hz) | [1-$^{13}$C]-AcONa[a] | [2-$^{13}$C]-AcONa[a] | [1,2-$^{13}C_2$]-AcONa[a] $J_{CC}$ (Hz) | [1-$^{13}$C]-AcONa[a] | [2-$^{13}$C]-AcONa[a] | [1,2-$^{13}C_2$]-AcONa[a] $J_{CC}$ (Hz) |
| C-2 | 3.33 | 0.56 | 0.67 | 63.6 | 5.57 | *g* | 63.6 | 6.52 | 0.34 | 62.5 | 5.67 | 1.00 | 63.2 |
| C-3 | 0.75 | 2.95 | 0.85 | 37.2 | 0.70 | 6.05 | 37.9 | 0.84 | 3.74 | 31.9 | 0.82 | 3.27 | 37.7 |
| C-4 | 6.48 | 0.82 | 0.83 | 37.2 | 7.83 | 0.82 | 37.9 | 7.22 | 0.44 | 31.9 | 5.02 | 0.51 | 37.7 |
| C-5 | 0.95 | 3.11 | 0.65 | 45.6 | 1.00 | 4.32 | 45.3 | 0.91 | 2.58 | 50.0 | 0.84 | 2.25 | 45.2 |
| C-6 | 3.65 | 0.57 | 0.72 | 45.6 | 3.87 | 0.91 | 45.3 | 6.01 | 0.39 | 50.0 | 5.57 | 0.29 | 45.2 |
| C-7 | 0.91 | 2.54 | 0.84 | 58.9 | *g* | 1.82 | 57.8 | 0.46 | 3.00 | 40.6 | *g* | 1.71 | 58.2 |
| C-8 | 4.68 | 1.00 | 0.86 | 58.9 | 7.65 | *g* | 57.8 | 5.37 | *g* | 40.6 | 5.79 | *g* | 58.2 |
| C-9 | 0.54 | 1.93 | 0.55 | 65.3/65.3 | *g* | 3.43 | 64.3/64.3 | 1.00 | 2.52 | 62.5/62.5 | *g* | 1.85 | 64.4/64.4 |
| C-10 | 3.28 | 0.58 | 0.67 | 65.3/70.4 | 4.93 | 0.46 | 64.3/70.9 | 4.74 | 0.60 | 62.5/70.3 | 4.85 | *g* | 64.4/71.2 |
| C-11 | 0.67 | 2.24 | 0.66 | 70.4 | *g* | 3.31 | 70.9 | 0.60 | 1.96 | 70.3 | 0.37 | 2.42 | 71.2 |
| C-12 | 3.28 | 0.55 | 0.65 | 42.7 | 5.59 | 0.76 | 42.6 | 7.40 | 0.45 | 43.0 | 4.20 | 0.34 | 42.9 |
| C-13 | 0.63 | 2.39 | 0.70 | 71.4 | 0.71 | 5.16 | 71.2 | 0.77 | 2.71 | 71.3 | *g* | 1.94 | 70.5 |
| C-14 | 3.75 | 0.62 | 0.73 | 65.3/71.4 | 4.23 | 0.60 | 64.3/71.2 | 6.83 | 1.00 | 62.5/71.3 | 5.51 | 0.65 | 64.4/70.5 |
| C-15 | 0.70 | 3.26 | 0.28 | 63.6 | *g* | 6.18 | 63.6 | 0.37 | 3.87 | 62.5 | *g* | 2.22 | 63.2 |
| C-16 | 0.50 | 0.54 | 11.74 | s[f] | 0.80 | 0.98 | s[f] | 0.35 | 0.43 | s[f] | 0.56 | 0.58 | s[f] |
| C-1′ | 0.74 | 2.93 | 0.60 | 43.2 | *g* | 2.72 | 42.4 | 0.55 | 1.86 | 42.8 | *g* | 2.57 | 43.0 |
| C-2′ | 3.55 | 0.48 | 0.73 | 62.4 | 5.86 | *g* | 61.7 | 4.81 | *g* | 62.7 | 3.01 | *g* | 62.0 |
| C-3′ | 0.82 | 3.14 | 0.59 | 62.4 | 0.88 | 3.64 | 61.7 | 0.79 | 2.19 | 62.7 | 0.75 | 2.81 | 62.0 |
| C-4′ | 5.92 | 0.85 | 0.82 | 43.2 | 4.86 | 0.63 | 43.4 | 6.35 | 0.44 | 43.0 | 4.95 | *g* | 43.2 |
| C-5′ | 0.64 | 2.46 | 0.60 | s[f] | 0.85 | 3.19 | s[f] | 0.79 | 2.02 | s[f] | 0.72 | 2.62 | s[f] |
| C-6′ | 3.86 | 0.71 | 0.65 | 63.1 | 5.53 | 0.71 | 63.2 | 5.93 | 0.37 | 63.8 | 6.76 | *g* | 63.9 |
| C-7′ | 0.68 | 1.97 | 0.68 | 63.1 | *g* | 2.72 | 63.2 | 0.73 | 2.02 | 63.8 | *g* | 1.82 | 63.9 |
| C-8′ | 4.26 | 0.66 | 0.83 | 62.2 | 7.41 | 0.96 | 62.3 | 7.51 | *g* | 61.0 | 7.86 | *g* | 62.0 |
| C-9′ | 1.00 | 1.99 | 0.90 | 62.2 | *g* | 2.55 | 62.3 | 0.94 | 2.64 | 61.0 | *g* | 1.44 | 62.0 |
| C-10′ | 4.22 | 0.58 | 1.00 | 43.5 | 7.27 | *g* | 44.2 | 7.87 | *g* | 44.0 | 8.10 | *g* | 42.9 |
| C-11′ | 0.66 | 1.98 | 0.80 | 43.5 | 0.94 | 4.07 | 44.2 | 0.32 | 2.05 | 44.0 | 1.00 | 3.28 | 42.9 |
| C-12′ | 5.19 | 0.71 | 0.87 | 70.8 | 7.60 | 1.00 | 70.5 | 6.32 | 0.49 | 70.7 | 5.59 | 0.47 | 70.7 |
| C-13′ | 0.60 | 2.62 | 0.73 | 70.8 | 0.98 | 4.40 | 70.5 | 0.95 | 2.96 | 70.7 | 0.63 | 2.70 | 70.7 |
| C-14′ | 2.48 | 0.80 | 0.60 | 43.2 | 4.47 | 0.95 | 42.4 | 3.82 | 0.21 | 42.8 | 3.63 | 0.79 | 43.0 |
| C-15′ | 0.71 | 2.71 | 0.65 | 42.7 | 0.73 | 4.27 | 42.6 | 0.81 | 3.00 | 43.0 | 0.67 | 1.72 | 42.9 |
| C-16′ | 0.78 | 3.02 | 0.74 | 43.2 | 0.80 | 3.26 | 43.4 | 0.21 | 2.22 | 43.0 | 0.99 | 2.94 | 43.2 |

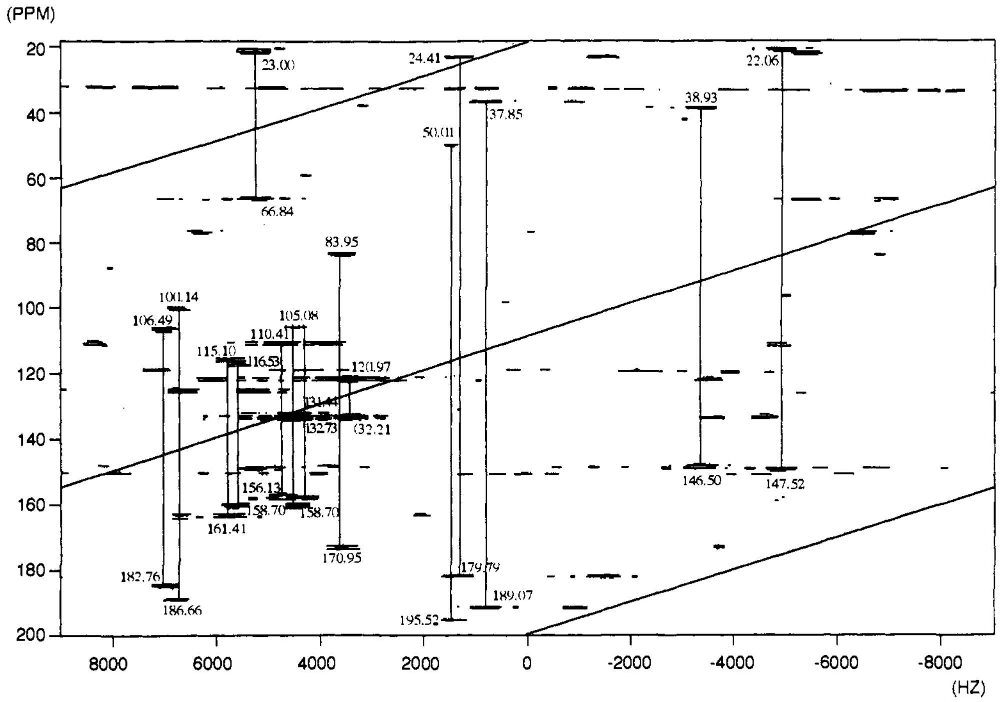
[a] AcONa = sodium acetate, Met = methionine. [b] Enrichment ratios were relative to the C-9′, C-8, and C-10′ signals ([1-$^{13}$C]acetate, [2-$^{13}$C]acetate, and [*Me*-$^{13}$C]Met) at 1.00, respectively. [c] Enrichment ratios were relative to the C-5 and C-12′ signals ([1-$^{13}$C]acetate and [2-$^{13}$C]acetate) at 1.00, respectively. [d] Enrichment ratios were relative to the C-9 and C-14 signals ([1-$^{13}$C]acetate and [2-$^{13}$C]acetate) at 1.00, respectively. [e] Enrichment ratios were relative to the C-11′ and C-2 signals ([1-$^{13}$C]acetate and [2-$^{13}$C]acetate) at 1.00, respectively. [f] Signal was singlet, so the carbon had no coupling with others. [g] Signals were not detected.

xanthoquinodin A1 and the $^{13}$C-labeling patterns after incorporation of [1-$^{13}$C]-, [2-$^{13}$C]-, and [1,2-$^{13}C_2$]acetates and L-[*methyl*-$^{13}$C]methionine are shown in Figure 5.

**Structures of Xanthoquinodins A2, A3, B1, and B2.** All the molecular formulas of xanthoquinodins A2 (**2**), A3 (**3**), B1 (**4**), and B2 (**5**) were determined to be $C_{31}H_{24}O_{11}$ (HREI-MS *m/z* calcd 572.1317, found **2** 572.1319, **3** 572.1324, **4** 572.1301, and **5** 572.1317), which is the same as that of **1**.

The structures of xanthoquinodins A2, A3, B1, and B2 were also determined by NMR analyses of $^1$H and $^{13}$C NMR (Table I), $^1$H–$^1$H COSY, $^{13}$C–$^1$H COSY, HMBC, and $^{13}$C-labeled precursor incorporation experiments (Table II, except for **4**). The results are summarized in Figure 6. It was found that both **1** and **2**, and **4** and **5** have the same general structure. All these xanthoquinodins are heterodimers of a xanthone and an anthraquinone bound in a unique fashion. A large chemical shift difference was observed at the C-3 position between **1** and **2** as well as between **4** and **5**, suggesting that the compounds in each pair are stereoisomers. Xanthoquinodin A3 (**3**) has a γ-lactone ring, evidence of which was supported by the absorption at 1783 cm$^{-1}$ in the IR spectrum.

**Stereochemistry of Xanthoquinodin B2.** Xanthoquinodin B2 (**5**) has four chiral carbons (C-2, C-3, C-11′, and C-14′) in its structure. Modeling of the bridgehead carbons of C-11′ and C-14′ linked *via* the (*Z*)-ethylene bridge of C-12′ and C-13′ ($J_{HH}$ = 8.3 Hz) revealed that the relative configurations at C-11′ and C-14′ must be *S** and *R**, respectively. Furthermore, NOE was observed between $H_3$-16 (δ 3.74) and H-12′ (δ 6.54) ($H_3$-16 to H-12′, 2.0%; H-12′ to $H_3$-16′, 1.3%), indicating that both positions are mutually accessible (Figure 7a). The configuration at C-2 was assigned as *S**. The configuration at C-3 was determined as follows. The proton H-3 has coupling constants of 12.3 and fig3




4q; y = 262q

**Figure 3.** INADEQUATE spectrum (400 MHz) of the [1,2-$^{13}C_2$]acetate-labeled xanthoquinodin A1.

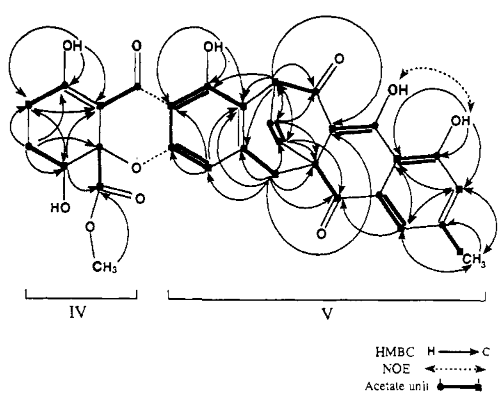



**Figure 4.** Two partial structures IV and V.

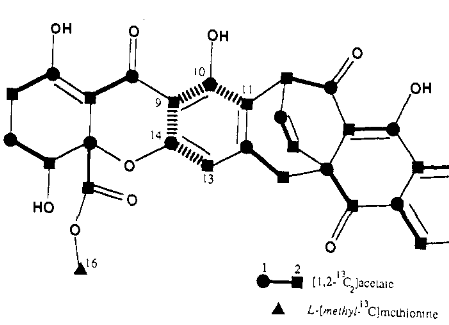



**Figure 5.** Incorporation patterns of $^{13}$C-labeled precursors of xanthoqinodin A1.

4.9 Hz in the $^1$H NMR spectrum (Table I). The larger coupling constant (12.3 Hz) is due to axial–axial coupling. The methyl ester residue was axial because it was at the angular position. Accordingly, the configuration at C-3 was deduced to be $S^*$ (Figure 7b). Taken together, the stereochemistry of C-2, C-3, C-11′, and C-14′ in **5** was determined to be $S^*$, $S^*$, $S^*$, and $R^*$, respectively.

**Interconversion of Xanthoquinodins by Heat Treatment.** Heat treatment of xanthoquinodin A1 resulted in a mixture of xanthoquinodins A1 (11 mol %), A2 (14 mol %), A3 (34 mol %), B1 (9 mol %), and B2 (32 mol %). Similarly, heat treatment of xanthoquinodins A2, A3, B1, or B2 produced xanthoquinodins A1 (10, 0, 4, and 10 mol %), A2 (55, 0, 12, and 21 mol %), A3 (0, 100, 0, and 0 mol %), B1 (8, 0, 68, an 13 mol %), and B2 (27, 0, 10, and 56 mol %), respectively. However, xanthoquinodin A3 was very stable toward heat treatment without any conversion to the other xanthoquinodins.

These data indicate that all xanthoquinodins except xanthoquinodin A3 thermodynamically interconvert to other components and that the stable xanthoquinodin A3 is produced only from xanthoquinodin A1.

## Discussion

**Biosynthesis and Structure of Xanthoquinodins.** The origin of all the carbons of xanthoquinodin A1 was unambiguously determined by $^{13}$C-labeled precursor feeding experiments (Table II and Figure 5). It was demonstrated that xanthoquinodin A1 is a heterodimer of octaketide-derived xanthone and anthraquinone moieties linked in a unique fashion. Biosynthesis of octaketide-derived xanthones and anthraquinones have been studied extensively.[5] The proposed biosynthetic sequence of **1** is shown in Scheme I. Two anthraquinones (or anthrones) **7** are produced from two octaketides *via* decarboxylation of the tail. In the [1,2-$^{13}C_2$]acetate-enriched **1**, the isolated signal of the C-5′ carbon could be explained as a result of this decarboxylation. One molecule of **7** is subjected to oxidative cleavage at the B ring to yield a hypothetical benzophenone intermediate. Similar decarboxylation and oxidative cleavage were reported in a biosynthetic study on related compounds.[6,7] The phloroglucinol moiety (C ring) of this intermediate rotates between **9a** and **9b**. The two pairs of doublet–doublet signals (C-9 to C-10 and C-14, C-10 to C-9 and C-11, and C-14 to C-9 and C-13) in the [1,2-$^{13}C_2$]acetate feeding experiments (Figure 5) can be explained as a result of this rotation. An analogous rotation has been reported in the biosynthesis of diversonol[8] and purpactin A.[7] The tricyclic xanthone **10** is formed from this intermediate **9**. Next, xanthone **10** and anthraquinone **8** are connected at the two sites

(5) Turner, W. B.; Aldridge, D. C. *Fungal Metabolites II*; Academic Press: London, 1983; pp 140–172.

(6) (a) Hamasaki, T.; Sato, Y.; Hatsuda, Y. *Agric. Biol. Chem.* **1975**, *39*, 2341. (b) Assaante, G.; Camarda, L.; Nasini, G. *Gazz. Chim. Ital.* **1980**, *110*, 629.

(7) Nishida, H.; Tomoda, H.; Okuda, S.; Ōmura, S. *J. Org. Chem.* **1992**, *57*, 1271.

(8) Turner, W. B. *J. Chem. Soc., Perkin Trans. 1* **1978**, 1621.

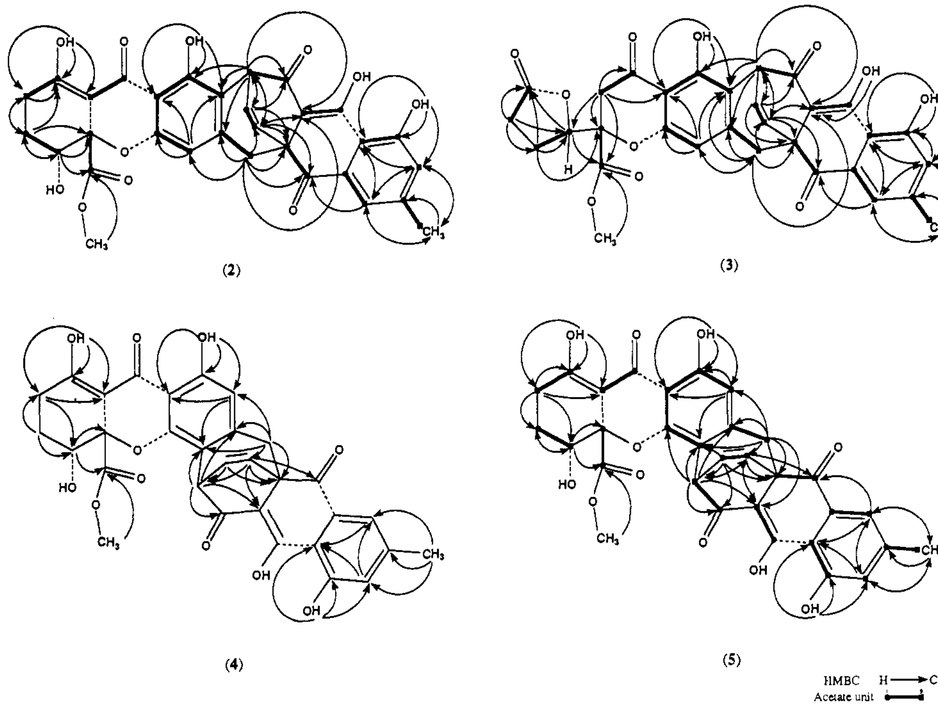



**Figure 6.** Structure determination of xanthoquinodins A2, A3, B1, and B2.

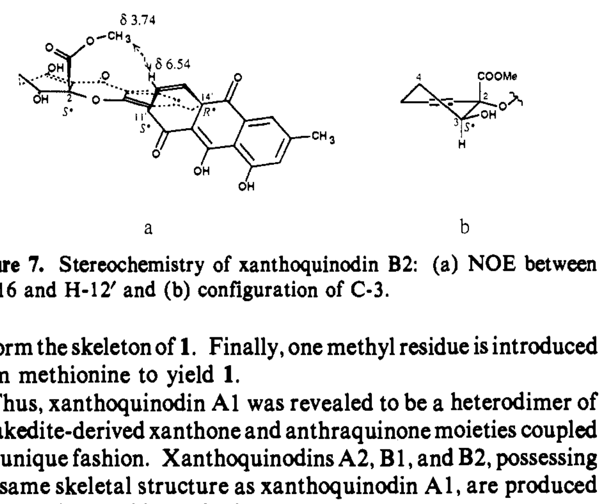



**Figure 7.** Stereochemistry of xanthoquinodin B2: (a) NOE between $H_3$-16 and H-12′ and (b) configuration of C-3.

to form the skeleton of **1**. Finally, one methyl residue is introduced from methionine to yield **1**.

Thus, xanthoquinodin A1 was revealed to be a heterodimer of octakedite-derived xanthone and anthraquinone moieties coupled in a unique fashion. Xanthoquinodins A2, B1, and B2, possessing the same skeletal structure as xanthoquinodin A1, are produced in an analogous biosynthetic sequence.

Many compounds have been reported to be dimers comprised of octaketide-derived anthraquinones[9] and/or anthrones.[10] Deoxyrubroskyrin[11] and flavoskyrin[12] are classified into a homodimer of anthraquinones, and eunitrins A1 and A2[13] are homodimers of xanthones. Beticolins[3] and cebetins,[4] both reported as fungal toxins, appear to be very similar to the xanthoquinodins in their heterodimeric xanthone–anthraquinone structure, although their biosynthesis has not been studied. The binding modes of the two monomers are quite different from the xanthoquinodins. In beticolins and cebetins, the anthraquinone and xanthone moieties are linked in an "end-to-end" fashion, while the two monomers of the xanthoquinodins are bound in an "end-to-body" manner. Rubellins, homodimers of two anthraquinones, are bound in a similar fashion,[14] although their biosynthesis has not been determnined either.

Taken together, the xanthoquinodins, a new family of heterodimers comprised of an octaketide-derived anthraquinone and xanthone linked in an "end-to-body" fashion, are the first compounds of this class whose biosyntheses are well characterized.

**Stereochemistry of Xanthoquinodins.** The same general structure was established for xanthoquinodins A1 and A2 and xanthoquinodins B1 and B2. It seems reasonable that the cleavage of the bond between O-1 and C-2 of xanthoquinodin A (A1 and A2) and recombination of C-2 with O-10 would produce xanthoquinodin B (B1 and B2). Since these four xanthoquinodins are interconvertible by heat treatment, a common intermediate for xanthoquinodin formations was proposed (Scheme II). The intermediate has a carbonium cation at the carbon bound to the methoxy carbonyl group of the A ring. Nucleophilic attack of the two distinct phenol hydroxy groups in the C ring proceeds from the two directions, *re*- and *si*-faces. When the C-10 phenolate anion attacks the carbonium ion of C-2 from the *si*-face (route d in Scheme II), xanthoquinodin B2 is formed *via* a nucleophilic reaction, resulting in the 2*S**, 3*S**, 11′*S**, and 14′*R** configurations described above. Similarly, xanthoquinodins A1, A2, and B1 are formed *via* routes a, b, and c, respectively, leading to the configurations shown at positions 2, 11′, and 14′. The configuration at C-3 is conserved in the xanthoquinodins. In fact, the NMR spectral data support the proposed structures. Only the data concerning the C-3 position displayed significant differences between **1** and **2** as well as between **4** and **5**. The coupling constant of the H-3 methine proton indicates an equatorial conformation for **1** (1.9 and 3.9 Hz) and **4** (1.9 and 4.0 Hz) and an axial one for **2** (5.0 and 12.5 Hz) and **5** (4.9 and 12.3 Hz). The difference

(9) (a) McGrath, D. *Chem. Ind. (London)* **1970**, 1353. (b) Foo, L. Y.; Tate, K. R. *Experientia* **1977**, *33*, 1271. (c) Gluchoff, K.; Aprin, N.; Danagye-Caye, M.-P.; Lebreton, P.; Steglich, W.; Töpper, E.; Pourrat, H.; Regerat, F.; Deruaz, D. *C. R. Acad. Sci., Ser. D* **1972**, *274*, 1739. (d) Ejiri, H.; Sankawa, U.; Shibata, S. *Phytochemistry* **1975**, *14*, 277. (e) Sedmera, P.; Podojil, M.; Vokoun, J.; Betina, V.; Nemec, P. *Folia Microbial (Prague)* **1978**, *23*, 64.

(10) (a) Bachmann, M.; Luthy, J.; Schlatter, C. *J. Agric. Food Chem.* **1979**, *27*, 1342. (b) Steglich, W.; Töpfer-Petersen, E.; Reininger, W.; Gluchoff, K.; Arpin, N. *Phytochemistry* **1972**, *11*, 3299. (c) Steglich, W.; Töpfer-Petersen, E. *Z. Naturforsch., Teil B* **1972**, *27*, 1286. (d) Besl, H.; Bresinsky, A. *Z. Pilzkd*, **1977**, *43*, 311. (e) Streglich, W.; Töpfer-Petersen, E.; Pils, I. *Z. Naturforsch., Teil C* **1973**, *28*, 354. (f) Streglich, W.; Töpfer-Petersen, E. *Ibid.* **1973**, *28*, 255.

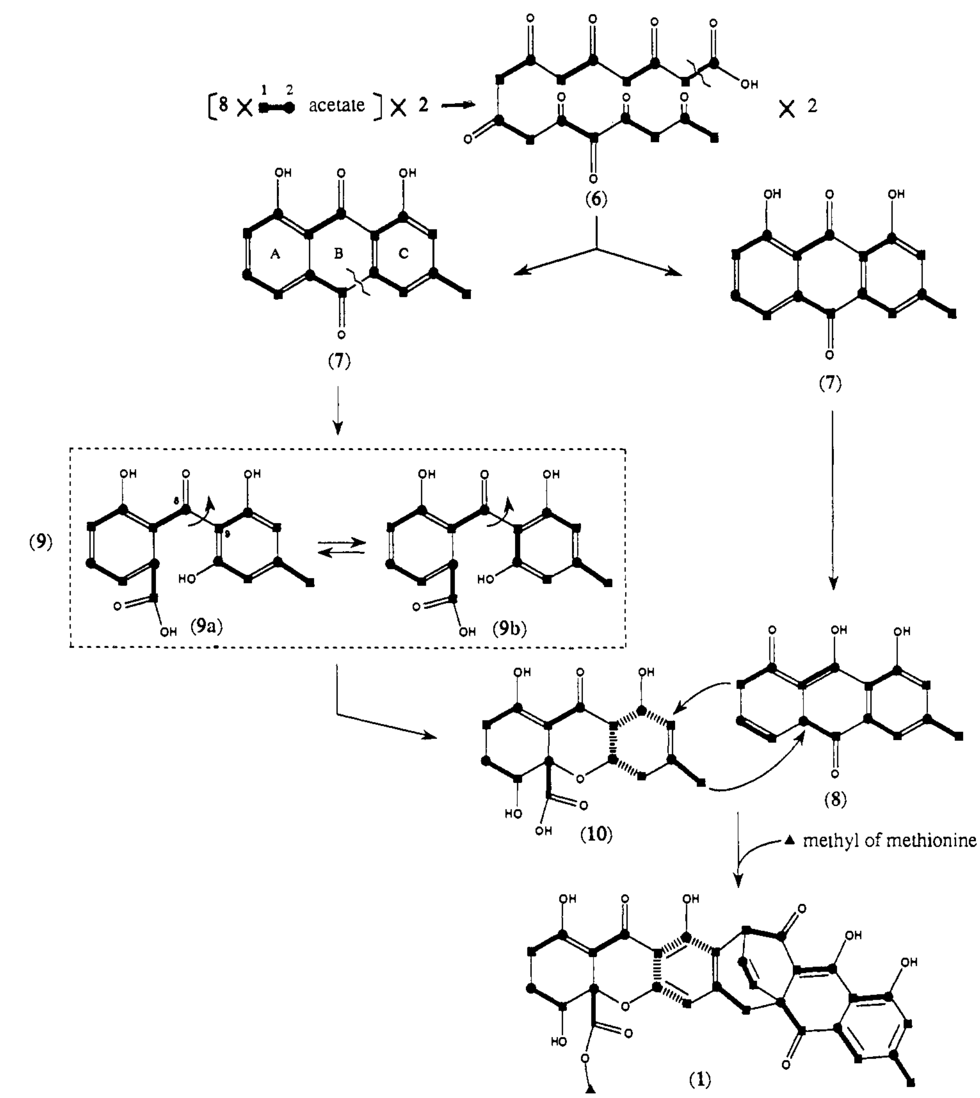
(11) Takeda, N.; Seo, S.; Ogihara, Y.; Sankawa, U.; Iitaka, I.; Kitagawa, I.; Shibata, S. *Tetrahedron* **1973**, *29*, 3703.

(12) (a) Howard, B. H.; Raistrik, H. *Biochem. J.* **1954**, *56*, 56. (b) Shibata, S.; Ikekawa, T.; Kishi, T. *Chem. Pharm. Bull.* **1960**, *8*, 889. (c) Seo, S.; Ogihara, Y.; Sankawa, U.; Shibata, S. *Tetrahedron Lett.* **1972**, 735. (d) Seo, S.; Sankawa, U.; Ogihara, Y.; Iitaka, Y.; Shibata, S. *Tetrahedron* **1973**, *29*, 3721.

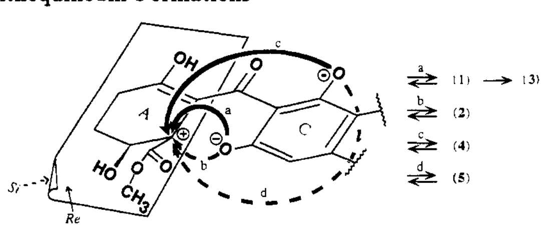
(13) Yang, D.-M.; Takeda, N.; Iitaka, Y.; Sankawa, U.; Shibata, S. *Tetrahedron* **1973**, *29*, 519.

(14) Arnone, A.; Camarda, L.; Nasini, G. *J. Chem. Soc., Perkin Trans. 1* **1986**, 255.

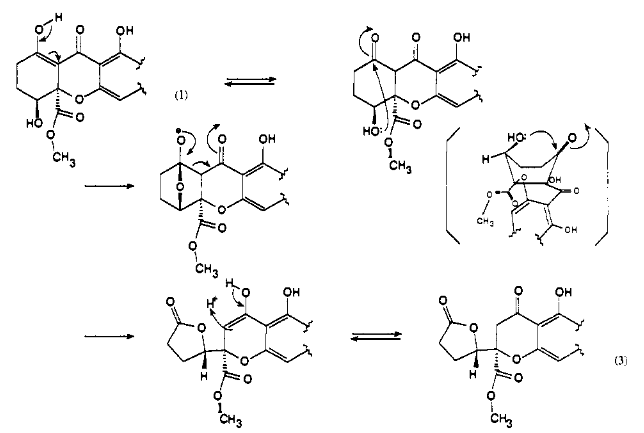
**Scheme I.** Proposed Biosynthetic Pathway of Xanthoquinodin A1




**Scheme II.** Hypothetical Common Intermediate for Xanthoquinodin Formations



**Scheme III.** Conversion of Xanthoquinodin A1 to Xanthoquinodin A3



in the $^{13}$C NMR chemical shifts at C-3 (4.98 and 4.82 ppm) between **1** and **2** and between **4** and **5** shows excellent agreement with the additivity rule as reported by Booth,[15] indicating that the hydroxy residues at C-3 are axial for **1** and **4** and equatorial for **2** and **5**. Accordingly, the complete configurations of xanthoquinodins A1, A2, and B1 are deduced as shown in Figure 1.

Xanthoquinodin A3, possessing a $\gamma$-lactone ring, originated from xanthoquinodin A1. A possible synthetic sequence is shown in Scheme III. Further studies are necessary to demonstrate conclusively the proposed stereochemistry of **3**.

## Experimental Section

**Spectroscopic Measurements.** Various NMR spectra were recorded on a Varian XL-400 (400 MHz) NMR spectrometer. NOE experiments were measured at 500 MHz on a JEOL JUM-GX-500 NMR spectrometer. HMBC experiments were measured at 400 and 270 MHz on JEOL JNM-EX-400 and JNM-EX-270 NMR spectrometers. Low-resolution mass spectra were obtained on a JEOL Model JMS-D 100 mass spectrometer. Fast atom bombardment mass spectrometry (FAB-MS) using argon or xenon was performed on a JEOL Model JMS-DX-300 instrument. UV-visible spectra were measured on a Shimadzu UV-200S spectrometer. IR spectra were recorded on a JASCO A-102 diffraction infrared spectrometer.

(15) Booth, H. *Org. Magn. Reson.* **1979**, *12*, 63.

**Materials.** More than 90%-enriched stable isotope precursors were purchased as follows: sodium [1-$^{13}$C]acetate, sodium [2-$^{13}$C]acetate, sodium [1,2-$^{13}C_2$]acetate, and L-[*methyl*-$^{13}$C]methionine from ISOTEC.

**Xanthoquinodins Production.** A *Humicola* sp. FO-888 strain was grown on slants adjusted to pH 6.0 and containing soluble starch (1.5%), yeast extract (0.4%), $K_2HPO_4$ (0.1%), $MgSO_4{\cdot}7H_2O$ (0.05%), and agar (2.0%) in distilled water. The slants were incubated at 27 °C and stored in tubes sealed with screw caps at room temperature. Prior to xanthoquinodin production, *Humicola* sp. FO-888 was precultivated in a 50-mL test tube (20 × 200 mm) containing 10 mL of a medium consisting of glucose (2.0%), yeast extract (0.2%), $MgSO_4{\cdot}7H_2O$ (0.05%), polypeptone (0.5%), $KH_2PO_4$ (0.1%), and agar (0.1%) in distilled water, adjusted to pH 6.0 before sterilization. The culture was shaken reciprocally at 300 rpm at 27 °C for 72 h. Ten milliliters of this culture broth was inoculated into each of three 500-mL Erlenmeyer flasks containing 100 mL of a medium consisting of saccharose (2.0%), glucose (1.0%), corn steep liquor (1.0%), meat extract (0.5%), $KH_2PO_4$ (0.1%), $CaCO_3$ (0.3%), and agar (0.1%) in distilled water, adjusted to pH 6.0 before sterilization, and shaken with a rotatory shaker at 27 °C for 164 h. Maximum yield of xanthoquinodin A1 under this condition was estimated by HPLC to be 120 mg/L.

**Isolation Procedures.** The fermentation broth (300 mL) was centrifuged at 3000 rpm for 5 min. To the supernatant was added $H_3PO_4$ (600 $\mu$L), and the acidified supernatant was extracted with an equal volume of ethyl acetate. To the mycelium were added 70% aqueous acetone (300 mL) and $H_3PO_4$ (600 $\mu$L). After centrifugation of this suspension at 3000 rpm for 5 min, the supernatant was concentrated to remove acetone. The aqueous solution was extracted with an equal volume of ethyl acetate. Both organic layers were concentrated, and the crude extracts, dissolved in methanol, were purified by preparative HPLC using an ODS column (column, YMC pack D-ODS-5 AM 343 (20 × 250 mm i.d.); detection, UV at 340 nm; eluant, aqueous acetonitrile (70% in 0.05% $H_3PO_4$) at 6 mL/min). Pure xanthoquinodins A1, A2, A3, B1, and B2 were isolated in a yield of ca. 244, 52, 36, 3, and 55 mg/L, respectively.

**Biosynthetic $^{13}$C-Labeling of Xanthoqninodins A1, A2, A3, and B2.** Biosynthetically $^{13}$C-labeled xanthoquinodins were prepared by adding a labeled precursor core once (at 72-h cultivation) or twice (at 31- and 55-h cultivation) after filtration–sterilization of the sample solution, adjusted to pH 6.0. The incorporation patterns and rates were determined by $^{13}$C NMR spectra using the Varian XL-400 spectrometer. $^{13}$C-Enriched xanthoquinodin samples (ca. 1.0 mg each) were dissolved in 0.5 mL of $CDCl_3$ (Merck). INADEQUATE was measured by $J_{CC}$ = 60.0 Hz, acquisition time 96 h (9.0 mg/tube in $CDCl_3$).

**[$^{13}$C]Acetate Incorporation.** As a precursor, 100 mg of sodium [1-$^{13}$C]-, [2-$^{13}$C]-, or [1,2-$^{13}C_2$]acetate solution was added at 72-h cultivation to each of three flasks containing 100 mL of culture medium (saccharose (1.3%), glucose (0.7%), corn steep liquor (1.0%), meat extract (0.5%), $KH_2PO_4$ (0.1%), $CaCO_3$ (0.3%), and agar (0.1%), and incubation was conducted for 6 days. Peak intensity enhancements at the specific carbon signals were 2- to 8-fold.

**L-[*methyl*-$^{13}$C]Methionine Incorporation.** To a flask containing 100 mL of the same culture medium as that in the [$^{13}$C]aetate incorporation experiment was added 12.5 mg and 2.5 mg of $^{13}$C-labeled methionine twice at 31- and 55-h cultivation, and incubation was conducted for 4 days. Peak intensity enhancement of the methoxy methyl signal was ca. 12-fold.

**Interconversion of Xanthoquinodins by Heat Treatment.** Each of the pure xanthoquinodins (0.3 mg) dissolved in 0.3 mL of MeOH in a test tube sealed with a screw cap (10 × 30 mm i.d.) was heated at 80 °C for 85 min. Each sample of the heat-treated xanthoquinodins was analyzed by HPLC using an ODS column (YMC, R-ODS-5, 4.6 × 200 mm) eluted with 70% acetonitrile in 0.05% $H_3PO_4$ at 0.7 mL/min. Under these conditions, xanthoquinodins A1, A2, A3, B1, and B2 were eluted with retention times of 27.5, 30.5, 21.0, 23.5, and 26.0 min, respectively. At time = 0, each xanthoquinodin had no contamination, showing only one peak by HPLC.

**Acknowledgment.** We express our thanks to Dr. H. Noguchi and Mr. H. Kobayashi for measuring NMR spectra and to Dr. A. B. Smith III, Dr. K. Shiomi, and Dr. T. Sunazuka for useful comments and discussion.