JOC*Article*

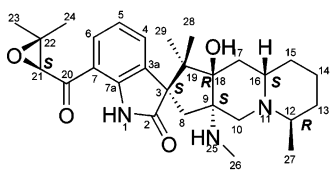
Absolute Stereochemistry of Citrinadins A and B from Marine-Derived Fungus

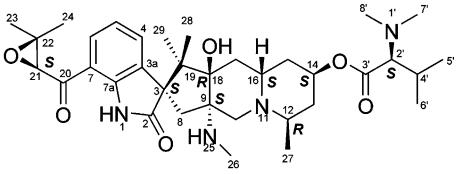
Takao Mugishima,[†] Masashi Tsuda,[†] Yuu Kasai,[†] Haruaki Ishiyama,[†] Eri Fukushi,[‡] Jun Kawabata,[‡] Masayuki Watanabe,[§] Kenichi Akao,[§] and Jun'ichi Kobayashi*,[†]

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan, and Spectrometry Instrument Division, JASCO Corporation, Hachioji 192-0032, Japan

jkobay@pharm.hokudai.ac.jp

Received July 19, 2005

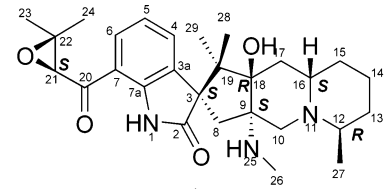




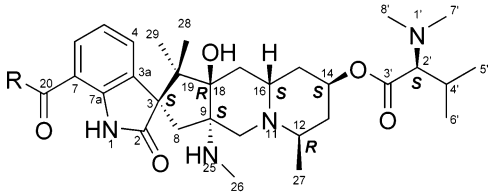
Citrinadin A (**2**) is a pentacyclic indolinone alkaloid isolated from the cultured broth of a fungus, *Penicillium citrinum*, which was separated from a marine red alga. The absolute stereochemistry of the pentacyclic core in **2** and its new congener, citrinadin B (**1**), was elucidated by analysis of the ROESY spectrum for the chlorohydrin derivative (**3**) of **1** as well as comparison of the electronic circular dichroism (ECD) spectra for **1** and **2** with those of known spirooxiindole alkaloids. On the other hand, the absolute configuration at C-21 bearing an epoxide ring was assigned as *S* by comparison of the vibrational circular dichroism (VCD) spectra of **1** with those of model compounds 2*S*- and 2*R*-2,3-epoxy-3,3-dimethyl-1-phenylpropan-1-one (**4a** and **4b**, respectively).

Introduction

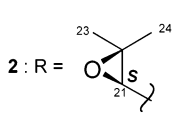
Marine-derived fungi have proven to been a rich source of new compounds with high chemical diversity.[1] In our search for new metabolites from marine-derived fungi,[2] a novel pentacyclic spiroindolinone alkaloid, citrinadin A (**2**), with an *N*,*N*-dimethylvaline residue and an α,*β*-epoxy carbonyl unit was isolated from the cultured broth of a fungus, *Penicillium citrinum* N059 strain, which was separated from a marine red alga.[3] The gross structure and relative stereochemistry for the pentacylic core have been elucidated by 2D NMR data, whereas the absolute configurations remained unsolved. Recently, we have isolated a new citrinadin congener, citrinadin B (**1**), from the same strain and elucidated the absolute stereochemistry of citrinadins A (**2**) and B (**1**) on the basis of NMR, ECD, and VCD[4] data.



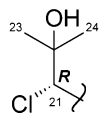
1



2 : R =



3 : R =



Results and Discussion

Isolation and Structure of Citrinadin B (1). The fungus *P. citrinum* (strain N059) was separated from a

* To whom correspondence should be addressed. Phone: +81 11 706 4985. Fax: +81 11 706 4989.
† Graduate School of Pharmaceutical Sciences, Hokkaido University.
‡ Graduate School of Agriculture, Hokkaido University.
§ JASCO Corp.

(1) Bugni, T. S.; Ireland, C. M. *Nat. Prod. Rep.* **2004**, *21*, 143−163 and references therein.
(2) (a) Kasai, Y.; Komatsu, K.; Shigemori, H.; Tsuda, M.; Mikami, Y.; Kobayashi, J. *J. Nat. Prod.* **2005**, *68*, 777−779. (b) Shigemori, H.; Kasai, Y.; Komatsu, K.; Tsuda, M.; Mikami, Y.; Kobayashi, J. *Marine Drugs* **2004**, *2*, 164−169.
(3) Tsuda, M.; Kasai, Y.; Komatsu, K.; Sone, T.; Tanaka, M.; Mikami, Y.; Kobayashi, J. *Org. Lett.* **2004**, *6*, 3087−3089.

10.1021/jo051499o CCC: $30.25
Published on Web 10/07/2005

TABLE 1. ^{1}H and ^{13}C NMR Data (ppm) of Citrinadin B (1) in $CDCl_3$

position		^{13}C	^{1}H	multiplicity, *J* (Hz)
1			9.61	s
2		185.3		
3		60.5		
3a		134.6		
4		133.3	7.64	d, 7.4
5		122.5	7.16	dd, 7.4, 8.0
6		127.6	7.75	d, 8.0
7		117.5		
7a		142.6		
8	α	41.7	2.17	d, 13.9
	β		2.10	d, 13.9
9		68.0		
10	α	51.1	3.62	d, 11.5
	β		3.18	d, 11.5
11			10.6	brs
12		58.2	3.61	m
13	α	28.7	2.95	m
	β		1.60	m
14	α	17.3	1.70	m
	β		1.66	m
15	α	29.0	2.50	m
	β		1.66	m
16		52.5	3.71	brt, 11.4
17	α	31.7	2.18	m
	β		1.66	m
18		82.4		
18-OH			5.25	brs
19		51.0		
20		194.8		
21		64.0	4.02	s
22		61.6		
23		24.3	1.57	s
24		18.6	1.25	s
25				
26		30.4	2.45	s
27		12.0	1.38	d, 6.7
28		21.7	0.97	s
29		27.5	1.36	s

marine red alga, *Actinotrichia fragilis*, collected at Hedo Cape, Okinawa Island, and grown in PYG liquid medium containing seawater for 14 days at 25 °C. The mycelium (258 g) of the culture broth (12 L) was extracted with MeOH. The extract was partitioned between hexane and 90% aqueous MeOH, and the MeOH-soluble portion was extracted with *n*-BuOH. The *n*-BuOH-soluble portions were subjected to LH-20 and SiO_2 column chromatographies to afford citrinadin B (**1**, 4.1 mg, 0.0016%, wet weight) together with citrinadin A (**2**, 6.7 mg) and a known mycotoxin, citrinin.[5]

Citrinadin B {**1**, $[\alpha]^{20}_D$ +8° (*c* 1.0, MeOH)} showed the pseudomolecular ion peak at *m/z* 482 in the FABMS, and the molecular formula was revealed to be $C_{28}H_{40}O_4N_3$ by HRFABMS [*m/z* 482.3022, $(M + H)^+$, 0.4 mmu]. The IR spectrum suggested the presence of OH/NH (3390 and 3301 cm^{-1}) and carbonyl group(s) (1701 and 1671 cm^{-1}). The UV absorption at 333 nm (ϵ 3100) was attributed to a conjugated benzenoid chromophore. The ^{13}C NMR (Table 1) spectrum disclosed the existence of two carbonyls (δ_C 194.8 and 185.3), three sp^2 quaternary carbons (δ_C 142.6, 134.6, and 117.5), three sp^2 methines (δ_C 133.3, 127.6, and 122.5), five sp^3 quaternary carbons (δ_C 82.4, 68.0, 61.6, 60.5, and 51.0), three sp^3 methines (δ_C 64.0, 58.2, and 52.5), six sp^3 methylenes (δ_C 51.1, 41.7, 31.7, 29.0, 28.7, and 17.3), and six methyls (δ_C 30.4, 27.5, 24.3, 21.7, 18.6, and 12.0). Since 5 out of 11 unsaturations were accounted for, **1** was inferred to contain six rings. The ^{1}H NMR (Table 1) spectrum of **1** disclosed proton signals due to three D_2O-exchangeable ones (δ_H 10.6, 9.61, and 5.25), three benzenoid methines (δ_H 7.75, 7.64, and 7.16), five singlet methyls (δ_H 2.45, 1.57, 1.36, 1.25, and 0.97), and a doublet methyl (δ_H 1.38), which was similar to that of citrinadin A (**2**), except for the lack of signals due to the *N*,*N*-dimethylvalyloxy group and a methine signal at C-14 observed for **2**.

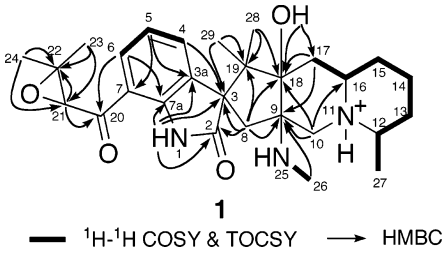


FIGURE 1. Selected 2D NMR data for citrinadin B (**1**).

The structure of citrinadin B (**1**) was elucidated to be a 14-des(*N*,*N*-dimethyl)valyloxy form of **2** on the basis of spectroscopic data including 2D NMR data such as the ^{1}H−^{1}H COSY, ROESY, and HMBC spectra (Figure 1). The ^{1}H−^{1}H COSY, TOCSY, and HSQC spectra revealed three connectivities from C-4 to C-6, from C-12 to C-17 and C-27, and from N-25 to C-26. The presence of a 7-substituted 3-spiroindolinone ring (C-1−C-7a) was suggested by HMBC correlations as follows: NH-1/C-2, NH-1/C-3, NH-1/C-3a, NH-1/C-7a, and H-4/C-3, H-5/C-3a, H-5/C-7, and H-6/C-7a. HMBC correlations for H-6/C-20, H-21/C-20, H_3-23/C-21, H_3-23/C-22, H_3-24/C-21, and H_3-24/C-22 indicated the existence of a 2,3-epoxy-3-methyl-1-oxobutyl side chain (C-20−C-24) at C-7. A cyclopenta[*b*]quinolizidine moiety (N-11, C-3, and C-8−C-19) was revealed by HMBC correlations as follows: H-8/C-3, H-8/C-9, H-8/C-18, H-8/C-19, H-10/C-9, H-10/C-16, H-10/C-18, H-17/C-9, H_3-28/C-18, H_3-28/C-19, H_3-29/C-3, and H_3-29/C-19. HMBC correlations for a D_2O-exchangeable proton (OH-18) at δ_H 5.25 to C-17 and C-18 indicated that a hydroxyl group was attached to C-18. It was revealed that an *N*-methylamino group (N-25−C-26) was connected to C-9, since the HMBC correlation was observed for H_3-26/C-9. HMBC correlations for H_2-8/C-3 and H_3-29/C-3 indicated that the cyclopenta[*b*]quinolizidine moiety and the indolinone ring were connected to each other through the spiro carbon (C-3). Therefore, the gross structure of citrinadin B was concluded to be **1**.

The relative stereochemistry of the pentacyclic core in citrinadin B (**1**) was elucidated on the basis of ROESY data and ^{1}H−^{1}H coupling constants (Figure 2). ROESY correlations for H-4/H_3-26 and H-4/H_3-29 indicated that

(4) (a) Sugeta, H. *Jasco Report* **1991**, *33*, 99−107. (b) Dukor, R. K.; Nafie, L. A. In *Encyclopedia of Analytical Chemistry: Instumentation and Aplications*; Meyer, R. A., Ed.; John Wiley and Sons: Chichester, U.K., 2000; pp 662−676. (c) Nafie, L. A.; Freedman, T. B. In *Infrared and Raman Spectroscopy of Biological Materials*; Gremlich, H.-U., Yan, B., Eds.; Marcel Dekker: New York, 2000; pp 15−54. (d) Keiderling, T. A. In *Circular Dichroism: Principles and Applications*, 2nd ed.; Berova, N., Nakanishi, K., Woody, R. W., Eds.; Wiley-VCH: New York, 2000; pp 621−666. (e) Kuppens, T.; Bultinck, P.; Langenaeker, W. *Drug Discovery Today: Technologies* **2004**, *1*, 269−275. (f) Taniguchi, T.; Miura, N.; Nishimura, S.; Monde, K. *Mol. Nutri. Food Res.* **2004**, *48*, 246−254.

(5) Turner, W. B. In *Fungal Metabolites*; Academic Press: London, 1971; p 121.

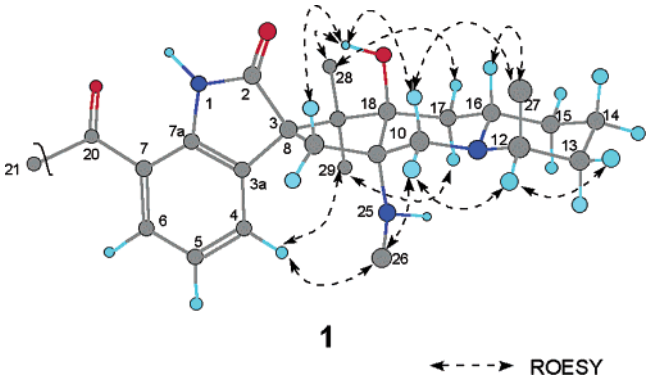


FIGURE 2. Selected ROESY correlations and relative stereochemistry for citrinadin B (**1**).

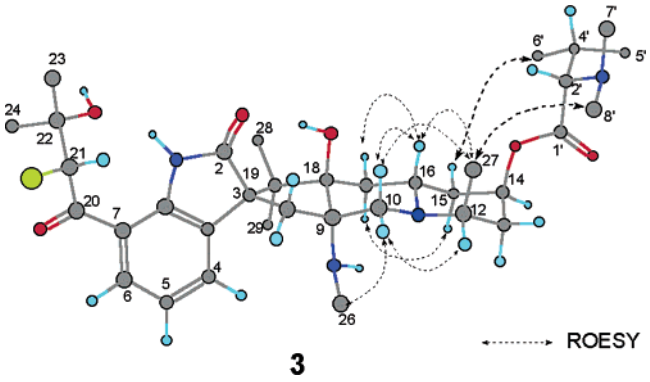
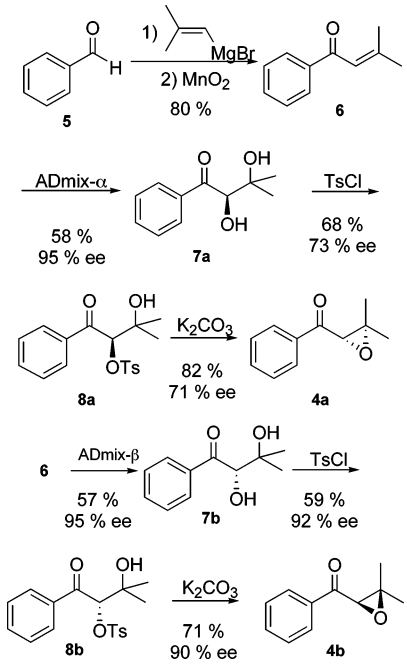


FIGURE 3. Selected ROESY correlations for chlorohydrin derivative (**3**) of citrinadin A (**2**).

one (C-29) of two methyl groups at C-19, the C-3−C-3a bond, and the methylamino group (C-25−C-26) were α-oriented. On the other hand, ROESY correlations for H-10β/H_3-27, H-10β/OH-18, H-16/H_3-27, and OH-18/H_3-28 and proton signal patterns for H-16 (brt, J = 11.4 Hz) suggested β-axial orientations for H-10β, H-16, OH-18, and H_3-27. Since ROESY correlations were observed for H-10α/H-12α, H-12α/H-13β, and H-17β/H_3-28, H-10α, H-12α, H-13β, and H-17β were considered to have equatorial orientations. Therefore, the relative configuration of the cyclopenta[*b*]quinolizidine moiety in **1** was elucidated to be anti/syn/anti and chair forms for the two six-membered rings.

Absolute Stereochemistry of the Pentacyclic Core in Citrinadins A (2) and B (1). To determine the absolute stereochemistry of six stereogenic centers in the pentacyclic core of citrinadin A (**2**), ROESY correlations from protons of the L-*N*,*N*-dimethylvalyl group to those of the pentacylic core in **2** were analyzed. Measurement of the ROESY spectrum was performed using the chlorohydrin derivative (**3**) of citrinadin A (**2**), which was obtained by treatment of **2** with 50 mM HCl in MeOH. In the ROESY spectrum of **3**, ROE correlations were observed for H-15β/H_3-6′ and H_3-27/H_3-8′ (Figure 3). Considering the *S*-configuration at C-2′, the absolute stereochmistry at C-14 was elucidated to be *R*. The ECD spectrum of citrinadin A (**2**) showed the negative first Cotton effect at λ_{ext} 340 nm ($\Delta\epsilon$ −1.5), which was longer in wavelength by conjugation with a carbonyl group than those (λ_{ext} ca. 280 nm) of usual spirooxindole alkaloids.[6−8] This suggested that the spiro carbon at C-3 had *S*-

SCHEME 1



configuration. Therefore, the absolute configurations at six chiral centers, C-3, C-9, C-12, C-14, C-16, and C-18, in citrinadin A (**2**) were assigned as *S*, *S*, *R*, *R*, *S*, and *R*, respectively. Citrinadin B (**1**) showed a similar Cotton curve to that of **2**, thus indicating that the absolute configurations at C-3, C-9, C-12, C-16, and C-18 in **1** were *S*, *S*, *R*, *S*, and *R*, respectively.

Absolute Stereochemistry of C-21 in Citrinadin A (2). As described above, the absolute configuration at C-21 remained unsolved. To determine the absolute configuration at C-21, model compounds (**4a** and **4b**) for the 2,3-epoxy-3-methyl-1-oxobutyl side chain in **1** and **2** were synthesized from benzaldehyde **5** as shown in Scheme 1. Grignard reaction of **5** with isobutenylmagnesium bromide afforded aryl alcohol, which was converted into an aryl ketone **6** by oxidation with manganese(IV) oxide. Compound **6** was subjected to asymmetric dihydroxylation[9] with AD-mix-α to give a diol **7a** in a 58% yield and 95% ee. Enantiomer excesses for all chiral synthetic products were determined by chiral HPLC analysis. Tosylation of the diol **7a** gave **8a**, which was treated with potassium carbonate to afford 2*S*-2,3-epoxy-3,3-dimethyl-1-phenylpropan-1-one (**4a**) in a 26% yield and 71% ee in five steps total. By a manner similar to that described above, 2*R*-2,3-epoxy-3,3-dimethyl-1-phenylpropan-1-one (**4b**) was prepared in the 40% yield and 90% ee from compound **6** using AD-mix-β.

The VCD spectra of compounds **4a** and **4b** showed weak Cotton effects at 1230 cm^{-1} and were the mirror

(6) ECD spectra of uncarines C and F possessing 3*R*-configuration showed the positive first Cotton effect, while those of uncarines D and E possessing 3*S*-configuration disclosed the negative first Cotton effect. Beecham, A. F.; Hart, N. K.; Johns, S. R.; Lamberton, J. A. *Aust. J. Chem.* **1968**, *21*, 491−504.

(7) (a) Pousset, J.-L.; Poisson, J.; Legrand, N. *Tetrahedron Lett.* **1966**, 6283−6289. (b) Pousset, J.-L.; Poisson, J.; Shine, R. J.; Shamma, M. *Bull. Soc. Chim. Fr.* **1967**, 2766−2779.

(8) Takayama, H.; Shimazu, T.; Sada, H.; Harada, Y.; Kitajima, M.; Aimi, N. *Tetrahedron* **1999**, *55*, 6841−6846.

(9) Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Zu, D.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768−2771.

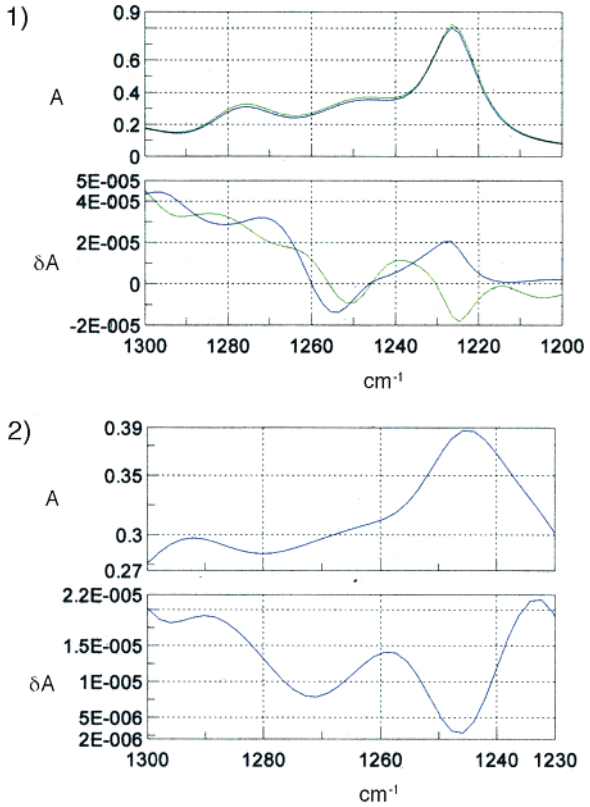


FIGURE 4. IR (upper) and VCD (lower) spectra of 1) model compounds **4a** (green) and **4b** (blue) and 2) citrinadin A (**2**).

image of each other, although undurations were observed for the baselines, probably due to a limitation of the instrument. Compound **4a** possessed a negative Cotton effect for the absorption at 1230 cm^{-1} due to symmetrical ring expansion of an epoxide, while compound **4b** showed a positive Cotton effect at 1230 cm^{-1} (Figure 4). The Cotton curve at 1245 cm^{-1} for citrinadin A (**2**) revealed the negative sign, thus indicating that the absolute configuration at C-21 was *S*. Although the VCD spectrum of citrinadin B (**1**) could not be measured, due to the small amount of sample, citrinadin B (**1**) probably possessed the same absolute configuration at C-21 as that of **2**, since the ECD spectra of **1** and **2** were similar.

Plausible Biosynthetic Pathway and Bioactivity. Citrinadins A (**2**) and B (**1**) belong to a novel class of pentacyclic spiroindolinone alkaloids with an epoxy isoprene unit for **1** and **2** and an *N*,*N*-dimethylvaline residue for **2**. Although several spiroindolinone alkaloids such as brevianamides,[10] paraherquamides,[11] marcfortines,[12] sclerotamide,[13] and asperparalins[14] have been isolated from

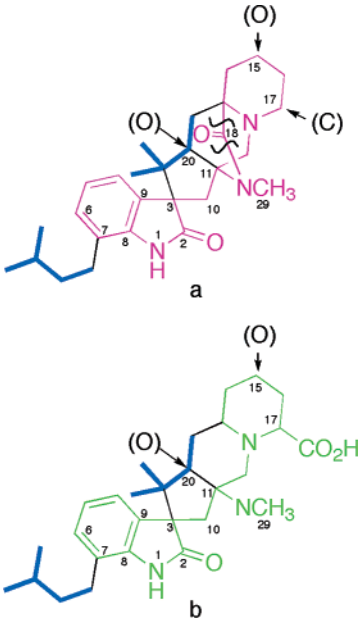


FIGURE 5. Two plausible biogenetic precursors (**a** and **b**) of citrinadins. Diketopiperazine and dipeptide portions and the isoprene unit are shown in magenta, green, and blue.

fungi of the genuses *Penicillium* or *Aspergillus*, the pentacyclic skeleton of **1** is unique. The citrinadin skeleton may be generated by loss of amide carbonyl carbon (C-18) from the marcfortine-type skeleton, such as **a**, in which C-15 and C-20 are oxidized and C-17 is methylated (Figure 5). Alternatively, a dipeptide-like precursor such as **b** may be converted into citrinadins. Although some alkaloids with an epoxy isoprene unit at C-4 position of a indole ring have been isolated from *Penicillium* spp.,[15] alkaloids possessing those at C-7 position of a indole ring such as **1** and **2** were rare.[16] Citrinadin B (**1**) showed modest cytotoxicity against murine leukemia L1210 cells (IC_{50}, 10 μg/mL).

Experimental Section

Citrinadin B (1): pale yellow solid; $[\alpha]^{20}_D$ +8° (*c* 1.0, MeOH); UV (MeOH) λ_{max} 333 (ϵ 3100), 248 (9800), 223 (7600), and 203 nm (12 500); IR (neat) ν_{max} 3390, 3301, 1702, and 1671 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m/z* 482 $(M + H)^+$; HRFABMS *m/z* 482.3022 [$(M + H)^+$, calcd for $C_{28}H_{40}O_4N_3$, 482.3018].

Chlorohydrin (3) of Citrinadin A (2). A solution of citrinadin A (**2**, 0.5 mg) in 50 mM HCl/MeOH (1 mL) was stirred at room temperature for 30 min. After evaporation of the solvent, chlorohydrin (**3**) was afforded as a pale yellow solid: IR (film) ν_{max} 3323, 2926, 1736, 1701 and 1601 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 13.02 (1H, brs, H-1′), 11.41 (1H, brs, H-11), 9.67 (1H, brs, H-1), 7.87 (1H, d, J = 8.0 Hz, H-6),

(10) (a) Birch, A. J.; Wright, J. J. *J. Chem. Soc. D* **1969**, 644−645. (b) Birth, A. J.; Wright, J. J. *Tetrahedron* **1970**, *26*, 2329−2344. (c) Birch, A. J.; Russell, R. A. *Tetrahedron* **1972**, *28*, 2999−3008.
(11) (a) Yamazaki, M.; Okuyama, E.; Kobayashi, M.; Inoue, H. *Tetrahedron Lett.* **1981**, *22*, 135−136. (b) Ondeyka, J. G.; Goegelman, R. T.; Schaeffer, J. M.; Keleman, L.; Zitano, L. *J. Antibiot.* **1990**, *43*, 1375−1379. (c) Blanchflower, S. E.; Banks, R. M.; Everett, J. R.; Manger, B. R.; Reading, C. *J. Antibiot.* **1991**, *44*, 492−497. (d) Blanchflower, S. E.; Banks, R. M.; Everett, J. R.; Reading, C. *J. Antibiot.* **1993**, *46*, 1355−1363.
(12) (a) Polonsky, J.; Merrien, M.-A.; Prangé, T.; Pascard, C.; Moreau, S. *J. Chem. Soc., Chem. Commun.* **1980**, 601−602. (b) Prangé, T.; Billion, M.-A.; Vuilhorgne, M.; Pascard, C.; Polonsky, J.; Moreau, S. *Tetrahedron Lett.* **1981**, *22*, 1977−1980.
(13) Whyte, A. C.; Gloer, J. B. *J. Nat. Prod.* **1996**, *59*, 1093−1095.
(14) (a) Hayashi, H.; Nishimoto, Y.; Nozaki, H. *Tetrahedron Lett.* **1997**, *38*, 5655−5658. (b) Hayashi, H.; Nishimoto, Y.; Akiyama, K.; Nozaki, H. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 111−115.
(15) (a) Numata, A.; Takahashi, C.; Ito, Y.; Takada, T.; Kawai, K.; Usami, Y.; Matsumura, E.; Imachi, M.; Ito, T.; Hasegawa, T. *Tetrahedron Lett.* **1993**, *34*, 2355−2358. (b) Jadulco, R.; Edrada, R. A.; Ebel, R.; Berg, A.; Schaumann, K.; Wray, V.; Steube, K.; Proksch, P. *J. Nat. Prod.* **2004**, *67*, 78−81. (c) Hayashi, H.; Matsumoto, H.; Akiyama, K. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 753−756. (d) Dalsgaard, P. W.; Blunt, J. W.; Munro, M. H. G.; Frisvad, J. C.; Christophersen, C. *J. Nat. Prod.* **2005**, *68*, 258−261.
(16) Domínguez, X. A.; Cano, G.; Luna, I.; Dieck, A. *Phytochemistry* **1977**, *16*, 1096.

7.67 (1H, d, J = 7.4 Hz, H-4), 7.20 (1H, dd, J = 7.4 and 8.0 Hz, H-5), 5.44 (1H, brs, H-14), 5.12 (1H, s, H-21), 4.03 (1H, m, H-16), 3.79 (1H, m, H-10α), 3.67 (2H, m, H-12 and H-2′), 3.50 (1H, m, H-13α), 3.25 (1H, m, H-10β), 3.16 (1H, m, H-15α), 2.99 (3H, brs, H_3-7′), 2.91 (3H, brs, H_3-8′), 2.53 (3H, s, H_3-26), 2.41 (1H, m, H-4′), 2.22 (2H, m, H_2-8), 2.18 (1H, m, H-17α), 2.02 (1H, m, H-15β), 1.89 (1H, m, H-13β), 1.79 (1H, m, H-17β), 1.70 (3H, s, H_3-23), 1.61 (1H, s, H_3-24), 1.58 (3H, d, J = 6.7 Hz, H_3-27), 1.44 (3H, s, H_3-29), 1.38 (3H, d, J = 6.5 Hz, H_3-6′), 1.08 (3H, d, J = 6.5 Hz, H_3-5), and 1.03 (3H, s, H_3-29); ESIMS m/z 661 $(M + H)^+$; HRESIMS m/z 661.3721 [$(M + H)^+$, calcd for $C_{35}H_{53}N_4O_6{}^{35}Cl$, 661.3732].

3-Methyl-1-phenylbut-2-en-1-one (6). A solution of 1-bromo-2-methylpropene (7.60 g, 56.5 mmol) in THF (5 mL) was added slowly to a suspension of magnesium (1.35 g, 56.3 mmol) in THF (10 mL). This solution was kept at reflux by heating for 1 h, and then THF (10 mL) was added to this mixture after magnesium disappeared. After the mixture was cooled in an ice bath, a solution of benzaldehyde (2.09 g, 19.7 mmol) in THF (2 mL) was added dropwise to the mixture, and stirring was continued for 1 h. The reaction mixture was poured onto saturated aqueous NH_4Cl, and the mixture was extracted with Et_2O. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated. The crude product was subjected to silica gel column chromatography (hexane/EtOAc, 3:1) to afford an aryl alcohol (3.19 g, 19.6 mmol, 99%) as a pale yellow oil: UV (MeOH) λ_{max} 216 (sh ϵ 9000) and 205 (13 200) nm; IR (neat) ν_{max} 3349 and 1027 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40−7.32 (4H, m), 7.26 (1H, tt, J = 2.0 and 7.3 Hz), 5.44 (1H, d, J = 9.1 Hz), 5.41 (1H,m), 1.80 (3H, s), and 1.76 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.1, 134.8, 128.2, 127.6, 127.0, 125.7, 70.6, 25.8, and 18.2; EIMS m/z 162 (M^+); HREIMS m/z 162.1044 (M^+, calcd for $C_{11}H_{14}O$, 162.1044).

To a stirred solution of aryl alcohol (0.6 g, 3.70 mmol) in CH_2Cl_2 (20 mL) was added MnO_2 (5.0 g, 57.1 mmol). The reaction mixture was stirred at room temperature for 3 h. After filtration and then evaporation of the solvent, a residue was subjected to silica gel column chromatography (hexane/EtOAc, 8:1) to give compound **6** (477.6 mg, 2.98 mmol, 81%) as colorless liquid: UV (MeOH) λ_{max} 260 (ϵ 12 600) and 203 nm (10 200); IR (neat) ν_{max} 1662 and 1615 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 (2H, d, J = 7.2 Hz), 7.52 (1H, t, J = 7.2 Hz), 7.43 (2H, t, J = 7.2 Hz), 6.75 (1H, s), 2.22 (3H, s), and 2.03 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.7, 156.4, 138.8, 131.8, 128.0, 127.7, 120.7, 27.6, and 20.8; EIMS m/z 160 (M^+); HREIMS m/z 160.0886 (M^+, calcd for $C_{11}H_{12}O$, 160.0888).

(2*R*)-2,3-Dihydroxy-3-methyl-1-phenylbutan-1-one (7a). To a suspension of AD-mix-α (3.1 g) in t-BuOH/H_2O (1:1, 8 mL) were added potassium osmate dihydrate (6.0 mg, 16 μmol), $NaHCO_3$ (392 mg, 4.7 mmol), and methanesulfonamide (155 mg, 1.6 mmol) at room temperature, and the mixture was stirred for 10 min. A solution of compound **6** (249 mg, 1.6 mmol) in t-BuOH/H_2O (1:1, 2 mL) was added to this mixture at 4 °C, and stirring was continued at 4 °C for 15 h. After addition of Na_2SO_3 (4.0 g, 31.7 mmol), the reaction mixture was stirred at room temperature for 1 h. After addition of $CHCl_3$ (20 mL), the reaction mixture was filtrated. The filtrate was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was subjected to silica gel column chromatography ($CHCl_3$/EtOAc, 95:5) to give compound **7a** (249.0 mg, 0.90 mmol, 58%, 95% ee) as a colorless solid; $[\alpha]^{21}_D$ −58° (c 1.0, $CHCl_3$); UV (MeOH) λ_{max} 247 (ϵ 7900) and 203 nm (12 000); IR (film) ν_{max} 3534, 3339, 1672, and 1087 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.92 (2H, d, J = 8.0 Hz), 7.60 (1H, t, J = 7.6 Hz), 7.47 (2H, brt, J = 8.0 Hz), 4.94 (1H, s), 1.16 (3H, s), and 1.12 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.9, 136.2, 133.9, 128.9, 128.7, 78.1, 72.7, 26.4, and 25.9; FABMS m/z 217 $(M + Na)^+$; HRFABMS m/z 217.0849 [$(M + Na)^+$, calcd for $C_{11}H_{14}O_3Na$, 217.0841].

The enantiomeric excess was determined by HPLC analysis using a CHIRALCEL OD column (Dicel Chemical, 0.46 × 250 mm; 2-propanol/hexane, 1:9; flow rate 0.5 mL/min; UV detection at 270 nm). Major and minor constituents of **7a** were found at t_R 20.8 and 21.8 min, respectively.

(2*S*)-2,3-Dihydroxy-3-methyl-1-phenylbutan-1-one (7b). Compound **6** (300 mg, 1.9 mmol) was treated with AD-mix-β (3.7 g) by the same procedure as described above to afford compound **7b** (205.6 mg, 1.1 mmol, 57%, 95% ee) as colorless solid: $[\alpha]^{21}_D$ +51° (c 1.0, $CHCl_3$); UV (MeOH) λ_{max} 247 (ϵ 7900) and 203 nm (1200); IR (film) ν_{max} 3535, 3339, 1673, and 1089 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 (2H, d, J = 8.0 Hz), 7.63 (1H, t, J = 7.6 Hz), 7.50 (2H, brt, J = 8.0 Hz), 4.95 (1H, d, J = 7.2 Hz), 1.17 (3H, s), and 1.14 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.9, 136.2, 134.0, 128.9, 128.7, 78.1, 72.7, 26.4, and 25.9; FABMS m/z 217 $(M + Na)^+$; HRFABMS m/z 217.0844 [$(M + Na)^+$, calcd for $C_{11}H_{14}O_3Na$, 217.0841].

The enantiomeric excess was determined by the same method as described above. Major and minor constituents of **7b** were found at t_R 21.8 and 20.8 min, respectively.

(2*R*)-3-Hydroxy-3-methyl-1-phenyl-2-(toluenesulfonyloxy)butan-1-one (8a). Toluenesulfonyl chloride (504.0 mg, 2.62 mmol) was added to a solution of diol **7a** (173.3 mg, 0.89 mmol) in pyridine (7 mL), and stirring was continued at room temperature for 6 h. After addition of saturated aqueous $CuSO_4$, the mixture was extracted with $CHCl_3$, and the organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was subjected to silica gel column chromatography (hexane/EtOAc, 4:1) to afford compound **8a** (210.4 mg, 0.60 mmol, 68%, 73% ee) as pale yellow oil: $[\alpha]^{21}_D$ −37° (c 1.0, $CHCl_3$); UV (MeOH) λ_{max} 251 (ϵ 8500), 228 (11 600), and 203 nm (20 100); IR (neat) ν_{max} 3524, 1687, 1363, and 1181 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.87 (2H, d, J = 8.5 Hz), 7.67 (2H, d, J = 8.5 Hz), 7.57 (1H, t, J = 7.6 Hz), 7.43 (2H,t, J = 7.6 Hz), 7.19 (2H, d, J = 8.5 Hz), 5.56 (1H, s), 2.37 (3H, s), 1.28 (3H, s), and 1.26 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.1, 145.1, 136.1, 133.7, 132.6, 129.6, 129.0, 128.5, 128.0, 83.6, 72.0, 26.6, 26.4, and 21.6; FABMS m/z 349 $(M + H)^+$; HRFABMS m/z 349.1111 [$(M + H)^+$, calcd for $C_{18}H_{21}O_5S$, 349.1110].

The enantiomeric excess was determined by the same method as described above. Major and minor constituents of **8a** were found at t_R 30.6 and 28.8 min, respectively.

(2*S*)-3-Hydroxy-3-methyl-1-phenyl-2-(toluenesulfonyloxy)butan-1-one (8b). Compound **7b** (200.1 mg, 1.03 mmol) was treated with toluenesulfonyl chloride (3.7 g) by the same procedure as described above to afford compound **8b** (212.3 mg, 0.61 mmol, 59%, 92% ee) as pale yellow oil: $[\alpha]^{21}_D$ +33° (c 1.0, $CHCl_3$); UV (MeOH) λ_{max} 251 (ϵ 8500), 228 (11 600), and 203 nm (20 100); IR (neat) ν_{max} 3521, 1687, 1363, and 1181 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.87 (2H, d, J = 8.5 Hz), 7.67 (2H, d, J = 8.5 Hz), 7.57 (1H, t, J = 7.6 Hz), 7.43 (2H,t, J = 7.6 Hz), 7.19 (2H, d, J = 8.5 Hz), 5.56 (1H, s), 2.37 (3H, s), 1.28 (3H, s), and 1.26 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.1, 145.1, 136.1, 133.7, 132.6, 129.6, 128.9, 128.5, 128.0, 83.6, 72.0, 26.5, 26.4, and 21.6; FABMS m/z 349 $(M + H)^+$; HRFABMS m/z 349.1101 [$(M + H)^+$, calcd for $C_{18}H_{21}O_5S$, 349.1110].

The enantiomeric excess was determined by the same method as described above. Major and minor constituents of **8b** were found at t_R 28.8 and 30.6 min, respectively.

(2*S*)-2,3-Epoxy-3-methyl-1-phenylbutan-1-one (4a). To a solution of tosylate **8a** (200.4 mg, 0.58 mmol) in MeOH (2.2 mL) was added K_2CO_3 (100 mg), and stirring was continued at 0 °C for 3 h. After filtration, the filtrate was evaporated in vacuo. The crude product was purified by silica gel column chromatography (hexane/EtOAc, 4:1) to afford compound **4a** (82.7 mg, 0.47 mmol, 82% yield, 71%ee) as colorless solid: $[\alpha]^{17}_D$ −11° (c 1.0, $CHCl_3$); UV (MeOH) λ_{max} 248 (ϵ 8500) and 203 nm (12300); IR (film) ν_{max} 1691 and 1231 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.98 (2H, d, J = 8.5 Hz), 7.62 (1H, t, J = 7.3 Hz), 7.50 (2H, t, J = 7.3 Hz), 4.04 (1H, s), 1.60 (3H, s), and 1.25 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.1, 135.7, 133.7, 128.8, 128.1, 64.5, 61.2, 24.4, and 18.6; EIMS m/z 176 (M^+); HREIMS m/z 176.0833 (M^+, calcd for $C_{11}H_{12}O_2$,176.0829).

The enantiomeric excess was determined by the same method as described above. Major and minor constituents of **4a** were found at t_R 12.2 and 14.0 min, respectively.

(2*R*)-2,3-Epoxy-3-methyl-1-phenylbutan-1-one (4b). Tosylate **8b** (202.3 mg, 0.58 mmol) was treated with AD-mix-β (3.7 g) by the same procedure as described above to afford compound **4b** (72.5 mg, 0.41 mmol, 71%, 90% ee) as colorless solid: $[\alpha]^{17}_D$ +12° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} 248 (ϵ 8500) and 204 nm (12300); IR (film) ν_{max} 1691 and 1231 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.97 (2H, d, J = 8.5 Hz), 7.62 (1H, t, J = 7.3 Hz), 7.51 (2H, t, J = 7.3 Hz), 4.04 (1H, s), 1.60 (3H, s), and 1.25 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.1, 135.7, 133.7, 128.8, 128.1, 64.5, 61.2, 24.4, and 18.6; EIMS *m/z* 176 (M^+); HREIMS *m/z* 176.0832 (M^+, calcd for $C_{11}H_{12}O_2$,176.0829).

The enantiomeric excess was determined by the same method as described above. Major and minor constituents of **4b** were found at t_R 14.0 and 12.2 min, respectively.

Acknowledgment. We thank M. Kiuchi and S. Oka, Center for Instrumental Analysis, Hokkaido University, for mass spectra measurements, and M. Iha, Tropical Technology Center, for his help with red alga collection. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Supporting Information Available: Spectral data for citrinadin B (**1**), **4a**, **4b**, **6**, **7a**, **7b**, **8a**, and **8b**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO051499O