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

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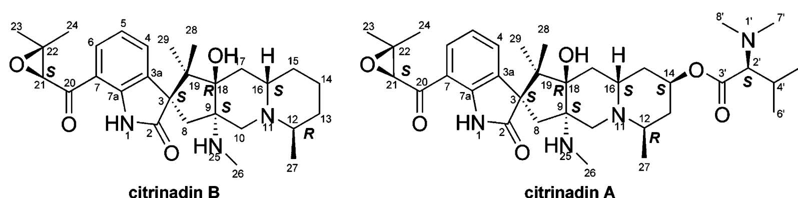
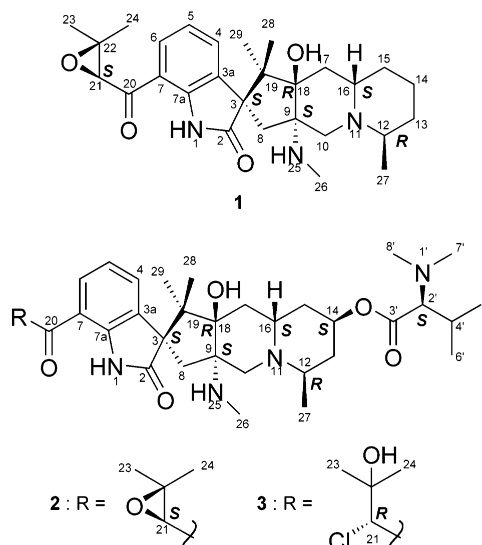


Citrinadin A (**2**) is a pentacyclic indolinone alkaloid isolated from the cultured broth of a fungus, *Penicillium citrinum*, which was separated from a marine red alga. The absolute stereochemistry of the pentacyclic core in **2** and its new congener, citrinadin B (**1**), was elucidated by analysis 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.



Results and Discussion

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

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(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.

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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

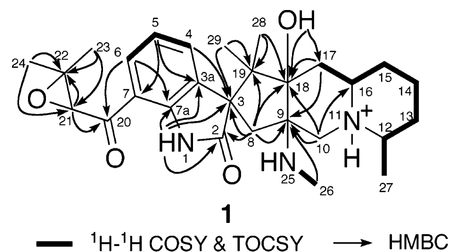


FIGURE 1. Selected 2D NMR data for citrinadin B (**1**).

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**.

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.

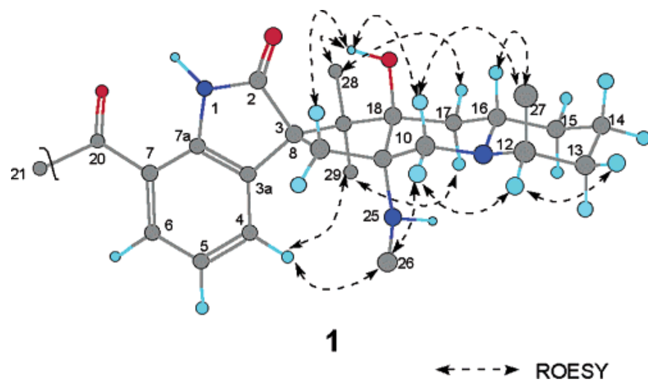


FIGURE 2. Selected ROESY correlations and relative stereochemistry for citrinadin B (**1**).

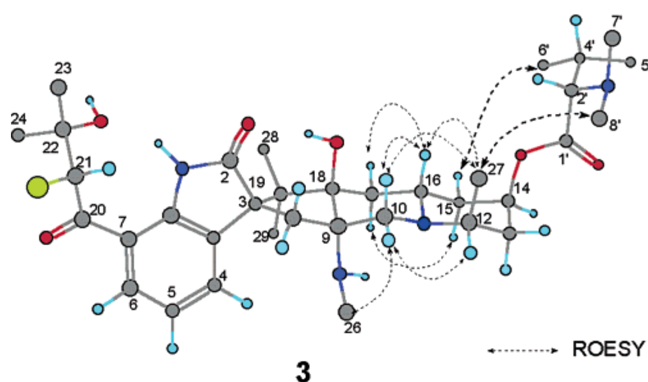
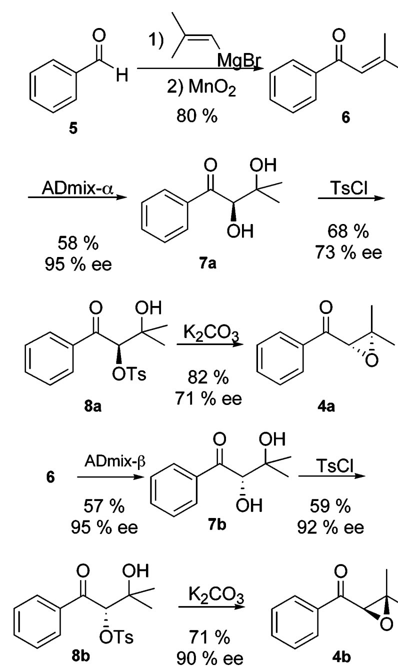


FIGURE 3. Selected ROESY correlations for chlorohydrin derivative (**3**) of citrinadin A (**2**).

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*-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.

SCHEME 1



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.

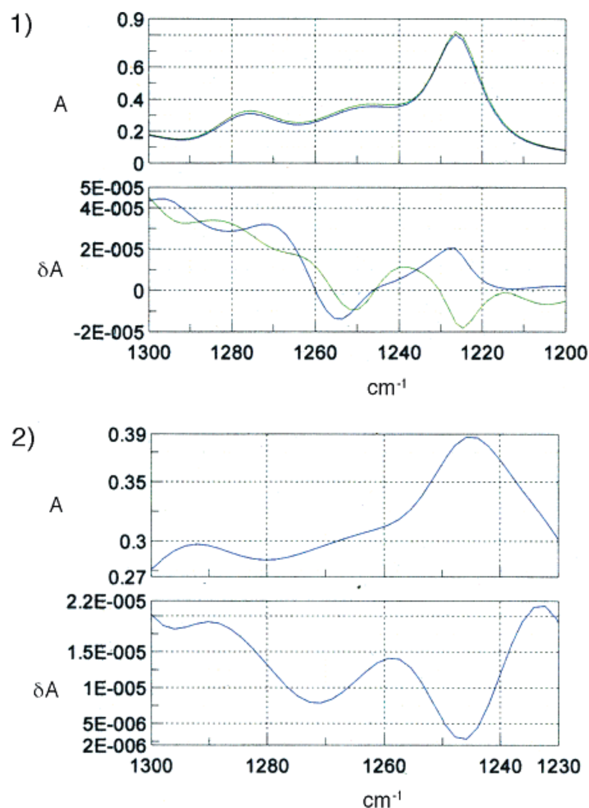


FIGURE 4. IR (upper) and VCD (lower) spectra of 1) model compounds **4a** (green) and **4b** (blue) and 2) citrinadin A (**2**).

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 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).

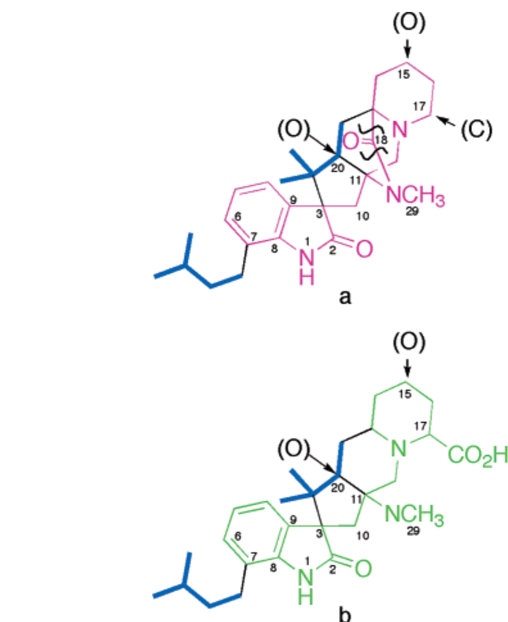


FIGURE 5. Two plausible biogenetic precursors (**a** and **b**) of citrinadins. Diketopiperazine and dipeptide portions and the isoprene unit are shown in magenta, green, and blue.

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}; ^{1}H 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}; ^{1}H 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.

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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.

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