Stereochemistry of the Roridins. Diastereomers of Roridin E

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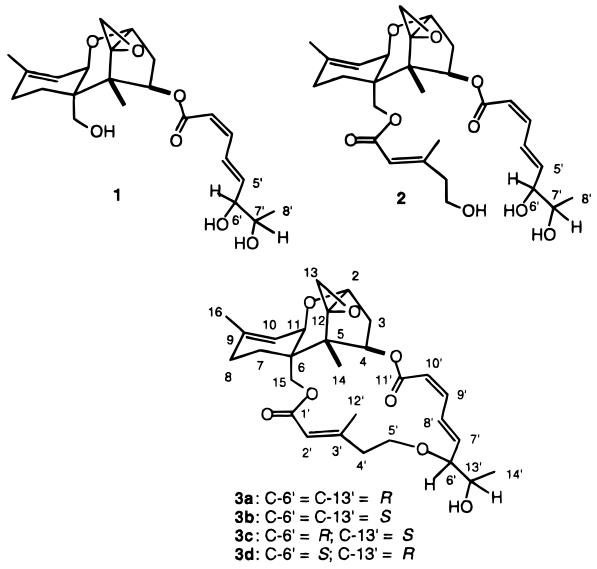
Received June 4, 1999

A careful investigation of cultures of *Myrothecium verrucaria* has shown that this fungus produces all four roridin E isomers (**3a**−**d**), diastereomeric at the C-6′ and C-13′ centers. The stereochemistries at these centers were established by a combination of X-ray crystallographic analysis, NMR spectroscopy, and chemical transformations. NMR data from these and other macrocyclic trichothecenes allows for the assignment of configurations at the C-6′ and C-13′ centers for most of these compounds, the exceptions being those congeners having a C-4′ ketone group in the macrolide ring.

The trichothecene complex of antibiotics is a well-known class of mycotoxins associated with numerous cases of animal and human intoxications.[1] This group of sesquiterpenes can be divided into two classes: simple[2] and macrocyclic.[3] The latter are produced by a variety of fungi including *Myrothecium roridum*, *Myrothecium verrucaria*, *Stachybotrys atra* (*S. chartarum*), *Cylindrocarpon* sp., *Verticimonosporium diffractum*, *Cryptomola acutispora*, *Phomopsis leptostromiformis*,[4] *Cercophora areolata*,[5] and *Ceratopicnidium baccharidicola*.[6] The trichoverroids are esters of the simple trichothecenes, verrucarol and/or trichodermol, which have at least one element of the macrocyclic ring but lack the ring itself.

The trichoverroids lie along the biosynthetic path leading from the simple to the macrocyclic trichothecenes.[7] The trichochoverrins appear to undergo ring closure to yield roridin E, which is further elaborated to the other roridins.[7] Initially, the trichoverroids produced by *Myrothecium* species of fungi were obtained as sets of diastereomers, epimeric at C-7′. Trichoverrin A is C-6′(*S*),C-7′(*S*) (*threo*), and trichoverrin B is C-6′(*S*),C-7′(*R*) (*erythro*).[7] Whereas the C-6′ centers in the trichoverrols (**1**) and in the trichoverrins (**2**) are *S*, the corresponding centers in the ring-closed macrocyclic trichothecenes (e.g., roridins and baccharinoids) are *R*. There are many examples of roridins (especially the plant-derived baccharinoids)[4] that are epimeric at C-13′, the stereogenic center that corresponds to the C-7′ center in the trichoverroids. However, the only macrocyclic trichothecene reported to have the C-6′(*S*) configuration is isororidin E (**3b**), where both C-6′ and C-13′ are *S*.[8] These centers are both *R* in roridin E (**3a**). Furthermore, whereas roridin E (**3a**) is produced commonly by *M. verrucaria* and *M. roridum*,[2] the only *Myrothecium* species reported to produce isororidin E is *M. verrucaria* ATCC 20540,[9] although **3b** was first isolated from a culture of *Cylindrocarpon* species.[10]

Upon investigation of an extract of a culture of *M. verrucaria* ATCC 20540, we showed that the trichoverroids produced by this fungus were C-6′(*R*) (isotrichoverroids)[11] rather than the C-6′(*S*) trichoverroids that are commonly produced by other *Myrothecium* cultures.[4,7] Thus, it appears that isororidin E is derived from an isotrichoverrin that has the *R* configuration at C-6′. Furthermore, because we had found all four possible C-6′/C-7′ diastereomers of the trichoverrins, it was reasoned that a careful search of *Myrothecium* cultures would eventually lead to the isolation of the two missing C-6′/C-13′ diastereomers of roridin E. Herein, we report the isolation and characterization of these two missing diastereomers, epiroridin E (**3c**) and epiisororidin E (**3d**).



Results and Discussion

Based on past experience,[7] we felt that *M. verrucaria* (ATCC 24571) would be a likely source for the missing diastereomers of roridin E. In fact, we were able to isolate a small amount of a new diastereomer of roridin E, epiroridin E (**3c**), from a chromatography fraction stored in the freezer for many years.[7] Two 4-L fermentations of submerged cultures of *M. verrucaria* (ATCC 24571) were conducted, one with and one without added Amberlite XAD-7 resin. Earlier, we had shown that adding resin to liquid cultures of *Myrothecium* could sometimes increase the level of trichothecene production,[12] but in the present case, both cultures yielded essentially the same quantities and types of trichothecenes. The combined crude extracts (7.5 g) of the above cultures were processed by a series of chromatographic procedures (see Experimental Section) to yield 400 mg of a 3:1 mixture of roridin E (**3a**) and isororidin E (**3b**) and 20 mg each of epiroridin E (**3c**) and epiisororidin E (**3d**).

Compounds **3a** and **3b** were separated by preparative HPLC and shown to be identical to authentic samples.

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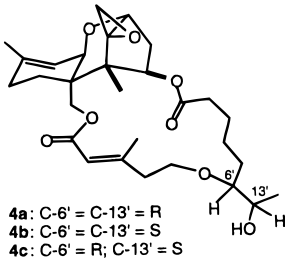
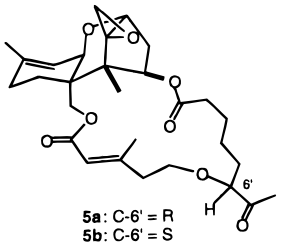
10.1021/np990272j CCC: $18.00
Published on Web 08/26/1999

HRMS confirmed that compounds **3a**−**d** had the same molecular formulas, and ^{1}H and ^{13}C NMR spectroscopy indicated that **3c** and **3d** were very close in structure to roridin E (**3a**) and isororidin E (**3b**). Because roridin E [C-6′(*R*)/C-13′(*R*)] and isororidin E [C-6′(*S*)/C-13′(*S*)] are *threo*, **3c** and **3d** are *erythro*, with one isomer C-6′(*R*)/C-13′(*S*) and the other isomer C-6′(*S*)/C-13′(*R*). The *erythro* configurations of **3c** and **3d** were confirmed by ^{1}H NMR spectroscopy of their acetates, both of which exhibited eight-line multiplets for the H-13′ protons; whereas, these signals in roridin E acetate and isororidin E acetate are five-line multiplets, characteristic of **3a** and **3b** having the C-6′/C-13′ *threo* configurations.[4,7,11]

To establish the absolute stereochemistries of **3c** and **3d**, we followed a method used previously in this laboratory to determine the sterochemistry of isororidin A.[8] Roridin E (**3a**), isororidin E (**3b**), and epiroridin E (**3c**) were first hydrogenated to their 7′,8′,9′,10′-tetrahydro derivatives **4a**−**c**, which were then oxidized to their C-13′ ketone derivatives **5a** and **5b**. It was best to first reduce the diene system in **3** prior to oxidation, because direct oxidation of roridins usually leads to oxidative cleavage of the pendant hydroxyethyl group to yield the corresponding verrucarins.[8] Thus, roridin E (**3a**) and epiroridin E (**3c**) gave **5a** and isororidin E gave **5b**, establishing the absolute stereochemistries of all four C-6′/C-13′ epimeric roridin Es as shown in structures **3a**−**d**.



4a: C-6' = C-13' = R
4b: C-6' = C-13' = S
4c: C-6' = R; C-13' = S



5a: C-6' = R
5b: C-6' = S

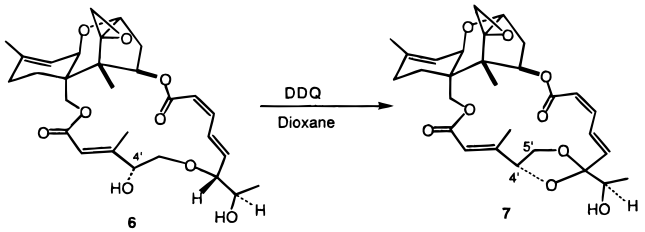
With the structural assignments for the roridin Es in hand, an analysis of their NMR data provides a clear basis for making structural assignments for the C-6′ and C-13′ centers in the macrocyclic trichothecenes. The NMR data for **3a**−**d** are all very similar, with two notable exceptions. For those epimers that are C-6′(*R*), the $J_{6',7'}$ values are about 3 Hz (compounds **3a** and **3c**); whereas, for those epimers that are *S* at this center, $J_{6',7'}$ is about 6 Hz. The second significant difference is found in the chemical shift differences between the C-7′ and C-8′ carbons. This difference is about 12 ppm for the C-6′(*R*) epimers but only about 4 ppm for the C-6′(*S*) epimers (see Table 1). These data hold true for a wide range of structural changes in the A, B, C rings of the trichothecene central ring system as well as for a wide variation in the substitution patterns at C-2′, C-3′, and C-4′ of the macrolide ring (Table 1). However, there are two apparent exceptions to these correlations: miotoxins B[13] and E.[14] Like the baccharinoids, the miotoxins are derived from Brazilian *Baccharis* species, and like both the baccharinoid and roridin classes of mycotoxins, they are reported to have C-6′(*R*) configurations.[13]

Miotoxins B and E are unique in being the only macrocyclic trichothecenes with a C-4′ ketone group, and it is possible that this functionality affects the conformation of the macrolide ring such as to alter $J_{6',7'}$ as well as the $\Delta\delta_{C-7'/C-8'}$ values. To test this hypothesis, we attempted to oxidize miotoxin A (**6**) (available from earlier work),[14] which is known to be C-6′(*R*).[13] Oxidation of the C-4′ hydroxyl group in **6** would give a C-4′ ketone with which we could test this hypothesis. However, **6** also has a C-13′ hydroxyl group, the oxidation of which, as noted earlier, gives rise to oxidative cleavage. Because the C-4′ alcohol is allylic, we first attempted oxidation with dichlorodicyanobenzoquinone (DDQ). However, this reaction gave ketal **7** in 15% yield along with 65% recovered starting material. The structure assignment for **7** is based on HRMS and NMR spectroscopy. For **7**, H-7′ is a doublet and H-13′ a quartet, indicating the loss of H-6′. The H-4′ resonance is a doublet of doublets at 4.6 ppm, which is 0.24 ppm downfield from where this proton resonates in **6**. The diastereotopic H-5′ protons exhibit an ABX pattern and are found at δ 3.42 and 4.35, suggesting that these two protons are in quite different environments.

Table 1. $J_{6',7'}$ Values and ^{13}C Chemical Shift Data for Various Macrocyclic Trichothecenes

compound	variations relative to roridin E	$J_{6',7'}$ (in Hz)	δ ^{13}C C-7′	δ ^{13}C C-8′	$\Delta\delta_{C7'/C8'}$ (Δppm)
roridin E		3	138.1	126.6	11.5
isororidin E		6	135.2	131.0	4.2
epiroridin E		3	137.8	126.8	11.0
epiisororidin E		6	134.7	131.0	3.7
isororidin K	[a]	6	135.1	131.2	3.9
roridin K acetate	[a]	3	138.5	126.5	12.0
roridin A	[b,c]	3	139.0	126.0	13.0
roridin D	[d]	3	138.1	126.2	11.9
baccharanoid B1	[b,e,f]	3	139.8	126.5	13.3
baccharanoid B9	[e,g]	3	138.0	126.6	11.4
miotoxin A	[e]	3	137.4	126.8	10.6
miotoxin B	[b,h]	6	136.2	129.8	6.4
miotoxin D	[b,e]	3	138.8	126.9	11.9
miotoxin E	[b,h,i]	6	135.9	130.7	5.2
miotoxin F	[b,e,i]	3	138.8	126.1	12.7

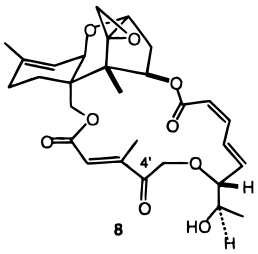
[a] 8α-hydroxy or acetoxy group. [b] 2′,3′-Single bond. [c] 2′-Hydroxy. [d] 2′,3′-Epoxide. [e] 4′-Hydroxy. [f] 8β-Hydroxy. [g] 9β,10β-Epoxide. [h] 4′-Ketone. [i] 3′-Hydroxy.



6 **7**

Although oxidations of roridins often lead to verrucarins,[8] careful oxidation of the C-8 allylic hydroxyl group in baccharinoid B4, a plant-derived roridin, by pyridinium chlorochromate (PCC) gave the corresponding C-8 ketone without concomitant oxidative cleavage of the C-6′ hydroxylethyl group.[15] Thus, treatment of **6** with PCC gave a complex mixture from which we got a 15% yield of what appeared to be, from TLC and HPLC analyses, a single less polar compound. However, NMR analysis showed clearly that the product was a 50−50 mixture of isomers; HRMS analysis showed that they had the correct molecular formula for the expected C-4′ ketone **8**. Careful analysis of the NMR spectra of this mixture, especially the ^{1}H−^{1}H COSY spectrum revealed that this mixture consisted of the target ketone **8** and the cyclic hemiketal **9**. These analyses were greatly facilitated by the coincidence that at 500 MHz, all of the proton signals of the macrolide chains in isomers **8** and **9** exhibit first-order patterns, and that none of these signals in **8** and **9** overlapped. These conditions allowed us to assign with confidence the individual proton signals of the macrolide portions for each compound. The most

important signals were those for H-5′ and H-6′. In ketone **8**, the diastereotopic H-5′ protons exhibit an AB pattern at δ 4.50 and 4.60 with J_{gem} = 16.5 Hz, while for **9** these protons are found at δ 3.43 and 4.18, with J_{gem} = 12.1 Hz. These data are consistent with the H-5 in **8** being attached to a carbon atom next to a carbonyl group, while in **9** these protons are on a C-2 carbon of a 1,4-dioxane ring. Consistent with the hypothesis above, the $J_{6',7'}$ coupling constant in both **8** and **9** is 5.7 Hz, even though the C-6′ center is *R*. Because of signal overlap in the carbon spectrum, we were unable to make complete carbon assignments for the **8**/**9** mixture. However, the C-4′ signals for ketone **8** and hemiketal **9** were evident at δ 199.7 and 94.4, respectively. Furthermore, the sp^2 carbons in the macrolide portions of **8** and **9** could be assigned through an $^1H-^{13}C$ COSY experiment. The $\Delta\delta_{C-7'/C-8'}$ for **8** is 4.0 ppm, which demonstrates that the C-4′ ketone congeners do not exhibit either the $J_{6',7'}$ or the $\Delta\delta_{C-7'/C-8'}$ values typically observed with the other C-6′(*R*) macrocyclic trichothecenes (Table 1). It is interesting that neither miotoxin B nor miotoxin E, unlike ketone **8**, appears to form hemiketals analogous to that observed with **8**/**9**. In miotoxins B and E, the C-2′/C-3′ centers are saturated, which means that the C-2′/C-3′ double bond in **8** facilitates the ring closure to hemiketal **9**.



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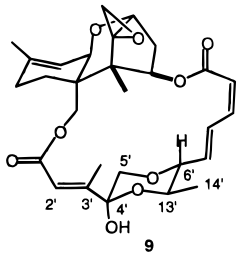
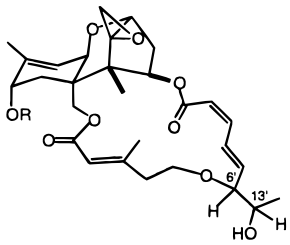


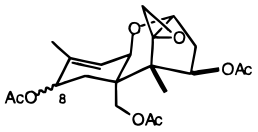
9

During the course of this work, we isolated roridin K acetate (**10a**) and, what appeared at first to be roridin K itself. Roridin K acetate (**10a**) was reported previously by us, and Kobayashi et al.[16] reported isolating from a *Myrothecium* species "8β-hydroxyroridin E," the 8β-hydroxy epimer of roridin K. Our "roridin K" (**10b**) and their 8β-hydroxyroridin E exhibited the same proton NMR spectra. Of note is that the $J_{6',7'}$ value for this compound is 6.3 Hz, which reveals that **10b** is of the C-6′(*S*) series and is in fact isororidin K. Furthermore, upon acetylation, **10a** and **10b** did not give the same diacetate, but they were both of the C-6′/C-13′ *threo* series (*R*/*R* or *S*/*S* at these centers), because both exhibit five-line multiplets ($J_{6',13'} = J_{13',14'}$ ca. 6.5 Hz) for their C-13′ protons. Therefore, both **10b** and the 8-hydroxyroridin E reported by Kobayahi et al. are derivatives of isororidin E.

An unusual aspect of the original structure assignment for "8β-hydroxyroridin E" was the configuration of the C-8 hydroxyl group. Fungal-produced trichothecenes bearing hydroxyl groups at the C-8 position, with this lone exception, have the C-8α substitution configuration.[3,4] The assignment of the β-configuration of this hydroxyl group was based on NOE data, and we sought a firmer basis for this assignment. To this end, compound **10b** was hydrolyzed and peracetylated to give a triacetate of 8-hydroxyverrucarol. Comparison of the GC−MS of this triacetate with those of 8α-acetoxyverrucarol diacetate (**11a**) and 8β-acetoxyverrucarol diacetate (**11b**) showed that the triacetate derived from (**10b**) was 8α-acetoxyverrucarol diacetate (**11a**). Thus, the correct structure of the compound originally called "8β-hydroxyroridin E" by Kobayashi et al. is **10b**, 8α-hydroxyisororidin E. They also reported isolating "roridin A" from their culture. However, the ^1H NMR data suggest that their "roridin A" is actually the C-6′(*S*)/C-13′(*S*) diastereomer of roridin A [C-6′(*R*)/C-13′(*R*)]. Unfortunately this diastereomer cannot be called "isororidin A" (cf. roridin E/isororidin E) because this name was used for the C-6′(*R*)/C-13′(*S*) diastereomer of roridin A.[8]



10a: R = Ac; C-6' = *R*; C-13' = *R*
10b: R = H; C-6' = *S*; C-13' = *S*



11a: C-8α
11b: C-8β

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns hot-stage mp apparatus and are uncorrected. Optical rotations were determined on a JASCO digital polarimeter model DIP-370. IR spectra (IR) were recorded on Nicolet 5DXC FTIR spectrometer or Perkin−Elmer 1600 FTIR. UV spectra were determined on a Gilford UV−vis spectrophotometer. NMR spectra were obtained on a Bruker AM 200, a Bruker AM 400, or a Bruker AMX 500 spectrometer in $CDCl_3$ with the $CHCl_3$ peak at 7.24 ppm as an internal standard. $^1H-^1H$ COSY and $^{13}C-^1H$ COSY spectra were obtained on a Bruker AMX-500 spectrometer. ^1H and ^{13}C NMR assignments were done by a combination of comparisons with literature chemical shift data, DEPT, and COSY experiments. Mass spectra were acquired on a VG 7070E instrument using electron impact (El), chemical ionization (Cl, ammonia), or FAB (positive ion). TLC was carried out on prepared Si gel plates (0.25 mm, E. Merck or Whatman), and visualization was effected with short wavelength UV light or by spraying with a solution of H_2SO_4−EtOH−vanillin (40 g/L of vanillin in EtOH−H_2SO_4 1:4) and heating with a heat gun until colored spots appeared. Preparative TLC was performed on the Chromatotron (Harrison Research Laboratories) model 7942 with preparative TLC circular glass plates coated with Si gel (thickness of 1, 2, or 4 mm) according to the instructions in the product manual. Flash chromatography was carried out on Si gel 60 (70−230 mesh, E. Merck or Whatman LPS-2) in glass columns. Medium-pressure chromatography was carried out in Michel−Miller (glass) columns packed with 13−24 mm Whatman LPS-1 Si gel. Normal-phase HPLC was carried out on a Gilson model 302 gradient liquid chromatograph with an LKB 2140 rapid spectral detector (diode array detector) or a Knauer variable wavelength detector set at 260 nm (analytical) or 280 nm (preparative); analytical columns measured 4.6 × 250 mm and semipreparative columns measured 10 × 250 mm, both packed with 5 μm material. Reversed-phase HPLC was carried out on an Altex model 332 gradient instrument with a Gilson variable wavelength detector set at 260 nm (analytical) or 280 nm (preparative). Both systems employed a Shimadzu C−R3A integrator−recorder. HPLC columns employed were analytical: PEI (Bakerbond wide-pore), Zorbax silica, Supelco (C_{18}), and Rainin (C_{18}); semipreparative: C_{18} Dynamax (Rainin), phenyl Dynamax (Rainin), and Supelco silica.

All high-speed countercurrent chromatography (CCC) separations were performed with a high-speed countercurrent chromatograph, model CCC-1000 (Pharm-Tech. Research Corp., Baltimore, MD), equipped with interchangeable columns. The three columns used in this work were analytical (Vc = 55 mL), semipreparative (Vc = 355 mL), and preparative (Vc = 850 mL). Each column consisted of three multiple-layer coils of

PTFE tubing (i.d. 0.85 mm for analytical, 1.6 mm for semipreparative, and 2.6 mm for preparative). General operation conditions were as follows (if not otherwise noted): lower organic layer was the mobile phase with solvent flow from head to tail [H−(T)]. Rotatory speeds were 1200 rpm (analytical column) and 1000 rpm (semipreparative and preparative columns). Samples were dissolved in the organic layer, and the volume of samples for each injection was 0.5 mL (analytical column), 5.0 mL (semipreparative column), and 5−10 mL (preparative column). The eluate from the outlet of the column was continuously monitored by a Knauer variable wavelength monitor connected to a Fisher recorder. The wavelength of the monitor was at 260−290 nm, depending on sample size. The solvent was delivered by a LDC/Milton minipump. The organic phase was used as the mobile phase. The operating procedure of the CCC was as follows. The column was first filled with stationary phase, then the mobile phase was pumped into the column, while the column rotated at the operation speed. After a certain amount of the stationary phase was displaced (the amount varied with column size and solvent system) and the flow of the mobile phase became steady, the sample was loaded on to the column.

All the solvents used in CCC and TLC were commercial grade and were glass distilled before use, except MeOH, which was reagent grade (Fisher Scientific). For HPLC, Fisher Scientific HPLC-grade solvents were used. The two solvent phases used in CCC were thoroughly equilibrated in a separatory funnel before use.

Sample 6 (S6) was a polar fraction from an initial Si gel chromatography column from a fermentation of *M. verrucaria* ATCC 24571 rich in trichoverrins and roridin E.

General Procedure for Acetylation. Trichothecene (5 mg) was dissolved in 0.5 mL each of pyridine and Ac_2O and allowed to stand at room temperature overnight. Removal of solvent in vacuo followed by passing the residue through a small silica column (0.1 g in a pipet) with 2% MeOH in CH_2Cl_2.

Isolation of Epiroridin E from *M. verrucaria* ATCC 24571. Sample 6 (S6) from *M. verrucaria* ATCC 24571 (1 g)[7] was subjected to CCC (Vc = 850 mL) with a solvent system of CCl_4−MeOH−H_2O (5:3:2), lower organic phase was the mobile phase, and the flow rate was 3.3 mL/min, to give verrucarin A (23 mg), roridin D (11 mg), a fraction rich in roridin E (400 mg), and roridin A (67 mg). The roridin E fraction was subjected to CCC (Vc = 355 mL) with a solvent system of CCl_4−hexane−MeOH−H_2O (4:1:3:2), lower organic phase was the mobile phase, and the flow rate was 1.8 mL/min, to give 310 mg of a mixture of roridin E (**3a**) and isororidin E (**3b**) and a later fraction of pure epiroridin E (**3c**, 50 mg).

Epiroridin E (3c): amorphous solid, $[\alpha]^{30}_D = +0.70°$ (*c* 1.8, $CHCl_3$); IR ($CHCl_3$) 2491, 1713, 1652, 1601 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.76 (3 H, s, H-14),1.13 (3 H, d, J = 6.0 Hz, H-14′), 1.67 (3 H, s, H-16), 1.92−2.20 (5 H, m, H-7, H-8, H-3β), 2.24 (3 H, s, H-12′), 2.42−2.54 (3 H, m, H-4′, H-3α), 2.83 (1 H, d, J = 4.0 Hz, H-13B), 3.15 (1 H, d, J = 4.0 Hz, H-13A), 3.50 (1 H, dt, J = 15.0, 6.0 Hz, H-5B), 3.69 (1 H, dt, J = 15.0, 6.0 Hz, H-5A), 3.87 (1 H, d, J = 5.0 Hz, H-2), 3.79−3.90 (2 H, m, H-6′, H-13′), 3.89 (1 H, d, J = 12.0 Hz, H-15B), 3.91 (1 H, d, J = 5.0 Hz, H-11), 4.31 (1 H, d, J = 12.0 Hz, H-15A), 5.44 (1 H, d, J = 5.0 Hz, H-10), 5.70 (1 H, d, J = 11.0 Hz, H-10′), 5.86 (1 H, dd, J = 3.0, 15.0 Hz, H-7′), 5.98 (1 H, s, H-2′), 6.15 (1 H, dd, J = 4.0, 8.0 Hz, H-4), 6.54 (1 H, dd, J = 11.0 Hz, H-9′), 7.45 (1 H, dd, J = 11.0, 15.0 Hz, H-8′); ^{13}C NMR (50 MHz, $CDCl_3$) δ 6.7 (C-14), 17.8 (C-14′), 19.8 (C-12′), 21.5 (C-7), 23.2 (C-16), 27.2 (C-8), 35.7 (C-3), 41.4 (C-4′), 42.7 (C-6), 48.1 (C-13), 48.4 (C-5), 63.6 (C-15), 65.6 (C-12), 67.2 (C-11), 69.1 (C-13′), 69.5 (C-5′), 74.2 (C-4), 79.2 (C-2), 82.5 (C-6′), 117.2 (C-10′), 117.6 (C-10), 118.9 (C-2′), 126.8 (C-8′), 137.8 (C-7′), 140.8 (C-9), 143.6 (C-9′), 159.0 (C-3′), 165.9 (C-1′), 166.4 (C-11′); HREIMS *m/z* 514.2580 (calcd for $C_{29}H_{38}O_8$, 514.2567).

Fermentation and Extraction of Cultures of *M. verrucaria* ATCC. The fermentations were carried out using two methods: (a) with XAD-7 resin added to the liquid culture,[12] and (b) without added XAD-7 resin; each was on a 4-L scale. The detailed procedures were the same as those described in the literature.[12]

With Resin. Resin and mycelium were separated from the fermentation broth by filtration. The resin and mycelium were soaked in 2 L of MeOH overnight, and the methanol−resin−mycelium mixture was filtered. The residue was extracted again with 2 × 2 L of MeOH (sonicated for 30 min). The combined MeOH extracts were concentrated by rotary evaporation to give an aqueous mixture (500 mL). This aqueous mixture was extracted with 3 × 300 mL of EtOAc, which, upon drying (Na_2SO_4) and concentrating by rotary evaporation, gave 4.0 g of crude extract.

Without Resin. The fermentation broth was filtered and residue (mycelium) extracted with 2 × 200 mL of MeOH. The MeOH extract was filtered, concentrated in vacuo, and the resulting aqueous solution extracted with 3 × 100 mL of EtOAc. The filtrate from fermentation broth was extracted with 3 × 1 L of EtOAc, and the total EtOAc extracts were combined, dried (Na_2SO_4), and concentrated in vacuo to give 3.5 g of crude extract.

Isolation of Epiroridin (3c) and Epiisororidin E (3d). The combined crude extract from *M. verrucaria* (7.5 g) was fractionated on Si gel flash chromatography (increasing amounts of MeOH in CH_2Cl_2) to give seven fractions (A−G) based on TLC analysis. Fraction C (0.8 g) was subjected to CCC (Vc = 355 mL) with a solvent system of CH_2Cl_2−CCl_4−hexane−MeOH−H_2O (1:3:1:3:2), lower organic phase was the mobile phase, and the flow rate was 1.8 mL/min, to give a fraction rich in roridin E (440 mg). This fraction was further chromatographed on CCC with a less polar organic mobile phase [CCl_4−hexane−MeOH−H_2O (7:3:6:4)] to give 8 mg of roridin K acetate (**10a**); 353 mg of a mixture of roridin E and isororidin E; 17 mg of mixture of roridin E, isororidin E, and epiisororidin E, and 20 mg of pure epiisororidin E.

The roridin E diastereomers can be separated by reversed-phase HPLC on a phenyl column (Phenomenex, 4.6 × 250 mm) with 40% MeCN in H_2O at a flow rate of 1.2 mL/min. The observed retention times under these conditions were: **3a** (27.7 min), **3b** (23.3 min), **3c** (26.1 min), and **3d** (22.1 min).

Epiisororidin E (3d): an amorphous solid, $[\alpha]^{30}_D$ −29.2° (*c* 1.5, $CHCl_3$); IR ($CHCl_3$) 3486, 1713, 1647, 1599 cm^1; ^1H NMR (400 MHz, $CDCl_3$) δ 0.77 (3 H, s, H-14), 1.12 (3 H, d, J = 6.5 Hz, H-14′), 1.68 (3 H, s, H-16), 1.96−2.13 (5 H, m, H-3β, H-7, H-8), 2.21 (3 H, d, J = 1.2 Hz, H-12′), 2.29 (1 H, m, H-4′B), 2.50−2.56 (2 H, m, H-3α, H-4′A), 2.81 (1 H, d, J = 4.0 Hz, H-13B), 3.13 (1 H, d, J = 4.0 Hz, H-13A), 3.56 (1 H, ddd, J = 5.0, 7.4, 10.1 Hz, H-5′B), 3.73 (1 H, ddd, J = 7.4, 7.5, 10.1 Hz, H-5′A), 3.83 (1 H, d, J = 5.0 Hz, H-2), 3.89 (1 H, m, H-6′), 3.98 (1 H, dq, J = 3.0, 6.5 Hz, H-13′), 4.01 (1 H, d, J = 12.5 Hz, H-15B), 4.03 (1 H, d, J = 5.5 Hz, H-11), 4.17 (1 H, d, J = 12.5 Hz, H-15A), 5.47 (1 H, d, J = 5.5 Hz, H-10), 5.80 (1 H, d, J = 11.0 Hz, H-10′), 5.82 (1 H, d, J = 1.2 Hz, H-2′), 5.87 (1 H, dd, J = 6.1, 15.6 Hz, H-7′), 6.29 (1 H, dd, J = 4.1, 8.0 Hz, H-4), 6.60 (1 H, dd, J = 11.0, 11.0 Hz, H-9′), 7.54 (1 H, dd, J = 11.0, 15.6 Hz, H-8′); ^{13}C NMR (50 MHz, $CDCl_3$) δ 6.5 (C-14), 17.9 (C-14′), 19.2 (C-12′), 22.3 (C-7), 23.2 (C-16), 27.7 (C-8), 36.4 (C-3), 40.3 (C-4′), 42.6 (C-6), 47.8 (C-5), 48.4 (C-13), 64.3 (C-15), 66.3 (C-5′), 66.6 (C-12), 67.0 (C-11), 68.5 (C-13′), 75.0 (C-4), 79.2 (C-2), 81.9 (C-6′), 117.2 (C-10′), 117.6 (C-10), 119.0 (C-2′), 131.0 (C-8′), 134.7 (C-7′), 140.2 (C-9), 142.3 (C-9′), 157.9 (C-3′), 166.3 (C-1′), 166.5 (C-11′); HREIMS *m/z* 514.2545 (calcd for $C_{29}H_{38}O_8$, 514.2567).

Roridin E acetate: an amorphous solid, ^1H NMR (200 MHz, $CDCl_3$) δ 0.77 (3 H, s, H-14), 1.13 (3 H, d, J = 6.0 H z, H −14′), 1.68 (3 H, s, H-16), 2.02 (3 H, s, acetate), 2.24 (3 H, s, H −12′), 2.79 (1 H, d, J = 4.0 Hz, H-13B), 3.11 (1 H, d, J = 4.0 Hz, H-13A), 3.50 (1 H, m, H-5′B), 3.72 (1 H, m, H-5′A), 3.81 (1 H, d, J = 5.0 Hz, H-11), 3.82 (1 H, d, J = 5.0 Hz, H-2), 3.91 (1 H, d, J = 13.0 Hz, H-15B), 3.99 (1 H, m, H-6′), 4.32 (1 H, d, J = 13.0 Hz, H-15A), 5.10 (1 H, dq, J = 6.0 Hz, H-13′), 5.44 (1 H, d, J = 5.0 Hz, H-10), 5.72 (1 H, d, J = 11.0 Hz, H-10′), 5.85 (1 H, dd, J = 3.0, 15.0 Hz, H-7′), 5.87 (1 H, s, H-2′), 6.22 (1 H, dd, J = 4.0, 8.0 Hz, H-4), 6.55 (1 H, dd, J = 11.0 Hz, H-9′), 7.47 (1 H, dd, J = 11.0, 15.0 Hz, H-8′).

Isororidin E acetate: an amorphous solid, ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.89 (3 H, s, H-14), 1.12 (3 H, d, J = 6.0 Hz, H-14′), 1.69 (3 H, s, H-16), 2.05 (3 H, s, acetate), 2.22 (3 H, s, H-12′), 2.81 (1 H, d, J = 4.0 Hz, H-13B), 3.12 (1 H, d, J = 4.0 Hz, H-13A), 3.49 (1 H, m, H-5′B). 3.68 (1 H, m, H-5′A), 3.82 (1 H, d, J = 5.0 Hz, H-2), 3.95 (1 H, d, J = 5.0 Hz, H-11), 3.98 (1 H, d, J = 12.5 Hz, H-15B), 4.01 (1 H, m, H-6′), 4.26 (1 H, d, J = 12.5 Hz, H-15A), 5.08 (1 H, dq, J = 6.0 Hz, H-13′), 5.46 (1 H, d, J = 5.0 Hz, H-10), 5.79 (1 H, d, J = 11.0 Hz, H-10′), 5.81 (1 H, dd, J = 5.0, 15.0 Hz, H-7′), 5.84 (1 H, s, H-2′), 6.21 (1 H, dd, J = 4.0, 8.0 Hz, H-4), 6.56 (1 H, dd, J = 11.0, 11.0 Hz, H-9′), 7.42 (1 H, dd, J = 11.0, 15.0 Hz, H-8′).

Epiroridin E acetate: an oil, ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.77 (3 H, s, H-14), 1.16 (3 H, d, J = 6.0 Hz, H-14′), 1.68 (3 H, s, H-16), 2.05 (3 H, s, acetate), 2.25 (3 H, s, H-12′), 2.79 (1 H, d, J = 4.0 H z, H-13B), 3.11 (1 H, d, J = 4.0 H z, H-13 A), 3.45 (1 H, m, H-5′B), 3.79 (1 H, m, H-5′A), 3.82 (1 H, d, J = 5.0 Hz, H-2), 3.83 (1 H, d, J = 5.0 Hz, H-11), 3.93 (1 H, d, J = 12.5 Hz, H-15B), 4.02 (1 H, m, H-6′), 4.30 (1 H, d, J = 12.5 Hz, H-15A), 4.95 (1 H, dq, J = 3.0, 6.0 Hz, H-13′), 5.44 (1 H, d, J = 5.0 Hz, H-10), 5.79 (1 H, d, J = 11.0 Hz, H-10′), 5.86 (1 H, s, H-2′), 5.86 (1 H, dd, J = 3.0, 15.0 Hz, H-7′), 6.10 (1 H, dd, J = 4.0, 8.0 Hz, H-4), 6.53 (1 H, dd, J = 11.0 Hz, H-9′), 7.47 (1 H, dd, J = 11.0, 15.0 Hz, H-8′); HREIMS m/z 556.2652 (calcd for $C_{31}H_{40}O_9$, 556.2672).

Fraction E (285 mg), which contained mainly roridin A, was subjected to CCC (Vc = 355 mL) with a solvent system of CH_2Cl_2–CCl_4–hexane–MeOH–H_2O (1:8:1:6:4), organic phase was the mobile phase, and the flow rate was 1.8 mL/min, gave 60 mg of roridin A and a fraction containing mainly isororidin K, which was further purified on CCC (Vc = 55 mL) with a solvent system of CH_2Cl_2–CCl_4–hexane–MeOH–H_2O (1:8:1:6:4); organic phase was the mobile phase, and the flow rate was 1.0 mL/min, gave 7 mg of isororidin K (**10b**).

Isororidin K (10b): an oil; IR ($CHCl_3$) 3487, 2931, 1718, 1675, 1650 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.81 (3 H, s, H-14), 1.15 (3 H, d, J = 5.8 Hz, H-14′), 1.64 (1 H, br d, J = 14.6 Hz, H-7β), 1.84 (3 H, s, H-16), 1.97 (1 H, ddd, J = 4.0, 5.0, 14.0 Hz, H-3β), 2.19 (3 H, d, J = 1.0 Hz, H-12′), 2.27 (1 H, ddd, J = 8.0, 8.0, 12.5 Hz, H-4′B), 2.37 (1 H, dd, J = 5.8, 14.6 Hz, H-7α), 2.56 (1 H, m, H-4′A), 2.58 (1 H, dd, J = 8.0, 14.0 Hz, H-3α), 2.85 (1 H, d, J = 4.0 Hz, H-13B), 3.13 (1 H, d, J = 4.0 Hz, H-13A), 3.45 (1 H, ddd, J = 4.6, 8.5,10.3 Hz, H-5′B), 3.72 (2 H, m, H-6′, H-13′), 3.80 (1 H, m, H-5′A), 3.82 (1 H, d, J = 5.0 Hz, H-2), 4.12 (1 H, br d, J = 6.1 Hz, H-11), 4.13 (1 H, d, J = 13.3 Hz, H-15B), 4.15 (1 H, br d, J = 5.8 Hz, H-8), 4.41 (1 H, d, J = 13.3 Hz, H-15A), 5.59 (1 H, br d, J = 6.1 Hz, H-10), 5.68 (1 H, dd, J = 6.1, 15.7 Hz, H-7′), 5.80 (1 H, d, J = 1.0 Hz, H-2′), 5.83 (1 H, d, J = 11.2 Hz, H-10′), 6.39 (1 H, dd, J = 4.0, 8.0 Hz, H-4), 6.60 (1 H, dd, J = 11.0, 11.2 Hz, H-9′), 7.57 (1 H, dd, J = 11.0, 15.7 Hz, H-8′); ^{13}C NMR (125 MHz, $CDCl_3$), δ 6.3 (C-14), 18.5 (C-14′), 19.8 (C-12′), 20.4 (C-16), 31.7 (C-7), 36.7 (C-3), 39.8 (C-4′), 42.9 (C-6), 47.6 (C-13), 48.5 (C-5), 65.6 (C-15), 65.7 (C-12), 66.7 (C-5′), 66.8 (C-8), 66.9 (C-11), 69.7 (C-13′), 75.3 (C-4), 79.1 (C-2), 83.0 (C-6′), 117.0 (C-2′), 119.5 (C-10′), 121.1 (C-10), 131.2 (C-8′), 135.1 (C-7′), 139.5 (C-9), 142.0 (C-9′), 157.8 (C-3′), 166.0 (11′), 166.3 (1′); HRCIMS m/z 531.2620 (calcd for $C_{29}H_{38}O_9$ + H, 531.2594).

Isororidin K diacetate: an oil, ^{1}H NMR (500 MHz, $CDCl_3$), δ 0.77 (3 H, s, H-1 4), 1.09 (3 H, d, J = 6.5 Hz, H-14′), 1.69 (1 H, br d, J = 14.0 Hz, H-7β), 1.73 (3 H, s, H-16), 1.99 and 2.05 (3 H each, s, acetates), 2.23 (3 H, d, J = 1.0 Hz, H-12′), 2.39 (3 H, m, H-7α, H-4′), 2.50 (1 H, dd, J = 8.0, 15.4 Hz, H-3α), 2.82 (1 H, d, J = 4.0 Hz, H-13B), 3.09 (1 H, d, J = 4.0 Hz, H-13A), 3.69 (2 H, m, H-5′), 3.81 (1 H, d, J = 5.0 Hz, H-2), 3.94 (1 H, br d, J = 5.2 Hz, H-11), 3.96 (1 H, m, H-6′), 4.07 (1 H, d, J = 12.7 Hz, H-15B), 4.51 (1 H, d, J = 12.7 Hz, H-15A), 5.10 (1 H, dq, J = 6.5 Hz, 6.5 Hz, H-13′), 5.19 (1 H, d, J = 4.8 Hz, H-8), 5.69 (1 H, br d, J = 5.2 Hz, H-10), 5.77 (1 H, d, J = 11.1 Hz, H− 10′), 5.79 (1 H, d, J = 1.0 Hz, H-2′), 5.84 (1 H, dd, J = 4.9, 15.7 Hz, H-7′), 6.09 (1 H, dd, J = 4.3, 8.0 Hz, H-4), 6.54 (1 H, dd, J = 11.0, 11.1 Hz, H-9′), 7.31 (1 H, dd, J = 11.0, 15.7 Hz, H-8′).

GC–MS Analysis of Isororidin K. Isororidin K (**10b**) (1 mg) was dissolved in 0.5 mL of a solution of 5% NaOH in MeOH. After a period of 18 h, the solution was passed through a short column of mixed H^+/OH^- resin (ca. 0.5 g), and the column was washed with 1 mL of MeOH. The eluate was dried in vacuo, and the residue was dissolved in a mixture of 0.5 mL of pyridine and 0.5 mL of Ac_2O. The mixture was stirred for 24 h and was taken to dryness in vacuo to give a residue for GC–MS analysis, instrument conditions: HP 5988A/MSD system equipped with a 25 m/0.25 mm/0.33 mm HP-1 (cross-linked methyl silicone) fused-silica capillary column. Retention time of 8α-hydroxyverrucarol triacetate is 15.7 min. Retention time of 8β-hydroxyverrucarol triacetate is 15.5 min.

Hydrogenation of Roridin Es. To a solution of roridin E (**3a**, 52 mg, 0.1 mmol) in EtOH (20 mL) was added 18 mg of 10% palladium on carbon, and the mixture was stirred under hydrogen at atmospheric pressure and room temperature for 10 min. Filtration through a Celite pad was followed by solvent removal in vacuo. The reaction mixture was cleaned on a small silica column (0.2 g in pipet) and then purified on HPLC (5-μm silica column, 250 × 10 mm, 50% EtOAc–hexane, flow rate = 3.5 mL/min) to give 21 mg of 7′,8′,9′,10′-tetrahydrororidin E (**4a**) (41%), amorphous solid; IR ($CHCl_3$) 3550, 1717, 1650 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.78 (3 H, s, H-14), 1.11 (3 H, d, J = 6.2 Hz, H-14′), 1.40–1.64 (10 H, m, H-7, H-8, H-7′, H-8′, H-9′), 1.68 (3 H, s, H-16), 1.90 (1 H, m, H-3β), 2.20 (3 H, s, H-12′), 2.30–2.46 (4 H, m, H-4′, H-10′), 2.51 (1 H, dd, J = 8.0, 15.5 Hz, H-3α), 2.79 (1 H, d, J = 4.0 Hz, H-13B), 3.00 (1 H, dd, J = 3.0, 7.5 Hz, H-6′), 3.11 (1 H, d, J = 4.0 Hz, H-13A), 3.49 (1 H, ddd, J = 5.2, 8.0, 8.0 Hz, H-5′B), 3.68 (1 H, dq, J = 6.0 Hz, H-13′), 3.77 (1 H, m, H-5′A), 3.79 (1 H, d, J = 5.0 Hz, H-2), 3.90 (1 H, d, J = 5.5 Hz, H-11), 3.94 (1 H, d, J = 12.5 Hz, H-15B), 4.32 (1 H, d, J = 12.5 Hz, H-15A), 5.44 (1 H, d, J = 5.5 Hz, H-10), 5.77 (1 H, s, H-2′), 6.04 (1 H, dd, J = 3.8, 8.0 Hz, H-4); HREIMS m/z 518.2883 (calcd for $C_{29}H_{42}O_8$, 518.2880).

The same procedure with 52 mg (0.01 mmol) of isororidin E gave 31 mg of 7′,8′,9′,10-tetrahydroisororidin E (**4b**) (60%), mp 178–180 °C (CH_2Cl_2–hexane); IR ($CHCl_3$) 3550, 1718, 1653 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.79 (3 H, s, H-14), 1.09 (3 H, d, J = 6.3 Hz, H-14′), 1.13–1.59 (10 H, H-7, H-8, H-7′, H-8′, H-9′), 1.69 (3 H, s, H-16), 1.89 (1 H, ddd, J = 3.9, 5.0, 15.3 Hz, H-3β), 2.19 (3 H, d, J = 1.2 Hz, H-12′), 2.31–2.41 (4 H, m, H-4′, H-10′), 2.52 (1 H, dd, J = 8.0, 15.3 Hz, H-3α), 2.81 (1 H. d, J = 4.0 Hz, H-13B), 3.08 (1 H, m, H-6′), 3.12 (1 H, d, J = 4.0 Hz, H-13A), 3.60–3.66 (2 H, m, H-5′), 3.80 (1 H, m, H-13′), 3.80 (1 H, d, J = 5.0 Hz, H-2), 3.90 (1 H, d, J = 13.0 Hz, H-15B), 3.92 (1 H, d, J = 5.5 Hz, H-11), 4.34 (1 H, d, J = 13.0 Hz, H-15A), 5.45 (1 H, d, J = 5.5 Hz, H-10), 5.80 (1 H, d, J = 1.2 Hz, H-2′), 6.08 (1 H, dd, J = 3.8, 8.0 Hz, H-4); HREIMS m/z 518.2880 (calcd for $C_{29}H_{42}O_8$, 518.2880).

A similar procedure was carried with epiroridin E (**3c**, 25 mg, 0.05 mmol), except that the reaction temperature was kept at 0 °C and the reaction time was 60 min, to give 12 mg of 7′,8′,9′,10′-tetrahydroepiroridin E (**4c**) (48%), amorphous solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.80 (3 H, s, H-14),1.10 (2 H, d, J = 6.5 Hz, H-14′),1.47–1.62 (10 H, m, H-7, H-8, H-7′, H-8′, H-9′), 1.69 (3 H, s, H-16), 2.19 (3 H, d, J = 1.0 Hz, H-12′), 2.30–2.47 (4 H, m, H-4′, H-10′), 2.53 (1 H, dd, J = 7.8, 15.5 Hz, H-3α), 2.80 (1 H, d, J = 4.0 Hz, H-13B), 3.03 (1 H, dt, J = 3.2, 10.4 Hz, H-6′), 3.12 (1 H, d, J = 4.0 Hz, H-13A), 3.41 (1 H, dt, J = 3.4, 9.5 Hz, H-5′B), 3.80 (1 H, d, J = 5.0 Hz, H-2), 3.86 (1 H, dd, J = 4.7, 9.5 Hz, H-5′A), 3.90 (1 H, d, J = 5.4 Hz, H-11), 3.96 (1 H, dq, J = 3.0, 6.5 Hz, H-13′), 4.08 (1 H, d, J = 12.4 Hz, H-15B), 4.17 (1 H, d, J = 12.4 Hz, H-15A), 5.46 (1 H, d, J = 5.4 Hz, H-10), 5.77 (1 H, d, J = 1.0 Hz, H-2′), 5.97 (1 H, dd, J = 3.8, 7.8 Hz, H-4); HREIMS m/z 518.2893 (calcd for $C_{29}H_{42}O_8$, 518.2880).

Preparation of 7′,8′,9′,10′-Tetrahydro-13′-oxororidin Es. To a solution of **4a** (20 mg, 0.04 mmol) in CH_2Cl_2 (2 mL) were added anhydrous sodium acetate (8 mg) and PCC (12 mg), and the mixture was stirred at room temperature for 90 min. The reaction mixture was filtered through a Celite pad followed by solvent removal in vacuo to give 15 mg of residue. This residue was cleaned on a small silica column (5% MeOH–CH_2Cl_2), and subjected to HPLC (silica column, 5 μm, 10 × 250 mm, 40% EtOAc in hexane) to give 7 mg of 7′,8′,9′,10′-tetrahydro-13′-oxororidin E (**5a**) (34%), amorphous solid: IR

(CCl_4) 3505, 1729, 1718, 1653 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.80 (3 H, s, H-14),1.52−1.63 (10 H, m, H-7, H8, H-7′, H-8′, H-9′), 1.69 (3 H, s, H-16), 2.13 (3 H, s, H-14′), 2.18 (3 H, d, J = 1.0 Hz, H-12′), 2.34 (3 H, m, H-4′, H-10′B), 2.49 (1 H, m, H-10′A), 2.53 (1 H, dd, J = 8.0, 15.3 Hz, H-3α), 2.80 (1 H, d, J = 4.0 Hz, H-13B), 3.12 (1 H, d, J = 4.0 Hz, H-13A), 3.36 (1 H, dt, J = 3.0, 9.0 Hz, H-5′B), 3.52 (1 H, m, H-6′), 3.66 (1 H, m, H-5′A), 3.79 (1 H, d, J = 5.0 Hz, H-2), 3.87 (1 H, d, J = 5.0 Hz, H-11), 4.12 (2 H, s, H-15), 5.45 (1 H, d, J = 5.0 Hz, H-10), 5.77 (1 H, J = 1.0 Hz, H-2′), 5.93 (1 H, dd, J = 3.9, 8.0 Hz, H-4); ^{13}C NMR (50 MHz, $CDCl_3$) δ 7.1 (C-14),19.3 (C-12′), 21.6 (C-7), 23.2 (C-16), 24.7 (C-9′), 24.7 (C-8′), 25.2 (C-14′), 27.8 (C-8), 32.1 (C-7′), 33.6 (C-10′), 36.5 (C-3), 41.0 (C-4′), 42.9 (C-6), 48.1 (C-13), 48.3 (C-5), 63.6 (C-15), 65.5 (C-12), 67.0 (C-5′), 67.0 (C-11), 75.0 (C-4), 79.2 (C-2), 86.2 (C-6,), 117.0 (C-10), 118.8 (C-2′), 140.2 (C-9), 157.5 (C-3′), 166.2 (C−l′), 172.8 (C-11′), 211.4 (C-13′); HREIMS m/z 516.2732 (calcd for $C_{29}H_{40}O_8$, 516.2723).

A similar procedure carried out with **4b** (20 mg, 0.04 mmol) gave 6 mg of ketone **5b** (30%), amorphous solid: IR (CCl_4) 3510, 1726, 1719, 1654 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.79 (3 H, s, H-14), 1.49−1.61 (10 H, H-7, H-8, H-7′, H-8′, H-9′),1.69 (3 H, s, H-16), 2.10 (3 H, s, H-14′), 2.20 (3 H, d, J = 1.0 Hz, H-12′), 2.33 (2 H, m, H-10′), 2.38 (2 H, t, J = 4.5 Hz, H-4′), 2.53 (1 H, dd, J = 8.0, 15.4 Hz, H-3α), 2.80 (1 H, d, J = 4.0 Hz, H-13B), 3.12 (1 H, d, J = 4.0 Hz, H-13A), 3.54 (1 H, m, H-5′B), 3.62−3.69 (2 H, m, H-6′, H-5′A), 3.80 (1 H, d, J = 5.0 Hz, H-2), 3.90 (1 H, br d, J = 5.4 Hz, H-11), 3.92 (1 H, d, J = 12.4 Hz, H-15B), 4.29 (1 H, d, J = 12.4 Hz, H-15A), 5.45 (1 H, d, J = 5.4, H-10), 5.82 (1 H, d, J = 1.0 Hz, H-2′), 6.05 (1 H, dd, J = 3.8, 8.0 Hz, H-4); ^{13}C NMR (50 MHz, $CDCl_3$) δ 6.7 (C-14), 18.7 (C-12′), 21.9 (C-7), 23.2 (C-16), 23.8 (C-9′), 24.4 (C-8′), 25.7 (C-14′), 27.7 (C-8), 31.7 (C-7′), 33.7 (C-10′), 36.7 (C-3), 41.0 (C-4′), 42.7 (C-6), 47.9 (C-5), 48.2 (C-13), 63.7 (C-15), 65.5 (C-12), 66.7 (C-5′), 67.0 (C-11), 75.0 (C-4), 79.1 (C-2), 85.3 (C-6′), 116.8 (C-10), 118.7 (C-2′), 140.3 (C-9), 157.5 (C-3′), 166,1 (C−l′), 173.0 (C-11′), 211.4 (C-13′); HREIMS m/z 516.2713 (calcd for $C_{29}H_{40}O_8$, 516.2723).

In a like manner, **4c** (15 mg, 0.03 mmol) gave 6 mg of **5b** (40%).

Oxidation of Miotoxin A (6) with DDQ. To a solution of miotoxin A[14] (**6**, 20 mg, 0.04 mmol) in 3 mL of dioxane was added 11 mg of DDQ (0.05 mmol). The mixture was refluxed for 16 h. The reaction mixture was concentrated in vacuo, and the residue was taken up with 10 mL of CH_2Cl_2. The CH_2Cl_2 mixture was filtered through Celite pad, and the Celite was washed with 2 mL of CH_2Cl_2. The filtrate was concentrated in vacuo to give a residue (25 mg). The residue was subjected to MPLC (5 g Si gel, 1% MeOH−CH_2Cl_2) to give 3 mg (15%) of ketal **7** and 12 mg of starting material. For **7**, an oil: ^1H NMR (500 MHz, $CDCl_3$), δ 0.80 (3 H, s, H-14), 1.21 (3 H, d, J = 6.5 Hz, H-14′), 1.43−1.57 (3 H, m, H-7B, H-8), 1.70 (3 H, s, H-16), 1.97 (1 H, ddd, J = 4.3, 4.5, 15.2 Hz, H-3β), 2.04 (3 H, s, H-12′), 2.18 (1 H, m, H-7A), 2.69 (1 H, dd, J = 7.9, 15.2 Hz, H-3α), 2.83 (1 H, d, J = 4.0 Hz, H-13B), 3.15 (1 H, d, J = 4.0 Hz, H-13A), 3.42 (1 H, dd, J = 8.1, 8.9 Hz, H-5′B), 3.82 (1 H, q, J = 6.5 Hz, H-13′), 3.83 (1 H, d, J = 4.5 Hz, H-2), 4.04 (1 H, d, J = 12.8 Hz, H-15B), 4.13 (1 H, d, J = 4.5 Hz, H-11), 4.15 (1 H, d, J = 12.8 Hz, H-15A), 4.35 (1 H, dd, J = 6.0, 8.1 Hz, H-5′A), 4.60 (1 H, dd, J = 6.0, 8.9 Hz, H-4′), 5.50 (1 H, d, J = 4.5 Hz, H-10), 5.95 (1 H, d, J = 15.4 Hz, H-7′), 5.99 (1 H, d, J = 11.0 Hz, H-10′), 6.15 (1 H, dd, J = 4.3, 7.9 Hz, H-4), 6.17 (1 H, s, H-2′), 6.52 (1 H, dd, J = 11.0, 11.6 Hz, H-9′), 7.58 (1 H, dd, J = 11.6, 15.4 Hz, H-8′); HRCIMS m/z 529.2428 (calcd for $C_{29}H_{36}O_9$ + H, 529.2438).

Oxidation of Miotoxin A (6) with PCC. To a solution of miotoxin A (**6**, 34 mg, 0.06 mmol) in 3 mL of CH_2Cl_2 was added 2 mg of anhydrous NaOAc and 22 mg (0.1 mmol) of PCC. The mixture was stirred at room temperature for 3 h. The reaction mixture was passed through a short silica column (packed in pipet, ca. 0.3 g of silica) and eluted with 5 mL of 5% MeOH−CH_2Cl_2. The eluate was concentrated in vacuo to give 32 mg of residue. The residue was subjected to HPLC (semipreparative C_{18}, 60% MeOH−H_2O, 4 mL/min) to give two portions: miotoxin A (**6**, 8 mg) and a crude mixture of **8** and **9** (9 mg). This material was further purified on HPLC (semipreparative phenyl, 50% CH_3CN−H_2O, 4 mL/min) to give 5 mg of the 4′-oxomiotoxin **8** and hemiketal **9** as an oily mixture: ^1H NMR (500 MHz, $CDCl_3$), for H-1 through H-15 of **8** and **9**, δ 0.79 and 0.86 (3 H each, s, H-14), 1.70 and 1.72 (3 H each, s, H-16), 1.88−2.19 (H-3β, H-7, H-8), 2.46 and 2.49 (1 H each, dd, J = 8.3, 15.3 Hz, H-3α), 2.80 and 2.82 (1 H each, d, J = 4.0 Hz, H-13B), 3.10 and 3.14 (1 H each, d, J = 4.0 Hz, H-13A), 3.62 and 3.98 (1 H each, d, J = 5.0 Hz, H-11), 3.82 and 3.86 (1 H each, d, J = 5.0 Hz, H-2), 3.95 and 4.02 (1 H each, d, J = 12.5 Hz, H-15B), 4.41 and 4.46 (1 H each, d, J = 12.5 Hz, H-15A), 5.42 and 4.49 (1 H each, br d, J = 5.0 Hz, H-10), 5.89 and 6.38 (1 H each, dd, J = 3.7, 8.1 Hz, H-4); for **8**: d 1.17 (3 H, d, J = 6.3 Hz, H-14′), 2.26 (1 H, d, J = 1.0 Hz, H-12′), 3.65 (1 H, dd, J = 5.7, 6.3 Hz, H-6′), 3.78 (1 H, dq, J = 6.3, 6.3, H-13′), 4.50 (1 H, d, J = 16.5 Hz, H-5′B), 4.60 (1 H, d, J = 16.5 Hz, H-5′A), 5.78 (1 H, dd, J = 5.7, 16.0 Hz, H-7′), 5.90 (1 H, d, J = 11.0 Hz, H-10′), 6.67 (1 H, dd, J = 10.0, 11.0 Hz, H-9′), 6.77 (1 H, d, J = 1.0 Hz, H-2′), 7.41 (1 H, dd, J = 10.0, 16.0 Hz, H-8′); for **9**: δ 1.10 (3 H, d, J = 6.2, H-14′), 2.23 (1 H, s, H-12′), 3.43 (1 H, dq, J = 6.2, 8.8, H-13′), 3.43 (1 H, d, J = 12.1 Hz, H-5′B), 3.74 (1 H, dd, J = 5.7, 8.8, H-6′), 4.18 (1 H, d, J = 12.1 Hz, H-5′A), 5.83 (1 H, d, J = 11.0 Hz, H-10′), 6.11 (1 H, dd, J = 5.7, 15.8 Hz, H-7′), 6.19 (1 H, s, H-2′), 6.49 (1 H, dd, J = 11.0, 11.0 Hz, H-9′), 7.16 (1 H, dd, J = 11.0, 15.8 Hz, H-8′); partial ^{13}NMR (125 MHz, $CDCl_3$) macrolide sp^2 carbons: δ 165.7, 166.0, 166.9, 167.2 (C-1′ and C-11′ of **8** and **9**); for **8**: δ 120.9 (C-10′), 125.5 (C-2′), 129.9 (C-8′), 133.9 (C-7′), 143.4 (C-9′), 151.1 (3′), 199.7 (C-4′); for **9**: δ 94.2 (C-4′), 119.3 (C-10′), 120.2 (C-2′), 131.5 (C-8′), 134.6 (C-7′), 140.3 (C-3′), 140.6 (C-9′); HREIMS m/z 528.2344 (calcd for $C_{29}H_{36}O_9$, 528.2360).

Acknowledgment. This work was supported by NIH grant R01-GM-43724.

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NP990272J