A New Macrocyclic Trichothecene, 12,13-Deoxyroridin E, Produced by the Marine-Derived Fungus *Myrothecium roridum* Collected in Palau

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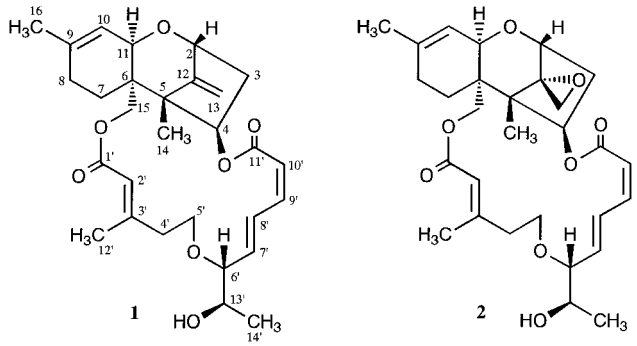
A new macrocyclic trichothecene, 12,13-deoxyroridin E (**1**), and three known compounds, roridin E (**2**), verrucarin A (**3**), and verrucarin J (**4**), were obtained as cytotoxic components from the marine-derived fungus *Myrothecium roridum*, isolated in Palau. 12,13-Deoxyroridin E is the second example of a macrocyclic trichothecene possessing a double bond at C-12−C-13 and was about 80-fold less cytotoxic than roridin E, the epoxide variant.

Filamentous fungi isolated from marine environments are a prolific source of biologically active natural products.[1] During our systematic study on bioactive metabolites from marine-derived fungi,[2] we found that a culture broth of *Myrothecium roridum*, isolated in Palau, showed strong cytotoxicity to human (HL-60) and murine (L1210) leukemia cell lines. Bioassay-guided separation of the culture broth yielded a new macrocyclic trichothecene, 12,13-deoxyroridin E (**1**), together with three known compounds, roridin E (**2**), verrucarin A (**3**), and verrucarin J (**4**). We report here the isolation and structure assignment of cytotoxic macrocyclic trichothecenes.



The filamentous fungus was isolated from a small woody material collected on the bottom (−5 m) of a coral reef during our Palau expedition using the training vessel Umitaka-maru in 1998. Identification of the fungus was done in the usual manner, and the strain was identified as *M. roridum*. The fungus was cultured for bioassays with a half nutrient potato dextrose medium (1/2 PD) on a plate for 3 weeks at 20 °C, and the broth showed strong cytotoxicity to HL-60 and L1210.

For the separation of bioactive components, *M. roridum* was cultured in six 500 mL Erlenmeyer flasks for 3 weeks at 20 °C. Acetone was added to the cultured broth, and the mixture was filtered. The filtrate was extracted with EtOAc, and bioactive components were separated by silica gel column chromatography and then by HPLC to afford 12,13-deoxyroridin E (**1**, 0.8 mg), roridin E[3,4] (**2**, 0.3 mg), verrucarin A[3,4] (**3**, 0.1 mg), and verrucarin J[3,4] (**4**, 0.2 mg).

Structures of three known compounds **2**−**4** were assigned based on their spectral data and confirmed by comparison of their NMR data with the reported values.[4]

The ^{1}H NMR spectrum (Table 1) of **1** was similar to that of roridin E (**2**). Differences, however, were observed in the chemical shifts of two proton signals ascribable to the C-13 position. Roridin E (**2**) showed two one-proton doublets at δ 2.83 and 3.15 (J = 4.0 Hz) due to an epoxy methylene, while **1** gave two one-proton singlets at δ 4.70 and 5.14 in their ^{1}H NMR spectra. ^{13}C NMR data for **1** (Table 1) was also similar to those for **2** (Experimental Section), except for the signals due to the C-12 and C-13 positions. The ^{1}H and ^{13}C signals ascribable to C-12 and C-13 revealed that **1** has an exo double bond instead of the epoxide present in roridin E (**2**). The structure of **1** was, therefore, presumed to be a deoxy derivative of roridin E. The molecular formula and weight ($C_{29}H_{38}O_7$, 498) of **1**, deduced from its ^{1}H and ^{13}C NMR and HRFABMS data, agreed with the above presumption.

The ^{1}H and ^{13}C NMR signals of **1** were assigned by 2D NMR experiments (HMQC, HMBC, and ^{1}H−^{1}H COSY spectra). The ^{1}H−^{1}H COSY spectrum (Table 1) of **1** revealed partial structures of 2-3-4, 10-11, 4′-5′, and 14′-13′-6′-7′-8′-9′-10′, and an HMBC experiment confirmed the total connectivity of carbons (Table 1).

The stereochemistry of **1** was assigned by comparison of ^{13}C NMR data for **1** with those for roridin E (**2**), isororidin E[4,5] [(6′*S*,13′*S*)-isomer of roridin E], and verrucarin K[4,6] (12,13-deoxy derivative of verrucarin A) and confirmed by the NOESY spectrum of **1**. ^{13}C NMR data for **1** at C-1′ to

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Table 1. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Data ($CDCl_3$) for 12,13-Deoxyroridin E (**1**)

C#	^{13}C signal	^{1}H signal δ, mult. (J in Hz)	$^{1}H-^{1}H$ COSY	HMBC	NOESY
2	79.0	4.45, d (5.1)	3a	4, 5, 11, 13	3a, 13b
3	37.2	(a) 1.75, m	2, 3b, 4	2, 4	2, 3b
		(b) 2.56, m	3a, 4	2, 5, 12	3a, 4, 11
4	74.4	6.26, dd (7.8, 3.7)	3a, 3b	2, 5, 6, 12, 11′	3b, 11, 15a, 15b
5	51.0				
6	42.5				
7	20.9	(a) 1.50, m	7b	6, 8, 9, 11	7b, 8, 14
		(b) 1.97, m	7a	6, 8, 9, 15	7a
8	27.7	2.00, 2H, m		6, 7, 9, 10	7a, 15b, 16
9	139.8				
10	119.1	5.44, br d (5.6)	11, 16	6, 8, 11, 16	11, 16
11	67.0	3.98, d (5.6)	10	5, 7, 9, 10	3b, 4, 10
12	152.7				
13	105.3	(a) 4.70, s	13b	2, 5, 12	13b, 14
		(b) 5.14, s	13a	2, 5, 12	2, 13a
14	11.3	1.02, 3H, s		4, 5, 6, 12	7a, 13a, 15a, 2′
15	64.2	(a) 3.96, d (12.5)	15b	5, 6, 7, 11, 1′	4, 7a, 14, 15b
		(b) 4.28, d (12.5)	15a	5, 6, 7, 11, 1′	4, 8, 15a
16	23.2	1.68, 3H, s	10	8, 9, 10	8, 10
1′	166.5				
2′	117.5	6.02, br s	12′	1′, 4′, 12′	13, 4′, 5′a
3′	158.7				
4′	41.3	2.53, 2H, m	5′a, 5′b	2′, 3′, 5′, 12′	2′, 5′a, 5′b, 12′
5′	69.8	(a) 3.55, m	4′, 5′b	3′, 4′, 6′	4′, 5′b, 8′
		(b) 3.65, m	4′, 5′a	3′, 4′, 6′	4′, 5′a
6′	83.9	3.70,m	7′, 13′	7′, 13′	7′
7′	137.8	5.90, dd (15.7, 3.3)	6′, 8′	6′, 8′, 9′	6′, 8′, 9′, 14′
8′	126.6	7.56, dd (15.7, 11.3)	7′, 9′	6′	5′a, 7′, 9′
9′	143.5	6.57, dd (11.3, 11.2)	8′, 10′	7′, 8′, 11′	7′, 8′, 10′
10′	117.7	5.66, d (11.2)	9′	8′, 11′	9′
11′	165.6				
12′	20.0	2.25, 3H, d (1.2)	2′	1′, 2′, 3′, 4′	4′
13′	70.6	3.64, m	6′, 14′	6′, 7′	14′
14′	18.3	1.20, 3H, d (6.1)	13′	6′, 13′	7′, 13′

C-14′ (Table 1) were very similar to those for **2** (Experimental Section). On the other hand, the ^{13}C NMR signals of isororidin E due to C-6′ to C-10′, C-13′, and C-14′ were observed at δ 83.2, 135.3, 131.0, 142.0, 117.1, 67.6, and 18.5, respectively.[4,5] Therefore, the stereochemistry of the macrocyclic ring in **1** was identical to that in roridin E (**2**). Verrucarin K is the 12,13-deoxy derivative of verrucarin A (**3**) and the only example thus far of a macrocyclic trichothecene possessing an exo double bond at C-12.[6] ^{13}C NMR data for verrucarin K at C-2 to C-16 [δ 78.5 (C-2), 35.9 (C-3), 75.5 (C-4), 52.0 (C-5), 44.2 (C-6), 18.7 (C-7), 27.4 (C-8), 140.5 (C-9), 118.2 (C-10), 66.5 (C-11), 151.6 (C-12), 106.3 (C-13), 12.1 (C-14), 63.5 (C-15), and 23.2 (C-16)][4,6] were similar to those for **1** (Table 1). These data suggested that the stereochemistry of the trichothecene ring in **1** and in verrucarin K are identical. NOESY data for **1** (Table 1) confirmed the above assignment.

Thus, compound **1** was assigned the structure as shown above and named 12,13-deoxyroridin E. 12,13-Deoxyroridin E (**1**) is the second example of a macrocyclic trichothecene possessing a double bond at C-12−C-13.[6]

The IC_{50} values of 12,13-deoxyroridin E (**1**), roridin E (**2**), verrucarin A (**3**), and verrucarin J (**4**) to HL-60 were 25, 0.3, 0.2, and 2.5 ng/mL, respectively, and to L1210 were 15, 0.2, 0.35, and 2.5 ng/mL, respectively. The new compound (**1**) showed reduced cytotoxicity about 80-fold less than that of roridin E (**2**). The epoxide at C-12−C-13 will, therefore, affect the cytotoxicity of macrocyclic trichothecenes.

Experimental Section

General Experimental Procedures. NMR spectra were measured on a Varian Unity Inova-500 spectrometer using a Nano NMR probe. Mass spectra were obtained by a JEOL HX-110 mass spectrometer (FAB mode). UV spectra were recorded with a Shimadzu UV-300. Optical rotation was recorded with a JASCO DIP-370 digital polarimeter. HPLC was performed using a Shimadzu LC-VP HPLC system with a PEGASIL ODS column (10 × 250 mm, particle size 8 μm, pore size 120 Å) or a Mightysil RP-18 GP (10 × 250 mm, particle size 5 μm, pore size 120 Å).

Isolation of Fungus. A submerged woody material was collected by scuba diving and sealed in a sterile plastic bag in the water. The substrate was cut into small pieces and washed with sterile seawater, and three pieces were placed on an agar plate (0.02% yeast extract, 0.1% soluble starch, 1.5% agar, and 200 ppm chloramphenicol in 90% natural seawater). Treatment of the substrate was done in a laboratory of the training vessel. The plate was placed in the laboratory (25 °C) of the ship and then incubated at 20 °C in an incubator after returning to the university. The mycelia grown on an agar plate from the substrate were inoculated on a slant in a culture tube (1/2 PDA: hot water (500 mL) extract of potato (100 g), glucose (10 g), agar (15 g), and 500 mL of natural seawater).

The fungus was grown on an agar plate (0.1% glycerol, 0.05% $NaNO_3$, 0.02% KH_2PO_4, 0.01% yeast extract, and 1.5% agar) and identified as *Myrothecium roridum* from the shape of hyphae, conidiophores, and conidia. The culture is maintained as TUF strain number 98F42.

Bioassay. *M. roridum* was cultured on a plastic plate for screening bioassay with 15 mL of 1/2 PD (50% natural seawater) for 3 weeks at 20 °C. MeOH (8 mL) was added to the broth, and the mixture was filtered. One milliliter of the filtrate was evaporated to dryness, dissolved in EtOH, and filtered. The EtOH extract was evaporated and dissolved again with EtOH to the required concentration for bioassay.

The human (HL-60) and murine (L1210) leukemia cell lines were incubated in RPMI 1640 and minimum essential medium, respectively, using 24-well assay plates. The above EtOH solution (10 μL) of each concentration was poured in a well and the solvent evaporated in a clean bench. The

suspension (1 mL, 1×10^4 cells/mL) of L1210 or HL-60 was added to each well and incubated at 37 °C for 72 h in a CO_2 incubator. The shape of the cells was observed after 24, 48, and 72 h under an inverted microscope. The number of vital cells in the sample wells after 72 h was compared with those in the control wells using WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt][7] (Cell Counting Kit-8).

Separation of Metabolites. Acetone (450 mL) was added to the cultured broth of *M. roridum* (900 mL), and the mixture was filtered and extracted with EtOAc. The extract was chromatographed on a Si gel column (benzene−EtOAc (gradient), benzene−acetone (gradient), and then MeOH), and the 40% EtOAc−benzene fraction was further separated by HPLC (ODS, 99% MeOH) to give 0.8 mg of 12,13-deoxyroridin E (**1**) and a mixture of three components (1.22 mg). 12,13-Deoxyroridin E was further purified by HPLC (ODS, 90% MeOH). The mixture was separated by HPLC (ODS, 70% MeOH) to afford 0.3 mg of roridin E (**2**), 0.1 mg of verrucarin A (**3**), and 0.2 mg of verrucarin J (**4**).

12,13-Deoxyroridin E (1): $[\alpha]_D$ −204° (*c* 0.025, $CHCl_3$); UV (MeOH) λ_{max} 263 nm (ϵ 15 700); ^{1}H (500 MHz, $CDCl_3$) and ^{13}C NMR data (125 MHz, $CDCl_3$), Table 1; HRFABMS *m*/*z* 499.2696 (calcd for $C_{29}H_{39}O_7$, 499.2698).

Roridin E (2): ^{13}C NMR (125 MHz, $CDCl_3$) δ 79.2 (C-2), 35.8 (C-3), 74.2 (C-4), 48.4 (C-5), 42.7 (C-6), 21.6 (C-7), 27.7 (C-8), 140.2 (C-9), 118.9 (C-10), 67.2 (C-11), 65.6 (C-12), 48.1 (C-13), 6.7 (C-14), 63.7 (C-15), 23.2 (C-16), 166.4 (C-1′), 117.2 (C-2′), 159.2 (C-3′), 41.2 (C-4′), 69.6 (C-5′), 83.9 (C-6′), 138.0 (C-7′), 126.6 (C-8′), 143.6 (C-9′), 117.8 (C-10′), 165.8 (C-11′), 20.3 (C-12′), 70.7 (C-13′), 18.3 (C-14′).

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Supporting Information Available: Spectral data for known compounds Roridin E (**2**), Verrucarin A (**3**), and Verrucarin J (**4**). This material is available free of charge via the Internet at http://pubs.acs.org.

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