Two New Roridins Isolated from *Myrothecium* sp.

MELISSA M. WAGENAAR and JON CLARDY*

Department of Chemistry and Chemical Biology,
Cornell University,
Ithaca, New York 14853-1301, USA

In the course of screening for potential anticancer compounds, the extract F210653 from a species of *Myrothecium* exhibited potent activity (mean $GI_{50} < 0.01$ μg/ml) in the NCI 60-cell line cytotoxicity screen. Species of *Myrothecium* are prolific producers of bioactive secondary metabolites with over 20 compounds reported from *Myrothecium roridum* and more than 30 described compounds produced by *Myrothecium verrucaria*.[1] In addition to the anticancer activity, the crude extract F210653 possessed activity against *Saccharomyces cerevisiae*, *Candida albicans*, and *Geotrichum candidum*. However, the crude extract did not inhibit the growth of either *Staphylococcus aureus* or *Bacillus subtilis*.

Bioassay-guided fractionation of the extract F210653 resulted in the isolation of five known compounds identified as verrucarin J,[2] 8-acetylverrucarin L,[3] roridin J,[4] roridin E,[5] isororidin E,[6] and two previously undescribed macrocyclic trichothecenes, 8-acetoxyroridin H (**1**) and 8-acetoxyroridin E (**2**). In this paper, the production, isolation, biological activity, and structural characterization of these compounds are reported.

The fungus *Myrothecium* sp. (Phylum, Ascomycotina; Class, Deuteromycetes) was isolated from a soil sample by the Michigan Department of Health (sample number MDH-37514). It was cultured at the National Cancer Institute using SGSM (Starch (10 g/liter), glucose (40 g/liter), soy peptone (10 g/liter)) at 25°C and 250 rpm. The broth was extracted with ethyl acetate. NCI reference number for the extract is F210653.

The crude extract (340 mg) was partitioned using a modified KUPCHAN[7] scheme as follows: the crude extract was resuspended at 5 mg/ml in 90 : 10 MeOH : H_2O and extracted 3× with hexanes, then diluted to 80 : 20 MeOH : H_2O and extracted 3× with CCl_4, and finally diluted to 60 : 40 MeOH : H_2O and extracted 3× with CH_2Cl_2. The active fractions (CCl_4 and CH_2Cl_2, 300 mg) were pooled and subjected to a flash silica column ($CHCl_3$: MeOH step gradient). The active fraction (52 mg) eluted with 95 : 5 $CHCl_3$: MeOH and was subjected to a second flash silica column (hexanes : EtOAc step gradient). Final purification was obtained by normal phase HPLC (Sigma-Aldrich Nucleosil Silica 5 μm, 25.0 cm×4.6 mm) with a solvent system of 3 : 2 hexanes : EtOAc (flow rate = 1.0 ml/minute) yielding 2.85 mg **1** (Rt 8.8 minutes) and 1.93 mg **2** (Rt 20.7 minutes).

HRMS-CI indicated $C_{31}H_{38}O_{10}$ (HRMS-CI (*m/z*): $[M+H]^+$ calcd for $C_{31}H_{39}O_{10}$, 571.2543; found, 571.2525) to be the molecular formula for **1** (white crystal; IR ($CHCl_3$) v_{max} 3514 (br), 1720, 1650, 1602, 1368, 1172, 1076, 1000, 968 cm^{-1}). The presence of 5 methyls, 5 methylenes, 13 methines, 5 quaternary carbons, and 3 ester carbonyls can be deduced from a combination of ^1H, ^{13}C, and ^1H-^{13}C DEPT NMR experiments. Based on the molecular formula of $C_{31}H_{38}O_{10}$, compound **1** must contain 13 degrees of unsaturation. Six spin systems were identified from a ^1H-^1H RelayH experiment as shown in Figure 2. Long-range HMBC correlations were used to establish the connectivities of the various spin systems of compound **1** as described below.

Partial structure **a** connects to C-12 at C-2 by long-range HMBC correlations from H-2 and H-3 to C-12. The methyl group (C-14) shows correlations to C-4, C-5, C-6, and C-12. The five-membered ring is closed based on the HMBC correlations of H-2 to C-5 and H-4 to C-12. The C-7 methylene protons and the C-8 methine proton of partial structure **b** correlate to C-6. Partial structure **b** links to spin system **c** through C-9 based on the correlations of H-8 to C-9 and C-10, H-16 to C-9 and C-10, and H-11 to C-9. Partial structure **a** attaches to partial structure **c** through an ether linkage based on the correlation of H-2 to C-11 and the downfield chemical shift of both C-2 (δ_C 79.2) and C-11 (δ_C 67.4). The correlations of H-10 and H-15 to C-6 and H-11 to C-15 were used to establish the remainder of the tricyclic trichothecane skeleton. The 12,13-epoxide was suggested by the correlation of the C-13 methylene protons to C-12 and C-2 and the downfield chemical shifts of both C-12 and C-13 (δ_C 65.6 and 48.1, respectively). The downfield chemical shift of C-8 and the correlations of H-8 and the C-18 methyl group to C-17 indicate the presence of an acetoxy group at C-8. A macrocyclic lactone ring is

* Corresponding author: jcc12@cornell.edu

Fig. 1.   Structures of 8-acetoxyroridin H (**1**) and 8-acetoxyroridin E (**2**).

1   2
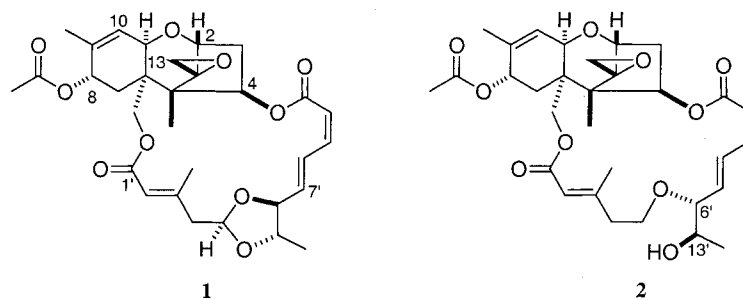
Fig. 2.   Spin systems **a~f** as determined by ¹H-¹H RelayH and HMQC experiments are shown with bolded bonds for compound **1**.

Arrows indicate HMBC correlations. Computer-generated perspective drawing of compound **1** (H-atoms have been omitted for clarity).
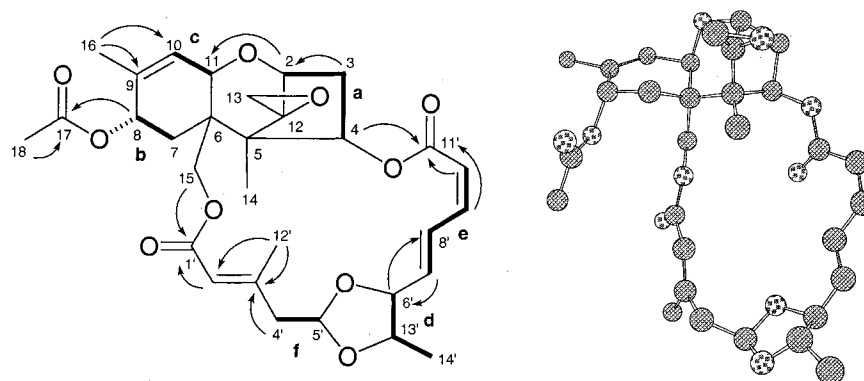
formed through ester linkages at C-4 and C-15 of the trichothecane ring system. Spin system **d** connects to the tricyclic system at C-4 based on the correlations of H-4, H-9′, and H-10′ to C-11′. Partial structure **e** attaches to partial structure **d** based on the correlation of H-7′ to C-6′. The ester linkage at C-15 is supported by the correlation of the C-15 methylene protons to C-1′ and the downfield chemical shift of C-15 (δ_C 64.7). The correlations of H-2′ to C-1′ and C-3′ and the correlations of the C-12′ methyl protons to both C-2′ and C-3′ along with the carbon chemical shifts of C-2′ and C-3′ (δ_C 118.9 and 155.4, respectively) indicate the presence of an α,β-unsaturated ester with a methyl group off the β-position. Spin system **f** connects to C-3′ by the correlation of the C-4′ methylene protons to C-3′. To accommodate the molecular formula and the degree of unsaturation, the macrocyclic ring must close through a ring containing two oxygens. The downfield chemical shifts

of C-6′ and C-13′ (δ_C 82.2, and 76.6) indicate each is oxygenated and the chemical shift of C-5′ (δ_C 100.9) suggests it has two oxygen substituents. Therefore, the macrocyclic ring is closed by a cyclic acetal system and the structure of compound **1** is as shown in Figure 1.

The stereochemistry at C-8 in compound **1** was assigned as α based on the following evidence. The methyl C-16 is shifted upfield by 2.2 ppm and C-15 is shifted downfield by 1.7 ppm in the ¹³C-NMR spectrum of **1** in comparison to the literature values for roridin H.[8,9] If the configuration at C-8 was β, the resonance for C-16 would be expected to shift upfield by 4~5 ppm and little to no effect would be observed for C-15.[10] This stereochemistry is the same as that observed in the previously described verrucarin L acetate which was also isolated from this extract.[3]

The structure of compound **1** including the stereochemistry was confirmed by single crystal X-ray

Table 1. ^{1}H and ^{13}C NMR data of 1 and 2 in CDCl$_3$ (δ in ppm).

position	**1** ^{13}C	**1** ^{1}H (500 MHz, mult, J in Hz)	**2** ^{13}C	**2** ^{1}H (500 MHz, mult, J in Hz)
2	79.2	3.84 (1H, d, 5.0)	79.3	3.84 (1H, d, 5.0)
3	34.9	2.19 (1H, m)	35.6	2.12 (1H, ddd, 4.0, 5.0, 15.5)
		2.48 (1H,dd, 8.5, 15.5)		2.50 (1H, m)
4	73.7	5.90 (1H, dd, 4.5, 8.5)	74.0	6.10 (1H, dd, 4.0, 7.5)
5	49.3		48.8^b	
6	42.4		42.3	
7	26.6	2.11 (1H, m)	27.6	2.06 (1H, m)
		2.24 (1H, m)		2.28 (1H, dd, 5.0, 15.0)
8	69.0	5.19 (1H, d, 4.5)	69.0	5.21 (1H, d, 5.0)
9	136.6		136.6	
10	124.4	5.70 (1H, dt, 0.5, 5.5)	124.4	5.72 (1H, d, 6.0)
11	67.4	3.76 (1H, d, 5.5)	67.2	3.93 (1H, d, 6.0)
12	65.6		65.6	
13	48.1	2.84 (1H, d, 4.0)	48.2^b	2.84 (1H, d, 4.0)
		3.11 (1H, d, 4.0)		3.12 (1H, d, 4.0)
14	7.5	0.84 (3H, s)	7.0	0.78 (3H, s)
15	64.7	4.36 (1H, d, 12.5)	64.9	4.11 (1H, d, 12.5)
		4.41 (1H, d, 12.5)		4.53 (1H, d, 12.5)
16	20.7	1.76 (3H, s)	20.6	1.76 (3H, s)
17	171.1		171.0^a	
18	21.1	1.93 (3H, s)	21.3	2.01 (3H, s)
1'	166.0		166.1	
2'	118.9	5.64 (1H, s)	116.8	5.86 (1H, s)
3'	155.4		160.1	
4'	47.9	2.24 (1H, m)	41.4	2.50 (2H, m)
		2.66 (1H, dd, 3.5, 12.0)		
5'	100.9	5.53 (1H, dd, 3.5, 8.5)	70.5	3.60 (1H, m)
6'	82.2	4.07(1H, dt, 2.5, 8.5)	84.3	3.71(1H, ddd, 1.5, 3.5, 7.0)
7'	135.1	5.96(1H, dd, 2.5, 15.5)	138.7	5.92(1H, dd, 3.5, 16.0)
8'	126.3	7.65(1H, ddt, 1.0, 11.5, 15.5)	126.6	7.46(1H, ddt, 1.5, 11.0, 16.0)
9'	143.3	6.57(1H, dd, 11.5)	144.2	6.58(1H, dd, 11.0)
10'	118.6	5.80(1H, dd, 1.0, 11.5)	117.8	5.74(1H, d, 11.0)
11'	166.4		166.1	
12'	18.4	2.28 (3H, d, 1.0)	21.0	2.31 (3H, d, 1.5)
13'	76.6	3.67 (1H, dq, 6.0, 8.5)	70.9	3.60 (1H, m)
14'	16.7	1.35 (3H, d, 6.0)	18.5	1.20 (3H, d, 6.0)

^{a}Chemical shift determined from HMBC experiment.
^{b}Signals may be interchanged.

Table 2.   Antimicrobial activity and cytotoxicity.

	zones of inhibition^a (mm)			IC$_{100}$ (ng/mL)	
	S. cerevisiae	C. albicans	G. candidum	HCT116	A2780S
verrucarin J	19	13	10	9.77	2.44
8-acetylverrucarin L	21	22	9	9.77	9.77
roridin J	19	10	no zone	2.44	2.44
roridin E	18	16	10	<0.153	0.610
isororidin E	18	18	11	2.44	2.44
8-acetoxyroridin H (1)	20	20	12	9.77	9.77
8-acetoxyroridin E (2)	14	15 hazy	no zone	2.44	2.44
nystatin^b	21	21	17		

^{a}Zones of inhibition resulting from 150 μg/disk.

^{b}Nystatin control disk contains 100 units (approximately 30 μg).

crystallography to be 8α-acetoxyroridin H. In the crystal structure (Figure 2), the macrocyclic ring is fully extended away from the tricyclic trichothecane skeleton and is almost planar with the epoxide pointing out of the plane.

Mass spectral data for compound **2** (HRMS-CI (m/z): [M+H]$^+$ calcd for $C_{31}H_{41}O_{10}$, 573.2670; found, 573.2696) indicated a molecular formula of $C_{31}H_{40}O_{10}$, a difference of two mass units between compound **1** and compound **2** (white solid; IR (CHCl$_3$) λ_{max} 3514 (br), 1728, 1650, 1602, 1374, 1174, 1078, 966 cm^{-1}). The ^{1}H NMR of compound **2** is similar to the ^{1}H NMR of 8α-acetoxyroridin H (**1**) except for the upfield chemical shift of H-5′ from δ_H 5.53 (1H) to 3.60 (2H) and H-6′ from δ_H 4.07 to 3.71. The ^{13}C NMR of compound **2** in comparison to compound **1** showed an upfield chemical shift of C-5′ from δ_C 100.9 to 70.5 suggesting the opening of the acetal ring. Other significant shifts in the ^{13}C NMR of compound **2** compared to **1** were observed for C-4′, C-6′, C-13′, and C-14′. In addition, both the ^{1}H and ^{13}C NMR spectra of **2** compared favorably to those of roridin E[5] with a few exceptions. The presence of an additional singlet (3H) at δ_H 2.01, the loss of the methylene protons of C-8, and the appearance of a deshielded proton at δ_H 5.21 were observed in the ^{1}H-NMR of **2** in comparison to the ^{1}H-NMR of roridin E. The ^{13}C NMR spectrum of compound **2** differed from that of roridin E by the substantial downfield chemical shift of C-8 to δ_C 69.0 and the presence of two additional signals at δ_C 171.0 and δ_C 21.3 indicative of an acetate. In addition, the chemical shifts of C-7, C-9, and C-10 were significantly affected suggesting oxidation at C-8. Therefore, compound **2** was proposed to be the 8-acetoxy derivative of roridin E.

The configuration at C-8 in **2** is proposed to be α based on similar arguments as used for **1**. The C-16 methyl resonance of **2** is shifted upfield by 2.2 ppm and the C-15 signal is shifted downfield by 1.2 ppm in the ^{13}C NMR spectrum of **2** in comparison to roridin E. The remaining stereochemistry is proposed to be identical to that previously established for roridin E.[5]

The isolated macrocyclic trichothecenes exhibited antifungal activity in agar diffusion assays against *Saccharomyces cerevisiae*, *Candida albicans*, and *Geotrichum candidum* (Table 2). Oxidation of roridin E to 8-acetoxyroridin E (**2**) results in a decrease in activity against each organism tested. Because roridin H was not isolated from the extract, no comparison of activity can be made between roridin H and 8-acetoxyroridin H (**1**). In cytotoxicity assays against HCT-116 human colon tumor

cell line, roridin E showed the greatest activity (IC$_{100}$<0.153 ng/ml) in comparison to 8-acetoxyroridin E (IC$_{100}$=2.44 ng/ml) and 8-acetoxyroridin H (IC$_{100}$=9.77 ng/ml).

References

1) BUCKINGHAM, J.: Dictionary of Natural Products; Chapman and Hall: NewYork, 1994
2) FETZ, E.; B. BOHNER & C. TAMM: Die konstitution von verrucarin J. Helv. Chim. Acta 48: 1669~1679, 1965
3) JARVIS, B. B.; J. O. MIDIWO, T. DESILVA & E. P. MAZZOLA: Verrucarin L, a new macrocyclic trichothecene. J. Antibiotics 34: 120~121, 1981
4) JARVIS, B. B.; G. P. STAHLY & G. PAVANASASIVAM: Structure of roridin J, a new macrocyclic trichothecene from *Myrothecium verrucaria*. J. Antibiotics 33: 256~258, 1980
5) TRAXLER, P.; W. ZURCHER & C. TAMM: Die struktur des antibioticums roridin E. Helv. Chim. Acta 53: 2071~2085, 1970
6) MATSUMOTO, M.; H. MINATO, K. TORI & M. UEYAMA: Structures of isororidin E, epoxyisororidin E, and epoxy- and diepoxyroridin H, new metabolites isolated from *Cylindrocarpon* species determined by carbon-13 and hydrogen-1 NMR spectroscopy. Tetrahedron Lett. 1977: 4093~4096
7) KUPCHAN, S. M.; R. W. BRITTON, M. F. ZIEGLER & C. W. SIGEL: Bruceantin, a new potent antileukemic simaroubolide from *Brucea antidysenterica*. J. Org. Chem. 38: 178~179, 1973
8) BREITENSTEIN, W. & C. TAMM: ^{13}C-NMR. —Spectroscopy of the trichothecane derivatives verrucarol, verrucarins A and B and roridins A, D and H. Helv. Chim. Acta 58: 1172~1180, 1975
9) TRAXLER, P. & C. TAMM: Die struktur des antibioticums roridin H. Helv. Chim. Acta 53: 1846~1869, 1970
10) JARVIS, B. B.; G. P. STAHLY, G. PAVANASASIVAM, J. O. MIDIWO, T. DESILVA, C. E. HOLMLUND, E. P. MAZZOLA & R. F. GEOGHEGAN, Jr.: Isolation and characterization of the trichoverroids and new roridins and verrucarins. J. Org. Chem. 47: 1117~1124, 1982