COMMUNICATIONS TO THE EDITOR

# Roridin L, M and Verrucarin M, New Macrocyclic Trichothecene Group Antitumor Antibiotics, from *Myrothecium verrucaria*

Sir:

In the course of our screening to find antitumor antibiotics from microorganisms, we have isolated novel macrocyclic trichothecene antibiotics[1], roridin L (**1**), M (**2**) and verrucarin M (**3**) from the culture broth of *Myrothecium verrucaria*. Here we report the fermentation, purification, structure elucidation and biological activities of **1**, **2** and **3**.

The producing strain was identified as *Myrothesium verrucaria* (Alb. & Schw.) Ditm.: Fr. based on the dark green fusiform conidia (5.5~8.0×2.5~3.0 $\mu$m) born on the sporodochia without setae[2]. It was isolated from soil sample collected at Takasaki-shi in Gunma prefecture. The fermentation was carried out in a 500-ml Erlenmeyer flask containing 100 ml medium with following composition: corn steep liquor 1.5%, soluble starch 2.0%, corn meal 0.1%, soybean meal 0.4%, glucose 0.25%, $(NH_4)_2SO_4$ 0.4%, $KH_2PO_4$ 0.03%, $CaCO_3$ 0.6% and agar 0.1%. The pH of the medium was adjusted to 6.7 before sterilization. The seed culture was inoculated to the flasks, and the fermentation was carried out at 27°C for 7 days on a rotary shaker.

**1**, **2** and **3** were isolated according to the scheme as shown in Fig. 2. In brief, the fermentation broth (10 liters) were centrifuged to give a mycelium cake and supernatants. A mycelium cake was extracted with acetone (3 liters) and the extract was filtered and concentrated *in vacuo* to yield an aqueous solution. An aqueous solution and supernatants were mixed and extracted twice with EtOAc. The organic layer was dehydrated over anhydrous $Na_2SO_4$ and then concentrated *in vacuo*. The residue was applied to a silica gel column (Silica gel 60, 5×50 cm) developed with $CHCl_3$-MeOH (80 : 1). The active fractions were further chromatographed on a HW40 column (2×50 cm) with $CHCl_3$-MeOH (1 : 1) and then purified by preparative HPLC (YMC ODS-AM323, 70% MeOH). The active fractions were pooled and concentrated *in vacuo* to afford **1** (5.6 mg), **2** (2.2 mg) and **3** (2.5 mg) as colorless powders.

The physico-chemical properties of **1**, **2** and **3** were summarized in Table 1. The molecular formula of **1** was determined to be $C_{29}H_{38}O_9$ by HRFAB-MS. The $^1H$ and $^{13}C$ NMR spectrum of **1** showed that **1** was closely similar to roridin E[3]. Analyses of $^1H$ and $^{13}C$ NMR including 2D NMR spectra (DQF COSY, C-H COSY and HMBC) revealed that the macrocyclic chain (C-1′~C-14′) in **1** was identical with roridin E including the geometries of C-2′ (*E*, C-12′ ($\delta_C$ 19.5)[3,4]), C-7′ (*E*, $J_{7',8'}$=15.5 Hz) and C-9′ (*Z*, $J_{9',10'}$=11.0 Hz). In while, the methylene singal at C-3 ($\delta_H$ 2.04, 2.53, $\delta_C$ 35.8) in roridin E was disappered and a methyne signal ($\delta_H$ 4.22, $\delta_C$ 78.2) coupled with H-2 ($\delta_H$ 3.77) and H-4 ($\delta_H$ 6.02) (DQF COSY) was appeared in **1**.

Table 1. Physico-chemical propeties of roridin L (**1**), roridin M (**2**) and verrucarin M (**3**).

| | 1 | 2 | 3 |
|---|---|---|---|
| Appearance | Colorless powder | Colorless powder | Colorless powder |
| mp(℃, dec) | 138-140 | 180-182 | 210-212 |
| Molecular formula | $C_{29}H_{38}O_9$ | $C_{29}H_{36}O_9$ | $C_{27}H_{30}O_9$ |
| HRFAB-MS Calcd: | 531.2594 | 529.2438 | 501.2125 |
| Found: | 531.2581 $(M+H)^+$ | 529.2433$(M+H)^+$ | 501.2108$(M+H)^+$ |
| UV $\lambda_{max}^{MeOH}$( $\varepsilon$ ) | 224(25,000) | 224(24,200) | 262(23,000) |
| | 263(19,500) | 263(19,100) | |
| IR $\nu$ (KBr)$cm^{-1}$ | 3450,1720,1650 | 3460,1715,1645 | 3460,1710,1650 |

Table 2. $^{13}C$ and $^{1}H$ NMR data of roridin L (**1**), M (**2**) and verrucarin M (**3**)[a].

| | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| | $^{13}C$ | $^{1}H$ | $^{13}C$ | $^{1}H$ | $^{13}C$ | $^{1}H$ |
| 2 | 79.1 | 3.77m | 77.3 | 3.66d4.9 | 79.1 | 3.66d4.9 |
| 3 | 78.1 | 4.22m | 77.2 | 4.40m | 77.2 | 4.40m |
| 4 | 84.3 | 6.02d3.1 | 82.4 | 5.61d3.6 | 83.8 | 5.71d3.6 |
| 5 | 47.6 | | 49.0 | | 47.6 | |
| 6 | 43.0 | | 43.8 | | 43.7 | |
| 7a | 22.9 | 1.47m | 20.9 | 1.75m | 20.9 | 1.74m |
| 7b | | 2.01m | | 2.01m | | 2.01m |
| 8a | 27.5 | 2.01m | 27.6 | 2.00m | 27.6 | 2.01m |
| 8b | | 2.01m | | 2.00m | | 2.01m |
| 9 | 139.7 | | 140.4 | | 139.2 | |
| 10 | 119.0 | 5.69d4.9 | 118.6 | 5.53d4.5 | 118.7 | 5.52d5.5 |
| 11 | 68.0 | 4.50d4.9 | 68.4 | 4.12[c] | 68.1 | 4.17d5.5 |
| 12 | 64.7 | | 64.6 | | 64.5 | |
| 13a | 47.6 | 2.77d4.0 | 47.2 | 2.76d4.3 | 47.3 | 2.77d3.7 |
| 13b | | 3.08d4.0 | | 3.04d4.3 | | 3.04d3.7 |
| 14 | 6.3 | 0.80 | 7.1 | 0.82 | 6.8 | 0.78 |
| 15a | 64.8 | 3.97d12.6 | 63.2 | 4.12d12.1 | 63.4 | 3.99d13.8 |
| 15b | | 4.17d12.6 | | 4.26d12.1 | | 4.36d13.8 |
| 16 | 23.1 | 1.62 | 23.3 | 1.70 | 23.3 | 1.70 |
| 1' | 167.8[b] | | 165.9[b] | | 166.2[b] | |
| 2' | 116.5 | 5.61 | 118.8 | 5.67 | 119.0 | 5.79 |
| 3' | 158.1 | | 155.5 | | 156.8 | |
| 4'a | 39.2 | 2.23m | 46.9 | 2.29dd12.3,7.3 | 40.1 | 2.48m |
| 4'b | | 2.55m | | 2.63d12.3 | | 2.48m |
| 5'a | 65.9 | 3.43m | 101.1 | 5.49dd7.3,1.0 | 60.5 | 4.12m |
| 5'b | | 3.71[c] | | | | 4.39m |
| 6' | 83.0 | 3.71[c] | 81.8 | 4.05br.d8.6 | 166.0[b] | |
| 7' | 135.8 | 5.68dd15.5,7.9 | 134.9 | 5.94d15.3 | 127.4 | 5.98d15.3 |
| 8' | 132.0 | 7.58dd15.5,11.0 | 125.9 | 7.56dd15.3,11.0 | 138.9 | 7.95dd15.3,11.0 |
| 9' | 142.0 | 6.64dd11.0,11.0 | 142.4 | 6.54dd11.0,11.0 | 139.2 | 6.66dd11.0,11.0 |
| 10' | 119.0 | 5.90d11.0 | 118.9 | 5.84d11.0 | 125.4 | 6.13d11.0 |
| 11' | 165.8[b] | | 166.7[b] | | 165.4[b] | |
| 12' | 19.5 | 2.17d1.2 | 20.9 | 2.25 | 17.2 | 2.23d1.2 |
| 13' | 69.4 | 3.71a | 76.6 | 3.63m | | |
| 14' | 18.5 | 1.15d6.7 | 16.6 | 1.31d5.4 | | |

a) Taken in $CDCl_3$

b) The assignments may be interchanged.

c) Resonance in one-dimentional spectra obscured by overlapping signals.

Considering the molecular formula of **1** was roridin E plus one oxygen atom, **1** was deduced to be 3-hydroxyl roridin E. The planer structure of **1** was shown in Fig. 1.

The $^{1}H$ and $^{13}C$ NMR spectrum of **2** were closely resembled to those of **1**. Especially, the signals due to trichothecene ring were completely identical with **1**. Detailed comparison of $^{1}H$ and $^{13}C$ NMR data between **2** and roridin H[5] indicated that the macrocyclic chain (C-1'~C-14') of

Fig. 1. Total structures of roridin L (**1**), roridin M (**2**) and verrucarin M (**3**).

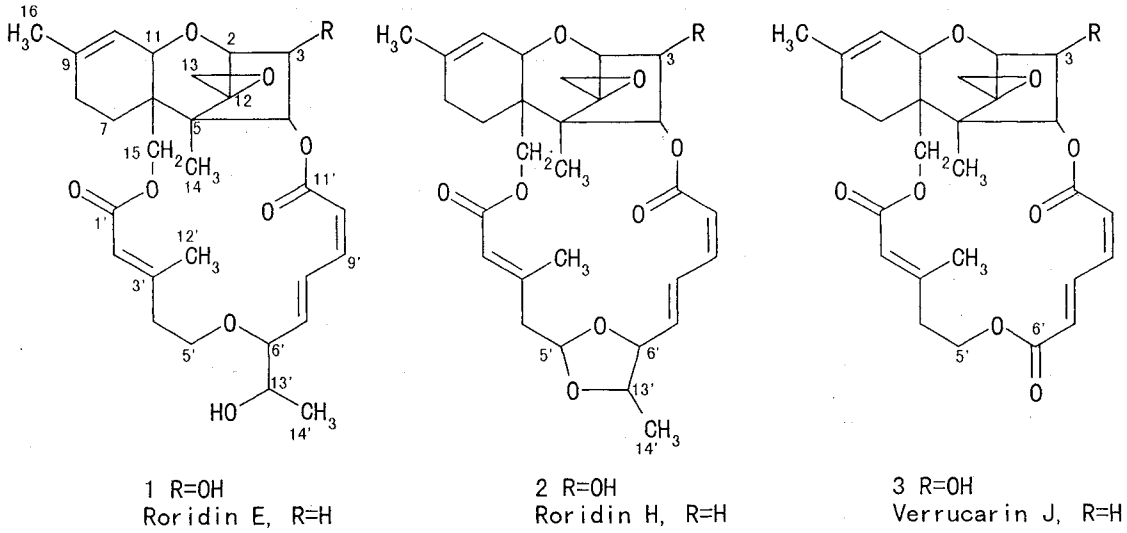
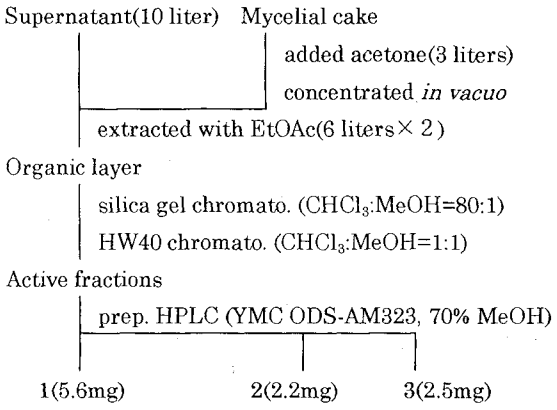



Fig. 2. Isolation scheme of roridin L (**1**), roridin M (**2**) and verrucarin M (**3**).




them were completely idenitical. Thus, the planar structure of **2** (3-hydroxy roridin H) was determined as shown in Fig. 1.

In the $^1H$ and $^{13}C$ NMR spectrum of **3**, the signals due to trichothecene ring in **1** was completely preserved. Analyses of $^1H$ and $^{13}C$ NMR revealed that the macrocyclic chain (C-1′~C-12′) of **3** was idenitical to that of verrucarin J[1,6] including the geometries. From these finding, **3** was determined to be 3-hydroxy verrucarin J (Fig. 1).

**1**, **2** and **3** were the first examples of macrocyclic trichothecene possessing a hydroxyl group at C-3 position. Further experiments to determine the stereochemistry of **1**, **2** and **3** were in progress.

**1**, **2** and **3** were tested for their *in vitro* cytotoxicity. $IC_{50}$ values against P388 (murine leukemia) were 1.6, 4.6 and 4.8 ng/ml, respectively. Since some trichothecenes (PD113,325 and PD113,326) were shown to possess *in vivo* antitumor activity against P388 lymphocytic leukemia model[7], **1**, **2** and **3** were also expected to be new candidates for antitumor agents. Further biological studies are in progress.

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