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NOTE

12′-Hydroxyl group remarkably reduces Roridin E cytotoxicity

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Abstract Roridin E is a well-known macrocyclic trichothecene mycotoxin possessing potent antiproliferative activity against cancer cell lines. 12′-Hydroxyroridin E was isolated from a marine-derived fungus, *Myrothecium roridum* 98F42. The cytotoxicities of these two compounds were tested against human monocytic THP-1, human promyelocytic leukemia HL-60, and Chinese hamster V79 cells, and roridin E exhibited more than 1000-fold stronger cytotoxicity than its 12′-OH derivative; therefore, it was suggested that the 12′-position is closely involved in the cytotoxicity of these compounds.

Keywords HL-60 · 12′-Hydroxyroridin E · Marine-derived fungus · *Myrothecium roridum* · THP-1

Roridin E is a well-known mycotoxin possessing a macrocyclic trichothecene structure, and it has been reported to possess significant biological activities, including cytotoxicity against cancer cell lines (Traxler et al. 1970; Zhang et al. 2002; Abbas et al. 2001, 2002; Garcia et al. 2002: Isaka et al. 1999; Hughes et al. 1989). We have isolated a new 12′-OH derivative of roridin E and three new trichothecenes from a marine-derived fungus, *Myrothecium roridum* strain 98F42 (Namikoshi et al. 2001; Xu et al. 2006). One of four new compounds, a 12,13-deoxy derivative of roridin E, showed reduced cytotoxicity—about 80-fold less than that of roridin E against human promyelocytic (HL-60) and murine leukemia (L1210) cell lines (Namikoshi et al. 2001).

Therefore, the inhibitory activities of 12′-hydroxyroridin E (**1**) (Fig. 1) (Namikoshi et al. 2001) against HL-60 cells, human monocytic leukemia THP-1, and Chinese hamster V79 cell lines were compared with those of roridin E (**2**) in the same experiment. The 12′-hydroxylated derivative (**1**) was more than 1000-fold less cytotoxic than roridin E (**2**).

Fetal bovine serum (FBS) was obtained from GIBCO after checking the lot, and all other reagents and chemicals were of the highest grade available commercially. *M. roridum* strain 98F42 was isolated from submerged woody material collected in Palau (Namikoshi et al. 2001). The fungus was cultured in 500-ml Erlenmeyer flasks containing 150 ml of a half-nutrient potato dextrose medium (50% natural seawater) for about 3 weeks at 20°C, as described in previous papers (Namikoshi et al. 2000, 2001). The culture broth of strain 98F42 (900 ml) was filtered and extracted with EtOAc. The EtOAc extract was chromatographed on a silica gel column with EtOAc–hexane (gradient elution) and then with MeOH. The EtOAc–hexane (2:3) fraction was further purified by HPLC (ODS, 75% MeOH–H_2O) to give roridin E (**2**, 7.5 mg). 12′-Hydroxyroridin E (**1**, 35.0 mg) was isolated from the MeOH eluate by HPLC (ODS, 65% MeOH–H_2O).

Chinese hamster V79 cells were grown as a monolayer culture in Eagle's MEM (Nissui Seiyaku Co., Ltd., Tokyo, Japan) with 10% heat-inactivated FBS. HL-60 and THP-1 cell lines were obtained from the Japanese Cancer Research Resources Bank (JCRB, Tokyo, Japan) and maintained in tissue culture dishes in RPMI 1640 medium (Nissui Seiyaku) supplemented with 10% heat-inactivated

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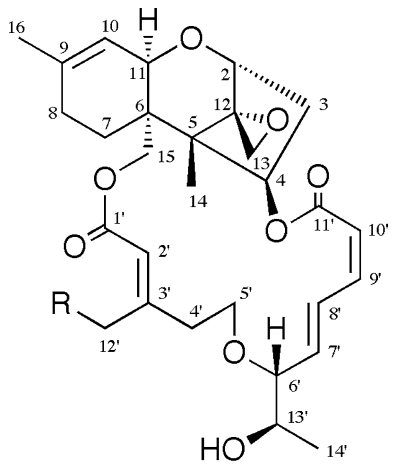

Fig. 1 Structures of 12′-hydroxyroridin E (**1**) and roridin E (**2**)

FBS, 2 mM glutamine, 100 U/ml penicillin G, and 100 μg/ml streptomycin.

The relative plating efficiencies against V79 cells were determined as the ratio of the number of colonies obtained at various sample concentrations to that in the sample-free control, as described in previous papers (Sakakibara et al. 1991; Sato et al. 1992). Two hundred cells were seeded on a 60/15-mm plastic plate with 4 ml culture medium and incubated overnight at 37°C. After each sample in DMSO (4 μl) was added to the culture medium, cells were further cultured for 4 days. The numbers of colonies in the sample plates were counted and compared with those in the control cultures.

Cell proliferation was evaluated by enumerating viable cells using the MTT formazan production method in 96-well microtiter plates (Carmichael et al. 1987). HL-60 or THP-1 cells (1×10^5 and 5×10^5 cells/ml) were treated with the DMSO solution of each test compound. The same volume of DMSO was added as a control. After incubating for 24 h, 20 μl MTT reagent (5 mg/ml in PBS) were added to each well which was then further incubated for 3 h. The optical density (OD_{570} nm) of each well was measured to assess formazan production. Data are shown as values relative (%) to each control optical density.

Three independent experiments were performed for each bioassay, and the mean values are shown.

The inhibitory activities of 12′-hydroxyroridin E (**1**) and roridin E (**2**) on colony formation of adherent cells, Chinese hamster V79, were investigated at 1×10^{-4} to 10 μM. This bioassay reflects the direct action of compounds on the cells. A remarkable difference in activity in the colony formation of V79 cells between two compounds was detected (Fig. 2). The 50% inhibitory concentrations (IC_{50} values) of **1** and **2** calculated from the results were 4.6 μM and 0.74 nM,

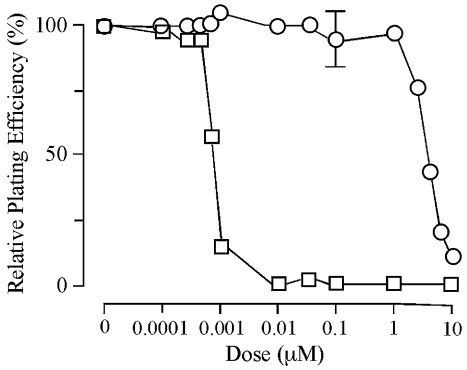

Fig. 2 Relative plating efficiencies of Chinese hamster V79 cells treated with 12′-hydroxyroridin E (**1**) (*open circles*) and roridin E (**2**) (*open squares*) for 48 h

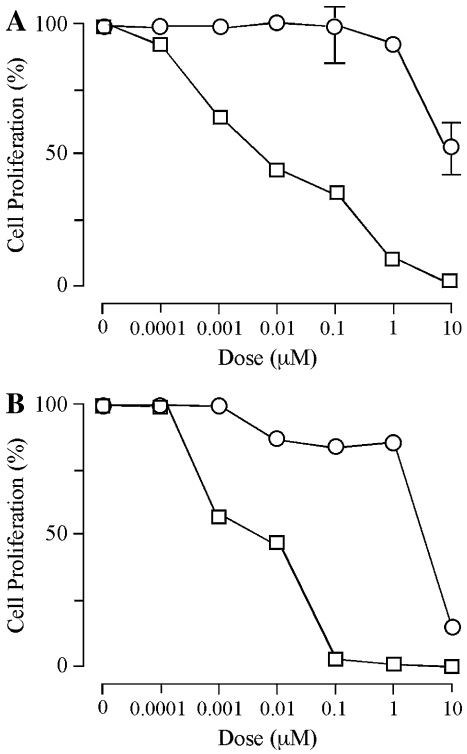

Fig. 3 Cell proliferation rates (%) of HL-60 cells treated with 12′-hydroxyroridin E (**1**) (*open circles*) and roridin E (**2**) (*open squares*) for 24 h. **a** Initial cell number of HL-60 cells: 5×10^5 cells/ml. **b** Initial cell number of HL-60 cells: 1×10^5 cells/ml

respectively. The IC_{50} values of **1** and **2** revealed that hydroxylation at the 12′-position reduced the cytotoxicity of roridin E (**2**) by more than 6000-fold.

The cytotoxicities of **1** and **2** against the floating cells, HL-60 and THP-1, were investigated in order to determine whether the two compounds showed a significant difference in activity, as observed in the case of V79. Two initial cell concentrations were used to detect the effects of **1** and **2** against the different numbers of cells. Compounds **1** and **2**

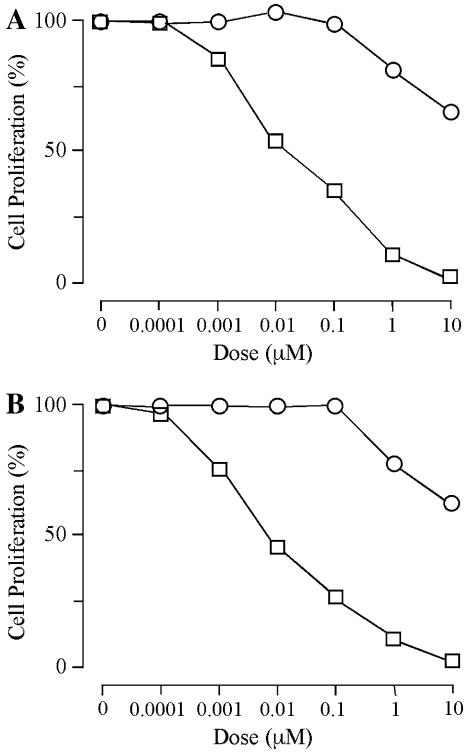

Fig. 4 Cell proliferation rate (%) of THP-1 cells treated with 12′-hydroxyroridin E (**1**) (*open circles*) and roridin E (**2**) (*open squares*) for 24 h. **a** Initial cell number of HL-60 cells: 5×10^5 cells/ml. **b** Initial cell number of HL-60 cells: 1×10^5 cells/ml

exhibited similar dose–response curves against HL-60 and THP-1 cells for two initial cell numbers (Figs. 3, 4).

The IC_{50} value of **2** against HL-60 at 5×10^5 cells/ml was 7.9 nM (Fig. 3b). In the same experiment, **1** did not give an IC_{50} value (>10 μM), which revealed that the 12′-OH derivative was more than 1000-fold less cytotoxic than roridin E (**2**). Similar dose-dependent effects were detected when activity was tested using the lower initial cell number (Fig. 3b), and **2** gave a similar IC_{50} value (8.8 nM) to above. The calculated IC_{50} value of **1** was 5.7 μM in this experiment.

On the other hand, **2** showed a three times lower IC_{50} value in the experiment with 1×10^5 cells/ml ($IC_{50} = 9.1$ nM) than with 5×10^5 cells/ml (33.8 nM) (Fig. 4). The IC_{50} value of **1** in both experiments was >10 μM. The dose-dependent effects of **1** and **2** against different initial cell numbers of THP-1 were also very similar.

From the results of the above experiments, it can be concluded that the 12′-hydroxyl group remarkably decreased the cytotoxicity of roridin E (**2**). The 12′-positions of these compounds may be involved in their growth inhibitory activities against adherent and floating cancer cell lines. It is also possible that the increase in hydrophilicity of **1** due to the presence of a hydroxyl group at C-12′ resulted in a low incorporation of the compound into the cells.

A 12′-hydroxylated derivative of (2′*Z*)-verrucarin J was isolated from *M. roridum*, but its in vitro cytotoxicity has not been reported (Smitka et al. 1984). Therefore, we cannot compare differences in in vitro cytotoxicity between (2′*Z*)-verrucarin J and its 12′-OH derivative with those between **1** and **2**. Nevertheless, the findings in this study would be significant with regard to the control of various biological activities.

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