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Anti-tumor Activity of Lemnalol isolated from the Soft Coral *Lemnalia tenuis* Verseveldt

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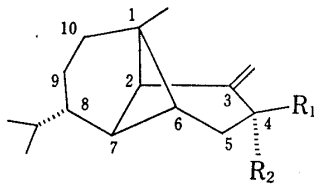
Anti-tumor activities of lemnalol (I), a new ylangene-type sesquiterpenoid isolated from the Japanese soft coral *Lemnalia tenuis* Verseveldt, its stereoisomer (II) and ketone derivative, lemnalone (III), were studied. Lemnalol (I) renders murine peritoneal exudate cells (PEC) cytotoxic, though it has no direct cytotoxicity itself, to syngeneic tumor cells *in vitro*, while it also inhibits tumor growth *in vivo*. The stereoisomer (II) does not show PEC-mediated or direct cytotoxicity, while lemnalone (III) exhibits strong direct cytotoxicity *in vitro*. It is suggested that the 4α-configuration of lemnalol (I) is essential for its anti-tumor activity by PEC activation *in vitro*.

Keywords——*Lemnalia tenuis*; lemnalol; anti-tumor activity; fibrosarcoma; peritoneal exudate cell activation

In the course of our investigations of biologically active substances in Japanese marine organisms, lemnalol (I), a new ylangene-type sesquiterpenoid having anti-tumor activity, was isolated from the soft coral *Lemnalia tenuis* Verseveldt. It is the first example from marine organisms, which activates peritoneal exudate cells (PEC) to exhibit anti-tumor effect *in vitro*. In this paper, we report the *in vitro* PEC-mediated and direct cytotoxicity of lemnalol (I), in comparison with those of its stereoisomer (II) and lemnalone (III), the ketone derivative, as well as the *in vivo* anti-tumor activity of lemnalol (I).



I: R_1=H, R_2=OH
II: R_1=OH, R_2=H
III: R_1=R_2=O

Chart 1

Experimental

Agents——Lemnalol (I) was obtained from *Lemnalia tenuis* Verseveldt collected at the coral reefs of Ishigaki Island (Okinawa, Japan).[1] The stereoisomer (II) and lemnalone (III) were chemically derived from III and I, respectively.[1] Poly(I)·Poly(C) (P.L Biochemicals, Inc.) and BCG (Japan BCG Laboratory, Tokyo) were used as positive controls.

Animals——Male DBA/2 and CDF_1 (BALB/c×DBA/2) F_1 mice were obtained from Charles River Japan, Inc., Kanagawa.

Target Cells——Used cell line was established from DBA/MC fibrosarcoma, a syngeneic fibrosarcoma induced by methylcholanthrene in male DBA/2 mice in our laboratory and maintained in male DBA/2 mice by transplantation of small fragments of the tumor. This cell line was maintained in Eagle's MEM medium supplemented with 20% heat-inactivated (56°C for 30 min) calf serum and 0.4% lactalbumin hydrolysate. These cells adhered to a plastic dish. The population exponentially proliferated with a doubling time of 13 h.

***In vitro* PEC-Mediated and Direct Cytotoxicity Test**——Effector Cells: (1) Preparation of PEC: Normal DBA/2 mice were injected intraperitoneally with 0.2 ml of saline solution containing 1 mg of glycogen. Two days later, PEC were harvested by washing the peritoneal cavity with 3—4 ml of heparinized Hanks' solution, washed with the culture medium at 1000 rpm for 5 min, and then resuspended in the medium to be adjusted to a required concentration.

(2) Preparation of Adherent and Nonadherent Cells: PEC collected from normal DBA/2 mice were placed into Falcon tissue culture plastic dishes and incubated at 37°C for 30 min. Nonadherent cells were decanted and adherent cells were gently scraped from the dishes with a soft rubber policeman. These cells were resuspended in the medium to be adjusted to a required concentration.

Methods——Target cells (5×10^3) were cultured in the wells of a microplate (Falcon Micro Test II tissue culture plate) for 24 h, then the agent was added with or without effector cells at the ratio of 50: 1 of effector cells to target cells. The plate was incubated for 48 h to examine PEC-mediated or direct cytotoxicity. Control target cells in wells became confluent at 72 h. All cultures were incubated at 37°C in an atmosphere of 5% CO_2 in air. After fixation of the cells in methanol, the microplate was stained with giemsa for microscopic examination to observe the growth inhibition of tumor cells.

***In Vivo* Growth Inhibition Test**——Target cells (10^6) were inoculated intradermally into the back region of 6-week-old CDF_1 mice on day 0. Six days later, the agent dissolved in 0.05 ml of 0.5% (v/v) ethanol was injected into the tumor site. The growth of the tumor was assessed by measuring the long diameter and short diameter at seven-day intervals. The tumor size was represented by the mean value of the long and short diameters. The experiments were carried out with 10 mice in each group.

Results and Discussions

In vitro, lemnalol (I) in the presence of PEC inhibited the growth of DBA/MC fibrosarcoma cells at concentrations of 10 to 40 μg/ml (Table I). Of course, PEC alone had no effect.

Fractionation of PEC revealed that plastic surface-adherent cells (mainly consisting of macrophages) were effective, and nonadherent cells (mainly consisting of lymphocytes) were not effective. From these results, it is considered that lemnalol (I) renders PEC (mainly macrophages) cytotoxic to DBA/MC fibrosarcoma cells *in vitro*. There are several papers[2-4] reporting that various agents including poly(I)·Poly(C) activate macrophages *in vitro*, whereby the macrophages exhibit enhanced cytostatic and cytocidal effects on tumor cells. Lemnalol (I) can now be included among these agents.

Next, the stereoisomer (II) and lemnalone (III) were also examined *in vitro*. The stereoisomer (II) had no effect, and lemnalone (III), which has an α,β-unsaturated ketone moiety,

TABLE I. Effects of Poly(I)·Poly(C), Lemnalol, Its Stereoisomer and Lemnalone on the Growth of DBA/MC Fibrosarcoma Cells *in Vitro*

Agent	Concentration (μg/ml)	Growth inhibition[a]	
		Agent+PEC	Agent only
Poly(I)·Poly(C)	40	(±)	(−)
	20	(±)	(−)
	10	(∓)	(−)
	5	(−)	(−)
Lemnalol(I)	40	(+)	(−)
	20	(±)	(−)
	10	(∓)	(−)
	5	(−)	(−)
Stereoisomer(II)	40	(−)	(−)
	20	(−)	(−)
	10	(−)	(−)
	5	(−)	(−)
Lemnalone(III)	40	(+)	(+)
	20	(+)	(+)
	10	(+)	(±)
	5	(+)	(±)
	2.5	(∓)	(−)

a) The effects of growth inhibition are graded and expressed as follows: (+) indicates more than 95 % growth inhibition; (±) between 50 and 95 %; (∓) between 30 and 50 %; and (−) less than 30 % compared with the control culture, respectively.

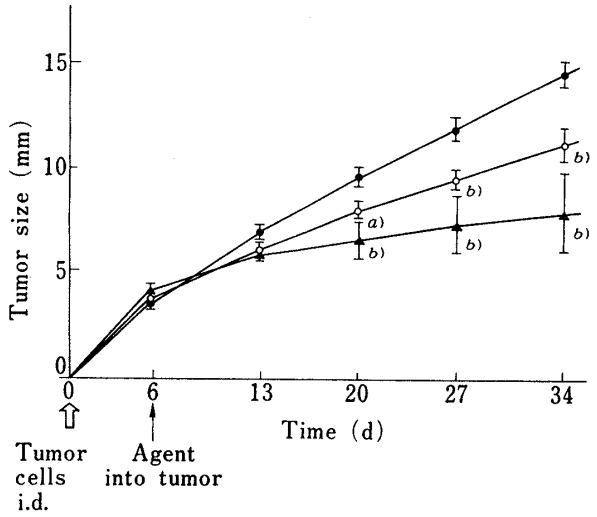


Fig. 1. Effects of Lemnalol and BCG on the Growth of DBA/MC Fibrosarcoma Cells *in Vivo*

●, control (0.5% (v/v)EtOH); ○, lemnalol (400 μg/site); ▲, BCG (400 μg/site).
Each point represents the mean ± S.E. of 10 mice.
a) p 0.05.
b) $p<0.01$ *vs.* 0.5% (v/v) EtOH-administered control.

exhibited remarkably strong cytotoxicity (Table I). This finding suggests that the 4α-configuration of lemnalol (I) may be important for the anti-tumor activity induced by PEC activation *in vitro*.

In the *in vivo* growth inhibition test, lemnalol (I) as well as BCG significantly inhibited the growth of DBA/MC fibrosarcoma cells at 400 μg/site (Fig. 1). In view of this finding of PEC activation by lemnalol (I) *in vitro*, we will further study to elucidate the mechanism of the anti-tumor activity of lemnalol (I) *in vivo* from the point of activation of PEC.

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References and Notes

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