

Note

Effect of Eucommiol Isolated from Aucuba (*Aucuba japonica* Thunb) on the Growth of Human Lymphoid Leukemia Cells

Yumiko ACHIWA,¹ Hiroshige HIBASAMI,² Hirotaka KATSUZAKI,¹ Kunio IMAI,¹ Kazumi YOSHIOKA,² Kimiko NAKANISHI,² Teruko KOJIMA,² Yaeko ISHII,² Kazuko OHNISHI² and Takashi KOMIYA¹

Faculties of ¹Bioresource and ²Medicine, Mie University, Tsu-city, Mie 514-8507, Japan

Received September 18, 1998; Accepted December 16, 1998

We have investigated the effect of crude extract prepared from leaves of Japanese aucuba (*Aucuba japonica* Thunb) on the growth of human lymphoid leukemia Molt 4B cells. The extract suppressed the growth of these cells by 60% at the concentration of 200 $\mu\text{g/ml}$. Fraction III obtained by separation with HW40S gel chromatography inhibited the growth of the cells by 95% at the same concentration. We analyzed the structure of the compound contained in Fraction III, and identified it as eucommiol by NMR and MS. The antiproliferative effect of eucommiol on the growth of Molt 4B cells was examined, and it was found to moderately suppress the proliferation of Molt 4B cells by 41% at the concentration of 200 $\mu\text{g/ml}$.

Keywords: eucommiol, growth inhibition, leukemia, aucuba (*aucuba japonica* Thunb)

We purified several compounds from plant leaves and evaluated their antitumor activity. In previous papers we demonstrated inhibitory effects of persimmon extract (Achiwa *et al.*, 1997), and oleanolic and ursolic acids isolated from leaves of loquat (*Eriobotrya*) (Komiya *et al.*, 1998) on the growth of human lymphoid leukemia Molt 4B cells, respectively. Bianco *et al.* (1974) and Deyama *et al.* (1986) also reported the isolation of eucommiol, a cyclopentenoidtetrol, from eucommiaceae (*Eucommia ulmoides* Oliv).

In the present study, we obtained some fractions having antiproliferative activity from the leaves of aucuba (*Aucuba japonica* Thunb) and determined the structure of the compounds contained in the fractions. We report here the identification of eucommiol for the first time from the leaves of Japanese aucuba (*Aucuba japonica* Thunb) and the inhibitory effects of the compounds on the growth of human lymphoid leukemia Molt 4B cells.

Materials and Methods

Chemicals HW 40S gel was purchased from Toyo Soda Co., Ltd, Tokyo. All other chemicals were of the highest grade available from Wako Chemicals, Kyoto.

Apparatus ¹³C NMR spectra were taken with a JEOL, JNM- δ 500. Field desorption mass spectra (FD-MS) were done by a Hitachi M-80B instrument. The preparation of eucommiol as described in the next item utilized high pressure liquid chromatography (HPLC) with a JASCO 880-PV.

Isolation and identification of eucommiol The leaves of aucuba (160 g) were harvested on the experimental farm of Mie University and homogenized in distilled water by a juicer. The homogenate was centrifuged at 10,000 $\times g$ for 20 min at 0°C. The resulting supernatant was frozen and dried to give 8.0 g of extract. This extract (1.0 g) was dissolved in

distilled water, applied to a HW 40S gel column, and eluted with distilled water. Thus obtained solution was analyzed by a UV spectrophotometer with 280 nm and divided into three fractions F I (31 mg), F II (576 mg) and F III (219 mg) according to the elution number. These fractions were tested for their antiproliferative effects on human lymphoid leukemia Molt 4B cells. Fraction III having antiproliferative activity was subjected to preparative HPLC (column, ODS-5; detection wavelength, 210 nm; Nomura Chemicals, Aichi); flow rate (0.5 ml/min) eluted with methanol-water (90:10) to give two substances, A and B whose peaks had a retention time of 6.5 and 12.0 min, respectively, by HPLC. Compound B gave a peak at m/z 189 (M+1) in FD-MS. The compound was identified as eucommiol by comparison of the spectral

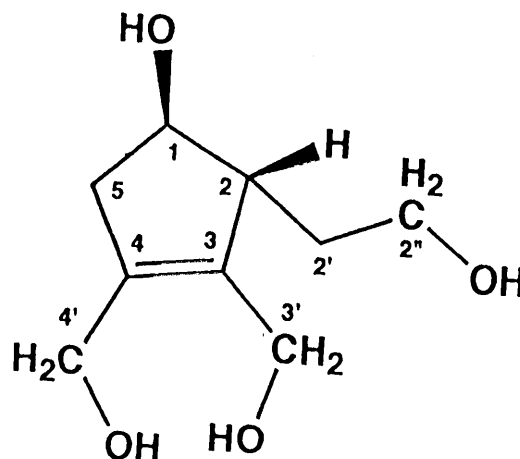


Fig. 1. Structure of eucommiol.

Table 1. ^{13}C -NMR (in D_2O) for eucommiol and compound B.

| carbon | eucommiol (ppm) | compound B (ppm) |
|--------|-----------------|------------------|
| 1 | 75.3 | 75.2 |
| 2 | 53.0 | 52.9 |
| 3 | 137.1 | 137.1 |
| 4 | 138.9 | 139.0 |
| 5 | 42.3 | 42.2 |
| 2' | 33.3 | 33.1 |
| 2'' | 60.9 | 60.9 |
| 3' | 56.2 | 56.2 |
| 4' | 58.0 | 57.9 |

Table 2. Effects of eucommiol on the growth of Molt 4B cells.

| Compound | Concentration ($\mu\text{g}/\text{ml}$) | Inhibition rate (%) |
|-----------|---|-----------------------------|
| Eucommiol | 0 | — |
| | 50 | 7.8 \pm 0.8 |
| | 100 | 20.2 \pm 2.6 ^a |
| | 200 | 41.3 \pm 5.2 ^a |

Each value is the mean \pm SD of triplicate experiments. ^a $p < 0.01$ versus the control (without eucommiol).

^{13}C -NMR data with those reported in the literature (Deyama *et al.*, 1986), as shown in Fig. 1 and Table 1.

Cell culture Human lymphoid leukemia Molt 4B cells were obtained from Dainippon Pharmaceutical Co., Osaka, and cultivated as previously described (Hibasami *et al.*, 1989). Molt 4B cells were grown in RPMI 1640 medium (GIBCO Laboratories, Grand island, NY) with 10% fetal calf serum, penicillin G (50 IU/ml) and streptomycin (50 $\mu\text{g}/\text{ml}$) at 37°C in a humidified 95% air-5% CO_2 atmosphere and passaged every 7 days. Mycoplasma testing was routinely negative.

Assay for growth inhibition Exponentially growing cells were placed in triplicate at 3 to 4 \times 10⁵ cells/ml in the culture flask and cultivated in the presence of vehicle (10 μl) or a solution (10 μl) of the compounds dissolved in distilled water. After cultivation for 4 days, the number of viable cells was counted by staining with trypan blue.

Statistical analysis Results are expressed as mean \pm

SD. The level of significance in the difference between groups was calculated by the Student's *t*-test.

Results and Discussion

Findings on the effect of crude extract from leaves of Japanese aucuba on the growth of human lymphoid leukemia Molt 4B cells were that crude extract suppressed the proliferation of these cells by 7.6% and 60.3% at 100 $\mu\text{g}/\text{ml}$ and 200 $\mu\text{g}/\text{ml}$, respectively. Crude extract was separated by HW40S gel column to give three fractions, F-I, F-II and F-III. Fraction FIII inhibited the proliferation of Molt 4B cells dose-dependently, while F I and F II did not inhibit them at all. The compound contained in F III was elucidated by field desorption mass spectroscopy (FD-MS), and identified as eucommiol. The structure of eucommiol is shown in Fig. 1. This is the first time, eucommiol was isolated from the leaves of aucuba (*Aucuba japonica* Thunb). We investigated the effects of aucummiol on the growth of human lymphoid Molt 4B cells. As shown in Table 2, inhibition rates of cell growth by eucommiol increased as the concentration of eucommiol was increased. Our results indicate that eucommiol may be an antiproliferative compound on human leukemia cells.

References

- Achiwa, Y., Hibasami, H., Katsuzaki, H., Imai, K. and Komiya, T. (1997). Inhibitory effect of persimmon (*Diospyros kaki*) extract and related polyphenol compounds on growth of human lymphoid leukemia cells. *Biosci. Biotech. Biochem.*, **61**, 1099-1101.
- Bianco, A., Iavarone, C. and Trogolo, C. (1974). Structure of eucommiol, a new cyclopentenoid-tetrol from *eucommia ulmoides*. *Tetrahedron*, **30**, 4117-4121.
- Deyama, T., Ikawa, T., Kitagawa, S. and Nishibe, S. (1986). The constituents of *eucommia ulmoides* Oliv. IV. Isolation of a new sesquignan glycoside and iridoids. *Chem. Pharm. Bull.*, **34**, 4933-4938.
- Hibasami, H., Maekawa, S., Murata, T. and Nakashima, K. (1989). Antitumor effect of a new multienzyme inhibitor of polyamine synthetic pathway, methylglyoxal-bis(cyclopentylamidinohydrazone), against human and mouse leukemia cells. *Cancer Res.*, **49**, 2065-2068.
- Komiya, T., Achiwa, Y., Katsuzaki, H., Imai, K., Adachi, T., Yamada, T. and Hibasami, H. (1998). Effect of oleanolic- and ursolic acids isolated from loquat (*Eriobotrya*) on the growth of human lymphoid leukemia cells. *Food Sci. Technol. Int., Tokyo*, **4**, 282-284.