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Short Communication

In vitro cytotoxic activity of (-)-ent-costunolide (Notes)

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SUMMARY

The cytotoxic activity was strongly shown by (-)-ent-costunolide (1) (P388; IC_{50}: 687 ng/mL) which isolated from Hepatostolonophora paucistipula. These results suggest that (-)-ent-costunolide (1) has a potential cytotoxic activity.

Key words: (-)-ent-Costunolide (1); Cytotoxic activity; Murine leukaemia cell lines

INTRODUCTION

Liverworts have been a rich source of sesquiterpenes, including several new skeletal types (Allison *et al*., 1975; Asakawa, 1995). This sequiterpene lactone (1) which isolated from the whole plant of *Hepatostolonophora paucistipula* (Rodw.) J.J. Engel (family Geocalycaceae) (*H. paucistipula*) inhibited the growth of the dermatophytic fungus Trichophyton mentagrophytes ATCC 28185, (4 mm inhibition zone at 15 μg/disc). The activities are expressed by the diameter of the developed inhibition zones and compared with those of the widely antibious chloramphenicol, gentamycin and nystatin (Kim *et al*., 2005).

In this study, *in vitro* the cytotoxic activity of (-)-ent-costunolide (1) from *H. paucistipula* has investigated by the MTT method.

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MATERIALS AND METHODS

General experimental procedures

All solvents were distilled before use. Removal of solvents from chromatography fractions were removed the *H. paucistipula* by rotary evaporation at temperature up to 40 °C. Initial fractionation of crude plant extract using reverse phase column chromatography was performed with octadecyl-functionalized silica gel (C18 Aldrich) as the adsorbent. Further column fractionation was performed using Davisil silica 60 (35 - 70 μm silica gel, Allth) as adsorbent. TLC was carried out using Merck DC-plastikfolien Kieselgel 60 F254 visualized first with a UV lamp, then by dipping in a vanillin solution (1% vanillin, 1% H_2SO_4 in EtOH) followed by heating. Microanalyses were performed by Marianne Dick and Bob McAllister (Campbel

Microanalytical Laboratory, Chemistry Department, University of Otago). MS, UV and IR spetra were recorded on Krato MS-80, Shimadzu UV 240, and Perkin-Elmer 1600 FT-IR instruments respectively. NMR spectra, of $CDCl_3$ solutions at 25 °C, were recorded at 300 MHz for 1H-NMR and 75 MHz for 13C-NMR on a Varian VXR-300 spectrometer. Chemical shifts are given in parts per million on the δ scale referenced to the solvent peak $CHCl_3$ at 7.25 ppm and $CDCl_3$ at 77.08 ppm and are referenced to TMS at 0.00 ppm.

Plant material

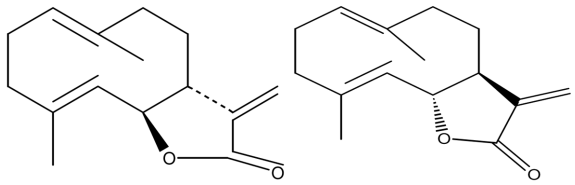
H. paucistipula was collected from Port Adventure, Stewart Island, in January 1994. This was identified by D. Glenny, Landcare Research, and a voucher specimen, OTA 046764, has been kept in the Otago University herbarium.

Screening for cytotoxic activity

This is a measure of the ability of a sample to inhibit the multiplication of murine leukaemia cells. The sample was dissolved in a suitable solvent, usually ethanol, at 0.5 mg/mL, and 30 μL of this solution was placed in the first well of a multiwell plate. Seven two-fold dilutions were made across the plate. After addition of the cell solution, the concentration range in the test wells was 25,000 down to 195 ng/mL. After incubation for three days, the plates were read using an ELISA palte reader at 540 nm wavelength. Automated reading of the plates was possible with the addition of a MTT tetrazolium salt (yellow color). Healthy cells reduce this salt to MTT formazan (purple color). The concentration of the sample required to inhibit cell growth to 50% of a solvent control was determined using the absorbance obtained upon staining with MTT tetrazolium. Mytomycin C (concentration 0.075 μg/mL) was used as a positive control and inhibited the growth of P388 cells by 43 - 75%.

RESULTS AND DISCUSSION

Normal-phase flash column chromatography concentrated the cytotoxic activity in fraction 3 eluted with 5% and 10% ethyl acetate - hexane. Chromatography on silica gel (0.5 g) with an ethyl acetate - cyclohexane gradient gave four fractions. The second silica gel column chromatography of subfraction 2 gave most of the mass in the less polar fraction, eluted with 5% and 10% ethyl acetate - hexane. Among them, subfraction 2 that eluted with 5% and 10% ethyl acetate - hexane is the most cytotoxic activity to murine leukaemia cells ATCC CCL 46 P388D1 (37.1 mg, P388 IC_{50} 945 ng/mL at 7.5 μg/disc). A comparison of IC_{50} (ng/mL) values of these subfractions in cancer cells showed that their susceptibility to these subfractions decreased in the following order; subfraction 2 > subfraction 4 > subfraction 1 = subfraction 3 (Fig. 2) (Baek *et al.*, 2003). Fig. 2 shows the potent cytotoxic activity of (-)-ent-costunolide (1) (Kim *et al.*, 2005) from *H. paucistipula* against P388 murine leukaemia cell



(1) (2)

Fig. 1. Structures of (-)-ent-costunolide (1) and (+)-costunolide (2).

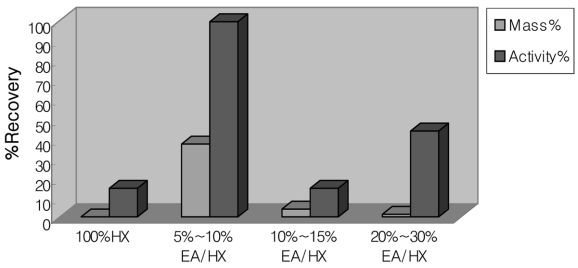


Fig. 2. *In vitro* cytotoxic activity of (-)-ent-costunolide (1) from *H. paucistipula* by the MTT method. This compound (1) was serially diluted in RPMI-1640 with 10% FBS and mixed with equal volume of murine leukaemia cells ATCC CCL 46 P388D1 (7.5 μg/disk). The colorimetric method was performed as described in materials and methods. Data are mean values of results obtained from three sets of experiments.

Table 1. The cytotoxic activity of (-)-ent-costunolide (1) from *H. paucistipula*

Assay	Tested material	
	1	Mitomycin C
Cytotoxicity BSC-1 cells[a]	++++	
P388 IC_{50}	687[b]	34.6[c]

[a]% of well showing cytotoxic effects. @ 0.5 mg/mL, 15 μg/disk; ++++: 100% activity. BSC-1 cells; African green monkey kidney cells. [b]Toxicity of sample to murine leukaemia cell lines (ATCC CCL 46 P388D1) in ng/mL at 7.5 μg/disk. P388; Concentration of the sample required to inhibit cell growth to 50% of a solvent control. [c]Toxicity of sample to murine leukaemia cells (ATCC CCL 46 P388D1) in ng/mL at 0.075 μg/disk.

lines (P388 IC_{50} 687 ng/mL). The cytotoxic activity of this sesquiterpene lactone was in a dose-dependent inhibition of cell proliferation. This compound showed a dose-dependent increase of cell antiproliferation after treatment with of (-)-ent-costunolide (1) (Baek *et al*., 2000; Shin *et al*., 2001). This compound- mediated cytotoxicity was rapidly increased in the MTT method when its concentrations or absorbances were raised from 2.591 to 3.494. However, the other absorbances were most strong cytotoxic. An IC_{50} of 0.57 μg/mL against KB carcinoma cells has been reported for (+)-costunolide (2) (Fig. 1) (Kupchan *et al*., 1971; Saunders *et al*., 1994; Williams, 1995; Perry *et al*., 2001; Pretsch *et al*., 2002; Lim, *et al*., 2004).

In conclusion, (-)-ent-costunolide (1) was isolated from the whole plant of *H. paucistipula*, and we determined its structure by spectroscopic analysis. The strong cytotoxicity of this compound was shown by the MTT method (P388D1, IC_{50} 687 ng/mL).

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