Cytotoxic Activity of 13(E)-Labd-13-ene-8α,15-diol

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The cytotoxic activity of 13(E)-labd-13-ene-8α,15-diol (1) was evaluated against tumor cell lines. A comparison of IC_{50} values of this compound in cancer cell lines showed that their susceptibility to this compound decreased in the following order: P388 > B16/F10 > MDA-MB-231> A549 > KB > SNU-C4 by the MTT method. 13(*E*)-Labd-13-ene-8α,15-diol (1) was the most effective growth inhibitor of P388 murine leukaemia cell lines, producing approximately 8.3 μg/mL of IC_{50} in the MTT method.

Key words : Cytotoxicity, 13(*E*)-labd-13-ene-8,15-diol (1), P388 murine leukaemia cell lines, MTT method.

Introduction

Brachyglottis monroi (Hook. f) B. Nordenstam (*Asteraceae compositae*), previously *Senecio monroi*, is a shrub endemic to New Zealand[1-2]. Cytotoxic activity against a range of murine leukaemia cell lines (IC_{50}'s 11.4 to > 50g/mL) has been reported for 13(*E*)-labd-13-ene-8α,15-diol (1) isolated from another plant, *Cistus creticus* (Cistaceae)[3]. This is the first report of any compounds from *B. monroi.*[4]. *B. repanda* was used by Maori for treatment of sores and wounds[4], but 13(*E*)-labd-13-ene-8α,15-diol (1) has been found in *B. bidwillii*[5]. The structure characterization of 1 and cytotoxic effect of 1 against tumor cell lines are reported in this paper.

Materials and Methods

1. General experimentalp Procedures

All solvents were distilled before use. Removal of solvents from chromatography fractions were removed by rotary evaporation at temperature up to 40℃. Initial fractionation of crude plant extract using reverse phase column chromatography was performed with octadecylfunctionalized silica gel (C-18, Aldrich) as the adsorbent. Further column fractionation was performed using Davisil silica 60 Å (35-70 μm silica gel, Allth) as adsorbent. TLC was carried out using Merck DC-plastikfolien Kieselgel 60 F_{254} visualized first with a UV lamp, then by dipping in a vanillin solution (1% vanillin, 1% H_2SO_4 in EtOH) followed by heating. NMR spectra of $CDCl_3$ solutions at 25℃ of extracts and fractions were recorded at 200 ㎒ for ^{1}H-NMR on a Varian Germini 200 spectrometer, or at 300 ㎒ for ^{1}H-NMR and 75 ㎒ for ^{13}C-NMR on a Varian Inova 300, or a Varian VXR-30 spectrometer. Chemical shifts are given in ppm on the δ scale and are referenced to TMS at 0.00 ppm (1H) or to solvent peaks at either: $CDCl_3$ (^{1}H at 7.25 ppm, ^{13}C at 77.0 ppm). Two-dimensional (2-D) NMR experiments were recorded on a Varian VXR-300 fitted with a Nalorac inverse-detection probe, or a Varian Inova 300 fitted with a Varian "Autoswitchable" 5 mm Z-axis PFG Direct 300㎒ probe (in both cases). Proton and carbon shifts were referenced as described for 1-D NMR experiments. Proton-carbon coupling correlations were observed from gCOSY experiments; one bond ^{1}H - ^{13}C correlation (^{13}C resonances with directly attached protons) were assigned using results from HMQC experiments; the ^{13}C resonance's of quaternary carbons and two and three bond 1H - 13C correlations were assigned using HMBC and DEPT experiments[5-6]. Solids were ground with KBr to a fine powder and pressed into a KBr disk. Spectra were recorded using a Perkin-Elmer 1600 Fourier Transform spectrophotometer. Melting point was determined in duplicate using a Mettler Toledo electronic melting point apparatus to an accuracy of ± 0.2o. High resolution mass spectra was recorded by Bruce Clark (Chemistry Department, University of Canterbury, Christchurch, NZ), on a Kratos MS80 (electron impact, 70eV) mass spectrometer. Microanalyses were performed by Marianne Dick and Bob McAllister (Campbell Microanalytical Laboratory, Chemistry Department, University

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of Otago). All other chemicals were of reagent grade.

2. Cell Culture

Tumor cells were grown at 37 ℃ in RPMI medium supplemented with 10% FBS penicillin (100 units/mL) and streptomycin (100 μg/mL). The cells were grown in a humidified atmosphere of 95% air / 5% CO_2. Cells were dissociated with 0.25% trypsin and were counted using a Hemacytomer just before transferring them for the experiment.

3. Plant Material.

B. monroiwas collected from the Dunedin Botanical Gardens, Dunedin, New Zealand, in June 1998. This was identified by D.Glenny, Landcare Research, and a voucher specimen, OTA 980309-63, has been kept in the Otago University herbarium.

4. Extraction and Isolation.

Air-dried *B. monroi* (26.88 g) was macrate in redistilled ethanol (200 mL) in a Waring Blender, and then filtered. the residual marc was reextracted in the same way with more ethanol (2 x 150 mL) and chloroform (100 mL). The combined filtrates were evaporated under reduced pressure to give a dark green gum (2.60 g). A sample was subjected to flash chroatoghraphy on C 18 (10 g) with a H_2O : MeCN : $CHCl_3$ gradient. The most cytotoxic fraction was eluted with 1 : 9 H_2O : MeCN (245 mg, IC_{50} 8.286 μg/mL). A sub-sample (245 mg) was subjected to flash chromatography on sillica gel (2.5 g) with an hexane : EtOAc gradient. A fraction eluted with 1 : 9 hexane : EtOAc was subjected to give 13(*E*)-labd-13-ene-8 α,15-diol (1, 51 mg). mp. 138.1 - 139.90C (lit, 131 - 134^0C, [6]), [α]18 -2.256 (C, 3.0 $CHCl_3$), High-resolution mass spectrum m/z, 308.2724 ($C_{20}H_{36}O_2$ requires 308.4986), TLC, CH_3CN : H_2O 3 : 1, R_f 0.37, black spot on H_2O : H_2SO_4 : MeOH 85 : 5 : 10 dip; $UV_{\lambda max}$ (MeOH) log ε 266 (3.01)nm; $IR_{\nu max}$(film), 3341, 2931, 2861, 1455, 1384, 1214, 1014, 756 cm^{-1} EI-MS (70 eV), 308.2724 (0.1, M^+, $C_{20}H_{36}O_2$ required 308.4986), 290 (M^+- H_2O), 275 (10), 257 (8), 245 (12), 204 (9), 192 (91), 177 (100), 163 (12), 149 (22), 137 (34), 123 (45), 109 (45), 95 (54), 81 (66), 69 (66), 55 (47), 43 (51); ^{1}H-NMR ($CDCl_3$) δ5.38 (1H, qt, J= 1.2, 6.9 Hz), 4.08 (2H, dd, J= 2.4, 7.1 Hz), 2.03 (2H, t, J= 8.1. Hz), 1.80 (2H, dt, J= 2.0, 12.3 Hz), 1.63 (3H, t, J= 0.6 Hz), 1.60 (2H, t, J= 1.0 Hz), 1.56 (1H, t, J= 2.7 Hz), 1.38 (2H, m), 1.33 (1H, dd, J= 1.4, 3.9 Hz), 1.29 (1H, dd, J= 1.4, 3.9 Hz), 1.07 (3H, t, J= 0.6 Hz), 0.99 (2H, t, J= 3.9 Hz), 0.99 (1H, t, J= 3.9 Hz), 0.90 (1H, t, J= 2.7 Hz), 0,86 (1H, dd, J= 1.4, 9.6 Hz), 0.80 (3H, s), 0.73 (3H, s), 0.72 (3H, s); ^{13}C-NMR ($CDCl_3$) δ42.04 (C-1), 18.51 (C-2), 39.80 (C-3), 33.31 (C-4). 56.17 (C-5), 20.64 (C-6), 44.60 (C-7), 74.22 (C-8), 61.25 (C-9), 39.30 (C-10), 23.64 (C-11), 42.96 (C-12), 141.01 (C-13), 123.23 (C-14), 59.35 (C-15), 16.53 (C-16), 23.99 (C-17), 33.46 (C-18), 21.58 (C-19), 15.55 (C-20).

5. 4,5-Dimethylthiazol-2-yl-2,5-diphenyl-2*H*-tetrazoliumbromide (MTT) assay

The assay is dependent on the cellular reduction of water-soluble MTT (Sigma Chemical Co. St. Louis, M.O.) by mitochondrial dehydrogenase of vial cells to a blue water-insoluble formazan crystal product which can be measured spectrophotometrically [3,4]. Tumor cell lines were cultured in RPMI-1640 medium (Gubco Laboratories) containing 10 % fetal bovine serum. Exponentially growing tumor cells (5 × 105) were cultured for 48 hrs at 37 ℃ in a humidified 5 % CO_2 incubator in the presence or absence of sample.

6. Cytotoxicity and Antiviral assays.

Antiviral (*Herpes simplex, Polio*) and anti-tumor (P388) biological assays were performed by Gill Ellis at the Chemistry Department, University of Canterbury, Christchurch [7]. For the antiviral assay, the samples were dried onto 8 mm filter paper disks and placed directly onto BSC-1 cells (African Green Monkey kidney), which were then infected with either *Herpes simplexType* I *virus* ATCC VR 733 or *Polio Type* I *virus* Pfizer vaccine strain. After incubation for 24 hours, the cells were examined using an inverted microscope. Two results were obtained : the proportion of cells around the disc that did not show the cytopathic effect of the viruses, and the proportion of cells around the disc that showed a cytotoxic effect.

7. Statistical Analysis

All values, expressed as the mean ± S.D., were statistically analyzed through analysis of Student's t-test. The P value less than 0.05 was considered as significant.

Results and Discussion

Air dired plant material (26. 88 g) was ground and blended with EtOH (200 mL, 2 x 150 mL) and $CHCl_3$ (100 mL) to give a crude extract (2.60 g, P388 IC_{50} 24 g/mL). A portion of the plant extract (2.60 g) was absorbed on octadecylfunctionalized silica gel (5.20 g, Aldrich Cat. 37, 763-5) by slurrying the absorbent in a solution of the extract in EtOH - H_2O (Ca 5.2 mL, mainly EtOH) and removing the solvent under reduced pressure. This was then loaded on a cotton wool pad at the top of a column of the C_{18}-silica gel (26 g, 20 × 2 cm) which had been dry-packed and then washed successively with CH_3CN, CH_3CN - H_2O (1 : 1), and H_2O (each ca. 50 mL). An

extract of *B. monroi* showed cytotoxic effects (P 388, IC_{50} 23.96 μg/mL at 75 mg/disc) against monkey kidney (BSC) cell lines. Chromatography on C-18 with a CH_3CN - H_2O, CH_3CN, $CHCl_3$ gradient gave a major active fractions which eluted with CH_3CN - H_2O, 9 : 1 (245 mg, IC_{50} 8.0 μg/mL). Silica column chromatography using an EtOAC : hexane gradient gave pure 13(*E*)-labd-13-ene-8α,15-diol (1) (51 mg). Structure (1) was derived from a combination of NMR experiments and confirmed by comparison with published NMR data[8]. Both (1) and its enantiomer have been reported from a variety of natural sources; the absolute stereochemistry shown was supported by a small negative optical rotation[9]. Compound (1) was toxic to P388 murine leukaemia cells (IC_{50} 8.29 μg/mL) and to BSC cells (25 % of well at 60 μg/well). Cytotoxic activity against a range of murine leukaemia cell lines (IC_{50}'s 11.4 to > 50 g/mL) has been reported for 13(*E*)-labd-13 -ene-8,15-diol (1) isolated from another plant, *Cistus creticus* (Cistaceae)[3]. This is the first report of any compounds from *B. monroi*[4]. *B. repanda* was used by Maori for treatment of sores and wounds[4], but 13(*E*)-labd-13-ene-8α,15-diol (1) has been found in *B. bidwillii* (Fig. 1)[5].

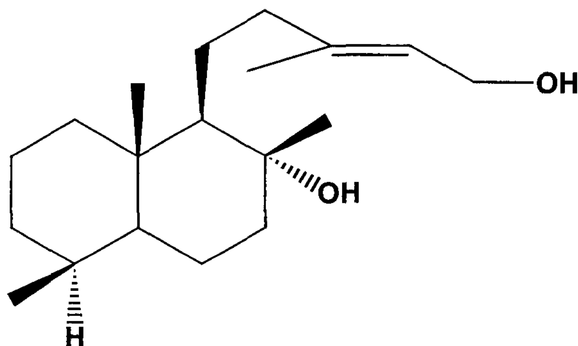

Fig. 1. The structure of 13(*E*)-labd-13-ene-8α,15-diol (1)

Table 1. Inhibition of tumor cell proliferation by 13(*E*)-labd- 13-ene-8 α,15-diol (1) from *B. monroi* by the MTT method.

1[a]	IC_{50} (μg/mL)[b]				
P388	B16/F10	MDA-MB-231	A549	KB	SNU-C4
8.29	16.32	16.33	20.27	20.43	21.30

a It was examined in three concentration in triplicate experiments. bIC50value of 13(E)-labd-13-ene-8α,15-diol (1) against each cancer cell lines, defined as the concentration that caused 50 % inhibition of cell proliferation *in vitro*.

Table 1 shows the potent cytotoxic activity of 13(*E*)-labd-13-ene-8α,15-diol (1) against cancer tumor cell lines. In general, the cytotoxic activity of this compound was in a dose-dependent manner, and the susceptibility of the cancer cell lines to 13(*E*)-labd-13-ene-8α,15-diol (1) was quite sensitive. The values of MTT_{50} were determined at 8.29 μg/mL against P388 murine leukaemia cell lines. A colorimetric assay was used to detect the *in vitro* cytotoxicity mediated by 13(*E*)-labd-13-ene-8 α,15-diol(1). 13(*E*)-Labd-13-ene-8α,15-diol (1)-mediated cytotoxicity gradually increased in the MTT method against P388murine leukaemia cell lines when its concentration was increased from control to 8.29 μg/mL. However, this compound showed a changeable in the MTT method against B16/F10 and MDA-MB-231 cancer cell lines when their concentrations were increased from 16 to 32 μg/mL. A comparison of IC_{50} values of this compound in cancer cell lines showed that their susceptibility to this compound decreased in the following order: P388 > B16/F10 > MDA-MB-231> A549 > KB > SNU-C4 by the MTT method. B16/F10 and MDA-MB-231 tumor cell lines (approximately IC_{50} = 16 μg/mL) showed similar cytotoxicities such as A549 and SNU-C4 (approximately IC_{50}= 20 μg/mL). 13(*E*)-Labd-13-ene-8α,15 -diol (1) was the most effective growth inhibitor of P388 murine leukaemia cell lines, producing approximately 8.3 μg/mL of IC_{50} in the MTT method.

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References

1. Connor, H. E. and Edger, E. Name changes in the indigenous New Zealand flora, 1960-1986 and Nomina Nova IV, 1983-1986, N.Z. J. Bot. 25, 115-170, 1987.
2. Allan, H. H. Flora of New Zealand. Indigenous Tracheophyta. Psilopsida, Lycopsida, Filicopsida, Gymnospermae, Dicotyledones; DSIR: Wellington; Vol. I. 1961.
3. Dimas, K., Demetzos, C., Marsellos, M., Sotiriadou, R., Malamas, M. and Kokkinipoulos, D. Cytotoxic activity of labdane type deterpenes against human leukemic cell lines *in vitro*, Planta Med. 64, 208-211, 1998.
4. Riley, M. Maori Healing and Herbal. Paraparaumu, New Zealand: Viking Sevenseas N.Z.Ltd., 1994.
5. Bloor, S. J. and Gainsford, G. J. A novel clerodane-ascorbate adduct from *Brachyglottis bidwillii*. Aust. J. Chem. 46, 1099-1104, 1993.
6. Calabuig, M. T., Cortes, M., Francisco, C. G., Hernandez, R. and Suarez, E. Labdane diterpenes from Cistus synphytifolius. Phytochemistry, 20, 2255-2258, 1981.
7. Lorimer, S. D., Barns, G., Evans, A. C., Foster, L. M., May, B. C.H., Perry, N. B. and Tangney, R. S. Cytotoxicity and antimicrobial activity of plants from New Zealand's subantarctic islands. Phytomedicine, 2, 327-333, 1996.
8. Zahra, J. P., Chauvet, F., Coste-Maniere, I., Martres, P.,

Perfetti, P. and Waegall, B. Oxidative degradation of the sclareol side chain: Hemisyntheses of ambergris derivatives using in the key steps palladium complexes or ruthenium tetroxide generated situ. Bull. Soc. Chim. Fr. 134, 1001, 1997.
9. Carman, R. M. Optical rotation and structure in the labdane series of diterpenoids. Aust. J. Chem. 19, 629-642, 1966.