SHORT PAPER

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ent-Costunolide from the liverwort *Hepatostolonophora paucistipula*†

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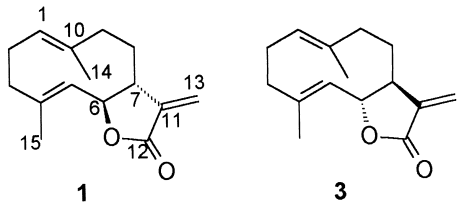
Bioactivity-directed isolation work on the New Zealand liverwort *Hepatostolonophora paucistipula* afforded the sesquiterpene lactones (-)-*ent*-costunolide (**1**) and (-)-*ent*-arbusculin B (**2**) as cytotoxic compounds. This is the first report of **1**, enantiomeric to the known germacranolide (+)-costunolide (**3**).

Keywords: Hepatostolonophora paucistipula, liverwort, *ent*-costunolide, sesquiterpenes

Liverworts have been a rich source of sesquiterpenes, including several new skeletal types.[1] When the same compounds have been isolated from liverworts and vascular plants, they are often enantiomeric.[1–3] On the other hand, both enantiomers of some sesquiterpene lactones have been reported from related liverwort species.[1] For example, (-)-*ent*-arbusculin B (**2**) has been isolated from *Frullania diltata* and *F. usamiensis*[4,5] and (+)-arbusculin B (**4**) from *F. serratta* and *F. muscicola*.[6,7] (+)-Costunolide (**3**) is known from vascular plants[8-10] and from liverworts.[11] We now report the isolation of (-)-*ent*-costunolide (**1**) for the first time.



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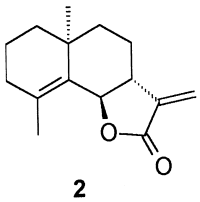

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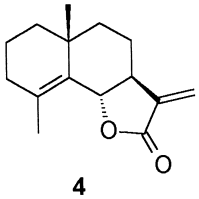

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Our search for bioactive compounds from New Zealand liverworts[3] led us to a cytotoxic extract of *Hepatostolonophora paucistipula* (Rodw.) J.J. Engel (family Geocalycaceae). There are no literature reports on the chemistry of this genus. Bioactivity-directed isolation work revealed two sesquiterpene lactones as the main active compounds.

The major sesquiterpene lactone had ^{1}H and ^{13}C NMR data matching those reported for (+)-costunolide (**3**).[8,9] However, the optical rotation ($[\alpha]_D$ –127°) was opposite to the literature values for **3** (+117°, +129°).[9-11] Therefore we propose that the compound is (-)-*ent*-costunolide (**1**), which does not seem to have been reported before (search of *Chemical Abstracts* Registry file).

The minor sesquiterpene lactone was identified as (-)-*ent*-arbusculin B (**2**) since it showed the same ^{1}H and ^{13}C NMR data as those reported for (+)-arbusculin B (**4**),[7,12] but had a negative optical rotation. We could only find two previous reports of the isolation of **2**, also known as (-)-γ-cyclocostunolide, from two *Frullania* species of liverwort.[4,5] It is not surprising that germacranolide **1** and eudesmanolide **2**, with the same rare 7α stereochemistry should co-occur, since eudesmanes are thought to be biosynthesised via germacrane intermediates.[13]

(-)-*ent*-Costunolide (**1**) was the main cytotoxic component in the extract with an IC_{50} of 0.7 μg/ml against P388 murine leukemia cells. (-)-*ent*-Arbusculin B (**2**) was also cytotoxic with an IC_{50} of 1.1 μg/ml. An IC_{50} of 0.57 μg/ml against KB carcinoma cells has been reported for (+)-costunolide (**3**).[14] The previous reports of **2** did not mention biological activity, but (+)-arbusculin B (**4**) showed toxic effects against Chinese hamster ovary cells at 12.5 μg/ml, as did (+)-costunolide (**3**).[15] There is a report that enantiomers of another sesquiterpene lactone had significantly different cytotoxicities against KB cells, but the difference was not great: IC_{50}'s of 3.3 ± 0.3 and 2.1 ± 0.7 μg/ml.[2]

Experimental

General procedures: All solvents were distilled before use and were removed by rotary evaporation at temperatures up to 35°C. Octadecyl functionalised silica gel (C18, Aldrich) was used for reversed-phase flash chromatography, and Davisil, 35-70 μm, 150 Å was used for silica gel flash chromatography. Preparative silica gel TLC was carried out using Merck DC-Fertigplatten Kieselgel 60 F_{254}. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Mass, UV, and IR spectra were recorded on Kratos MS-80, Shimadzu UV 240, and Perkin-Elmer 1600 FTIR instruments, respectively. NMR spectra, at 25°C, were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C on a Varian VXR-300 spectrometer. Chemical shifts are given in ppm on the δ scale referenced to the solvent peaks C**H**Cl_3 at 7.25 and **C**DCl_3 at 77.0. For the P388 assay a two-fold dilution series of the sample was incubated for 72 hrs with murine leukemia cells (ATCC CCL 46 P388D1). The concentration of the sample required to inhibit cell growth to 50% of the growth of a solvent control (IC_{50}) was determined using the absorbance obtained upon staining with MTT tetrazolium. As a positive control for this assay, mitomycin C at a concentration of 0.06 μg/ml inhibited the growth of P388 cells by 43-75%.

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.

Extraction and isolation: Air dried whole plant (76.3 g) was ground and blended with EtOH (1000 ml, 3 × 300 ml) to give a crude extract (1.585 g, IC_{50} 2.5 μg/ml). A sub-sample (0.836 g) was subjected to flash chromatography on C18 (10 g) with a H_2O : MeCN : $CHCl_3$ gradient. The most cytotoxic fraction was eluted with 1 : 3 H_2O : MeCN (217 mg, IC_{50} 0.6 μg/ml). A sub-sample (96 mg) was

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† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M).*

subjected to flash chromatography on silica gel (1 g) with a hexane : EtOAc gradient. A fraction eluted with 1 : 19 hexane : EtOAc (6 mg, IC_{50} 1.5 μg/ml) was subjected to preparative silica gel TLC (1 : 4 hexane : EtOAc) to give arbusculin B **2** (4 mg, R_F 0.56). A silica column fraction eluted with 1 : 9 hexane : EtOAc (44 mg, IC_{50} 0.3 μg/ml) was subjected to preparative silica gel TLC (1 : 9 hexane : EtOAc) to give costunolide **1** (14 mg).

(-)-*ent-Arbusculin B (2) (CAS Registry No. 62929-54-8):* colourless gum; $[\alpha]^{25}_{D}$ –97°, $[\alpha]^{25}_{577\ nm}$ –128°, $[\alpha]^{25}_{546\ nm}$ –164°, $[\alpha]^{25}_{435\ nm}$ –424°, $[\alpha]^{25}_{405\ nm}$ –502° (*c* 0.1, $CHCl_3$), lit. $[\alpha]_D$ –35° ($CHCl_3$);[5] ^{1}H and ^{13}C NMR spectra as published.[7,12]

(-)-*ent-Costunolide* (1): colourless gum; $[\alpha]^{25}_{D}$ –127°, $[\alpha]^{25}_{577\ nm}$ –175°, $[\alpha]^{25}_{546\ nm}$ –233°, $[\alpha]^{25}_{435\ nm}$ –621°, $[\alpha]^{25}_{405\ nm}$ –748° (*c* 0.075, $CHCl_3$), lit. $[\alpha]_D$ –35° ($CHCl_3$); UV (MeOH) λ_{max} (log ε) 224 (4.00) nm; IR (film) ν_{max} 2921, 2856, 1763, 1665, 1442, 1382, 1289, 1246, 1137, 968, 755 cm^{-1}; ^{1}H NMR ($CDCl_3$) 6.27 (1H, d, *J*=3.6 Hz, H-13), 5.53 (1H, d, *J*=3.3 Hz, H-13), 4.86 (1H, br dd, *J*=6.6, 10.8 Hz, H-1), 4.75 (1H, br d, *J*=10.2 Hz, H-5), 4.58 (1H, dd, *J*=8.7, 9.6 Hz, H-6), 2.58 (1H, m, H-7), 2.46 (1H, br dd, *J*=3.9, 13.5 Hz), 2.4 – 2.0 (6H, m), 1.71 (3H, d, *J*=1.2 Hz, H-15), 1.67 (1H, m), 1.43 (3H, br s, H-14); ^{13}C NMR spectrum as published;[8] EIMS *m/z* 232.1464 $[M]^+$ (26, calcd for $C_{15}H_{22}O_2$ 232.1463), 217 (25), 149 (36), 121 (100), 81 (61).

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References

1 Y. Asakawa, *Progr. Chem. Org. Nat. Prod*. 1995, **65**, 1.
2 Y. Ohta, N.H. Andersen and C.-B. Liu, *Tetrahedron*, 1977, **33**, 617.
3 S.D. Lorimer, E.J. Burgess and N.B. Perry, *Phytomedicine*, 1997, **4**, 273.
4 Y. Asakawa, R. Matsuda and A. Cheminat, *Phytochemistry*, 1987, **26**, 1117.
5 Y. Asakawa, J.-C. Muller, G. Ourisson, J. Foussereau and G. Ducombs, *Bull. Soc. Chim. Fr. (Chim. Mol.)*, 1976, 1465.
6 Y. Asakawa, X. Lin, K. Kondo and Y. Fukuyama, *Phytochemistry*, 1991, **30**, 4019.
7 L. Kraut, R. Mues and M. Sim-Sim, *Phytochemistry*, 1994, **37**, 1337.
8 F.S. El-Feraly, Y.-M. Chan, G.A. Capiton, R.W. Doskotch and E.H. Fairchild, *J. Org. Chem.*, 1979, **44**, 3952.
9 H.-J. Park, W.-T. Jung, P. Basnet, S. Kadota and T.J. Namba, *Nat. Prod.*, 1996, **59**, 1128.
10 V. Kumar and S. Saminathan, *Planta Med.*, 1995, **61**, 587.
11 Y.C. Kim, V.d.S. Bolzani, N. Baj, A.A.L. Gunatilaka and D.G.I. Kingston, *Planta Med.*, 1996, **62**, 61.
12 A.E. Greene, J.-C. Muller and G. Ourisson, *J. Org. Chem.*, 1974, **39**, 186.
13 D.E. Cane, *In Isoprenoids including carotenoids and steroids*; D.E. Cane, ed.; Elsevier; Amsterdam, 1999, p 155-pp 200.
14 S.M. Kupchan, M.A. Eakin and A.M. Thomas, *J. Med. Chem.*, 1971, **14**, 1147.
15 B.F. Abeysekera, Z. Abramowski and G.H.N. Towers, *Biochem. Syst. Ecol.*, 1985, **13**, 365.