Antibacterial and Antifungal Polyine Compounds from *Glehnia littoralis* ssp. *leiocarpa*

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Abstract: *Glehnia littoralis* F. Schmidt ssp. *leiocarpa* (Mathias) Hult. (Apiaceae), a species of ethnopharmacological interest in British Columbia, has antibacterial and antifungal properties. Antibacterial and antifungal compounds include two hitherto unreported polyine compounds, (9*Z*)1,9-heptadecadiene-4,6-diyne-3,8,11-triol and (10*E*)1,10-heptadecadiene-4,6-diyne-3,8,9-triol.

Key words: *Glehnia littoralis*, Apiaceae, antibiotic activity, traditional medicine.

Introduction

Glehnia littoralis F. Schmidt ssp. *leiocarpa* (Mathias) Hult. (Apiaceae) is a perennial herb growing on sandy beaches of Pacific countries. The root, described in the Chinese and Japanese Pharmacopoeias, is used as a diaphoretic, antipyretic, and analgesic medicine. This species was also used by native peoples of North America. The Haida people used the roots of this herb to treat bladder infections and other ailments (Turner, N. personal comm.).

In the course of our research on antibiotics from British Columbian plants of ethnobotanical interest, we report here the isolation and characterization of antibacterial and antifungal compounds **1**–**6** from *Glehnia littoralis*. Compounds **5** and **6**, hitherto unreported compounds, were identified as (9*Z*)1,9-heptadecadiene-4,6-diyne-3,8,11-triol (**5**), and (10*E*)1,10-heptadecadiene-4,6-diyne-3,8,9-triol (**6**). The minimal inhibitory concentrations (MICs) of each compound were determined by the broth dilution method.

Materials and Methods

IR spectra were acquired with a Perkin-Elmer 1600 spectrophotometer, and UV spectra with a Philips PU 8720 UV/VIS scanning spectrophotometer. EI- and EI-HR-MS were measured on a Kratos MS 50. ^{1}H- and ^{13}C-NMR were determined on a Bruker WH-400 and a Varian XL-300 with TMS as an internal standard. HMQC and HMBC experiments were carried out with a Bruker AM-500 (500 MHz, $CDCl_3$). Silica gel (Art 60, 230–400 mesh) for column chromatography (CC), silica gel (Kieselgel 60 F_{254}) coated on aluminum plates for thin layer chromatography (TLC), and silica gel (Kieselgel 60 F_{254}) coated on glass plates (20 cm × 20 cm) for preparative thin layer chromatography (pTLC) were supplied by E. Merck. Sephadex LH-20 for CC was obtained from Pharmacia Fine Chem., and the μBONDAPAK C_{18} steel column (7.8 × 300 mm) for high performance liquid chromatography (HPLC) was supplied from Waters.

Plant material

Roots of *Glehnia littoralis* were collected from the beach at Masset, B. C. in July, 1993. A voucher specimen was deposited in the herbarium of the University of British Columbia (Accession No. 208 174).

Bioassay

Complete details for a bacterial overlay thin layer chromatography assay and minimal inhibitory concentration (MIC) measurements have been described previously (1, 2).

Test organisms

Laboratory strains of bacteria and fungi were obtained from the University of British Columbia collection.

Extraction and separation of compounds

A dried and powdered root (814 g) of *Glehnia littoralis* was extracted with MeOH. The syrupy liquid remaining after rotary evaporation was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was chromatographed by silica gel CC [180 g, MeOH : $CHCl_3$, 0 : 100 (1000 ml), 5 : 95 (1000 ml), 10 : 90 (1000 ml), 20 : 80 (1500 ml), v/v] to give 4 fractions (A–D). Growth inhibitory effects against *Bacillus subtilis* Vernon, methicillin-sensitive *Staphylococcus aureus* K147, *Pseudomonas aeruginosa* H188, and *Candida albicans* 105 were detected in fraction A, eluted with a solvent system of MeOH : $CHCl_3$ (0 : 100, v/v), fraction B, eluted with a solvent system of MeOH : $CHCl_3$ (5 : 95, v/v), and fraction C, eluted with a solvent system of MeOH : $CHCl_3$ (10 : 90, v/v), using a bacterial overlay thin layer chromatography assay (1). The fraction A contained at least three active compounds, and each compound exhibited UV absorption at 254 nm. R_f values were 0.66, 0.58, and 0.50, respectively, by silica gel TLC, using EtOAc : benzene (4 : 6, v/v). These compounds showed a pale brown color on treatment with a vanillin-sulfuric acid reagent. One of the active compounds in fraction B exhibited brown color on treatment with

Planta Medica 62 (1996) 256–259

the vanillin-sulfuric acid reagent and had an R_f value of 0.3, using a solvent system of MeOH : $CHCl_3$ (10 : 90, v/v). Two of the active compounds existing in fraction C had R_f values of 0.30 and 0.25, respectively, by silica gel TLC, using a solvent system of MeOH : $CHCl_3$ (10 : 90, v/v). Based on these observations and monitoring the activity by a bacterial thin layer chromatography assay for each purification step, rechromatography of fraction A was carried out on silica gel CC (200 g, Et_2O : *n*-hexane, 50 : 50, v/v), Sephadex LH-20 (20 g, MeOH : $CHCl_3$, 50 : 50, v/v), and pTLC (MeOH : $CHCl_3$, 1 : 99, v/v, R_f = 0.56) to afford **1** (10.8 mg) which had an R_f value 0.38 on silica gel TLC, using $CHCl_3$ as a solvent system. Further rechromatography of another active fraction derived from the fraction A by pTLC ($CHCl_3$, R_f = 0.25) and HPLC (μBONDAPAK C_{18}, MeOH : H_2O, 80 : 20, v/v, 2 ml/min, detection by UV light absorption at 210 nm) gave **2** (12.4 mg) and **3** (7.9 mg). Rechromatography of fraction B by silica gel CC (100 g, Et_2O : *n*-hexane, 50 : 50, v/v), (70 g, MeOH : $CHCl_3$: AcOH, 3 : 97 : 1, v/v/v), and (30 g, MeOH : $CHCl_3$, 4 : 96, v/v) gave **4** (450 mg). Rechromatography of fraction C by silica gel CC (100 g, MeOH : $CHCl_3$, 5 : 95, v/v), (150 g, MeOH : $CHCl_3$: AcOH, 3 : 97 : 1, v/v/v), (150 g, EtOAc : C_6H_6 : AcOH, 40 : 60 : 1, v/v/v), (20 g, MeOH : $CHCl_3$, 6 : 94, v/v), and pTLC /MeOH : $CHCl_3$, 10 : 90, v/v, R_f = 0.25) gave **6** (47 mg). Another active fraction derived from fraction C was purified by silica gel CC (150 g, EtOAc : C_6H_6 : AcOH, 40 : 60 : 1, v/v/v), Sephadex LH-20 (20 g, MeOH : $CHCl_3$, 50 : 50, v/v), and silica gel CC (30 g, MeOH : $CHCl_3$, 5 : 95, v/v) to afford **5** (74 mg) which had an R_f value of 0.25 on silica gel TLC, using MeOH : $CHCl_3$ (4 : 96, v/v) as a solvent system.

(9Z)1,9-Heptadecadiene-4,6-diyne-3,8-diol (falcarindiol) (**4**): $[\alpha]_D^{24}$: +219.4° ($CHCl_3$, *c* 4.6); ^{1}H-NMR: Table **1**; ^{13}C-NMR: Table **2**.

(9Z)1,9-Heptadecadiene-4,6-diyne-3,8,11-triol (**5**): $[\alpha]_D^{24}$: +83.7° ($CHCl_3$, *c* 0.09); EI-MS: *m/z* (rel. int.) = 276 $[M]^+$ (0.4), 221 (2), 118 (100), 105 (31), 91 (38), 77 (30), 69 (31), 55 (40); EI-HR-MS: 276.1729 (calcd. for $C_{17}H_{24}O_3$: 276.1725); IR: ν_{max}^{film} = 3382, 2930, 2858, 2150, 1434, 1286, 1118, 1016, 933 cm^{-1}; ^{1}H-NMR: Table **1**; ^{13}C-NMR: Table **2**.

(10E)1,10-Heptadecadiene-4,6-diyne-3,8,9-triol (**6**): $[\alpha]_D^{24}$: −19.0° ($CHCl_3$, *c* 0.06); EI-MS: *m/z* (rel. int.) = 258 $[M - H_2O]^+$ (0.3), 141 (22), 118 (100), 105 (13), 81 (30), 69 (19), 57 (47); EI-HR-MS: 258.1621 $[M - H_2O]^+$ (calcd. for $C_{17}H_{22}O_2$: 258.1620); IR: ν_{max}^{film} = 3338, 2926, 2856, 2152, 1708, 1670, 1651, 1410, 1283, 1013 cm^{-1}; ^{1}H-NMR: Table **1**; ^{13}C-NMR: Table **2**.

Table 2 ^{13}C-NMR for **4**, **5**, and **6** (50.3 MHz[a], 75.0 MHz[b], $CDCl_3$).

	4[a,c]	5[b]	6[b]
C-1	117.2	117.4	117.7
C-2	135.8	135.9[d]	135.9
C-3	63.3	63.3	63.6
C-4	78.8	79.2[e]	78.3[f]
C-5	70.3	70.1	70.5[g]
C-6	68.8	69.1	70.4[g]
C-7	78.3	78.5[e]	78.4[f]
C-8	58.4	67.8	66.8
C-9	127.7	129.5	75.8
C-10	134.3	135.7[d]	126.6
C-11	31.8	58.5	136.8
C-12	29.3	37.0	32.7
C-13	29.2	31.8	32.0
C-14	29.1	29.2	29.2
C-15	27.6	25.2	29.1
C-16	22.6	22.6	22.9
C-17	14.2	14.1	14.4

[c] Assignments were given according to literature (8).
[d,e,f,g] Interchangeable.

Table 1 ^{1}H-NMR assignments for **4** (200 MHz, $CDCl_3$) and **5**, **6** (400 MHz, $CDCl_3$).

^{1}H (ppm)	4	5	6
Ha-1	5.47 (dt, *J* = 17.0, 1.0 Hz)	5.45 (d, *J* = 17.0 Hz)	5.48 (d, *J* = 17.1 Hz)
Hb-1	5.25 (dt, *J* = 10.0, 1.0 Hz)	5.23 (d, *J* = 9.9 Hz)	5.24 (d, *J* = 10.2 Hz)
H-2	5.94 (ddd, *J* = 17.0, 10.0, 5.8 Hz)	5.92 (ddd, *J* = 17.0, 9.9, 5.1 Hz)	5.92 (ddd, *J* = 17.1, 10.2, 5.3 Hz)
H-3	4.95 (br. d, *J* = 5.8 Hz)	4.92 (br. d, *J* = 5.1 Hz)	4.92 (br. d, *J* = 5.0 Hz)
H-8	5.20 (d, *J* = 8.0 Hz)	5.27 (br. d, *J* = 8.5 Hz)	4.23 (br. d, *J* = 6.3 Hz)
H-9	5.51 (m)	5.64 (dd, *J* = 10.9, 7.3 Hz)	4.11 (br. t, *J* = 6.5 Hz)
H-10	5.51 (m)	5.58 (dd, *J* = 10.9, 7.3 Hz)	5.50 (dd, *J* = 15.4, 6.8 Hz)
H-11	2.10 (m)	4.42 (br. q, *J* = 7.0 Hz)	5.85 (dt, *J* = 15.4, 6.8 Hz)
Ha-12	1.25 (m)	1.59 (m)	2.05 (m)
Hb-12	1.25 (m)	1.47 (m)	2.05 (m)
H-13 | H-16	1.25 (8H, m)	1. 34 (8H, m)	1.45–1.15 (8H, m)
H-17	0.89 (t, *J* = 6.1 Hz)	0.88 (t, *J* = 6.1 Hz)	0.86 (t, *J* = 6.6 Hz)
-OH		3.34 (br. s)	2.68 (br. s)
-OH		2.47 (br. s)	2.48 (br. s)
-OH		2.47 (br. s)	2.24 (br. s)

Results and Discussion

The root of *Glehnia littoralis* extracted with MeOH and subsequently with $CHCl_3$ showed inhibitory properties against *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Candida albicans.* The $CHCl_3$ soluble layer was further fractionated to give purified compounds **1**–**6**.

Compounds **1**–**4** were determined to be psoralen (**1**), bergapten (**2**), xanthotoxin (**3**), and falcarindiol (**4**) by direct comparison of ^{1}H-NMR spectra with published data (3, 4, 5, 6, 7).

Compounds **5** and **6** were isolated as pale yellow syrups. By means of EI-HR-MS experiments the formulae of **5** and **6** were both determined to be $C_{17}H_{24}O_3$ {276.1729 $[M]^+$ (calcd. for $C_{17}H_{24}O_3$: 276.1725) for **5** and 258.1621 $[M - H_2O]^+$ (calcd. for $C_{17}H_{22}O_2$: 258.1620) for **6**}. The IR spectrum showed absorptions of hydroxy (3382 cm^{-1} for **5** and 3338 cm^{-1} for **6**) and acetylenic (2150 cm^{-1} for **5** and 2152 cm^{-1} for **6**) groups. It was proved that both compounds had three -OH moieties which were exchanged with D_2O. The ^{1}H- and ^{13}C-NMR spectra of both compounds showed a close resemblance to those of falcarindiol (**4**) (Tables **1** and **2**). This observation suggested the structures of **5** and **6** contained a polyine skeleton like falcarindiol (**4**). By means of the ^{1}H-^{1}H COSY experiments the partial structures were proved (Hab-1/H-2/H-3 and H-8/H-9/H-10/H-11/H-12 for **5** and H-1/H-2/H-3 and H-8/H-9/H-10/H-11/H-12 for **6**). The configurations of the olefin bonds of -^{9}CH=^{10}CH- of **5** and -^{10}CH=^{11}CH- of **6** were determined to be *Z* and *E*, respectively, by the coupling constants ($J_{10\text{-}9}$ = 10.9 Hz for **5** and $J_{11\text{-}10}$ = 15.4 Hz for **6**). Considering the δ values of H-3, H-8, and H-11 of **5** and H-3, H-8, and H-9 of **6** in ^{1}H-NMR spectrum, the -OH moieties were proved to be attached with the carbons of C-3, C-8, and C-11 of **5** and C-3, C-8, and C-9 of **6**. Because the multiplicities of H-3 and H-8 of **5** and **6** were br d, it was determined that the carbons C-3 and C-8 of **5** and **6** were directly attached with acetylenic carbons (Fig. **1**). In order to confirm these structures, HMQC and HMBC experiments were carried out. The close peaks of the HMQC and HMBC spectra supported the structures of **5** and **6**. Therefore structures of **5** and **6** were determined to be (9*Z*)1,9-heptadecadiene-4,6-diyne-3,8,11-triol (**5**) and (10*E*)1,10-heptadecadiene-4,6-diyne-3,8,9-triol (**6**) (Fig. **1**). The partial assignments of protons and carbons were determined by means of HMQC and HMBC experiments and comparing the assignments of falcarindiol (8). (Table **1**).

The antimicrobial activities of **1**–**6** were examined by a broth dilution method (2). Psoralen (**1**), bergapten (**2**), and xanthotoxin (**3**) did not have any growth inhibitory effect under 400 μg ml^{-1} against all test microorganisms. Falcarindiol (**4**), which has been previously reported as an antibiotic by Muir et al. (9), showed a growth inhibitory effect against *Escherichia coli* DC2 (MIC = 2.5 μg ml^{-1}), methicillin-sensitive *Staphylococcus aureus* K147 (MIC = 12.5 μg ml^{-1}), methicillin-resistant *S. aureus* SAP0017 (MIC = 25.0 μg ml^{-1}), and *Bacillus subtilis* Vernon (MIC = 12.5 μg ml^{-1}). (9*Z*)1,9-Heptadecadiene-4,6-diyne-3,8,11-triol (**5**) and (10*E*)1,10-heptadecadiene-4,6-diyne-3,8,9-triol (**6**) showed weak growth inhibition with MICs of around 200–400 μg ml^{-1} against *Pseudomonas aeruginosa* H188, *Escherichia coli* DC2, *Staphylococcus aureus* K147, *S. aureus* SAP0017, *Bacillus subtilis* Vernon, and *Candida albicans* 105.

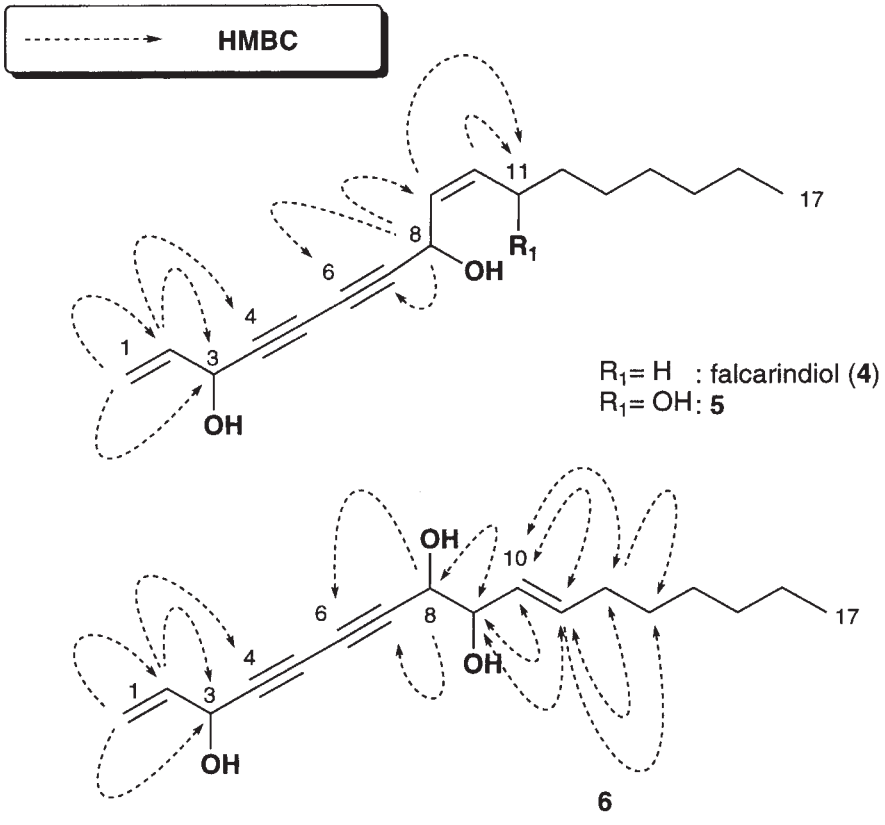

Fig. 1 The structures of falcarindiol (**4**), **5**, and **6** and HMBC experiments for **5** and **6**.

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