Sesquiterpene Quinones and Related Metabolites from *Phyllosticta spinarum*, a Fungal Strain Endophytic in *Platycladus orientalis* of the Sonoran Desert[1]

E. M. Kithsiri Wijeratne,[†] Priyani A. Paranagama,[†,§] Marilyn T. Marron,[†] Malkanthi K. Gunatilaka,[‡] A. Elizabeth Arnold,[‡] and A. A. Leslie Gunatilaka*[,†]

SW Center for Natural Products Research and Commercialization, Office of Arid Lands Studies, College of Agriculture and Life Sciences, University of Arizona, 250 E. Valencia Road, Tucson, Arizona 85706-6800, and Division of Plant Pathology and Microbiology, Department of Plant Sciences, College of Agriculture and Life Sciences, University of Arizona, Tucson, Arizona 85721-0036

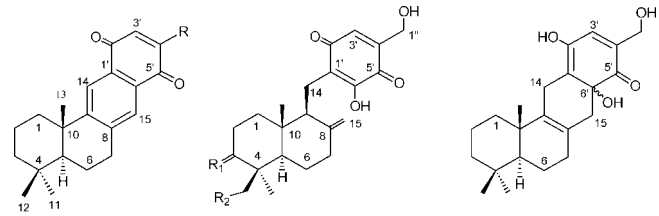
Received October 25, 2007

Five new metabolites, (+)-(5*S*,10*S*)-4′-hydroxymethylcyclozonarone (**1**), 3-ketotauranin (**3**), 3α-hydroxytauranin (**4**), 12-hydroxytauranin (**5**), and phyllospinarone (**6**), together with tauranin (**2**), were isolated from *Phyllosticta spinarum*, a fungal strain endophytic in *Platycladus orientalis*. The structures of the new compounds were determined on the basis of their 1D and 2D NMR spectroscopic data and chemical interconversions. All compounds were evaluated for inhibition of cell proliferation in a panel of five cancer cell lines, and only tauranin (**2**) showed activity. When tested in a flow cytometry-based assay, tauranin induced apoptosis in PC-3M and NIH 3T3 cell lines.

Plant-associated fungal strains are a rich source of structurally diverse and biologically active natural products.[2] In a continuation of our studies on plant-associated microorganisms of the Sonoran Desert for potential anticancer agents,[1] we have investigated an antiproliferative EtOAc extract of *Phyllosticta spinarum* (Botryosphaeriaceae), a fungal strain endophytic in the leaf tissue of oriental arbor-vitae (*Platycladus orientalis*; Cupressaceae), which is cultivated as an ornamental in southeastern Arizona. In this paper we report the isolation and structure elucidation of five new metabolites, (+)-(5*S*,10*S*)-4′-hydroxymethylcyclozonarone (**1**), 3-ketotauranin (**3**), 3α-hydroxytauranin (**4**), 12-hydroxytauranin (**5**), and phyllospinarone (**6**), along with tauranin (**2**). We also report the antiproliferative and apoptotic activity of tauranin (**2**) toward several cancer cell lines. Previous investigations of fungal strains of the genus *Phyllosticta* have resulted in the isolation of phycarone,[3,4] elsinochromes A−C,[5] phyllosinol,[6] phyllostine,[7] phyllostine II,[8] phyllosticta III,[8] several derivatives of cyclohex-2-en-1-one,[9,10] brefeldin A,[11] PM-toxins B and C,[12] and cholesterol.[13] Tauranin (**2**), a sesquiterpene quinone previously encountered in the mycelium of *Oospora aurantia*, a mold that grows on seeds of Japanese tea (*Thea japonica*),[14] has been reported to inhibit cholesterol biosynthesis.[15] It is noteworthy that a number of marine-derived sesquiterpene quinones have been reported to display inhibitory properties toward tyrosine kinases involved in cell signaling and proliferation.[16] This is the first report of metabolites from *P. spinarum* and the potential anticancer activity of tauranin (**2**).

Results and Discussion

Bioassay-guided fractionation of an antiproliferative EtOAc extract of *P. spinarum* involving solvent−solvent partitioning, size-exclusion chromatography, and preparative TLC furnished **1**−**6**. Compound **1** was obtained as a red crystalline solid that analyzed for $C_{22}H_{26}O_3$ by a combination of HRFABMS, ^{13}C NMR, and DEPT spectra and indicated 10 degrees of unsaturation. Its IR spectrum had absorption bands at 3425, 1690, and 1657 cm^{-1}, suggesting the presence of hydroxyl and carbonyl groups. Absorption bands at 272, 362, and 432 nm in its UV spectrum were



indicative of a 1,4-naphthoquinone moiety.[17] The presence of a 2(or 3),6,7-trisubstituted 1,4-naphthoquinone moiety in **1** was supported by its ^1H NMR signals at δ 7.97 (1H, s), 7.32 (1H, s), and 6.96 (1H, s) and the ^{13}C NMR signals for two carbonyl carbons (δ 181.7 and 178.8) and eight aromatic carbons (δ 153.2, 145.2, 141.4, 137.3, 131.4, 130.9, 128.6, and 127.5). A detailed analysis of the ^{13}C NMR spectrum of **1** with the help of HSQC data revealed the presence of three methyl carbons (δ 33.2, 24.4, and 21.6), six methylene carbons (δ 60.4, 41.4, 38.4, 30.9, 18.9, and 18.5) of which one is oxygenated, one methine carbon (δ 49.9), and two quaternary carbons (δ 38.2 and 33.5), in addition to the carbons assignable to a 1,4-naphthoquinone moiety (see above). The $^1H-^1H$ correlations observed in the DQF-COSY spectrum suggested the presence of $CH_2CH_2CH_2$ and CH_2CH_2CH spin systems. The ^1H and ^{13}C NMR spectroscopic data of **1** resembled those of (+)-cyclozonarone (**7**),[18] except that in **1** a CH_2OH group was found in place of the aromatic proton at δ 6.90 (d, J = 10.3 Hz), suggesting that **1** is a derivative of **7**. In the HMBC spectrum (Figure 1), the aromatic proton at δ 7.97 showed correlations with the quaternary carbon at δ_C 38.2, two quaternary aromatic carbons at δ_C 145.2 and 131.4, and the carbonyl carbon at δ_C 178.8. Thus, the remaining carbonyl carbon signal at δ 181.7 in the ^{13}C NMR spectrum of **1** was assigned to C-5′. The proton at δ 6.96 showed HMBC correlations with C-7 (δ 30.9), C-9 (δ 153.2), and C-1′ (δ 128.6), placing this proton at C-15. The presence of HMBC correlations from H_2-1″ (δ_H 4.49) to the carbonyl carbon (C-5′, δ_C 181.7), and the protonated aromatic carbon (C-3′, δ_C 141.4), unambiguously placed the CH_2OH group at C-4′. The absolute configuration of the naturally occurring (−)-cyclozonarone[19] has been established as 5*R*,10*R* by comparison of its spectroscopic data and optical rotation (−89.1) with those of (+)-cyclozonarone.[20] The optical rotation of **1** (+87.2) suggested that it is a derivative of (+)-cyclozonarone. The structure of **1** was thus elucidated as

* To whom correspondence should be addressed. Tel: (520) 741-1691. Fax: (520) 741-1468. E-mail: leslieg@ag.arizona.edu.
† SW Center for Natural Products Research and Commercialization, University of Arizona.
§ Present address: Department of Chemistry, University of Kelaniya, Sri Lanka.
‡ Department of Plant Sciences, University of Arizona.

10.1021/np070600c CCC: $37.00
Published on Web 02/05/2008