Metabolites from the Xylariaceous Fungus PSU-A80

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The family xylariaceae is a rich source of antimicrobial and/or antioxidant secondary metabolites, for example, carneic acids A—B,1a cohaerins C—F,2a daldinins C—F,3a ramulosin,4 and sassafrins A—D.5 During our ongoing search for biologically active metabolites from the endophytic fungi, the broth extract of the xylariaceous fungus PSU-A80, isolated from the leaves of Garcinia atroviridis, showed the antioxidant activity in 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical assay. To search for antioxidant metabolites, the ethyl acetate extracts from the culture broth and cell of the xylariaceous fungus PSU-A80 were subjected to various chromatographic separation. Three new compounds, one hypoxylonol, xylarenol (1), one hexadienoic acid, xylarenoic acid (2), and one tetralone, xylarenone (3), were isolated from the xylariaceous fungus PSU-A80 together with ten known compounds. The structures were established by analysis of spectroscopic data. 8-Methoxy-1-naphthol, one of the known metabolites, displayed good radical scavenging potency with an IC50 value of 30 µg/ml.

Key words Xyraliacea; hypoxylonol; hexadienoic acid; tetralone; antioxidant activity

Results and Discussion

Xylarenol (1) with the molecular formula C20H16O4 from HR-EI-MS,1H- and 13C-NMR data was isolated as a yellow gum. The UV and IR spectra were almost identical to those of 4,6 thus indicating that they possessed the same chromophore and functional groups. The 1H-NMR spectrum displayed two hydroxyls (δ 12.60, 8.22), three aromatic protons of a 1,2,3-trisubstituted benzene (δ 7.53, 7.36, 6.92), two ortho-coupled aromatic protons of a 1,2,3,4-tetrasubstituted benzene (δ 7.24, 6.77), two oxymethine protons (δ 5.67, 5.28), one methine proton (δ 4.05) and two sets of nonequivalent methylene protons (δ 3.39, 2.39 and 5.28, 2.02). These data were very similar to those of 4 except for the disappearance of a methoxyl signal in 1, suggesting the replacement of the methoxyl group in 4 with a hydroxyl group in 1. These results are in agreement with the molecular formula which indicated CH7 less than 4. Irradiation of H-3 (δ 5.28, dd, J=10.0, 4.5 Hz) in the NOE experiment affected the intensity of H-2 (δ 2.80, dt, J=12.5, 4.5 Hz) and 4-OH (δ 8.22, s) signals, but did not enhance the H-1 (δ 5.67, t, J=4.5 Hz) signal, indicating trans relationship between H-1 and H-3. Since H-1 was coupled with H, 2 and H-3 with a small coupling constant of 4.5 Hz, it was located at β-equatorial position. These results established the location of H-3 at α-axial. This assignment was supported by small and large coupling constants (J=4.5, 10.0 Hz) between H, 2 and H-3. H-6b appeared as a doublet of doublet at δ 4.05 with J values of 14.5 and 5.5 Hz. Accordingly, it was placed at axial position. However, the NOE experiment could not determine the relative configuration of H-6b. Consequently, xylarenol (1) was identified as a new hypoxylonol derivative.

Xylarenoic acid (2) with the molecular formula C9H12O4 from HR-EL-MS was isolated as a colorless gum. The IR spectrum showed hydroxyl (3373 cm−1) and two carboxyl (1720, 1689 cm−1) absorption bands. The 1H-NMR spectrum contained signals of trans-1,3-pentadienyl unit [δ 7.47 (1H, d, J=11.5 Hz), 6.54 (1H, d, J=15.0, 11.5, 1.5 Hz), 6.32 (1H, d, J=15.0, 6.9 Hz) and 1.94 (dd, J=6.9, 1.5 Hz)], one oxymethylene group (δ 4.92, s) and one methyl group (δ 2.07, 3H, s). The 13C-NMR spectrum showed two carboxyl carbons (δ 170.5, 169.5), one quaternary carbon (δ 121.0), three olefinic methine carbons (δ 145.5, 142.4, 125.6), one oxymethylene carbon (δ 56.9) and two methyl carbons (δ 19.9, 18.1). 3J HMBC correlations of H-3 (δ 7.47)C-1 (δ 169.5) and C-7 (δ 56.9) constructed a 2,4-hexadienoic acid skeleton bearing the oxymethylene group at C-2. The remaining methyl group (δ 2.07) was connected with the ester carbonyl carbon (δ 170.5) which gave a HMBC correlation with H-7 (δ 4.92). These results established the substituent at C-7 to be an acetoxyl group. Irradiation of H-4 (δ 6.54), in the NOE experiment, enhanced signal intensity of H-7 and H-6, indicating that both double bonds had E configuration.

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tion. Thus, xylarenoic acid (2) was identified as a new 2,4-hexadienoic acid.

Xylarenone (3) was obtained as a colorless gum with the molecular formula C11H12O3 from HR-ESI-MS. The UV and IR spectra were almost identical to those of 5. The 1H-NMR spectral data were very similar to those of 5 except that a hydroxyl signal at δ 12.43 (s) in 5 was replaced in 3, by a methoxyl signal at δ 3.86 (s). A HMBC correlation of the methoxy protons with C-8 (δ 160.1) and signal enhancement of these protons after irradiation of H-7 (δ 6.91, d, J=9.0 Hz) in the NOEDIFF experiment supported the assignment. The configuration of C-4 was assigned as S, identical to that of 5 ([α] +19),10 on the basis of their similar optical rotation. Therefore, xylarenone (3) was identified as a methyl ether of 5.

The crude ethyl acetate extracts of the broth and mycelia showed weak antioxidant activity using DPPH assay with IC50 values of 0.20 and 1.37 mg/mL, respectively. At the concentration of 50 μg/mL, compounds 1, 3, 4 and 8-methoxy-1-naphthol were able to trap the DPPH radical with % scavenging of 0.88, 2.65, 1.74 and 63.50, respectively. The remaining compounds showed no activity. 8-Methoxy-1-naphthol, the most potent antioxidant, gave the IC50 value of 30 μg/mL while the standard 2,6-di-tert-butyl-4-hydroxytoluene gave the IC50 value of 20 μg/mL. It is worth to note that 1, the demethylated derivative of 4, was less active than 3 whereas 3, the methylated derivative of 5, displayed better activity than 5.

A molecular identification of the xylariaceous fungus PSU-A80 was performed based on ITS sequence analysis because it did not produce conidia or spores. Its sequence matched with seven fungal sequences in the Family Xylariaceae, *Annulohypoxylon stygium* (DQ223761), *Hypoxylon stygium* (AJ390409), *H. atroroseum* (AF201712), *A. atroroseum* (DQ223734), and *A. atroroseum* (DQ223733) but with low bootstrap values (57%). It showed only 67% homology to *A. stygium* (DQ223761) and *A. stygium* (DQ223760). Since there are not yet significant molecular data in GenBank for meaningful comparisons to be made for this fungus, the isolate PSU-A80 was then assigned to Xylariaceae species (GenBank accession number EF350147).
(1H, m, H₂-3), 2.10 (1H, m, H₃-3). ¹³C-NMR (75 MHz) δ: 197.0 (s, C-1), 160.1 (s, C-8), 147.8 (s, C-4a), 134.7 (d, C-6), 118.8 (d, C-5), 112.0 (d, C-7), 68.5 (d, C-4), 56.1 (q, C-9), 36.3 (t, C-2), 31.3 (t, C-3).

FT-IR (neat)
nmax cm⁻¹: 3419, 1663. UV lmax (MeOH) nm (log e): 214 (3.99), 254 (3.64), 317 (3.34).

EI-MS m/z (% relative intensity): 192 (100), 164 (61), 135 (31), 136 (23); HR-EI-MS m/z: 192.0778 [M]⁺ (Calcd for C₁₁H₁₂O₃ 192.0786). [α]D₂⁸ 21.3° (c/0.03, CHCl₃).

Free Radical Scavenging Activity  This was carried out according to that of Yen and Hsieh.²² To different concentrations of a sample in methanol (0.5 ml each) was added 1 ml of a methanolic solution of 0.2 mM DPPH. After mixing thoroughly, the mixture was allowed to stand in the dark for 30 min and the absorbance at 523 nm was measured using methanol for the baseline correction. The results were then compared with that of the control prepared as above but without any sample. Radical scavenging activity was expressed as percentage and was calculated using the following formula:

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\text{%scavenging} = \left( \frac{A_{\text{sample}} - A_{\text{control}}} {A_{\text{control}}} \right) \times 100.
\]

For 8-methoxy-1-naphthol, the result was also presented as IC₅₀ (sample concentration that produced 50% scavenging of DDPH radical).

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References and Notes