

## SECONDARY METABOLITES WITH PLANT GROWTH REGULATOR ACTIVITY PRODUCED BY AN ENDOPHYTIC FUNGUS *Purpureocillium* sp. FROM *Solanum rostratum*

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Endophytic fungi are broadly distributed among plant tissues and associated with various parts such as leaves, branches, stems, roots, and shoots [1]. Common pathogenic species of endophytic fungi are isolated from different cultures.

Secondary metabolites are isolated from various living sources including plants, bacteria, and fungi. They protect plants from the effects of herbicides, herbivores, and phytopathogens and stimulate growth by inducing morphological, physiological, and biochemical changes in their hosts [2, 3]. Furthermore, many secondary fungal metabolites possess antibacterial, antifungal, and antitumor activity [4].

An potentially new endophytic fungus *Purpureocillium* sp. was isolated from leaves of *Solanum rostratum* Dunal. The fungi were cultivated in a shaker at 25°C at 140 rpm for 30 d. The fungal culture in 24 flasks with potato dextrose broth (PDB) was extracted with EtOAc (4 × 700 mL). The obtained extract was filtered and condensed under vacuum. The EtOAc extract (5.73 g) was chromatographed over a column of silica gel (180 g, 100–200 mesh) with elution by *n*-hexane–EtOAc (40:1→0:1) and EtOAc–MeOH (4:1→1:1) to afford 21 subfractions (SR84-1–Sr84-21) that were analyzed by TLC. Fraction SR84-15–16 (324.2 mg) was chromatographed over a column of silica gel (200–300 mesh) with gradient elution by *n*-hexane–EtOAc (25:1→1:1) and then sequentially rechromatographed over Sephadex LH-20 with elution by CHCl<sub>3</sub>–MeOH (1:1) to isolate compound **1** (33 mg). Chromatography of fraction SR84-17-19 (0.699 g) over a column of silica gel (200–300 mesh) with gradient elution by *n*-hexane–EtOAc (15:1→1:1) followed by separation by preparative HPLC using MeOH–H<sub>2</sub>O (67:33) isolated **2** (0.6 mg) and **3** (2.7 mg).

A comparison of spectral data (PMR, <sup>13</sup>C NMR, HSQC, HMBC) of the isolated compounds with the literature and a direct comparison with authentic samples identified them as adenosine (**1**) [5], cerevisterol (**2**) [6], and thymine (**3**) [7].

**Adenosine (1)**, light-orange powder (MeOH), C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>. <sup>1</sup>H NMR spectrum (600 MHz, CD<sub>3</sub>OD, δ, ppm, J/Hz): 3.75 (1H, dd, J = 12.5, 2.6, H-5'a), 3.89 (1H, dd, J = 12.5, 2.4, H-5'b), 4.17 (1H, dd, J = 5.1, 2.6, H-4'), 4.32 (1H, dd, J = 5.0, 2.6, H-3'), 4.74 (1H, dd, J = 6.1, 5.3, H-2'), 5.96 (1H, d, J = 6.4, H-1'), 8.18 (1H, s, H-2), 8.31 (1H, s, H-8). <sup>13</sup>C NMR spectrum (150 MHz, CD<sub>3</sub>OD, δ, ppm): 63.48 (C-5'), 72.66 (C-3'), 75.47 (C-2'), 88.18 (C-4'), 91.26 (C-1'), 121.05 (C-5), 142.00 (C-8), 150.02 (C-4), 153.50 (C-2), 157.60 (C-6) [5].

**Crevisterol (2)**, white powder (MeOH), C<sub>28</sub>H<sub>46</sub>O<sub>3</sub>. <sup>1</sup>H NMR spectrum (600 MHz, CD<sub>3</sub>OD, δ, ppm, J/Hz): 0.65 (3H, s, H-18), 0.85 (3H, d, J = 6.8, H-26), 0.87 (3H, d, J = 6.8, H-27), 0.94 (3H, d, J = 6.8, H-28), 1.04 (3H, d, J = 6.7, H-21), 1.06 (3H, s, H-19), 3.98 (1H, s, H-6), 4.58 (1H, m, H-3), 5.22 (1H, t, J = 7.2, H-7), 5.27 (1H, m, H-22), 5.27 (1H, m, H-23). <sup>13</sup>C NMR spectrum (150 MHz, CD<sub>3</sub>OD, δ, ppm): 12.81 (C-18), 18.22 (C-28), 18.90 (C-19), 20.09 (C-27), 20.47 (C-26), 21.66 (C-21), 23.04 (C-11), 24.03 (C-15), 29.17 (C-16), 31.77 (C-2), 33.92 (C-25), 34.39 (C-1), 38.16 (C-10), 40.52 (C-12), 40.73 (C-20), 41.81 (C-4), 44.37 (C-9), 44.37 (C-24), 44.71 (C-13), 55.91 (C-14), 57.41 (C-17), 68.40 (C-3), 74.24 (C-6), 76.95 (C-5), 119.07 (C-7), 133.24 (C-23), 137.02 (C-22), 143.76 (C-8) [6].

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**Thymine (3)**, light-yellow powder (MeOH), C<sub>5</sub>H<sub>6</sub>N<sub>2</sub>O<sub>2</sub>. <sup>1</sup>H NMR spectrum (600 MHz, CD<sub>3</sub>OD, δ, ppm, J/Hz): 1.85 (3H, d, J = 1.0, H-7), 7.22 (1H, d, J = 1.0, H-6). <sup>13</sup>C NMR spectrum (150 MHz, CD<sub>3</sub>OD, δ, ppm): 12.10 (C-7), 110.44 (C-5), 139.12 (C-6), 153.71 (C-2), 167 (C-4) [7].

All compounds exhibited plant growth inhibitory activity. Compound **3** had the strongest activity and decreased the root length of the control plant (*Amaranthus retroflexus*) up to 63.8% at the lowest tested concentration (4 μg/mL) and completely inhibited seed sprouting if the concentration was increased from 100 to 500 μg/mL. Compound **1** showed moderate activity at 100 μg/mL and suppressed root development by 53.3% as compared to the control while treatment at 500 μg/mL completely inhibited seed sprouting. Compound **2** showed the weakest activity at 100 and 500 μg/mL, decreasing root length up to 56.6% and 32.1%, respectively, as compared to the control. The growth of *A. retroflexus* sprouts corresponded to root growth but to a lesser extent. Thymine at concentrations of 100 and 500 μg/mL showed strong inhibitory activity as compared to the other compounds.

All compounds were isolated for the first time from the genus *Purpureocillium* and the *Purpureocillium* sp.

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