In the course of screening rhizosphere microflora of Sonoran desert plants for potential anticancer agents, an unidentified Penicillium sp. (Trichonaceae) isolated from the rhizosphere of the apache plume (Fallugia paradoxa D. Don; Rosaceae) was found to produce several cytotoxic substances. Fractionation of the cytotoxic EtOAc extract led to the isolation of a new anthraquinone (1), a known anthraquinone (2), and cytotoxic curvularins, 3–5. This is the first report of the natural occurrence of 2. Curvularins 3–5 have been reported as metabolites of several fungi including Penicillium sp. This paper briefly describes the isolation of 1–5, structure elucidation of 1, and evaluation of 1–5 for their ability to inhibit the growth of three sentinel tumor cell lines.
The cytotoxic 80% aqueous MeOH fraction was evaporated hexane and 80% aqueous MeOH (MeOH/H2O-80:20). A dark brown solid (1.18g), which was partitioned between extracts were evaporated under reduced pressure to afford a gel (2.2g) by elution with CH2Cl2/MeOH (92:8) afforded (9.3mg). Chromatography of fraction D (70.0mg) on silica gel (6.0g) by elution with hexane/acetone (4:1) to furnish 1 (45.6mg) and 5 (270.0mg) was further fractionated on silica gel (6.0g) to furnish 2 (8.1mg) and 3 (27.4mg). Fraction C separated by reversed phase TLC (eluant: 25% H2O in CH2Cl2/acetone (1:4) (300ml) and finally with MeOH (100ml). Forty two liters) was concentrated to one forth of its volume and extracted with EtOAc (3~1000ml). Combined EtOAc extracts were evaporated under reduced pressure to afford a dark brown solid (1.18g), which was partitioned between hexane and 80% aqueous MeOH (MeOH/H2O-80:20). The cytotoxic 80% aqueous MeOH fraction was evaporated under reduced pressure to obtain a dark brown solid (1.02g) which was subjected to gel permeation chromatography on a column of Sephadex LH-20 (30.0g) in hexane/CH2Cl2 (4:1) and eluted with hexane/CH2Cl2 (3:2) (600ml), CH2Cl2/acetone (1:4) (300ml), CH2Cl2/MeOH (1:1) (300ml) and finally with MeOH (100ml). Forty two fractions (50ml each) were collected and combined on the basis of their TLC profiles to yield five major fractions [A (219.2 mg), B (153.6 mg), C (276.1 mg), D (72.2 mg), and E (45.7 mg)] of which the fractions B, C and D were found to be cytotoxic. Chromatography of fraction B (150.0 mg) on silica gel (4.0 g) by elution with hexane/acetone (4:1) afforded a yellow solid (36.5 mg) which was further separated by reversed phase TLC (eluant: 25% H2O in MeOH) to furnish 2 (8.1mg) and 3 (27.4 mg). Fraction C (270.0 mg) was further fractionated on silica gel (6.0 g) using CH2Cl2/MeOH (95:5) to furnish 1 (45.6 mg) and 5 (9.3 mg). Chromatography of fraction D (70.0 mg) on silica gel (2.2 g) by elution with CH2Cl2/MeOH (92:8) afforded 4 (7.9 mg).

1, 3-Dihydroxy-6-hydroxymethyl-7-methoxyanthraquinone (1): Yellow powder; mp 272~274°C; UV λmax MeOH nm 218, 249, 432; IR νmax IR cm⁻¹ 3402, 2932, 1628, 1450, 1404, 1342, 1312, 1250, 1173, 1103; ¹H NMR (DMSO-d6, 500 MHz) δ 13.26 (1H, s, OH-1) 11.10 (1H, brs, OH-3), 7.76 (1H, s, H-5), 7.50 (1H, s, H-8), 7.05 (1H, d, J=2.0 Hz, H-4), 6.56 (1H, d, J=2.0 Hz, H-2), 5.58 (1H, brs, OH-15), 4.65 (2H, s, CH2-15), 3.94 (3H, s, CH-16); ¹³C NMR (DMSO-d6, 125 MHz) δ 186.3 (s, C-9), 182.3 (s, C-10), 164.4 (s, C-1), 164.2 (s, C-3), 160.6 (s, C-7), 151.4 (s, C-6), 134.7 (s, C-11), 134.1 (s, C-13), 118.3 (s, C-14), 116.8 (d, C-5), 116.2 (d, C-8), 110.1 (s, C-12), 108.2 (d, C-2), 107.0 (d, C-4), 62.2 (t, C-15), 56.4 (q, C-16); APCIMS+ve mode m/z 301 [M+1]+; APCIMS−ve mode m/z 299 [M−1]+; HRFAB-MS: calcld. for C16H13O6 [M+H] 301.0712; found: m/z 301.0712.

1,3-Dihydroxy-6-methyl-7-methoxyanthraquinone (2): Yellow powder; ¹H NMR (acetone-d6, 500 MHz) δ 7.66 (1H, s, H-5), 7.41 (1H, s, H-8), 7.16 (1H, d, J=2.4 Hz, H-4), 6.63 (1H, d, J=2.4 Hz, H-2), 3.93 (3H, s, OCH3-7), 2.49 (3H, s, H-15); ¹³C NMR (acetone-d6, 125 MHz) δ 187.7 (s, C-9), 183.3 (s, C-10), 167.9 (s, C-1), 161.5 (s, C-3), 162.0 (s, C-7), 147.8 (s, C-6), 136.1 (s, C-11), 135.5 (s, C-13), 121.0 (d, C-5), 120.3 (d, C-8), 119.2 (s, C-14), 111.6 (s, C-12), 109.2 (d, C-2), 107.8 (d, C-4), 56.8 (q, OCH3-7), 22.0 (t, C-15); APCIMS+ve mode m/z 285 [M+1]+; APCIMS−ve mode m/z 283 [M−1]+.

Dehydrocurvularin (3): Pale yellow needles; ¹H NMR (acetone-d6, 500 MHz) δ 6.78 (1H, d, J=15.5 Hz, H-10), 6.57 (1H, dq, J=15.5, 4.8 Hz, H-11), 6.36 (1H, d, J=2.4 Hz, H-4), 6.31 (1H, d, J=17.3 Hz, H-2), 3.61 (1H, d, J=17.7 Hz, H-2), 2.42 (1H, m, H-12a), 2.35 (1H, m, H-12b), 1.99 (1H, m, H-13), 1.85 (1H, m, H-14), 1.67 (1H, m, H-13), 1.62 (1H, m, H-14), 1.19 (3H d, J=6.4 Hz, CH3-16); ¹³C NMR (acetone-d6, 125 MHz) δ 197.7 (s, C-9), 172.3 (s, C-1), 166.5 (s, C-7), 163.6 (s, C-5), 150.1 (d, C-11), 140.0 (s, C-3), 133.1 (d, C-10), 116.2 (s, C-8), 114.2 (d, C-4), 103.3 (d, C-6), 73.4 (d, C-15), 44.2 (t, C-9), 35.3 (t, C-14), 33.7 (t, C-12), 25.5 (t, C-13), 22.8 (q, C-16); APCIMS+ve mode m/z 291 [M+1]+; APCIMS−ve mode m/z 289 [M−1]+.

11-Methoxycurvularin (4): Yellow oil; ¹H NMR (acetone-d6, 500 MHz) δ 9.13 (2H, brs), 6.42 (1H, d, J=2.0 Hz), 6.41 (1H, d, J=2.0 Hz), 6.34 (2H, m), 4.94 (1H, m), 4.79 (1H, m), 3.93 (2H, d, J=15.5 Hz), 3.63 (2H, d, J=15.5 Hz), 3.66 (1H, m), 3.60 (1H, m), 3.40 (1H, m), 3.30 (3H, s), 3.25 (3H, s), 3.05 (2H, m), 2.95 (1H, m), 1.64 (2H, m), 1.27−1.53 (1H, m), 1.09 (6H, m); APCIMS+ve mode m/z 323 [M+1]+; APCIMS−ve mode m/z 321 [M−1]+.

11-Hydroxycurvularin (5): Yellow oil; ¹H NMR (acetone-d6, 500 MHz) δ 9.13 (2H, brs), 6.40 (1H, d, J=2.4 Hz), 6.39 (1H, d, J=2.4 Hz), 6.33 (2H, m), 4.94 (1H, m), 4.81 (1H, m), 4.09 (1H, m), 3.99 (1H, m), 3.83 (2H, d,
\[ J = 15.5 \text{ Hz} \], 3.67 (2H, d, \( J = 15.5 \text{ Hz} \)), 3.53 (1H, m), 3.29 (1H, dd, \( J = 13.8, 3.0 \text{ Hz} \)), 3.05 (1H, m), 2.87 (1H, m), 1.69 (2H, m), 1.27–1.60 (8H, m), 1.10 (6H, m); APCIMS+ve mode m/z 309 [M+1]+; APCIMS−ve mode m/z 308 [M−1]+.

Cytotoxicity Bioassays

The tetrazolium-based colorimetric assay (MTT assay)\(^5\) was used for the in vitro evaluation of cytotoxicity to human non-small cell lung carcinoma (NCI-H460), human breast carcinoma (MCF-7), and human glioma (SF-268) cells.

Results and Discussion

The molecular formula of compound 1 was determined to be \( \text{C}_{16}\text{H}_{12}\text{O}_{6} \) on the basis of HRFABMS and NMR data. Its UV spectrum had absorption bands at 218, 249, 286 and 432 nm indicating it to be a 9,10-anthraquinone\(^6\), which was further supported by its \(^{13}\text{C}\) NMR spectrum with signals at \( \delta \) 186.3 and 182.3 for quinone carbonyl carbons\(^7\). \(^{1}\text{H}\) NMR spectrum of 1 had three \( \text{D}_{2}\text{O} \) exchangeable protons at \( \delta \) 13.26, 11.10, and 5.58, two aromatic 1H singlets at \( \delta \) 7.76 and 75.0, two aromatic meta-coupled 1H doublets (\( J = 2.0 \text{ Hz} \)) at \( \delta \) 7.05 and 6.56, a 2H singlet at \( \delta \) 4.65 and a singlet due to \( \text{OCH}_{3} \) at \( \delta \) 3.94. The chemical shift (\( \delta \) 4.65) of the 2H singlet indicated that it may be due to the \( \text{CH}_{3} \) of a \( \text{CH}_{2}\text{OH} \) group on an aromatic system. \(^{13}\text{C}\) NMR spectrum of 1, in addition to the two carbonyl carbon signals (see above) had signals due to 12 aromatic/olefinic carbons [of which 3 were oxygenated (\( \delta \) 164.4, 164.2, and 160.6), and 4 were protonated (\( \delta \) 116.8, 116.2, 108.2 and 107.0)], a \( \text{OCH}_{3} \) carbon (\( \delta \) 56.4), and a \( \text{CH}_{2}\text{OH} \) carbon (\( \delta \) 62.2). These data suggested that 1 is a 9,10-anthraquinone bearing two \( \text{OH} \), a \( \text{OCH}_{3} \), and a \( \text{CH}_{2}\text{OH} \) substituent. The multiplicities of the aromatic protons suggested that one of the aromatic rings of the 9,10-anthraquinone system is ortho disubstituted and the other meta disubstituted. The substitution pattern was determined by the analysis of NOESY and HMBC spectra. In the NOESY spectrum, the \( \text{OCH}_{3} \) protons and the \( \text{CH}_{2} \) protons (of the \( \text{CH}_{2}\text{OH} \)) showed strong cross-peaks to aromatic singlets at \( \delta \) 7.50 and 7.76, respectively, suggesting that these two substituents are in the ortho-disubstituted aromatic ring. In the HMBC spectrum of 1, the carbonyl carbon at \( \delta \) 182.3 showed strong correlations with the aromatic protons at \( \delta \) 7.76 (s) and 7.05 (d, \( J = 2.0 \text{ Hz} \)). Other key HMBC and NOESY correlations are depicted in Fig. 1. On the basis of the foregoing evidence, the structure of 1 was determined to be 1,3-dihydroxy-6-hydroxymethyl-7-methoxyanthraquinone.

Comparison of the \(^{1}\text{H}\) and \(^{13}\text{C}\) NMR data of 2 with those of 1 indicated that they are structurally related, the major difference being the presence of a \( \text{CH}_{3} \) in 2 in place of the \( \text{CH}_{2}\text{OH} \) in 1. This was supported by mass spectral data, which showed that the \( M^+ \) of 2 is 16 mass units less than that of 1. Identification of 2 as 1,3-dihydroxy-6-methoxy-7-methylanthaquinone was further supported by a detailed analysis of its HMQC and HMBC spectra. Although this anthraquinone has been synthesized previously\(^8\) this is the first report of its natural occurrence. Comparison of the \(^{1}\text{H}\) and \(^{13}\text{C}\) NMR spectral data of compounds 3–5 with those reported in the literature allowed these to be identified as dehydrocurvularin (3)\(^9\), 11-methoxycurvularin (4)\(^3\), and 11-hydroxycurvularin (5)\(^3\). Co-occurrence of the anthraquinones 1 and 2, and curvularins 3–5 in this \( \text{Penicillium} \) sp. is interesting as all these metabolites may arise from the same octa-ketide biogenetic precursor.

Compounds 1–5 were evaluated for in vitro cytotoxicity against a panel of three sentinel human cancer cell lines, NCI-H460 (non-small cell lung), MCF-7 (breast), and SF-268 (CNS glioma), recently recommended by the U.S. National Cancer Institute\(^10\). Cells were exposed to serial dilutions of test compounds for 72 hours in RPMI 1640 media supplemented with 10% fetal bovine serum, and cell viability was evaluated by the MTT assay\(^5\). As shown in Table 1, only curvularins 3–5 were found to be cytotoxic. As measured by the MTT assay, the concentrations resulting in 50% inhibition of cell proliferation/survival (IC\(_{50}\)) were found to range between 1.8 and 13.3 \( \mu \text{M} \). Compounds 3–5 showed moderate cytotoxic activity toward NCI-H460, and MCF-7 cell lines, but were less active against the SF-268 cell line. Although compounds
3–5 have been reported to inhibit sea urchin embryogenesis by acting on components of the mitotic apparatus and to effectively inhibit cell division4), this constitutes the first report of their ability to inhibit proliferation of human cancer cell lines. Studies to elucidate their molecular mechanism(s) of anticancer action are currently in progress.

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Table 1. Cytotoxicities of the compounds 1–5 against a panel of human tumor cell linesa.

<table>
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<th>Compound No.</th>
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<th>SF-268</th>
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*aResults are expressed as IC50 values in μM except for Taxol, which is in nM.

References


