Mycophenolic Derivatives from *Eupenicillium parvum*

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A new compound, euparic acid (1, C_{14}H_{18}O_{6}), and the known compounds 5,7-dihydroxy-4-methylphthalide (2), 6-(3-carboxybutyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (3), 6-(5-carboxy-3-methylpent-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (4), and 6-(5-carboxy-4-hydroxy-3-methylpent-2-enyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (5) were isolated from the EtOAc extract of *Eupenicillium parvum*. The structure of 1 was determined by interpretation of MS and homo- and heteronuclear 2D NMR spectroscopic data and confirmed by X-ray crystallography. The absolute configuration of 5 was determined via MPA ester derivatization.

Humate soil is organic matter that has a positive effect on the physical, chemical, and biological reactions of the soil, leading to an increase in soil fertility. Humate is comprised of humic acid, fulvic acid, some minerals, bacteria, and fungi, which facilitate the development of deep root systems, enhance beneficial microbial activity, and improve overall plant health and resistance to diseases.

Continuing our earlier studies on sourcing fungi for bioactive natural products, we have investigated the constituents of *Eupenicillium parvum*, isolated from a humate sample. Four known derivatives (2–5) of the antibiotic mycophenolic acid (MPA), previously isolated from the fermentation broths of several *Penicillium* species, and a new derivative (1) were found. Mycophenolic acid (4, C_{13}H_{16}O_5) is a potent inhibitor of human inosine 50'-monophosphate dehydrogenase (IMPDH), a key enzyme in the de novo biosynthesis of guanine nucleotide. Its prodrug, mycophenolate mofetil (MMF), is used as an immunosuppressive agent to prevent kidney allograft rejection. MPA has also been shown to possess cytotoxicity against cancer cell lines.

![Chemical structure of compounds](image)

Table 1. 1H and 13C NMR (δ, J in Hz in parentheses) Dataa of Compounds 1 and 3

| Compound | δH | δC |
|----------|-----|-----|
| 1        | 1H  | 13C |
| 2        | 1H  | 13C |
| 3        | 1H  | 13C |

*Based on COSY, HSQC, HMBC, and ROESY experiments.

1. ESIQTOFMS yielded a parent mass at m/z 263.0919 corresponding to the pseudomolecular ion [M – OH]+ (calcd C_{14}H_{18}O_5, m/z 263.0919). The 1H NMR spectrum of 1 (Table 1) exhibited signals for a secondary methyl group at δ 1.18 (3H, d, J = 6.8 Hz), an aromatic methyl group at δ 2.10, and a singlet signal at δ 5.24 (2H) corresponding to an aliphatic methylene bearing oxygen. These data were closely comparable to those of the known compound 5,7-dihydroxy-4-methylphthalalde (2) and suggested that compound 1 contains this nucleus in its structure.

2. The 13C NMR (Table 1) and DEPT spectra of 1 disclosed 14 carbons, which were indicative of a saturated carboxylic acid at δ 179.3 (C-5'), two aliphatic methylenes at δ 20.6, and 33.0, an aliphatic methine at δ 38.9, and a secondary methyl group at δ 17.0, and suggested a chain in the position C-6 of the nucleus, in which the 1H−1H-COSY experiment allowed the sequential assignment. On the basis of the above data, 1 was identified as a derivative of mycophenolic acid.
and its structure was established as 6-(3-carboxybutyl)-5,7-dihydroxy-4-methylphthalan-1-one (euparvic acid, 6). The HMBC and COSY spectra supported the structure assignments. Finally, the postulated structure was further validated by a single-crystal X-ray study (Figure 1).

With compound 3, both the $^{1}H$ and $^{13}C$ NMR (Table 1) spectroscopic data are similar to those of 1, but one difference in their $^{1}H$ NMR spectra was noted. An additional signal at $\delta 3.67$ suggests the presence of a methoxy group at C-5. The $^{13}C$ NMR (Table 1) and DEPT NMR spectra confirmed this observation. The structure was assigned and confirmed using HMBC and ROESY data. A literature search led to the identification of this compound by comparison of its morphological and DNA profile data with the library in the National Centre for Advanced Technologies, Lincoln University, New Zealand. A voucher specimen (UM-042106) has been deposited in the culture collection of the Medicinal Chemistry Department, University of Mississippi. The fungus was inoculated in 50 mL of potato-dextrose broth and kept for two weeks in stationary phase at 25 °C; then the mycelium and sporulation broth (1 mL) were seeded onto a medium in each flask consisting of 100 g of shredded wheat, 200 mL of low-pH Oxoid mycological broth, 2% yeast extract, and 20% sucrose in 2.8 L Fernbach flasks (22 flasks) followed by incubation for 22 days at 24 °C.

**Figure 1.** ORTEP-3 projection of compound 1, with the displacement ellipsoids drawn at the 50% probability level. The atoms of the minor component of the disorder in the molecule and the hydrogens of both components have been omitted for clarity.

**Table 2.** $^{1}H$ NMR Shifts of the MPA Ester of Compound 6 before and after the Addition of the Ba(ClO$_4$)$_2$ Salt

| $\delta_{1}^{t}$ MPA ester | $\delta_{1}^{t}$ MPA ester + Ba(ClO$_4$)$_2$ | $\Delta$ $\delta_{1}$ |
|---------------------------|------------------------------------------|---------------------|
| 1 $'$                      | 5.241                                    | +0.075              |
| 2 $'$                      | 5.530                                    | 0.002               |
| 3 $'$                      | 6.037                                    | 0.005               |
| 4 $'$                      | 6.012                                    | -0.006              |
| 5 $'$                      | 5.324                                    | +0.084              |

* 500 MHz, 6 in ppm.

**Figure 2.** Ba$^{2+}$ complex of the (S)-MPA ester of 6.

**Experimental Section**

**General Experimental Procedures.** The melting point was determined on a Opti-Melt MPA 100 instrument (Stanford Research Systems) and is uncorrected. Optical rotations were measured using a Rudolph Research Analytical Autopol V polarimeter. IR spectra were recorded using a Perkin-Elmer model spectrum-100 spectrophotometer. $^{1}H$ and $^{13}C$ NMR spectra were obtained on Bruker model AMX 500 and 400 NMR spectrometers with standard pulse sequences, operating at 500 and 400 MHz in $^{1}H$ and 125 and 100 MHz in $^{13}C$. Acetone-$d_{6}$, CD$_3$OD, and CD$_3$CN were used as solvents, and TMS was used as internal standard. High-resolution mass spectra (HRMS) were recorded on a Micromas Q-Tof Micro mass spectrometer with a lock spray source. Column chromatography was carried out on silica gel (70–230 mesh, Merck). Fractions obtained from column chromatography were monitored by TLC (silica gel 60 F$_{254}$, and preparative TLC was carried out on silica gel 60 PF$_{254}$+366 plates (20 × 20 cm, 1 mm thick).

**Fungal Material.** The particular humate used was collected in Cuba, NM, in March 2008 and was plated out on potato-dextrose agar (PDA) that was maintained at 24 °C, until discrete fungal colonies appeared. Samples were taken from colonies and kept on PDA slants in test tubes at 24 °C, then placed in a 4 °C refrigerator until used. The fungus was identified as *Esperculium parvum* by comparison of its morphological and DNA profile data with the library in the National Centre for Advanced Technologies, Lincoln University, New Zealand. A voucher specimen (UM-042106) has been deposited in the culture collection of the Medicinal Chemistry Department, University of Mississippi.

**Extraction and Isolation.** Following incubation, 300 mL of acetone was added to each flask, and the mycelia and the substrate were homogenized (Super Dispex, Tekmark Co., SD-45). The suspension was filtered and the filtrate concentrated under a vacuum at 50 °C. The residue was mixed with H$_2$O (200 mL), then extracted with EtOAc (500 mL × 3). The combined EtOAc extracts were dried over anhydrous Na$_2$SO$_4$ and concentrated under a vacuum. The EtOAc extract (7.0 g) was chromatographed on silica gel 60, 70–230 mesh (400 g), with fractions stepwise eluted with CH$_2$Cl$_2$–Me$_2$CO mixtures (8:2; 7.5:2.5; 7:3; 1:1), Me$_2$CO, and Me$_2$CO–MeOH (9:1) (each 200 mL), yielding four fractions. Fraction 1 (150 mg) was rechromatographed over silica gel, eluted with CHCl$_3$–EtOAc (9:1), to yield 5,7-dihydroxy-4-methylphthalide (2, 50 mg) and mycophenolic acid (3, 12 mg). Fraction 2 (200 mg) and fraction 3 (350 mg) were chromatographed over a silica gel 60 column eluted with a CHCl$_3$–MeOH gradient to yield five subfractions. Subfractions 3–5 were chromatographed by preparative TLC with CHCl$_3$–MeOH (19:1) three times, affording 6-(3-carboxybutyl)-7-hydroxy-5-methoxy-4-methylphthalan-1-one (4, 10 mg) and 6-(3-carboxybutyl)-5,7-dihydroxy-4-methylphthalan-1-one (4, 8 mg). From the last fraction, a solid was recovered and purified on a silica gel column eluted with CHCl$_3$–MeOH (50:1), followed by preparative...
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+ (M software.14 Hydrogen atoms were placed in their expected chemical projection. refinement cycles and were left as large ellipsoids in the final disordered pairs were too close to be separated after several attempts to isolate suitable quantities of euparvic acid will be performed to test its effects in a broader range of bioassays.

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