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Antibacterial and Radical Scavenging Epoxycyclohexenones and Aromatic Polyols from a Marine Isolate of the Fungus *Aspergillus*

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Abstract – Bioassay-guided fractionation of an organic extract of the broth from the marine-derived fungus of the genus *Aspergillus* led to the isolation of the polyketides, (+)-epoxydon (**1**), (+)-epoxydon monoacetate (**2**), gentisyl alcohol (**3**), 3-chlorogentisyl alcohol (**4**), and methylhydroquinone (**5**). Compounds **1-5** showed a potent antibacterial activity against the methicillin-resistant and multidrug-resistant *Staphylococcus aureus* (MRSA and MDRSA) with MIC (minimum inhibitory concentration) values of 12.5, 12.5, 12.5, 50.0, and 6.2 μg/mL, respectively. Compounds **1-4** also exhibited a significant radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) with IC_{50} values of 6.0, 15.0, 7.0, and 1.0 μM, respectively.
Keywords – *Aspergillus*, (+)-epoxydon, (+)-epoxydon monoacetate, gentisyl alcohol, 3-chlorogentisyl alcohol, methylhydroquinone, antibacterial activity, radical scavenging activity

Introduction

Multidrug-resistant strains of many clinically important pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Staphylococcus aureus* (MDRSA) strains, are posing a worldwide health problem (Doern *et al.*, 1998; Spencer *et al.*, 1997). There is an urgent need to discover new agents to treat the patients infected with methicillin-resistant and multidrug-resistant bacteria (Jones, 1996).

Since the discovery of penicillin from *Penicillium notatum* in the 1940s, terrestrial microorganisms have been a key source of many important products in the drug industry. These encouraging results obtained from terrestrial microorganisms suggest that their marine counterparts might also have the potential to be useful sources of new drug leads.

As part of an effort to discover biologically active natural products from marine microorganisms (Li *et al.*, 2005), we have investigated bioactive constituents of the marine-derived algicolous fungus *Aspergillus* sp., and isolated bioactive epoxycyclohexenones, (+)-epoxydon (**1**) and (+)-epoxydon monoacetate (**2**) (Assante *et al.*, 1981), and aromatic polyols, gentisyl alcohol (**3**) (Son *et al.*, 2002), 3-chlorogentisyl alcohol (**4**) (McCorkindale *et al.*, 1972), and methylhydroquinone (**5**) (Buckingham *et al.*, 1994).

This paper briefly describes the isolation of **1-5**, and evaluation of **1-5** for their antibacterial and radical scavenging activities.

Experimental

Fungal isolation and culture – The fungal strain, *Aspergillus* sp., was isolated from the surface of the marine red alga *Hypnea saidana* collected in the Tongnyeong, Gyeongnam Province, Korea in 1999 and identified based on the morphological evaluation. A voucher specimen is deposited at Pukyong National University with the code MFA292.

The fungus was cultured (20 L) for 30 days (static) at 29°C in SWS medium: soytone (0.1%), soluble starch (1.0%), and seawater (100%).

Extraction and isolation – The culture broth and mycelium were separated, and the broth (10 L) was extracted with ethyl acetate to provide a crude extract (640 mg) which was subjected to silica gel flash chromatography and eluted with *n*-hexane/EtOAc (5 : 1), *n*-hexane/EtOAc (1 : 1), *n*-hexane/EtOAc (1 : 5), *n*-hexane/EtOAc (1 : 10) and finally with EtOAc. Each collections (30 mL each) were combined on the basis of their TLC profiles to yield five major fractions. Medium pressure liquid chromatography (MPLC) of each fractions on ODS by elution with MeOH afforded compounds **1-5**, respectively. The isolated compounds were further

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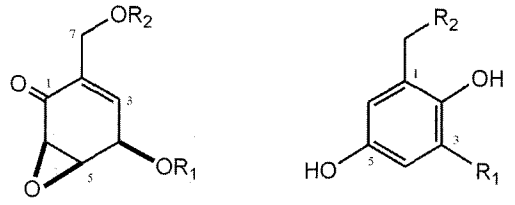

1: R_1, R_2 = H
2: R_1 = H, R_2 = $COCH_3$
3: R_1 = H, R_2 = OH
4: R_1 = Cl, R_2 = OH
5: R_1, R_2 = H

Fig. 1. The structure of bioactive metabolites (**1-5**).

purified by HPLC (YMC ODS-A, MeOH) utilizing a 30 min gradient program of 50% to 100% MeOH in H_2O to furnish (+)-epoxydon (**1**, 5.0 mg), (+)-epoxydon monoacetate (**2**, 12.0 mg), gentisyl alcohol (**3**, 10.0 mg), 3-chlorogentisyl alcohol (**4**, 20.0 mg), and methylhydroquinone (**5**, 5.0 mg), respectively.

(+)-Epoxydon (1): a colorless oil; $[\alpha]_D$ + 71.6° (*c* 0.3, MeOH); IR (neat) ν_{max} 3356, 1680, 1400, 1236, 1027, 903, 867 cm^{-1}; UV (MeOH) λ_{max} (log ε) 203 (3.7), 237 (3.6) nm; CD (MeOH) (Δε) 338 (+0.95), 245 (−1.76) nm; ^1H NMR (400 MHz, DMSO-d_6) δ 6.39 (1H, dddd, *J* = 2.7, 2.6, 2.2, 2.1 Hz, H-3), 4.70 (1H, ddddd, *J* = 6.2, 2.8, 2.8, 2.6, 2.1 Hz, H-4), 5.79 (1H, d, *J* = 6.2 Hz, 4-OH), 3.40 (1H, d, *J* = 4.2 Hz, H-5), 3.76 (1H, ddd, *J* = 4.2, 2.8, 2.7 Hz, H-6), 3.96 (1H, dddd, *J* = 15.2, 5.5, 2.8, 2.2 Hz, H_a-7), 4.07 (1H, dddd, *J* = 15.2, 5.5, 2.6, 2.1 Hz, H_b-7), 5.01 (1H, t, *J* = 5.5 Hz, 7-OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 193.9 (s, C-1), 133.8 (s, C-2), 141.4 (d, C-3), 63.7 (d, C-4), 52.9 (d, C-5), 54.0 (d, C-6), 57.3 (t, C-7); CIMS *m/z* (rel. int.) 156 $[M]^+$ (100), 138 $[M - H_2O]^+$ (7), 122 $[M- H_2O-O]^+$ (2), 110 $[M-CO-H_2O]^+$ (3).

(+)-Epoxydon monoacetate (2): a yellow oil: $[\alpha]_D$ +66° (*c* 0.5, MeOH); IR (KBr) ν_{max} 3392, 1733, 1683, 1374, 1240, 1032, 902, 866, 785, 665 cm^{-1}; UV (MeOH) λ_{max} (log ε) 233 (3.7), 213 (3.6) nm; ^1H NMR (DMSO-d_6, 400 MHz) δ 6.52 (1H, m, H-3), 4.72 (1H, m, H-4), 5.90 (1H, d, *J* = 6.7 Hz, 4-OH), 3.47 (1H, d, *J* = 4.3 Hz, H-5), 3.78 (1H, ddd, *J* = 4.3, 2.7, 2.6 Hz, H-6), 4.63 (1H, ddd, *J* = 13.0, 1.6, 1.3 Hz, H_a-7), 4.56 (1H, ddd, *J* = 13.0, 1.3, 1.0 Hz, H_b-7), 2.02 (3H, s, 7-$OCOCH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 193.1 (s, C-1), 128.7 (s, C-2), 145.8 (d, C-3), 63.6 (d, C-4), 52.7 (d, C-5), 54.2 (d, C-6), 60.1 (t, C-7), 169.9 (s, 7-$O\underline{C}OCH_3$), 20.5 (q, 7-$OCO\underline{C}H_3$); HREIMS *m/z* 198.0532 $[M]^+$ (calcd for $C_9H_{10}O_5$, 198.0528); LREIMS *m/z* (rel. int.) 198 $[M]^+$ (1), 156 $[M-COCH_2]^+$ (4), 138 $[M-COCH_2-H_2O]^+$ (100), 110 $[M-COCH_2-H_2O-CO]^+$ (55).

Gentisyl alcohol (3): a yellow oil; ^1H NMR (400 MHz, DMSO-d_6) δ 8.54 (1H, s, 2-OH), 6.53 (1H, d, *J* = 8.4 Hz, H-3), 6.41 (1H, dd, *J* = 8.4, 2.9 Hz, H-4), 8.52 (1H, s, 5-OH), 6.73 (1H, d, *J* = 2.9 Hz, H-6), 4.39 (2H, d, *J* = 5.7 Hz, H_2-7), 4.87 (1H, d, *J* = 5.7 Hz, 7-OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 129.3 (s, C-1), 149.7 (s, C-2), 113.9 (d, C-3), 113.2 (d, C-4), 146.3 (s, C-5), 115.0 (d, C-6), 58.2 (t, C-7); LREIMS *m/z* (rel. int.) 140 $[M]^+$ (52), 122 $[M-H_2O]^+$ (100), 110 (3), 94 (80), 81 (5), 65 (51), 55 (25).

3-Chlorogentisyl alcohol (4): a red solid; mp 127-129 °C (MeOH); IR (KBr) ν_{max} 3435, 1627, 1599, 1478, 1451, 1306, 1167, 1112, 1026 cm^{-1}; UV (MeOH) λ_{max} (log ε) 220 (3.70), 297 (3.60) nm; ^1H NMR (400 MHz, MeOH-d_4) δ 6.66 (1H, d, *J* = 2.8 Hz, H-4), 6.72 (1H, d, *J* = 2.8 Hz, H-6), 4.60 (2H, s, H_2-7), ^{13}C NMR (100 MHz, MeOH-d_4) δ 122.7 (s, C-1), 152.5 (s, C-2), 132.8 (s, C-3), 116.2 (d, C-4), 145.1 (s, C-5), 115.3 (d, C-6), 61.9 (t, C-7).

Methylhydroquinone (5): a colorless needle; ^1H NMR (400 MHz, DMSO-d_6) δ 8.44 (1H, s, 2-OH), 6.53 (1H, d, *J* = 8.5 Hz, H-3), 6.36 (1H, dd, *J* = 8.5, 2.8 Hz, H-4), 8.48 (1H, s, 5-OH), 6.45 (1H, d, *J* = 2.8 Hz, H-6), 2.02 (3H, s, H_3-7); ^{13}C NMR (100 MHz, DMSO-d_6) δ 124.3 (s, C-1), 147.6 (s, C-2), 117.1 (d, C-3), 115.0 (d, C-4), 149.5 (s, C-5), 112.6 (d, C-6), 16.1 (q, C-7).

Antibacterial assay – The *in vitro* antibiotic activity in fermentation broth and purification samples were evaluated by the conventional a 2-fold serial dilution method using *S. aureus*, methicillin-resistant *S. aureus*, and multidurg-resistant *S. aureus* as indicator strains. A 5-mL suspension containing 105 cells per mL was used as inoculum of the test organism. The MIC values were determined after the inoculation for 18 hours at 37°C (Li *et al.*, 2003).

Radical scavenging assay – Samples to be tested were dissolved in MeOH and the solution (160 μL) was dispensed into wells of a 96-well microtiter tray. 40 μL of the DPPH solution in MeOH (1.5×10^{-4} M) was added to each well. The mixture was shaken and left to stand for 30 min, and the absorbance of the resulting solution was measured at 520 nm with microplate reader (Packard Co., Spectra Count™). The scavenging activity on DPPH radical was expressed as IC_{50}, which is the concentration of the tested compound required to give a 50% decrease of the absorbance from that of the blank solution [consisting of MeOH (160 μL) and DPPH solution (40 μL)] (Li *et al.*, 2002).

Results and Discussion

The broth extract showed an antibacterial activity

against MRSA and MDRSA with MIC value of 12.5 μg/mL, and also exhibited a radical scavenging activity against DPPH with IC_{50} value of 13.9 μg/mL. Isolation of active compounds **1-5** were accomplished through bioassay-guided purification employing various chromatographic methods from the broth extract.

(+)-Epoxydon (**1**), $[\alpha]_D$ +71.6° (*c* 0.3, MeOH), was isolated as a colorless oil. The IR spectrum of **1** suggested the presence of hydroxyl (3356 cm^{-1}), enone (1680, 1027 cm^{-1}), and epoxy (1236, 903, 867 cm^{-1}) groups. The UV spectrum of **1** showed the presence of an α,β-disubstituted enone chromophore [203 nm (log ε 3.7), 237 (3.6)]. Detailed analyses of the ^1H and ^{13}C NMR spectra of **1**, including the results from DEPT, COSY, HMQC, HMBC, and NOESY experiments, suggested the metabolite (**1**) is (+)-epoxydon (Assante *et al.*, 1981).

(+)-Epoxydon monoacetate (**2**) was isolated as a yellow oil, which was deduced to have the molecular formula $C_9H_{10}O_5$ from the HREIMS and ^{13}C NMR data. The IR spectrum of **2** suggested the presence of hydroxyl (3392 cm^{-1}), ester (1733, 1240 cm^{-1}), enone (1683, 1032 cm^{-1}), and epoxy (1240, 902, 866 cm^{-1}) groups. The UV spectrum of **2** showed the presence of a α,β-disubstituted enone chromophore [213 nm (log ε 3.6), 233 (3.7)].

The general features of its UV, IR and NMR spectra closely resembled those of (+)-epoxydon (**1**), except that the NMR signals of a new acetyl group [δ 2.02 (3H, s), δ 20.5 (q), 169.9 (s)] have appeared due to the acetylation of one hydroxyl group of compound **2**.

Detailed analyses of the ^1H and ^{13}C NMR spectra of **2** suggested that the metabolite (**2**) is the monoacetate of compound **1**. The downfield shift of H-7 and C-7 from δ_H 3.96, 4.07 (H_2-7) and δ_C 57.3 (C-7) for compound **1** to δ_H 4.63, 4.56 (H_2-7) and δ_C 60.1 (C-7) for compound **2** revealed the 7-OAc. On the basis of foregoing evidence, the stereostructure of compound **2** was assigned to be (+)-epoxydon monoacetate (Assante *et al.*, 1981). Compounds **3-5** showed spectral data virtually identical to those reported in the literature (Son *et al.*, 2002; McCorkindale *et al.*, 1972; Buckingham *et al.*, 1994).

Compounds **1-5** exhibited a potent antibacterial activity against the methicillin-resistant and multidrug-resistant *Staphylococcus aureus* with MIC values of 12.5, 12.5, 12.5, 50.0, and 6.2 μg/mL, respectively. Compounds **1-4** also exhibited a significant radical scavenging activity against DPPH with IC_{50} values of 6.0, 15.0, 7.0, and 1.0 μM, respectively, which are more potent than the positive control, ascorbic acid (IC_{50}, 20 μM). However, compound **5** showed moderate activity (IC_{50}, 32 μM) in the radical scavenging assay.

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