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MR-93A, A NEW OXAZOLE FROM *TRICHODERMA HARZIANUM* KCTC 0114BP

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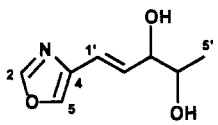
ABSTRACT.—The ethylacetate extract of *Trichoderma harzianum* KCTC 0114BP cultures afforded a novel oxazole, MR-93A [**1**]. The structure of **1** was elucidated as 4-(3,4-dihydroxy-(*E*)-1-pentenyl)-oxazole by spectroscopic methods.

Trichoderma harzianum has attracted considerable attention for use in the biological control of plant pathogens. Recent studies by several groups have implicated various metabolites from *Trichoderma* spp. (1–4). Although oxazole metabolites have been found mainly in plants, marine sponges, and actinomycetes (5), only a few such compounds have been found to date in fungi, with no previous report from *Trichoderma* spp.

Cultures of *Trichoderma harzianum* KCTC 0114BP yielded a new oxazole metabolite, MR-93A [**1**], [4-(3,4-dihydroxy-(*E*)-1-pentenyl)-oxazole]. An EtOAc extract of *T. harzianum* KCTC 0114BP was separated by Sephadex LH-20 cc and prep. hplc to yield **1** as a colorless oil. The eims showed the parent peak $[M]^+$ at *m/z* 169 while cims gave a $[MH]^+$ peak at *m/z* 170. The hreims gave a $[M]^+$ at *m/z* 169.0751 for $C_8H_{11}NO_3$ (calcd 169.0739). In the uv spectrum of **1** only a single maximum was observed at 226 nm (ε 8991). The ^{1}H-nmr spectrum exhibited two singlets for H-2 and H-5, two doublets for methine proton H-1′ and methyl protons H-5′, two doublets of doublets for methine protons H-2′ and H-3′, and a multiplet for methine proton H-4′ (Table 1). The ^{13}C- and DEPT nmr spectra exhibited signals for six CH carbons, one CH_3 group, and one quaternary carbon. It became clear that the monosubstituted oxazole was joined to dihydroxypentenyl, but the nature of the



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substitution pattern in the oxazole ring was still ambiguous. The possibilities to be considered included monosubstitution at either C-4 or C-5. The HMBC nmr spectrum established that the dihydroxypentenyl moiety was linked to the C-4 or C-5 carbon since one quaternary carbon correlated to the signals at δ 7.84, δ 7.60, and δ 6.50. These spectral properties of the oxazole part of the molecule of **1** are similar to those reported for rhizoxin, suggesting that the side-chain of **1** is attached to C-4 (6). To confirm the substitution pattern of the oxazole, the $^1J_{CH}$ values were measured by PFG-HMQC nmr without ^{13}C decoupling (7). From the obtained $^1J_{CH}$ values, $^1J_{C2H2}$=230.5 Hz and $^1J_{C5H5}$=206.0 Hz, the position of the side-chain was assigned to C-4 (8,9). An *E*-orientation of the 1′, 2′-double bond was inferred from a large coupling constant between H-1′ and H-2′ (15.6 Hz). Based on the above-mentioned spectral data, the structure of MR-93A [**1**] was determined as 4-(3,4-dihydroxy-(*E*)-1-pentenyl)-oxazole. MR-93A [**1**] showed no significant antimicrobial activity against *E. coli* AB 11513, *S. typhimurium* TV 119, *S. aureus* IFO 12732, *C. albicans*

TABLE 1. ^{13}C-, ^{1}H- (J in Hz), and HMBC Nmr Data for Compound **1** in $CDCl_3$.

Position	^{1}H δ (ppm)	^{13}C δ (ppm)	HMBC[a]
2	7.84 (1H, s)	151.3	C-4, C-5
4		137.8	
5	7.60 (1H, s)	135.5	C-2, C-4
1′	6.50 (1H, d, J=15.6)	131.2	C-4, C-2′, C-3′
2′	6.60 (1H, dd, J=15.6, 6.0)	119.9	C-4, C-1′, C-3′, C-4′
3′	4.04 (1H, dd, J=6.0, 6.0)	76.5	C-1′, C-2′, C-4′, C-5′
4′	3.78 (1H, m)	70.8	C-2′ C-3′, C-5′
5′	1.25 (3H, d, J=6.0)	19.0	C-3′, C-4′

[a]Carbon resonances that were long-range correlated with protons.

IFO 1594, or *R. solani* at 1 mg/ml.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The uv spectrum was determined on a Shimazu uv-260 instrument, and the hrms was measured on a JEOL JMS-DX303 mass spectrometer. The optical rotation and the cd spectrum were taken in MeOH solution on an Autopol III polarimeter and a Jasco J720 spectropolarimeter, respectively. Nmr spectra were obtained in $CDCl_3$ (δ 7.26 was used as internal reference) on Varian Unity 500 and JEOL JNM-A600 spectrometers. Chemical shift values are reported in ppm, and coupling constants (J values) in Hz.

FUNGAL MATERIAL.—A leaf sample, collected from Che-Ju, Korea in 1993, yielded a fungus, that was isolated using potato dextrose agar (Difco Labs, Detroit). The fungus was identified as *Trichoderma harzianum* and has been deposited with the Korean Collection for Type Cultures, and assigned as KCTC 0114BP.

FERMENTATION CONDITIONS.—Spores of KCTC 0114BP were inoculated into 1-liter shake flasks containing 200 ml of a medium consisting of sucrose (20 g/liter), lactose (10 g/liter), peptone (3 g/liter), NaCl (5 g/liter), corn steep liquor (3 g/liter), KNO_3 (2.3 g/liter), and mineral solution (1 ml/liter). The flask was placed on a rotary shaker at 200 rpm, at 25°. After 3 days, 1 liter of seed culture was inoculated into 50 liters of fermenter containing 35 liters of a medium described above. The fermenter was agitated at 250 rpm with aeration at 25° for 7 days prior to being harvested.

EXTRACTION AND ISOLATION.—The mycelium of the fungal strain was removed by filtration at the end of the growth period, and the metabolites present in the filtrate were fractionated on a Diaion HP-20 (Mitsubishi Kasei Co.) column; with 100% H_2O, H_2O-MeOH (3:7), H_2O-MeOH (7:3), and 100% MeOH as solvents. The 3:7 fraction was concentrated and extracted with EtOAc. After concentration, the extract was further fractionated on a Sephadex LH-20 (Pharmacia LKB) column using an isocratic mobile phase of H_2O-MeOH (1:1). The fractions containing **1** were taken to dryness and chromatographed on a MCI gel (CHP20P, 75–150 μm, Mitsubishi Kasei Co.) column using a gradient from 100% H_2O to 100% MeOH and chromatographed further on a PLRP-S column (300×7.5 mm, Phenomenex) using an isocratic mobile phase of H_2O-MeCN (9:1) at a flow rate of 1.5 ml/min. Fractions eluting at about 16 min yielded 6.5 mg of **1** as a colorless oil.

MR-93A [**1**].—$[\alpha]^{25}D$ −6.0° (c=0.1, CH_3OH); hreims m/z $[M]^+$ 169.0751 (calcd 169.0739 for $C_8H_{11}NO_3$); cims m/z $[MH]^+$ 170, eims m/z $[M]^+$ 169; uv [MeOH] λ max 226 (ε 8991); cd (MeOH) (nm) λ ext 258 (Δε −0.04), 256 (0), 254 (Δε +0.08), 251 (0), 246 (Δε −0.19), 234 (0), 227 (Δε +0.10), 224 (0), 220 (Δε +0.16), 210 (0), 208 (Δε −0.06), 119 (Δε −0.47); ^{1}H- (500 MHz, $CDCl_3$) and ^{13}C- (300 MHz, $CDCl_3$) nmr data, see Table 1.

BIOASSAYS.—Purified **1** was dissolved in MeOH and diluted to the test concentrations. One yeast, *Candida albicans* (IFO 1594), two Gram-negative bacterial species, *Escherichia coli* (AB 11513) and *Salmonella typhimurium* (TV 119), one Gram-positive bacterial species, *Staphylococcus aureus* (IFO 12732), and one fungus, *Rhizoctonia solani*, were spread evenly over the surface of nutrient agar (Difco Labs, Detroit, MI) plates. Samples (40 μl) were added to paper disks and plates were incubated for 2 days at 30° prior to measuring inhibition.

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