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A NEW DIHYDROOXAZOLE ANTIBIOTIC FROM THE FERMENTATION BROTH OF *STREPTOMYCES DJAKARTENSIS*

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Abstract – A new antibiotic was isolated from the fermentation broth of *Streptomyces djakartensis* by bioassay-guided fractionation. It was elucidated as *(S)*-2-(2-hydroxyphenyl)-4-hydroxymethyl-4,5-dihydrooxazole mainly by analyses of 2D NMR and MS spectral data and the absolute configuration of the antibiotic was established by X-ray crystal data and proved to be an enantiomer of known antibiotic with name of Nocazoline A or spoxazomicin C. It exhibited broad spectrum antibacterial activities with MIC values of 7.81~31.25 μg/mL.

Soil actinomycetes is still important sources of discovery novel antibiotics. The vast majority (70%) of the known antibiotics was isolated from actinomycetes.[1,2] In the past decades, although many species which produced biologically active metabolites have been obtained from the soil samples, the chance of isolating a new actinomycete strain from a usual terrestrial habitant has markedly reduced.[3,4] To meet the increased demands on the discovery of new bioactive compounds, researchers have to look for novel microorganisms in unusual environment.[5] Chemical polluted soil is a sort of unusual environment. In fact, chemical polluters, especial some pesticides, could be a mutagen. Some of the microorganisms stressed by chemical polluters might be induced mutations. These mutant strains might give rise to increase in productivity of bioactive metabolite, even produce new bioactive compounds. In the course of a screening program for new antibiotics in our research group, NW35 strain of *Streptomyces djakartensis* which was isolated from a pesticidal polluted soil sample has been investigated and afforded a new antibiotic Yanglingmycin. In this paper, isolation, structure elucidation of Yanglingmycin (Figure 1) and its

antibacterial activities against *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Escherichia coil, Pseudomonas aeruginosa, Pseudomonas syringae* pv. *actinidiae, Erwinia carotovora, Ralstonia solanacearum* and *methicillin-resistant S. aureus*. will be reported.

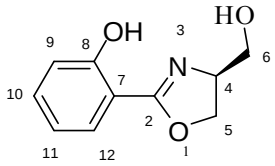

Figure 1. Structure of Yanglingmycin

Yanglingmycin was obtained in form of colourless needles with mp 78-80 °C. Its molecular formula was determined as $C_{10}H_{11}NO_3$ based on the result of HR-ESI-MS with a *quasi* molecular ion peak of $[M+H]^+$ at *m/z* 194.08171 (calcd. 194.08172). The IR spectrum of Yanglingmycin showed absorptions of hydroxyl (3385 cm^{-1}) and imine (1643 cm^{-1}) moieties. The ^1H NMR spectrum of Yanglingmycin revealed the presence of four aromatic protons at δH 6.90-6.94 (m, 1H), 6.98 (dd, 1H, *J*=8.0 Hz, 0.5 Hz), 7.41-7.45 (m, 1H), 7.60 (dd, 1H, *J*=7.5 Hz, 1.5 Hz), one phenolic hydroxyl proton at δH 12.26 (s, 1H), one methine proton at δH 4.39-4.44 (m, 1H), two methylene at δH 4.30 (t, 1H, *J*=7.5 Hz), 4.49 (dd, 1H, *J*=7.5 Hz, 9.5 Hz) and 3.56 (t, 2H, *J*=5.0 Hz) and one hydroxyl proton at δH 4.95 (t, 1H, *J*=5.5 Hz) indicated that Yanglingmycin was a primary alcohol. The ^{13}C NMR spectrum of Yanglingmycin exhibited 10 carbon signals, which were resolved through a DEPT experiment into two methylene, five methine, and three quaternary carbons. HMBC correlations from imine δc 165.06 only to aromatic proton at δH 7.60 (dd, 1H, *J*=7.5 Hz, 1.5 Hz) and methylene proton at δH 4.30 (t, 1H, *J*=7.5 Hz), 4.49 (dd, 1H, *J*=7.5 Hz, 9.5 Hz) indicated that Yanglingmycin was a *ortho*-substituted benzene (Figure 3). Based on the above analysis, Yanglingmycin was assigned as 2-(2-hydroxyphenyl)-4-hydroxymethyl-4,5-dihydrooxazole. A survey of the literature revealed that the ^1H and ^{13}C NMR spectra of Yanglingmycin were very similar to those of Nocazoline A [6] (Table 2) and Spoxazomicins C.[7] The specific rotation of Yanglingmycin was $[\alpha]^{28}_D$ -16.2 (c 0.1, MeOH) but the specific rotation of *(R)*-2-(2-hydroxyphenyl)-4-hydroxymethyl-4,5-dihydrooxazole (Nocazoline A) was $[\alpha]^{25}_D$ +15. Spoxazomicins C and Nocazoline A had no antimicrobial activities but Yanglingmycin exhibited broad spectrum antibacterial activities (Table 3). So Yanglingmycin was assigned as *(S)*-2-(2-hydroxyphenyl)-4-hydroxymethyl-4,5-dihydrooxazole based on these data and X-ray structure data analyses (Figure 2). The crystal structure was deposited at the Cambridge Crystallographic Data Centre and the deposition numbers were CCDC 1002189.

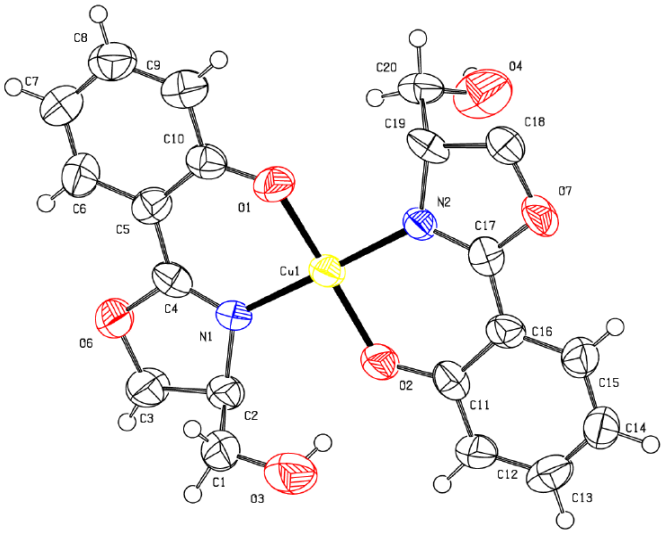

Figure 2. Structure of Yanglingmycin

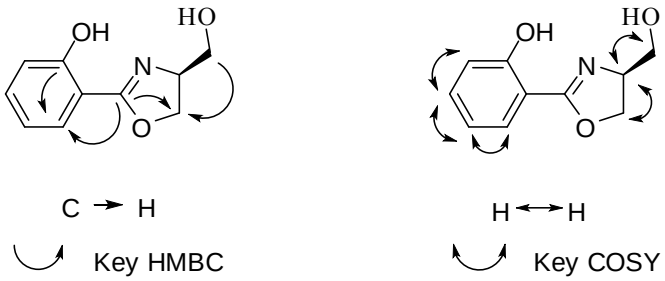

Figure 3. Key HMBC and COSY of Yanglingmycin

Table 1. NMR data of Yanglingmycin (^{1}H 500 MHz, ^{13}C 125 MHz DMSO-d_6)

Position	^{1}H NMR	^{13}C NMR	COSY	HMBC
2		166.99		
4	4.50-4.52 (m, 1H)	66.86	5-H	
5	4.36-4.38 (m, 1H), 4.47-4.49 (m, 1H)	68.61	4-H, 6-H	2-C, 6-C
6	3.72 (dd, 1H, *J*=3.5 Hz, 11.5 Hz) 3.90 (dd, 1H, *J*=3.5 Hz, 11.5 Hz)	63.97	5-H	
7		110.38		
8		159.84		
9	6.99 (1H, d, *J*=8.0 Hz)	116.78	10-H	7-C
10	7.37 (1H, m)	133.72	9-H, 11-H	8-C
11	6.86 (1H, m)	118.81	10-H, 12-H	7-C
12	7.65 (1H, m)	128.25	11-H	2-C, 8-C

Table 2. NMR data of Yanglingmycin and Nocazoline A (^{1}H 500 MHz, ^{13}C 125 MHz $CDCl_3$)

Position	^{1}H NMR Nocazoline A	^{1}H NMR Yanglingmycin	^{13}C NMR Nocazoline A	^{13}C NMR Yanglingmycin
2			166.9	166.99
4	4.46, m	4.54-4.50 (2H), m	67.0	66.86
5	4.44, dd (9.9, 1.8); 4.33, t (6.1)	4.47-4.50 (1H), m 4.36, t (6.0)	68.7	68.61
6	3.86, dd (11.5, 3.3); 3.68, dd (11.6, 3.8)	3.90, dd (11.5, 3.0) 3.71, dd (11.5, 3.5)	64.0	63.97
7			110.5	110.38
8			159.8	159.84
9	6.99, dd (8.2, 1.1)	7.00, d (8.0)	116.8	116.78
10	7.36, dt (8.2, 1.1)	7.38, dt (7.5, 1.5)	133.7	133.72
11	6.86, dt (7.7, 1.1)	6.88, dt (7.5, 0.5)	118.9	118.81
12	7.64, dd (8.3, 1.6)	7.65, dd (8.5, 1.5)	128.3	128.25

Table 3. MIC of Yanglingmycin against the tested bacteria

Name of the test bacteria	MIC of Yanglingmycin (μg/mL)	MIC of Ampicillin(μg/mL)
Bacillus cereus	15.625	50
Bacillus subtilis	15.625	25
Staphylococcus aureus	15.625	25
Escherichia coil	31.25	100
Pseudomonas aeruginosa	31.25	＞100
Pseudomonas syringae pv. *actinidiae*	7.8125	＞100
Erwinia carotovora	31. 25	＞100
Ralstonia solanacearum	15.625	＞100
Methicillin-resistant *Stphylococcus aureus*	15.625	＞100

EXPERIMENTAL

General. Solvents were of anal.reagent (AR) grade unless otherwise mentioned. TLC: E. Merck 60 F_{254} silica gel plates. Column chromatography: HPD100 macroporous resin (Baoen Co., Ltd., Cangzhou,

Hebei, China), and then eluted with H_2O and MeOH in sequence. HPLC: Waters 600E HPLC apparatus (Waters Co., Ltd., Milford, MA, USA) equipped with a Hypersil ODS-BP (20 × 250 mm, 10 μm) reverse phase column, using methanol-water as the mobile phase, flow rate of 3.0 mL/min, monitored by UV detector at 240 nm. Mp Yanagimoto apparatus; uncorrected. ^{1}H NMR and ^{13}C NMR Spectra: Bruker-Avance-500 spectrometer; DMSO-d_6 or $CDCl_3$ as solvent and $SiMe_4$ as internal standard.

Fermentation of the actinomycete. *Streptomyces djakartensis* NW35 was isolated from a pesticidal polluted soil sample collected from the Shandong province of China. The voucher specimen of this streptomycete was deposited in China General Microbiological Culture Collection Center(CGMCC NO.6817). *Streptomyces djakartensis* NW35 was cultivated at 28 °C in starch casein agar medium, which contained soluble starch (1%), K_2HPO_4 (0.2%), KNO_3 (0.2%), NaCl (0.2%), Casein (0.03%), $MgSO_4$ (0.005%), $CaCO_3$ (0.002%), $FeSO_4$ (0.001%) and agar (1.5%). Fermentation was performed in two stages: seed growth and antibiotics production. The spores of *S. djakartensis* NW35 grown on starch casein agar were used to inoculate a 250 mL flask containing 60 mL of a sterile seed medium consisting of glucose (1.0%), millet steep liquor (1.0%), peptone (0.5%), $(NH_4)_2SO_4$ (0.1%), NaCl (0.25%), and $CaCO_3$ (0.05%); pH 7.2. The flask was shaken on a shaker at 180 rpm for 18 h at 28 °C. 6 mL of the seed culture were transferred to 250 mL flasks containing 60 mL of a sterile production medium consisting of glucose (1.0%), millet steep liquor (1.0%), peptone (0.3%), $(NH_4)_2SO_4$ (0.1%), NaCl (0.25%) and $CaCO_3$ (0.1%); pH 7.2. Fermentation was carried out at 180 rpm for 4 days at 28 °C on a rotary shaker.

Extraction and Isolation. The culture of 90 L of *Streptomyces djakartensis* NW35 was filtered through cheesecloth to separate the medium and culture liquid at 25 °C, pH 7.0. The filtrate was absorbed onto HPD−100 macroporous resin (Baoen Co., Ltd., Cangzhou, Hebei, China), and then eluted with H_2O and MeOH in sequence (from 40% MeOH/H_2O to 60% MeOH/H_2O then MeOH). The MeOH fraction was evaporated in vacuum. The concentrate was subjected to column chromatography and eluted with EtOAc and MeOH in sequence. The antimicrobial fraction was concentrated under vacuum, and further purified on a Waters 600E HPLC apparatus (Waters Co., Ltd., Milford, MA, USA) equipped with a Hypersil ODS-BP (20 × 250 mm, 10 μm) reverse phase column, using methanol-water as the mobile phase (70% MeOH/H_2O), flow rate of 3.0 mL/min, monitored by UV detector at 240 nm and at the retention time of 45min to afford Yanglingmycin (106 mg).

***(S)*-2-(2-Hydroxyphenyl)-4-hydroxymethyl-4,5-dihydrooxazole** (Yanglingmycin):colourless needles; ($[\alpha]^{28}_D$ -16.2 (c 0.1, MeOH); IR (KBr) cm^{-1}: 3385,1643; ^{1}H and ^{13}C NMR, see Table 1; HR-ESI-MS $[M+H]^+$ at *m/z* 194.08171 (calcd. 194.08172).

Antimicrobial Assay. Antibacterial activities were measured by the micro-broth dilution method in 96-well culture plates using the Mueller-Hinton (MH) broth (Hangzhou Microbial Reagent Co. Ltd, Hangzhou City, China), according to the Standard of National Committee for Clinical Laboratory.[8] The

standard bacterial strains were obtained from the China General Microbiological Culture Collection center. A clinical isolate of Methicillin-resistant *Stphylococcus aureus* strain was obtained from Nanjing Medical University. Ampicillin (Sigma, Shanghai, China) was used as positive control. The tested bacteria were incubated in the MH broth for 12 h at 30 °C at 190 rpm, and the spore concentration was diluted to approximately 1×10^5–1×10^6 CFU/mL with MH broth. After incubation for 24 h at 30 °C, the MICs were examined.

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REFERENCES AND NOTES

1. A. Lazzarini, L. Cavaletti, G. Toppo, and F. Marinelli, *Antonie Van Leeuwenhoek*, 2000, **78**, 399.
2. M. G. Watve, R. Tickoo, M. M. Jog, and B. D. Bhole, *Arch. Microbiol.*, 2001, **176**, 386.
3. B. Spellberg, R. Guidos, D. Gilbert, J. Bradley, H. W. Boucher, W. M. Scheld, J. G. Bartlett, and J. Edwards, *Clin. Infect. Dis.*, 2008, **46**, 155.
4. S. Donadio, S. Maffioli, P. Monciardini, M. Sosio, and D. Jabes, *J. Antibiot.*, 2010, **63**, 423.
5. M. E. Juhnke, D. E. Mathre, and D. C. Sands, *Appl. Environ. Microbol.*, 1987**, 53**, 2793.
6. P. Fu, P. P. Liu, H. J. Qu, Y. Wang, D. F. Chen, H. Wang, J. Li, and W. M. Zhu, *J. Nat. Prod.*, 2011, **74**, 2219.
7. Y. Inahashi, M. Iwatsuki, A. Ishiyama, M. Namatame, A. Nishihara-Tsukashima, A. Matsumoto, T. Hirose, T. Sunazuka, H. Yamada, K. Otoguro,Y. Takahashi, S. Omura, and K. Shiomi, *J. Antibiot.*, 2011, **64**, 303.
8. J. H. Jorgensen, S. A. Crawford, C. C. Kelly, and J. E. Patterson, *Antimicrob. Agents Chemother.*, 2003, **47**, 3760.