SHORT REPORT

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Secondary Metabolites of *Curvularia oryzae* MTCC 2605

Siddhardha Busi[1*], Prabhakar Peddikotla[2], Suryanarayana M. Upadyayula[1*], Venkateswarlu Yenamandra[2]

[1]*Biology Division, Indian Institute of Chemical Technology, Tarnaka, Hubsiguda, Hyderabad 500 007, Andhra Pradesh, India*

[2]*Organic Chemistry Division-I, Indian Institute of Chemical Technology, Tarnaka, Hubsiguda, Hyderabad 500 007, Andhra Pradesh, India*

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Abstract: *Curvularia oryzae* MTCC 2605 was exploited for the production of secondary metabolites. The major compounds from the crude extract were purified by silica gel column chromatography and identified to be 11-α-methoxycurvularin and (*S*)-5-ethyl-8, 8-dimethylnonanal by NMR and Mass spectral data. Bioassays showed that 11-α-methoxycurvularin was active against bacteria, fungi and 4th instar *Spodoptera litura* larvae.

Keywords: *Curvularia oryzae*; 11-α-methoxycurvularin; antibacterial; antifungal; antilarval.

1. Fungal Source

Curvularia oryzae is a filamentous fungus and develops black, velvet colonies with an abundant septate mycelium. Species of *Curvularia* mostly occur as tropical and subtropical facultative plant pathogens with teleomorphic states in *Cochliobolus* and *Pseudocochliobolus. Curvularia oryzae* originally reported from rice grains and causes a fruit rot in okra (*Abelmoschus esculentus*). Many varieties of *C. oryzae* were known to cause infection to different varieties of rice (*Oryza sativa*) [1, 2]. *Curvularia oryzae* Bugnicourt MTCC 2605 was procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India and maintained on potato dextrose agar slants at 27 ºC prior to cultivation.

* Corresponding author: E- Mail: siddhardha.busi@gmail.com., murty_usn@yahoo.com

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2. Previous Studies

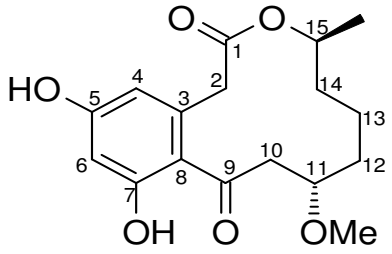
There has been no antimicrobial investigation of *Curvularia oryzae* (MTCC 2605) reported previously. However, isolation of 11-α-methoxycurvularin was previously reported from *Penicilium citroviridae* and some other *Penicilium* species [3-6].

3. Present Study

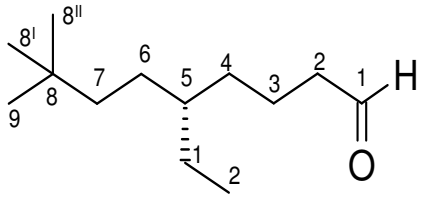
The fungal strain *Curvularia oryzae* Bugnicourt MTCC 2605 was procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India, was cultivated on 8 L of potato dextrose broth medium at room temperature (29 ºC) for 9 days. The cultures were then extracted with ethyl acetate to afford 2.4 g of residue after removal of the solvent under reduced pressure. The extract was separated into two fractions by column chromatography on silica gel, using a gradient of n-hexane: ethyl acetate (90:10, 50:50, 0:100). Fractions that showed homogenity on TLC plates were combined and concentrated together to give pure compounds. Fraction 1 (51 mg) and Fraction 2 (38 mg) were obtained. ^{1}H and ^{13}C NMR were recorded on Bruker UXNMR by dissolving in $CDCl_3$, and Mass spectrum on Finnigan MAT 1020-B. The optical rotation was measured on a JASCO DIP-360 polarimeter. The metabolites were identified as 11-α-methoxycurvularin (fraction 1) and (*S*)-5-ethyl-8, 8-dimethyl nonanal (fraction 2).

11-α-methoxycurvularin ($C_{17}H_{22}O_6$): $[\alpha]_D^{25}$-17.2°; ^{1}H NMR (300 MHz, $CDCl_3$): δ: 6.29 (1H, d, *J*=2.0Hz, H-4), 6.22 (1H, d, *J*=1.6Hz, H-6), 4.92 (1H, t, *J*=6.8Hz, H-15), 3.89 (1H, d, *J*=15.6Hz, H-2), 3.81 (1H, d, *J*=3.6Hz, H-2), 3.70 (1H, dd, *J*=15.6Hz, 6.8Hz, H-10), 3.39 (1H, d, *J*=12.0Hz, H-11), 3.36 (3H, s, OMe), 3.01 (1H, dd, *J*=14.8Hz , 8.8Hz, H-11), 1.53-1.62 (6H, m, H-12,13 and 14), 1.19 (3H, d, *J*=7.2Hz,Me). ^{13}C NMR (300 MHz, $CDCl_3$): δ: 172.2 (C-1), 48.8 (C-2), 135.6 (C-3), 112.4 (C-4), 159.3 (C-5), 102.7 (C-6), 158.3 (C-7), 119.6 (C-8), 205.1 (C-9), 39.9 (C-10), 77.0 (C-11), 31.9 (C-12), 20.9 (C-13), 32.6 (C-14), 73.9 (C-15), 20.9 (C-16), 55.9 (C-OMe). EIMS (*m/z*) 322 [M^+].

(S)-5-Ethyl-8, 8-dimethyl nonanal ($C_{13}H_{26}O$): $[\alpha]_D^{25}$-12.1°; ^{1}H NMR (300 MHz, $CDCl_3$): δ: 0.9 (3H, t, C-2,C-5), 1.3 (13H, s, H-9,H-7 and H-6), 1.6 (4H, t,H-3 and H-4), 2.1 (3H, s,H-5 AND H-1), 2.4 (2H, t,H-2). ^{13}C NMR (300 MHz, $CDCl_3$): δ: 178.96 (C-1), 29.66 (C-2,C-3), 29.58 (C-4), 31.92 (C-5), 29.42 (C-6), 29.35 (C-7), 33.85(C-8), 22.68(C-9), 29.23 (C-10), 14.1(C-11), 24.69(C-12), 29.05 (C-13). EIMS (*m/z*) 183 [M^+].



(1)



(2)

Figure 1. Chemical structures of 11-α-methoxycurvularin (**1**) and (*S*)-5-Ethyl-8, 8-dimethyl nonanal (**2**).

Bioactivity Tests

The cytotoxic activity of the compound 11-α-methoxycurvularin was previously reported against NCI-H460, MCF-7, and SF-268 cell lines [7]. This is the first report for antibacterial, antifungal and larvicidal activity of 11-α-methoxycurvularin. The antibacterial and antifungal

Table 1. Antibacterial, antifungal and larvicidal activities of 11-α-methoxycurvularin

	Zone of inhibition			MIC (µg/mL)	
	(1)		Control 30 µg	(1)	Control
Bacteria	50 µg	100 µg	Penicillin-G		Nitrofurantoin
Gram Positive Bacteria					
S. aureus MTCC 96	12	14	18	100	50 µg/mL
S. epidermides MTCC 435	10	12	18	200	50 µg/mL
B. subtilis MTCC 441	10	12	20	>200	100 µg/mL
B. sphericus MTCC 511	14	16	20	100	100 µg/mL
Gram Negative Bacteria			Streptomycin		
E. coli MTCC 443	10	12	29	>200	50 µg/mL
P. aeruginosa MTCC 741	12	14	34	200	75 µg/mL
P. oleovorans MTCC 617	12	14	30	200	75 µg/mL
K. pneumoniae MTCC 39	10	12	30	>200	50 µg/mL
Fungi				**Larvicidal assay**	
Filamentous fungi			Clotrimazole	(1)	
A. niger MTCC 1344	12	14	22	LD_{50}	205.59 µg/mL
A. parasiticus MTCC 411	12	14	22	LD_{90}	645.33 µg/mL
R. oryzae MTCC 262	10	12	23	Control (Pyrethrum)	
C. cladosporides MTCC 2607	10	12	20	LD_{50}	1.6 µg/mL
Unicellular fungi				LD_{90}	3.0 µg/mL
C. albicans MTCC 227	12	14	18		
C. albicans MTCC 3018	12	14	18		
S. cerevisiae MTCC 170	12	14	19		
S. cerevisiae MTCC 171	12	14	19		

(1)= 11-α-methoxycurvularin; LD_{50} = Lethal concentration (µg/mL) at which 50 % of the larvae showed mortality; Negative control DMSO-No activity.

activities of the compounds were determined according to Linday [8]. The tested compounds were dissolved in dimethylsulfoxide (DMSO) at a concentration of 1 mg/mL. 50 µL and 100 µL of the solutions were pipetted into agar wells which were bored on appropriate growth medium (PDA and NA) spreader with respective test organism. The radius of zone of inhibition was measured in mm. The minimum inhibitory concentration was determined according to the method described by Andrews [9]. *Spodoptera litura* is an economically important polyphagous pest in India, China and Japan, causing considerable economic loss to many vegetable and field crops. Crop loss due to insect pests varies between 10% and 30% for major crops [10]. Larvicidal activity (measured as mortality after 24 h) of the compounds was determined by topical application to early fourth instars according to Luria *et.al* [11]. Lethality was estimated by applying different concentrations (100 to 1000 µg/mL) of the metabolites. Two replicates of 10 larvae were tested per dose. A probit analysis was carried out to calculate LD_{50} and LD_{90} [12].

The antimicrobial activity of the isolated compounds against all the test organisms is given in the Table 1. 11-α-methoxycurvularin showed strong antibacterial activity against gram-positive bacteria i.e. *Staphylococcus aureus, Bacillus sphericus* and gram-negative bacteria i.e. *Pseudomonas aeruginosa, Pseudomonas oleovorans* with zone of inhibition between 12 to 16 mm. The MIC value of the 11-α-methoxycurvularin was 100µg/mL against *Staphylococcus aureus* and *Bacillus sphericus*. 11-α-methoxycurvularin was tested for antifungal activity against eight fungi and showed moderate activity against all fungi except *Rhizopus oryzae* and *Cladosporium cladosporides*. Against *Spodoptera litura* 4th instar larvae LD_{50} was determined to be 205.59 µg/mL while (*S*)-5-Ethyl-8, 8-dimethyl nonanal doesn't showed any biological activity.

The present study of screening bioactive secondary metabolites from fungi revealed that *Curvularia oryzae* as a source for the production of two secondary metabolites. Fungi are remarkable organisms that readily produce a wide range of natural products called secondary metabolites. Microbial secondary metabolites form an immense reservoir of natural chemical diversity, providing us with an enormous diversity of unique carbon skeletons and functional group modifications. The significance of these compounds is considerable, as many natural products are of medical, industrial and/or agricultural importance [13]. Two compounds were isolated from *Curvularia oryzae* and their antimicrobial and larvicidal activities reported. 11-α-Methoxycurvularin showed potent antibacterial, antifungal and larvicidal activities. These compounds can be further exploited for biotechnological applications.

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