NOTES

Mathemycin A, a New Antifungal Macrolactone from Actinomycete sp. HIL Y-8620959

I. Fermentation, Isolation, Physico-chemical Properties and Biological Activities†

S. R. NADKARNI*, TRIPTIKUMAR MUKHOPADHYAY, R. G. BHAT and S. V. GUPTE

Research Centre, Hoechst Marion Roussel Limited, Mulund (W), Mumbai-400 080, India

B. SACHSE

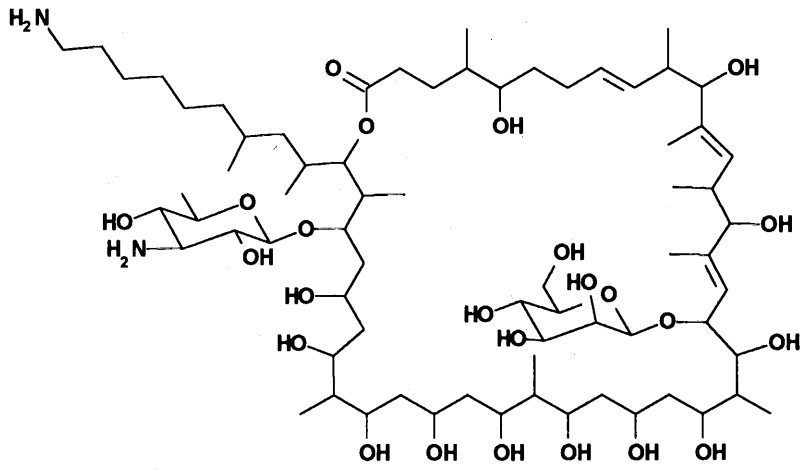
AgrEvo GmbH, Biological Research, D-65926, Frankfurt and Main, Germany

(Received for publication March 23, 1998)

During the course of our screening for new antifungal metabolites active against plant pathogens, we isolated a new macrolactone named mathemycin A from an unidentified Actinomycete species HIL Y-8620959. Herein, we report the production, isolation and biological activities of mathemycin A.[1)]

Strain HIL Y-8620959 was isolated from a soil sample collected from Matheran, Maharashtra, India. The strain belongs to the Actinomycetes as seen by its characteristic branching of the mycelium and spore formation as well as its resistance to amphotericin B. A loopful of mature slant culture of Y-8620959 was inoculated into Erlenmeyer flasks (25 × 1 liter) containing 100 ml each of seed medium consisting of glucose 0.5%, soluble starch 2.0%, proteose peptone 0.5%, yeast extract 0.3% and $CaCO_3$ 0.3% (pH adjusted to 7.2 before autoclaving). The flasks were shaken on a rotary shaker at 240 rpm for 72 hours at 28°C. The resultant seed culture was inoculated (15%) into a 10-liter fermenter containing 9 liters of the above seed medium. The aeration and agitation of the fermentation were maintained at 10 lpm and 200 rpm respectively and the temperature at 28°C. The fermentation was carried out for 48 hours and the resultant seed culture was inoculated (6%) into a 100-liter fermenter containing 80 liters of production medium consisting of glucose 1.5%, starch 2.0%, corn steep liquor 0.2%, soyatone 0.3%, peptone 0.3%, NaCl 0.2%, $Mg_2(PO_4)_2$ 0.5% and $CaCO_3$ 0.2% (pH 7.0 before autoclaving). The aeration and agitation were maintained at 50 lpm and 70 rpm respectively and the temperature at 28°C. The fermentation was carried out for 138 hours. The production of the antibiotic and its purification were monitored by activity against *Fusarium culmorum* 100.

The culture broth (81 liters) was harvested and centrifuged to separate the mycelium. The culture filtrate (72 liters) was passed through a column of Amberlite IRC-50 (H^+) (1.75 liters). The column was washed with



(1)

† Dedicated to Prof. Dr. D. SEEBACH, Laboratorium für Organische Chemie, ETH Zentrum-Universitätstrasse 16, CH-8092, Zürich, Switzerland on the occasion of his 60th birthday.

water (5 liters) and water-MeOH (2:8) (10 liters) and then eluted with MeOH-1 N HCl (8:2) (12 liters). The active eluates were combined and neutralized with aq. $NaHCO_3$. The MeOH was removed under vacuum and the concentrate (2 liters) extracted with *n*-BuOH (8 × 250 ml). The organic extract was separated, concentrated under reduced pressure and lyophilized to obtain crude antibiotic (25 g).

The crude antibiotic was dissolved in water (500 ml) and the pH was adjusted to pH 3.5 with dilute HCl. The acidic solution was passed through a column of CM-sephadex (Na^+) (750 ml). The column was washed with water and eluted successively with 0.1 M (3 liters), 0.2 M (5 liters), 0.3 M (5 liters), 0.4 M (5 liters) and 0.5 M (3 liters) of aq. NaCl solution. The active fractions, which eluted out in 0.2 M ~ 0.4 M aq. NaCl solution, were combined (12 liters) and desalted by passing through a column of Diaion HP-20 (2 liters). The column was washed with water (20 liters) and then eluted with acetone-0.1 M aq. ammonia (1:1) (15 liters). The active eluates were concentrated and lyophilized to obtain pure mathemycin A (7.2 g). The 0.1 M ~ 0.2 M eluants were found to contain another active metabolite. Further work on this metabolite is in progress.

The physico-chemical properties of **1** are given in Table

Table 1. Physico-chemical characteristics of mathemycin A (**1**).

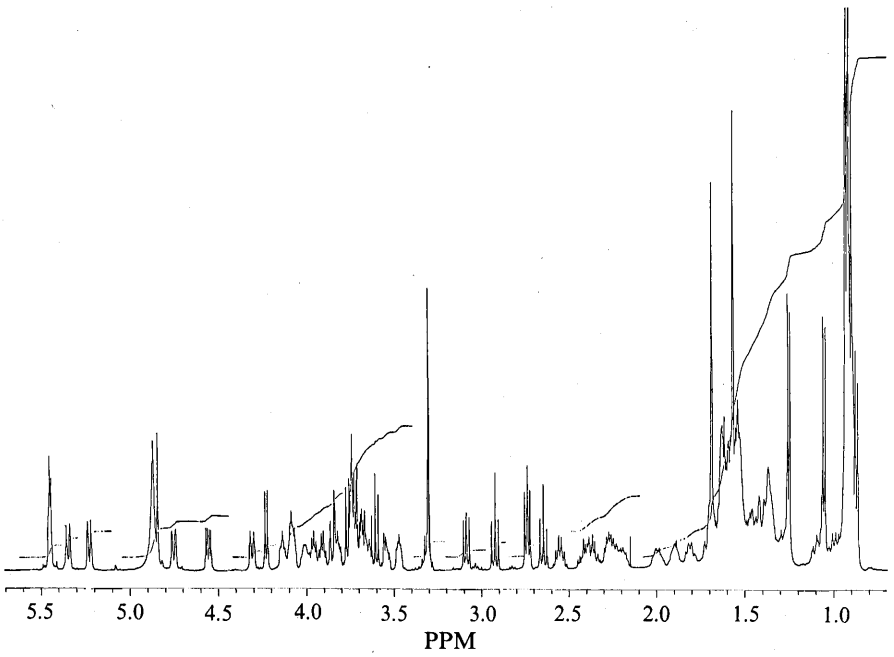
Appearance	White solid
Solubility	Water, MeOH and DMSO
MP	129 ~ 131°C
$[\alpha]_D$	+4.24° (*c* 0.253, water)
Molecular weight	1396 (FAB-MS)
Molecular formula	$C_{71}H_{132}N_2O_{24}$ (HRFAB-MS)
Found	1397.9212 $(M+H)^+$
Calcd. for $C_{71}H_{133}N_2O_{24}$	1397.9248 $(M+H)^+$
HPLC Rt	6.1 minutes
UV (MeOH) nm	End absorption
IR (KBr) cm^{-1}	3400, 2950, 1730, 1660, 1620, 1470, 1070

Table 2. *In-vitro* activity of mathemycin A (**1**).

Test organism	MAC[a] (mg/liter)
Fusarium culmorum 100	31.25
Alternaria mali P37	15.60
Botrytis cinerea AO6	15.60
Botrytis cinerea DO_1	15.60
Pellicularia sasakii JO3	15.60
Leptosphaeria nodorum JO2	31.25
Pyricularia oryzae KO2	31.25
Pseudocercosporella herpotrichoides 008	62.50
Phytophthora infestans JO8	7.80

[a] Concentration, at which a paper disc (6 mm diameter) impregnated with 20 μl of test solution and dried shows an inhibition zone of 10 mm when tested by agar diffusion method, is considered as MAC.

Fig. 1. 500 MHz ^1H NMR spectrum of mathemycin A in CD_3OD.



1 and the 500 MHz ^1H spectrum in CD_3OD is shown in Figure 1.

The *in vitro* activity [minimum active concentration (MAC)] of mathemycin A (**1**) is shown in Table 2. The MAC values against phytopathogens are in the range of 7.8 ~ 62.5 mg/liter. The activity against *Phytophthora infestans* (7.8 mg/liter) is better than that shown by Maneb® (125 mg/liter) and Mancozeb® (62.5 mg/liter). However, in green house trials, the LC_{50} and LC_{90} of **1** against *Phytophthora infestans* JO8 were found to be 42 and 500 ppm respectively, which are inferior to that shown by the commercial standards *viz.* Maneb®[2] (LC_{50}: 20 ppm; LC_{90}: 250 ppm) and Mancozeb®[2] (LC_{50}: 10 ppm; LC_{90}: 110 ppm).

Acknowledgments

We thank Mr. E. SREEKUMAR and Mr. S. D. NAKER for technical assistance and Mr. R. R. CHANDRAN for fermentation. We are grateful to Dr. K. ROY for providing us the *in-vitro* data. We acknowledge the support of Dr. P. K. INAMDAR for optical rotation and IR spectrum.

References

1) NADKARNI, S. R.; S. V. GUPTE, R. G. BHAT, T. MUKHOPADHYAY, B. N. GANGULI, H. KOGLER & B. SACHSE: M 881615B, a new antifungal antibiotic produced by an *Actinomycete* sp. HIL Y-8620959. Program and Abstracts of BMP Japan 95, Oiso, Japan, p. 50, April 23 ~ 26, 1995
2) WORTHING, C. R.: 'The Pesticide Manual', 9th Edition, pp. 529 ~ 532, 1991