Mathemycin B, a New Antifungal Macrolactone from Actinomycete Species HIL Y-8620959

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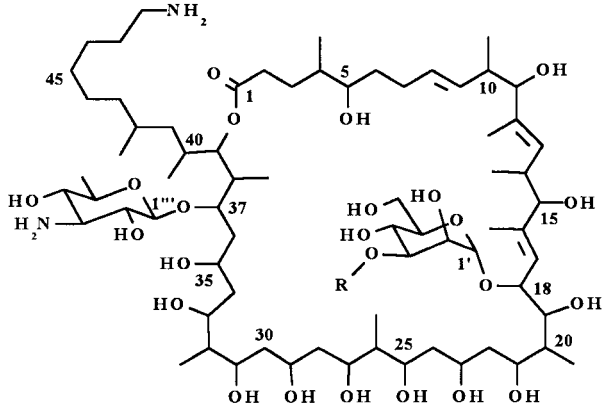
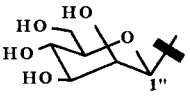
A new macrocyclic lactone antibiotic mathemycin B (**1**) was isolated from the fermentation broth of an *Actinomycete* sp. culture Y-8620959. The structure of **1** was elucidated by high-resolution MS and interpretation of 2D NMR results. Mathemycin B is active against a variety of phytopathogenic organisms.

In continuation of our search for new antifungal metabolites active against plant pathogens, we isolated a new macrolactone named mathemycin B (**1**) from an unidentified actinomycete species HIL Y-8620959. Mathemycin B was coproduced with another macrolactone named mathemycin A[1,2] as a minor compound. Here, we report the isolation, characterization, and biological properties of mathemycin B (**1**).



Mathemycin A R = H

Mathemycin B (**1**) R = 

Compound **1** was obtained as white amorphous powder. The IR spectrum of **1** showed bands at 3400 and 1735 cm^{-1} indicating the presence of hydroxyl and amine and of carbonyl groups, respectively. The molecular formula of **1** was determined to be $C_{77}H_{142}N_2O_{29}$ by high-resolution FABMS. The ^{13}C NMR data of **1** is given in the Experimental Section. The carbon multiplicities were determined by a DEPT-135 spectrum.[3] All the protons were assigned by analysis of phase sensitive double quantum filtered $^1H-^1H$ COSY[4] and HMQC−TOCSY[5,6] spectra. The protonated carbon resonances were identified by analysis of the proton-detected $^{13}C-^1H$ shift correlation (HSQC)[7] NMR experiment. The quaternary carbons were identified by interpretation of a proton-detected long-range $^{13}C-^1H$ shift correlation (HMQC)[8] NMR experiment optimized for $^nJ_{CH}$ values of 7 Hz and a broadband decoupled ^{13}C NMR spectrum.

The ^{13}C NMR and DEPT-135 spectra of **1** revealed 12 × CH_3, 19 × CH_2 [16 × CH_2, 1 × NCH_2 and 2 × OCH_2], 43 × CH [9 × CH, 1 × NCH, 26 × OCH, 3 × OCHO and 4 × =CH], and 3 × C [2 × =C and 1 × CO] signals accounting for all 77 carbon atoms and 117 protons bound to carbon. The remaining 25 protons are linked to heteroatoms.

The ^1H NMR spectroscopic comparison of the two mathemycins was carried out initially in CD_3OD, but the severe overlap of resonances impeded the assignments, especially for the carbohydrate residues. Therefore, spectra were recorded in DMSO-d_6 where 22 exchangeable protons (bound to oxygen) could be identified and assigned (out of 23 exchangeable protons).

The NMR spectral data together with the molecular ion at *m/z* 1559 $(M + H)^+$ in FABMS indicated that **1** was closely related to mathemycin A[1,2] except for **1** having an additional hexose unit ($C_6H_{10}O_5$,162 amu). However, acid hydrolysis of **1** and mathemycin A (2 N HCl, 100 °C, 2 h) followed by TLC [silica/acetone−*n*-butanol−0.02 M, pH 7 phosphate buffer (4:1:1)/*p*-anisaldehyde-sulfuric acid spray)] comparison indicated the presence of only mannose and 3-amino-3-desoxyrhamnose in both mathemycin A and **1**. This suggested that the additional hexose unit was another mannose. The ^1H NMR data of the additional mannose unit is given in the Experimental Section.

The location of the second mannose unit was established by long-range correlations in the HMQC[8] spectrum. The anomeric carbon signal (δ 101.9) of the second mannose unit gave a $^3J_{CH}$ correlation to a proton signal at δ 3.66 assigned to the 3′-H of mannose-1 establishing the linkage of the second mannose to mannose-1 at C-3′.

Thus, the structure of mathemycin B was established as represented by **1**. The stereochemistry of the basic carbohydrate residue was established by Smith degradation[9] to be 3-amino-3-desoxy-D-rhamnopyranose. The mannoside anomers were determined to be α as determined from $^1H-^1H$ ($^3J_{H1\text{-}H2}$ = 0.8 Hz/1.0 Hz), $^1H-^{13}C$ ($^1J_{CH}$ = 168 Hz/170 Hz,[10] $^3J_{C-3H\text{-}1}$ = 6.0 Hz/6.1 Hz,[11,12] and $^3J_{C-5H\text{-}1}$ = 6.7 Hz/6.8 Hz[11,12]) coupling constants and ^{13}C chemical shifts[10] (δ_{C5} 73.7, 73.6). The anomeric center of the 3-amino-3-desoxyrhamnose residue was determined to be β from $^1J_{CH}$ ($^1J_{C1\text{-}H}$ = 158 Hz) and $^3J_{HH}$ ($^3J_{H1\text{-}H2}$ = 7.6 Hz) coupling constants, both indicating the axial orientation of H-1.[10−12]

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Stereochemistry of all other asymmetric centers was not established.

A shake flask fermentation study using HPLC for detection (Experimental Section) was undertaken to improve the production of **1**. When starch (2%) in the production medium was replaced by glucose (2%), the ratio of mathemycin A and B changed to 55:45 from the usual ratio of 80:20. The MAC[1] (minimum active concentration in mg/L) values against phytopathogens are listed in the Experimental Section. The activity against *Phytophthora infestans* (62.5 mg/L) was better than that shown by Maneb (125 mg/L), and was similar to that of Mancozeb (62.5 mg/L). However, **1** was less active than mathemycin A[1].

Experimental Section

General Experimental Procedures. The HPLC analysis was carried out on a 4 mm × (30 + 100) mm ODS-hypersil (10 μ) column using 0.05 M phosphate buffer, pH 7.6−CH_3CN (50:50) as the mobile phase at a flow rate of 2 mL/min and detection at 210 nm. Optical rotation was measured using a Rudolph autopol III polarimeter. The UV spectrum was recorded on a UVIKON 810 double beam spectrophotometer. The IR spectrum was obtained on a Perkin-Elmer 157 spectrophotometer. NMR experiments were recorded on a Bruker DRX 600 NMR spectrometer using a concentration of 4.5 mg per 0.6 mL of DMSO-d_6. FABMS spectrum was recorded on a VG-ZAB SEQ spectrometer.

Organism, Fermentation, and Isolation. The organism (actinomycete species HIL Y-8620959) and fermentation conditions used are the same as described earlier[1] for mathemycin A. HPLC analysis of the processed culture filtrate sample showed the presence of mathemycin B (**1**) in addition to the major metabolite mathemycin A, in the ratio of 20:80.

The culture broth (81 L) was harvested and processed as described earlier[1] to obtain the crude antibiotic (25 g). The crude antibiotic was dissolved in H_2O (0.5 L), and the pH was adjusted to pH 3.5 with dilute HCl. The acidic solution was passed through a column of CM-Sephadex (Na^+) (0.75 L). The column was washed with H_2O and eluted successively with 0.1 M (3 L), 0.2 M (5 L), 0.3 M (5 L), 0.4 M (5 L), and 0.5 M (3 L) of aqueous NaCl solution. The active fractions, which eluted with 0.1−0.2 M aqueous NaCl solution, were combined (4 L) and desalted by passing through a column of Diaion HP-20 (0.4 L). The column was washed with H_2O (3 L) and then eluted with acetone−0.1 M aqueous ammonia (1:1) (2 L). The active eluates were concentrated to 20 mL and chromatographed on Sephadex G-10 (0.4 L) with H_2O as the eluting solvent. The active fractions were concentrated and lyophilized to obtain pure **1** (0.6 g). Further elution of the CM-Sephadex (Na^+) column with 0.2−0.4 M aqueous NaC1 solution and subsequent desalting yielded mathemycin A[1] (7.2 g).

Mathemycin B (1): isolated as white amorphous powder; soluble in H_2O, MeOH and DMSO; mp 184−187 °C; $[\alpha]^{25}_D$ +11.76° (*c* 0.273, MeOH); UV (MeOH) λ_{max} end absorption; IR (KBr) ν_{max} 3400, 2950, 1735, 1650, 1530, 1470, 1400, and 1080 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz, chemical shifts of the additional mannose unit) δ 4.88 (1H, d, J = 0.8 Hz, H-1″), 4.59 (1H, d, J = 4.5 Hz, 4″-OH), 4.53 (1H, d, J = 4.5 Hz, 2″-OH), 4.48 (1H, t, J = 6.0 Hz, 6″-OH), 4.42 (1H, d, J = 4.5 Hz, 3″-OH), 3.74 (1H, ddd, J = 4.5, 2.5, 0.8 Hz, H-2″), 3.64 (1H, ddd, J = 10.8, 6.0, 2.2 Hz, H_1-6″), 3.60 (1H, ddd, J = 9.5, 5.5, 2.2 Hz, H-5″), 3.58 (1H, ddd, J = 9.0, 4.5, 2.5 Hz, H-3″), 3.44 (1H, ddd, J = 10.8, 6.0, 5.5 Hz, H_2-6″), 3.37 (1H, ddd, J = 9.5, 9.0, 4.5, H-4″); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 172.6 (s, C-1), 143.9 (s, C-16), 135.1 (s, C-12), 133.5 (d, C-9), 128.4 (d, C-8), 128.1 (d, C-13), 120.4 (d, C-17), 101.9 (d, C-1″), 99.7 (d, C-1‴), 95.8 (d, C-1′), 80.7 (d, C-15), 79.0 (d, C-3′), 78.6 (d, C-11), 77.3 (d, C-39), 74.7 (d, C-19), 74.1 (d, C-37), 73.7 (d, C-5′), 73.6 (d, C-5″), 72.2 (d, C-5‴), 71.9 (d, C-5), 71.8 (d, C-25), 71.5 (d, C-4‴), 70.7 (d, C-3″), 70.5 (d, C-33), 70.3 (d, C-18), 70.2 (d, C-2″), 69.7 (d, C-2‴), 69.6 (d, C-2′), 68.9 (d, C-31), 68.6 (d, C-35), 68.1 (d, C-27), 67.2 (d, C-4″), 66.4 (d, C-23), 65.9 (d, C-4′), 65.1 (d, C-21), 63.9 (d, C-29), 61.2 (t, C-6″), 61.0 (t, C-6′), 58.0 (d, C-3‴), 43.4 (d, C-32), 43.1 (d, C-26), 42.3 (t, C-30), 41.9 (t, C-24), 41.8 (t, C-22), 41.8 (t, C-28), 39.7 (d, C-20), 39.6 (d, C-10), 39.1 (t, C-34), 38.7 (t, C-48), 37.3 (d, C-4), 37.0 (t, C-36), 36.5 (t, C-41), 35.9 (d, C-38), 35.2 (d, C-14), 34.3 (t, C-43), 34.1 (t, C-6), 31.7 (t, C-2), 30.9 (d, C-40), 29.0 (t, C-7), 29.0 (d, C-42), 28.7 (t, C-45), 28.3 (t, C-3), 26.8 (t, C-47), 26.0 (t, C-44), 25.7 (t, C-46), 20.8 (q, 42-CH_3), 17.5 (q, C-6‴), 17.1 (q, 14-CH_3), 16.8 (q, 40-CH_3), 15.2 (q, 10-CH_3), 13.2 (q, 4-CH_3), 12.5 (q, 12-CH_3), 11.5 (q, 16-CH_3), 11.1 (q, 26-CH_3), 10.2 (q, 32-CH_3), 9.9 (q, 20-CH_3), 9.4 (q, 38-CH_3); HRFABMS *m/z* 1559.9777 (calcd for $C_{77}H_{142}N_2O_{29}$,-1559.9776); HPLC (above-mentioned conditions) t_R 4.6 min.

In-Vitro Assay. The in vitro activity of **1** was determined by the method described earlier[1] for mathemycin A. Compound **1** showed moderate activity against phytopathogens with minimum active concentration (MAC) values of 62.5 mg/L (*Fusarium culmorum* 100), 62.5 mg/L (*Alternaria mali* P37), 62.5 mg/L (*Botrytis cinerea* AO6), 62.5 mg/L (*Botrytis cinerea* DO_1), 62.5 mg/L (*Pellicularia sasakii* JO3), 125 mg/L (*Leptosphaeria nodorum* JO2), 125 mg/L (*Pyricularia oryzae* KO2), 250 mg/L (*Pseudocercosporella herpotrichoides* 008), and 62.5 mg/L (*Phytophthora infestans* JO8).

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Supporting Information Available: ^1H and ^{13}C NMR data for Mathemycin B (**1**) in DMSO-d_6 and CD_3OD (Table S1). Ordering information is given on any current masthead page.

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