Azalomycin F Complex from *Streptomyces hygroscopicus*, MSU/MN-4-75B

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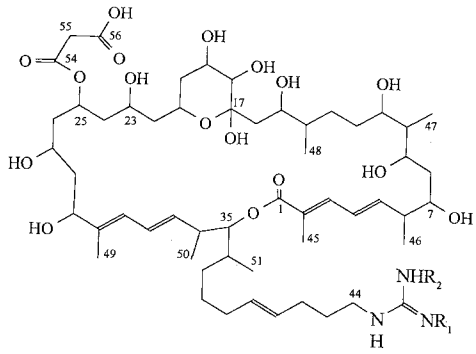
Our ongoing research for antimicrobial compounds from soil borne microorganisms[1,2] have resulted in the repeat isolation of a complex (**1**) azalomycins F_{3a}, F_{4a} and F_{5a}[3] from *Streptomyces hygroscopicus*, MSU/MN-4-75B. The compounds showed a broad spectrum antibacterial and antifungal activities similar to the activities reported earlier[4,5]. Also, the excellent zones of inhibition were obtained against asparagus (*Asparagus officinalis*) pathogens *Fusarium moliniforme* and *Fusarium oxysporum* as well as powdery mildew pathogen *Botrytis* spp. The structural work of the azalomycin F complex isolated in our laboratory revealed some additional information which is not reported earlier. Therefore, we propose a revised structure for azalomycins F_{3a}, F_{4a} and F_{5a}.

Cultures of *Streptomyces hygroscopicus* (6 liters), were grown in 2-liter baffle bottomed Erlenmeyer flasks, each containing 400 ml of A-9 medium (peptone 5 g, glucose 10 g, "Brer Rabbit green label" molasses 20 g, distilled H_2O 1 liter). The inoculated flasks were placed on a rotary shaker at 130 rpm at 26°C for 7 days and centrifuged to obtain the mycelial cake. The mycelial cake was extracted with MeOH (2.5 liters) to afford a crude extract (4.8 g) which was fractionated by vacuum liquid chromatography (VLC) on silica gel. Three fractions, I (500 ml, $CHCl_3$), II (500 ml, $CHCl_3$ - MeOH 4:1, v/v) and III (750 ml, MeOH), were evaporated *in vacuo* separately to afford 580 mg, 218 mg and 3.4 g of powdered products, respectively. Bioassay of these fractions revealed that fraction III was the only fraction with the antimicrobial activity. It was further purified on a preparative liquid chromatograph LC-20 (Japan Analytical Co., Ltd., Tokyo, Japan). Two serially connected GS-310 2F columns (13 μm, 21.5 × 300 mm; Asahi Chemical Industrial Co., Ltd., Kawasaki-shi, Japan) were used separation. The guard column was GS10P (7.6 × 50 mm). The mobile phase MeOH - H_2O (80:20,



Azalomycin F complex, **1**.

1: $R_1=R_2=CH_3$ (F_{5a})
$R_1=H$; $R_2=CH_3$ (F_{4a})
$R_1=R_2=H$ (F_{3a})

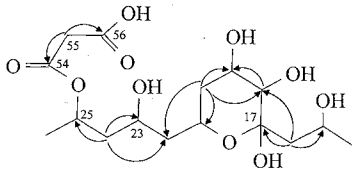
Table 1. ^1H NMR chemical shifts for compound **1** (J in Hz).

Proton	δ	Proton	δ
H-3	7.08 (d, 11.49)	H-29	4.17 (ddd, 8.17, 3.09, 2.65)
H-4	6.44 (dd, 14.58, 11.49)	H-31	6.00 (d br, 11.05)
H-5	6.07 (dd, 14.80, 8.40)	H-32	6.22 (dd, 14.00, 11.05)
H-6	2.44 m	H-33	5.44 m
H-7	3.76 (t br, 3.80)	H-34	2.56 (dd, 7.51, 7.07)
H-8	1.55 m	H-35	4.78 (dd, 8.10, 4.19)
H-9	3.88 m	H-36	1.82 m
H-10	1.78 m	H-37	1.55 m
H-11	3.88 m	H-38	1.55 m
H-12	1.55 m	H-39	2.03 m
H-13	1.55 m	H-40	5.44 m
H-14	1.78 m	H-41	5.44 m
H-15	3.88 m	H-42	2.03 m
H-16	1.55 m	H-43	1.55 m
H-18	3.35 (d, 8.00)	H-44	3.15 (t, 6.85)
H-19	3.88 m	H-45	1.92 s
H-20	1.44 m	H-46	1.10 (d, 6.85)
H-21	4.08 (t br, 10.83, 9.94)	H-47	0.86 (d, 6.84)
H-22	1.44 m	H-48	0.88 (d, 6.63)
H-23	3.88 m	H-49	1.65 s
H-24	1.55 m	H-50	0.98 (d, 6.62)
H-25	5.23 m	H-51	0.94 (d, 6.63)
H-26	1.55 m	H-53	2.85 s
H-27	4.02 m	H-55	3.24 s
H-28	1.55 m	H-57$^{(5a)}$	2.87 s

Table 2. ^{13}C NMR chemical shifts for compound **1**.

Carbon	δ	Carbon	δ	Carbon	δ
C-1	170.02	C-21	65.58	C-41	130.15
C-2	126.76	C-22	41.89	C-42	29.77
C-3	140.07	C-23	65.71	C-43	30.52
C-4	127.50	C-24	44.55	C-44	42.14
C-5	145.98	C-25	70.72	C-45	12.84
C-6	40.64	C-26	46.26	C-46	16.89
C-7	75.78	C-27	66.32	C-47	10.54
C-8	39.27	C-28	44.02	C-48	14.94
C-9	75.19	C-29	74.28	C-49	13.33
C-10	44.10	C-30	140.07	C-50	17.68
C-11	72.33	C-31	125.09	C-51	14.38
C-12	39.27	C-32	128.47	C-52$^{(3a)}$	158.69
C-13	29.77	C-33	136.11	C-52$^{(4a)}$	158.27
C-14	40.64	C-34	40.84	C-52$^{(5a)}$	157.30
C-15	72.49	C-35	80.85	C-53	28.34
C-16	41.99	C-36	33.52	C-54	171.59
C-17	99.79	C-37	28.35	C-55	46.26
C-18	77.39	C-38	27.85	C-56	173.98
C-19	69.69	C-39	30.52	C-57$^{(5a)}$	28.40
C-20	41.16	C-40	132.49		

Fig. 1. ^{1}H-^{13}C HMBC correlations observed for the hemiketal and malonyl moieties in compound **1**. Arrows are directed from H to C.

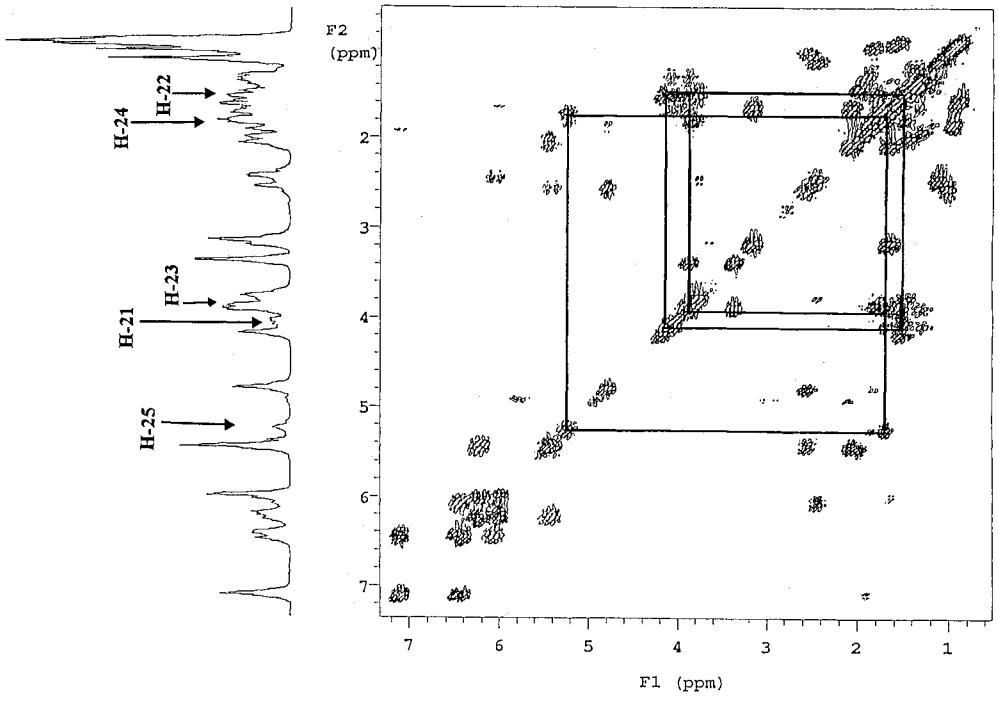


v/v) was used under isocratic conditions at a flow rate of 5 ml/minute and detected at 254 nm and afforded the active fraction (710 mg) as a single peak at 25.54 minute. This fraction was purified again on an ODS column (Jaigel S-343-15; 15 μm, 20 × 250 mm; Japan Analytical Co., Ltd., Tokyo, Japan) using LC-20 at a flow rate of 3 ml/minute. The single peak at 52.19 minute (358 mg) was active and recrystallized from EtOH/H_2O. The azalomycin F complex, **1** (284 mg) gave a single spot on TLC (Silica gel; $CHCl_3$ - MeOH - H_2O 1 : 1 : 0.1; Rf 0.45).

Azalomycin F complex, **1**, white amorphous solid melted at 130~133°C, gave the molecular ion peaks at (FAB, NBA) *m/z* (% intensity, molecular formula): 1097.3 (24, $C_{57}H_{97}N_3O_{17}+2H$), 1083.3 (48, $C_{56}H_{95}N_3O_{17}+2H$), 1069.2 (34, $C_{55}H_{93}N_3O_{17}+2H$), 136.2 (100). The difference of 14 mass units among the observed molecular ion peaks suggested that the isolated antibiotic is a mixture of azalomycins F_{5a} (MW 1095), F_{4a} (MW 1081) and F_{3a} (MW 1067)[3] at the ratio of 22.64%, 45.28% and 32.07%, respectively.

^{1}H, ^{13}C, DEPT, DQFCOSY, HMQC and HMBC NMR were carried out on Varian VXR 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) using standard pulse sequences and CD_3OD as the internal standard (Tables 1 and 2). The assignments for the hemiketal moiety were confirmed by the HMBC correlations as shown in Fig. 1. Long-range couplings were observed for proton at C-16 (δ 1.55) to the hemiketal carbon at C-17 (δ 99.79). Further assignments of the ^{1}H and ^{13}C NMR were accomplished by the detailed analyses of these spectra as well as the comparison of the data with those of related antibiotics[6,7]. In the earlier report, the signal for a hemiketal carbon in azalomycin F was reported at δ

Fig. 2. DQFCOSY ^{1}H NMR spectrum of compound **1**.



99.78 ppm (C-17) in its ^{13}C NMR spectrum, but the corresponding proton signal (21-H) was missing in the ^{1}H NMR spectrum. Hence the structure of azalomycin F was represented by assigning carbonyl and hydroxy groups at C-17 and C-21, respectively[3,4,8~10]. In addition, the position of the malonyl moiety was left unambiguous.

The ^{1}H NMR signals for H-15 and H-16 appeared as multiplets at 3.88 and 1.55 ppm, respectively. The H-18 proton appeared as a doublet at 3.35 ppm due to the hemiketal moiety at C-17. Except for H-21, H-19 through H-28 protons appeared as multiplets (Table 1). The ^{1}H NMR spectrum of **1** showed a broad triplet at δ 4.08 for H-21, correlating to the multiplet at δ 1.44 of 20-H and 22-H, in the DQFCOSY spectrum of the compound (Fig. 2). The multiplicity of the signal at δ 4.08 along with the 2D NMR correlations established that H-21 is part of a hemiketal ring similar to copiamycin and malolactomycins A and B[6,7]. The ^{1}H NMR spectrum also showed multiplets at δ 5.22 and 3.88, which were accounted for the methine protons attached to the malonyl and hydroxyl groups at C-25 and C-23, respectively[7]. The signal at δ 3.88 showed cross peaks with the multiplet at δ 1.55 (24-H) and δ 1.44 (20-H, 22-H), while signal at δ 5.22 was correlated only to the signals at δ 1.55 in the DQFCOSY spectrum of **1** as shown in Fig. 2. These observations confirmed the correct position of the malonyl moiety at C-25 and the presence of a 6-membered ring hemiketal between C-17 and C-21. Similar ^{1}H and ^{13}C NMR spectral data were reported for malolactomycin B[7] which contain 6-membered hemiketal ring and malonyl moieties. Therefore, the structure of azalomycin F complex isolated from *Streptomyces hygroscopicus* MSU/MN-4-75B has been established by our NMR analysis as shown in **1**.

Acknowledgments

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