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

II. Structure Elucidation†

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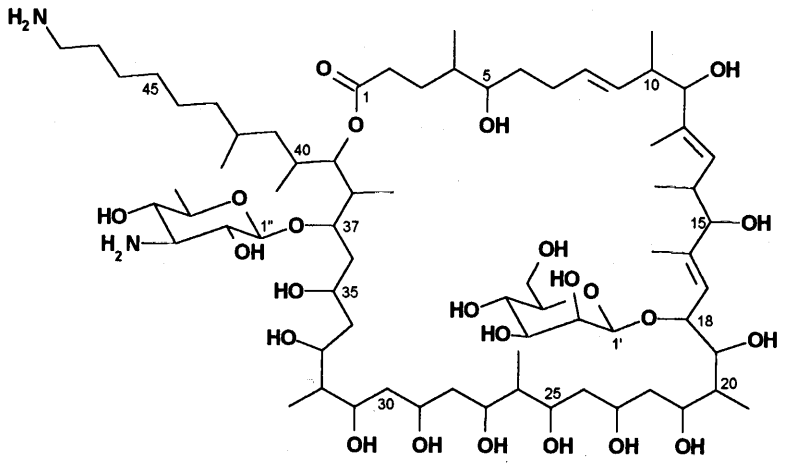
In the preceding paper[1], we reported the fermentation, isolation, and biological properties of an antifungal metabolite mathemycin A active against plant pathogens. Herein, we report the structure elucidation of mathemycin A (**1**) by a combination of 2D-NMR techniques and chemical degradations.

Mathemycin A was obtained as a white powder, m.p. 129~131°C; $[\alpha]_D$ +4.24° (*c* 0.253, water). The IR spectrum of **1** showed absorption bands at 3460 and 1725 cm^{-1} indicating the presence of hydroxyls/amines and at least one carbonyl respectively. The molecular formula of **1** was determined to be $C_{71}H_{132}N_2O_{24}$ by the analysis of the HRFAB-MS [$(M+H)^+$: Found *m/z* 1397.9212; calcd. for $C_{71}H_{133}N_2O_{24}$ *m/z* 1397.9248]. Acid hydrolysis (2 N TFA, 100°C, 2 hours) followed by GLC analysis of the hydrolysate as trimethylsilyl derivatives indicated the presence of mannose and an unidentified sugar.

Table 1 summarizes the ^1H and ^{13}C NMR spectra of mathemycin A (**1**) in CD_3OD. The carbon multiplicities were determined by DEPT-135 spectrum[2]. All the protons were assigned by the analysis of phase-sensitive double quantum filtered ^1H-^1H COSY[3] and HSQC-TOCSY[4]. The protonated carbon resonances were identified by the analysis of the proton-detected ^{13}C-^1H shift-correlation (HSQC)[5] NMR experiment. The quaternary carbons were identified by the interpretation of proton-detected long-range ^{13}C-^1H shift-correlation (HMBC)[6] NMR experiment optimized for $^nJ_{CH}$ values of 6 Hz.

The ^{13}C NMR and DEPT-135 spectra of **1** revealed the presence of $12\times CH_3$, $18\times CH_2$ [$16\times CH_2$, $1\times NCH_2$ and $1\times OCH_2$], $38\times CH$ [$9\times CH$, $1\times NCH$, $22\times OCH$, $2\times OCHO$ and $4\times =CH$] and $3\times C$ [$2\times =C$ and $1\times CO$] accounting for 110 protons. The remaining 22 protons are linked to hetero atoms and got exchanged in CD_3OD. The molecular formula of **1** required 7 degrees of unsaturation, out of which 6 were accountable in the form of three double bonds, one carbonyl and two sugars, indicating that mathemycin A was a cyclic compound with two sugars. The spectral properties bore close similarities to those reported for desertomycin A,



(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.

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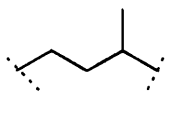
Table 1. ^{1}H and ^{13}C NMR spectral data of mathemycin A (**1**) in CD_3OD.

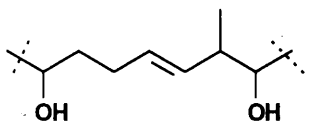
Carbon	δ_C	δ_H	
		δ_H	HSQC-TOCSY
1	175.73 (s)	—	
2	34.01 (t)	2.411 (dt, 15.8, 7.8, 5.6 Hz)	3-H_2, 4-H, 4-CH_3
		2.372 (ddd, 15.8, 8.0, 6.2 Hz)	
3	30.35 (t)	1.804 (dt, 13.0, 7.9, 6.3 Hz)	2-H_2, 4-H, 4-CH_3
		1.518 (m)	
4	39.91 (d)	1.520 (m)	2-H_2, 3-H_2, 4-CH_3
5	75.39 (d)	3.469 (ddd, 7.6, 4.6, 3.0 Hz)	6-H_2, 7-H_2
6	35.84 (t)	1.471 (m), 1.445 (m)	5-H, 7-H_2, 8-H
7	31.43 (t)	2.202 (m), 1.992 (m)	5-H, 6-H_2, 8-H
8	130.89 (d)	5.435 (m)	9-H, 10-CH_3
9	135.68 (d)	5.444 (m)	8-H, 10-H
10	41.84 (d)	2.270 (ddq, 6.6, 6.3, 6.0 Hz)	9-H, 10-CH_3, 11-H
11	81.58 (d)	3.731 (dq, 6.0 Hz)	9-H, 10-H, 10-CH_3
12	137.04 (s)	—	
13	130.16 (d)	5.219 (dq, 9.5, 1.2 Hz)	12-CH_3, 14-H, 14-CH_3, 15-CH_3
14	37.36 (d)	2.553 (tq, 9.5, 8.8, 6.6 Hz)	13-H, 14-CH_3, 15-H
15	84.28 (d)	3.753 (dd, 8.8 Hz)	13-H; 14-H, 14-CH_3
16	146.95 (s)	—	
17	124.10 (d)	5.337 (dq, 9.8, 1.0 Hz)	16-CH_3, 18-H, 19-H
18	73.19 (d)	4.553 (dd, 9.8, 4.5 Hz)	17-H, 19-H
19	78.69 (d)	3.675 (dd, 9.8, 4.5 Hz)	17-H, 18-H, 20-H, 20-CH_3
20	40.68 (d)	1.590 (m)	19-H, 20-CH_3
21	69.26 (d)	4.305 (dt, 10.2, 1.6 Hz)	22-H_2
22	43.63 (t)	1.699 (dt, 10.2, 2.3 Hz)	21-H, 23-H, 24-H_2
		1.370 (m)	
23	69.68 (d)	4.005 (m)	22-H_2, 24-H_2, 25-H
24	43.41 (t)	1.672 (m), 1.601 (m)	22-H_2, 23-H, 25-H, 26-H
25	75.23 (d)	3.819 (ddd, 9.4, 6.4, 3.0 Hz)	23-H, 24-H_2, 26-H
26	45.00 (d)	1.637 (m)	24-H_2, 25-H, 26-CH_3
27	72.69 (d)	3.905 (q, 6.5 Hz)	28-H_2
28	43.44 (t)	1.648 (m), 1.610 (m)	26-H, 27-H, 29-H, 30-H_2
29	66.98 (d)	4.077 (m)	28-H_2, 30-H_2
30	44.12 (t)	1.624 (m)	28-H_2, 29-H, 31-H
31	70.99 (d)	4.070 (dt, 9.0, 3.0 Hz)	30-H_2, 32-H
32	44.84 (d)	1.565 (m)	31-H, 32-CH_3, 34-H_2
33	73.56 (d)	4.133 (dt, 7.3, 2.5 Hz)	32-H, 34-H_2, 35-H
34	41.23 (t)	1.540 (m)	32-H, 33-H, 35-H, 36-H_2
35	72.15 (d)	3.953 (quint., 6.2 Hz)	33-H, 34-H_2, 36-H_2, 37-H
36	38.55 (t)	1.601 (m), 1.580 (m)	34-H_2, 35-H, 37-H, 38-H
37	77.76 (d)	3.647 (dt, 9.8, 2.6 Hz)	35-H, 36-H_2'
38	38.45 (d)	2.252 (ddq, 10.1, 9.8, 6.8 Hz)	36-H_2, 37-H, 38-CH_3, 39-H, 40-H
39	80.48 (d)	4.749 (dd, 10.1, 1.9 Hz)	38-H, 38-CH_3
40	33.38 (d)	1.896 (m)	40-CH_3, 41-H_2
41	38.53 (t)	1.394 (m)	40-H, 40-CH_3, 42-H, 42-CH_3
		1.090 (ddd, 14.0, 11.1, 4.0 Hz)	
42	31.48 (d)	1.515 (m)	41-H_2, 42-CH_3, 43-H_2
43	36.67 (t)	1.425 (m), 0.995 (m)	42-H, 44-H_2, 45-H_2
44	28.25 (t)	1.420 (m), 1.270 (m)	43-H_2, 45-H_2
45	31.32 (t)	1.357 (m), 1.304 (m)	43-H_2, 44-H_2, 46-H_2
46	28.37 (t)	1.372 (m), 1.349 (m)	45-H_2, 47-H_2, 48-H_2
47	32.07 (t)	1.542 (quint., 7.9 Hz)	46-H_2, 48-H_2
48	42.27 (t)	2.737 (t, 7.3 Hz)	46-H_2, 47-H_2
4-CH_3	14.68 (q)	0.895 (d, 6.6 Hz)	3-H, 4-H
10-CH_3	16.12 (q)	0.908 (d, 6.6 Hz)	9-H, 10-H, 11-H
12-CH_3	13.66 (q)	1.565 (d, 1.0 Hz)	13-H
14-CH_3	18.51 (q)	1.050 (d, 6.6 Hz)	13-H, 14-H, 15-H
16-CH_3	12.68 (q)	1.685 (d, 1.2 Hz)	17-H
20-CH_3	11.95 (q)	0.903 (d, 6.6 Hz)	19-H, 20-H

Table 1. (Continued)

Carbon	δ_C	δ_H	
		δ_H	HSQC-TOCSY
26-CH_3	12.17 (q)	0.927 (d, 6.6 Hz)	25-H, 26-H
32-CH_3	12.08 (q)	0.923 (d, 6.6 Hz)	31-H, 32-H
38-CH_3	10.75 (q)	0.928 (d, 6.8 Hz)	39-H, 38-H
40-CH_3	18.32 (q)	0.870 (d, 6.7 Hz)	40-H, 41-H_2
42-CH_3	22.08 (q)	0.929 (d, 6.6 Hz)	42-H, 43-H_2
1′	98.08 (d)	4.840 (d, 1.5 Hz)	2′-H
2′	73.22 (d)	3.743 (dd, 1.5 Hz)	1′-H, 3′-H, 4′-H, 5′-H
3′	72.85 (d)	3.716 (dd, 9.7 Hz)	1′-H, 4′-H, 6′-H_2
4′	69.30 (d)	3.603 (dd, 9.7, 9.5 Hz)	3′-H, 5′-H, 6′-H_2
5′	75.47 (d)	3.541 (ddd, 9.5, 5.6, 2.1 Hz)	4′-H, 6′-H_2
6′	63.46 (t)	3.844 (dd, 11.4, 2.1 Hz)	4′-H, 5′-H
		3.706 (dd, 11.4, 5.6 Hz)	
1″	102.73 (d)	4.225 (d, 7.8 Hz)	2″-H, 3″-H
2″	75.08 (d)	3.086 (dd, 9.6, 7.8 Hz)	1″-H, 3″-H, 4″-H
3″	60.09 (d)	2.646 (t, 9.6 Hz)	1″-H, 2″-H, 4″-H, 5″-H
4″	77.09 (d)	2.920 (dd, 9.6, 9.2 Hz)	2″-H, 3″-H, 5″-H, 6″-H
5″	75.06 (d)	3.308 (quint., 9.2, 6.3 Hz)	3″-H, 4″-H, 6″-H
6″	19.10 (q)	1.252 (d, 6.3 Hz)	4″-H, 5″-H



(A)



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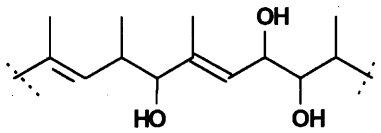

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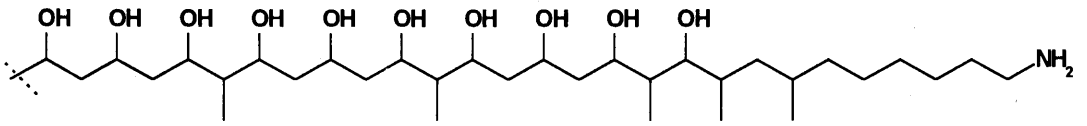

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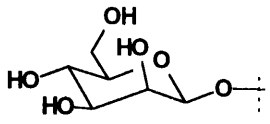

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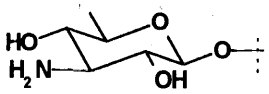

(F)

B[7,8] and oasomycins A～D[9].

Due to the severe multiple overlap of the proton resonances, only 36 proton resonances could be explicitly identified in the ^{1}H NMR spectrum of **1**. However, the identification of all the remaining proton resonances could be achieved by the analysis of HSQC spectrum. Therefore, additionally a HSQC-TOCSY experiment was carried out in CD_3OD, where the dispersion of proton-proton resonances was very much improved by sorting out proton signals along their attached carbon chemical shifts, which led to 71 resolved proton resonances (out of 86). However, unambiguous correlations would require the proton-coupling partner to be resolved at the proton scale, where only 36 individual resonances are clearly distinguishable (42%). The correlations derived from HSQC-TOCSY are listed in Table

1 (272 correlations, 37.5% of these are unambiguous). The analysis of HSQC-TOCSY ($^3J_{HH}$, $^4J_{HH}$) in addition to the HH COSY ($^2J_{HH}$, $^3J_{HH}$, 83 correlations, 18 unambiguous) led to six spin systems A~F.

The spin system (F) resembles rhamnose, except that 3-H appeared at a relatively high field (δ 2.646) which required a different heteroatom at this position, most probably nitrogen. The spin system (F) could, therefore, be a 3-amino-3-desoxyrhamnose moiety. The absolute configuration of 3-amino-3-desoxyrhamnose was established to be D by using a modified Smith degradation followed by chiral investigation of the reduced and hydrolyzed fragments[10].

Finally, the sequence of all the spin systems was deduced from topological reasons as well as by proton-carbon long correlations (HMBC). The fact that all the methyl groups were attached to olefinic carbons or methine groups suggested that the main chain was not terminated by a methyl group. Further, the IR band at 1725 cm^{-1} indicated that the nature of ring closure of the sub-unit (D) was through an ester linkage, but not an amide linkage. In the HMBC spectrum of **1**, the carbonyl at δ 175.73 gave correlations to C2-H_2 (δ 2.411 and δ 2.372) and C39-H (δ 4.749) suggesting that the ester linkage was between C1 and C39. The carbon signal at δ 73.19 assigned to C18 showed a correlation to a proton signal at δ 4.840 assigned to the anomeric proton of mannose suggesting that the mannose was linked to C18. Further, the coupling constant of the anomeric proton (d, 1.5 Hz) of mannose as well as the ^{13}C chemical shifts of C3′ and C5′ (δ 72.9 and 75.5) suggested that the glycosidic linkage was β[11]. The carbon signal at δ 77.76 showed a correlation with the anomeric proton at δ 4.225 (d, J=7.8 Hz) of 3-amino-3-desoxyrhamnose indicating that 3-aminorhamnose was connected to C37 through β-glycosidic linkage.

Thus, the structure of mathemycin A was established as represented by **1**. The stereochemistry of all the asymmetric centres was not established. The desertomycins A, B[7,8] and oasomycins A, B[9] are 42-membered macrolactones and oasomycins C, D[9] are 44-membered, while mathemycin A is the first member of similar macrolactones bearing a 40-membered macrolactone skeleton.

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