SPOREAMICIN A, A NEW MACROLIDE ANTIBIOTIC

II. STRUCTURE DETERMINATION

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(Received for publication October 7, 1991)

Structure of a novel antibiotic, sporeamicin A (SRM-A), was determined by a combination of spectroscopic and X-ray crystallographic studies. SRM-A has a unique structure containing a 2,3-dihydro-3-oxofuran moiety as part of a 14-membered macrolide ring.

Sporeamicin A (SRM-A) isolated from a culture broth of *Saccharopolyspora* sp. The taxonomy of the producing strain, the fermentation, isolation and characterization of this antibiotic have been reported in the previous paper[1]. We will report herein the structure determination of SRM-A.

Results and Discussion

The fast atom bombardment mass spectra (FAB-MS) of SRM-A showed quasi-molecular ion peaks at *m/z* 714 $(M+H)^+$ in the positive ion mode and at *m/z* 712 $(M-H)^-$ in the negative ion mode, and also the chemical ionization mass spectrum (CI-MS) showed the quasi-molecular ion peak at *m/z* 714 $(M+H)^+$. The molecular formula was found to be $C_{37}H_{63}NO_{12}$ by high resolution FAB-MS (found: 714.4419, calcd: 714.9121 $(M+H)^+$) and element analysis.

Homonuclear shift correlation spectroscopy (COSY)[2,3], heteronuclear shift correlation spectroscopy (^{1}H-^{13}C COSY)[4,5] and long-range heteronuclear shift correlation spectroscopy (long-range ^{1}H-^{13}C COSY)[4,5] are shown in Figs. 1, 2 and 3, respectively. The structure of SRM-A obtained by the present NMR study is shown in Fig. 4 which includes the numbering scheme. The ^{1}H and ^{13}C chemical shifts are shown in Tables 1 and 2, respectively.

In the ^{1}H NMR spectrum, characteristic signals due to one $N(CH_3)_2$, one OCH_3, one $=C-CH_3$ (20-H) and 2 anomeric protons (1′-H, 1″-H) were observed.

The ^{13}C NMR spectrum showed the expected signals for 37 carbons. The distortionless enhancement by polarization transfer (DEPT) experiment[6] assigned them to 13 methyls including one $N(CH_3)_2$ and one OCH_3, 4 methylenes, 13 methines including two anomeric (C-1′, C-1″), and 7 quarternary carbons including 2 carbonyl groups (C-1, C-11) and one $O-C=$ (C-9) carbon.

The ^{1}H-^{13}C COSY of SRM-A (Fig. 2) showed the following: The C-20 (6.00 ppm), C-15 (10.72 ppm), C-17 (10.93 ppm), C-16 (14.06 ppm), C-6″ (17.67 ppm), C-21 (20.62 ppm), C-6′ (21.01 ppm), C-19 (21.10 ppm), C-7″ (21.53 ppm) and C-18 (26.37 ppm) carbons showed the connectivity with resonances of methyl protons 20-H at 1.74 ppm, 15-H at 0.89 ppm, 17-H at 1.05 ppm, 16-H at 1.19 ppm, 6″-H at 1.30 ppm, 21-H at 1.37 ppm, 6′-H at 1.27 ppm, 19-H at 1.37 ppm, 7″-H at 1.23 ppm and 18-H at 1.25 ppm, respectively. The C-7 (41.82 ppm), C-14 (21.34 ppm), C-4′ (29.13 ppm) and C-2″ (35.05 ppm) carbons showed the

Fig. 1. COSY spectrum.

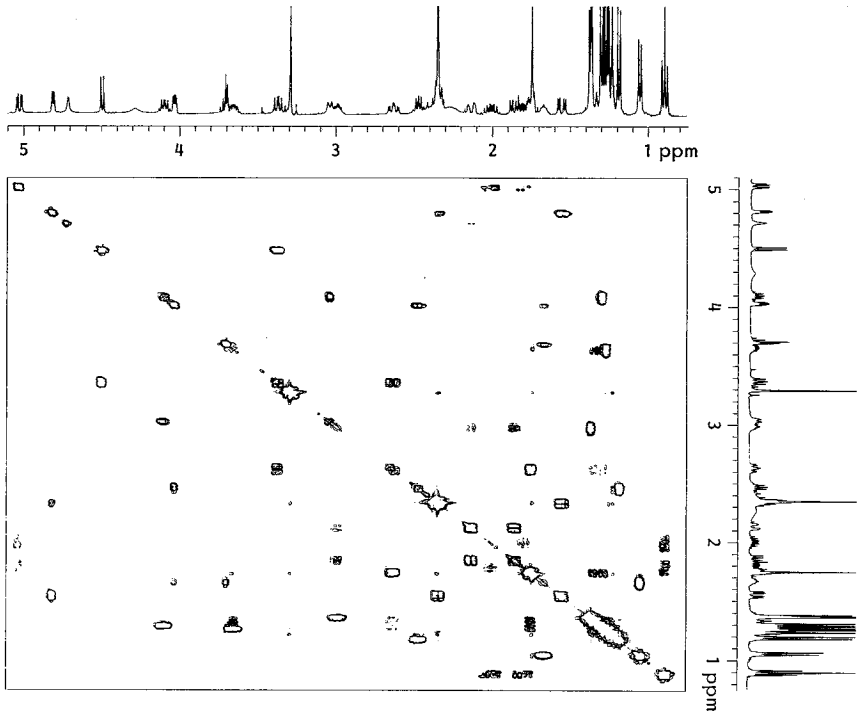


Fig. 2. ^{1}H-^{13}C COSY spectrum.

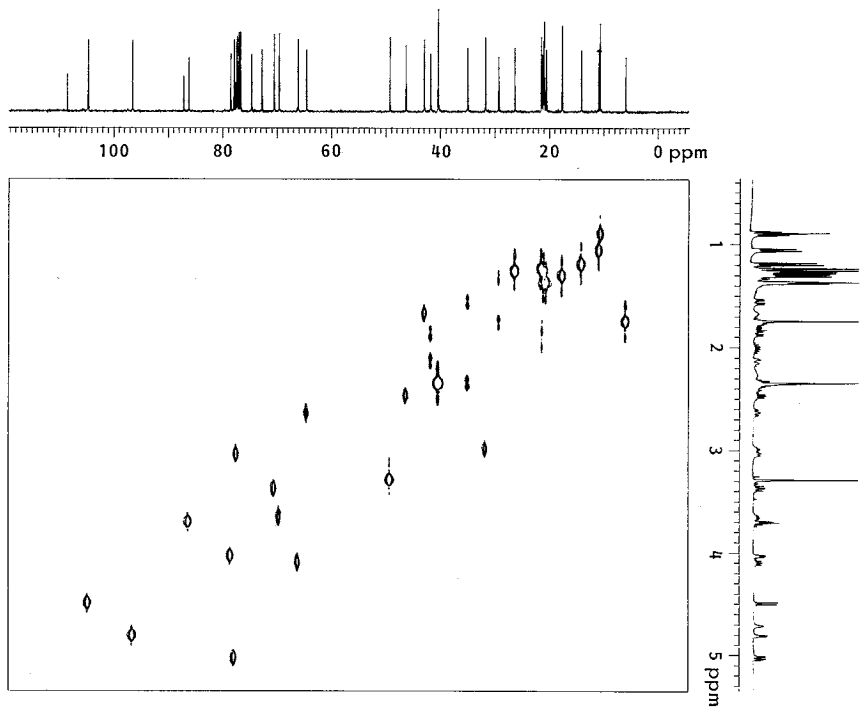


Fig. 3. Long-range ^{1}H-^{13}C COSY spectrum.

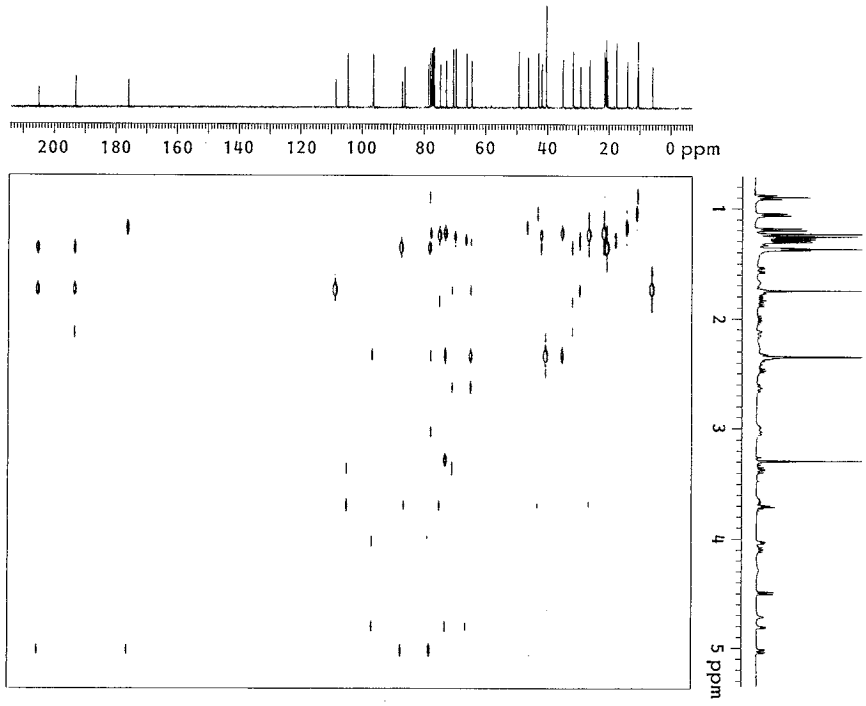
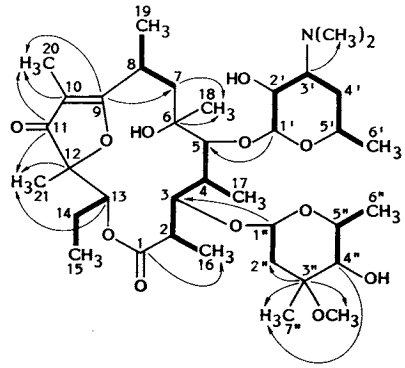


Fig. 4. The structure of SRM-A.



connectivity with resonances of a nonequivalent methylene protons 7-H_a (1.86 ppm) and 7-H_b (2.12 ppm), 14-H_a (1.80 ppm) and 14-H_b (2.00 ppm), 4'-H_a (1.34 ppm) and 4'-H_b (1.71 ppm), and 2''-H_a (1.55 ppm) and 2''-H_b (2.31 ppm), respectively. The C-8 (31.79 ppm), C-4 (43.05 ppm), C-2 (46.31 ppm), C-3' (64.65 ppm), C-5'' (66.17 ppm), C-5' (69.69 ppm), C-2' (70.58 ppm), C-4'' (77.54 ppm), C-13 (77.93 ppm), C-3 (78.55 ppm), C-5 (86.28 ppm), C-1'' (96.57 ppm) and C-1' (104.80 ppm) carbons were connected with resonances of methine protons 8-H at 2.99 ppm, 4-H at 1.66 ppm, 2-H at 2.46 ppm, 3'-H at 2.60 ppm, 5''-H at 4.09 ppm, 5'-H at 3.64 ppm, 2'-H at 3.36 ppm, 4''-H at 3.03 ppm, 13-H at 5.01 ppm, 3-H at 4.03 ppm, 5-H at 3.71 ppm, 1''-H at 4.81 ppm and 1'-H at 4.50 ppm, respectively.

In the COSY spectrum (Fig. 1), the methine proton of 8-H at 2.99 ppm shows cross peaks with resonances of methylene protons (7-H_a at 1.86 ppm and 7-H_b at 2.12 ppm) and methyl protons (19-H at 1.37 ppm). The methylene protons (14-H_a at 1.80 ppm and 14-H_b at 2.00 ppm) were coupled with the methine proton (13-H at 5.01 ppm) and methyl protons (15-H at 0.89 ppm). The methine proton (3-H at 4.03 ppm) shows two cross peaks with resonances of methine protons (2-H at 2.46 ppm and 4-H at 1.66 ppm). The methine protons of 2-H and 4-H show further connectivity with methyl protons (16-H at 1.19 ppm) and each of methine proton (5-H at 3.71 ppm), methyl protons (17-H at 1.05 ppm), respectively. From

Table 1. ^{1}H NMR chemical shifts of SRM-A ($CDCl_3$, 400 MHz, 27°C).

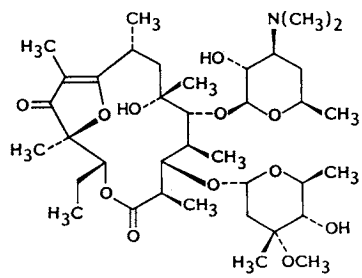
Position	δ (ppm)	J (Hz)	Position	δ (ppm)	J (Hz)
15	0.89 (3H, t)	7.32	7b	2.12 (1H, dd)	2.69, 14.90
17	1.05 (3H, d)	7.32	2″b	2.31 (1H, m)	
16	1.19 (3H, d)	6.84	$N(CH_3)_2$	2.33 (6H, s)	
7″	1.23 (3H, s)		2	2.46 (1H, dq)	5.86, 6.83
18	1.25 (3H, s)		3′	2.60 (1H, ddd)	3.91, 10.25, 12.21
6′	1.27 (3H, d)	6.34	8	2.99 (1H, m)	
6″	1.30 (3H, d)	6.34	4″	3.03 (1H, d)	9.28
4′a	1.34 (1H, m)		OCH_3	3.29 (3H, s)	
21	1.37 (3H, s)		2′	3.36 (1H, dd)	7.33, 10.25
19	1.37 (3H, d)	6.83	5′	3.64 (1H, m)	
2″a	1.55 (1H, dd)	4.88, 15.14	5	3.71 (1H, d)	4.88
4	1.66 (1H, m)		3	4.03 (1H, dd)	2.93, 5.37
4′b	1.71 (1H, m)		5″	4.09 (1H, dq)	6.35, 9.28
20	1.74 (3H, s)		1′	4.50 (1H, d)	7.32
14a	1.80 (1H, m)		1″	4.81 (1H, dd)	
7a	1.86 (1H, dd)	6.84, 15.14	13	5.01 (1H, dd)	3.42, 10.74
14b	2.00 (1H, m)				

Table 2. ^{13}C NMR chemical shifts of SRM-A ($CDCl_3$, 100 MHz, 27°C).

Position	δ (ppm)	Position	δ (ppm)	Position	δ (ppm)
20	6.00 (q)	8	31.79 (d)	6	74.76 (s)
15	10.72 (q)	2″	35.05 (t)	4″	77.54 (d)
17	10.93 (q)	$N(CH_3)_2$	40.42 (q)	13	77.93 (d)
16	14.06 (q)	7	41.82 (t)	3	78.55 (d)
6″	17.67 (q)	4	43.05 (d)	5	86.28 (d)
21	20.62 (q)	2	46.31 (d)	12	87.14 (s)
6′	21.01 (q)	OCH_3	49.29 (q)	1″	96.57 (d)
19	21.10 (q)	3′	64.65 (d)	1′	104.80 (d)
14	21.34 (t)	5″	66.17 (d)	10	108.58 (s)
7″	21.53 (q)	5′	69.69 (d)	1	175.90 (s)
18	26.37 (q)	2′	70.58 (d)	9	193.02 (s)
4′	29.13 (t)	3″	72.80 (s)	11	204.96 (s)

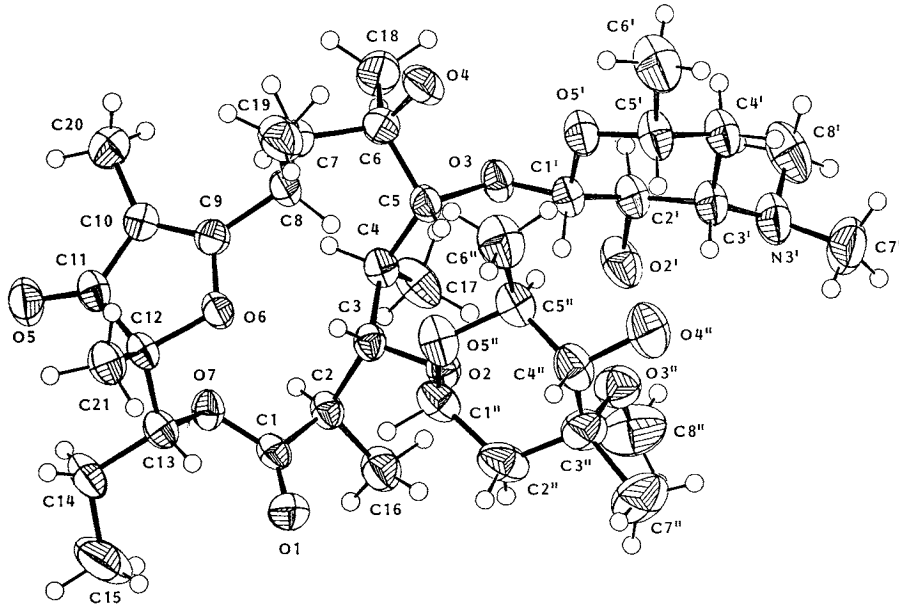
these data, partial structures of the aglycon moiety were assigned. Similarly, cross peaks were observed from anomeric proton 1′-H at 4.50 ppm to the methyl protons 6′-H at 1.27 ppm through 2′-H (3.36 ppm), 3′-H (2.60 ppm), 4′-H_a (1.34 ppm), 4′-H_b (1.71 ppm) and 5′-H (3.64 ppm). From these data, the skeletal structure of desosamine was deduced. By repeating the same procedure, the connectivities between the anomeric proton 1″-H at 4.81 ppm and the methylene protons 2″-H_a (1.55 ppm), 2″-H_b (2.31 ppm) and from the methine proton 4″-H at 3.03 ppm to the methyl protons 6″-H (1.30 ppm) through 5″-H (4.09 ppm) were observed in cladinose.

Fig. 5. The absolute structure of SRM-A.



The connectivities of these partial structures were solved by clear correlations observed in the ^{1}H-^{13}C long-range COSY spectrum (Fig. 3). That is, long-range couplings were observed between carbonyl carbon

Fig. 6. An ORTEP drawing of SRM-A.



C-1 and 16-H; anomeric carbon C-1″ and 3-H; C-3″ and each of 2″-H_b, 7″-H, OCH_3; C-4″ and 7″-H; anomeric carbon C-1′ and 5-H; C-3′ and $N(CH_3)_2$; C-6 and 18-H; C-7 and 18-H; enone carbon C-9 and each of 7-H_b, 20-H; quarternary carbon C-10 and 20-H; carbonyl carbon C-11 and each of 20-H, 21-H; quarternary carbon C-12 and 21-H; C-13 and 21-H (Fig. 4).

Based on the data presented, we proposed the structure shown in Fig. 4 for SRM-A, and all the signals were assigned as shown in Tables 1 and 2.

In the CI-MS spectrum, the fragment ions *m/z* 556, 381 and 158 may be attributable to the aglycon-*O*-desosamine (loss of cladinose), aglycon-H and desosamine, respectively. Strong absorption bands at 1740 and 1690, 1620^{-1} found in the IR spectrum were assignable to ester carbonyl and enone functions, respectively.

In order to confirm the molecular structure, X-ray crystallographic analysis was carried out on a single crystal. The molecular structure of SRM-A is shown in Fig. 5. An ORTEP drawing of SRM-A is shown in Fig. 6. The absolute structure of SRM-A was concluded to be that of 9,10-didehydro-9-deoxo-11,12-dideoxy-9,12-epoxy-11-oxoerythromycin A.

After the completion of this work, we became aware of abstracts (No. 1028, page 276) of the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, reported by Abott Laboratories[7]. This abstract summary describes a macrolide antibiotic of the same structure as that described here for SRM-A. Although, SRM-A was discovered in the fermentation broth of *Sacchropolyspora* sp. L53-18, this compound has also been synthesized by chemical modification of erythromycin A[7].

Experimental

Spectroscopic Studies

FAB-MS and CI-MS were measured on a Jeol JMS-SX 102 and a Jeol JMS-D 300 double focusing spectrometer, respectively. IR spectra were recorded on a Shimadzu FTIR-4200 spectrophotometer.

^{1}H and ^{13}C NMR spectra were recorded on a Jeol JNM-GSX 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer in $CDCl_3$.

X-Ray Crystallographic Study

SRM-A was recrystallized from methanol as transparent prisms. A crystal of the approximate dimensions $0.4 \times 0.2 \times 0.2$ mm was mounted on a Mac Science MXC-18 X-ray diffractometer with graphite monochromated CuKα radiation ($\lambda = 1.54178$ Å) and a 15 kW rotating anode generator.

The crystal data are as follows: $C_{37}H_{63}O_{12}N \cdot H_2O$, MW = 731.93, orthorhombic, space group $P2_12_12_1$, $a = 13.715$ (5), $b = 30.51$ (1), $c = 9.882$ (3) Å, $V = 4{,}135$ (4) Å^3, $Z = 4$, $D_{calc} = 1.18\ g \cdot cm^{-3}$.

Intensities were measured in the $2\theta/\omega$ scan mode with the scan speed 12°/second in ω. Backgrounds were measured at each end of the scan for half of the total scan time. A total of 3,380 reflections in the 2θ range $3° \sim 120°$ were measured. The structure was solved by direct method using MULTAN 84[8] program. In the final refinement, the non-hydrogen atoms were refined anisotropically by full matrix least square. Hydrogen positions were located by difference Fourier techniques. The hydrogen parameters were not refined. The final R value was 0.067.

Acknowledgments

We are grateful to Drs. T. SAITOH, K. MIZUNO and J. MURASE for their encouragement throughout this work.

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